ELECTROPHYSIOLOGICAL STUDIES ON THE 
PHARMACOLOGY OF THE PROCESSING OF 
INFLAMMATORY NOCICEPTION IN THE RAT 

by 
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ABSTRACT

I have recorded the responses of nociceptive dorsal horn neurones to acute electrical and prolonged inflammatory stimuli in intact halothane anaesthetised rats. Dorsal horn neuronal responses to peripheral injection of formalin were biphasic: the first phase lasted from 0-10 minutes and the second from 10-60 minutes.

Studies with peripheral local anaesthesia showed the second phase of the formalin response to be driven by afferent activity. Both phases of the formalin response were equally inhibited by intrathecal local anaesthetic.

Peripherally, both phases of the formalin response were partly mediated by bradykinin acting at the B\textsubscript{2} receptor, with prostaglandins contributing to the second phase only.

Spinal B\textsubscript{2} receptor antagonism reduced the second phase of the formalin response. Prostaglandins were shown to contribute to the spinal processing of both phases of the response.

The AMPA but not the NMDA receptor for the excitatory amino-acids plus the neurokinin-1 receptor were shown to be involved in the spinal processing of the first phase. NMDA mediated wind up and NK1 receptor activation contributed to the second phase of the formalin response. Intrathecal administration of somatostatin and the analogue, sandostatin, did not influence acute nociceptive responses but inhibited the formalin response. Intrathecal morphine inhibited both the acute nociceptive responses and the formalin response.

Multiple peripheral and central transmitters therefore contribute to the full manifestation of the formalin response.

The responses of spinal neurones after ultraviolet irradiation of the hindpaw were studied and compared to controls. The dorsal horn neurones now exhibited spontaneous activity and whereas the C-fibre related responses were unaltered the A\textsubscript{B}-fibre responses were enhanced above the control. Drug effects were not different from control animals except that A\textsubscript{B}-fibre evoked responses were inhibited by NMDA receptor antagonism and wind up of the neurones was inhibited by B\textsubscript{2} receptor antagonism.

The aim of these studies was to investigate the peripheral and spinal pharmacology of nociceptive transmission in different animal models of pain.
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CHAPTER 1:

INTRODUCTION
Activation of nociceptors in the periphery results in the generation of noxious inputs which converge in the dorsal horn of the spinal cord. The deep convergent dorsal horn neurones provide a central focus for the incoming noxious inputs and play a major role in the processing of nociceptive information. Within the spinal cord nociceptive processing is not static and noxious messages can be modulated by inhibitory systems and enhanced by excitatory systems. Consequently, the processing of nociceptive information can be modified and this capacity to change, termed plasticity, can occur over a short time course and may occur in pain states which cannot be relieved by conventional therapy. Studies of the peripheral and spinal pharmacology of nociceptive processing in different animal models of pain may lead to novel analgesic therapies for clinical pain states.

1.1 The properties of the primary afferents

The primary afferents have been divided into three categories, Aβ-fibres, Aδ-fibres and C-fibres. C-fibres are small diameter, 0.2-15 μm (Martin 1989) unmyelinated afferents (Gasser and Erlanger 1927) which mainly transmit noxious information. The C-fibres have conduction velocities in the region of 0.49-0.89 m/s in the rat (Lynn and Carpenter 1982, Handwerker et al. 1987, Leem et al. 1993) and do not show spontaneous activity. It has been shown that 73% of C-fibres are polymodal nociceptors with small receptive fields which respond to noxious heat and mechanical stimulation (Lynn and Carpenter 1982). The remainder are sensitive mechanoreceptors, cold thermoreceptors or insensitive (Lynn and Carpenter 1982).

Recently, a class of nociceptors has been identified which do not respond to conventional noxious stimuli and under normal conditions are non-responsive. Current opinion suggests 20-40% of unmyelinated afferents in rat skin are silent (Handwerker et al. 1989). These nociceptors have been termed silent nociceptors (or sleeping nociceptors or mechanically insensitive nociceptors). The silent nociceptors are responsive to noxious chemical stimuli and become responsive during inflammation (see refs. in MacMahon and Koltzenburg 1990). Under normal circumstances the mechanism for signalling an enhanced stimulus intensity involves temporal summation of a constant population of nociceptors. In contrast, during inflammation the silent nociceptors are activated and spatial summation of these normally silent nociceptors may enhance the intensity of the
noxious input from the periphery to the spinal cord.

The Aδ-fibres have been classified as high threshold mechanoreceptive (HTM) neurones, responding to moderately intense noxious mechanical stimuli although some also respond to chemical or thermal stimuli (Besson and Chaouch 1987, Perl 1968). The Aδ-fibres are larger diameter (1-5 μm) myelinated afferents (Martin 1989) with conduction velocities in the range of 1.9-11.2 m/s (Handwerker et al. 1987).

The Aβ-fibres are large diameter (6-12 μm) myelinated afferents which respond to innocuous stimuli such as brush, touch and pressure (Martin 1989). These afferents have the lowest thresholds of the primary afferents and conduction velocities in the range of 4-44 m/s with rapidly adapting responses (Lynn and Carpenter 1982).

1.2 Activation of the peripheral C-fibre nociceptor

The polymodal C-fibre nociceptors are fine undifferentiated free afferent nerve endings which respond to high intensity stimulation such as noxious heat, noxious mechanical and noxious chemical stimuli. They have the capacity to increase their discharge to repetitive stimulation and may continue responding after stimulation has ceased (see refs. in Besson and Chaouch 1987).

Noxious stimuli can result in tissue injury or trauma and low tissue pH. Protons have an excitatory effect on neurones and produce a brief depolarisation of the neurones which inactivates after a few seconds. In contrast, protons not only briefly depolarize C-fibre afferents but also produce a maintained depolarisation of these afferents. This maintained depolarisation is associated with an increased ionic conductance and an inward current (see refs. in Rang et al. 1991). Inflammatory exudates are usually acidic and these elevated levels of protons probably contribute to the activation of C-fibres and the relay of noxious signals from the periphery to the spinal cord. Tissue injury after a noxious stimulus also results in the generation of bradykinin (Dray and Perkins 1993). Bradykinin is an inflammatory mediator which is liberated from vascular kininogen by the plasma kallikreins after tissue injury or trauma (see refs. in Steranka et al. 1988). Bradykinin is released into the surrounding tissue due to the increased permeability of microvasculature and has been shown to be algesic (Lim et al. 1969, Whalley et al. 1987, Jensen et al. 1990). Bradykinin has been shown to induce oedema formation via an
action at the B_2 receptor and this effect is potentiated by prostaglandins (Neppl et al. 1991). In addition, the algesic effect of bradykinin is mediated via actions at the bradykinin B_2 receptors on the C-fibre afferents (see refs. in Regoli and Barabe 1980 and Bathon and Proud 1991). The B_2 receptors have been shown to be coupled to phospholipase C and phospholipase A_2 which then leads to the modulation of ion channels, resulting in depolarisation of the nociceptors and finally the relay of noxious signals from the periphery to the spinal cord (see refs. in Dray and Perkins 1993). In addition to this direct action of bradykinin on the C-fibres, bradykinin also induces the release of histamine from mast cells (Lawrence et al. 1989) and stimulates the production of the prostaglandins via phospholipase A_2 activation (Lembeck et al. 1976, Juan 1977).

Activation of the peripheral nociceptors by noxious mediators, such as bradykinin, can evoke an axon reflex release of neuropeptides such as substance P, neurokinin A and CGRP from the peripheral endings of the C-fibres (see refs. in Yaksh and Hammond 1982, Dray and Perkins 1993). The peripherally released neuropeptides, substance P and neurokinin A have been shown to sensitize nociceptors and contribute to the development of neurogenic inflammation (see refs. in Yaksh and Hammond 1982, Dray and Perkins 1993, Levine et al. 1993).

5-hydroxytryptamine (5HT), released from platelets and mast cells during tissue damage has been shown to sensitize nociceptors (Lang et al. 1990). In addition, subthreshold doses of 5HT have been shown to sensitize nociceptors to bradykinin (Lang et al. 1990), this effect can be inhibited by 5HT_3 receptor antagonists (see refs. in Rang et al. 1991). Moreover, the 5HT_3 receptor has been shown to be directly coupled to Na^+ channels and via this action can directly activate the peripheral nociceptors (see refs. Rang et al. 1991). There appears to be a functional role of peripheral 5HT during inflammatory nociception since 5HT_3 receptor antagonists have been shown to inhibit carrageenan induced hyperalgesia without affecting the associated oedema (see refs. Rang et al. 1991).

The role of the prostaglandins in the sensitization of peripheral nociceptors during inflammation is well established (Moncada et al. 1975). There are 5 natural prostaglandins: prostaglandin D, prostaglandin F, prostacyclin, thromboxane and prostaglandin E, with their respective receptors being termed DP, FP, IP, TP and EP_1,2,3. The second messenger systems linked to the activation of the prostaglandin EP receptors have been established; the prostaglandin EP_1 receptor is coupled to IP_3 / DG, the prostaglandin EP_2 receptor is coupled to increased cAMP and the prostaglandin EP_3
receptor is coupled to both IP$_3$ / DAG and decreased cAMP (TIPS Receptor Nomenclature 1993). Prostaglandins do not always evoke pain responses but are thought to sensitize the C-fibre nociceptors to bradykinin and natural stimulation (Chahl and Iggo 1977, Lang et al. 1990, see refs. in Levine et al. 1993). Prostaglandin induced hyperalgesia has been shown to be dependent on cAMP but not protein kinase C (see refs. in Simone 1992).

The importance of nerve growth factor (NGF) during inflammatory nociceptive processing has become increasingly apparent and has recently been reviewed (Lewin and Mendell 1993). One of the major sources of NGF are the skin keratinocytes. Levels of NGF have been shown to be dramatically increased during inflammation and systemic administration of NGF has been shown to result in profound heat and mechanical hyperalgesia (see refs. in Lewin and Mendell 1993). The effects of NGF on sensory neurones during inflammation are generally considered to be two fold. Firstly, NGF stimulates mast cell degranulation and the subsequent release of mast cell products activate and / or sensitize primary sensory afferents. The second effect is more direct. It has been proposed that NGF may bind to its receptor on the primary afferent terminals and be retrogradely transported to the cell body. This has been suggested to result in the upregulation of neuropeptides, such as SP and CGRP, in the cell body of the primary afferent. Increased levels of neuropeptides in sensory neurones have been observed in models of inflammatory pain (see refs. in Lewin and Mendell 1993). This theory is important, since it suggests a possible mechanism by which peripheral inflammatory nociceptive events which do not cause ongoing C-fibre discharge may influence the spinal processing of nociceptive information. This may result in long lasting changes or plasticity in the processing of nociceptive information in the spinal cord.

The discovery of a role of nitric oxide (NO) during nociceptive transmission has resulted in considerable scientific excitement. NO is generated by bradykinin and histamine in peripheral tissue and it has been proposed that NO may be involved in activation of nociceptors by bradykinin (Haley et al. 1992). Peripheral administration of L-NAME has been shown to inhibit the second phase of the formalin response, but this effect may be due to L-NAME causing vasoconstriction and consequently decreasing the inflammatory response as opposed to L-NAME having a direct antinociceptive effect.
1.3 Termination of the primary afferents in the dorsal horn

The cell bodies of the primary afferents are located in the dorsal root ganglia. Lissauer's tract (dorsolateral fasiculus) overlies the grey matter of the spinal cord and contains fine myelinated and unmyelinated axons which traverse into the grey matter of the spinal cord (see refs. in Fitzgerald 1989). Over two thirds of the axons of Lissauer's tract have been shown to be primary afferents at the lumbosacral level of the spinal cord in the rat (Chung et al. 1979). The primary afferents have been shown to enter the spinal cord via both the dorsal and ventral roots (see refs. in Fitzgerald 1989).

The dorsal horn is organised into ten laminae in the cat (Rexed 1954). Studies have shown a similar organization of the spinal cord cells in the rat (Molander et al. 1984). Lamina I neurones form a thin rim of the dorsal curvature, underlying this lamina II consists of a tightly packed outer zone (IIo) and a more loosely packed inner zone of neurones (IIIi). Lamina III to V then follow in a ventral progression in sequential order. The remainder of laminae are more scattered.

Generally it is thought the C-fibre collaterals terminate superficially within laminae I and III (Light et al. 1979, see refs. in Hunt et al. 1982a). The Aδ-fibres terminate within Laminae I and IIo and in the deeper laminae IV-VI (Light and Pearl 1979, Mense and Prabhakar 1988). Aβ-fibre collaterals form longitudinal sheets through the rostral caudal axis of the spinal cord and terminate in laminae III-V (see refs. in Fitzgerald 1989).

1.4 The relay of information from the primary afferents to the dorsal horn neurones

Numerous studies have shed light on the arrangement of synaptic connections between the primary axons and the lamina I and II neurons (see refs. Gobel et al. 1982, Rethelyi et al. 1989). The afferents relay inputs to the dendritic spines and small dendritic shafts of the lamina I and II neurons via axodendritic synapses. Some of these dendrites contain vesicles and form dendrodendritic synapses on adjacent dendrites and dendroaxonic synapses onto the primary ending (Gobel et al. 1980). The dendritic arbors of lamina I neurons are largely confined to within this lamina and the axons of the lamina I neurones enter the white matter and ascend to higher brain centres (Kumazawa et al.
Interneurones exist in discrete populations within lamina II (substantia gelatinosa) of the dorsal horn, these interneurones have been divided into stalk cells and islet cells (see refs. in Gobel et al. 1982). The cell bodies of the stalk cells are found in lamina Ilo and at the I / IIo border. The dendrites of the stalk cells not only traverse lamina II but may also enter lamina III and their axons ascend into lamina I and form extensive axonal arbors (Gobel et al. 1980, Bennett et al. 1980). Stalk cells have been shown to be either nociceptive specific or wide dynamic range neurons (Bennett et al. 1980) which may serve to be excitatory interneurones transferring information from lamina II and lamina III neurons to the dendrites of lamina I neurons.

The islet cell bodies are located in clusters within lamina Ilo and IIi, both the dendritic and axonal arbors of these cells are confined to lamina II and extend rostrocaudally (Gobel et al. 1980, Bennett et al. 1980). Islet cells are thought to function as inhibitory interneurones, inhibiting the transfer of inputs by stalk cell dendrites to the lamina I neurons via dendrodendritic synapses and axodendritic synapses on stalk cells (Gobel et al. 1980). In addition, islet cells are thought to diminish or inhibit transmitter release from primary endings via dendroaxonic synapses (Gobel et al. 1980). Since the islet cells could modulate the stalk cells which in turn influence the projection neurons, the islet cells may indirectly modify the output from the dorsal horn. More recently, islet and stalk cells in the rat and monkey have been shown to send deep projecting myelinated parent axons and unmyelinated branches into laminae III-VI which arborize and terminate on the deep cell dendrites (Light and Kavookjian 1988).

Deeper in the dorsal horn, laminae V-VII neurones send dendrites both dorsally into the laminae I, II, III and IV and ventrally (Woolf and King 1987). The dendrites bifurcate generating many fine branches within the superficial laminae of the dorsal horn (Ritz and Greenspan 1985, Woolf and King 1987). These dendrites receive inputs from the superficial neurones (Ritz and Greenspan 1985, Woolf and King 1987). Since not all deep convergent neurones have dendrites which extend into laminae I and II these neurones must also receive nociceptive inputs via interneurones (Ritz and Greenspan 1985). Dendritic branches are also sent into the dorsal columns and Lissauer’s tract as well as traversing ventrally through the neck of the dorsal horn. The axons of these deep neurones have many collaterals throughout the deeper laminae which finally cross the midline and ascend to higher brain centres (see refs. in Fitzgerald 1989).
1.5 The primary afferent transmitters

The excitatory amino acids and a number of peptides fulfill many of the criteria required for neurotransmitters and there is considerable evidence for the involvement of the excitatory amino acids, glutamate and aspartate, and a number of peptides in nociceptive transmission in the dorsal horn of the spinal cord (see refs. in Salt and Hill 1983, Besson and Chaouch 1987, Evans 1989). The peptides implicated in nociception consist of the tachykinin family of peptides, calcitonin gene-related peptide, somatostatin, vasoactive intestinal polypeptide, cholecystokinin, galanin, bombesin and neurotensin (Yaksh and Hammond 1982, Salt and Hill 1983, Morton and Hutchinson 1989, Merighi et al. 1991, Levine et al. 1993).

The spinal cord is known to have high levels of glutamate immunoreactivity, originating from myelinated and unmyelinated primary afferent fibres in addition to intrinsic interneurones and projection neurones (Battaglia and Rustioni 1988, Miller et al. 1988, Maxwell et al. 1990). The actions of the excitatory amino acids have been extensively shown to be mediated via the N-methyl-D-aspartate (NMDA) receptor and non-NMDA receptors: the \(\alpha\)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor, the metabotropic and the kainate receptor (see refs. in Watkins et al. 1991, Barnard and Henley 1991).

In vitro studies have shown that both A\(\beta\) and C-fibre activation increases aspartate and glutamate outflow at the level of the spinal cord (Jeffinija et al. 1991, Kangrga and Randic 1991, see refs. in Headley and Grillner 1991). These results were confirmed by in vivo microdialysis studies which have shown increased extracellular concentrations of glutamate and aspartate in the lumbar spinal cord during formalin induced nociceptive behaviour (Skilling et al. 1988).

Substance P (SP) has long been implicated as one of the neurotransmitters involved in nociceptive transmission (see refs. in Henry 1980, Pernow 1983, Otsuka and Yanagisawa 1987). Dorsal horn SP has been shown to originate from primary afferent fibres (Hokfelt et al. 1975) and intrinsic neurones together with a contribution from descending fibres (Jessell et al. 1979, Yaksh et al. 1988). Currently there are three subclasses of tachykinin receptors: neurokinin-1, neurokinin-2, neurokinin-3. SP has been shown to be the preferred tachykinin at the neurokinin-1 receptor (see refs. in
The neurokinin receptors have been shown to be postsynaptic to the afferent fibre terminals, located in laminae I, II and X of the dorsal horn of the spinal cord (Yashpal et al. 1991). The release of SP into the dorsal horn by peripheral noxious stimuli has been shown in a number of studies (Kuraishi et al. 1985a, Duggan and Hendrey 1986, Duggan et al. 1988, Go and Yaksh 1987).

The distribution of somatostatin within the dorsal horn of the spinal cord has led to speculation that somatostatin has a role in the processing of nociceptive inputs. The peptide is present in small diameter cells in the dorsal root ganglion (Tuscherer and Seybold 1985) and afferent terminals in the substantia gelatinosa of the spinal cord (Hokfelt et al. 1976). Spinal somatostatin originates from the primary sensory neurons, in addition to the ascending and descending pathways (Stine et al. 1982). The dorsal horn somatostatin content has been shown to be fifteen times higher than the level of somatostatin in the ventral horn (Stine et al. 1982). The highest content of somatostatin in the dorsal horn has been shown in lamina II (Polack and Bloom 1986). Noxious thermal stimulation has been shown to increase the release of somatostatin within the dorsal horn of the spinal cord (Kuraishi et al. 1985a). In vitro studies have shown that iontophoretic application of somatostatin results in the hyperpolarisation of dorsal horn neurones and a reduction in spontaneous firing (Murase et al. 1982). This suggests somatostatin has an inhibitory role in the dorsal horn.

Galanin is a putative inhibitory peptide which is co-localised with SP and CGRP in a large proportion of primary afferents. Studies of thermal nociception have indicated galanin has antinociceptive effects, but there is also evidence that galanin has pro-nociceptive effects (see refs. in Levine et al. 1993). Further studies of the role of galanin during nociceptive transmission and the development of selective antagonists are necessary for the further understanding of the role of this peptide during nociceptive transmission.

Vasoactive intestinal polypeptide (VIP) is known to be in sacral visceral afferents (see refs. Fitzgerald 1989). Surprisingly electrical stimulation of the sciatic nerve has been shown to increase VIP immunostaining in the dorsal horn (Klein et al. 1992). Therefore although VIP is present in the primary sensory neurones (Ju et al. 1987) its role during the spinal nociceptive processing of noxious cutaneous stimuli is unknown. Only with the development of selective VIP antagonists will this mystery be unravelled.
A comparatively new putative nociceptive transmitter is nitric oxide (NO). Recently many studies have provided much indirect evidence for a spinal role of NO during prolonged nociceptive transmission, recently reviewed by Meller and Gebhart 1993. NO appears to have a role during prolonged chronic pain states which are associated with NMDA receptor activation. For example, the nitric oxide synthase antagonist, L-NAME and the NMDA receptor antagonist AP5 have been shown to abolish facilitated reflexes without affecting baseline nociceptive reflexes. In addition, intrathecal L-NAME has been shown to inhibit thermal hyperalgesia in neuropathic animals but not sham animals (see refs. in Meller and Gebhart 1993). Moreover, intrathecal L-NAME has been shown to inhibit the response of single dorsal horn neurones to a peripheral injection of formalin (Haley et al. 1992). It has been proposed that NMDA receptor activation and the associated Ca^{2+} influx results in the generation of NO. Currently there are a number of proposed sites of action of NO: as a retrograde transmitter influencing pre-synaptic neurones, on adjacent glial cells or neurones (see refs. in Meller and Gebhart 1993). It has been suggested that NO may be involved in the long term changes associated with spinal hyperalgesia such as early gene expression, but there is little direct evidence.

1.6 The dorsal horn nociceptive neurones

The nociceptive neurones are the nociceptive-specific and the convergent neurones. The nociceptive specific neurones are located in laminae I and II and to a much lesser extent in the deeper laminae. These nociceptive specific neurones are activated exclusively by noxious stimulation of the Aδ- and C-fibres. The more numerous convergent neurones are found both in the deep laminae IV-VI as well as in the superficial laminae (see refs. in Besson and Chaouch 1987). In contrast with the nociceptive specific neurones, the convergent neurones respond to both innocuous and noxious stimuli since they receive Aβ-, Aδ- and C-fibre inputs. The convergent neurones exhibit temporal and spatial summation which encodes the intensity of the inputs received (Woolf 1991). With repetitive C-fibre stimulation the convergent neurones exhibit enhanced responses which are not in proportion to the C-fibre afferent input. In addition, these responses outlast the duration of the noxious stimulus. These enhanced nociceptive responses have been termed 'wind up' (Mendell 1966). The convergent neurones have been shown by antidromic stimulation to give rise to ascending axons (see refs. in Willis 1988, 1989).
1.7 The relay of noxious inputs from the spinal cord to higher brain centres

The projection or spinothalamic neurones of the dorsal horn have been shown by antidromic activation to be located in laminae I, V and VI. The spinothalamic neurones respond to noxious mechanical, noxious heat and noxious chemical stimuli but the greatest proportion also respond to innocuous stimuli and are therefore convergent neurones (see refs. in Willis 1988, 1989). The anterolateral quadrant contains three separate somatosensory pathways, the spinoreticular tract, spinothalamic tract and the spinotectal tract (see refs. in Martin 1989). These pathways have been shown to terminate in the medial and lateral thalamus and reticular formation (Giesler et al. 1979 and see refs. in Kevetter et al. 1983). In particular, the spinothalamic tract transmits information to the ventral posterior lateral nucleus of the thalamus and then to the primary somatic sensory cortex in the parietal lobe (see refs. in Martin 1989). The projection of the spinothalamic tract to the ventral posterior lateral nucleus of the thalamus is thought to be important in the discriminative aspects of somatic sensations. In addition, a number of the spinothalamic tract neurones terminate more medially in the intralaminar nuclei of the thalamus. This projection and the spinoreticular tract neurones which terminate in the reticular formation are thought to be important in the motivational and affective aspects of somatic sensation and in maintaining arousal. The spinotectal tract terminates in the midbrain tectum (see refs. in Martin 1989).

1.8 The effects of descending controls on nociceptive transmission

Descending controls from the higher brain centres to the spinal cord provide a mechanism for the modulation of sensory events in the dorsal horn by the higher brain centres. Both supraspinal opiate produced analgesia and brain stimulation produced analgesia operate via these descending pathways (see refs. in Besson and Chaouch 1987). The descending pathways are located in the dorsolateral funiculus (DLF) of the spinal cord and have been shown to originate from numerous brainstem neuronal groups (see refs. in Willis 1988, 1989). Neurones in the nucleus raphe magnus (NRM) send descending fibres via the DLF, the source of serotonergic (5-HT) actions on the dorsal horn neurones (see refs. Willis 1982, Dubner et al. 1984). In addition, there is a small
direct contribution of descending fibres from the periaqueductal grey (PAG) but the bulk of the ventral projections of the PAG are to the NRM (Basbaum and Fields 1979). Stimulation of the PAG and NRM can selectively inhibit the responses of the dorsal horn neurones to noxious inputs without influencing the innocuous responses suggesting a pre-synaptic inhibition of noxious inputs (see refs. in Carstens 1987). In addition, direct descending 5-HT postsynaptic inhibitions directly modify the activity of lamina II interneurones as well as lamina I projection neurones (Dubner et al. 1984).

There is also a descending inhibitory system which originates from the catecholamine containing nuclei in the locus coeruleus, subcoeruleus region with small contributions from the A5 neurone group, the Kolliker-Fuse nuclei and parabrachial nuclei (see refs. in Westlund 1992). The catecholamine terminals have been shown to terminate in the superficial dorsal horn of the spinal cord (see refs. in Headley 1992, Fleetwood Walker 1992). Intrathecally administered catecholamines have been shown to selectively inhibit the nociceptive responses of spinothalamic tract neurones (see refs. in Besson Chaouch 1987). There is evidence that the α2 receptor subtype has a role in the modulation of nociceptive transmission (see refs. in Headley 1992, Fleetwood Walker 1992). The superficial laminae have been shown to have a high concentration of α2 receptors of which approximately 20-30% were located pre-synaptically on the primary afferents. The location of the α2 receptors possibly explains the selective action of the catecholamines on nociceptive processing (see refs. in Headley 1992, Fleetwood-Walker 1992). Although there is indirect evidence for an antinociceptive role of the catecholamine descending inhibitory system, the functional role of the catecholamines and their contribution to the tonic descending inhibitory systems still remains unknown.

Diffuse noxious inhibitory controls (DNIC) are partly serotonergic descending controls proposed to differentiate and filter the noxious responses of convergent neurones from the innocuous background responses. The initial noxious relay from the spinal cord to the higher brain centres activates DNIC which selectively inhibits the background activity of the convergent neurones not receiving a noxious input. Consequently there is a dampening effect on the background innocuous activity, producing a greater contrast of the noxious response of the convergent neurones (Le Bars et al. 1983, 1986).
1.9 The intrinsic pharmacology of the dorsal horn

Glutamate immunoreactivity has been observed in cell bodies and dendrites within laminae I and II of the dorsal horn, indicating the presence of intrinsic glutamate within the dorsal horn (Miller et al. 1988). A more recent study has separated metabolic glutamate from transmitter pools within the dorsal horn and in some instances glutamate co-exists with GABA (Merighi et al. 1991). Both glutamate and SP are thought to be the major intrinsic excitatory transmitters of the dorsal horn especially since CGRP is not found intrinsically within the dorsal horn (see refs. in Levine et al. 1993). Cytochemical studies of the dorsal horn have shown some lamina II stalk cells contain GAD immunocytochemistry indicating that they are inhibitory GABA-ergic neurones (Hunt et al. 1982b). In addition, some stalk cells contain enkephalin-like immunoreactivity and may function as inhibitory interneurones (see refs. in Gobel et al. 1982). More recently, colocalised SP and enkephalin-like immunoreactivity have been shown in lamina II neurones thought to be stalk cells (Ribeiro-da-Silva et al. 1991). Histochemical studies have also shown that some islet cells are GABAergic and some also contain glycine but there is also a population of smaller islet cells which do not contain GABA or glycine (Spike and Todd 1992). In addition, there is evidence for intrinsic SP, somatostatin and neurotensin-positive cells within lamina II (see refs. Hunt et al. 1982b).

GABAergic neurones have also been characterized in lamina III. These neurones have dendritic trees orientated along the rostrocaudal axis with some extensions into lamina IV and II. These neurones resemble the islet cells of lamina II (Powell and Todd 1992). Many of the lamina III neurones have been shown to contain both GABA and glycine (Todd 1990). These neurones are thought to be activated mainly by low threshold mechanoreceptors and exert inhibitory effects on the deep convergent neurones as well as having a pre-synaptic action on the primary afferents (Powell and Todd 1992).

Enkephalin is a naturally occurring opioid with a preferential binding affinity for the delta receptor. It is found throughout the dorsal horn but is most heavily concentrated in the superficial laminae (Hokfelt et al. 1977, Gibson et al. 1981). The majority of enkephalin is derived from intrinsic neurones since there is very little change in the dorsal horn content after spinal transection (see refs. in Besson and Chaouch 1987). Importantly the lamina I enkephalin-containing neurons are not projection neurons (see...
The enkephalin-containing lamina I neurons in addition to the stalk cells may modulate the outputs of the laminae I projection neurons. Modulation of nociceptive response by non-noxious inputs may involve the activation of the low threshold mechanoreceptive enkephalin-containing islet cells (Dubner et al. 1984). The levels of spinal enkephalin may change in certain circumstances. A greater than 200% increase in the levels of preproenkephalin mRNA has been shown within laminae I-II, V-VI and VII during carrageenan inflammation (Noguchi et al. 1992).

Dynorphin, the preferential endogenous agonist at the kappa receptor is also present in the superficial dorsal horn. Under normal conditions the level of dynorphin within the dorsal horn is up to twenty times lower than the level of enkephalin (see refs. in Noguchi et al. 1992). Increased levels of preprodynorphin mRNA and the dynorphin peptide have been observed on the ipsilateral side following the development of peripheral inflammation. The expression of dynorphin parallels the development of behavioural hyperalgesia in models of chronic inflammation (Dubner and Ruda 1992). The elevated levels of dynorphin within the dorsal horn are thought to contribute to the development of central hypersensitivity and the enhancement of the peripheral receptive fields observed in models of chronic inflammation (Dubner and Ruda 1992).

1.10 The localisation of the endogenous opioids and binding sites within the dorsal horn

The endogenous opioids are composed of three families of peptides: enkephalins, dynorphins and endorphin (see refs. in Yaksh 1987, Dickenson 1991). Predominantly there are three classes of opioid receptors: \(\mu\), \(\delta\) and \(\kappa\) (Kosterlitz and Paterson 1985). The preferential endogenous ligands for the \(\mu\), \(\delta\) and \(\kappa\)-receptors are \(\beta\)-endorphin, met-enkephalin and dynorphins respectively (see refs. in Dickenson 1991, Yaksh 1987). Interestingly although high levels of \(\mu\)-receptors are found in the spinal cord the endogenous ligand endorphin is not found. The enkephalins have been shown to be present in the dorsal horn of the spinal cord (Hokfelt et al. 1977, Gibson et al. 1981).

The opioid receptors are concentrated in laminae I and II with smaller amounts in the deeper laminae (LaMotte et al. 1976, Atweh and Kuhar 1977, Gouarderes et al. 1985). The ratio of \(\mu\), \(\delta\) and \(\kappa\)-receptors in laminae I-II of the rat dorsal horn has been
estimated to be 70% / 24% / 6% (Besse et al. 1991). Whereas other studies have shown that the proportion of μ-receptors in this region to be considerably higher (Stevens et al. 1991). Rhizotomy studies have indicated that approximately 50-60% of the opioid receptors are pre-synaptic in the rat and primate (Ninkovic et al. 1981, Besson and Chaouch 1987, Zajac et al. 1989, Gouarderes et al. 1991).

1.11 The actions of opioid receptor activation on the dorsal horn neurones

Both μ- and δ-receptor activation has been shown to decrease C-fibre transmitter release, demonstrating a pre-synaptic loci of these receptors (Kangraga and Randic 1991). In vivo perfusion of morphine has been shown to dose-dependently inhibit capsaicin evoked release of substance P in the rat (Aimone and Yaksh 1989) and mechanically evoked release of substance P in the rabbit (Hirota et al. 1985). Since capsaicin selectively activates C-fibres, the study utilizing capsaicin evoked release of substance P implicates a pre-synaptic action of opioids. In addition, intrathecal morphine dose-dependently inhibited the electrically evoked release of spinal substance P in the cat (Yaksh et al. 1980). Inhibitory effects of enkephalin have been observed in superficial spinal cord slices, these effects consisted of a decreased frequency of miniature excitatory post synaptic potentials (mEPSP's) but the amplitude of the mEPSP's were not altered. In addition, enkephalin did not alter the responses to exogenous glutamate, suggesting a pre-synaptic site of action of enkephalin (Hori et al. 1992). In contrast, systemic morphine has been shown not to reduce the noxious evoked release of immunoreactive neurokinins in the dorsal horn of the spinal cat (Lang et al. 1991). The discrepancy between this last study and the numerous other studies which have indicated a pre-synaptic role of spinal opiates may be due to an insufficient dose of opiate being used or an insensitive probe for the neurokinins. Overall there is good evidence that activation of opioid receptors located pre-synaptically on the C-fibres directly inhibits transmitter release from the C-fibre terminals in the dorsal horn.

There is also considerable evidence for a post-synaptic site of the opioid receptors. Combined retrograde horseradish peroxidase and enkephalin immunocytochemical studies have suggested that 30% of lamina I and 50% of lamina V spinothalamic projection neurones receive enkephalin-immunoreactive varicosities (Ruda et al. 1984). In addition, rhizotomy studies have indicated that approximately 24% of μ-opioid receptors, 39% of
δ-opioid receptors and 47% of κ-opioid receptors are post-synaptic to the C-fibre terminals (Zajac et al. 1989, Besse et al. 1990). Post-synaptic opioid receptors are present in the superficial layers of the dorsal horn, located on the dendrites of the deep neurones which penetrate into the superficial layers to receive C-fibre inputs. In addition, post-synaptic receptors may also be located on interneurones. Therefore the relay of noxious inputs to the deep neurones by the dendrites and interneurones can be inhibited by the post-synaptic opiate receptors. Systemic morphine has been shown to inhibit hyperactivity of dorsal horn neurones in the deafferented rat and since all pre-synaptic opioids receptors are absent in this preparation this illustrates a post-synaptic action of opioids (Lombard and Besson 1989). Importantly, the inhibitory effect of systemic morphine on the spontaneous activity of dorsal horn neurones in the intact arthritic rat was found to be twice as effective as that observed in the deafferented rat (Lombard and Besson 1989).

The mechanism of action of the μ- and δ-receptors is via a G-protein or cAMP link which opens K^+ channels resulting in the hyperpolarisation of the neurones and/or decreased transmitter release at pre-synaptic sites (North et al. 1987, McFadzean 1988, Schroeder et al. 1991, Wimpey et al. 1991 and see refs. in Dickenson 1991). The κ-receptors produce inhibitory effects by closing Ca^{2+} channels (see refs. in Dickenson 1991). Both in vitro and in vivo studies have shown low doses of μ-preferring opioid agonists produce facilitations (Higashi et al. 1982, Mauborgne et al. 1987, Wiesenfeld and Duranti 1987 and refs. in Dickenson 1991). These facilitations observed with opioids may be due to a direct presynaptic action which results in a prolongation of action potentials (see refs. in Crain and Shen 1990, Fan et al. 1991). These effects of opioids on the action potential may then lead to the observed increase in release of excitatory transmitters by low doses of opioid. Recently it has been shown that some opioid receptors are directly linked to a stimulatory guanine nucleotide-binding protein (Gs) and stimulate adenylate cyclase (Cruciani et al. 1993). In addition, it has been proposed that μ-autoreceptors which modulate the release of opioid peptides may account for these low dose facilitations (Kayser et al. 1987, 1988). At higher opioid doses, which inhibit output neurones, SG interneurones are facilitated. The mechanism for this may be via opioid inhibition of inhibitory controls on inhibitory neurones, resulting in a release of inhibition (disinhibition) which further reinforces direct opioid inhibitions at pre- and
post-synaptic sites (Magnuson and Dickenson 1991).

1.12 Central hypersensitivity of the dorsal horn

The systems involved in the transmission of noxious messages within the dorsal horn are not static but have the capacity to change so exhibiting plasticity. One of the consequences of plasticity is the development of central hypersensitivity, the enhancement of dorsal horn neuronal responses to noxious inputs (Dickenson 1991, Woolf and Thompson 1991). One of the manifestations of central hypersensitivity is the development of wind up, which is comparable to long term potentiation (LTP) in the hippocampus (Madison et al. 1991). Both wind up and LTP mechanisms involve NMDA receptor mediated events (Dickenson 1991, Collingridge et al. 1983). The consequences of central hypersensitivity include the induction of early genes (Gogas et al. 1991) and the enhanced expression of mRNA for the endogenous opioids (Dubner and Ruda 1992). These intracellular changes in the level of expression of some genes may result in profound alterations in nociceptive transmission within the dorsal horn.

1.13 Animal models of nociception

The experience and sensation of pain is unique for each individual and different types of pain may be transmitted differently. Behavioural and electrophysiological studies of animal models of pain are necessary for a better understanding of the neuronal mechanisms involved in different pain states and the development of suitable analgesics which specifically diminish pain without producing side effects or affecting the perception of other sensations. The models of pain currently in use have been reviewed (see refs. in Wood 1984) and can be broadly separated into models of transient (acute) and phasic (prolonged) nociception. Some models of acute pain use noxious heat stimuli such as the tail flick test and hot plate tests. Electrical stimulation is also employed for a number of models of acute pain, including stimulation of the tail and the assessment of tail movement and vocalization. In the flinch-jump assay, the floor of the cage is electrically stimulated and the subsequent flinch, jump or vocalization of the animal is monitored. Reflex responses to noxious stimuli do have some problems since they are not a measure of pain.
sensation but of reflex behaviour and these reflexes can be elicited in the spinalised animal (see refs. in Dubner 1989). An additional factor is the dependence of the reflex responses on motor function which if impaired would give a false positive for analgesia.

In the clinical situation pain may last for long periods of time and so there is a need for animal models of prolonged nociception. The use of chemical stimuli has been employed in numerous models of phasic pain which last for varying periods of time. The chemical stimuli utilized for models of inflammatory pain include carrageenan, yeast, and formalin. The formalin model of inflammatory pain has a time course of an hour whereas the carrageenan model has a slightly longer duration with peak hyperalgesia occurring between 4 and 6 hours. Injections of Freud's adjuvant or sodium urate crystals into the ankle joint are used as model of monoarthritis. The inflammation and hyperalgesia induced by the injection of Freud's adjuvant manifests at 4 hours, peaks within 1-2 days and lasts for between 7-10 days. A model of even more prolonged pain also utilizes the injection of Freud's adjuvant but in this case it is injected systemically. In this model of polyarthritis, Freud's adjuvant is injected into the rat tail. This model of polyarthritis is associated with a delayed hypersensitivity reaction with inflammation and hyperalgesia of multiple joints. The response commences 10 days after the injection and lasts for up to 3 weeks (see refs. in Dubner 1989). The procedure for the development of animal models of neuropathic pain involves either the sectioning, crushing or ligation of the sciatic nerve. More recently a more discrete animal model of peripheral nerve ligation has been developed (Bennett and Xie 1988). The behaviour associated with the ligation of the sciatic nerve peaks 10-14 days after the induction of the injury and lasts for up to 3 months (see refs. in Laird and Bennett 1993). All these models of prolonged nociception last for differing periods of time and are associated with different behavioural responses, which may reflect different peripheral and/or central changes in nociceptive processing.

1.4 The formalin response as a model of prolonged inflammatory nociception: characteristic behavioural responses

Formalin has been used for a number of decades to study the effects of anti-inflammatory drugs on oedema formation (see refs. in Brown et al. 1968). Formalin induced oedema has been shown to develop slowly, peaking at 4 hours with approximately a 40% increase in paw volume (Wheeler-Aceto and Cowan 1993). In
addition to the development of oedema, subcutaneous formalin produces a prolonged behaviour indicative of pain (see refs. in Dubuisson and Dennis 1977).

Thus the subcutaneous injection of formalin into the hind paw produces a well defined biphasic response which has been described in rats, cats (Dubuisson and Dennis 1977), mice (Shibata et al. 1989) and primates (Alreja et al. 1984). The relative duration of the two phases and the type of behaviour vary slightly between species, since all the experiments in this thesis have used rats, I will concentrate on the behavioural response of the rat to a peripheral injection of formalin.

Injection of formalin into the rat hind paw produces two behaviours indicative of pain (Wheeler-Aceto et al. 1990). The first behaviour was described as flinching, including paw shaking and/or paw lifting. The second behaviour is a licking response but this response has been shown to be considerably more variable (Wheeler-Aceto et al. 1990). The overall behavioural response to formalin is biphasic consisting of an initial acute phase (0-7min), a 10 minute quiescent period which is then followed by a second phase. The second phase flinching is maximal from 20 and 50 minutes and lasts up to 80 minutes. In comparison the licking behaviour is shorter in duration, peaking between 18 and 35 minutes. The early phase flinching response has been shown to be violent and vigorous whereas the second phase flinching response is slower and rhythmic. Licking behaviour is more cyclical and occurs in bursts (Wheeler-Aceto et al. 1990). A recent study has shown the flinching behaviour increases in a dose dependent manner with increasing concentrations of formalin, whereas the concentration-response curve for the licking response was bell shaped, with peak licking occurring with 1.5-5% formalin (Wheeler-Aceto 1993). Since the licking and flinching behaviours are not synonymous, there are inevitable problems interpreting studies which have grouped licking and flinching behaviour together.

The two phases of the formalin response are considered to be due to different mechanisms. The first phase is thought to be due to the direct activation of the sensory nociceptors by the formalin, whereas the second phase is due to the development of inflammatory mediators which activate the nociceptors (Dubuisson and Dennis 1977, Alreja et al. 1984, Hunskaar and Hole 1987, Tjolsen et al. 1992). The second phase of the behavioural response to formalin is phasic and is thought to be more representative of continuous pain than some of the other transient models of nociception (see section 1.13).
A recent behavioural study in mice has indicated the ambient temperature affects the second phase but not the first phase of the formalin response, with the second phase response being diminished at lower room temperatures (Rosland et al. 1991). A possible explanation for this effect was proposed by Tjolsen et al. 1992, based on the cooler ambient temperature decreasing tissue temperature resulting in a slower rate of development of the inflammation and consequently a reduced second phase response.

Morphine tolerance studies have indicated different neuronal mechanisms are involved in the formalin response as compared to the acute tail flick test (Abbott et al. 1982) and withdrawal reflex tests (Abbott et al. 1981). The use of phasic pain stimuli allows a more precise determination of the onset and duration of analgesic effects. Restraint of animals during the formalin response is not necessary and therefore the complication of stress induced analgesia is eliminated.

In this thesis I have studied the responses of single convergent dorsal horn neurones to acute and prolonged noxious stimuli. In particular I have studied the responses of these neurones to a peripheral injection of formalin. These electrophysiological studies record the neuronal response to formalin and consequently overcome some of the problems associated with the subjective analysis of the behavioural response of animals. I have studied the peripheral and central pharmacology of prolonged nociceptive transmission. In behavioural studies the response of the animal is dependent on the motor response (licking or flinching) and therefore drugs which influence the motor response give false positives for analgesia. These electrophysiological studies are not dependent on the motor response of the animal and therefore any effect of the drug on the motor responses will not influence the true sensory effect on the dorsal horn neurones.
CHAPTER 2:

METHODS
Single cell extracellular recordings were made from the convergent dorsal horn neurones of the spinal cord in the intact halothane anaesthetized rat.

**Animals**

The animals used were male Sprague Dawley rats (200-250g) obtained from University College London animal house and female Sprague Dawley rats (90-120g) obtained from Sandoz Institute for Medical Research.

**The choice of general anaesthetic**

In these studies since I have recorded the responses of dorsal horn neurones to noxious and innocuous stimuli in anaesthetized rats, it is necessary to use a general anaesthetic which will not have marked depressive effects on the responses of these neurones. The events observed in these studies of the mechanisms of nociceptive processing in the presence of an established clinical anaesthetic regime will also be applicable to the clinical situation and have implications for post-operative pain.

An important prerequisite is that the responses of the dorsal horn neurones are not overly affected by the anaesthetic. It is desirable that any effect of the anaesthetic on the neurones is constant over the course of the experiment, eliminating any variability of the responses due to the anaesthetic. The route of administration and the type of anaesthetic used may influence the results of the study.

Barbiturates are well established anaesthetics which are administered intravenously. Intravenous phenobarbital has been shown to nonspecifically depress both spontaneous and evoked activity of laminae IV, V, VI neurones. In addition, in some cases, barbiturates have been shown to briefly block neuronal responses to natural and electrical stimulation (see refs. Kitahata 1975). More recently barbiturates have been shown in electrophysiological studies to reduce the actions of the excitatory amino acid agonists, quisqualate, kainate and NMDA (see refs. in Lodge and Johnson 1990). In view of these effects of barbiturates and since part of this thesis studies the role of the excitatory amino acids during nociceptive transmission, barbiturates were not considered desirable anaesthetics. Another systemically active anaesthetic, α-Chloralose is also known to
depress neuronal activity of the spinal cord. (Shapovalov 1965).

Ketamine hydrochloride has been shown to produce profound anaesthesia associated with strong analgesic effects. The noxious evoked responses of neurones in laminae IV and V have been shown to be reduced by ketamine (Conseiller et al. 1972). In addition the spontaneous responses of laminae I and V are also inhibited by ketamine (Kitahata et al. 1973). Further studies have shown ketamine to reduce polysynaptic reflexes (Lodge and Anis 1984). In light of the realization that ketamine is a channel blocker of the NMDA receptor complex (see refs. in Watkins et al. 1991), the analgesic effects of ketamine are not unexpected and because of the NMDA blocking action is not suitable as a general anaesthetic for this study.

The use of an inhalational anaesthetic ensures that the level of anaesthesia is easy to control. Numerous studies have shown the effects of halothane on the dorsal horn neurones to be relatively minimal as compared to the anaesthetics listed above. Moreover, halothane is one of the main general anaesthetics used clinically. Halothane (1-2%) has been shown to suppress the responses of neurones in lamina IV in the decerebrated cat and monkey and higher levels of halothane (2-3%) may completely depress these responses (see refs. in Kitahata 1975). In the decerebrate spinal cat, halothane (0.5-1.5%) has been shown to dose dependently inhibit the spontaneous activity of laminae I, V and VI neurones and the evoked activity of the laminae I and V neurones (Kitahata et al. 1975). These studies by Kitahata were carried out in decerebrate spinalised animals and as such the spinal cord did not receive descending controls. Therefore these effects of halothane on the neurones may not represent an accurate picture of the effects of halothane in an intact animal. Moreover, a comparison of the responses of convergent dorsal horn neurones to radiant heat and repetitive innocuous mechanical stimulation were shown to be identical in the non anaesthetized spinal rat and the halothane anaesthetized intact rat (Le Bars and Chitour 1983). These results clearly show that the use of halothane as an anaesthetic does not markedly influence the responses of the dorsal horn neurones to innocuous and noxious stimuli.

Halothane was considered the best anaesthetic for these studies since noxious and innocuous evoked responses of the neurones could still be elicited in fully anaesthetized rats. In addition the responses of the neurones to formalin mirror the behavioural responses of animals. Halothane was given in combination with nitrous oxide and
oxygen. Since halothane is a gaseous anaesthetic it was easy to control the amount of anaesthetic received by the animal, therefore providing a stable basis for these studies. The animals were maintained in a state of aflexia for the duration of the experiment. Since halothane is used clinically for anaesthesia any effects of halothane on nociceptive processing will also occur in the clinical situation.

Nitrous oxide was delivered with oxygen. Nitrous oxide does not have anaesthetic properties but has some analgesic properties. Therefore a lower concentration of halothane was required to maintain anaesthesia, thus decreasing the probability of halothane overdose. In addition nitrous oxide along with oxygen served as a carrier gas for the vaporization of halothane. Nitrous oxide (75%) has been shown to dampen down spontaneous neuronal activity of lamina V neurones, with neurones in other laminae unaffected (Kitahata et al. 1971). Considerably lower levels of nitrous oxide (33%) were used in this study, probably eliminating any adverse effect of nitrous oxide on the neuronal responses.

**Induction of anaesthesia**

The halothane was delivered in a gaseous mixture of 33% oxygen and 66% nitrous oxide. The rate of flow of the oxygen (150 cm$^3 \cdot$min$^{-1}$) and nitrous oxide (300 cm$^3 \cdot$min$^{-1}$) was controlled by flow meters (Platon Ltd.). The animal was placed in a sealed perspex box and anaesthesia was induced with 3% halothane. When the animal had lost consciousness it was removed from the box and placed on a heating blanket and a nose cone was used to maintain the anaesthetic delivery. Once the animal had lost all reflex withdrawal behaviour, a tracheal cannula was inserted, providing a closed system for the delivery of constant anaesthetic and gases throughout the day, thus ensuring that minimal leakage occurred. The gas exchange system consisted of a Y-shaped plastic tube, with the halothane dispenser connected on to one tube and the exhaled gases from the animal being removed to an outside vent via the other tube. The animal was placed in the headholder of a stereotactic frame. All further surgical procedures were performed with the animal receiving 2-2.5% halothane, ensuring a state of areflexia.
Surgery; Lumbar Laminectomy

An incision of the skin of the dorsal surface of the animal was made and underlying connective tissue was removed. Incisions were made in the exposed dorsal musculature on both sides of the vertebral canal from the lower thoracic to the lower lumbar level. The rostral end of the vertebral canal and associated muscle was raised and clamped. The individual vertebrae were exposed by removing any muscle covering the vertebral canal, and a laminectomy was performed from L1-L3 using surgical rongeurs. It was necessary for the underlying dura mater to be gently removed to allow the insertion of the microelectrode into the spinal cord and easy drug access. The pia mater was left intact. The caudal end of the exposed vertebral column was clamped. Metal rods were placed laterally on either side of the musculature of the vertebrae of the animal, thus enhancing the stability of the recording conditions. The result of the laminectomy and removal of the associated muscles was a small well, allowing the application of drugs intrathecally which could bathe the spinal cord.

Maintenance of core temperature

The core temperature of the animal was monitored with a rectal thermal probe for the duration of the experiment. The core temperature was maintained between the limits of 36.5-37 °C by an automatic feedback control to the heating blanket (Animal Blanket Control Unit, Harvard) on which the animal was placed.

Electrophysiological recordings

Parylene coated tungsten microelectrodes were used to make extracellular single cell recordings. The microelectrode was fitted into the electrode holder which was attached to the A terminal of the recording headstage (Neurolog NL 100). The headstage was mounted on a micromanipulator and connected to a SCAT microdrive (Digitimer) system. Using the headstage (Neurolog NL 100, A-B configuration) a differential recording was made between the electrode inserted into the spinal cord and an indifferent electrode clipped to the skin of the animal. The voltage signal was amplified (Neurolog Nl
104x5K, Neurolog NL 106x80) and filtered (Neurolog NL 125). The filtered signal was fed into a storage oscilloscope (Gould, 20MHz Digital storage, 1421) providing a visual representation of the responses of the recorded neurones. The signal was also fed into a Neurolog NL 200 spike trigger, adjusted to count action potentials (spikes) of a specified height, therefore separating action potentials recorded from a single neurone from any recordings from other closely located neurones. Each action potential which crossed the predetermined amplitude threshold triggered a brightening pulse which fed into the second channel of the oscilloscope and appeared as an intense dot above each counted action potential. In addition the filtered signal was fed via an audio-amp module (NL 120) providing audio representation of the recorded neurone.

**Searching for neurones**

The electrode was gently lowered onto the surface of the exposed spinal cord. The surface of the dorsal horn was established by tapping the toes of the hindlimb and when spikes were no longer elicited by the tapping this was taken as the top of the dorsal horn. A microdrive was used to lower the electrode with measurement of the depth of the neurones being recorded. While applying non noxious tapping and noxious pinching of the toes the electrode was gradually lowered at 10\(\mu\)m steps through the dorsal horn in a search for dorsal horn neurones which received both a non noxious (light brush, prod) and noxious input (sustained response to pinch) from the receptive field.

Once it was established that the neurone was a convergent neurone, the responses of the neurone to electrical stimulation of the receptive field were recorded. A pair of fine stimulating needles were subdermally inserted into the receptive field. Single (2ms wide square wave) pulses (from a Neurolog NL 510 pulse buffer controlling an NL 800 constant current stimulator) were delivered at a frequency of 0.5Hz. This frequency of stimulation was used since higher frequencies may result in the failure of the C-fibres to conduct impulses. The electrically evoked C-fibre responses could be separated from the evoked A-fibre responses by virtue of the higher thresholds of the C-fibres to electrical stimulation and slower conduction velocities resulting in a longer latency of the C-fibre evoked response. The A-fibre responses arrived within 0-90 mseconds after stimulation, whereas the C-fibres arrived 90-300 mseconds after the stimulation. Stimulation of the
receptive field at 3x C-fibre threshold produced a consistent and highly reproducible A-fibre and C-fibre responses of the dorsal horn neurones which provided the basis for the experiments using transcutaneous electrical stimulation.

Quantification of the electrically evoked responses

The frequency and duration of the electrical stimulus was controlled by a period generator (Neurolog NL 304) and a digital width module (Neurolog NL 401). The period generator also triggered the sweep of the oscilloscope. The Neurolog NL 603 was set to sweep 16 times triggered by the period generator (Neurolog NL 304) which was in turn controlled by the Neurolog NL 603. The spike count was feed directly into the latch counter (Neurolog NL 606) and was fed via a delay module (Neurolog NL 403) into another latch counter. The Neurolog NL 403 was set to count spikes between 90 mseconds and 800 mseconds after the stimulus. Therefore counter 1 counted all spikes in a 1 second sweep, whereas counter 2 captured all spikes registered in the 90-800 msecond period after the sweep stimulus. The logic gates were controlled by the period generator and fed into the other modules so that all stimulating and counting modules were gated to only operate during the train of 16 stimuli. The output from the spike trigger was also fed into a CED 1401 interface (Cambridge Electronic design) and displayed using MRATE software. Using the software and cursor measurements quantitative analysis of the post-stimulus histograms and the formalin response of the neurones was made according to the temporal criteria defined above. A circuit diagram of the equipment used for the recording and analysing of the responses of single dorsal horn neurones is shown in diagram 1.
INPUT FROM THE OUTPUT OF THE RECORDING ELECTRODE AND HEADSTAGE

104 PRE-AMP
106 AC/DC AMP
123 FILTERS
201 SPIKE COUNT
120 AUDIO AMP
501 LOGIC
605 COUNTER

304 PERIOD GENERATOR
401 DIGITAL WIDTH
510 PULSE BUFFER
808 STIMULATOR

CEB 1401

Oscilloscope
Channel 1
Channel 2
Time Base

603 DELAY WIDTH
606 COUNTER

Diagram 1 A simplified circuit diagram of the equipment used to record, analyse and display the responses of single dorsal horn neurones
Administration of formalin

The animals used in these experiments were male Sprague-Dawley rats. A dilution of 5% formalin in saline, was made from 37-40% formaldehyde. Formalin was injected subcutaneously into the receptive field using a Hamilton syringe fitted with a 0.4 mm x 12 mm needle, the total volume injected was 50μl. The neuronal activity was recorded for one hour. The stimulating system was turned off for the duration of the response. The output from the averager was continuous, fed into the logic gates and then directly into the latch counter. The spike trigger output was fed via a pulse integrator (Neurolog NL 601) into a CED 1401 interface (Cambridge Electronic design) and displayed using MRATE software. A single toe only ever received one injection of formalin and a maximum of 5 formalin injections were injected per animal, each into a different toe. Each injection of formalin into separate toes was separated by an interval of 2 hours.

Administration of the bradykinin B2 receptor antagonist HOE140 into the peripheral receptive field

HOE140 (volume of 50μl) was injected peripherally with a 50μl Hamilton syringe fitted with 0.4 mm x 12 mm needle. The peripheral injection of HOE140 produced a short lasting firing of the dorsal horn neurones similar to the effect of a peripheral injection of saline (Dickenson and Sullivan 1987c). The short period of firing had ceased before the subsequent injection of the formalin.

Ultraviolet A irradiation of the hindpaw as a model of prolonged inflammatory nociception

Female Sprague-Dawley rats were used in this series of experiments and the ultraviolet irradiation of the hindpaw was carried out by the Sandoz Institute for Medical Research. The plantar surface of 1 hindpaw was exposed to ultraviolet light (intensity maximum: 365 nm, 69 mW/cm²) for 90 seconds and this was repeated 18 hours later. The first exposure to U.V.A light was taken as day 1.

Electrophysiological studies were carried out on these animals on day 3 and day 5.
after the first exposure to U.V.A irradiation. The procedures used to study the electrically evoked responses of the dorsal horn neurones of these animals were identical to those previously stated.

**Drugs**

The drugs used and there sources were: formalin (5% of formaldehyde solution) from B.D.H. Chemicals Ltd, lignocaine hydrochloride from Phoenix pharmaceuticals, HOE140 from Hoescht, B4162 donated by Prof. M. Schachter (UCL), indomethacin from Sigma, 6-cyano-7-nitroquinoxaline-2-3-dione (CNQX) and 7-chlorokynureenate (7CK) from Tocris Neuramin, morphine sulphate from Evans Medical Ltd., sandostatin (Octreotide) from Sandoz Institute for Medical Research (London), somatostatin donated by Dr. Chrubasik (Frieburg, Germany), \{(3aR,7aR)-7,7-diphenyl-2-[1-imino-2(2-methoxyphenyl)ethyl] perhydroisoindol-4-one\} (RP67580), \{(3aS, 7aS) -7,7-diphenyl-2-[1-imino-2(2-methoxyphenyl)ethyl] perhydroisoindol-4-one\} (RP68651) from Rhone-Poulenc, Dimethyl sulfoxide (DMSO) from Sigma, and Tween and alcohol from Sandoz Institute for Medical Research.

**Analysis of results**

The control electrically evoked responses of the dorsal horn neurones, including Aβ- and C-fibre responses, input and wind up of individual dorsal horn neurones were calculated as the mean number of action potentials elicited in the absence of any drugs. The mean maximal change of the response in the presence of a drug was expressed as a percentage of the pre-drug control response or as a percentage inhibition of the pre-drug control response. Statistical analysis of the effects of a drug on the electrically evoked responses used Student’s paired two tailed t-test.

The control response of the dorsal horn neurones to the peripheral injection of formalin was expressed as the mean number of action potentials ± s.e.m. for the first ten minutes of firing (first phase) and the mean number of action potentials ± s.e.m. for remaining 50 minutes of firing (second phase), for the neuronal population. The effects of drugs on the first phase and second phase of the formalin response were expressed as
percentage inhibition or facilitation of the control first phase of the formalin response and the control second phase of the formalin response. Statistical analysis of the effects of drugs on the two phases of the formalin response as compared to the control response used Student's unpaired two-tailed t-test.

Multiple comparisons between dose response curves was necessary for the studies of UV induced model of inflammatory nociception. Statistical comparisons between the control group, 3 day U.V.group and 5 day U.V.group used analysis of variance (ANOVA) and post-hoc tests where applicable.

Linear Regression was performed on some of the data to determine the correlation coefficient r. If the r value was positive, the Y variable increased with the X variable. With a negative r value the Y variable decreased with an increasing X variable. The p value reveals whether the correlation is significant.

To analyse the effects of the various intrathecal treatments over the 60 minute time course the mean areas under the curve (AUC) were calculated for some of the electrically evoked responses. Statistical analysis of the AUC used the Student's two tailed unpaired t-test.
CHAPTER 3:

THE RESPONSE OF THE DORSAL HORN NEURONES TO ACUTE NOXIOUS STIMULATION
The responses of the dorsal horn neurones to transcutaneous electrical stimulation (2ms wide pulses at 0.5Hz) of their receptive fields located on the ipsilateral hindpaw was utilized as a model of acute nociception. Electrical stimulation was used since it is easy to reproduce and may be applied repeatedly at a constant intensity. The electrical stimulus was applied at three times the electrically evoked C-fibre threshold of the dorsal horn neurone, as described in the methods. The mean depth of the neuronal population used in these studies was 816±22μm, n=169. The mean threshold of the dorsal horn neurones to electrical stimulation for the C-fibre evoked response was 1.19±0.05 mA. All cells studied had a clear short latency Aβ-fibre evoked response (0-20 msec) followed by a Aδ-fibre evoked response and a longer latency C-fibre evoked response (90-300 msec), a typical example is shown in figure 1.

![Figure 1](image_url)

Figure 1. A typical response of a single dorsal horn neurone to a single electrical stimulus at 3x the C-fibre threshold. The action potentials were of equal height when observed on the oscilloscope but due to the digitalised nature of this recording some of the action potentials appear smaller than others. The Aβ-, Aδ- and C-fibre evoked responses are still clearly distinguishable from the baseline activity.

The mean C-fibre, Aβ-fibre and Aδ-fibre evoked responses of the dorsal horn
neuronal population were 314±11 action potentials, 118±4 action potentials and 59±6 action potentials respectively. Any remaining response of the neurone between 300msec and 800msec was also counted as a measure of the after discharge (post-discharge) of the neurone. The mean post-discharge of the neuronal population was 166±11 action potentials. In the absence of electrical stimulation I did not observe any spontaneous activity of the neurones.

Examples of typical post stimulus histograms of the responses of single dorsal horn neurones to a train of sixteen stimuli at three times the C-fibre threshold are shown in figure 2A, figure 2B and figure 3.
Figure 2A and 2B. The responses of two different single dorsal horn neurones to a train of 16 stimuli at three times the C-fibre threshold. The short latency Aβ-fibre evoked response can be clearly separated from the longer latency C-fibre evoked response. A small amount of post discharge was also observed.
Figure 3. This post stimulus histogram of the response of a single dorsal horn neurone to a train of 16 stimuli illustrates a large post discharge response, as compared to the previous two examples. The amount of neuronal post-discharge represents the degree of wind up exhibited by the neurone.

The ability of the neurones to exhibit wind up (Mendell 1966), an enhanced response to repetitive but constant C-fibre stimulation, was also measured. A typical response of a single neurone to the first, forth, eight, twelfth and sixteenth stimulus is shown in figures 4A-E. A comparison between the response to the first stimulus and for example the 8th stimulus clearly indicates the enhancement of the neuronal response to repetitive stimulation.
Figure 4A. The response of a single dorsal horn neurone to the first stimulus.

Figure 4B. The response of the same dorsal horn neurone to the fourth stimulus.
Figure 4C. The response of the same dorsal horn neurone to the eighth stimulus.

Figure 4D. The response of the same dorsal horn neurone to the twelth stimulus.
Figure 4E. The response of the same dorsal horn neurone to the sixteenth stimulus.

Wind up was calculated as the difference between the total number of action potentials produced by the train of sixteen stimuli during the period of 90 msec-800 msec and the input; the number of action potentials produced by the first stimulation at C-fibre strength multiplied by sixteen, the total number of stimuli. The mean electrically evoked wind up of the neuronal population stimulated at a frequency 0.5Hz was 203±15 action potentials. Interestingly, considerably less wind up of the neuronal response was observed with electrical stimulation of the C-fibres at a frequency of 0.1Hz (93±18 action potentials, n=10). Figure 5 shows the electrically evoked wind up of a single dorsal horn neurone stimulated at a frequency of 0.5Hz and 0.1Hz. Therefore exemplifying the frequency dependency of wind up of the dorsal horn neurones.
Figure 5. The electrically evoked wind up of a single neurone stimulated at a frequency of 0.5Hz as compared to the wind up of the same dorsal horn neurone stimulated at a frequency of 0.1Hz. The absence of wind up of the neuronal response when stimulated at 0.1Hz illustrates the frequency dependent nature of wind up.
CHAPTER 4:

THE RESPONSE OF THE DORSAL HORN NEURONES TO PROLONGED NOXIOUS STIMULATION
4.1 Introduction

Electrophysiological studies in the intact anaesthetized rat (Dickenson and Sullivan 1987a) have shown that a peripheral injection of formalin into the receptive field of convergent dorsal horn neurones results in a biphasic excitation of these neurones which parallels the time course of the biphasic activity observed in behavioural studies (Dubuisson and Dennis 1977). Neurones which receive only innocuous Aβ-fibre and Aδ-fibre inputs have been shown not to respond to peripheral formalin (Dickenson and Sullivan 1987c). Studies in the decerebrate unanaesthetized cat have shown a peripheral injection of formalin produces an immediate and continuous firing of the wide dynamic range neurones (Banna et al. 1986). In comparison a peripheral injection of saline produces a small transient unsustained firing of the wide dynamic range neurones (Banna et al. 1986) and dorsal horn neurones (Dickenson and Sullivan 1987c).
4.2 Results

4.2.1 The response of the convergent dorsal horn neurones to a peripheral injection of formalin

The peripheral injection of formalin resulted in immediate firing of the dorsal horn neurones which previously had either a very low rate of spontaneous firing or were silent. The initial firing subsided within ten minutes, and was then followed by a silent period of 10-15 minutes. Approximately 25 minutes after the injection of formalin a more prolonged phase of neuronal firing began. The response to formalin lasted for one hour after the peripheral injection. The first and second phases of the response could be clearly separated for all cells, a typical example of a response of a single dorsal horn neurone to formalin is shown in figure 6.

The control response (in the absence of any drugs) of the dorsal horn neurones to a peripheral injection of formalin was studied continuously to allow for any seasonal changes in the response. Individual groups of control responses were determined for each series of experiments to ensure that changes in the responses due to external factors did not influence the results.

The mean first phase and mean second phase of the dorsal horn neurones responses to a peripheral injection of formalin are shown in table 1. The neurones were separated in terms of the depth of the recorded neurone; superficial laminae n=5, substantia gelatinosa n=23 and deep laminae n=144.
Table 1. A comparison of the relative sizes of the two phases of the formalin responses of the superficial (0-250\(\mu\)m), substantia gelatinosa (250-500\(\mu\)m) and deeper laminae dorsal horn neurones (500-1000\(\mu\)m).

<table>
<thead>
<tr>
<th>Depth</th>
<th>First Phase Action potentials</th>
<th>Second Phase Action potentials</th>
<th>Depth (\mu)m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superficial Laminae</td>
<td>2913±447</td>
<td>13011±4008</td>
<td>232±23</td>
</tr>
<tr>
<td>Substantia Gelatinosa</td>
<td>3936±550</td>
<td>27492±6735</td>
<td>412±15</td>
</tr>
<tr>
<td>Deep Laminae</td>
<td>6034±442</td>
<td>23364±1758</td>
<td>846±17</td>
</tr>
</tbody>
</table>

The majority of my studies were performed on deep dorsal horn neurones and therefore a further analysis of the responses of a population of deep dorsal horn neurones to a peripheral injection of formalin was made.
Figure 6. An example of the response of a single dorsal horn neurone to a peripheral injection of formalin. The spikes per second are plotted on the vertical axis and the time in seconds is plotted on the horizontal scale. The response is biphasic with an initial first phase followed by a silent period and then a second phase of prolonged firing.
4.2.2 The response of a population of deep dorsal horn neurones to a peripheral injection of formalin

The mean first phase and mean second phase control responses of a population of dorsal horn neurones (n=62) to a peripheral injection of formalin were 5466±632 action potentials and 21171±2659 action potentials. The mean depth of the neuronal population was 780±36μm. Analysis revealed a significant correlation between the first phase and second phase of the formalin response (r=0.34, p=0.0063). This correlation is interesting in view of the fact that a complete peripheral local anaesthetic block of the first phase of the response does not influence the size of the second phase of the formalin response (Haley et al. 1990). The correlation may reflect the contribution of the initial action of formalin at the peripheral nociceptors to the degree of inflammation at the local site of the formalin injection. The peripheral inflammation will determine the degree of activation of the nociceptors and therefore the magnitude of noxious inputs relayed into the spinal cord and the size of the second phase response of the dorsal horn neurones.

Further analysis was conducted to ascertain whether the magnitude of the response to formalin correlated with the response of the same neurone to electrical stimulation (n=62). The first phase of the response was positively correlated with both the electrically evoked wind up (r=0.5, p=0.0002) and the C-fibre evoked response (r=0.3, p=0.006) of the dorsal horn neurone. Therefore as one would expect, a greater responsivity of the neurone to electrical stimulation is paralleled with a larger first phase response to the injection of formalin. The first phase of the formalin response was not correlated with the threshold of the neurones to electrical stimulation (r=-0.2, p=0.13). Presumably this reflects the high intensity (supra-threshold) of the initial activation of the neurone by formalin. During the first phase of the response the direct action of formalin at the C-fibre nociceptors can maximally activate the dorsal horn neurones with relatively high thresholds, as well as those with lower thresholds to electrical stimulation.

The second phase of the response was not correlated with the electrically evoked C-fibre response (r=0.2, p=0.08), therefore the magnitude of the second phase of the formalin response was not influenced by the general responsivity of the neurone to electrical stimulation. This is probably due to the activation of the NMDA receptor during the second phase of the formalin response (Haley et al. 1990) and the response no longer...
being in proportion to the initial input. In contrast with the first phase, the second phase of the response was inversely correlated with the C-fibre threshold to electrical stimulation ($r=-0.1$, $p=0.01$). Therefore dorsal horn neurones with lower C-fibre evoked electrical thresholds exhibited larger second phase responses to formalin.

There was no correlation between the electrically evoked wind up of the dorsal horn neurones and the size of the second phase of the formalin response. This is unexpected since it has previously been shown that both the second phase of the formalin response (Haley et al. 1990) and the electrically evoked wind up (Dickenson and Aydar 1991) of the dorsal horn neurones involves NMDA receptor mediated events. This lack of correlation may be due to the level and duration of activation of the peripheral nociceptors which in turn determines the degree of central hypersensitivity of the dorsal horn neurones during the second phase of the formalin response. During the second phase of the formalin response the peripheral input is maintained for a longer period of time (30-40 minutes) than during electrical stimulation. Neurones which exhibited little wind up to acute repetitive C-fibre stimulation may be maximally activated by the noxious inputs associated with the second phase of the formalin response and exhibit large responses to the peripherally injected formalin.
CHAPTER 5

THE EFFECT OF A PRIOR PERIPHERAL INJECTION OF FORMALIN ON THE ELECTRICALLY EVOKED C-FIBRE AND A-FIBRE RESPONSES OF THE DORSAL HORN NEURones
5.1 Introduction

The effect of formalin on the excitability of the dorsal horn neurones was assessed. It was impossible to gauge whether neuronal responsitivity had altered as a consequence of formalin induced activity whilst the neurones were firing as a result of the formalin injection. A peripheral injection of lignocaine into the toe containing the site of the formalin injection has previously been shown to block the response of the dorsal horn neurones to formalin within the 30 second injection time (Dickenson and Sullivan 1987a). Local anaesthetics act via the blockade of sodium channels to dampen down or inhibit neuronal activity without interacting with a specific receptor complex (Strichartz 1976).

In this experimental paradigm the electrically evoked C-, Aδ- and Aβ-fibre responses and the wind up of a single dorsal horn neurone which had a receptive field over two toes were recorded. Formalin was injected into the adjacent toe which also contained part of the receptive field. The response of the neurone to formalin was recorded and during the second phase of the response 50 μl of 2% lignocaine (weight / volume: 1000 μg / 50 μl) was injected into the same site as the formalin, blocking the peripheral inputs into the spinal cord. Immediately after the blockade of the peripheral inputs, electrical stimulation of the receptive field on the adjacent toe was applied, as described earlier, at 5 minute intervals for 30 minutes. The electrically evoked C-, Aδ- and Aβ-fibre responses and the wind up of the dorsal horn neurones after the temporary block of the response to formalin was used as a measure of central excitability and was expressed as a percentage of the control responses (pre formalin injection).

In addition, the electrically evoked responses of the dorsal horn neurones after the injection of lignocaine alone into the adjacent toe was studied. Thus controlling for any spread of the effect of peripheral lignocaine between the two toes.
5.2 Results

5.2.1 The effect of a peripheral injection of formalin on the response of the dorsal horn neurones to electrical stimulation

The effect of a prior peripheral injection of formalin on the electrically evoked C-fibre, Aδ-fibre, Aβ-fibre and wind up responses of 27 dorsal horn neurones (mean depth 703±61μm) was studied. The electrically evoked C-, Aδ-, Aβ-fibre and wind up responses of the dorsal horn neurones after the peripheral injection of lignocaine into the same site as the formalin injection were not significantly different from the control responses (table 2). The formalin induced firing of the dorsal horn neurones restarted 15 to 30 minutes after the peripheral injection of the lignocaine.

Table 2. The mean maximal effect of a peripheral injection of formalin on the response of the dorsal horn neurones to electrical stimulation.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-fibre</th>
<th>Wind up</th>
<th>Aδ-fibre</th>
<th>Aβ-fibre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Formalin</td>
<td>93±12%</td>
<td>132±32%</td>
<td>114±15%</td>
<td>107±10%</td>
</tr>
<tr>
<td>+Lignocaine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lignocaine Alone</td>
<td>117±17%</td>
<td>130±50%</td>
<td>122±39%</td>
<td>108±25%</td>
</tr>
</tbody>
</table>

Results are expressed as mean maximal percentage change from the control response.

The electrically evoked responses of the dorsal horn neurones after the injection of lignocaine alone into the adjacent toe was studied on a total of 5 dorsal horn neurones (mean depth 904±161). The injection of lignocaine alone into the toe adjacent to the toe receiving electrical stimulation did not alter the electrically evoked responses as compared to control responses (table 2). Therefore the local anaesthetic block of one toe did not spread to the adjacent toe.
5.3 Discussion

The electrically evoked C-, Aδ- and Aβ-fibre responses of the dorsal horn neurones during the peripheral lignocaine block of the formalin induced activity were not significantly different from control values. In addition, wind up of the dorsal horn neurones was not significantly affected by the prior activation of the dorsal horn neurone by a peripheral injection of formalin. It was ensured that a peripheral lignocaine injection into the adjacent toe did not spread, verifying that the lignocaine block only encompassed the site of the formalin injection.

The dorsal horn neuronal response to formalin did not influence the electrically evoked responses of the same dorsal horn neurones. Therefore although the second phase of the formalin response is associated with the development of a NMDA receptor mediated central hypersensitivity (Haley et al. 1990), when the formalin evoked activity was temporarily blocked by peripheral lignocaine, the electrically evoked responses of the same neurone to stimulation of the adjacent toe were not different from the pre-formalin control responses. Therefore suggesting the NMDA receptor mediated responses are homosynaptic and restricted to those connections activated by the formalin.

The firing of the dorsal horn neurones during the second phase of the formalin response was completely blocked by peripheral lignocaine injection, therefore the second phase of the response and the associated NMDA receptor mediated central hyperalgesia is dependent on a peripheral input. The ability of peripheral local anesthesia to inhibit the response to formalin has previously been reported (Dickenson and Sullivan 1987a). However, behavioural studies have shown that the peripheral injection of bupivacaine (0.5%, 150 μl) during the second phase of the formalin response resulted in a maximum inhibition of 72% of the behavioural score (Coderre et al. 1990a). The difference between this behavioural study and the findings of this electrophysiological study may be due to the site of the formalin injection. In my study the injection was localized into the toe, whereas in the behavioural study the formalin was injected into the plantar region. In the behavioural study it may be more difficult to block all the C-fibres activated by formalin due to the more diffuse site of injection in the behavioural studies. This could explain the incomplete block of the behaviour in these animals. Alternatively, in my studies the presence of general anaesthesia may have contributed to the greater block of
the response to formalin. Since halothane and related volatile anaesthetics are widely used for intraoperative anaesthesia, if such an interaction occurs it is relevant to the treatment of post-operative pain. The results of my electrophysiological study are in agreement with another recent behavioural study. Peripheral administration of bupivicaine after nerve ligation was shown to reduce thermal hyperalgesia (Mao et al. 1992a), thus agreeing with my study that peripheral inputs are required to maintain central hypersensitivity. Moreover a clinical study of painful neuropathy has shown that peripheral local anaesthesia blocks mechano-allodynia and spontaneous pain in patients, indicating the necessity of a peripheral input to maintain altered sensory processing (Gracely et al. 1992).

In conclusion the results of this study are two-fold. Firstly, the second phase of the response of the dorsal horn neurones to a peripheral injection of formalin was dependent on a peripheral input. Secondly these results showed that the peripheral injection of formalin did not alter the electrically evoked responses of the same dorsal horn neurone.
CHAPTER 6:

THE EFFECT OF INTRATHECAL LOCAL ANAESTHETIC ON THE NEURONAL RESPONSE TO FORMALIN
6.1 Introduction

Pain hypersensitivity has been shown to have both a peripheral and spinal component (Woolf 1983). Chapter 5 showed the response of dorsal horn neurones to a peripheral injection of formalin required a peripheral input. The susceptibility of the formalin response to central sodium channel blockade, using intrathecal administration of a local anaesthetic, has been studied in this chapter.

Local anaesthetics do not influence specific receptor systems but reduce neuronal transmission by binding to voltage dependent Na⁺ channels (Strichartz 1976). Therefore local anaesthetics dampen down neuronal activity and prevent Na⁺ channels opening in response to excitatory stimuli.

Systemic administration of local anaesthetics has been shown to selectively inhibit nociceptive responses of flexor motoneurones to noxious heat and chemical stimuli (Woolf and Wiesenfeld-Hallin 1985). In addition, systemic administration of the local anaesthetic lidocaine has been shown to block ectopic impulse discharge from neuromas and dorsal root ganglia of nerve injured rats without altering the propagation of impulses along the axon, or the initiation of electrically evoked or mechanically evoked impulses (Devor et al. 1992). These findings confirm an earlier study in which clinically effective concentrations of lidocaine were shown to reduce tonic injury discharge in Aδ- and C-fibre endings in the rabbit corneal preparation, whereas the noninjured nerve discharge was not altered (Tanelian and MacIver 1991). A theory for this selectivity of local anaesthetics was proposed by Devor et al. 1992. The voltage gated Na⁺ channels are important for the regenerative membrane current which contributes to the generation of action potentials. Local anesthetics by blocking Na⁺ channels reduce the number of available channels, therefore increasing the threshold of the fibre to repetitive firing. Fibres which have a low drive for sustained discharge are therefore more susceptible to local anaesthetics than fibres which are firing well above their threshold to repetitive firing.

Intrathecal administration of local anaesthetic has been shown to preferentially inhibit the electrically evoked C-fibre responses and wind up of the deep convergent dorsal horn neurones. The inhibitions of Aβ-fibre evoked responses by local anaesthetic were less extensive and were shorter in duration than the inhibitions of the C-fibre evoked
responses (Fraser et al. 1992). Electrically evoked wind up of the dorsal horn neurones was more sensitive to intrathecal local anaesthetic than the C-fibre evoked responses. This increased sensitivity of wind up to intrathecal local anaesthetic may be explained by the theory proposed by Devor et al. 1992. NMDA receptor mediated wind up has been shown to be dependent on a repetitive C-fibre input. Devor et al. proposed that local anaesthetics act to decrease the frequency of neuronal firing, it has previously been shown that NMDA receptor activation is dependent on a repetitive input for the removal of the Mg\(^{2+}\) blockade of the NMDA receptor channel (see refs. in Dickenson 1990). Therefore it can be envisaged that a consequence of local anaesthetics decreasing the frequency of the input is a decreased rate of removal of the Mg\(^{2+}\) block of the NMDA receptor complex. This would result in a decreased activation of the NMDA receptor complex. In addition, local anaesthetics may block the NMDA receptor channel. Therefore these two effects of local anaesthetics will result in an increased sensitivity of the NMDA receptor mediated wind up to the inhibitory effects of local anaesthetics. In a recent in vitro study subanaesthetic doses of local anaesthetic were shown to selectively inhibit the slow ventral root potential, whereas monosynaptic ventral root potentials were unaffected (Jaffe and Rowe 1993). This finding shows low doses of local anaesthetic selectively inhibit nociceptive responses. Furthermore, a clinical study of the effect of epidural lidocaine on the blockade of noxious sensation indicated that pain evoked by short localised stimuli is more readily blocked than pain elicited by prolonged widespread stimuli (Brennum et al. 1992). Therefore both the temporal and spatial summation of the afferent input dictate the effectiveness of spinal local anaesthetic block. The findings of these studies with spinal administration of local anaesthetic are in agreement with studies of systemic local anaesthetic which also observed a preferential inhibition of nociceptive responses.

I have looked at the effect of intrathecal local anaesthetic (lignocaine) on the dorsal horn neuronal response to a peripheral injection of formalin. In addition, the importance of the timing of the administration of lignocaine relative to the peripheral injection of formalin was also studied. In the first series of experiments lignocaine (0.5, 1 and 2%, in a volume of 50μl) was applied intrathecally 5 minutes before the peripheral injection of formalin (pre-administration). In the second series of experiments the same doses of lignocaine were applied intrathecally 5 minutes after the peripheral injection of formalin (post-administration).
6.2 Results

The control response of the dorsal horn neurones to a peripheral injection of formalin was a first phase of 6724±758 action potentials and a second phase of 23249±1880 action potentials (n=9). The mean depth of the neuronal population was 830±70μm.

6.2.1 The effect of pre-administered lignocaine on the response of the dorsal horn neurones to a peripheral injection of formalin

The effect of pre-administered intrathecal lignocaine on the formalin response was studied on a total of 21 dorsal horn neurones. The mean depth of the neuronal population was 821±49μm. Pre-administration of 0.5% lignocaine and 1% lignocaine did not significantly alter the first phase of the formalin response (37±22% inhibition, 31±15% inhibition respectively). The highest dose of pre-administered intrathecal lignocaine (2%) significantly inhibited first phase of the formalin response (56±7% inhibition p<0.001).

The second phase of the formalin response was not inhibited by intrathecal pre-administration of 0.5% and 1% lignocaine but was significantly inhibited by intrathecal pre-administration of 2% lignocaine (53±17% inhibition p<0.01) (table 3).

An example response of a single dorsal horn neurone to a peripheral injection of formalin after intrathecal pre-administration of 2% lignocaine is shown in appendix 1.

6.2.2 The effect of post-administered lignocaine on the response of the dorsal horn neurones to a peripheral injection of formalin

The effect of post-administered intrathecal lignocaine on the formalin response was studied on a total of 22 dorsal horn neurones. The mean depth of the neuronal population was 764±52μm. Lignocaine was applied intrathecally 5 minutes after the peripheral injection of formalin and therefore was without effect on the first phase of the formalin response.

The lower doses of post-administered intrathecal lignocaine, 0.5% and 1%, did not significantly alter the second phase of the formalin response. In contrast post-administration of 2% lignocaine significantly inhibited the second phase of the
formalin response (57±12% inhibition, p=0.001) (table 3).

An example response of a single dorsal horn neurone to a peripheral injection of formalin in the presence of 2% lignocaine given intrathecally as a post-administration is shown in appendix 1.

Table 3. The effect of pre-administered versus post-administered intrathecal lignocaine on the second phase of the formalin response

<table>
<thead>
<tr>
<th>Dose</th>
<th>Pre-administration</th>
<th>Post-administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>11±43%</td>
<td>7±47%</td>
</tr>
<tr>
<td>1%</td>
<td>4±32%</td>
<td>27±21%</td>
</tr>
<tr>
<td>2%</td>
<td>53±17%*</td>
<td>57±12%**</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the control second phase response to a peripheral injection of formalin. Statistical analysis used Student’s unpaired two-tailed t-test, *p< 0.05, **p< 0.001. n=5-8

6.2.3 Comparison of the effect of pre-administered lignocaine versus post-administered lignocaine on the second phase of the formalin response

Only the highest dose of intrathecal lignocaine studied significantly inhibited the second phase of the formalin response. The inhibition of the second phase of the formalin response observed in the presence of post-administered lignocaine (2%) was equivalent to the inhibition observed with pre-administration of the same dose of lignocaine (p=0.83) (table 3).
6.3 Discussion

6.3.1 The effect of intrathecal lignocaine on the formalin response

Lower doses of lignocaine did not significantly inhibit the formalin response. Pre-administration of 2% lignocaine significantly inhibited both phases of the formalin response. Therefore the response of the dorsal horn neurones to a peripheral injection of formalin is dependent on the activity of sodium channels, thus indicating the neuronal basis of this activity. These results are in agreement with a previous electrophysiological study in which systemic administration of a local anaesthetic was shown to inhibit responses to the noxious chemical stimuli (Woolf and Wiesenfeld-Hallin 1985).

The doses of lignocaine used in this study were comparable to those used in a previous electrophysiological study in which intrathecal lignocaine significantly reduced the electrically evoked NMDA receptor mediated wind up of the dorsal horn neurones (Fraser at al. 1992). The ability of 2% lignocaine to inhibit the second phase of the formalin response is in accordance with this previous electrophysiological study. The dose response relationship for lignocaine on the formalin response was very steep and this possibly reflects the mode of action of local anaesthetics as inhibitors of sodium ion channel conductance.

6.3.2 Comparison of the effect of pre- versus post-administered lignocaine on the formalin response

Intrathecal post-administration of 2% lignocaine was as effective at inhibiting the second phase of the formalin response as the same dose given as a intrathecal pre-administration. Therefore inhibition of the first phase of the formalin response, as produced by pre-administered lignocaine, was not necessary for the inhibition of the second phase of the formalin response. Since the inhibitory effects of intrathecal lignocaine did not depend on the timing of the application (pre- or post-administration) there is little evidence of peripheral formalin causing central changes which alter the efficacy of intrathecal local anaesthetic. Therefore if peripheral inputs into the dorsal horn during the first phase of the formalin response result in a general increase in central neuronal sensitivity which contributes to the second phase of the formalin response, this
can still be inhibited by spinal blockade of sodium channels.

These results are not in agreement with behavioural studies in which pre-administration of intrathecal lignocaine was more effective at inhibiting the formalin response than the same dose given as a post-administration (Coderre et al. 1990a). This difference between my study and the study by Coderre et al. may be due to slightly different experimental procedures. In the behavioural study formalin was injected into the plantar of the foot and therefore a larger number of C-fibres would have been recruited as compared to our study in which the formalin was discretely injected into a single toe. The size of the injection area and hence the number of C-fibres activated may determine the extent of central hypersensitivity which would then form a basis for differential effects of the same treatment depending on the timing of administration. Therefore the ability of intrathecal lignocaine to inhibit the formalin response may be dependent on the size of the injection site and explain why our study failed to observe supporting evidence for this behavioural study by Coderre et al. 1990a.

Our results where timing of the spinal lignocaine administration did not determine the degree of analgesia produced are in agreement with recent clinical studies in which the degree of analgesia produced by pre and post-administration of local anaesthetics were not significantly different (Dahl et al. 1992, Dierking et al. 1992).

The roles of higher brain centres in the development of central hypersensitivity are an important consideration. The effects of pre- and post-administration of lignocaine into higher brain centres on the behavioural response to subcutaneous formalin differ depending on the site of injection (cingulum bundle, fornix bundle and the medial bulboreticular formation) (Vaccarino and Melzack 1992).

In conclusion, intrathecal pre-administration of 2% lignocaine significantly inhibit both the first and second phase of the formalin response. Intrathecal administration of 2% lignocaine, after the first phase of the formalin response (post-administration), significantly inhibited the second phase of the formalin response. There was no difference between the inhibitory effect of pre-administered lignocaine and post-administered lignocaine. Therefore the timing of the intrathecal administration of lignocaine did not alter its ability to inhibit the second phase of the formalin response.
CHAPTER 7:

THE ROLE OF BRADYKININ DURING NOCICEPTIVE TRANSMISSION
7.1 Introduction

**The role of bradykinin in the periphery**

Bradykinin is a nonapeptide, formed from kininogen by the enzyme kallikrien in response to tissue injury and is one of a number of mediators involved in the production of the cardinal signs of inflammation, vasodilation and increased vascular permeability, which result in oedema formation (see refs. in Schachter 1964, Regoli and Barabe 1980, Bathon and Proud 1991). In addition to the role of bradykinin in the inflammatory response, it is a well established pain producing substance (see refs. in Regoli and Barabe 1980, Dray and Perkins 1993).

There is plenty of evidence for the algesic nature of bradykinin. Injection of bradykinin into the paw of mice has been associated with nociceptive behaviour (Shibata et al. 1986) and clinical studies have shown bradykinin to be a potent algesic in humans (Lim et al. 1969, Whalley et al. 1987, Jensen et al. 1990). In addition, intra-arterial injection of bradykinin activates the fine articular afferents which relay both noxious and innocuous information (Kanaka et al. 1985). Moreover, intra-arterial injection of bradykinin also evokes firing of the spinal nociceptive neurones in the cat (Besson et al. 1972, Belcher 1979). A recent study has indicated that intradermal administration is more effective than subcutaneous administration of bradykinin at producing hyperalgesia, thought to be due to the proximity of the injection relative to the bradykinin receptors (Khasar et al. 1993).

During inflammatory pain states bradykinin is generated in injured tissue and activates primary afferents (see refs. in Yaksh and Hammond 1982, Martin et al 1987) via the bradykinin B\textsubscript{2} receptor (see refs. in Dray and Perkins 1993, Bathon and Proud 1991). Bradykinin has been shown to dose-dependently increase vasodilation and plasma extravasation in the rat hind footpad, these effects were significantly reduced in capsaicin treated animals, thus indicating a small diameter primary afferent contribution to the response (Khalil and Helme 1992). In a more recent ex-vivo study, bradykinin evoked CGRP release from the C-fibre terminals in the rat trachea preparation was shown to be blocked by a bradykinin B\textsubscript{2} receptor antagonist, the NSAID indomethacin and capsaicin desensitization (Hua and Yaksh 1993). These results suggest the action of bradykinin on
the primary afferents of the rat trachea is indirect and mediated by the prostaglandins.
With the in vitro neonatal rat spinal cord with attached tail preparation, peripheral
bradykinin was shown to produce bradykinin $B_2$ receptor sensitive ventral root
depolarisations (Dray et al. 1992). In the same preparation, bradykinin has been shown
to activate capsaicin sensitive C-fibres via a protein kinase C mechanism (Dray et al.
1988a). An in vivo electrophysiological study has shown peripherally applied bradykinin
evokes firing of single convergent dorsal horn neurones of the rat spinal cord, this
bradykinin evoked response was sensitive to the bradykinin $B_2$ receptor antagonist
B4162 (Haley et al. 1989).

The peripheral role of bradykinin during nociceptive inflammatory responses has
been confirmed with the selective $B_2$ receptor antagonist B4162. Peripheral
administration of B4162 reduced the second phase, but not the first phase, of the formalin
response (Haley et al. 1989). This indicates that peripheral bradykinin, via $B_2$ receptor
activation, contributes to the second phase of the formalin response, but not the first
phase of the response. In another model of inflammatory pain, the carrageenan model of
inflammatory nociception, the associated hyperalgesia, hyperthermia and oedema were
significantly blocked by bradykinin antagonism (Costello and Hargreaves 1989). In
addition, the responsiveness of the tissue to bradykinin has been shown to be greater
within an inflamed area than outside of the inflamed area (Kirchhoff et al. 1990).

The recent development of the highly potent and long lasting bradykinin $B_2$ receptor
1991) has provided a useful tool for a more extensive study of the roles of bradykinin. In
vivo studies have shown HOE140 to be 250-700 times more potent and to have a
considerably longer half life than earlier $B_2$ receptor antagonists (Bao et al. 1991). This
has been suggested to be due to HOE140 having a higher affinity for $B_2$ receptors and a
greater stability against enzyme degradation. Intravenous administration of HOE140 has
been shown to inhibit carrageenan, bradykinin, prostaglandin and urate crystal induced
paw oedema in the rat but not to modify oedema induced by paw heating (Damas and
Remacle-Volon 1992). A behavioural study in mice confirmed these inhibitory actions of
HOE140. In addition, intraplantar administration of HOE140 was shown to suppress the
behavioural response to the peripheral injection of formalin (Beresford and Birch 1992).
Further studies have shown that HOE140 exhibits potent antinociceptive effects on
bradykinin, kaolin and acetic acid induced abdominal constriction assay, but is ineffective against zymosan induced responses (Heapy et al. 1993). Overall, there is overwhelming evidence for a role of peripheral bradykinin during inflammatory nociceptive processing.

The role of bradykinin in the spinal cord

Bradykinin has been shown to be present in the spinal cord (Perry and Synder 1984) and bradykinin receptor binding sites have been localised within the superficial laminae of the dorsal horn (Steranka et al. 1988). Irrespective of these anatomical indicators of bradykinin and bradykinin receptors in spinal areas associated with high proportions of nociceptive sensory neurones, the role of bradykinin during nociceptive transmission at the spinal level is less clear. A number of studies have shown an excitatory effect of spinal bradykinin in the cat (Henry 1976, Randic and Yu 1976) and rat (Dray 1988b, Dunn and Rang 1990). In contrast, behavioural studies (tail flick latency test) have demonstrated an antinociceptive effect of intrathecal bradykinin, but these antinociceptive actions may be attributed to an excitatory presynaptic action of bradykinin on the descending noradrenergic inhibitory system (Laneuville and Couture 1987, 1989). An electrophysiological study, using the in vitro neonatal rat spinal cord with attached tail preparation, showed spinal bradykinin produced ventral root depolarisations which were unaltered by selective bradykinin B1 receptor antagonism but were inhibited by a nonselective bradykinin antagonist (Dray et al. 1988b). These results give indirect evidence for a spinal action of bradykinin at the B2 receptor. Further studies, using the same preparation, showed spinal bradykinin evoked depolarisations were diminished by capsaicin, implicating a direct action of bradykinin on the primary afferent fibres (Dunn and Rang 1990). Recently an in vitro release study has shown bradykinin not to alter the basal release of CGRP but to potentiate dorsal root stimulation evoked release of CGRP, this effect was sensitive to indomethacin (Andreeva and Rang 1993). Since CGRP is present only in the primary afferents this study demonstrates an excitatory action of bradykinin on the primary afferents. The sensitivity of this effect to indomethacin suggests this effect is indirect and via the generation of prostaglandins, similar to the effects observed in the periphery.

Although there are indicators that exogenous spinal bradykinin has pro-nociceptive effects, whether spinal bradykinin contributes to the physiological processing of
nociceptive information is another issue. Moreover, it has been shown that neither a selective bradykinin B₁ receptor antagonist nor a non-selective bradykinin antagonist influenced the ventral root response to peripheral heat or capsaicin, suggesting that bradykinin is not a physiological mediator of acute nociception (Dray et al. 1988b). Therefore although spinally applied bradykinin has a reversible excitatory effect on nociceptive neurones, there is little evidence so far for a physiological role of bradykinin during acute nociception in the spinal cord.

In this series of experiments, I have studied the effect of peripheral administration of the bradykinin B₂ receptor antagonist, HOE140, on the response of dorsal horn neurones to the peripheral injection of formalin. In addition, the effect of spinal administration of HOE140 and a less potent B₂ receptor antagonist, B4162, on the responses of the dorsal horn neurones to the peripheral injection of formalin was studied. Finally the effect of intrathecal HOE140 on the responses of the dorsal horn neurones to acute electrical stimulation was also studied.
7.2 Results

For this series of experiments the control response of the dorsal horn neurones to the peripheral injection of formalin was a mean first phase of 5670 ± 857 action potentials and a mean second phase of 19648 ± 1925 action potentials (n=9). The mean depth of the neuronal population was 695±83μm.

7.2.1 The effect of peripheral administration of HOE140 on the response of dorsal horn neurones to a peripheral injection of formalin

The effect of bradykinin B$_2$ receptor antagonism at the site of the formalin injection was investigated. HOE140 was injected directly into the receptive field of the dorsal horn neurone ten minutes prior to the injection of formalin into the same site. The effect of peripheral HOE140 on the formalin response was recorded from a total of 21 neurones. The mean depth of the neuronal population was 556±72μm.

The lower doses of HOE140 did not influence the first phase of the formalin response. The highest dose of peripherally administered HOE140 (10μg) significantly inhibited the first phase of the formalin response (83±4% inhibition of control, p=0.0004) (figure 7). This result implicates an important contribution of peripheral bradykinin, via an action at the B$_2$ receptor, during the first phase of the formalin response.
Figure 7. The effect of peripheral administration of HOE140 on the first phase of the formalin response. Results are expressed as percentage inhibition of the control first phase response of the dorsal horn neurones to a peripheral injection of formalin. Statistical analysis used Student’s two tailed unpaired t-test, ***p<0.0001

The second phase of the formalin response was more sensitive to the peripherally injected HOE140 (figure 8). The second phase of the formalin response was significantly inhibited by 1μg of HOE140 (55±15% inhibition of control, p=0.008) and 10 μg of HOE140 (84±10% inhibition of control, p<0.0001). These results suggest bradykinin, via an action at the B2 receptor, contributes to the peripheral generation of the second phase of the formalin response.
Figure 8. The effect of peripheral administration of HOE140 on the second phase of the formalin response. Results are expressed as percentage inhibition of the control second phase response of the dorsal horn neurones to a peripheral injection of formalin. Statistical analysis used Student’s two tailed unpaired t-test. **p< 0.001, ***p<0.0001.

7.2.2 The effect of intrathecal administration of HOE140 on the response of dorsal horn neurones to the peripheral injection of formalin

The effects of intrathecal HOE140 on the responses of the dorsal horn neurones to the peripheral injection of formalin were studied to investigate the possibility of a physiological spinal role of bradykinin during inflammatory nociception.

The responses of 20 dorsal horn neurones to the peripheral injection of formalin in the presence of intrathecal HOE140 (doses 1-500µg) were studied. HOE140 was applied intrathecally 20 mins prior to the peripheral injection of formalin. The mean depth of neuronal population was 602±72µm.

The first phase of the formalin response was not significantly altered from control by intrathecal administration of HOE140 (table 4).
Table 4. The effect of intrathecal HOE140 on the first phase of the formalin response.

<table>
<thead>
<tr>
<th>Dose</th>
<th>First phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μg</td>
<td>-29±27% (facilitation)</td>
</tr>
<tr>
<td>10μg</td>
<td>7±19%</td>
</tr>
<tr>
<td>100μg</td>
<td>15±26%</td>
</tr>
<tr>
<td>500μg</td>
<td>37±22%</td>
</tr>
</tbody>
</table>

The effect of intrathecal HOE140 on the first phase of the formalin response. Results are expressed as percentage inhibition of the control first phase response of the dorsal horn neurones to the peripheral injection of formalin. n=5 per group.

The second phase of the formalin response was dose-relatedly inhibited by intrathecal HOE140 (figure 9). With the lower doses of intrathecal HOE140 the second phase of the formalin response was non-significantly facilitated. Whereas with 100μg of HOE140 there was a tendency towards inhibition of the second phase of the formalin response. The highest dose of HOE140, 500 μg, significantly inhibited the second phase of the formalin response (44±19% inhibition, p=0.04). An example response of a single dorsal horn neurone to a peripheral injection of formalin after intrathecal administration of 500μg of HOE140 is shown in appendix 2. These results provide evidence for a physiological role of spinal bradykinin via an action at the B_2 receptor during prolonged nociceptive transmission.
Figure 9. The effect of intrathecal HOE140 on the second phase of the formalin response. Results are expressed as percentage inhibition or facilitation of the control second phase response of the dorsal horn neurones to the peripheral injection of formalin. Statistical analysis used Student’s two tailed unpaired t-test, *p< 0.05.

7.2.3 The effect of intrathecal administration of B4162 on the response of dorsal horn neurones to the peripheral injection of formalin

The effect of intrathecal administration of the early bradykinin B₂ receptor antagonist, B4162, on the response of the dorsal horn neurones to the peripheral injection of formalin was recorded from a total of 18 dorsal horn neurones. The mean depths of the neuronal population was 703±30μm.

Spinal administration of the early bradykinin antagonist, B4162, did not inhibit either phases of the formalin response (table 5). This is in contrast to the inhibitory effect of the equivalent dose of HOE140 on second phase of the formalin response. The differential effects of the two bradykinin B₂ receptor antagonists on the formalin response may be explained on the basis of HOE140 being considerably more potent and having a longer duration of action than B4162.
Table 5. The effect of intrathecal B4162 on the first and second phase of the formalin response.

<table>
<thead>
<tr>
<th>Dose</th>
<th>First phase</th>
<th>Second phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>50μg</td>
<td>72±10%</td>
<td>107±18%</td>
</tr>
<tr>
<td>100μg</td>
<td>117±25%</td>
<td>74±21%</td>
</tr>
<tr>
<td>500μg</td>
<td>76±37%</td>
<td>110±30%</td>
</tr>
</tbody>
</table>

Results are expressed as percentages of the first phase and second phase of the control response of the dorsal horn neurones to the peripheral injection of formalin. n=4-7

7.2.4 The mean maximal effect of intrathecal HOE140 on the electrically evoked C-fibre, Aβ-fibre and wind up response of the dorsal horn neurones

The effect of intrathecal HOE140 (500 μg) on the electrical evoked responses was studied on a total of 6 dorsal horn neurones. The mean depth of the neuronal population was 685±153μm. The mean maximal changes in the electrically evoked C- and Aβ- fibre neuronal responses observed with this treatment were 36±27% facilitation and 26±8% inhibition respectively. The mean maximal change in the windup was 52±79% facilitation, illustrating that the effect of HOE140 on this response was more variable. This lack of effect of HOE140 on the electrically evoked C-fibre and Aβ-fibre responses of the dorsal horn neurones shows HOE140 has no effect on acute non-inflammatory nociceptive processing. This is in contrast to the clear inhibition of the second phase of the formalin response by spinal administration of the antagonist. Moreover, since the electrically evoked responses of the neurones were not altered by this treatment, this high dose of HOE140 did not produce non-specific effects on these neurones.
7.3 Discussion

The role of peripheral bradykinin during prolonged nociception

The algesic action of bradykinin at peripheral levels is well established (see refs. in Bathon and Proud 1991). The ability of bradykinin to activate peripheral sensory nerve endings which subsequently transmit nociceptive information into the dorsal horn of the spinal cord has been attributed to an action at the bradykinin B$_2$ receptor on the peripheral sensory nerve endings (Steranka et al. 1988, Haley et al. 1989, see refs. Dray and Perkins 1993). Recent studies have shown that the ability of bradykinin to excite C-fibres is not dependent on an intact sympathetic nervous system nor due to a secondary vascular reaction (Koltzenberg et al. 1992).

The intraplantar administration of HOE 140 into the same site as the formalin injection resulted in a marked reduction of both the first phase and second phase of the formalin response with the effect on the second phase being greater than that on the first. There was no difference between the degree of inhibition by HOE 140 during the first 5 minutes as compared to the second 5 minutes of the first phase of the response. Overall, these results are in concordance with recent behavioural studies of intraplantar HOE 140 on the formalin response (Beresford and Birch 1991, Correa and Calixto 1993). By contrast, previous studies with peripherally applied B4162 only observed a reduction in the second phase of the response (Haley et al. 1989). These differences may be due to a lower potency of the earlier antagonists, especially in view of my findings that the second phase of the formalin responses was more sensitive to B$_2$ receptor antagonism than the first phase. From my results and behavioural studies it can be concluded that bradykinin has a peripheral role in both early events following the administration of formalin as well as a major pro-nociceptive role in the generation of the second phase of the formalin response.

The role of the B$_1$ receptor in the peripheral mechanisms of nociception has previously been investigated. The bradykinin B$_1$ receptor antagonist, Des-Arg$^9$-(Leu$^8$)-bradykinin has been shown not to alter either phase of the formalin response (Haley et al. 1989). In comparison, a behavioural study in mice using Des-Arg$^9$-(Leu$^8$)-bradykinin indicated a role of the peripheral B$_1$ receptor during both phases of the formalin response.
(Shibata et al. 1989). The discrepancy between the electrophysiological and behavioural study may be due to species differences or the relatively high dose of Des-Arg⁹-(Leu⁸)-bradykinin used in the behavioural study acting non specifically. Recent evidence suggests bradykinin B₁ receptors are induced during longer term inflammatory states such as in the ultraviolet induced model of hyperalgesia (Perkins et al. 1993a). It has been shown that the induction of B₁ receptors is enhanced by interleukin-1, and as desArg⁹Bk stimulates interleukin-1 release from macrophages a positive feed back system is created (see refs. in Perkins et al. 1993a). The formalin response lasts for one hour and this is probably not sufficient time for the induction of B₁ receptors, thus explaining the inability of peripheral bradykinin B₁ receptor antagonists to inhibit the formalin response.

Studies using the human blister base have shown that B₁ receptor antagonism is not effective against bradykinin induced pain whereas B₂ receptor antagonists significantly reduce the response (Whalley et al. 1987).

The role of spinal bradykinin during prolonged nociception

As indicated in the introduction, previous studies of the role of bradykinin in the spinal cord have not been coherent. I have shown that spinally administered HOE140 significantly inhibited the second phase of the response of the dorsal horn neurones to the peripherally injected formalin. These results indicate bradykinin has a spinal role in the transmission of nociceptive information during the second phase of the formalin response. Spinal administration of B4162 did not influence either phases of the formalin response, and this may be due to this early B₂ receptor antagonist being rapidly degraded or insufficiently potent. A comparison of the effects of HOE140 and B4162 on the formalin response shows HOE140 to be about 50 times more potent than B4162, and as previously stated HOE140 has a longer duration of action, lasting for at least three hours. The ability of HOE140 to inhibit the second phase of the formalin response probably reflects its greater potency as compared to B4162. The dose of intrathecal HOE140 (500μg) which inhibited the second phase of the formalin response did not alter the electrically evoked responses of the dorsal horn neurones. Therefore non-specific side effects are unlikely to underlie the effect of HOE140 on the formalin response. These results suggest that endogenous bradykinin is present in the spinal cord during
inflammatory pain states but not during acute nociception. A possible source of central bradykinin is from the vascular innervation of the dura, pia and other non-neuronal structures surrounding the spinal cord.

Studies of cultured rat sensory neurones have shed light on the cellular mechanisms mediating bradykinin evoked excitability. Bradykinin has been shown to increase the opening of sodium channels of sensory neurones, resulting in depolarisation of the neurones with the response being dependent on protein kinase C activation (Burgess et al. 1989). In another in vitro study, the response to bradykinin was shown to be mediated via a G-protein activation (McGuirk and Dolphin 1992).

To summarize, bradykinin appears to be involved in the peripheral generation of both phases of the formalin response but predominantly in the second phase. Spinally, bradykinin is only involved in the second phase of the response of the dorsal horn neurones to the peripheral injection of formalin. Thus the relative roles of bradykinin at the two sites will vary with time as the inflammation develops. Spinal bradykinin did not appear to be involved in the processing of acute electrically evoked responses of the dorsal horn neurones. Overall, these findings may be of importance not only to events underlying the processing of cutaneous nociceptive information but in other circumstances where central vascular changes occur such as in migraine. Although the triggers for migraine are unknown, it is associated with neurogenic inflammation of the dura mater. It has been shown that during migraine perivascular trigeminal axons are activated and release peptides within the dura mater. These peptides cause vasodilation and plasma extravasation which may result in the generation of inflammatory mediators, such as bradykinin and prostaglandins which activate the C-fibre afferents (see refs. in Moskowitz 1992). Since bradykinin is generated during neurogenic inflammation and activates C-fibres, the bradykinin receptor antagonists may have a therapeutic role in the management of migraine as well as in inflammatory pain arising elsewhere in the body.
CHAPTER 8:

THE ROLE OF PROSTAGLANDINS IN NOCICEPTIVE PROCESSING
8.1 Introduction

The prostaglandins are products of cyclooxygenase enzyme activity (see refs. in Samuelsson et al. 1978). Prostaglandin E2 (PGE2), prostaglandin D2 (PGD2) and prostaglandin I2 (PGI2) are generated and present in tissue during inflammatory responses (see refs. in Rang et al. 1991). The relative contributions of PGE2 and PGI2 during nociceptive transmission has recently been addressed by a number of studies. As discussed in this chapter, there is considerable evidence for an important role of peripheral PGE2 and PGI2 during inflammatory nociceptive processing. The development of selective tools for the prostaglandins has started to shed light on the differential roles of the prostaglandins during nociceptive transmission. The recent studies which have used selective antagonists of prostaglandins to investigate the role of prostaglandins during nociceptive transmission are discussed below.

The peripheral role of the prostaglandins during nociceptive transmission

Generally prostaglandins alone evoke negligible pain responses except at very high doses (Crunkchorn and Willis 1971). However, prostaglandins have been shown to sensitize peripheral primary afferents (Moncada et al. 1975), in particular to bradykinin (Ferreira 1972, 1973, Willis and Comelson 1973). One of the consequences of this sensitization was shown to be the lowering of nociceptive flexion reflex thresholds (Taiwo and Levine 1986, Taiwo et al. 1987). A more recent study has shown PGE2 and PGI2 are the dominant hyperalgesic metabolites of the cyclooxygenase pathway (Taiwo and Levine 1990). Intra-arterial PGE2 has been shown to excite and sensitize the medial articular nerve afferents of the joint to noxious and innocuous movement and bradykinin (Schaible and Schmidt 1988, Schepelmann et al. 1992). In addition, in the study by Schepelmann et al. 1992, PGI2 was also shown to produce excitatory and sensitizing effects. Differential effects of PGE2 and PGI2 were observed. PGI2 was shown to excite and sensitize the nociceptive afferents for a shorter duration than PGE2, but PGI2 was able to excite a greater proportion of afferents (Schepelmann et al. 1992).

There is an intricate relationship between the prostaglandins and bradykinin. Bradykinin is known to stimulate the generation of prostaglandins (Lembeck et al. 1976).
The mechanism of this effect has been shown to be via bradykinin B2 receptor activation, which in turn activates phospholipase A2 resulting in the generation of prostaglandin precursors from membrane phospholipids (Juan 1977). Since prostaglandins sensitize the nociceptive afferents to the effects of bradykinin, bradykinin by causing the production of the prostaglandins can augment its own algesic action (Lembeck et al. 1976, Chahl and Iggo 1977). In the periphery, both bradykinin and prostaglandins have been shown to evoke a dose dependent secretion of CGRP-like immunoreactivity with this effect being blocked by the cyclooxygenase inhibitor indomethacin (Geppetti et al. 1991). The effect of bradykinin and prostaglandins was sensitive to capsaicin, therefore indicating either a direct or indirect action of the prostaglandins on the primary afferents (Geppetti et al. 1991). In addition, there is evidence for the production of prostaglandins by sympathetic postganglionic neurones, since an intact post-ganglionic neurone terminal is required for arachidonic acid-induced hyperalgesia (Gonzales et al. 1989).

The non steroidal anti-inflammatory drugs (NSAID) inhibit the cyclooxygenase enzyme and therefore inhibit the generation of the prostaglandins (Vane 1971, see refs. in Samuelsson et al. 1978). The inhibition of peripheral prostaglandin production by the NSAIDs has been shown to prevent the sensitization of the afferent endings and consequently decrease hyperalgesia (Ferreira et al. 1973, Moncada et al. 1975, Gonzales et al. 1989). Moreover, intraperitoneal administration of NSAIDs has been shown to inhibit hyperalgesia associated with the reperfusion of the rat tail following a period of ischaemia (Gelgor et al. 1992a). In contrast, intraperitoneal NSAIDs did not alter the behavioural response to the ischaemic stimulus nor did they influence tailflick latencies in the absence of ischaemia (Gelgor et al. 1992a).

With the development of a selective PGI2 receptor agonist, cicaprost, the role of PGI2 in nociceptive processing is becoming more apparent. A recent study which utilized cicaprost provided evidence for a role of PGI2 in the sensitization of high-threshold joint mechanoreceptors during mild adjuvant-induced arthritis (McQueen et al. 1991).

The role of the prostaglandins at the spinal level during nociceptive transmission

In addition to a peripheral role, there is considerable evidence that prostaglandins
have a spinal role during nociceptive transmission. PGE$_2$ has been shown to sensitize cultured sensory neurones dissociated from the trigeminal and dorsal root ganglia (Baccaglini and Hogen 1983). The release of spinal prostaglandins during nociceptive processing has been demonstrated in a number of studies. Increased levels of prostaglandins have been shown to be present in the perfusate of the spinal cord upon hindlimb stimulation (Ramwell et al. 1966) and following noxious peripheral thermal stimulation but not innocuous thermal stimulation (Coderre et al. 1990b). The increased levels of PGE$_2$ within the spinal cord have been shown to be dependent on the presence of noradrenergic terminals within the spinal cord, implicating the bulbospinal noradrenergic terminals as the site of origin of spinal prostaglandins (Coderre et al. 1990b).

Further evidence for a spinal role of prostaglandins during nociceptive transmission has been acquired indirectly with studies which utilize the intrathecal administration of prostaglandins. Intrathecal administration of PGE$_2$ and PGF$_2\alpha$ has been shown to produce hyperalgesia in the Randall-Selitto paw withdrawal test (Taiwo and Levine 1986). In addition, intrathecal PGE$_2$ has been shown to have an hyperalgesic effect on the hot plate test and the acetic acid writhing test in mice and this effect was dose dependently blocked by a prostaglandin EP$_1$ receptor antagonist (Uda et al. 1990). In a more recent behavioural study intrathecal prostaglandins were shown to produce dose-dependent hyperalgesia in the thermal tail flick test and intrathecal indomethacin was shown to produce a dose-dependent analgesia (Taiwo and Levine 1988). The behavioural studies by Taiwo and Levine is in agreement with a recent electrophysiological study which has implicated a central role of prostaglandins during acute nociceptive transmission. In this study intrathecal administration of indomethacin was shown to reduce the C-fibre evoked activity but was without effect on the A$\beta$-fibre evoked activity (Jurna et al. 1992). The analgesic effect of indomethacin on acute nociceptive transmission reported by Taiwo and Levine 1988 is in contrast to another study in which intrathecal administration of a number of NSAIDs, including indomethacin, were shown to be without effect on acute nociception, using in this case, the hot plate latency test (Malmberg and Yaksh 1992a). Therefore the role of central prostaglandins during acute nociceptive transmission remains unclear. Evidence for a spinal role of prostaglandins during prolonged nociceptive transmission has been acquired by a recent behavioural study. Intrathecal NSAIDs were shown to inhibit excitatory amino acid and SP-induced
thermal hyperalgesia, whereas NSAIDs had no effect on normal thermal response latencies (Malmberg and Yaksh 1992b). It has been suggested by Malmberg and Yaksh that NSAID’s function as spinal analgesics in pain models in which there is a spinally mediated hyperalgesic state evoked by a protracted barrage from small afferents.

The study by Taiwo and Levine 1988 indicated the mechanism of action of spinal prostaglandins, it was shown that intrathecal prostaglandins blocked stimulation produced analgesia and analgesia produced by supraspinal opioids. In addition, intrathecal prostaglandin antagonists were shown to synergise with supraspinal morphine to produce analgesia. Taken together, these results implicate an inhibitory action of the prostaglandins on the descending noradrenergic bulbospinal projection neurones (Taiwo and Levine 1988).

In this series of experiments I have studied the role of spinal prostaglandins during acute and prolonged nociceptive transmission. For comparative purposes, the role of peripheral prostaglandins during prolonged nociceptive transmission was also studied. The non steroidal anti-inflammatory drug, indomethacin, was used. To gauge whether spinal roles of prostaglandins parallel those seen in the periphery during inflammatory pain states I have compared the effects of subcutaneous indomethacin and intrathecal indomethacin on the responses of the dorsal horn neurones to a peripheral injection of formalin. In addition, I have studied the effect of intrathecal indomethacin on the dorsal horn neuronal response to non-inflammatory non-tissue damaging electrical stimuli.
8.2 Results

The control values of the first and second phase of the formalin response for these experiments were 4547±655 and 23555±2753 action potentials respectively (n=15). The mean depth of neuronal population was 690±81 μm.

8.2.1 The effect of subcutaneous indomethacin on the response of the dorsal horn neurones to a peripheral injection of formalin

The effect of a subcutaneous injection of indomethacin on the response of the dorsal horn neurones to a peripheral injection of formalin was recorded from a total of 14 dorsal horn neurones. The mean depth of the neuronal population was 1019±119 μm. Indomethacin was subcutaneously injected into the scruff of the neck twenty minutes prior to the peripheral injection of formalin.

The lower dose studied, 250 μg/kg of indomethacin, did not alter either the first phase (120±27% control) or second phase (134±34% control) of the formalin response. In comparison, the higher dose, 5mg/kg indomethacin, significantly inhibited the second phase of the response, 71±17% inhibition of the control second phase response, p=0.006. The first phase of the formalin response was not altered by 5mg/kg indomethacin.

8.2.2 The effect of intrathecal indomethacin on the response of dorsal horn neurones to a peripheral injection of formalin

The effect of intrathecal indomethacin on the response of the dorsal horn neurones to a peripheral injection of formalin was recorded from a total of 45 dorsal horn neurones. The mean depth of the neuronal population was 791±35 μm. Indomethacin was administered 20 minutes prior to the peripheral injection of formalin.

The lower doses of intrathecal indomethacin (0.1-50 μg) did not alter the first phase of the formalin response. In contrast, the highest dose of intrathecal indomethacin, 250 μg, significantly inhibited the first phase of the formalin response (54±13% inhibition, p=0.04) (figure 10).
Figure 10. The effect of intrathecal indomethacin on the first phase of the formalin response. Results are expressed as percentage inhibition of the control first phase response of the dorsal horn neurones to a peripheral injection of formalin. Statistical analysis used Student’s unpaired two tailed t-test, *p< 0.05.

The second phase of the formalin response was not inhibited by the lower doses of intrathecal indomethacin (0.1-50 μg) but was significantly inhibited by intrathecal administration of 250μg of indomethacin (76±12% inhibition, p=0.001) (figure 11). An example of the inhibitory effect of intrathecal administration of 250μg of indomethacin on the response of a single dorsal horn neurone to a peripheral injection of formalin is shown in appendix 2.
Figure 11. The effect of intrathecal indomethacin on the second phase of the formalin response. Results are expressed as percentage inhibition of the control second phase response of the dorsal horn neurones to a peripheral injection of formalin. Statistical analysis used Student’s unpaired two tailed t-test, *p< 0.05, **p<0.001.

The lower dose of subcutaneous indomethacin (250μg/kg) did not influence either phase of the formalin response. Therefore the effects of the highest dose of spinal indomethacin (250μg) on the formalin response are probably due to actions restricted to the spinal cord and are not as a consequence of leakage into the systemic circulation. The dose response curve for indomethacin on the first phase and the second phase was steep, possibly be due to the mechanism of action of indomethacin. Indomethacin inhibits cyclooxygenase which generates prostaglandins and may have an all or nothing effect on this enzyme, possibly explaining why the effect of indomethacin was not dose-dependent.

8.2.3 The mean maximal effect of intrathecal indomethacin on the electrically evoked responses of the dorsal horn neurone

The effects of intrathecal indomethacin (125μg and 250μg) on the electrically evoked C-fibre, Aβ-fibre and wind up responses of the dorsal horn neurones was studied on a total of 12 dorsal horn neurones. The mean depth of the neuronal population was 858±42μm.
The acute electrically evoked responses of the dorsal horn neurones were not significantly altered from the control values by intrathecal indomethacin (table 6). These results suggest the prostaglandins does not play an observable role during the spinal transmission of nociceptive inputs associated with non inflammatory states.

Table 6. The mean maximal effect of intrathecal indomethacin on the electrically evoked responses of the dorsal horn neurones.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
</tr>
</thead>
<tbody>
<tr>
<td>125μg</td>
<td>109±11%</td>
<td>98±7%</td>
<td>146±57%</td>
</tr>
<tr>
<td>250μg</td>
<td>97±10%</td>
<td>95±4%</td>
<td>182±68%</td>
</tr>
</tbody>
</table>

The mean maximal effect of intrathecal indomethacin on the electrically evoked responses of the dorsal horn neurones are expressed as percentages of the control (pre-drug) responses. n=6 per group

Since the doses of indomethacin used in this study did not alter the electrically evoked responses of these neurones it may be inferred that these doses of indomethacin are not producing non-specific actions on the dorsal horn neurones. Importantly the same doses of indomethacin inhibited the formalin response indicating that the effects of indomethacin on the on the dorsal horn neurones are not due to non-specific effects.
8.3 Discussion

The peripheral role of prostaglandins during the formalin response

There was a tendency towards an inhibition of the first phase of the formalin response by the highest dose of subcutaneous indomethacin studied but this effect was not significant. The second phase of the response was significantly inhibited by the higher dose of subcutaneous indomethacin (5 mg/kg). These findings are in agreement with a recent behavioural study which has shown subcutaneous indomethacin inhibits the second phase of the formalin response (Correa and Calixto 1993). Taken together these results implicate a contribution of peripheral prostaglandins in the generation of the second phase but not the first phase of the formalin response. It should be noted that the effects of subcutaneously administered indomethacin on the formalin response may be due to actions at central sites as well as peripheral sites.

The results of this study with subcutaneous indomethacin confirm previous studies which have shown only the second phase of the formalin response to be inhibited by intraplantar (Shibata et al. 1989) and intraperitoneal (Hunskaar et al. 1986, Malmberg and Yaksh 1992a) indomethacin. In addition, a peripheral role of prostaglandins during the behavioural response to formalin and during the acetic acid writhing response has been shown with oral prostaglandin antagonists (Drower et al. 1987). The contribution of peripheral prostaglandins during prolonged inflammatory nociception has been observed in another model of prolonged nociception where intraperitoneal NSAIDs have been shown to inhibit the hyperalgesia associated with reperfusion of the rat tail following ischaemia (Gelgor et al. 1992a).

The spinal role of prostaglandins during the formalin response

In these studies intrathecal indomethacin significantly reduced both the first and second phase of the formalin response, this finding implicates a physiological role of spinal prostaglandins during prolonged nociception. However, the acute electrically evoked responses of the dorsal horn neurones were not altered in the presence of intrathecal indomethacin. The results of the electrically evoked study are in agreement
with the study by Malmberg and Yaksh 1992a but contradict studies which implicate a role of the prostaglandins during acute nociceptive transmission (Taiwo and Levine 1988, Jurna et al. 1992). The dose of intrathecal indomethacin used in the study by Jurna et al. 1992 was 100μg. Therefore there are large dose discrepancies between this study and my results, which have shown the electrically evoked responses of the dorsal neurones to be unaltered by intrathecal indomethacin (250μg). A possible explanation for these differences may be due to the use of the anaesthetic urethane by Jurna et al.

A full recovery of the inhibitory effect of indomethacin on the formalin response was observed despite the relatively high dose of indomethacin (250μg) required. In addition, the same dose of indomethacin did not influence the electrically evoked responses. Taken together this suggests the inhibitory effects of indomethacin on the formalin response are not due to toxic effects and are selective for inflammatory pain states. Comprably high doses of indomethacin are required for peripheral analgesia in human studies and access problems particularly after intrathecal application may explain why high doses were required in my study.

This study implicating a spinal role of prostaglandins during the formalin response is in agreement with recent behavioural studies of the effect of intrathecal indomethacin on the response to formalin (Malmberg and Yaksh 1992a, Malmberg and Yaksh 1992b). In addition, post-administration of NSAIDs was shown to be effective at inhibiting the second phase of the behavioural response to formalin, therefore indicating a rapid and ongoing generation of prostaglandins during the formalin response (Malmberg and Yaksh 1992a, Malmberg and Yaksh 1992b). Moreover, intrathecal administration of prostaglandin PGE receptor antagonists has been shown to reduce the second phase of the behavioural response to formalin (Yaksh et al. 1993). Further evidence for a role of prostaglandins during the formalin response has been obtained by a recent microdialysis study. Peripheral injection of formalin was shown to be associated with a biphasic release of PGE2 at the spinal level which parallels the biphasic behavioural response to formalin (Malmberg and Yaksh, personal communication). In contrast, an earlier behavioural study of the response of mice to formalin concluded that acetylsalicylic acid, but not indomethacin, had central actions on nociceptive transmission in the spinal cord (Hunskaar et al. 1986, Hunskaar and Hole 1987). The discrepancy between the study by Hunskaar et al. 1986, Hunskaar and Hole 1987 and the other studies listed above, may be due to
access problems with systemically administered indomethacin.

Therefore it appears there is a spinal role of prostaglandins during prolonged protracted nociceptive transmission but not acute nociceptive transmission. In the present study I did not further increase the dose of intrathecal indomethacin to determine whether complete inhibition of the formalin response was possible. Other studies have shown there to be a ceiling effect of both NSAIDs and prostaglandin antagonists on the formalin response (Drower et al. 1987, Malmberg and Yaksh 1992a). This is probably due to the multitude of other transmitters involved in the relay of noxious information within the dorsal horn of the spinal cord.

The generation and possible mechanisms of action of spinal prostaglandins

The potential sequence of events leading to an increased production of prostaglandins involves an increase in intracellular calcium, activation of phospholipase A\textsubscript{2} and consequently an increase in cytosolic arachidonic acid. Prostaglandins are generated by the action of cyclooxygenase on the free pools of arachidonic acid (Rang and Dale 1991). Interestingly spinal prostaglandins appear only to be involved in prolonged nociceptive processing such as the second phase of the formalin response. This response has previously been shown to be partly mediated by NMDA receptor activation (Haley et al. 1990), which increases cytosolic calcium (see refs. in Miller 1992). Therefore it is feasible that NMDA receptor activation is the starting point for the generation of prostaglandins during prolonged nociceptive transmission. It is possible that during acute electrically evoked stimulation NMDA receptor activation is insufficient to produce the required levels of intracellular free calcium to result in prostaglandin production. This hypothesis explains the inability of intrathecal indomethacin to inhibit the electrically evoked responses of the dorsal horn neurones.

Interestingly, in cerebellar granular cells, arachidonic acid has been shown to directly potentiate and make more transient the current through NMDA receptor channels therefore enhancing the NMDA receptor channel opening probability which in turn increases intracellular calcium levels (Miller et al. 1992). If similarities hold between this system and events in the spinal cord during the formalin response, the reinforcing effect of arachidonic acid on NMDA receptor mediated events may enhance the second phase of the formalin response. Since arachidonic acid generation requires increased levels of calcium
for its generation this action of arachidonic acid on the NMDA receptor channel which results in increased levels of intracellular calcium will have a self reinforcing effect on the generation of arachidonic acid. In addition, arachidonic acid has been shown to decrease glutamate uptake in glial cells (Barbour et al. 1989) and again if this occurs within the spinal cord this may also influence the formalin response.

Two mechanisms have been proposed for the actions of prostaglandins during nociceptive transmission. Firstly, the prostaglandins have been shown to increase calcium conductance and release substance P from avian sensory neurones, and it has been suggested that the sensitizing effects of prostaglandins may result as a consequence of this increased calcium current (Nicol et al. 1992). This effect was confirmed by a more recent study in which prostaglandins were shown to evoke substance P release from cultured dorsal root ganglia neurones (Chang et al. 1993). Secondly, the prostaglandins have been shown to inhibit the descending noradrenergic inhibitory controls (Taiwo and Levine 1988). The effects of prostaglandins within the spinal cord during prolonged nociceptive processing are probably a combination of these two mechanisms.

There is also evidence for a role of the prostaglandins at the level of the higher brain centres during nociceptive transmission. Microinjections of the NSAID, diclofenac, into the periaqueductal grey area, ventromedial thalamus and nucleus raphe magnus inhibits ethacrynic acid-induced writhing response in the rat (Bjorkman et al. 1992). In addition, intracerebroventricular administration of a prostaglandin antagonist was shown to inhibit the hyperalgesia evoked by carrageenan (Ferreira et al. 1978) and intracerebroventricular administration of NSAIDs has been shown to inhibit the development of post-ischemia reperfusion hyperalgesia of the rat’s tail (Gelgor et al. 1992b).

In conclusion, subcutaneous administration of indomethacin only inhibited the second phase of the formalin response, whereas intrathecal indomethacin inhibited both phases of the formalin response. From these results it may be inferred that peripheral prostaglandins contribute to the second phase of the response. However, at the spinal level prostaglandins appear to be involved in the processing of both the first and second phase of the formalin response. Spinal prostaglandins do not appear to be involved in the processing of acute electrically evoked responses of the dorsal horn neurones. From these studies and the literature prostaglandins appear to contribute to prolonged inflammatory nociceptive processing at the peripheral, spinal and supraspinal level.
CHAPTER 9:

THE CONTRIBUTION OF THE EXCITATORY AMINO ACIDS DURING NOCICEPTIVE TRANSMISSION
9.1 Introduction

Distribution of the excitatory amino acid glutamate and glutamate binding sites in the spinal cord

There is considerable anatomical evidence implicating the excitatory amino acid glutamate in the transmission of nociceptive information in the dorsal horn of the spinal cord. The spinal cord is known to have high levels of glutamate immunoreactivity, originating from myelinated and unmyelinated primary afferent fibres in addition to intrinsic interneurones and projection neurones (Battaglia and Rustioni 1988, Miller et al 1988, Maxwell et al 1990).

The actions of the excitatory amino acids have been extensively shown to be mediated via the N-methyl-D-aspartate (NMDA) receptor and non-NMDA receptors, primarily the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor and the metabotropic and kainate receptors (see refs. in Watkins et al. 1991 and Barnard and Henley 1991). Moreover, the glutamate binding sites and NMDA sites have been shown to be concentrated in the outer laminae of the dorsal horn in the human (Jansen et al. 1990), cat (Mitchell and Anderson 1991), mice (Gonzalez et al. 1993) and rat (Greenamyre et al. 1984, Monaghan and Cotman 1985, Henley et al. 1993) spinal cord. More recently the distribution of the expression of different subunits of the AMPA and NMDA receptor within the laminae of the dorsal horn has been investigated (Furuyama et al. 1993). Regional differences in the expressed AMPA receptor subunits were observed. The AMPA receptor subunits in the superficial laminae were different to those in the deeper laminae, suggesting different AMPA receptor subunits are involved in nociceptive transmission and innocuous transmission. In contrast the NMDA receptor subunit was shown to be evenly distributed within laminae II, III and IV-VI.

The development of selective excitatory amino acid antagonists

The importance of the excitatory amino acids during central excitatory transmission has become increasing evident with the advent of selective AMPA receptor and NMDA receptor antagonists. 6-cyano-7-nitroquinoxaline-2-3-dione (CNQX) has been shown to
be a selective antagonist of the AMPA receptor (Blake et al. 1988, Honore et al. 1988, Honore et al. 1989, Lodge and Johnson 1990). There are a number of antagonists of the NMDA receptor complex: competitive antagonists, AP5 and CG19755; non-competitive channel blockers, dizocilpine (MK801) and phencyclidine (TIPS Receptor Nomenclature 1993). In addition to a glutamate binding site, the NMDA receptor complex has a modulatory strychnine insensitive glycine site (see refs. in Kemp and Leeson 1993). Previous studies have shown glycine to be a co-agonist of the NMDA receptor (Thompson 1990). This has led to the development of novel antagonists for the glycine site on the NMDA receptor complex, including 7-chlorokynurenate (7CK) (Kemp et al 1988).

The role of the excitatory amino acids in the spinal processing of nociceptive information

In vitro studies have shown both Aβ-fibre and C-fibre activation increases aspartate and glutamate outflow in the dorsal horn of the spinal cord (see refs. in Headley and Grillner 1990, Jeftinija et al. 1991, Kangrga and Randic 1991). A recent microdialysis study has indicated non-NMDA receptors are present at the first synapse of primary afferent fibres in the dorsal horn. Sciatic nerve evoked amino acid release into the dorsal horn of the rat spinal cord was inhibited by the AMPA receptor antagonist CNQX. In contrast basal levels of amino acids were not altered by CNQX but were decreased by tetrodotoxin (Paleckova et al. 1992).

A number of in vitro rat spinal slice studies have provided evidence for the involvement of both non-NMDA and NMDA receptor activation during nociceptive transmission. Glutaminergic miniature excitatory postsynaptic currents (mEPSCs) recorded from rat spinothalamic tract neurones were shown to have both an early non-NMDA component and a later NMDA component (Hori and Endo 1992). In addition to this, repetitive stimulation of the primary afferent fibres has been shown to produce an excitatory synaptic response which consisted of two depolarizing components. The initial component was sensitive to both NMDA and non-NMDA receptor antagonists whereas the slower component was thought to be peptidergic (Gerber et al. 1991). Moreover, the AMPA receptor antagonist CNQX has been shown to inhibit dorsal root evoked

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extracellular ventral root reflexes and intracellular excitatory postsynaptic potentials in ventral horn neurones, in addition to inhibiting longer latency synaptic components (King et al. 1992).

Early in vivo studies showed that excitatory amino acid antagonists depress amino acid induced excitations and synaptic excitations of cat dorsal horn neurones (Davies and Watkins 1983). Iontophoretic application of NMDA and AMPA has been shown to dose-dependently activate spinal neurones, and these excitations were blocked by their respective selective antagonist. In the same studies, subthreshold doses of NMDA and AMPA enhanced spinal neuronal responses to peripheral noxious stimuli (Aanonsen et al 1990, Dougherty et al 1992).

Electrophysiological studies have shown acute electrically evoked A- and C-fibre evoked responses of the dorsal horn neurones to be dose-dependently inhibited by a non-selective excitatory amino acid receptor antagonist, whereas NMDA antagonists did not influence these responses (Davies and Watkins 1983, Schouenborg and Sjolund 1986, Dickenson and Sullivan 1990). In contrast, wind up (Mendell 1966), the frequency dependent potentiation of dorsal horn neuronal responses to repetitive C fibre stimulation has been shown to be inhibited by NMDA receptor antagonists (Davies and Lodge 1987, Dickenson and Sullivan 1987b, 1990, Haley et al. 1990, Dickenson and Aydar 1991, Woolf and Thompson 1991). Similarly in anaesthetized mice, the AMPA receptor antagonist CNQX has been shown to selectively inhibit the monosynaptic Hoffman-reflex, whereas the polysynaptic flexor reflex was selectively inhibited by the NMDA receptor antagonist 3-((±)-2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP) (Turski et al. 1990).

Plasticity, the capacity for change within neuronal systems, may result in enhanced or altered nociceptive responses of the dorsal horn neurones in the spinal cord. The NMDA receptor has been shown to be involved in the induction of amplified neuronal responses (central spinal hypersensitivity) (see refs. in Dickenson 1991, Dubner and Ruda 1992) associated with inflammatory (Haley et al. 1990), neuropathic (Seltzer et al. 1991, Mao et al. 1992b) and ischaemic (Sher and Mitchell 1990) nociception and also the induction of the facilitated flexor reflex (Woolf and Thompson 1991). In addition, the maintenance of amplified neuronal responses have been shown to be dependent on NMDA receptor mediated events (Dickenson 1991, Dubner and Ruda 1992) during inflammatory (Haley et al. 1990, Schaible et al. 1991).
and neuropathic nociception (Mao et al. 1992b) and the maintenance of the facilitated flexor reflex (Woolf and Thompson 1991).

The role of the excitatory amino acids during inflammatory nociceptive transmission has attracted much attention. The first and second phases of the formalin response are reduced by pre-administration of the non-selective amino acid antagonist γ-D-glutamylglycine (DGG), whereas post-administered DGG was ineffective (Haley et al. 1990). Interestingly, pre-administration of the NMDA receptor channel blocker, MK801, inhibited only the second phase of the formalin response. Post-administration of the same doses of MK801 produced similar inhibitions of the second phase response as observed with the pre-administration (Haley et al. 1990). Surprisingly, in a recent behavioural study of the formalin response, intrathecal application of NMDA or SP was shown to decrease nociceptive behaviour during the second phase of the response (Mjellem-Joly et al. 1992). This implies that the activation of inhibitory mechanisms by exogenously administered NMDA or SP can occur and suggests endogenous mechanisms can be brought into play to counteract high levels of NMDA receptor mediated excitation (Mjellem-Joly et al. 1992). There are a number of problems with the study by Mjellem-Joly et al. 1992. Firstly, the control behavioural response to formalin was not biphasic and a silent phase was not observed. Secondly, the inhibitory effect of intrathecal NMDA or SP was only observed during the 10-20 minute period of the response and this time point is normally associated with the silent phase of the response. Thirdly, intrathecal co-administration of SP and NMDA enhanced the formalin response. A mechanism by which two agonists when administered independently inhibit the formalin response but when administered together enhance the formalin response is not easy to understand and therefore it is very difficult to draw any conclusions from this study.

In the carrageenan induced model of inflammatory nociception MK801 was shown to increase the ipsilateral paw withdrawal latencies to noxious heat, whereas the withdrawal latencies of the contralateral paw were unaltered (Ren et al. 1992). Therefore NMDA receptor mediated events appear to be involved in the generation of central hypersensitivity during nociceptive processing in the carrageenan model of inflammatory pain.

In the first series of experiments I have studied the effect of intrathecal CNQX on acute electrically evoked nociception. Since the role of the AMPA receptor during inflammation nociception has not previously been reported, I have studied the effect of
the selective AMPA receptor antagonist, CNQX, on the formalin response. The importance of the timing of the administration of CNQX relative to the peripheral formalin injection was also studied. Intrathecal CNQX was either administered 5 minutes before the peripheral injection of formalin or 5 minutes after the peripheral injection of formalin.

In the second series of experiments, I have studied the effect of intrathecal 7CK on the formalin response, with the importance of the timing of the administration of 7CK relative to the formalin injection again being studied. Intrathecal 7CK was either administered 5 minutes before the peripheral injection of formalin or 5 minutes after the peripheral injection of formalin.
9.2 Results

9.2.1 The mean maximal effect of CNQX on the electrically evoked C-fibre, Aβ-fibre and wind up response of the dorsal horn neurones

The effect of intrathecal CNQX (doses 5μg, 50μg and 500μg in 50μl volume) on the electrically evoked C-fibre and Aβ-fibre responses and wind up of the dorsal horn neurones was studied for 60 minutes, at 5 minute intervals post drug administration. A total of 22 deep dorsal horn neurones were studied in this series of experiments.

The electrically evoked C-fibre, Aβ-fibre and wind up responses of the dorsal horn neurones were not influenced by the lowest dose of intrathecal CNQX (5μg). Both the Aβ-fibre evoked response and wind up of the dorsal horn neurones were significantly inhibited by 50μg of CNQX. In contrast, the C-fibre evoked response was not influenced by 50μg of CNQX. The highest dose of CNQX studied (500μg) significantly inhibited the C-fibre evoked response as well as the Aβ-fibre evoked response and wind up of the dorsal horn neurones (figure 12). Therefore the Aβ-fibre evoked response was more sensitive than the C-fibre evoked response of the dorsal horn neurones to intrathecal CNQX. It is important to note that in this study the Aβ-fibre evoked responses of the dorsal horn neurones was to a supra-maximal stimulus at 3x the C-fibre threshold. It is possible that this supra-maximal activation of the Aβ-fibres results in maximal activation of AMPA receptors within the dorsal horn which in turn makes the response extremely sensitive to CNQX. The C-fibre evoked response of the dorsal horn neurones was only sensitive to the highest dose of CNQX, possibly due to the contribution of peptides as well as excitatory amino acids in the spinal transmission of nociceptive information.
Figure 12. The mean maximal effect of intrathecal CNQX on the electrically evoked responses of the dorsal horn neurones. Results are expressed as percentage inhibition of the control (pre-drug) electrically evoked responses. Statistical analysis used Student's two tailed paired t-test, *p< 0.05, **p< 0.001.

9.2.2 The effect of intrathecal CNQX on the response of the dorsal horn neurones to a peripheral injection of formalin

The control first phase and second phase responses of the dorsal horn neurones to a peripheral injection of formalin were 5762±898 and 20484±2016 action potentials respectively (n=10).

The effect of intrathecal CNQX (doses 0.5μg, 5μg, 50μg and 500μg in 50μl volume) on the response of the dorsal horn neurones to a peripheral injection of formalin was studied. CNQX was applied intrathecally either 5 minutes before (pre-administration) or 5 minutes after (post-administration) the peripheral injection of formalin.
9.2.3 The effect of intrathecal CNQX given 5 minutes prior to a peripheral injection of formalin on the first and second phase response of the dorsal horn neurones

The effect of intrathecal pre-administration of CNQX on the formalin response was studied on a total of 19 dorsal horn neurones. The lowest dose of CNQX did not alter the first phase of the formalin response. Higher doses of CNQX (5-500|g CNQX) significantly inhibited the first phase of the formalin response. The degree of the inhibitions of the first phase produced by CNQX (5-500|g) were not significantly different from each other, suggesting that the effects of these doses are at the plateau of the dose response curve (table 7).

The second phase of the formalin response was not inhibited by the lowest dose of CNQX. Dose-related inhibitions of the second phase of the formalin response were observed with the pre-administration of 5-50|g of CNQX. Both 50|g and 500|g of CNQX significantly inhibited the second phase response, but the magnitude of these inhibitions were not significantly different between the two doses, suggesting the effects of these doses are at the plateau of the dose response curve (table 7).
Table 7. The effect of intrathecal pre-administration of CNQX on the first and second phase of the formalin response.

<table>
<thead>
<tr>
<th>Dose</th>
<th>First Phase</th>
<th>Second Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5µg</td>
<td>-7±22</td>
<td>-1±19</td>
</tr>
<tr>
<td>5µg</td>
<td>40±22*</td>
<td>49±20*</td>
</tr>
<tr>
<td>50µg</td>
<td>52±20*</td>
<td>93±4***</td>
</tr>
<tr>
<td>500µg</td>
<td>40±28*</td>
<td>65±17*</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the first and second phase of the control formalin response as appropriate. Statistical analysis used Student’s two tailed unpaired t-test, *p< 0.05, **p< 0.001, ***p< 0.0001. n=4-5

9.2.4 The effect of the post-administration of intrathecal CNQX on the second phase response of the dorsal horn neurones to a peripheral injection of formalin

The effect of post-administration of intrathecal CNQX (0.5-500µg) on the formalin response was studied on a total of 19 dorsal horn neurones. The overall effects of post-administered CNQX on the second phase of the response were very different to those observed with pre-administered CNQX. With the lowest dose of CNQX (0.5µg) there was a tendency towards facilitation of the second phase of the formalin response (48±36% facilitation). With 5µg of CNQX the second phase of the formalin response was significantly facilitated (47±19% facilitation, p=0.03). Increasing doses of CNQX (50µg and 500µg) dose-relatedly inhibited the second phase of the formalin response (42±10% inhibition, p=0.02 and 51±20% inhibition, p= 0.02 respectively).
9.2.5 Comparison of the effect of pre- and post-administration of CNQX on the second phase of the formalin response

A comparison of the effect of pre-administered CNQX and post-administered CNQX on the second phase of the formalin response is shown in figure 13.

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Figure 13. The effect of pre-administered versus post-administered CNQX on the second phase of the formalin response. Results are expressed as percentage inhibition or percentage facilitation of the control second phase of the formalin response. Statistical analysis used Student's two tailed unpaired t-test, *p< 0.05, **p< 0.001.

The second phase of the formalin response was not significantly altered by pre- or post-administration of the lowest dose of CNQX (0.5µg). Pre-administration of 5µg CNQX significantly inhibited the second phase response whereas post-administration of this dose of CNQX facilitated the second phase response. Significantly different effects of this dose of CNQX were observed, depending on the timing of the administration (p=0.01). The time course of the effects of pre- and post-administered CNQX (5µg) are compared in figure 14. The facilitations of the second phase of the formalin response observed with post-administered CNQX (5µg) were significantly different to the inhibitions observed with pre-administered CNQX (5µg) for the 10-20 minute, 20-30 minute and 30-40 minute period of the formalin response (p=0.04, p=0.05 and p=0.02.
respectively).

Figure 14. A comparison of the time course of the effects of pre- and post-administered CNQX (5μg) on the formalin response. Significant differences were observed between 20 - 40 minutes. Statistical analysis used Student's unpaired two tailed t-test, *p<0.05.

Inhibition of the second phase of the formalin response by post-administration of 50μg CNQX was significantly less than that seen with pre-administration of this dose (p=0.002). The time course of the inhibitions produced by pre- and post-administered CNQX (50μg) are compared in figure 15. The inhibitions observed with post-administered CNQX (50μg) during the 40-50 minute and 50-60 minute period of the formalin response were significantly less than those observed with the same dose of pre-administered CNQX (p=0.01 and p=0.04 respectively).
Figure 15. A comparison of the time course of the effect of pre- and post-administered CNQX (50μg) on the formalin response. Significant differences in the degree of inhibitions produced were observed between 40-50 and 50-60 minutes. Statistical analysis used Student’s two tailed unpaired t-test, *p<0.05.

An example of the effect of pre-administration of 50μg of CNQX on the response of a single dorsal horn to a peripheral injection of formalin is shown in appendix 1. In addition, an example of the effect of post-administration of 50μg of CNQX on the response of a different single dorsal horn neurone to a peripheral injection of formalin is shown in appendix 1.

With the highest dose of CNQX (500μg) significant inhibitions of the second phase of the formalin response were produced by pre- and post-administration (p=0.003 and p=0.02 respectively), these inhibitions did not differ from each other (p=0.6).

Thus the ability of lower doses of CNQX to inhibit the second phase of the formalin response appears to be dependent on the timing of the intrathecal injection of CNQX relative to the peripheral injection of formalin.
9.2.6 The effect of intrathecal 7 chlorokynurenate on the response of the dorsal horn neurones to a peripheral injection of formalin

For this series of experiments, the control first phase response of the dorsal horn neurones to a peripheral injection of formalin was 7251±1031 action potentials. The control second phase response of the dorsal horn neurones to a peripheral injection of formalin was 29074±4999 action potentials (n=20). The mean depth of the neuronal population was 741±52μm.

The effect of intrathecal administration of 7CK was studied in a total of 47 dorsal horn neurones. The mean depth of the neuronal population was 756±40μm. 7CK was administered intrathecally either 5 minutes before (pre-administration) or 5 minutes after (post-administration) the peripheral injection of formalin.

Pre-administration of intrathecal 7CK (0.25-2.5μg) did not alter the first phase of the formalin response as compared to the control response (table 8).

Table 8. The effect of pre-administered 7CK on the first phase of the formalin response.

<table>
<thead>
<tr>
<th>Dose</th>
<th>First phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25μg</td>
<td>130±32%</td>
</tr>
<tr>
<td>1μg</td>
<td>81±13%</td>
</tr>
<tr>
<td>2.5μg</td>
<td>106±26%</td>
</tr>
</tbody>
</table>

Results are expressed as percentages of the control first phase response of the dorsal horn neurones to a peripheral injection of formalin, n=7-10

The second phase of the formalin response was significantly inhibited by intrathecal pre-administration of 7CK. In addition, intrathecal post-administration of 7CK significantly inhibited the second phase of the formalin response. Pre- and post-administration of 7CK produced identical inhibitions of the second phase of the formalin response (table 9). Therefore the timing of the administration of intrathecal 7CK did not
influence the ability of 7CK to inhibit the second phase of the formalin response.

An example of the effect of intrathecal pre-administration of 2.5μg of 7CK on the response of a single dorsal horn neurone to a peripheral injection of formalin is shown in appendix 1. In addition, an example of the effect of intrathecal post-administration of 2.5μg of 7CK on the response of a different single dorsal horn neurone to a peripheral injection of formalin is also shown in appendix 1.

Table 9. A comparison of the effect of pre- and post-administered 7CK on the second phase of the formalin response.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Pre-administration</th>
<th>Post-administration</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.25μg</td>
<td>-44±44% facilitation</td>
<td>20±20%</td>
</tr>
<tr>
<td>1μg</td>
<td>49±13%</td>
<td>40±18%</td>
</tr>
<tr>
<td>2.5μg</td>
<td>67±10%*</td>
<td>56±7%*</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the control second phase response of the dorsal horn neurones to a peripheral injection of formalin. Statistical analysis used Student's two tailed unpaired t-test, *p< 0.05; n=7-10
9.3 Discussion

The role of the AMPA receptor during acute nociceptive transmission

The dose-dependent inhibition of the electrically evoked Aβ-fibre response by CNQX indicates the involvement of the AMPA receptor during fast synaptic transmission at the Aβ-fibre synapses in the dorsal horn of the spinal cord. C-fibre evoked responses of the dorsal horn neurones were less sensitive to CNQX, with only the highest dose significantly inhibiting this response. A possible explanation for the C-fibre evoked responses being less sensitive to CNQX is based on both the excitatory amino acids and peptides having roles in the spinal transmission of C-fibre evoked responses of the dorsal horn neurones. It has previously been shown that electrical stimulation at A-fibre strength does not alter peptide immunostaining at the spinal level. In contrast, stimulation at C-fibre strength results in a significant reduction of peptide immunostaining at the spinal level (Klein et al. 1992). Therefore C-fibre stimulation but not A-fibre stimulation results in a release of peptides at the spinal level. With the blockade of only some of the AMPA receptors with lower doses of CNQX there will still be AMPA receptors in addition to the peptidergic receptors being activated after C-fibre stimulation and therefore this dose of CNQX will not influence the C-fibre evoked response of the dorsal horn neurones. With the highest dose of CNQX it could be envisaged that virtually all of the AMPA receptors are blocked, leaving only the peptidergic component. In the case of substance P (SP), as will be shown in chapter 10, a selective SP receptor antagonist has minimal effects on acute C-fibre evoked responses of the dorsal horn neurones. Thus SP is probably not a factor although other peptides such as CGRP, neurokinin A and neurokinin B could still transmit the nociceptive messages in the presence of a partial AMPA receptor block. Alternatively, the AMPA receptor may not have a role in the spinal processing of cutaneous nociceptive inputs. A recent study has suggested that NMDA receptors mediate cutaneous nociceptive transmission whereas AMPA receptors mediated nociceptive inputs originating from muscle (Song and Zhao 1993). The high dose (500μg) of CNQX inhibited the electrically evoked C-fibre response. It is possible that this dose of CNQX may be acting non-selectively at sites other than the AMPA receptor such as at the NMDA receptor (Birch et al. 1988).
Wind up which is mediated by NMDA receptor mechanisms (Dickenson and Sullivan 1987b) was sensitive to CNQX, thus implicating the AMPA receptor in its development. If the AMPA receptor does contribute to wind up it may be via AMPA receptor mediated post-synaptic depolarisations removing the Mg\(^{2+}\) block of the NMDA receptor channel, which is the necessary prerequisite for the activation of the NMDA receptor complex. But the rapid time course of AMPA receptor mediated post-synaptic depolarisations makes this unlikely. Peptides have been implicated in the slow long-lasting depolarisation of dorsal horn neurones observed with repetitive stimulation of the dorsal root (Urban and Randic 1984). These peptide mediated events are thought to be important in the induction of NMDA receptor mediated excitability (Mjellem-Joly et al. 1992, Xu et al. 1992a). Moreover, it has recently been suggested that neuropeptide induced long lasting post-synaptic depolarisations may have a role in the removal of the Mg\(^{2+}\) block of the NMDA receptor channel (Nagy et al. 1993).

The results of this study are in agreement with a study on primate spinothalamic tract neurones. Microdialysis of CNQX into the lumbar region of the spinal cord was shown to significantly inhibit the background responses of the spinothalamic tract neurones. In addition the responses of these neurones to innocuous stimuli as well as to noxious pinch were also inhibited (Dougherty et al 1992). In a recent study the spinal responses to innocuous and noxious stimulation of rat joints were studied. Ionophoretic application of CNQX and AP5 (NMDA receptor antagonist) into the spinal cord reduced the responses to noxious compression of the joint, whereas CNQX, but not AP5, reduced the responses to innocuous stimulation (Neugebauer et al. 1993).

The differential affect of intrathecal CNQX on the formalin response

Overall, pre-administration of intrathecal CNQX significantly inhibited the first and second phases of the formalin response. Post-administration of CNQX had a biphasic effect on the second phase of the response, with low dose facilitation and higher doses producing significant inhibitions. The formalin response was more sensitive than the electrically evoked C-fibre response to CNQX (pre-administration). The results with the pre-administration are in agreement with a behavioural study in which post-administration was not studied (Nasstrom et al. 1992). However, in another behavioural study, CNQX
did not influence the formalin response (Coderre and Melzack 1992). A possible explanation for the differences between these studies may be that the doses of CNQX used in the second behavioural study were slightly lower than those used in this electrophysiological study.

In this study we observed a shift in the sensitivity of the formalin evoked neuronal responses to CNQX during the second phase of the response. Post-administered CNQX, except at the highest dose, was less effective at inhibiting the second phase response as compared to pre-administered CNQX. Low doses of post-administered CNQX facilitated the dorsal horn neuronal responses so that the second phase was enhanced. As a result of this facilitatory action of CNQX the silent period between the first and second phase was not observed. This is interesting since it has previously been shown that the silent period arises from peripheral mechanisms since recordings from C-fibres after formalin reveal the biphasic nature of the response (Russell et al. 1987). This effect of the low dose of post-administered CNQX may be explained by AMPA receptors mediating excitatory transmission early in the first phase which in turn induces inhibitory events which then dampen down neuronal activity. Post-administered CNQX may act to inhibit these AMPA receptor mediated inhibitory effects and a resultant facilitation then predominates.

Spinal administration of excitatory amino acids has been shown to result in an absence of the silent phase of the behavioural response to formalin (Coderre and Melzack 1992).

Post-administration of 50μg of CNQX was less effective at inhibiting the second phase of the formalin response than pre-administration of the same dose. It is possible that AMPA receptor activation during the first phase of the formalin response primes or sensitizes the AMPA receptors to the second phase nociceptive inputs. It could be envisaged that the presence of intrathecal CNQX (pre-administration) during the first phase of the response reduces the sensitization of the AMPA receptor enabling low doses of CNQX to inhibit the second phase response. With post-administered CNQX the AMPA receptor will already be sensitized by the first phase nociceptive inputs and therefore will be more sensitive to the second phase nociceptive inputs and less susceptible to CNQX. My results with the lower doses of CNQX are in agreement with a recent behavioural study of neuropathic pain. It was shown in the behavioural study of neuropathic pain that intrathecal administration of CNQX days after the lesion failed to inhibit the associated hyperalgesia whereas pre-administered CNQX was effective at inhibiting the hyperalgesic response (Mao et al. 1992b).
The highest dose of CNQX produced identical inhibitions of the second phase of the formalin response whether given as either a pre- or a post-administration. This was the only dose of CNQX to significantly inhibit the electrically evoked C-fibre response but this result from actions no longer selective for the AMPA receptor (Birch et al. 1988).

The role of the NMDA receptor during the formalin response, pre-administration of intrathecal 7CK

The first phase of the formalin response was unaltered by intrathecal 7CK indicating that NMDA receptor mediated events are not important in the development of the first phase of the formalin response. This is in agreement with previous behavioural studies of the effect of NMDA receptor antagonists on the first phase of the formalin response (Coderre and Melzack 1992, Coderre 1993, Vaccarino et al. 1993). In contrast, two behavioural studies showed weak inhibitions of the first phase of the formalin response in the presence of intrathecal NMDA receptor antagonists (Yamamoto and Yaksh 1992, Kristensen et al. 1993). In addition, a previous electrophysiological study has also observed weak inhibitions of the first phase of the formalin response with intrathecal administration of a NMDA receptor antagonist (Haley et al. 1990). This effect of NMDA receptor antagonists on the first phase of the formalin response was not significant in any of these studies and therefore the NMDA receptor probably does not play an important role during the first phase of the formalin response.

The second phase of the formalin response was inhibited by intrathecal 7CK indicating the involvement of NMDA receptor mediated events during the second phase of the formalin response. This effect of pre-administered 7CK on the second phase of the formalin response supports the role of the NMDA receptor in this prolonged response of the dorsal horn neurones. This finding is in agreement with a previous electrophysiological study (Haley et al. 1990) and behavioural studies on the role of the NMDA receptor during the second phase of the formalin response (Yamamoto and Yaksh 1992, Coderre and Melzack 1992, Millan and Sequin 1993, Kristensen et al. 1993, Vaccarino et al. 1993). However, the results of this electrophysiological study contrast with the results of a more recent behavioural study in which 7CK was found to be ineffective at inhibiting the second phase of the formalin response (Coderre 1993). This
is unexpected since in all areas of the CNS studied, the action of glycine at this site is essential for expression of NMDA mediated responses. The reason for the lack of effect of 7CK inCoderre’s study cannot simply be the behavioural testing of the responses to formalin, since two other behavioural studies using different strychnine-insensitive glycine site antagonists, systemic HA-966 (Millan and Sequin 1993) and systemic ACEA-1011 (Vaccarino et al. 1993), found marked drug-induced inhibitions of the second phase of the formalin response.

The role of the NMDA receptor in the maintenance of the second phase of the formalin response, post-administration of intrathecal 7CK

In contrast with the diminished effectiveness observed with post-administration of CNQX, the NMDA receptor antagonist 7CK was equally potent at inhibiting the second phase of the formalin response whether given either as a pre- or as a post-administration. These results are in agreement with a previous electrophysiological study of the effect of post-administered MK801, an NMDA receptor channel blocker (Haley et al. 1990) and a recent behavioural study of the effect of post-administered HA966, an antagonist at the glycine site of the NMDA receptor, on the second phase of the formalin response (Millan and Sequin 1993). In contrast, behavioural studies of the formalin response have shown post-administration of NMDA receptor antagonists, MK801 and APV, to be ineffective at inhibiting the second phase of the formalin response (Coderre and Melzack 1992, Yamamoto and Yaksh 1992, Vaccarino et al. 1993). These differences may be due to the behavioural response being an integrated response involving a number of different neuronal systems other than just the initial activation of the convergent dorsal horn neurones. In behavioural responses, supraspinal systems may have been activated by the initial activation of the convergent neurones during the first phase of the response and a resulting sensitization of the higher brain centres may occur. Once these systems are sensitized, only a minimal output from the spinal cord may be required to manifest a complete behavioural response to formalin. This could explain the lack of inhibitory effect of post-administered NMDA receptor antagonists on the second phase of the behavioural response to formalin. This is conceivable since electrophysiological studies have shown that complete inhibition of responses of the convergent neurones during the second phase of the formalin response is not observed with intrathecal NMDA
antagonists.

Interestingly, a recent clinical report has shown that NMDA receptor antagonists only influence pathological pain transmission due to peripheral and central sensitization and not physiological pain due to transient non-tissue damaging noxious stimuli (Kristensen et al. 1992).

**Comparison of the second phase of the formalin response and electrically evoked wind up events**

Wind up of the dorsal horn neurones has been shown to be sensitive to a variety of NMDA receptor antagonists including, NMDA receptor channel blockers AP5, MK801 (Dickenson and Sullivan 1987, 1990) and ketamine (Davies and Lodge 1987) and an antagonist at the glycine site of the NMDA receptor complex, 7CK, (Dickenson and Aydar 1991). The susceptibility of the second phase of the formalin response to NMDA receptor antagonists suggests NMDA receptor mediated wind up mechanisms are involved in the development of the second phase of the response. It is possible that the one of the reasons for the shift in sensitivity observed with post-administered CNQX is the development of NMDA receptor mediated wind up-like events during the second phase of the formalin response.

**Implications of NMDA receptor activation**

Recently the expression of the early gene c-fos in the dorsal horn neurones during nociceptive transmission has been implicated as a mediator or contributor to the generation of spinal hypersensitivity. Peripheral noxious stimuli have been shown to induce the expression of spinal c-fos (Hunt et al. 1987). In particular, the subcutaneous injection of formalin has been shown to produce c-fos expression in the dorsal horn of the spinal cord (Presley et al. 1990, Leah et al. 1992). The expression of c-fos has been shown to be sensitive to intrathecal pre-administration of the NMDA receptor antagonist MK801 (Kehl et al 1991). Thus both NMDA receptor mediated events and c-fos expression have been implicated in the generation of central hypersensitivity. However, since complete reduction of c-fos expression has been shown not to be necessary for antinociception
(Hammond et al. 1992), the connection between c-fos expression and central hypersensitivity is not absolutely clear.

To conclude from my studies on excitatory amino-acids, the electrically evoked Aβ-fibre response and wind up of the dorsal horn neurones were more sensitive to intrathecally administered CNQX than the electrically evoked C-fibre response of the dorsal horn neurones. The first phase of the formalin response was significantly inhibited by pre-administered CNQX, but this effect was not dose related. Pre-administration of CNQX dose-dependently inhibited the second phase of the formalin response. There was a shift in the sensitivity of the second phase of the formalin response to post-administered CNQX as compared to pre-administered CNQX. Thus, lower doses of post-administered CNQX facilitated the second phase of the response and a higher dose (50μg) produced smaller inhibitions than those observed with pre-administration of the same dose. The highest dose of CNQX studied produced identical inhibitions of the second phase of the formalin response, irrespective of the timing of administration. The NMDA receptor antagonist, 7CK, did not alter the first phase of the formalin response. In contrast the second phase of the formalin response was inhibited to a similar extent by both pre- and post-administration of intrathecal 7CK.
CHAPTER 10:

THE ROLE OF SUBSTANCE P DURING THE SPINAL PROCESSING OF NOCICEPTIVE INFORMATION
10.1 Introduction

The origins of spinal substance P

Substance P (SP) has been widely implicated as one of a large number of neurotransmitters involved in nociceptive transmission (see refs. in Pernow 1983, Otsuka and Yanagisawa 1987, Iversen et al. 1989). SP has been shown to be present in 10-20% of primary afferent neurones (Hokfelt et al. 1975, 1976). SP has been shown to be most concentrated in the dorsal root ganglia at the cervical and lumbar level (Smith et al. 1993). However, since both spinal cord transection and primary afferent rhizotomy reduces the amount of SP-like immunoreactivity in the dorsal horn of the spinal cord (Jessell et al. 1979, Yaksh et al. 1988), dorsal horn SP must originate from both the afferent and descending fibres. Only a small percentage of ascending tract cells contain neuropeptides including SP, with the majority in the lateral spinal nucleus which is predominantly involved in the processing of visceral but not cutaneous information (Leah et al. 1988).

The tachykinin receptors

Currently there are three subclasses of tachykinin receptors: Neurokinin-1, -2 and -3, (see refs. in Iversen 1989), SP has been shown to be the preferred tachykinin for the neurokinin-1 (NK1) receptor, neurokinin A is the preferred tachykinin for the neurokinin-2 (NK2) receptor and neurokinin B is the preferred tachykinin for the neurokinin-3 (NK3) receptor (see refs. in Iversen 1989). Dorsal horn SP receptors have been demonstrated in anatomical studies to be post-synaptic to the sensory neurones (Helke et al. 1986, Yashpal et al. 1991). Autoradiographic studies have shown that the three receptor subtypes are located in laminae I, II and X of the dorsal horn (Yashpal et al. 1991). The distribution of the NK1 receptor within the spinal cord has recently been shown to be on neurones some distance from the primary afferents (Brown et al. 1993, Liu et al. 1993). In addition, there is evidence that SP may diffuse to target sites (Liu et al. 1993).
The role of spinal substance P during spinal nociceptive transmission

In an early in vitro study, dorsal root-evoked slow depolarisations and SP-induced depolarisations were shown to be similar (Urban and Randic 1984). More recently, SP has been shown to mediate slow excitatory postsynaptic potentials in dorsal horn neurones (De Koninck and Henry 1991, Nagy et al. 1993), therefore suggesting a role of SP in longer term changes in neuronal excitability. Moreover a structural-functional link has been demonstrated between dorsal horn neurones responding to noxious inputs with a slow excitatory postsynaptic potentials and the number of SP inputs these neurones receive (De Koninck et al. 1992). The mechanism of action of SP was addressed by a nerve culture study in which SP was shown to raise neuronal excitability by reducing an inward rectifying potassium current suggesting a self-reinforcing element to the depolarization conductance produced by SP (Stanfield 1985). This effect of SP on the potassium current has been recently demonstrated in rat spinal motoneurones (Fisher and Nistri 1993).

The release of SP at the spinal level in response to the selective activation of the C-fibres has provided good evidence for the role of SP as a neurotransmitter involved in spinal processing of nociceptive information. Peripheral application of capsaicin, which selectively activates C-fibres, has been shown to evoke SP release from the SG region of the dorsal horn (Zhao et al. 1992). In addition, intrathecal administration of the tachykinin receptor antagonist spantide has been shown to dose-dependently block the behavioural responses of mice to peripheral capsaicin (Sakurada et al. 1992). Furthermore, intrathecal SP has been shown to produce a dose-related hyperalgesia in the tail-pressure assay (Matsumura et al. 1985).

A number of studies have demonstrated that noxious peripheral stimuli evoke SP release into the substantia gelatinosa of the lumbar region of the dorsal horn of the spinal cord (Kuraishi et al. 1985a, Duggan and Hendrey 1986, Go and Yaksh 1987, Duggan et al. 1988, Kuraishi et al. 1989). Electrical stimulation of the sciatic nerve of the rat at C-fibre strength has been shown to decrease immunostaining for a number of peptides including SP in the medial part of the superficial dorsal horn, thus providing indirect evidence for a role of the peptides during nociceptive transmission (Klein et al. 1992). In the spinalised cat electrical stimulation of C-fibres has been shown to release SP in and
around lamina II of the dorsal horn (Schaible et al. 1992). Moreover, CGRP was shown to produce intraspinal spreading of released SP throughout the dorsal horn (Schaible et al. 1992). The effect of CGRP on SP spreading may be attributed to the ability of CGRP to inhibit the endopeptidases responsible for peptide degradation, so protecting SP (Mao et al. 1992c). Anatomical studies have shown most SP positive cell bodies of the dorsal root ganglia to also be immunoreactive for CGRP (Ju et al. 1987), providing an anatomical substrate for the proposed mechanism of intraspinal spreading.

Formalin has been shown to evoke SP release into the dorsal horn of the spinal cord of the rabbit (Kuraishi et al. 1989) and rat (Kantner et al. 1986). The release of SP during the formalin response was shown to be dependent upon both electrical activity and axoplasmic transport of a chemical signal (Kantner et al. 1986). It seems surprising that formalin evoked release of SP is dependent on axoplasmic transport of a chemical signal since the time course of the formalin response is short and lasts for only one hour and at least 24 hours would be necessary for the effects of axonal transport blockade to influence chemical nociception (see refs. in Keynes 1980). In addition it has been shown that subcutaneous injection of colchicine into the paw does not influence the formalin response (Haley et al. 1990). Studies with early substance P antagonists indicated a role of neurokinin receptor activation during the first phase, but not the second phase of the formalin response (Ohkubo et al. 1990). The more recent development of potent selective non peptide neurokinin antagonists has provided an important tool for the study of the role of SP at the NK1 and NK2 receptors during nociceptive transmission (Picard et al. 1993). Behavioural studies with the recently developed potent selective non-peptide neurokinin-1 antagonist (2S,3S)-cis-2-(diphenylmethyl)-N-((2-methoxyphenyl) methyl)-1-azabicyclo[2,2,2]octan-3-amine, ((±)-CP-96,345) has indicated a role of SP at the NK1 receptor in different types of nociceptive transmission (Radhakrishnan and Henry 1991, Birch et al. 1992, Nagahisa et al. 1992a). CP-96,345 has been shown to block slow excitatory post synaptic potentials induced by noxious cutaneous stimulation and repetitive C-fibre stimulation and to reduce noxious heat neuronal responses without affecting innocuous inputs (DeKoninck and Henry 1991, Schmidt et al. 1992). Subcutaneous administration of CP-96,345 has been shown to significantly inhibit the second phase of the behavioural response to formalin (Yashpal et al. 1993). In addition, intrathecal CP-96,345 has been shown to inhibit SP and C-fibre induced reflex facilitation
but not the unconditioned flexor reflex (Xu et al. 1992b). Unfortunately recent evidence has indicated that (±)-CP-96,345 induces non specific suppression of neurotransmission (Wang and Hakanson 1992) and has non-specific activity in the formalin and carrageenan induced models of nociception (Nagahisa et al. 1992b). These non specific actions of CP-96,345 have been attributed to CP-96,345 being a calcium channel antagonist (Schmidt et al. 1992). Therefore the relevance of studies using CP-96,345 is uncertain.

The recently developed tachykinin antagonist RP67580 ((3aR,7aR)-7,7-diphenyl-2-[1-imino-2(2-methoxyphenyl)ethyl] perhydroisoindol-4-one) has been shown to be a potent competitive antagonist selective for the NK1 receptor (Garret et al. 1991, Peyronel et al. 1992).

In this series of experiments the role of the NK1 receptor during nociceptive transmission in the dorsal horn of the rat spinal cord was investigated using the selective NK1 receptor antagonist RP67580 and its inactive 3aS, 7aS enantiomer RP68651. The effects of intrathecal RP67580 and RP68651 on the electrically evoked C-fibre, Aβ-fibre and wind up responses of the deep dorsal horn neurones was studied. In addition, the effects of intrathecal RP67580 and RP68651 on the response of the deep dorsal horn neurones to a peripheral injection of formalin were studied. Since both compounds had to be dissolved in concentrated hydrochloric acid, a parallel study of the effect of the vehicle on the electrically evoked responses and on the response of the dorsal horn neurones to a peripheral injection of formalin was made. The effects of RP67580 (doses 10µg / 12.5% HCl and 50µg / 25% HCl) and the inactive isomer RP68651 (dose 50µg / 25% HCl) on the electrically evoked C- and Aβ- fibre responses of the dorsal horn neurones were studied for 60 minutes at 10 minute intervals post drug administration. The effect of intrathecal HCl (12.5 % and 25%) alone on the electrically evoked C- and Aβ-fibre responses of the dorsal horn neurones was studied for 60 minutes at 10 minute intervals post vehicle administration.

The effect of intrathecal RP67580 (doses 1µg, 5µg and 10µg) and RP68651 (5µg) on the dorsal horn neuronal responses to peripherally injected formalin were studied. RP67580 and RP68651 were administered intrathecally 5 minutes prior to the peripheral injection of formalin. The effect of the vehicle, HCl (3%,n=4 and 12.5%, n=4 corresponding to the % HCl present in 5µg and 10µg RP67580 respectively) on the formalin response was also studied.
10.2 Results

10.2.1 The mean maximal effect of intrathecal RP67580, a potent NK1 receptor antagonist on the electrically evoked responses of the dorsal horn neurones

The mean maximal effects of RP67580 on the electrically evoked responses of a total of 11 dorsal horn neurones was studied. The mean depth of the neuronal population was 900±35µm. The mean maximal effects of the corresponding vehicles alone, 12.5% and 25% HCl (total of 12 dorsal horn neurones, mean depth 637±100µm) on the electrically evoked responses of the dorsal horn neurones were also studied.

The lower dose of RP67580 did not alter the electrically evoked responses as compared to the pre-drug controls, nor were the responses different from those observed with the vehicle (12.5% HCl). The higher dose of RP67580 (50µg) significantly inhibited the electrically evoked C-fibre response (p=0.019) as compared to the effect of the vehicle (25% HCl) alone. Although inhibitions of the electrically evoked Aβ-fibre response and wind up of the dorsal horn neurones were observed with the higher dose of RP67580 these effects were not significantly different to effects observed in the presence of the vehicle (25% HCl). The mean maximal effects of RP67580 and the vehicle alone on the electrically evoked responses of the dorsal horn neurones are shown in table 10.
Table 10. A comparison of the mean maximal effect of intrathecal RP67580 on the electrically evoked responses of the dorsal horn neurones and the effect of the vehicle alone on these responses.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibres</th>
<th>Aβ-fibres</th>
<th>Wind up</th>
</tr>
</thead>
<tbody>
<tr>
<td>10μg (in 12.5% HCl)</td>
<td>22±16%</td>
<td>13±6%</td>
<td>40±21%</td>
</tr>
<tr>
<td>12.5% HCl alone</td>
<td>12±9%</td>
<td>7±6%</td>
<td>42±23%</td>
</tr>
<tr>
<td>50μg (in 25% HCl)</td>
<td>65±8%*</td>
<td>40±9%</td>
<td>74±15%</td>
</tr>
<tr>
<td>25% HCl alone</td>
<td>39±12%</td>
<td>28±8%</td>
<td>55±17%</td>
</tr>
</tbody>
</table>

The mean maximal effects of the intrathecal treatments are expressed as percentage inhibition of the control electrically evoked responses of the dorsal horn neurones. Statistical analysis used Student's unpaired two-tailed t-test, comparing the effect of the RP67580 to the effect of the vehicle alone, *p<0.05. | n=6-11

The mean areas under the curve (AUC) were calculated for the electrically evoked responses in order to analyse the effects of the various intrathecal treatments over the 60 minute time course. The lower dose of RP67580 (10μg) did not alter the AUC for the electrically evoked C-fibre, Aβ-fibre and wind up responses of the dorsal horn neurones as compared with the effects observed with the vehicle (12.5% HCl). On the basis of the mean AUC, the electrically evoked C-fibre response was significantly inhibited in the presence of 50μg RP67580 (p=0.02) as compared to the vehicle (25% HCl) (figure 16). This same dose of RP67580 did not significantly alter the AUC for the wind up or Aβ-fibre responses of the neurones as compared to the vehicle (25% HCl) (figure 16).
Figure 16. A comparison of the effect of 50μg of RP67580, 50μg of RP68651 and the vehicle (25% HCl) on the electrically evoked responses. The effects are expressed as the mean area under the curve, therefore taking into account the effects of the various intrathecal treatments over the 60 minutes of the study. Statistical analysis used Student's two tailed unpaired t-test, *p<0.05. Open columns—50μg of RP67580, hatched columns—25% HCl, filled columns—50μg of RP68651.

10.2.2 The mean maximal effect of intrathecal RP68651 on the electrically evoked responses

The effect of intrathecal administration of a single dose of the inactive isomer RP68651 (50μg) on the electrically evoked responses of 6 dorsal horn neurones was studied. The mean depth of the neuronal population was 689±125μm. The electrically evoked C-fibre response was inhibited by the vehicle (25% HCl) alone, 39±12% inhibition of pre-vehicle control, whereas intrathecal RP68651 (50μg/25% HCl) produced less of an inhibition of the C-fibre evoked response, 12±14% inhibition. The mean AUC for the C-fibre response after 25% HCl and the mean AUC for the C-fibre response after RP68651 (50μg/25% HCl) were significantly different (p=0.01), indicating that
RP68651 produces actions which oppose the inhibitory action of the vehicle (figure 16). The effects of RP68651 on the Aβ-fibre response (17±14% mean maximal inhibition) and wind up (-2±24% mean maximal inhibition) of the dorsal horn neurones were not significantly different to the effects of the vehicle alone on these responses (figure 16).

A statistical comparison between the mean maximal effects of 50μg RP67580 and 50μg RP68651 on the electrically evoked responses of the dorsal horn neurones was performed using Student’s unpaired two tail t-test. RP67580 significantly inhibited the C-fibre evoked response (p=0.007) and the wind up (p=0.01) of the dorsal horn neurones as compared to RP68651. The effects of RP67580 and RP68651 on the electrically evoked Aβ-fibre responses of the dorsal horn neurones were not significantly different.

The inhibitions of the electrically evoked responses observed with the vehicle were transient, with a maximum effect at 30 minutes. Complete reversal of these effects were observed within 60 minutes and further recordings were made from other dorsal horn neurones. The electrically evoked responses of neurones recorded subsequently were no different from those seen in untreated animals.

10.2.3 The effect of intrathecal RP67580 on the response of the dorsal horn neurones to a peripheral injection of formalin

The effects of the intrathecal administration of RP67580 (doses 1μg, 5μg, and 10μg), on the response of 18 dorsal horn neurones to a peripheral injection of formalin were studied. The mean depth of neuronal population was 775±38μm. In addition, the effects of intrathecal administration of 3% and 12.5% HCl (the corresponding vehicles for 5μg and 10μg RP67580 respectively) on the response of 8 dorsal horn neurones to a peripheral injection of formalin were studied. The mean depth of the neuronal population was 650±50μm.

In the absence of an intrathecal injection, the control values for the first and second phases of the formalin response were 6724±758 and 23249±1880 action potentials (n=9) respectively. The mean depth of the neuronal population was 818±74μm.

Both phases of the formalin response were dose-relatedly inhibited by intrathecal RP67580 (table 11) as compared to the responses of the dorsal horn neurones to formalin.
in the absence of an intrathecal injection (control response). The first phase of the formalin response was significantly inhibited by all 3 doses of RP67580 studied, whereas the second phase was only significantly inhibited by 5 and 10 µg of RP67580.

Table 11. The effect of intrathecal RP67580 on the response of the dorsal horn neurones to a peripheral injection of formalin.

<table>
<thead>
<tr>
<th>Dose</th>
<th>First phase</th>
<th>Second phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>1µg</td>
<td>50±12% *</td>
<td>25±22%</td>
</tr>
<tr>
<td>5µg</td>
<td>41±21% *</td>
<td>68±10% **</td>
</tr>
<tr>
<td>10µg</td>
<td>87±7% **</td>
<td>79±14% **</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of first phase and second phase of the control formalin response (in the absence of an intrathecal injection) as appropriate. Statistical analysis used Student's unpaired two tailed t-test, comparing the response to formalin in the presence of RP67580 to the control formalin response,* P < 0.05, ** P < 0.01, n=6 per group.

The mean first phase response and mean second phase response of the dorsal horn neurones to a peripheral injection of formalin in the presence of intrathecal HCl (3%) were 76±19% and 108±19% of the control response respectively. The inhibitory effect of RP67580 (5µg in 3% HCl) on the second phase of the formalin response was statistically significant as compared with the effect of the vehicle (p=0.008). In contrast, the effect of 5µg RP67580 on the first phase of the formalin response was not significantly different to the effect of 3% HCl on the first phase of the formalin response. With intrathecal administration of 12.5% HCl, the mean first phase and mean second phase response of the dorsal horn neurones to a peripheral injection of formalin were 87±19% and 162±48% of the control response respectively. The virtually maximum inhibitions of the first and second phase of the formalin response by RP67580 (10µg in 12.5% HCl) were significant (p=0.003 and p=0.01 respectively) as compared to the effect of the vehicle.
alone. An example of the effect of intrathecal administration of 10μg of RP67580 on the response of a single dorsal horn neurone to a peripheral injection of formalin is shown in appendix 2.

10.2.4 The effect of intrathecal RP68651 on the response of the dorsal horn neurones to a peripheral injection of formalin

The effect of intrathecal RP68651 (5μg) on the formalin response was studied on 5 dorsal horn neurones. The mean depth of the neuronal population was 598±130μm. This isomer produced no inhibitory effects. The mean facilitation of the first and second phase of the formalin response in the presence of intrathecal RP68651 (5μg/3% HCl) were 129±46% and 182±21% of the control first and second phase response respectively. Only the facilitation of the second phase of the formalin response was statistically significant, as compared with the effect of the vehicle (p= 0.04).
10.3 Discussion

Intrathecal RP67580 produced a mild but significant inhibition of the C-fibre evoked response and marked inhibitions of the formalin response which could be separated from vehicle effects. The same doses of RP68651 and RP67580, containing equal vehicle concentrations produced opposite effects on both the formalin response and the electrically evoked C-fibre response. RP68651 produced significant facilitations whereas the active isomer RP67580 produced significant inhibitions. Previous studies have shown RP68651 has no affinity for the NK1 binding site (Garrett et al. 1991). The facilitatory actions of RP68651 therefore cannot be attributed to an action at the NK1 receptor, and further studies are necessary to determine the underlying mechanism of this response. These opposing effects of RP67580 and RP68651 are of added interest in light of the recent report showing that CP-96,345 and the isomer CP-96,344 which is not a NK1 antagonist produce equal inhibitions of the formalin response, suggesting non specific actions of CP-96,345 (Nagahisa et al. 1992b).

It is unlikely that either intrathecal RP67580, RP68651 nor the vehicle had toxic or adverse side effects on the spinal cord since the formalin responses of neurones recorded after intrathecal administration of RP67580, RP68651 or the vehicle were not different from those seen in untreated animals. In addition, the electrically evoked Aβ-fibre responses were unaffected by both RP67580 and RP68651.

The inhibition of the formalin response by the NK1 receptor antagonist, RP67580, implies a role of the NK1 agonist, SP, during the formalin response. Peripheral injection of formalin has been shown to evoke SP release into the dorsal horn of the spinal cord of the rabbit (Kuraishi et al. 1989). In contrast, another release study has shown that the release of SP into the dorsal horn of the rat spinal cord is reduced in a biphasic manner by the peripheral injection of formalin (McCarson and Goldstein 1991). In addition, the peripheral injection of formalin has been shown to be associated with an increase in SP-like immunoreactivity of the tissue, indicating a decreased release of SP (Holland and Goldstein 1990, McCarson and Goldstein 1989, 1990). My results are in agreement with the study by Kuraishi et al. 1989 but are difficult to reconcile with the studies by Holland and Goldstein 1990, McCarson and Goldstein 1989, 1990 and 1991.

A number of studies are in agreement with the inhibitory effect of RP67580 on the
formalin response. Intrathecal spantide, a non selective tachykinin antagonist (Sakurada et al. 1992), subcutaneous RP67580 (Sequin et al. 1993) and systemic RP67580 (Garret et al. 1991) have been shown to inhibit both phases of the behavioural response to formalin. An early SP antagonist only inhibited the first phase of the formalin response (Ohkubo et al. 1990) but this may be due to the instability of the antagonist leading to breakdown before the development of the second phase of the response. Intrathecal CP96,345 has been shown to inhibit both phases of the behavioural response to formalin (Yamamoto and Yaksh 1991, Sakurada et al. 1993), but unfortunately as stated in the introduction the selectivity of this compound is somewhat dubious. In addition, there appears to be an overlap between the doses of CP-96,345 required for antinociception and those producing motor effects (Sakurada et al. 1993).

My results extend these studies to show that the spinal cord is an important site of action of NK1 antagonists. The acute C-fibre evoked responses were less sensitive to the intrathecal antagonist which may be explained by a more important contribution of the tachykinins to prolonged as compared to acute noxious responses. This view is confirmed by a recent study in which RP67580 was shown to inhibit a facilitated spinal reflex, but did not influence the non facilitated response (Laird et al. 1993). Moreover, in a recent in vitro study, RP67580 inhibited C-fibre evoked ventral root potentials in hyperalgesic animals but did not effect the ventral root potentials in non-hyperalgesic animals (Thompson et al. 1993). These studies and my results suggest that SP, via an action at the NK1 receptor, contributes predominantly in the processing of more prolonged noxious responses and less to the processing of acute nociceptive responses.

Interestingly, adjuvant induced inflammation has been shown to increase the level of mRNA encoding preprotachykinin A (the precursor protein of SP) on the ipsilateral side of the spinal cord (Minami et al. 1989). More recently, precise locations of the increased mRNA have been shown to be laminae I, II and V/VI, with a 200% increase in the number of projection neurones in lamina I exhibiting preprotachykinin A mRNA (Noguchi and Ruda 1992). Moreover, peripheral inflammation has been shown to induce an upregulation of NK1 receptor mRNA in lamina I/II dorsal horn neurones (Schafer et al. 1993). Taken together these recent studies infer an increased production of SP and an upregulation of binding sites for SP in the dorsal horn during prolonged inflammatory nociception, therefore indicating the importance of spinal SP during inflammatory

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nociceptive transmission.

The roles of the neurokinin NK2 and NK3 receptors in the spinal processing of nociceptive inputs are also an important issue. The NK2 receptor but not the NK1 receptor has been shown to be important in the transmission of thermal nociception in the superficial dorsal horn (Fleetwood-Walker et al. 1990). In addition, in a recent in vitro study, the NK2 receptor antagonist MEN, 10376 was shown to inhibit C-fibre evoked ventral root potentials in non-hyperalgesic animals but did not affect the ventral root potentials in hyperalgesic animals (Thompson et al. 1993). Therefore it is possible that different neurokinin receptors are involved in the processing of different noxious modalities. Recently ionophoresis of the NK2 receptor antagonist L-659874 was shown to inhibit carrageenan induced preprodynorphin mRNA expression whereas a NK1 receptor antagonist was without effect (Fleetwood-Walker et al. 1993). Therefore there seems to be conflicting evidence for the role of the NK1 receptor antagonist during prolonged inflammatory nociception. The effects of NK3 receptor agonists and antagonists during spinal processing of inflammatory nociception have not been studied and therefore the role of this receptor in the spinal processing of nociception remains unknown.

To conclude the results with RP67580 demonstrate a spinal role for NK1 receptors in both the acute C-fibre evoked response of dorsal horn nociceptive neurones and in their responses to peripheral inflammation. Since the Aβ-fibre responses were unaffected by intrathecal RP67580 the NK1 receptor does not appear to be involved in innocuous transmission. NK1 receptor activation appears to be involved in the spinal processing of both the first and second phases of the formalin response. Therefore the role of the tachykinins at the NK1 receptor should be a consideration for future therapies, in particular for longer term inflammatory pain states.
CHAPTER 11:

THE ROLE OF SOMATOSTATIN DURING NOCICEPTIVE TRANSMISSION
11.1. Introduction

The distribution and origins of somatostatin within the spinal cord have previously been investigated. Somatostatin-like immunoreactivity has been observed in laminae II, III, IV and VI of the dorsal horn of the spinal cord (Krukoff et al. 1986). After colchicine pretreatment, somatostatin-like immunoreactivity was found in discrete populations of cell bodies within laminae I-III (Hunt et al. 1981), this finding indicates the presence of intrinsic somatostatin within dorsal horn neurones of the spinal cord. The somatostatin content of the dorsal horn is fifteen times higher than that of the ventral horn (Stine et al. 1982) and the dorsal horn content of somatostatin is highest in lamina II (Polack and Bloom 1986). Somatostatin has been shown to be present in small diameter cells in the dorsal root ganglion (Tuscherer and Seybold 1985) and afferent terminals in the substantia gelatinosa of the spinal cord (Hokfelt 1976). Furthermore, the primary sensory neurons have been confirmed as one of the origins of spinal somatostatin (Stine et al. 1982). In addition, somatostatin was shown to originate from ascending and descending pathways (Stine et al. 1982). In contrast to the study by Stine (1982), spinal transection and surgical isolation of the lumbar segments has been shown not to lower the somatostatin content of the cord more than that observed with dorsal root section; implying the origins of somatostatin of the spinal cord are from the dorsal roots and from local spinal neurones (Tessler et al. 1986). Autoradiographic studies have shown somatostatin binding sites to be within the substantia gelatinosa of the dorsal horn (Reubi and Maurer 1985). Somatostatin has been shown to be co-localised with the inhibitory transmitter met-enkephalin in some axons of the superficial dorsal horn, some of which are derived from local neurones (Todd and Spike 1992). Taken together the evidence for the distribution of the peptide itself and binding sites for somatostatin within the dorsal horn of the spinal cord suggests somatostatin has a role during the transmission of nociceptive information.

There is considerable evidence that somatostatin produces inhibitory effects. Somatostatin has been shown to have inhibitory effects on the responses of hippocampal and cerebral cortex neurones (Renaud et al. 1975). More recently somatostatin has been shown to augment the inhibitory M-current in hippocampal cells (Moore et al. 1988, Schweitzer et al. 1990), this effect was mimicked by arachidonic acid and blocked by
lipoxygenase inhibitors, implicating arachidonic acid metabolites in this effect of somatostatin (Schweitzer et al. 1990). The dose response curve for somatostatin on cortical neurones is biphasic so that lower concentrations are excitatory whereas higher concentrations are inhibitory. However at higher doses long lasting and often irreversible neuronal depolarization indicative of neuronal membrane damage were observed (Delfs and Dichter 1983). At the spinal level, in vitro studies have shown that iontophoretic application of somatostatin results in the hyperpolarisation of dorsal horn neurones and a reduction in spontaneous firing (Murase et al. 1982).

Evidence for a physiological role of somatostatin during nociceptive processing has been based on the increased release of somatostatin in the dorsal horn following peripheral noxious thermal stimulation (Kuraishi et al. 1985). A behavioural study of the effect of intrathecal somatostatin on the tail flick latency test and hot plate test demonstrated initial excitatory effects followed by analgesic effects of somatostatin (Gaumann and Yaksh 1988). Unfortunately this latter effect was only observed in the presence of toxic effects. The excitatory response (biting and scratching) to intrathecally applied somatostatin has been observed in other behavioural studies (Seybold et al. 1982, Wiesenfeld-Hallin 1985) but in the absence of antinociception. It has previously been shown that a large array of intrathecally applied drugs including morphine result in a biting and scratching response (see references in Iversen 1989), therefore the basis of this behaviour has been difficult to ascertain and is not necessarily indicative of pain.

As a consequence of the earlier studies which indicated a toxic effect of somatostatin, a safety margin for the intrathecal administration of somatostatin in rats was defined by Mollenholt et al. (1988). The threshold intrathecal dose of somatostatin for antinociception was 10μg as compared to 30μg which gave rise to chronic motor impairment associated with necrotic changes and loss of an immunohistochemical marker for motoneurones. Subsequently it has been shown that intrathecally applied somatostatin (10μg) produces antinociception in the tail flick test which could be separated from a transient motor dysfunction (< 30 minutes) and high-dose induced neuronal necrosis (Mollenholt et al. 1990). Studies of the motor and neurodegenerative effects of intrathecal somatostatin have indicated species differences may also exist, since toxic effects of intrathecal somatostatin are not observed in guinea pigs (Mollenholt et al. 1992). Irrespective of the claims that somatostatin has toxic effects, human studies have shown
both intrathecal and epidural application of somatostatin to be effective in the management of acute, post-operative and chronic cancer pain (Chrubasik et al. 1985, Meynadier et al. 1985).

In this series of experiments I have investigated the effect of intrathecal somatostatin on the responses of nociceptive dorsal horn neurones to acute electrical stimuli and to a peripheral injection of formalin. In addition, I have also studied the effect of a stable analogue of somatostatin, sandostatin (Octreotide) (Penn et al. 1992), on the acute and prolonged nociceptive responses of the dorsal horn neurones.
11.2 Results

11.2.1 The mean maximal effect of spinal administration of somatostatin and sandostatin on the acute electrically evoked responses

The mean maximal effects of intrathecal somatostatin (15µg and 150µg) and sandostatin (2µg and 20µg) on the electrically evoked responses of a total of 25 dorsal horn neurones were studied. The mean depth of the neuronal population was 780±51µm.

The electrical evoked C-fibre and Aβ-fibre responses were not altered by either intrathecal somatostatin or sandostatin (table 12). The wind up of the neurones was more variable in the presence of the highest dose of somatostatin and sandostatin (table 12), with 20µg sandostatin there was a tendency towards inhibition of the wind up response, but this effect was not significant. Overall, the electrically evoked responses were not influenced by either intrathecal somatostatin or sandostatin, suggesting the relay of acute nociceptive messages at the spinal level are not susceptible to the inhibitory actions of somatostatin or sandostatin.
Table 12. The mean maximal effect of intrathecal somatostatin and sandostatin on the electrically evoked C-fibre, Aβ-fibre and wind up of single dorsal horn neurones

<table>
<thead>
<tr>
<th>Treatment</th>
<th>C-fibre Response</th>
<th>Aβ-fibre Response</th>
<th>Wind up Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Somatostatin</td>
<td>115±4%</td>
<td>98±6%</td>
<td>156±21%</td>
</tr>
<tr>
<td>(15µg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Somatostatin</td>
<td>108±9%</td>
<td>87±11%</td>
<td>80±9%</td>
</tr>
<tr>
<td>(150µg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandostatin</td>
<td>96±15%</td>
<td>88±21%</td>
<td>105±40%</td>
</tr>
<tr>
<td>(2µg)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sandostatin</td>
<td>109±29%</td>
<td>115±4%</td>
<td>65±23%</td>
</tr>
<tr>
<td>(20µg)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean maximal effects of sandostatin and somatostatin on the electrically evoked responses are expressed as percentages of the control (pre-drug) response. n=5-9

11.2.2 The effect of intrathecal sandostatin on the response of the dorsal horn neurones to a peripheral injection of formalin

The first phase and second phase of the control formalin response for this series of experiments were 8261±1581 action potentials and 27849±3227 action potentials respectively. The mean depth of neuronal population was 805±72µm. These controls were also used for all of the following experiments using intrathecal somatostatin, subcutaneous sandostatin and locally administered sandostatin.

The effects of intrathecal sandostatin (0.02-20µg) on the responses of a total of 23 dorsal horn neurones to a peripheral injection of formalin were studied. The mean depth of the neuronal population was 657±73µm. Sandostatin was administered intrathecally 30 minutes prior to the peripheral injection of formalin.
The effects of sandostatin on the second phase of the formalin response were considerably more marked than the effects on the first phase of the response (table 13). The first phase of the formalin response was significantly inhibited by the highest dose of sandostatin studied. Sandostatin dose-relatedly inhibited the second phase of the response, with the highest dose producing a virtual abolition of the response. An example of the effect of intrathecal administration of 20μg of sandostatin on the response of a single dorsal horn to a peripheral injection of formalin is shown in appendix 2.

Table 13. The effect of intrathecal sandostatin on the formalin response

<table>
<thead>
<tr>
<th>Dose</th>
<th>First Phase</th>
<th>Second Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.02μg</td>
<td>36±12%</td>
<td>16±29%</td>
</tr>
<tr>
<td>0.2μg</td>
<td>56±7%</td>
<td>55±5%*</td>
</tr>
<tr>
<td>2μg</td>
<td>27±14%</td>
<td>59±13%*</td>
</tr>
<tr>
<td>20μg</td>
<td>66±12%*</td>
<td>91±2%**</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the first and second phase of the control formalin response as appropriate. Statistical analysis used Student’s unpaired two tailed t-test, * p<0.05, **p<0.001 | n=4-6.

11.2.3 The effect of intrathecal somatostatin on the response of the dorsal horn neurones to a peripheral injection of formalin

The effects of somatostatin (15μg and 150μg) on the responses of a total of 18 dorsal horn neurones to a peripheral injection of formalin were studied. The mean depth of the neuronal population was 856±111μm. Somatostatin was administered intrathecally 30 minutes prior to the peripheral injection of formalin.

The lowest dose of intrathecal somatostatin studied did not alter either phases of the
formalin response. The higher dose of intrathecal somatostatin (150µg) significantly inhibited both the first and second phase of the formalin response (table 14).

Table 14. The effect of intrathecal somatostatin on the formalin response

<table>
<thead>
<tr>
<th>Dose</th>
<th>First Phase</th>
<th>Second Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>15µg</td>
<td>39±9%</td>
<td>-6±18%</td>
</tr>
<tr>
<td>150µg</td>
<td>52±13%*</td>
<td>39±16%*</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the first and second phase of the control formalin response as appropriate. Statistical analysis used Student’s unpaired two tailed t-test, * p<0.05. n=8-10

11.2.4 Comparison of the effect of intrathecal sandostatin and somatostatin on the formalin response

The degree of inhibition of the first phase of the formalin response by the higher dose of somatostatin (52±13% inhibition) and the highest dose of sandostatin (66±12% inhibition) were extremely similar. The second phase of the formalin response was more sensitive, than the first phase of the formalin response, to the inhibitory effects of sandostatin. Sandostatin was found to be approximately 400 times more potent than somatostatin as shown by the dose response relationship of somatostatin and sandostatin on the second phase of the formalin response (figure 17).
11.2.5 The effect of subcutaneous administration of sandostatin on the response of the dorsal horn neurones to a peripheral injection of formalin

The effect of subcutaneous administration of sandostatin (10mg/kg and 100mg/kg) on the response of a total of 14 dorsal horn neurones to the peripheral injection of formalin was studied. The mean depth of the neuronal population was 792±66μm. Sandostatin was administered subcutaneously into the scruff of the neck 30 minutes prior to the peripheral injection of formalin.

The lower dose of subcutaneous sandostatin did not inhibit either phases of the formalin response. In contrast, both the first and second phase of the formalin response were significantly inhibited by the higher dose (100mg/kg) of subcutaneous sandostatin (table 15).
Table 15. The effect of subcutaneous sandostatin on the response of the dorsal horn neurones to a peripheral injection of formalin

<table>
<thead>
<tr>
<th>Dose</th>
<th>First Phase</th>
<th>Second Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>10mg/kg</td>
<td>30±25%</td>
<td>31±22%</td>
</tr>
<tr>
<td>100mg/kg</td>
<td>54±10%*</td>
<td>63±8%*</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the first phase and second phase of the control formalin response. Statistical analysis used Student's unpaired two tailed t-test, *p<0.05. n=7 per group

11.2.6 The effect of local peripheral injection of sandostatin on the response of the dorsal horn neurones to a peripheral injection of formalin

The effect of local peripheral administration of sandostatin (2 μg, 20 μg) on the response of a total of 12 dorsal horn neurones to a peripheral injection of formalin was studied. The mean depth of the neuronal population was 843±67μm. Sandostatin was administered peripherally 20 minutes prior to the peripheral injection of formalin into the same site.

Neither doses of peripherally applied sandostatin influenced the first phase of the formalin response (table 16). In contrast, the second phase of the formalin response was altered by peripherally administered sandostatin. The lower dose of peripherally applied sandostatin significantly facilitated the second phase of the formalin response. In contrast, the higher dose of sandostatin significantly inhibited the second phase of the formalin response.
Table 16. The effect of peripheral sandostatin on the response of the dorsal horn neurones to a peripheral injection of formalin

<table>
<thead>
<tr>
<th>Dose</th>
<th>First Phase</th>
<th>Second Phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>2μg</td>
<td>-2±20%</td>
<td>-58±25%* facilitation</td>
</tr>
<tr>
<td>20μg</td>
<td>26±13%</td>
<td>51±11%*</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the first phase and second phase of the control formalin response. Statistical analysis used Student's unpaired two tailed t-test, * p<0.05, n=6 per group
11.3 Discussion

Sandostatin and somatostatin did not produce inhibitory effects on the acute electrically evoked responses of the dorsal horn neurones. In contrast, both phases of the formalin response were inhibited by intrathecal sandostatin and somatostatin.

The ineffectiveness of somatostatin and sandostatin on the acute electrically evoked responses is in agreement with a behavioural study in which intrathecal somatostatin did not influence the tail flick reaction time (Cridland and Henry 1988). In contrast, another electrophysiological study in the cat showed that the noxious heat-evoked responses of laminae I-VI dorsal horn neurones were depressed by intrathecal somatostatin, whereas the innocuous responses and background activity were unaffected (Sandkuhler et al. 1990). In the study by Sandkuhler et al. 1990, the dose of somatostatin used was considerably higher than that used by Cridland and Henry, possibly explaining the difference between these two studies.

In this study the highest dose of somatostatin studied was not within the safety margin as defined by Mollenholt et al. 1988. However, with this dose of somatostatin I did not observe any change in the response of the neurones to electrical stimulation as compared to the controls suggesting a lack of toxic effects on the primary afferent fibres or these sensory neurones, at least over the period of study. In addition, I was able to record from other neurones once the intrathecally applied somatostatin was removed and the remaining somatostatin was irrigated with saline.

Other studies have suggested that the neurotoxicity associated with somatostatin is selective for the motoneurones (Mollenholt et al. 1990). The electrophysiological model used in this study does not depend on motor responses and therefore even if either somatostatin or sandostatin did cause neurotoxic effects on motoneurones it would not influence my results.

Sandostatin was considerably more potent than somatostatin, which is likely to be a consequence of sandostatin not being enzymatically degraded and therefore having a long duration of action (Pless et al. 1986). Sandostatin did not appear to have any toxic effects on the spinal cord, nor any action on acute inputs since the electrically evoked C fibre, Aβ fibre and wind up responses of the dorsal horn neurones were unaltered (as compared to controls) in the presence of all doses studied.

The ability of intrathecal somatostatin and sandostatin to inhibit the formalin
response but not the response to electrical stimulation may reflect the different nociceptive intensities of these two models. The nociceptive response to formalin is of a lower intensity but longer duration of action and was inhibited by somatostatin and sandostatin. Electrical activation of the nociceptors results in a higher intensity of nociceptive transmission but over a shorter time course which was not inhibited by somatostatin and sandostatin.

Subcutaneous administration of a high dose of sandostatin resulted in a significant inhibition of both phases of the formalin response. In contrast, peripheral administration of sandostatin inhibited only the second phase of the formalin response and therefore it is likely systemic sandostatin was able to cross the blood brain barrier and have a central site of action on both phases of the formalin response, as seen with spinal administration. The subcutaneous dose of sandostatin used was substantially greater than the highest intrathecal dose studied but the intrathecal dose inhibited the formalin response to a greater degree and therefore it seems unlikely that the inhibitory action of spinal sandostatin can be attributed to leakage into the systemic circulation and a peripheral site of action. My results showing a spinal inhibitory action of somatostatin and sandostatin are in agreement with a previous in vitro study (Murase et al. 1982).

The peripheral administration of the low dose of sandostatin facilitated the second phase of the formalin response, but the mechanism for this effect is unknown. In contrast, the high dose of peripherally administered sandostatin inhibited the second phase of the formalin response. It is plausible that peripheral somatostatin receptors are only effective during prolonged nociceptive states associated with inflammation such as the second phase of the formalin response. Activation of the peripheral somatostatin receptors by the higher dose of sandostatin may counter the actions of proalgesic mediators such as bradykinin and prostaglandins which have been shown to be peripheral contributors to the second phase of the formalin response. My results with the higher dose of sandostatin agree with a plasma extravasation study which provided indirect evidence for a pre-synaptic inhibitory action of somatostatin on the capsaicin sensitive C-fibre afferents during neurogenic joint inflammation (Green et al. 1992). The study by Green et al. suggested somatostatin blocks the further release of pro-inflammatory mediators such as SP during inflammatory responses.

In conclusion, intrathecal administration of somatostatin or sandostatin did not
influence the responses of the dorsal horn neurones to acute nociceptive stimuli. In contrast, the response of these neurones to a prolonged inflammatory stimuli, the peripheral injection of formalin, were significantly inhibited by both intrathecal somatostatin and sandostatin. A high dose of sandostatin had a weaker inhibitory action at the peripheral nociceptor terminal compared to central effects in nociceptive states associated with peripheral inflammation. Overall my results support previous clinical studies which have shown that spinal somatostatin (Chrubasik et al. 1985, Meynadier et al. 1985) and sandostatin (Penn et al. 1990, Penn et al. 1992) are potent analgesics which are effective in the management of cancer pain. However, although our results show that a genuine antinociception can be observed without overt toxic effects on sensory neurones the neurotoxic effects of spinal somatostatin on motoneurones in the rat (Mollenholt et al. 1990) has to borne in mind in the clinical use of these agents.
CHAPTER 12:

THE EFFECT OF THE MU OPIOID RECEPTOR AGONIST MORPHINE DURING ACUTE NOCICEPTIVE PROCESSING
12.1 Introduction

The selective inhibition of nociceptive responses by spinal opioids

An action of the opioids at the level of the spinal cord was first established two decades ago. Systemic morphine was shown to produce naloxone reversible inhibitions of both spontaneous activity and supra-maximal electrically evoked responses of the lamina V neurones of the dorsal horn in the spinal cat (Le Bars et al. 1975). This study indicated a direct spinal action of morphine since the animals were spinalised and therefore the relay of descending inhibitory controls to the spinal cord was disrupted. Thus these inhibitory effects observed with morphine were due to an action at the spinal level and not due to an action of morphine at the higher brain centres. The spinal action of morphine was further demonstrated in a study by Johnson and Duggan 1981, where inhibitions of C-fibre evoked responses of the dorsal horn neurones by systemic morphine were reversed by microelectrophoretical administration of naloxone at the level of the substantia gelatinosa.

Systemic morphine has been shown to inhibit C-fibre evoked responses of the lamina V neurones (Le Bars 1976) and C-fibre evoked activity of the ascending axons (Jurna and Heinz 1979). Microelectrophorectic administration of morphine at the level of the substantia gelatinosa (S.G.) selectively inhibits noxiously evoked responses of deep dorsal horn neurones, whereas administration of morphine near the cell bodies was considerably less effective; again these effects were naloxone reversible (Duggan et. al 1977). The S.G has been shown to have a high density of opioid binding sites (LaMotte et al. 1976), therefore explaining the greater effectiveness morphine administered at the level of the S.G. as compared with administration near the cell bodies. This effect of morphine administered at the level of the S.G. was confirmed in a later study by Davies and Dray 1978. Subsequently it has been demonstrated that spinal μ-opioid agonists have an inhibitory effect on the spinothalamic tract neurones of the primate (Willcockson et al 1986) and rat (Hylden and Wilcox 1986).

Behavioural studies have shown intrathecal morphine to be effective at inhibiting the tail-flick, hot-plate and tail-pinch tests (Kuraishi et al. 1985b, see refs. Yaksh and Noueihed 1985). The inhibitory effects of μ- and δ-opioid agonists have been shown to
be selective for the nociceptive responses of the spinocervical tract neurones of the cat (Fleetwood-Walker 1988). Numerous in vivo electrophysiological studies have shown the inhibitory actions of spinal μ- and δ-opioid agonists to be selective for C-fibre evoked responses of the dorsal horn neurones (Dickenson and Sullivan 1986, Dickenson et al. 1987, Sullivan et al. 1989, Duggan et al. 1977, Woolf and Wall 1986). The preferential inhibition of nociceptive transmission over innocuous transmission by opioid agonists may be explained by the location of the opioid receptors.

The C-fibres terminate superficially in the dorsal horn of the spinal cord (see section 1.3) and as discussed earlier the majority of opioid binding sites are at the level of laminae I and II (see section 1.10). Thus the distribution of the opioid receptors in the dorsal horn mirrors the areas where C-fibre afferents terminate and the majority of receptors are found presynaptically on the fine afferent terminals (Besse et al 1990). Aβ-fibres terminate deeper in the dorsal horn and do not possess opioid receptors. The doses of opioids required to partially inhibit the Aβ-fibre evoked responses are considerably higher than those required for inhibition of noxious C-fibre evoked responses (Dickenson and Sullivan 1986). This may be due to there being only few opioid receptors on neurones in the deeper laminae of the dorsal horn. Overall, opioids generally inhibit the processing of noxious information and do not overtly influence the perception of other sensations.

Repeated electrical stimulation of C-fibres may result in a sudden increase in the response of dorsal horn neurones (wind up) to the constant peripheral stimulation (see refs. in Dickenson 1990, Dickenson and Sullivan 1990). As discussed in chapter 9.3, electrically evoked frequency dependent wind up (Mendell 1966) of dorsal horn nociceptive neurones is sensitive to a variety of NMDA receptor antagonists including NMDA receptor channel blockers AP5, MK801 (Dickenson and Sullivan 1987b, 1990) and ketamine (Davies and Lodge 1987) and an antagonist at the glycine site of the NMDA receptor complex, 7CK, (Dickenson and Aydar 1991). In addition, the facilitated flexor reflex has been shown to be inhibited by systemic administration of the non competitive NMDA receptor antagonist MK-801 and the competitive NMDA receptor antagonist D-CPP (Woolf and Thompson 1991). Although NMDA receptor antagonists effectively inhibit the electrically evoked wind up responses of the dorsal horn neurones, they have little effect on the initial (input) responses of the dorsal horn neurones (Dickenson and Sullivan 1990, Dickenson and Aydar 1991). In contrast, intrathecal opioids effectively
inhibit the steady C-fibre evoked input responses of the dorsal horn neurones, but are less
effective at inhibiting wind up (Dickenson and Sullivan 1986, Dickenson 1991). This is
likely to reflect the predominant presynaptic opioid control of afferent activity with
relatively few post-synaptic sites as discussed above.

I have looked at the ability of morphine to inhibit the acute electrically evoked C-
fibre and Aβ-fibre responses of the dorsal horn neurones. Previous studies have not
separately quantified the effect of morphine on the wind up of dorsal horn neurones from
its effect on the overall C-fibre evoked response. In this study the effects of morphine on
the input and wind up component of the C-fibre evoked response were analysed. In
addition, the effect of morphine on the overall C-fibre evoked response of the dorsal horn
neurones was studied. The input component was taken as the C-fibre evoked response of
the neurone to the first stimulus multiplied by the total number of stimuli (16). The wind
up component was taken as the difference between the total C-fibre evoked response of
the dorsal horn neurones to the train of stimuli and the input. In an attempt to study the
effect of morphine on the frequency dependent wind up of the dorsal horn neurones, two
frequencies of stimulation (0.5 Hz and 0.1 Hz) were used in this study. The higher
frequency of stimulation was sufficient to remove the Mg²⁺ block of the NMDA receptor
complex and wind up of the dorsal horn neurone response was observed. In contrast, the
lower frequency of stimulation did not result in wind up of the dorsal horn neuronal
response, this may reflect the inability of this frequency of stimulation to remove the
Mg²⁺ block of the NMDA receptor complex.

Since wind up is dependent on NMDA receptor activation, I have studied the effect
of a low dose of morphine co-administered intrathecially with a low dose of 7CK, an
antagonist at the glycine site of the NMDA receptor complex, on the electrically evoked
C-fibre and Aβ-fibre responses of the dorsal horn neurones. Again, the C-fibre evoked
response of the dorsal horn neurones was separated into the input and wind up response.
Therefore any differential effect of the co-administered morphine and 7CK on the input
versus wind up of the dorsal horn neurones was apparent.

Finally, the ability of 20μg of intrathecal naloxone to reverse any effects morphine
and of co-administered morphine and 7CK was also studied.
12.2 Results

12.2.1 The mean maximal effect of intrathecal morphine on the electrically evoked (0.5Hz) dorsal horn neuronal responses

The lower dose of intrathecal morphine inhibited the electrically evoked input (the initial response) of the dorsal horn neurones to a greater degree than the electrically evoked wind up of the dorsal horn neurones (table 17). Virtually equal inhibitions of the input and wind up of the dorsal horn neurones were observed with the high dose of intrathecal morphine (table 17). The electrically evoked C-fibre response of the dorsal horn neurones was dose-dependently inhibited by morphine. Since the C-fibre evoked response consists of both an input and wind up component the effect of the lower dose of morphine on the overall C-fibre evoked response (24±11% inhibition) appears to be less than that observed on the wind up response (35±9% inhibition). The Aβ-fibre response was only partially inhibited by the high dose of intrathecal morphine (table 17). The effects of intrathecal morphine on the electrically evoked responses of the dorsal horn neurones were reversed by intrathecal administration of the opioid receptor antagonist naloxone (20μg). Naloxone reversed the effects of morphine on the C-fibre evoked response, Aβ-fibre evoked response, wind up and input to 15±18% inhibition, 11±6% inhibition, 7±26% facilitation and 7±30% facilitation respectively.
Table 17. The mean maximal effect of intrathecal morphine on the electrically evoked (0.5Hz) responses of the dorsal horn neurones.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre Response</th>
<th>Aβ-fibre Response</th>
<th>Wind up Response</th>
<th>Input Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>5μg</td>
<td>24±11%*</td>
<td>18±10%</td>
<td>35±9%*</td>
<td>62±7%**</td>
</tr>
<tr>
<td>50μg</td>
<td>80±10%**</td>
<td>37±9%*</td>
<td>88±5%***</td>
<td>81±5%***</td>
</tr>
<tr>
<td>Plus Naloxone (20μg)</td>
<td>15±18%</td>
<td>11±6%</td>
<td>-7±26%</td>
<td>-7±30%</td>
</tr>
</tbody>
</table>

The mean maximal effect of intrathecal morphine on the electrically evoked responses are expressed as percentage inhibition of the control (pre-drug) response. Statistical analysis used Student's paired two-tailed t-test, *p≤0.05, **p≤0.001, ***p≤0.0001. n=10

The effect of 5μg of morphine on the electrically evoked wind up, elicited at a frequency of 0.5Hz, of a single dorsal horn neurone is shown in figure 18. The initial responses of the dorsal horn neurone were inhibited by morphine but after the sixth stimulation the wind up response of the neurone breaks through the morphine inhibition, thus illustrating the limited inhibition of wind up by this dose of morphine.
Figure 18. The effect of intrathecal administration of 5μg of morphine on the wind up of a single dorsal horn neurone. Wind up responses were elicited by electrical stimulation at a frequency of 0.5Hz and 0.1Hz. The control wind up response (in the absence of morphine) to stimulation at a frequency of 0.5Hz and 0.1Hz are illustrated with the filled boxes and open boxes respectively. The wind up responses of a single dorsal horn neurone, elicited by stimulation at a frequency of 0.5Hz and 0.1Hz, in the presence of 5μg of morphine are illustrated with the filled circles and open circles respectively.

The high dose of morphine completely inhibited both the input and wind up response of the dorsal horn neurones. Therefore it can be concluded from this study that morphine is less effective at inhibiting the wind up response than the input of the dorsal horn neurones but at sufficiently high doses morphine can effectively inhibit wind up.

12.2.2 The mean maximal effect of intrathecal morphine on the electrically evoked (0.1Hz) responses of the dorsal horn neurones

Overall the inhibitory effects of intrathecal morphine on the electrically evoked responses elicited at 0.1Hz (table 18) were similar to the effects of morphine on the electrically evoked responses elicited at 0.5Hz (table 17). The low-frequency of stimulation caused little or no wind up. The C-fibre evoked response, input and reduced wind up of the dorsal horn neurones were dose-dependently inhibited by intrathecal
morphine (table 18). All effects of intrathecal morphine were reversed by 20μg of naloxone. A typical response of a single dorsal horn neurone stimulated at 0.1Hz is shown in figure 18 where the response remains constant for the duration of the train of stimuli. In the presence of 5μg of morphine this response of the dorsal horn neurone is further reduced for the duration of the train of stimuli.

Table 18. The mean maximal effect of intrathecal morphine on the electrically evoked (0.1Hz) responses of the dorsal horn neurones.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre Response</th>
<th>Aβ-fibre Response</th>
<th>Wind up</th>
<th>Input</th>
</tr>
</thead>
<tbody>
<tr>
<td>5μg</td>
<td>44±10%*</td>
<td>-5±13%</td>
<td>62±10%**</td>
<td>50±12%*</td>
</tr>
<tr>
<td>50μg</td>
<td>84±11%**</td>
<td>40±4%*</td>
<td>82±10%**</td>
<td>87±5%**</td>
</tr>
<tr>
<td>Plus Naloxone (20μg)</td>
<td>15±23%</td>
<td>3±7%</td>
<td>17±11%</td>
<td>-24±35%</td>
</tr>
</tbody>
</table>

The mean maximal effect of intrathecal morphine on the electrically evoked responses are expressed as percentage inhibition of the control (pre-drug) response. Statistical analysis used Student's paired two tailed t-test, *p≤0.05, **p≤0.001, n=10

12.2.3 Comparison of the effect of 5μg of morphine on the wind up elicited at 0.5Hz and 0.1Hz

At a stimulation strength of 0.1Hz less wind up was exhibited by the dorsal horn neurones as compared to the control wind up response elicited at 0.5Hz. Overall, the mean wind up response elicited at 0.5Hz was 292±65 action potentials (n=10), whereas the mean wind up of the same population of neurones elicited at 0.1Hz was 93±18 action potentials. The mean wind up response elicited at 0.5Hz was reduced to 155±34 action potentials in the presence of 5μg of morphine. At a frequency strength of 0.1Hz, the
mean wind up response was reduced to 56±14 action potentials in the presence of 5μg of morphine.

The effect of 5μg of morphine on the reduced wind up response elicited at 0.1Hz was different to the effect observed on the wind up response elicited at 0.5Hz (figure 18). 5μg of morphine inhibited the response elicited at a frequency of 0.1Hz for the duration of the 16 stimuli. At this lower frequency of stimulation the effects of morphine on the input and reduced level of wind up are similar. In contrast, wind up elicited with a frequency strength of 0.5Hz was less sensitive to the same dose of morphine (5μg). At this higher frequency of stimulation, 5μg of morphine inhibited the input to a greater extent than the wind up. These results illustrate that morphine is less potent at inhibiting the electrically evoked wind up responses than the steady response of the dorsal horn neurones.

12.2.4 The mean maximal effect of a subthreshold dose of the NMDA receptor antagonist, 7CK, on the electrically evoked responses

A previous study of the effect of intrathecal 7CK on the electrically evoked responses of dorsal horn neurones indicated 2.5μg of 7CK was a subthreshold dose (Dickenson and Aydar 1991). Control experiments were performed to investigate the mean maximal effect of 2.5μg of intrathecal 7CK alone on the electrically evoked responses of the dorsal horn neurones.

The effect of 2.5μg of intrathecal 7CK on the electrically evoked responses was studied on a total of 5 dorsal horn neurones. The mean depth of the neuronal population was 810±62μm. This dose of 7CK did not alter the electrically evoked responses of the dorsal horn neurones. The electrically evoked C-fibre and Aβ-fibre response were not different from controls (9±8% and 10±5% inhibition of control respectively). In addition, the wind up and input responses were not different from control (18±6% increase from control, 27±26% inhibition of control respectively). From these results it can be concluded 2.5μg of 7CK was a submaximal dose, which did not alter the C-fibre and Aβ-fibre evoked response, wind up or input responses of the dorsal horn neurones.
12.2.5 The mean maximal effect of co-administration of 5μg of morphine and 2.5μg of 7CK on the electrically evoked responses

In this series of experiments a submaximal dose of intrathecal 7CK was co-administered with a low dose of morphine. The mean maximal effect of intrathecal administration of a mixture of 5μg of morphine and 2.5μg of 7CK (in a total volume of 50μl) on the electrically evoked responses of seven dorsal horn neurones was studied. The mean depth of the neuronal population was 633±35μm.

Co-administration of 5μg of morphine and 2.5μg of 7CK resulted in virtually a total inhibition of the electrically evoked C-fibre response, input and wind up responses of the dorsal horn neurones (table 19). The electrically evoked Aβ-fibre response was inhibited, but this effect was less than that observed on the electrically evoked C-fibre responses.

Table 19. The mean maximal effect of intrathecal co-administration of morphine and 7CK on the electrically evoked responses of dorsal horn neurones.

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>C-fibre Response</th>
<th>Aβ-fibre Response</th>
<th>Wind up</th>
<th>Input</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mor/7CK</td>
<td>92±4%**</td>
<td>52±10%*</td>
<td>94±4%***</td>
<td>92±5%**</td>
</tr>
<tr>
<td>Plus Naloxone</td>
<td>27±16%</td>
<td>4±20%</td>
<td>29±14%</td>
<td>20±9%</td>
</tr>
<tr>
<td>(20μg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The mean maximal effect of co-administered morphine and 7CK on the electrically evoked responses are expressed as percentage inhibition of the control (pre-drug) response. Statistical analysis used Student’s paired two tailed t-test, *p≤0.05, **p≤0.001, ***p≤0.0001. n=7

Intrathecal naloxone (20μg) partially reversed the inhibitions of the electrically evoked responses observed with co-administered 7CK and morphine (table 19). The remaining inhibitions observed in the presence of naloxone may be attributed to the
12.2.6 A comparison of the effect of morphine alone and of the same dose of morphine in combination with 7CK, on the input and wind up responses of the dorsal horn neurones

As discussed above in section 12.2.1, 5μg of morphine alone produced significant inhibitions of the electrically evoked C-fibre responses, 62±7% inhibition of the input and 35±9% inhibition of wind up. The dose of 7CK used in this study had negligible effects on the electrically evoked responses when applied alone.

Statistical comparisons between the effect of 5μg of morphine alone and of the same dose of morphine in combination with 2.5μg of 7CK were made. The inhibition of the input and wind up by co-administered morphine and 7CK was significantly greater than the inhibitions produced by the same dose of morphine alone (p<0.001, p<0.0001 respectively). An example of the effect of co-administered 7CK and morphine on the wind up of a single dorsal horn neurone is shown in figure 19.

Due to pharmacological constraints it was not possible to study the effect of morphine alone and co-administered 7CK and morphine on the same cell. Therefore the effects of these treatments were studied on different cells. The effect of morphine on the wind up of a single dorsal horn neurone is shown in figure 20. A comparison of figure 19 and figure 20 illustrates the similarity between the effect of intrathecal co-administration of 7CK and morphine and intrathecal administration of 50μg of morphine alone on the wind up of dorsal horn neurones.
Figure 19. The effect of intrathecal co-administration of morphine (5μg) and 7CK (2.5μg) on the electrically evoked wind up of a single dorsal horn neurone. The control wind up illustrates a phenomenon of wind down for the last 6 stimuli. This may be due to high levels of NMDA receptor activation inducing inhibitory systems which counteract wind up of dorsal horn neurones. Co-administered morphine and 7CK virtually abolished the electrically evoked wind up of this dorsal horn neurone.

Figure 20. The effect of intrathecal administration of 5μg and 50μg of morphine on the electrically evoked wind up of a single dorsal horn neurone.
12.3 Discussion

In this study morphine doses dependently inhibited the electrically evoked C-fibre responses of dorsal horn neurones. In contrast, the Aβ-fibre evoked responses of these neurones were only partly inhibited by the high dose of morphine. The preferential inhibition of the C-fibre evoked responses over the Aβ-fibre evoked responses by intrathecal morphine is in agreement with previous electrophysiological studies (see section 12.1).

Further analysis of the C-fibre evoked response elicited at a stimulation frequency of 0.5Hz showed the input was more strongly inhibited by 5μg of morphine than the wind up. However, with 50μg of morphine the input and wind up were similarly inhibited. Therefore it can be concluded that wind up of the dorsal horn neurones is less sensitive to low doses of morphine, but is inhibited by higher doses of morphine.

At a stimulation frequency of 0.1Hz, less wind up of the neuronal response was observed. With a reduced level of wind up, 5μg of morphine effectively inhibited the residual wind up response of the dorsal horn neurone for the duration of the 16 stimuli. Again, the electrically evoked wind up responses of the dorsal horn neurones elicited at a frequency of 0.1Hz were virtually abolished by 50μg of morphine.

The effect of morphine on the wind up of dorsal horn neurones

These differential effects of low doses versus high doses of morphine on the input and wind up may be explained by the location of the opioid receptors. The effects of morphine on wind up can be interpreted on the basis of the actions of morphine at pre- and post-synaptic sites (see section 1.11). It is feasible that submaximal doses of morphine (5μg) act mainly at receptors on the primary afferent terminals, pre-synaptic to the recorded neurone. The contribution of pre-synaptic opioid receptors to the analgesic effect of morphine has previously been demonstrated (Lombard and Besson 1989). This pre-synaptic action of morphine would reduce transmitter release from primary afferents and therefore inhibit the input onto the dorsal horn neurones. As illustrated by this study, morphine (5μg) does not completely block transmitter release from the primary afferents since the input onto the dorsal horn neurones was not abolished. This is in agreement
with studies which have shown that opiates reduce but do not completely abolish noxious evoked release of substance P into the dorsal horn of the spinal cord (Hirota et al. 1985, Aimone and Yaksh 1989, Yaksh et al. 1980). The reduced but not abolished input onto the dorsal horn neurones will activate post synaptic excitatory receptors. With sufficient depolarization of the post synaptic membrane the voltage dependent magnesium block of the NMDA receptor complex will be removed, leading to the activation of the NMDA receptor and the induction of wind up. In contrast, the high dose of morphine are needed to act at the post-synaptic opioid receptors (Lombard and Besson 1989). The pre-synaptic action of morphine results in a decreased transmitter release from the primary afferents and a post-synaptic action produces a hyperpolarisation of the post-synaptic membrane. The additional post-synaptic action of high doses of morphine will counter post-synaptic depolarisations produced by synaptic transmission. One of the consequences of this opiate evoked post-synaptic hyperpolarisation is a decrease in the degree of NMDA receptor activation. Therefore the combined activation of pre- and post-synaptic receptors by high doses of morphine may result in a more potent inhibition of wind up.

Overall, these results demonstrate the relative insensitivity of NMDA receptor mediated wind up to low doses of morphine as compared to the steady C-fibre evoked response which is readily inhibited by morphine. Importantly, this study also illustrates that wind up can be inhibited by higher doses of morphine.

Co-administration of morphine and 7CK profoundly inhibits wind up

Co-administration of a low dose of morphine and a sub-threshold dose of 7CK produced inhibitions of wind up which were comparable to those observed with 50μg of morphine. These profound inhibitions may be explained by the dual site of action of morphine at pre-synaptic sites and 7CK at post-synaptic sites, resulting in an inhibition of primary afferent transmitter release and antagonism of the NMDA receptor respectively. Thus the decreased input in association with a decreased NMDA receptor activation produces profound inhibitory actions on the wind up of the dorsal horn neurones.
Clinical Implications

The results of this study may have important clinical implications. Pains associated with peripheral nerve damage have been found to be relatively insensitive to opioids (Arner and Meyerson 1988, Portenoy et al. 1990, Jadad et al. 1992). The mechanisms involved in the development of opioid insensitive pain are of considerable interest (Dickenson 1991). It has been suggested that NMDA receptor mediated wind up may relate to far reaching alterations in central pain processing which may contribute to the development of opioid insensitive pain (Dickenson 1990, McQuay and Dickenson 1990, Woolf and Thompson 1991). The results of my study and a study by Dickenson and Sullivan 1986 have shown wind up of the dorsal horn neurones to be less sensitive to opioids and therefore excessive activation of the NMDA receptor may be one of the causes of opioid insensitive pain.

High doses of morphine are required to inhibit NMDA receptor mediated wind up, but in the clinical situation high systemic doses of opioids may result in undesirable side effects such as respiratory depression, psychotomimetic effects and constipation. The results of my study suggest the clinical use of combination therapy with a submaximal dose of morphine and a submaximal dose of a NMDA receptor antagonist may be useful in the relief of opioid insensitive pain with reduced side effects. The antagonists at the glycine site of the NMDA receptor complex may be suitable for this type of combination therapy since they have a large window between desired effects and unwanted effects of NMDA receptor blockade. The antagonists at the glycine site of the NMDA receptor complex, in particular (±) HA966, do not produce behavioural stimulation in animals. This behavioural stimulation is thought to underlie the psychotomimetic effect of NMDA receptor channel blockers which has been observed in humans. In addition, there is a clear separation between the anticonvulsant effects and sedative effects of antagonists at the glycine site of the NMDA receptor complex (see refs. in Kemp and Leeson 1993).
CHAPTER 13:

THE EFFECT OF INTRATHECAL MU OPIOID RECEPTOR AGONIST MORPHINE ON THE FORMALIN RESPONSE
13.1 Introduction

Inhibition of prolonged nociceptive transmission by opioids

In the clinical situation many pain states are prolonged, and so it is important to consider the ability of opioids to inhibit more prolonged nociception rather than simply the acute models of nociception. As discussed throughout this thesis the peripheral injection of formalin is a good model of more prolonged nociception which has an associated peripheral inflammation.

Numerous behavioural studies, using a menagerie, have shown both phases of the formalin response to be sensitive to the μ-opioid agonist morphine. Both aspects of the behavioural response to formalin are inhibited by intramuscular administration of morphine in the monkey (Alreja et al. 1984). Systemic and intracerebroventricular administration of morphine has been shown to inhibit both phases of the formalin response in the mouse (Oluyomi et al. 1992). Finally, intraperitoneal administration of morphine in the rat (Dubuisson and Dennis 1977, Hunskaar and Hole 1987, Hunskaar et al. 1985), cat (Dubuisson and Dennis 1977) and naked mole rat (Kanui et al. 1993) has also been shown to inhibit both phases of the formalin response.

The results of these behavioural studies are in agreement with electrophysiological studies of the effects of intrathecal opioids on the formalin response. Intrathecal administration of DAGO, the selective μ-opioid agonist has been shown to inhibit the response of dorsal horn neurones to the peripheral injection of formalin and this effect was reversed by naloxone (Dickenson and Sullivan 1987c, Haley et al. 1988). Similar inhibitions of the formalin response were also observed after intrathecal administration of DSTBULET, a selective δ-opioid agonist and kelatorphan, a full peptidase inhibitor which protects the enkephalins from degradation (Sullivan et al. 1989).

Results from both behavioural and electrophysiological studies have shown the doses of morphine required to inhibit the formalin response are less than those required to inhibit acute nociceptive responses. For example, Abbott et al. 1982 observed a greater sensitivity of the formalin response to morphine as compared to the tail flick test, a measure of acute nociceptive transmission. A comparison of the inhibitory effect of the μ-opioid receptor agonist DAGO on the formalin response (Dickenson and Sullivan
1987c) and the electrically evoked C-fibre response (Dickenson et al. 1987) also reveals an increased sensitivity of the formalin response to the μ-opioid receptor agonist. Similar effects were observed with both a δ-opioid agonist (DSTBULET) and a full peptidase inhibitor (kelatorphan). The response of the dorsal horn neurones to the peripheral injection of formalin was more sensitive to DSTBULET and kelatorphan than the responses of these neurones to acute electrical stimuli (Sullivan et al. 1989).

Currently there is a huge amount of controversy and clinical interest in the possible advantages of pre-emptive analgesia as compared with post-treatment (McQuay 1993). This is particularly so for the opioids. In view of this clinical interest I have investigated whether there is any basic evidence for pre-administered morphine providing a greater degree of analgesia than post-administered morphine. Previous behavioural and electrophysiological studies have compared the ability of pre- and post-administered morphine to inhibit the second phase of the formalin response. Unfortunately differing effects have been observed. In a recent behavioural study pre-administered morphine dose-dependently inhibited both the first and second phase of the response (Yamamoto and Yaksh 1992). Post-administered morphine also reduced the second phase of the formalin response, with no significant difference between the effect of pre- and post-administered morphine on the second phase of the formalin response being observed (Yamamoto and Yaksh 1992). In contrast an electrophysiological study has shown post-administered DAGO to be considerably less effective than pre-administered DAGO at inhibiting the second phase of the formalin response (Dickenson and Sullivan 1987c).

I have studied the effect of intrathecal morphine on the formalin response. The importance of the timing of the administration of morphine was also studied. Morphine was administered either before (pre-administration) or after (post-administration) the peripheral injection of formalin. To allow for direct comparison with a recent behavioural study (Yamamoto and Yaksh 1992), the timing of the pre-administered morphine was 15 minutes prior to the peripheral injection of formalin and post-administered morphine was given 9 minutes after the peripheral injection of formalin.
13.2 Results

For this series of experiments the control first phase and second phase response of the dorsal horn neurones to the peripheral injection of formalin were $5669 \pm 1295$ action potentials and $23516 \pm 7803$ action potentials ($n=11$). The mean depth of the neuronal population was $797 \pm 77 \mu m$.

The effect of intrathecal morphine ($0.0025 \mu g, 0.01 \mu g, 0.025 \mu g$ and $0.25 \mu g$) on the response of dorsal horn neurones to the peripheral injection of formalin was studied. The effect of pre-administered morphine, administered 15 minutes before the peripheral injection of formalin, was studied on a total of 24 dorsal horn neurones. The mean depth of the neuronal population was $676 \pm 50 \mu m$. The effect of post-administered morphine, administered 9 minutes after the peripheral injection of formalin, was studied on a total of 21 dorsal horn neurones. The mean depth of the neuronal population was $762 \pm 40 \mu m$.

13.2.1 The effect of intrathecal pre-administration of morphine on the response of dorsal horn neurones to a peripheral injection of formalin

The lowest dose of pre-administered morphine did not alter the first phase of the formalin response. The first phase of the formalin response was non-significantly facilitated by $0.01$ and $0.025 \mu g$ of pre-administered morphine. The highest dose of pre-administered morphine ($0.25 \mu g$) non-significantly inhibited the first phase of the formalin response (table 20).

The second phase of the formalin response was inhibited in a dose-related manner by pre-administered morphine. The highest dose of pre-administered morphine studied ($0.25 \mu g$) significantly inhibited the second phase of the formalin response (table 20).
Table 20. The effect of pre-administration of intrathecal morphine on the response of the dorsal horn neurones to a peripheral injection of formalin.

<table>
<thead>
<tr>
<th>Dose</th>
<th>First phase</th>
<th>Second phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0025</td>
<td>21±17%</td>
<td>14±29%</td>
</tr>
<tr>
<td>0.01µg</td>
<td>-19±23% facilitation</td>
<td>28±29%</td>
</tr>
<tr>
<td>0.025µg</td>
<td>-20±24% facilitation</td>
<td>47±26%</td>
</tr>
<tr>
<td>0.25µg</td>
<td>40±22%</td>
<td>61±16%*</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the first phase and second phase of the control formalin response. Statistical analysis used Student’s two tailed unpaired t-test, *p<0.05. n=5-8

13.2.2 The effect of intrathecal post-administration of morphine on the second phase of the response of dorsal horn neurones to a peripheral injection of formalin

In this series of experiments morphine was given as a post-administration, after the first phase of the formalin response. The second phase of the formalin response was inhibited by post-administered morphine in a dose-related manner with the highest dose of morphine significantly inhibiting the second phase of the response (table 21).
Table 21. The effect of intrathecal post-administration of morphine on the second phase of the formalin response.

<table>
<thead>
<tr>
<th>Dose</th>
<th>Second phase</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.0025µg</td>
<td>11±24%</td>
</tr>
<tr>
<td>0.01µg</td>
<td>32±17%</td>
</tr>
<tr>
<td>0.025µg</td>
<td>45±19%</td>
</tr>
<tr>
<td>0.25µg</td>
<td>70±10%*</td>
</tr>
</tbody>
</table>

Results are expressed as percentage inhibition of the second phase of the control formalin response. Statistical analysis used Student’s two tailed unpaired t-test, *p≤0.05. n=5-6

13.2.3 Comparison of the effect of pre- and post-administered morphine on the second phase of the formalin response

The effects of pre- and post-administered morphine on the second phase of the formalin response are compared in figure 21. The inhibitions of the second phase of the formalin response observed with post-administered morphine were identical to the inhibitions observed with pre-administered morphine. Therefore the timing of the administration of morphine did not influence the ability of morphine to inhibit the second phase of the formalin response.
Fig 21. A comparison of the inhibitory effects of pre-administered and post-administered intrathecal morphine on the second phase of the formalin response.
13.3 Discussion

The first phase of the formalin response was non-significantly inhibited by the highest dose of pre-administered morphine. In contrast, lower doses of pre-administered morphine facilitated the first phase of the response. Low to intermediate doses of morphine have previously been shown to produce early facilitations (0-30 minutes after intrathecal administration) followed by later (30-60 minutes) inhibitions of the C-fibre evoked responses (Dickenson and Sullivan 1986). If similar events occur during the formalin response it may be envisaged that the lower doses of pre-administered morphine produced early facilitations (0-25 minutes after intrathecal administration) of the first phase of the formalin response. The time points for the pre- and post-administration of morphine were identical to a previous behavioural study (Yamamoto and Yaksh 1992) to allow a direct comparison between the two studies. The aim of this study was to investigate the effect of pre- and post-administered morphine on the second phase of the response, and therefore higher doses of morphine were not studied and a significant inhibition of the first phase of the response was not observed. A previous electrophysiological study has shown intrathecal administration of the μ-opioid agonist, DAGO, inhibited the first phase of the formalin response (Dickenson and Sullivan 1987c).

A significant inhibition of the second phase of the formalin response was observed with the highest dose of pre-administered morphine. This illustrates the ability of μ-opioid receptor activation to inhibit the processing of prolonged nociceptive information at the spinal level. This finding is in agreement with previous behavioural and electrophysiological studies discussed in the section 13.1. The effects observed with the doses of morphine used in this study were comparable to the effects observed with a similar dose range of morphine in a recent behavioural study (Yamamoto and Yaksh 1992). A previous electrophysiological study showed 0.25μg of intrathecal morphine had negligible effect on the C-fibre evoked responses of the dorsal horn neurones (Stanfa et al. 1992). In contrast, 0.25μg of morphine inhibited the second phase of the formalin response, illustrating the enhanced sensitivity of the second phase of the formalin response, as compared to the acute C-fibre evoked response, to intrathecal morphine. A similar increase in the analgesic efficacy of spinal opioid agonists has been observed in
another model of peripheral inflammation, carrageenan induced hyperalgesia (Hylden et al. 1991, Stanfa et al. 1992). In my study and the study by Hylden et al. and Stanfa et al., drugs were administered at the spinal level. Therefore this increased opioid efficacy is most probably due to changes at the spinal level and not due to an action at peripheral opioid receptors which have been demonstrated to have a role during inflammation (Stein et al. 1989). It has been shown that the enhanced opioid efficacy observed during carrageenan inflammation is due to a decreased opiate modulation by spinal 'anti-opiate peptides' such as cholecystokinin (CCK) (Stanfa et al. 1993). Intrathecal CCK has been shown to reduce μ-opioid actions at the spinal level (Magnuson et al. 1990). The study by Stanfa et al. 1993 implicated a decreased release or synthesis of CCK at the spinal level during carrageenan induced inflammatory nociception. Decreased levels or release of spinal CCK during formalin evoked inflammatory nociception may also explain the increased sensitivity of the second phase of the formalin response to intrathecal morphine.

Alternatively, the enhanced potency of morphine may be due to increased levels of noradrenergic inhibitory descending controls to the spinal cord during the formalin response. The predominant receptor for noradrenaline at the spinal level is the alpha-2-adrenoceptor (see refs. in Dickenson 1993). There is considerable evidence that intrathecal μ-opioid agonists and alpha-2-agonists synergise and have supra-additive inhibitory effects on nociceptive transmission (see refs. in Dickenson 1993). If there is increased descending noradrenergic activity during the formalin response this may explain the enhanced potency of morphine at inhibiting the second phase of the formalin response as compared to the effect of morphine on the acute C-fibre evoked responses of the dorsal horn neurones.

The effect of pre- versus post-administration of morphine on the second phase of the formalin response

The effect of a full dose range of morphine on the second phase of the formalin response was studied. The dose-related inhibitions of the second phase of the formalin response observed with pre-administered morphine were identical to the inhibitions observed with post-administered morphine. Therefore the timing of the administration of intrathecal morphine did not effect the ability of morphine to inhibit the second phase of
the formalin response. These results are in agreement with a recent behavioural study which found that the timing of the administration of morphine did not influence the ability of morphine to inhibit the second phase of the formalin response (Yamamoto and Yaksh 1992). A previous electrophysiological study investigated the effect of intrathecal DAGO, administered 2 minutes after the first phase of the formalin response, as compared to pre-administered DAGO on the second phase of the formalin response (Dickenson and Sullivan 1987c). The second phase of the formalin response was less sensitive to post-administered DAGO as compared to pre-administered DAGO. In this previous study only one dose of DAGO was investigated (5μg), considerably higher than the doses of morphine used in this study. This discrepancy between my results and the study by Dickenson and Sullivan 1987c may be due to intrinsic differences between the two μ-opioid agonists used in these studies. There is evidence that morphine stimulates the release of CCK in the dorsal horn of the spinal cord (Zhou et al. 1993), whereas DAGO has been shown to inhibit the release of CCK (see refs. in Benoliel et al. 1991). Moreover it has been proposed by Stanfa et al. 1993, that under sub-chronic inflammatory conditions morphine no longer evokes the release of CCK. It could be envisaged that DAGO no longer inhibits the release of CCK under subchronic inflammatory conditions, thus explaining the reduced ability of DAGO as compared to morphine to inhibit the second phase of the formalin response. Since both morphine and DAGO act at the same receptor these suggested differential effects of morphine and DAGO remain unclear.

It has recently been shown that intrathecal administration of low doses of DAGO during the second phase of the formalin response were less effective at inhibiting the second phase of the formalin response than the same doses of DAGO given as a pre-administration (Dickenson unpublished). In contrast, high doses of DAGO administered during the formalin response were as effective as the same doses of DAGO given as a pre-administration at inhibiting the second phase of the formalin response (Dickenson unpublished). These findings are as one would expect since the second phase of the formalin response has been shown to be partly mediated by the NMDA receptor. It is important to realise the different experimental paradigms used in this unpublished study, my study and the behavioural study by Yamamoto and Yaksh 1992. Both in my study and the behavioural study post-administered morphine was administered at the end of the first phase, before the second phase of the response and therefore before NMDA receptor
activation had commenced. In the unpublished study, post-administered DAGO was administered during the second phase of the formalin response, that is once NMDA receptor activation had commenced. As discussed in chapter 12, low doses of μ-opioid agonists are less effective at inhibiting NMDA receptor mediated events than acute non-NMDA receptor mediated nociceptive events. Since NMDA receptor mediated events are active during the second phase of the formalin response this may explain the decreased ability of low doses of DAGO, administered during the second phase, to inhibit the second phase of the formalin response. High doses of μ-opioid agonists are effective at inhibiting NMDA receptor mediated wind up events (see chapter 12). If similarities exist between the second phase of the formalin response and electrically evoked wind up this may explain the equi-effectiveness of high doses of pre-administered DAGO and DAGO administered during the second phase at inhibiting the second phase of the formalin response.

In conclusion, the results of this study have shown that the second phase of the formalin response is sensitive to intrathecal pre-administration of morphine, the doses of morphine required to inhibit the second phase of the response were considerably lower than those required to inhibit the electrically evoked responses (see chapter 12). Administration of morphine early after the first phase but before the second phase of the formalin response (post-administration) inhibited the second phase of the formalin response to an equal degree as pre-administered morphine. This finding is in agreement with the recent behavioural study (Yamamoto and Yaksh 1992).
CHAPTER 14:

ULTRAVIOLET IRRADIATION OF THE HINDPAW AS A NOVEL ANIMAL MODEL OF THE INFLAMMATORY NOCICEPTION
14.1 Introduction

An ultraviolet (U.V.) model of persistent hyperalgesia has recently been developed by Sandoz Institute for Medical Research. In this experimental paradigm the hindpaw is exposed to U.V.A light on two consecutive days (Perkins et al. 1993a,b). This model of hyperalgesia has parallels with sunburn, it is associated with a peripheral inflammation characterized by erythema and blistering but is without evidence of swelling. From personal experience, pain associated with sunburn has symptoms similar to those of allodynia in that normally non-painful stimuli such as touch and brush are perceived as painful. The time course of the persistent hyperalgesia is up to 10 days and therefore is considerably longer in duration than that experienced by people with sunburn (Perkins et al. 1993a).

Behavioural studies of U.V.A induced hyperalgesia

Following exposure of the hind paw to U.V.A light, animals are hyperalgesic with a 70% decrease in latency of response to noxious heat. This hyperalgesia was maintained for up to 10 days (Perkins et al. 1993a). Peak hyperalgesia was observed on the third day after exposure to U.V.A light and remained constant for up to seven days (Perkins et al. 1993a). In addition, a mechanical hyperalgesia was also observed which was maximal on day 5 with a 40% reduction in paw withdrawal threshold (Perkins et al. 1993b). The peripheral mediators involved in the U.V.A light induced hyperalgesia have been studied (Perkins et al. 1993a). The hyperalgesia was shown be decreased by subcutaneous ibuprofen, suggesting it is partly mediated by prostaglandins. In addition, intraplantar administration of the bradykinin B\textsubscript{2} receptor antagonist HOE140 on the same day as the exposure to U.V.A light was shown to decrease the level of hyperalgesia. In contrast, HOE140 administered on the days following U.V.A light exposure did not alter the hyperalgesia. The bradykinin B\textsubscript{1} receptor antagonist des-Arg\textsuperscript{9} [Leu\textsuperscript{8}]-BK administered on either the same day as exposure to U.V.A light or the following days reduced the hyperalgesia. This suggests the involvement of peripheral bradykinin B\textsubscript{2} receptors at the very beginning of the development of the peripheral inflammation, with the bradykinin B\textsubscript{1} receptors playing an important role during all stages of the inflammation. There is little
evidence for a role of peripheral bradykinin $B_1$ receptors under normal conditions or during models of inflammatory pain, such as the formalin response (Haley 1989), therefore it is of considerable interest that U.V.A irradiation of the hindpaw results in the induction of these receptors. There is thought to be an important link between the induction of $B_1$ receptors and the level of inflammatory mediators such as IL-1 and immunocompetent cells (see refs. in Perkins et al. 1993a). As would be expected subcutaneous morphine was shown to dose-relatedly reduce the thermal hyperalgesia (Perkins et al. 1993b). From these two behavioural studies it appears U.V.A irradiation of the hindpaw results in the development of a peripheral inflammation and hyperalgesia to noxious heat and noxious mechanical stimuli.

The findings of this behavioural study are as one would expect since peripheral sensitisation is a major factor which contributes to the changes in thermal sensation elicited after sunburn (see refs. in Woolf 1991). The generation of bradykinin and prostaglandins at the site of U.V.A irradiation are important contributors to the development of a peripheral hyperalgesia. Both bradykinin and prostaglandins are well established sensitizers of primary afferents (see refs. in Dray and Perkins 1993).

As a preliminary investigation, I have studied the electrophysiological properties of the dorsal horn neurones 3 and 5 days after the second exposure to U.V.A light. Two groups were studied and were termed 3 day U.V. group and 5 day U.V. group. These two time points were used for the electrophysiological study since behavioural studies indicate hyperalgesia is maximal at these time points (Perkins et al. 1993a, b).

In addition, I have studied the effect of intrathecal administration of the following drugs: the opioid morphine, a local anaesthetic, lignocaine, a NMDA receptor antagonist, 7 chlorokynurenate and a bradykinin $B_2$ receptor antagonist, HOE140, on the electrically evoked responses of the dorsal horn neurones of the 3 day U.V. group and 5 day U.V. group. These four different classes of drugs were studied since they have been shown in this thesis to be effective at inhibiting the second phase of the formalin response which is also associated with a peripheral inflammation. The pharmacological properties of the above drugs have previously been discussed in this thesis.

A control group which was not exposed to U.V.A light, but was identical in every other aspect was used for comparative purposes for both the cell characteristics and drug effects.
14.2 Results

14.2.1 The electrically evoked responses of the dorsal horn neurones 3 days and 5 days after the irradiation of the hindpaw with U.V.A light as compared to the control responses

The electrically evoked responses of the dorsal horn neurones of the 3 day U.V. group, 5 day U.V. group and the control group are compared in table 22. The mean depth of the neuronal population of the 3 day U.V. group was significantly lower than the mean depth of the neuronal population of the 5 day U.V. group but was not different to the control group. The mean depth of the neuronal population of the 5 day U.V. group was not different to the control group. Overall all neurones studied were deep dorsal horn neurones, and therefore although there was a difference between the mean depth of the 3 day U.V. group and 5 day U.V. group this would not be expected to influence the results of this study.

The threshold for the electrically evoked C-fibre response of the dorsal horn neurones was not significantly different between the three groups. In addition, the mean electrically evoked C-fibre responses of the 3 day U.V. group, 5 day U.V group and control group were not significantly between the three groups.

The Aβ-fibre threshold of the 3 day U.V. group was significantly lower than the Aβ-fibre thresholds of the control group and the 5 day U.V. group. In addition, the Aβ-fibre threshold of the 5 day U.V. group was significantly less than the control group. In line with these decreased Aβ-fibre thresholds, the electrically evoked Aβ-fibre responses were significantly higher in the 3 day and 5 day U.V. group as compared to the control group.

The wind up of the dorsal horn neurones of the 3 day and 5 day U.V. group were not different from the control group. In contrast, the wind up of the dorsal horn neurones of the 3 day U.V. group was lower than the wind up of the 5 day U.V. group. The spontaneous activity of the dorsal horn neurones was significantly greater in the 3 day and 5 day U.V. group as compared to the control group which exhibited an extremely low level of spontaneous activity.
Table 22. The electrically evoked responses of the dorsal horn neurones 3 days and 5 days after U.V.A irradiation of the hindpaw as compared to control responses.

<table>
<thead>
<tr>
<th></th>
<th>3-Day n=61</th>
<th>5-Day n=35</th>
<th>Control n=35</th>
</tr>
</thead>
<tbody>
<tr>
<td>Depth (µm)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C-threshold (mA)</td>
<td>1.08±0.1</td>
<td>1.3±0.15</td>
<td>1.09±0.1</td>
</tr>
<tr>
<td>Aβ-threshold (mA)</td>
<td>0.05±0.007</td>
<td>0.1±0.01***</td>
<td>0.13±0.02</td>
</tr>
<tr>
<td>C-fibres</td>
<td>276±18</td>
<td>262±26</td>
<td>269±19</td>
</tr>
<tr>
<td>Wind up</td>
<td>140±18+</td>
<td>211±34</td>
<td>198±32</td>
</tr>
<tr>
<td>Aβ-fibres</td>
<td>89±5**</td>
<td>90±12*</td>
<td>64±5</td>
</tr>
<tr>
<td>Spontaneous (AP/sec)</td>
<td>2.5±0.55**</td>
<td>1.86±0.63*</td>
<td>0.37±0.13</td>
</tr>
</tbody>
</table>

Statistical Analysis used Student’s unpaired two tailed t-test
Comparisons to control responses, *p<0.05, **p<0.001, ***p<0.0001.
Comparisons between UV groups, +p<0.05, ++p<0.001.
14.2.2 The mean maximal effect of intrathecal morphine on the electrically evoked responses of dorsal horn neurones of the control group

Overall, intrathecal morphine produced dose-related inhibitions of the electrically evoked C-fibre and wind up of the dorsal horn neurones whereas the Aβ-fibre evoked responses were less susceptible to morphine (see table 23). In the presence of 0.5μg morphine the C-fibre evoked response was non significantly facilitated in 2 out of 8 neurones. The C-fibre evoked response of 1 out of the 6 neurones tested was facilitated by 5μg morphine. These facilitations were included in the full statistical analysis of the effect of morphine on the C-fibre evoked response. 50μg morphine inhibited the electrically evoked responses of all neurones studied. A full reversal of the effects of the inhibitory effects of 50μg morphine on the electrically evoked responses was observed with 50μg naloxone (table 23).

Table 23. The mean maximal effect of intrathecal morphine on the electrically evoked responses of dorsal horn neurones of the control group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5μg</td>
<td>21±13*</td>
<td>29±5*</td>
<td>49±16*</td>
<td>7</td>
</tr>
<tr>
<td>5μg</td>
<td>45±21*</td>
<td>28±11*</td>
<td>48±17**</td>
<td>7</td>
</tr>
<tr>
<td>50μg</td>
<td>80±20**</td>
<td>36±13*</td>
<td>89±8**</td>
<td>7</td>
</tr>
<tr>
<td>Plus Naloxone 50μg</td>
<td>28±9</td>
<td>20±23</td>
<td>-73±90</td>
<td>7</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student’s two tailed paired t-test, *:p ≤0.05, **:p ≤0.001.
14.2.3 The mean maximal effect of intrathecal morphine on electrically evoked responses of dorsal horn neurones of the 3 day U.V. group

The mean maximal inhibitions of electrically evoked responses by intrathecal morphine in 3 day U.V. group are shown in table 24. As with the control group, morphine generally produced dose-related inhibitions of the electrically evoked responses of the dorsal horn neurones. In the presence of 0.5μg morphine the C-fibre evoked response was non significantly facilitated in 1 out of 5 neurones. The C-fibre evoked response of 2 out of 9 neurones was facilitated by 5μg morphine. These facilitations were included in the full statistical analysis of the effect of morphine on the C-fibre evoked response. Only the highest dose of morphine significantly inhibited the C-fibre evoked response, whereas the electrically evoked wind up was inhibited by both 5μg and 50μg of morphine. In contrast to the control group, the Aβ-fibre response was not significantly inhibited by the lowest dose of morphine. The highest dose of morphine studied (50μg) significantly inhibited all of the electrically evoked responses of all dorsal horn neurones. A full reversal of the effects of the inhibitory effects of 50μg morphine on the electrically evoked responses was observed with 50μg naloxone (table 24).

Table 24. The mean maximal effect of intrathecal morphine on the electrically evoked responses of dorsal horn neurones 3 days after U.V.A irradiation.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5μg</td>
<td>-12±26</td>
<td>13±10</td>
<td>-26±35</td>
<td>6</td>
</tr>
<tr>
<td>5μg</td>
<td>29±23</td>
<td>20±6*</td>
<td>58±11*</td>
<td>10</td>
</tr>
<tr>
<td>50μg</td>
<td>96±2**</td>
<td>37±5**</td>
<td>95±4**</td>
<td>10</td>
</tr>
<tr>
<td>Plus Naloxone 5μg</td>
<td>22±21</td>
<td>4±8</td>
<td>-16±4</td>
<td>7</td>
</tr>
</tbody>
</table>

Mean maximal effects are expressed as percentage inhibition of the control response. Statistical analysis used Student's two tailed paired t-test, *:p ≤0.05, **:p ≤0.001.
14.2.4 The mean maximal effect of intrathecal morphine on electrically evoked responses of dorsal horn neurones of the 5 day U.V. group

The mean maximal inhibitions of electrically evoked responses of 5 day U.V. group by intrathecal morphine are shown in table 25. In the presence of 0.5μg morphine the C-fibre evoked response was non-significantly facilitated in 4 out of 9 neurones, 5μg morphine produced facilitations of the C-fibre evoked response in 3 out of 10 neurones. These facilitations were included in the full analysis of the data. As observed with the 3 day U.V. group, only the highest dose of morphine (50μg) produced significant inhibitions of the C-fibre evoked response. Both the Aβ-fibre evoked response and wind up of the dorsal horn neurones were inhibited in a dose-related manner by intrathecal morphine. A full reversal of the inhibitory effects of 50μg morphine on the electrically evoked responses was observed with 50μg of intrathecal naloxone (table 25).

Table 25. The mean maximal effect of intrathecal morphine on electrically evoked responses of the 5 day U.V. group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5μg</td>
<td>-8±22</td>
<td>23±12</td>
<td>28±27</td>
<td>11</td>
</tr>
<tr>
<td>5μg</td>
<td>24±21</td>
<td>36±11*</td>
<td>40±29*</td>
<td>10</td>
</tr>
<tr>
<td>50μg</td>
<td>88±7***</td>
<td>48±11**</td>
<td>92±5**</td>
<td>8</td>
</tr>
<tr>
<td>Plus Naloxone 5μg</td>
<td>3±29</td>
<td>11±17</td>
<td>-18±41</td>
<td>8</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student's two tailed paired t-test, *:p ≤0.05, **:p ≤0.001, ***p ≤0.0001.
14.2.5 Comparison between the effects of morphine on the electrically evoked responses of the control group, 3 day U.V. group and 5 day U.V. group

Intrathecal morphine dose-relatedly inhibited the electrically evoked C-fibre response of the dorsal horn neurones in the control group, 3 day U.V. group and 5 day U.V. group (figure 22). There appeared to be a slight shift at the lower end of the dose response curve of the 3 and 5 day U.V. group as compared to control, with 0.5μg of morphine having less of an inhibitory effect on the C-fibre evoked responses. Indeed 5μg of morphine did not significantly inhibit the C-fibre evoked responses in the 3 or 5 day U.V. group, whereas this dose produced significant inhibitions of this response in the control group. The inhibitory effects observed with 50μg of morphine were similar for the three groups.

![Figure 22. The effect of intrathecal morphine on the electrically evoked C-fibre response of the dorsal horn neurones.](image)

Statistical analysis (ANOVA) showed the inhibitions of the C-fibre evoked response of the dorsal horn neurones by intrathecal morphine were not significantly different between the control group and the 3 day post U.V. group ($F_{2,41} = 0.803, p = 0.4549$).
This was also true for the control group and the 5 day U.V. group ($F_{2,46}=0.461$, $p=0.633$). Finally no significant differences were observed between the effects of intrathecal morphine on the electrically evoked C-fibre response of the dorsal horn neurones of the 3 day U.V group and 5 day U.V. group ($F_{2,47}=0.15$, $p=0.8617$).

The dose response curves for the effect of morphine on the electrically evoked wind up of the control group, 3 day U.V. group and 5 day group are shown in figure 23. Again there appeared to be a shift at the lower end of the dose response curve, with 0.5μg of morphine producing more facilitations in the 3 and 5 day U.V. groups as compared to control. At the upper end of the dose range, morphine produced similar inhibitions of the electrically evoked wind up of the dorsal horn neurones of all three groups.

![Figure 23. The effect of intrathecal morphine on the electrically evoked wind up of the dorsal horn neurones.](image)

Statistical analysis (ANOVA) showed the effect of intrathecal morphine on the wind up of the dorsal horn neurones was not significantly different between the control group and the 3 day U.V. group ($F_{2,39}=2.3$, $p=0.1$), or between the control group and the 5 day U.V. group ($F_{2,39}=0.9$, $p=0.41$), or between the 3 day U.V. group and 5 day U.V. group ($F_{2,38}=1.16$, $p=0.32$).

Statistical analysis (ANOVA) showed the effect of intrathecal morphine on Aβ-fibre
evoked response of the dorsal horn neurones was not significantly different between the control group and the 3 day U.V. group ($F_{2,40}=0.182, p=0.84$), or between the control group and 5 day U.V. group ($F_{2,45}=0.057, p=0.94$), or between 3 day U.V. group and 5 day U.V. group ($F_{2,49}=0.083, p=0.92$).
14.2.6 The mean maximal effect of intrathecal lignocaine on the electrically evoked responses of dorsal horn neurones of the control group

Local anaesthetics reduce neuronal activity by blocking sodium channels. In a previous electrophysiological study, intrathecal lignocaine (0.1-2%) selectively inhibited the electrically evoked C-fibre responses and wind up of the dorsal horn neurones. The Aβ-fibre evoked responses were less sensitive to intrathecal lignocaine (Fraser et al. 1992). As discussed in chapter 6, spinal local anaesthetics selectively inhibit nociceptive transmission over innocuous transmission. I have studied the effect of intrathecal local anaesthetic, lignocaine, on the electrically evoked responses of the dorsal horn neurones. In addition, the effect of lignocaine on the spontaneous activity of the dorsal horn neurones. The doses of lignocaine used in this study were within the same dose range of those used by Fraser et al. 1992.

Overall, with intrathecal lignocaine I observed dose-related inhibitions of the electrically evoked C-fibre response and wind up of the dorsal horn neurones of the control group (table 26). In contrast the effect of lignocaine on the Aβ-fibre evoked response plateaued. The highest dose of lignocaine inhibited the C-fibre evoked response and wind up of the dorsal horn neurones of the control group with the Aβ-fibre evoked response being less sensitive to this dose of lignocaine. A small degree of spontaneous activity of the dorsal horn neurones was observed in the control group and this was also inhibited by lignocaine in a dose-related manner (table 26).
Table 26. The mean maximal effect of intrathecal lignocaine on electrically evoked responses of the dorsal horn neurones of the control group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>20±6</td>
<td>24±3*</td>
<td>56±25</td>
<td>49±17</td>
<td>8</td>
</tr>
<tr>
<td>1%</td>
<td>44±13*</td>
<td>33±5*</td>
<td>87±7*</td>
<td>70±17***</td>
<td>8</td>
</tr>
<tr>
<td>2%</td>
<td>54±12*</td>
<td>33±8**</td>
<td>91±8**</td>
<td>88±9***</td>
<td>8</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student’s two tailed paired t-test, *:p ≤0.05, **:p ≤0.001, *** p ≤0.0001.

14.2.7 The mean maximal effect of intrathecal lignocaine on the electrically evoked responses of the dorsal horn neurones of the 3 day U.V. group

The electrically evoked C-fibre response and the wind up of the dorsal horn neurones were dose-relatedly inhibited by intrathecal lignocaine (table 27). The electrically evoked Aβ-fibre response was less sensitive to lignocaine as compared to the electrically evoked C-fibre response and wind up of the dorsal horn neurones. In contrast to the control group, only the highest dose of lignocaine (2% ) produced significant inhibitions of the electrically evoked Aβ-fibre response (table 27). The spontaneous activity of the dorsal horn neurones was dose-relatedly inhibited by lignocaine (table 27).
Table 27. The mean maximal effect of intrathecal lignocaine on the electrically evoked responses of the 3 day U.V. group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>20±3*</td>
<td>10±10</td>
<td>67±14*</td>
<td>58±15</td>
<td>7</td>
</tr>
<tr>
<td>1%</td>
<td>33±12*</td>
<td>14±7</td>
<td>73±11*</td>
<td>60±13*</td>
<td>8</td>
</tr>
<tr>
<td>2%</td>
<td>79±13*</td>
<td>57±17*</td>
<td>100±0**</td>
<td>72±16*</td>
<td>7</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student's two tailed paired t-test, *:p ≤0.05, **:p ≤0.001, ***: p ≤0.0001.

14.2.8 The mean maximal effect of intrathecal lignocaine on the electrically evoked responses of the dorsal horn neurones of the 5 day U.V. group

The electrically evoked C-fibre response and the wind up of the dorsal horn neurones were dose-relatedly inhibited by intrathecal lignocaine (table 28). Again as observed with the 3 day U.V. group, the Aβ-fibre response was less sensitive to the effects of intrathecal lignocaine as compared to the control group, and was only significantly inhibited by 2% lignocaine. As observed with the control and 3 day U.V. groups, the spontaneous activity of the dorsal horn neurones of the 5 day U.V. group was inhibited by lignocaine in a dose-related manner (table 28).
Table 28. The mean maximal effect of intrathecal lignocaine on electrically evoked responses of the dorsal horn neurones of the 5 day U.V. group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5%</td>
<td>25±9*</td>
<td>11±6</td>
<td>39±13*</td>
<td>41±22*</td>
<td>10</td>
</tr>
<tr>
<td>1%</td>
<td>54±13*</td>
<td>30±9*</td>
<td>69±10*</td>
<td>49±20*</td>
<td>10</td>
</tr>
<tr>
<td>2%</td>
<td>61±11**</td>
<td>42±11*</td>
<td>76±9**</td>
<td>65±16*</td>
<td>10</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student’s two tailed paired t-test, *:p ≤0.05, **:p ≤0.001, ***: p ≤0.0001.
14.2.9 Comparison between the effects of lignocaine on the electrically evoked responses of the control group, the 3 day U.V. group and the 5 day U.V. group

The effect of intrathecal lignocaine on the electrically evoked C-fibre responses in the control group, 3 day group and 5 day was extremely similar (figure 24).

![Graph showing the effect of lignocaine on C-fibre responses](image)

Figure 24. The effect of intrathecal lignocaine on the electrically evoked C-fibre response of the dorsal horn neurones.

Statistical analysis (ANOVA) showed there was no significant difference between the effect of intrathecal lignocaine on C-fibre evoked responses of the dorsal horn neurones of the control group and the 3 day U.V. group ($F_{2,40} = 1.54$, p=0.22), or between the control group and the 5 day U.V. group ($F_{2,48} = 0.017$, p=0.98), or between the 3 day U.V. group and 5 day U.V. group ($F_{2,46} = 0.163$, p=0.20).

The effects of intrathecal lignocaine on the electrically evoked wind up of the dorsal horn neurones were similar for the control group, the 3 day U.V. group and 5 day U.V. group (figure 25).
Figure 25. The effect of intrathecal lignocaine on the electrically evoked wind up of the dorsal horn neurones.

Statistical analysis (ANOVA) showed there was no significant difference between the effect of intrathecal lignocaine on the wind up of the dorsal horn neurones of the control group and the 3 day U.V. group ($F_{2,36}=1.98$, $p=0.15$), or between the control group and the 5 day U.V. group ($F_{2.39}=1.101$, $p=0.342$), or between the 3 day U.V. group and 5 day U.V. group ($F_{2,41}=0.77$, $p=0.47$).

The Aβ-fibre evoked response of the 3 and 5 day U.V. was less sensitive to intrathecal lignocaine than the Aβ-fibre evoked response of the control group. Statistical analysis (ANOVA) showed the effect of lignocaine on the Aβ-fibre evoked response was not significantly different between the control group and 3 day U.V. group ($F_{2,40}=3.08$, $p=0.05$). In addition, the effect of lignocaine on the Aβ-fibre evoked response was not significantly different between the control group and the 5 day U.V. group ($F_{2,47}=1.018$, $p=0.37$), or between the 3 day U.V. group and 5 day U.V. group ($F_{2,45}=1.042$, $p=0.36$).

The spontaneous activity of the dorsal horn neurones of the control group, 3 day U.V. group and 5 day U.V. group was inhibited in a dose-related manner by lignocaine. Statistical analysis (ANOVA) showed the effect of lignocaine on the spontaneous activity was not significantly different between the control group and the 3 day U.V. group.
(F_{2,36}=0.66, p=0.52). Again the effects lignocaine on the spontaneous activity of the control group and the 5 day U.V. group were extremely similar (F_{2,34}=1.38, p=0.26) and for the 3 day and 5 day U.V. groups (F_{2,34}=0.32, p=0.73).
14.2.10 The mean maximal effect of 7 chlorokynurenate on the electrically evoked responses of the dorsal horn neurones of the control group

The effect of 7 chlorokynurenate (7CK) on the electrically evoked responses of the dorsal horn neurones of the control group was studied (table 29). Overall, intrathecal 7CK produced dose-related inhibitions of the electrically evoked C-fibre responses of the dorsal horn neurones. Clear dose-dependent inhibitions of the electrically evoked wind up responses of the dorsal horn neurones were observed with intrathecal 7CK. The electrically evoked Aβ-fibre responses were unaltered by 7CK. There was a tendency towards inhibition of the spontaneous activity of the dorsal horn neurones by 7CK, but significant inhibitions were only observed with the highest dose.

Table 29. The mean maximal effect of intrathecal 7CK on electrically evoked responses of the dorsal horn neurones of the control group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5μg</td>
<td>12±5*</td>
<td>-5±11</td>
<td>20±7*</td>
<td>12±18</td>
<td>7</td>
</tr>
<tr>
<td>10μg</td>
<td>21±9*</td>
<td>13±7</td>
<td>55±13*</td>
<td>42±20</td>
<td>7</td>
</tr>
<tr>
<td>50μg</td>
<td>41±13*</td>
<td>-6±8</td>
<td>74±13***</td>
<td>76±22*</td>
<td>7</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student's two tailed paired t-test, *:p ≤0.05, **:p ≤0.001 ***: p ≤0.0001.

14.2.11 The mean maximal effect of 7 Chlorokynurenate on the electrically evoked responses of the dorsal horn neurones of the 3 day U.V. group

The electrically evoked C-fibre responses and wind up of the dorsal horn neurones were dose-dependently inhibited by 7CK (table 30). In contrast to the control group the
electrically evoked Aβ-fibre evoked responses was inhibited by 7CK in a dose-related manner (table 30). The spontaneous activity of the dorsal horn neurones was inhibited by 7CK, but these inhibitions were not dose-related.

Table 30. The mean maximal effect of intrathecal 7CK on electrically evoked responses of the dorsal horn neurones of the 3 day U.V. group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5μg</td>
<td>11±5</td>
<td>12±3*</td>
<td>57±17*</td>
<td>55±15*</td>
<td>8</td>
</tr>
<tr>
<td>10μg</td>
<td>32±14*</td>
<td>24±6*</td>
<td>87±9*</td>
<td>55±16*</td>
<td>8</td>
</tr>
<tr>
<td>50μg</td>
<td>49±5**</td>
<td>48±10*</td>
<td>91±7***</td>
<td>72±15*</td>
<td>8</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student's two tailed paired t-test, *:p ≤0.05, **:p ≤0.001 ***: p ≤0.0001.

14.2.12 The mean maximal effect of intrathecal 7 Chlorokynurenate on the electrically evoked responses of the dorsal horn neurones of the 5 day U.V. group

The electrically evoked C-fibre responses and wind up of the dorsal horn neurones were inhibited by 7CK in a dose-related manner (table 31). In contrast to the 3 day U.V. group, the Aβ-fibre evoked responses were only inhibited by the highest dose of 7CK (table 31). The spontaneous activity of the dorsal horn neurones was inhibited by 7CK but this effect was not dose-related.
Table 31. The mean maximal effect of intrathecal 7CK on electrically evoked responses of the dorsal horn neurones of the 5 day U.V. group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5 µg</td>
<td>18±2*</td>
<td>14±1</td>
<td>46±11*</td>
<td>74±14*</td>
<td>6</td>
</tr>
<tr>
<td>10µg</td>
<td>24±4*</td>
<td>20±7</td>
<td>59±12*</td>
<td>55±20*</td>
<td>6</td>
</tr>
<tr>
<td>50µg</td>
<td>46±11*</td>
<td>37±6*</td>
<td>88±5**</td>
<td>84±8*</td>
<td>6</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student’s two tailed paired t-test, *:p ≤0.05, **:p ≤0.001, ***: p ≤0.0001

14.2.13 Comparison of the effects of 7 chlorokynurenate on the electrically evoked responses of the control group, the 3 day U.V. group and the 5 day U.V. group

The dose-related inhibitions of the C-fibre evoked responses of the dorsal horn neurones by 7CK were similar for the control group, 3 day U.V. group and the 5 day U.V. group (see figure 26).
Figure 26. The effect of intrathecal 7CK on the electrically evoked C-fibre response of the dorsal horn neurones.

Statistical analysis (ANOVA) showed there was no significant difference between the effect of 7CK on the C-fibre evoked responses of the control group and the 3 day U.V. group ($F_{2,35}=0.178$, $P=0.83$), the control group and the 5 day U.V. group ($F_{2,34}=0.187$, $P=0.83$), or the 3 and 5 day U.V. groups ($F_{2,34}=0.369$, $P=0.8$).

Intrathecal 7CK significantly inhibited the electrically evoked wind up of the dorsal horn neurones of all 3 groups (figure 27). The wind up of the 3 day U.V. group and the 5 day U.V. group was more sensitive to the lower doses of 7CK as compared to the control group.
Figure 27. The effect of 7CK on the electrically evoked wind up of the dorsal horn neurones.

Statistical analysis (ANOVA) showed there was no significant difference between the effect of 7CK on the electrically evoked wind up responses of the control group and the 3 day U.V. group ($F_{2,32}=0.17$, $P=0.84$), the control group and the 5 day U.V. group ($F_{2,36}=1.019$, $P=0.37$), or between the 3 day U.V. group and 5 day U.V. group ($F_{2,35}=0.768$, $P=0.55$).

The electrically evoked Aβ-fibre responses of the dorsal horn neurones of the control group were not significantly inhibited by 7CK. In contrast, 7CK dose-relatedly inhibited the Aβ-fibre evoked responses of the dorsal horn neurones of the 3 day U.V. group and 5 day U.V. group (figure 28). In particular, the highest dose of 7CK produced clear inhibitions of the Aβ-fibre evoked responses of the U.V. groups very different from the lack of effect of 7CK on the Aβ-fibre evoked responses of the control group.
Figure 28. The effect of 7CK on the electrically evoked Aβ-fibre response of the dorsal horn neurones.

Statistical analysis (ANOVA) showed there was a significant difference between the effect of 7CK on the electrically evoked Aβ-fibre responses of the control group and the 3 day U.V. group ($F_{2,36}=3.07$, $P=0.05$, Student Newman-keuls, Post-Hocs) and a marginally significant difference between the control group and the 5 day U.V. group ($F_{2,34}=2.69$, $P=0.08$, Student Newman-keuls, Post-Hocs). In addition comparison of the 3 groups revealed a significant difference ($F_{4,52}=2.54$, $P=0.05$, Student Newman-keuls, Post-Hocs).

Finally, the effect of 7CK on the spontaneous activity of the dorsal horn neurones of the 3 groups was compared. At the lower end of the dose range, the spontaneous activity of dorsal horn neurones of the 3 and 5 day U.V. group appeared to be more sensitive to 7CK than the spontaneous activity of the control group (tables 29, 30, 31). At the top end of the dose range the inhibition of the spontaneous activity by intrathecal 7CK was similar for all 3 groups. Statistical analysis (ANOVA) showed there was no significant difference between the effect of 7CK on the spontaneous activity of the control group and the 3 day U.V. group ($F_{2,36}=0.94$, $P=0.40$), or between the control group and the 5 day U.V. group ($F_{2,26}=1.8$, $P=0.18$), or between the 3 day and 5 day U.V. groups ($F_{2,29}=1.55$, $P=0.2$).
14.2.14 The mean maximal effect of HOE 140 on the electrically evoked responses of the control group

The electrically evoked C- and Aβ-fibre responses of the control group were not altered by intrathecal administration of the B2 receptor antagonist HOE140. In contrast the wind up of the dorsal horn neurones was significantly inhibited by both 100 and 500μg HOE140 (table 32). There was also a tendency towards inhibition of the spontaneous activity although this effect was not significant.

Table 32. The mean maximal effect of intrathecal HOE140 on the electrically evoked responses of the dorsal horn neurones of the control group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μg</td>
<td>-3±10</td>
<td>-11±10</td>
<td>-9±16</td>
<td>17±35</td>
<td>6</td>
</tr>
<tr>
<td>100μg</td>
<td>17±12</td>
<td>9±13</td>
<td>51±13*</td>
<td>31±34</td>
<td>7</td>
</tr>
<tr>
<td>500μg</td>
<td>11±16</td>
<td>8±21</td>
<td>56±13*</td>
<td>48±40</td>
<td>6</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student's two tailed paired t-test, *: p ≤0.05.

14.2.15 The mean maximal effect of HOE 140 on the electrically evoked responses of the 3 day U.V. group

The electrically evoked responses of the dorsal horn neurones were not significantly inhibited by intrathecal administration of HOE140 (table 33). There was a tendency towards inhibition of the wind up of the dorsal horn neurones, but this effect did not reach significance.
Table 33. The mean maximal effect of intrathecal HOE140 on the electrically evoked responses of the dorsal horn neurones of the 3 day U.V. group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μg</td>
<td>2±13</td>
<td>15±8</td>
<td>15±83</td>
<td>-7±32</td>
<td>7</td>
</tr>
<tr>
<td>100μg</td>
<td>3±9</td>
<td>31±17</td>
<td>23±16</td>
<td>-26±60</td>
<td>7</td>
</tr>
<tr>
<td>500μg</td>
<td>10±26</td>
<td>24±4</td>
<td>59±34</td>
<td>-36±61</td>
<td>7</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response.

14.2.16 The mean maximal effect of HOE 140 on the electrically evoked responses of the 5 day U.V. group

The electrically evoked C-fibre and Aβ-fibre responses of the dorsal horn neurones were not significantly inhibited by intrathecal administration of HOE140 (table 34). The electrically evoked wind up of the dorsal horn neurones was significantly inhibited by HOE140, but this effect was not dose-related. Although there was a tendency towards inhibition of the spontaneous activity of the dorsal horn neurones, this effect did not reach significance.
Table 34. The mean maximal effect of intrathecal HOE140 on the electrically evoked responses of the dorsal horn neurones of the 5 day U.V. group.

<table>
<thead>
<tr>
<th>Dose</th>
<th>C-fibre</th>
<th>Aβ-fibre</th>
<th>Wind up</th>
<th>Spon.</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td>1μg</td>
<td>7±13</td>
<td>2±11</td>
<td>68±14*</td>
<td>33±14</td>
<td>6</td>
</tr>
<tr>
<td>100μg</td>
<td>12±14</td>
<td>5±12</td>
<td>43±17*</td>
<td>61±12</td>
<td>10</td>
</tr>
<tr>
<td>500μg</td>
<td>15±14</td>
<td>1±17</td>
<td>68±12*</td>
<td>30±44</td>
<td>8</td>
</tr>
</tbody>
</table>

The mean maximal effects are expressed as percentage inhibition of the pre-drug control response. Statistical analysis used Student's two tailed paired t-test, *:p ≤0.05.

14.2.17 Comparison of the effects of HOE140 on the electrically evoked responses of the control group, the 3 day U.V. group and the 5 day U.V. group

Statistical analysis (ANOVA) showed there was no significant difference between the effect of HOE140 on the electrically evoked C-fibre response of the control group and 3 day U.V. group (F_{2,34}=0.22, p=0.8), or between the control group and 5 day group (F_{2,37}=0.22, p=0.8), or between the 3 day U.V. group and 5 day U.V. group (F_{2,35}=0.2, p=0.9).

Statistical analysis (ANOVA) showed there was no significant difference between the effect of HOE140 on the electrically evoked wind up response of the dorsal horn neurones of the control group and 3 day U.V. group (F_{2,30}=0.67, p=0.5). A significant difference was found between the control group and 5 day group (F_{2,34}=3.7, p=0.03, Student Newman-keuls, Post-Hocs). There was no significant difference between the effect of HOE140 on the electrically evoked wind up response of the dorsal horn neurones of the 3 day U.V. group and 5 day U.V. group (F_{2,33}=0.18, p=0.98).

Statistical analysis (ANOVA) showed there was no significant difference between the effect of HOE140 on the electrically evoked Aβ-fibre response of the control group
and 3 day U.V. group ($F_{2,33}=0.09$, $p=0.9$), or between the control group and 5 day group ($F_{2,37}=0.24$, $p=0.7$), or between the 3 day U.V. group and 5 day U.V group ($F_{2,35}=0.16$, $p=0.95$).

Finally, statistical analysis (ANOVA) showed there was no significant difference between the effect of HOE140 on the spontaneous activity of the control group and 3 day U.V. group ($F_{2,23}=0.2$, $p=0.82$), or between the control group and 5 day group ($F_{2,20}=0.3$, $p=0.74$), or between the two U.V. groups ($F_{2,20}=0.1$, $p=0.94$).
14.3 Discussion

In this series of experiments the animals used were female S.D. rats, weighing less than those used in the previous studies (see methods). This choice of sex and weight of the rats allowed direct comparisons with behavioural studies of U.V. induced hyperalgesia.

General cell characteristics

The mean threshold for the electrically evoked C-fibre responses of the dorsal horn neurones was not different between the control, 3 day U.V. group and 5 day U.V. group. In line with this the mean C-fibre evoked responses of the dorsal horn neurones was similar for all three groups. In contrast, the mean threshold for electrically evoked Aβ-fibre responses of the dorsal horn neurones were significantly lower for the 3 day U.V. group and the 5 day U.V. group as compared to controls. In addition, the mean threshold for the electrically evoked Aβ-fibre responses of the dorsal horn neurones of the 3 day U.V. group was significantly lower than the 5 day U.V. group. The mean electrically evoked Aβ-fibre responses of the dorsal horn neurones were significantly larger for the 3 day U.V group and 5 day U.V. group as compared to the control group. From these results to may be concluded that ultraviolet irradiation of the hindpaw results in changes in the Aβ-fibre thresholds and responses of the dorsal horn neurones but not in the C-fibre evoked responses, suggesting there are changes centrally which influence the Aβ-fibre evoked responses but not the C-fibre evoked responses of the dorsal horn neurones. The mean maximal change of the Aβ-fibre threshold was largest in the 3 day U.V. group, paralleling the finding of a recent behavioural study (Perkins et al. 1993a). The study by Perkins et al. 1993a suggested sensitization of the C-fibre afferents by bradykinin and prostaglandins contributed to the thermal hyperalgesia. In addition, the responses of polymodal nociceptors to noxious heat and bradykinin have been shown to be enhanced by U.V.A irradiation (Szolcsanyi 1987). With direct electrical stimulation the nociceptors are bypassed and therefore I would not expect to see any effect of these peripheral mediators on the electrically evoked C-fibre responses. Another factor which should also
be considered is the possibility that normally silent nociceptors may be recruited after U.V. irradiation.

The dorsal horn neurones of both the 3 and 5 day U.V. groups exhibited significantly higher levels of spontaneous activity as compared to the control group which exhibited extremely low levels of spontaneous activity. This is in agreement with a recent electrophysiological study of dorsal horn neurones after peripheral U.V. irradiation (Urban et al. 1993) and recordings of single C-fibre cutaneous units after peripheral U.V. irradiation (Szolcsanyi 1987). Rhizotomy 3 days after U.V. irradiation has been shown to significantly reduce spontaneous activity, whereas rhizotomy 5 days after U.V irradiation did not significantly reduce spontaneous activity of the dorsal horn neurones (Urban et al. 1993). These results implicate a significant contribution of peripheral inputs to the maintenance of spontaneous activity of the dorsal horn neurones during the initial (1-3) days after U.V. irradiation. It appears that peripheral inputs may become less important and a central component may be involved in the maintenance of spontaneous activity at later (5-7 days) time points. The spontaneous activity of the dorsal horn neurones during the initial days after U.V. irradiation may be due to peripheral inflammatory mediators activating the primary afferents resulting in a relay of inputs from the periphery to the spinal cord. Since the spontaneous activity of the dorsal horn neurones was not totally eliminated by rhizotomy (Urban et al. 1993) this suggests part of the spontaneous activity is due to changes within the dorsal horn, particularly at later time points. Spontaneous activity may represent the loss of spinal inhibitions or increased levels of excitability with the dorsal horn, or be a combination of both.

A recent electrophysiological study has shown that responses of dorsal horn neurones to noxious pinch and heat were enhanced and the thresholds to noxious heat were reduced after U.V. irradiation of the hind paw, and again maximal changes were observed at day 3 (Urban et al. 1993). Therefore although I have not observed any changes in the electrically evoked C-fibre responses, it has been shown that the responses of the dorsal horn neurones to noxious pinch are enhanced after U.V.A irradiation (Urban et al. 1993). One explanation for this may be sensitization of the nociceptors by peripheral mediators enhancing the responses to noxious pinch. As mentioned earlier electrical stimulation bypasses the nociceptors and any affect of peripheral mediators on the nociceptors would not be expected to alter the electrically evoked C-fibre response. In addition, the receptive fields of the wide dynamic range neurones were shown to be
increased on the ipsilateral hind paw after U.V.A irradiation of the hindpaw (Urban et al. 1993).

The central pharmacology of U.V.-induced persistent nociception

The effects of intrathecal morphine and the mechanisms of opiate action have previously been discussed in the section 1.11 and chapter 12. The effects of intrathecal morphine in this study are in line with previous studies (see chapter 12). Overall the inhibitory effects of intrathecal morphine on the electrically evoked C-fibre responses of the dorsal horn neurones of the 3 and 5 day U.V. groups were similar to the effects of intrathecal morphine on the electrically evoked C-fibre responses of the dorsal horn neurones of the control group. There appeared to be a subtle shift at the lower end of the dose range, with the 3 and 5 day U.V. group being less sensitive to low doses of morphine. Decreased opioid sensitivity has been observed in other models of prolonged nociception such as electrically evoked wind up (Dickenson and Sullivan 1986, see refs. in Dickenson 1991) and allodynia (Yaksh 1989). In contrast, some models of prolonged inflammatory nociception such as the formalin model and carrageenan model have increased sensitivities to opiates (Sullivan and Dickenson 1987, Stanfa et al. 1992 and chapter 13).

The electrically evoked Aβ-fibre responses and wind up of dorsal horn neurones of the control group were sensitive to all three doses of morphine studied. This is in contrast to the effects of intrathecal morphine observed in chapter 12. These differences may be due to the rats used in this study being female and / or being smaller in weight. Overall, there was no significant difference between the effect of intrathecal morphine on the electrically evoked wind up of the dorsal horn neurones of the 3 and 5 day U.V. group and the control group. This was also true for the Aβ-fibre evoked responses of the dorsal horn neurones. Again, there appeared to be a subtle decrease in the inhibitory effect of low doses of morphine on the electrically evoked wind up and the Aβ-fibre evoked responses of the dorsal horn neurones of the 3 and 5 day U.V. group as compared to the control group. The effect of intrathecal morphine on the spontaneous activity of the dorsal horn neurones was not studied.

As discussed in the results section there appeared to be more opioid produced
facilitations in the U.V. groups as compared to the control group. These facilitations may mask the inhibitory effects of morphine. Opioid produced facilitations have been discussed in section 1.11, possible mechanisms for these facilitations include an increased release of excitatory neuropeptides within the dorsal horn by low doses of morphine (Wiesenfeld-Hallin et al. 1991). There is also evidence that low doses of opiates have direct excitatory effects by decreasing potassium channel activity and directly decreasing the degree of hyperpolarisation of neurones (see refs. in Crain and Shen 1990, Fan et al. 1991). This decrease in the hyperpolarisation of the dorsal horn neurones, translates as an increased excitability of the dorsal horn neurones. Alternatively in these studies spontaneous activity of the dorsal horn neurones may activate inhibitory systems within the cord in an attempt to counter the increased levels of excitability. The lower doses of intrathecal morphine may inhibit these inhibitory systems resulting in opioid facilitations.

Intrathecal lignocaine inhibited the electrically evoked C-fibre response of the dorsal horn neurones to an equal extent in the 3 and 5 day U.V. groups as compared to the control group. The electrically evoked wind up of dorsal horn neurones was inhibited by intrathecal lignocaine, the effects of lignocaine on wind up were very similar for the control and 3 day U.V. group and similar to the effect seen in a previous study (Fraser et al. 1992). The wind up of the dorsal horn neurones of the 5 day U.V. group was less sensitive to the lower doses of lignocaine than the wind up of the dorsal horn neurones of the control group, this effect was not significant. In both U.V. groups there was a decreased sensitivity of the Aβ-fibre evoked responses of the dorsal horn neurones to the lower doses of lignocaine as compared to the control group, this effect was not significant. This finding ties in well with the decreased Aβ-fibre threshold to electrical stimulation since it is known if fibres are operating well above their rhythmic firing threshold the effects of local anaesthetics are less marked (Devor et al. 1992). In this series of experiments the effect of intrathecal lignocaine on the spontaneous activity of the dorsal horn neurones was studied. Intrathecal lignocaine reduced the spontaneous activity of the dorsal horn neurones of all three groups and therefore the spontaneous activity, which may reflect central hypersensitivity, is in part dependent on Na\(^+\) channel activity.

Intrathecal administration of 7CK inhibited the electrically evoked wind up of the dorsal horn neurones of the control group, 3 day U.V. group and 5 day U.V. group. The effects of 7CK on wind up were extremely similar for the three groups, with wind up
being virtual abolished by the highest dose of 7CK studied. 7CK reduced the overall C-fibre evoked responses of the dorsal horn neurones of the control group, 3 day U.V group and 5 day U.V. group. Since the electrically evoked C-fibre response consists of both the input response and wind up response of the dorsal horn neurones, this effect of 7CK on the C-fibre evoked response is expected. The effects of 7CK on C-fibre evoked responses were extremely similar for all three groups and the inhibitions of the C-fibre evoked responses were approximately 50% smaller than the parallel inhibitions of wind up of the dorsal horn neurones. These effects agree with a previous electrophysiological study of the effect of intrathecal 7CK on the electrically evoked C-fibre responses and wind up of the dorsal horn neurones (Dickenson and Aydar 1991).

The Aβ-fibre evoked responses of the dorsal horn neurones of the control group were not altered by any of the doses of 7CK studied, this finding is in agreement with a previous electrophysiological study (Aydar and Dickenson 1991). In contrast, the Aβ-fibre evoked responses of the dorsal horn neurones of the 3 day U.V. group and 5 day U.V. group were significantly inhibited by 7CK. Moreover the dose response curves of the effect of 7CK on the Aβ-fibre evoked responses of the U.V. groups were significantly different to the control dose response curve. This data is intriguing since under normal conditions the NMDA receptor is only activated by repetitive C-fibre activation and not Aβ-fibre activity. This is thought to be due to Aβ-fibre inputs producing fast synaptic depolarisations of the dorsal horn neurones which do not activate the NMDA receptor. Peptide mediated longer lasting post-synaptic depolarisations are thought to be required for the activation of the NMDA receptor and since peptides are not present in the Aβ-fibres this may explain why under normal conditions Aβ-fibre inputs do not activate the NMDA receptor. Therefore after U.V.A irradiation of the hindpaw there are changes in the spinal nociceptive systems and an overall increase in the excitability of the convergent neurones may account for Aβ-fibre evoked responses of the dorsal horn neurones being sensitive to NMDA receptor antagonism.. As discussed below, the spontaneous activity of the dorsal horn neurones was sensitive to NMDA receptor antagonism, this suggests there is ongoing NMDA receptor activation after U.V.A irradiation of the hindpaw. In view of these findings it is likely that Aβ-fibre inputs arrive on dorsal horn neurones which are already hyperexcitable due to NMDA receptor mediated events and therefore the resultant Aβ-fibre evoked responses of the dorsal horn
neurones are enhanced. With the blockade of the NMDA receptors the overall excitability of the dorsal horn neurones is decreased and A\textbeta- fibre evoked responses of the dorsal horn neurones are reduced. There appears to be similarities between the effects of U.V.A irradiation of the hindpaw and allodynia. Allodynia is characterized by tactile stimuli being perceived as noxious inputs and has previously been shown to be sensitive to NMDA receptor antagonists (Yaksh 1989).

The spontaneous activity of the dorsal horn neurones of all three groups was significantly inhibited by intrathecal 7CK. From these results it may be concluded that the increased level of spontaneous activity of the dorsal horn neurones after U.V.A irradiation are partially mediated by the NMDA receptor. The spontaneous activity of the dorsal horn neurones may represent the development of an NMDA receptor mediated central hypersensitivity, similar to that observed during the formalin response (Haley et al. 1990, see refs. in Dickenson 1991) and during the facilitated flexor reflex (Woolf and Thompson 1991).

Intrathecal administration of the B\textsubscript{2} receptor antagonist HOE140 did not influence the electrically evoked C-fibre response or A\textbeta-fibre evoked response of the dorsal horn neurones of the control, 3 day U.V. group or 5 day U.V. group. The wind up of the dorsal horn neurones of the control group was significantly inhibited by the higher doses of HOE140 studied, this is in contrast to the lack of effect of HOE140 on the electrically evoked wind up described in chapter 7. Again this may be due to differences in the rats used in this study and the previous study. There was a tendency towards inhibition of electrically evoked wind up of the dorsal horn neurones of the 3 day U.V. group. Due to a large degree of variability of the effect of HOE140 on the wind up response of the dorsal horn neurones of the 3 day U.V. group significant effects were not observed. In contrast, HOE140 significantly inhibited the wind up of dorsal horn neurones of the 5 day U.V. group. Moreover, these effects were significantly different to the effects observed with HOE140 on the wind up of the control group. Therefore it appears at this later time point spinal bradykinin contributes to the development of wind up of the dorsal horn neurones. This is similar to the inhibitory effect of intrathecal HOE140 on second phase of the formalin response (chapter 7). These results support the concept that spinal bradykinin plays a role during prolonged inflammatory nociception. The precise location of spinal bradykinin B\textsubscript{2} receptors is unknown and therefore the mechanism of action of
bradykinin can only be speculative. Since HOE140 did not influence the C-fibre evoked activity of the dorsal horn neurones it seems unlikely the site of action is pre-synaptic. Therefore bradykinin may contribute to wind up via an action at sites post-synaptic to the C-fibre afferents, this may be via a direct action on the convergent dorsal horn neurones or via an action on interneurones. There was a considerable variability in the effect of HOE140 on the spontaneous activity of the dorsal horn neurones, especially for the U.V. groups. Consequently significant effects were not observed, although there was a tendency towards inhibition of the spontaneous activity of the dorsal horn neurones of the 3 and 5 day U.V. groups by HOE140.

To conclude the changes in the dorsal horn neuronal responses observed after peripheral U.V.A irradiation consisted of a decrease in the electrically evoked Aβ-fibre thresholds and an increased electrically evoked Aβ-fibre responses of the dorsal horn neurones. The C-fibre evoked responses of the dorsal horn neurones were not altered as compared to the control group. In addition, the dorsal horn neurones of U.V.A irradiated groups exhibited spontaneous activity, which was sensitive to NMDA receptor antagonism and the local anaesthetic lignocaine. Considerably less spontaneous activity was observed in the control group. The spontaneous activity of the 5 day U.V. group was particularly sensitive to lower doses of the NMDA receptor antagonist 7CK. This is in agreement with the findings of a study by Urban et al. 1993 which showed dorsal rhizotomy did not reduce spontaneous activity of the 5 day U.V. group, suggesting the spontaneous activity at later time points is due to central plasticity and the manifestation of central hypersensitivity. A summary of the major pharmacological differences between the U.V. irradiated group and the control group are summarized in figure 29.
Figure 29

THE MAIN PHARMACOLOGICAL DIFFERENCES BETWEEN THE ELECTRICALLY EVOKED RESPONSES OF THE U.V. GROUPS AND CONTROL GROUP

AB-FIBRE EVOKED RESPONSE, SENSITIVE TO NMDA RECEPTOR ANTAGONIST, LESS SENSITIVE TO LOW DOSES OF LIGNOCAINE

C-FIBRE EVOKED RESPONSE, LESS SENSITIVE TO LOW DOSES OF MORPHINE

WIND UP

INCREASED SENSITIVITY TO LOW DOSES OF NMDA RECEPTOR ANTAGONISTS, IN 5 DAY U.V. GROUP, INHIBITED BY HOE140
In these studies I observed discrete spinal changes in nociceptive processing after U.V.A irradiation of the hindpaw. Behavioural studies have clearly demonstrated that peripheral events such as inflammation and sensitization contribute to the hyperalgesia to natural noxious stimuli. Taken together the results of previous studies (Perkins 1993a,b, Urban et al. 1993) and my electrophysiological studies suggest changes in nociceptive signaling both at the peripheral level and spinal level are important contributors in this model of persistent pain. Following U.V.A irradiation of the hindpaw the electrically evoked Aβ-fibre response of the dorsal horn neurones was sensitive to NMDA receptor antagonism, this has similarities to allodynia (Yaksh 1989). The sensitivity of wind up to the bradykinin B₂ receptor antagonist, HOE140, at later stages of the U.V.A induced persistent nociception implicates a role of spinal bradykinin in mediating central hypersensitivity. The evidence for a role of spinal bradykinin in the processing of prolonged nociceptive transmission has been discussed in chapter 7.

Finally, a recent study has shown that peripheral U.V.A irradiation results in an increase in \textit{junD} mRNA levels in the rat lumbar spinal cord and \textit{junD} proto-oncogene expression 6 hours after exposure to U.V. irradiation (Gillardon et al. 1992). The consequences of the induction of this intermediate-early gene may contribute to the changes observed in the dorsal horn neuronal responses after peripheral U.V.A irradiation of the hindpaw.
CHAPTER 15:

CONCLUSIONS
In these in vivo experiments I have used electrophysiological techniques to investigate the peripheral and spinal pharmacology of nociceptive responses. There are many types of clinical pains and in this thesis I have studied the responses of the dorsal horn neurones to three different types of noxious stimuli. Electrical stimuli of the hindpaw was used in these studies as a stable and reproducible model of acute pain. Generally acute pain management is not a clinical problem. Secondly, a peripheral injection of formalin into the hindpaw was used as a model of relatively prolonged inflammatory pain. Thirdly, irradiation of the hindpaw with ultraviolet A light was used as a model of persistent pain associated with burn injury.

Electrical stimulation of the hindpaw directly activates the primary afferents without producing tissue damage, trauma or inflammation. With electrical stimulation the responses of the dorsal horn neurone either lasts for only the duration of the electrical stimulation or for seconds following the cessation of stimulation. The noxious C-fibre evoked responses are clearly separable from the innocuous Aβ-fibre evoked responses of the dorsal horn neurones. Differential effects of drug treatments on C-fibre versus Aβ-fibre evoked responses of the dorsal horn neurones were easy to demonstrate.

The peripheral injection of formalin results in a biphasic firing pattern of the dorsal horn neurones lasting for 1 hour, with an associated inflammatory response at the site of the formalin injection. Importantly the response of a single dorsal horn neurone to a peripheral injection of formalin mirrors the behavioural response of an animal to the same injection. Therefore the use of halothane as an anaesthetic does not appear to overly influence the responses of these neurones to the peripherally injected formalin.

Irradiation of the hindpaw with U.V.A light has recently been developed as a model of persistent pain associated with burn injury. Behavioural studies have shown that U.V.A irradiation of the hindpaw induces a two phase hyperalgesia consisting of an initial inflammatory phase followed by a phase of persistent nociception in the absence of inflammation. The time course of the U.V.A-induced hyperalgesia is over a period of days (Perkins et al. 1993a).

I investigated the peripheral inflammatory mediators and spinal pharmacology involved in the processing of the nociceptive response of the dorsal horn neurones to a peripheral injection of formalin. The recent development of potent and selective antagonists has enabled a clear cut study of the pharmacology of the formalin response.
The effects of different drug treatments on the acute electrically evoked responses were studied in parallel with the studies on the formalin response allowing a comparison of drug effects on the prolonged nociceptive responses with the effects of these drug treatments on acute responses.

The response of the dorsal horn neurones during the second phase of the formalin response was immediately blocked by an injection of local anesthetic at the same site as the injection of formalin. It can be concluded that the second phase response of the dorsal horn neurones is dependent on a peripheral input. This finding contrasts a previous behavioural study in which peripheral local anaesthesia at the time of the formalin injection inhibited the response to formalin whereas peripheral local anaesthesia after the formalin was less effective at inhibiting the behavioural response (Coderre et al. 1990). Possible reasons for this difference have been discussed in chapter 5.

My results show that the second phase of the formalin response, associated with NMDA receptor mediated central hypersensitivity, requires a peripheral input even once the NMDA receptor is activated. This is not surprising since an inhibition of the C-fibre afferent activity will result in a dramatic reduction in the release of the C-fibre afferent transmitters into the dorsal horn of the spinal cord and a reduced level or zero activation of the post-synaptic NMDA receptors. A previous study has shown that spinal NMDA receptor antagonism during the second phase of the formalin response, that is once the NMDA receptor has been activated, still inhibits the second phase of the formalin response (Haley et al. 1990). From this and my results it may be concluded that ongoing NMDA receptor mediated events in the second phase can be inhibited by either a removal of the C-fibre afferent input into the dorsal horn or blockade of the NMDA receptor.

I then investigated the peripheral events involved in the activation of the nociceptors by formalin. One of the peripheral mediators shown to be involved in the generation of the formalin response is bradykinin. The peripheral bradykinin B2 receptor is involved during both the first and second phase of the formalin response, with a greater contribution to the second phase. In addition, with the use of indomethacin, I have shown that peripheral prostaglandins are involved in the generation of the second phase but not the first phase of the formalin response. Overall, these findings are consistent with current opinion that the first phase of the formalin response is primarily due to the direct activation of the nociceptors by formalin. The second phase of the response is due
to the generation of inflammatory mediators at the site of the formalin injection. These inflammatory mediators are thought to activate and/or sensitize the C-fibre nociceptors. In addition, bradykinin was found to contribute to the initial response. The injection of formalin results in an immediate firing of the dorsal horn neurones and this initial activity must be due to a direct action of formalin since there is insufficient time for the generation of bradykinin. Subsequently, bradykinin is generated within the first ten minutes after formalin and contributes to the activation of the nociceptors during the first phase response.

Intrathecal administration of drugs was used to study the effect of different drug treatments on the spinal processing of nociceptive information.

Intrathecal pre-administration of a local anaesthetic was shown to inhibit both phases of the formalin response. Intrathecal local anaesthetic administered after the peripheral injection of formalin (post-administration) blocked the second phase of the response to an equal degree. Therefore even if the first phase nociceptive inputs sensitize the dorsal horn neurones to the second phase of incoming nociceptive inputs this does not alter the ability of spinally administered local anesthetic to inhibit the second phase of the formalin response.

Since the formalin response is associated with a peripheral inflammatory response I investigated whether there was any evidence for similar events occurring at the level of the spinal cord during this response. Intrathecal administration of a bradykinin B₂ receptor antagonist (HOE140) inhibited the second phase of the formalin response. The first phase of the formalin response was not influenced by HOE140. Both the first and second phase of the formalin response were inhibited by intrathecal administration of indomethacin. These results implicate a role of spinal prostaglandins during the formalin response. The origins of spinal bradykinin and prostaglandins are unknown but it would be expected that bradykinin originates from the vasculature and prostaglandins originate from the conversion of free arachidonic acid by cyclooxygenase activity. Spinal events during the second phase of the formalin response may result in vasodilation and plasma extravasation resulting in the enhanced access of bradykinin to spinal bradykinin receptors. The second phase of the formalin response has been shown to be partly mediated by NMDA receptor activation which results in increased cytosolic levels of calcium. The potential sequence of events leading to an increased production of prostaglandins may involve an increased level of intracellular calcium, the activation of
phospholipase A2 and consequently an increase in free cytosolic arachidonic acid. Therefore it is possible that NMDA receptor activation is the starting point for the generation of prostaglandins during the second phase of the formalin response. The established generation of prostaglandins during the first ten minute phase of the formalin response cannot be attributed to this mechanism since there is a minimal role of the NMDA receptor during this phase. However, the high levels of AMPA and NK1 mediated firing during the first phase of the response may generate spinal prostaglandins. Spinal bradykinin and prostaglandins appear to be involved in the spinal processing of prolonged inflammatory, but not acute, nociceptive responses.

The role of spinal excitatory amino acids during the processing of acute and prolonged nociceptive responses was studied. The acute electrically evoked nociceptive responses of the dorsal horn neurones were not very sensitive to the AMPA receptor antagonist (CNQX). The C-fibre evoked response of the dorsal horn neurones were only inhibited by the highest dose of intrathecal CNQX studied. In contrast, intrathecal pre-administration of CNQX inhibited both phases of the response of the dorsal horn neurones to formalin supporting a role of the AMPA receptor during the spinal processing of prolonged inflammatory nociception. With intrathecal post-administration of CNQX a biphasic effect on the second phase of the response of the dorsal horn neurones was observed. Lower doses of post-administered CNQX facilitated the second phase of the formalin response whereas higher doses produced inhibitions which were smaller than those observed with the same doses of pre-administered CNQX. The selective antagonism of the NMDA receptor was studied with an antagonist at the glycine site of the NMDA receptor (7CK). Both intrathecal pre- and post-administration of 7CK inhibited the second phase of the formalin response to an equal degree. The first phase of the formalin response was not influenced by intrathecal 7CK. Overall, from these results it can be concluded that the AMPA receptor but not the NMDA receptor is involved in the spinal processing of the first phase of the formalin response. NMDA receptor mediated events were shown to play an important role in the spinal processing of the second phase of the formalin response.

I have used a selective neurokinin-1 receptor (NK1) antagonist (RP67580) to study the role of the NK1 receptor (and so substance P) during both acute and prolonged inflammatory nociceptive processing at the level of the spinal cord. The electrically
evoked C-fibre response of the dorsal horn neurones was only sensitive to the highest dose of intrathecally administered RP67580 suggesting a minor role of substance P in the processing of acute nociceptive information. In contrast, the formalin response was extremely sensitive to intrathecal RP67580 and both phases of the formalin response were inhibited but not abolished by RP67580. Considering the roles of AMPA and NMDA receptors these results suggest that both peptides and excitatory amino acids are neurotransmitters involved in the relay of nociceptive information at the level of the dorsal horn during the formalin response. The co-existence of glutamate and substance P in the dorsal root ganglion neurones has been long established (Battaglia and Rustioni 1988). More recently evidence for an co-operative interaction between NMDA receptor mediated events and tachykinin receptors in spinal nociception has been reported (Xu et al. 1992a, Nagy et al. 1993, Dickenson et al. unpublished, Mjellem-Joly et al. 1992). Furthermore, since both the tachykinins and CGRP have been shown to increase the basal and electrical evoked release of excitatory amino acids at the level of the spinal cord (Kangrga and Randic 1993) the interaction between NMDA receptor mediated events and tachykinin receptor mediated events may be even more complex than originally thought. Overall, there is conflict over which neurokinin receptors are involved in these interactions but there is most certainly an interaction between the tachykinin receptor mediated events and NMDA receptor mediated events. It is important to note that the in vivo studies have shown the importance of the NK1 receptor in this interaction with the NMDA receptor mediated events (Xu et al. 1992a, Dickenson et al. unpublished, Mjellem-Joly et al. 1992) whereas the in vitro study showed an interaction between the NK2 receptor mediated events and NMDA receptor mediated events (Nagy et al. 1993). It is extremely likely that further investigations will show that the tachykinins are not an exceptional case and other peptides such as CGRP may also be involved in such interactions. From a mechanistic point of view the following sequence of events can be envisaged. Slow postsynaptic depolarisations produced by peptides may aid the removal of the Mg²⁺ block of the NMDA receptor complex. Once the Mg²⁺ block has been lifted the NMDA receptor can be activated by the excitatory amino acids resulting in the observed NMDA receptor mediated responses of the dorsal horn neurones such as wind up.

Somatostatin has been shown to be present in the C-fibres. Whereas intrathecal administration of somatostatin or its stable analogue sandostatin did not influence the electrically evoked responses of the dorsal horn neurones they inhibited both phases of
the formalin response. However, as discussed in chapter 11, there is contradictory evidence for a anti-nociceptive role of somatostatin in behavioural studies but these previous studies may be confounded by neurotoxic effects of somatostatin. The differential effects on acute nociceptive responses and prolonged inflammatory nociceptive responses may reflect the different nociceptive inputs in the two models. The nociceptive responses to formalin are less time-locked than the synchronous activation produced by peripheral electrical stimulation. Alternatively this differential effect of somatostatin and sandostatin may reflect the ability of activation of the somatostatin receptor to influence different mediators involved in the spinal processing of acute as compared to prolonged nociceptive responses. The sites of action of somatostatin and its analogue sandostatin are unknown. It is interesting that all of the peptides (somatostatin and sandostatin) or their antagonists (RP67580 and HOE 140) studied in this thesis have affected most dramatically the responses of the dorsal horn neurones to prolonged inflammatory stimuli and not acute electrical stimuli. It is possible the inhibitory effect of somatostatin on the formalin response is due to it counteracting the excitatory effects of substance P, bradykinin and prostaglandins during the formalin response.

Opioids have been long established as analgesics but there are a number of clinical conditions in which opioids do not provide adequate pain relief. I have investigated the ability of intrathecal opioids to inhibit electrically evoked wind up of the dorsal horn neurones which as discussed in chapter 12, is mediated by the NMDA receptor. Intrathecal morphine effectively inhibited the electrically evoked input responses of the dorsal horn neurones but did not effectively inhibit the electrically evoked wind up of the dorsal horn neurones except at a high dose when both measures were inhibited. Since NMDA receptor mediated wind up of the dorsal horn neurones was less sensitive to morphine, one of the reasons for opiate insensitive pain may be a marked degree of NMDA receptor activation. I have shown that co-administration of a sub-threshold dose of morphine and a sub-threshold dose of an NMDA receptor antagonist virtually abolished the electrically evoked wind up of the dorsal horn neurones. From these results it may be predicted that combination therapy with low doses of morphine and low doses of an NMDA receptor antagonist may be effective at relieving opiate insensitive pains with low side-effect liability.

There is considerable clinical interest in whether pre-emptive opiates are more
effective than conventional therapy with opiates at producing pain relief. My studies showed that intrathecally post-administered morphine inhibited the second phase of the formalin response to an equal degree as intrathecally pre-administered morphine. This finding suggests that in the case of prolonged inflammatory pain there is little evidence for pre-emptive opiates producing more effective analgesia than conventional opiate therapy. Obviously this may not be true for all types of pain especially those associated with increased levels of anti-opiate peptides such as been suggested to be the case for neuropathic pain (Xu et al. 1993).

The recently developed animal model of persistent pain associated with burn injury has been used in these studies to investigate the mechanisms underlying this type of pain. After the irradiation of the hindpaw with ultraviolet light the dorsal horn neurones exhibited spontaneous activity. The spontaneous activity of the dorsal horn neurones following U.V.A irradiation has previously been shown to be partly due to peripheral inputs (Urban et al. 1993). In addition, after U.V.A irradiation there appeared to be changes in the dorsal horn neuronal responses to electrically stimuli. Fundamental changes in the innocuous Aβ-fibre evoked responses were observed, with the threshold of the Aβ-fibre evoked responses of the dorsal horn neurones being lower than the control thresholds. Moreover, the electrically evoked Aβ-fibre responses were larger than the control Aβ-fibre responses of the dorsal horn neurones. These changes may be due to an increased excitability of the dorsal horn neurones. The C-fibre evoked thresholds and C-fibre evoked responses of the dorsal horn neurones were unaltered but this may be due there being a ceiling effect on the C-fibre evoked responses of the dorsal horn neurones with inhibitory systems counteracting excessively high levels of C-fibre evoked activity. Alternatively there may be decreased levels of inhibitory systems which normally counter or reduce the Aβ-fibre evoked responses. Essentially the pharmacology of electrically evoked C-fibre and Aβ-fibre responses after U.V.A irradiation were similar to the controls. One exception to this was the effect of spinal NMDA receptor antagonism on the Aβ-fibre response. After U.V.A irradiation of the hindpaw the Aβ-fibre evoked responses of the dorsal horn neurones were significantly reduced by NMDA receptor antagonism. As discussed in chapter 14, after U.V.A irradiation of the hindpaw an overall increase in the excitability of the dorsal horn neurones was observed and therefore it is likely that Aβ-fibre inputs arrive on dorsal horn neurones which are already
hyperexcitable due to NMDA receptor mediated events and therefore the resultant Aβ-fibre evoked responses of the dorsal horn neurones are enhanced. With blockade of the NMDA receptors the overall excitability of the dorsal horn neurones is decreased and Aβ-fibre evoked responses of the dorsal horn neurones are reduced. Finally, the electrically evoked wind up of the dorsal horn neurones of the 5 day U.V. group was significantly inhibited by spinal antagonism of the bradykinin B2 receptor. Therefore bradykinin appears to be involved in the spinal mechanisms involved in the development of NMDA receptor mediated wind up of the dorsal horn neurones at later time points after U.V.A irradiation of the hindpaw. This is quite the opposite to events in the periphery. Behavioural studies have shown peripheral bradykinin B2 receptors to be involved in the initial stages of the inflammatory response with the B1 receptor being more important throughout the response (Perkins et al. 1993a). As yet a possible role of spinal B1 receptors after U.V.A irradiation has not be investigated.

My studies have illustrated the complexity of the spinal processing of prolonged inflammatory nociceptive responses. From my results together with previous studies it is apparent that a multitude of neurotransmitters and neuromodulators are involved in these spinal systems. The following scheme of events may explain some of the complex interactions between these different neurotransmitters and neuromodulators during the spinal processing of prolonged inflammatory nociception. The barrage of second phase inputs into the dorsal horn results in the activation of the NMDA receptor and the NK1 receptor. Activation of the NK1 receptor may contribute to the slow prolonged postsynaptic hyperpolarisation which as discussed above is necessary for the activation of the NMDA receptor. Activation of the NMDA receptor can result in an increase of intracellular calcium which in turn could activate nitric oxide synthase (NOS) and phospholipases. The roles of nitric oxide during nociceptive transmission are not fully understood. Nitric oxide may diffuse to the C-fibre terminals where it may influence the release of transmitters from the primary afferent. Activation of phospholipase results in an increased level of free arachidonic acid which is then converted by cyclooxygenase enzyme activity to the prostanoids including the prostaglandins. As discussed in chapter 8, prostaglandins have a number of actions at the level of the spinal cord. Prostaglandins have been shown to facilitate the release of substance P from the C-fibre terminals (Nicol et al. 1992). Therefore both prostaglandins and NO may enhance the release of substance
P from C-fibre afferents. This enhanced release of substance P by prostaglandins and possibly NO during the second phase of the formalin response may explain the enhanced role of substance P during prolonged nociceptive transmission as compared to acute nociceptive transmission. Prostaglandins may also sensitize post-synaptic neurones to the excitatory C-fibre input as well as inhibiting descending noradrenergic controls (see refs. in Malmberg and Yaksh 1992). If this sequence of events occurs, C-fibre afferents would release more transmitters at the first-order synapse which would then impinge upon hyperexcitable neurones in the presence of decreased supraspinal descending noradrenergic controls.

Preliminary in vivo dialysis studies have shown an increased release of spinal glutamate and aspartate in the first 10 minutes after the peripheral injection of formalin. In contrast, the levels of the release of these excitatory amino acids for the remaining 10-60 minutes were not greater than the control basal release (Malmberg and Yaksh personal communication). This finding may seem to contradict my results on the involvement of NMDA receptor mediated events during the second phase of the formalin response and the ability of pre-administered CNQX to inhibit this response. However in view of all the other excitatory systems involved in the spinal processing of prolonged nociceptive events the findings of Malmberg and Yaksh are not so surprising. It is feasible that the initial activation of the AMPA receptors initiates a series of events which result in the generation of a number of neuromodulators such as prostaglandins and bradykinin (see above). Together these neuromodulators and substance P may depolarize the post-synaptic membrane resulting in the removal of the NMDA receptor block and leaving it open to activation by basal levels of excitatory amino acids. It could be envisaged that the chain of events resulting in the generation of spinal bradykinin and prostaglandins are prevented or reduced by pre-administered CNQX. When these systems are already induced such as after the first phase, post-administered CNQX was less effective at inhibiting the second phase of the formalin.

Overall, there is strong evidence for a role of spinal bradykinin during prolonged inflammatory nociceptive processing as demonstrated by the effect of B2 receptor antagonism on the electrically evoked wind up following U.V.A irradiation of the hindpaw and during the second phase of the formalin response. This suggests that over a period of time, afferent inputs into the spinal cord result in the activation of plasma kallikreins and the generation of bradykinin. Both wind up and the second phase of the
formalin response are mediated by the NMDA receptor and as discussed above NMDA receptor activation is thought to result in the generation of NO. One of the well established actions of NO is vasodilation of blood vessels which would favour the arrival of bradykinin at the spinal level. There is evidence from studies of visceral sensory neurones that bradykinin activates cAMP. Activated cAMP inhibits calcium dependent K+ channels which underlie post spike hyperpolarisations (see refs in Dray and Perkins 1993). A consequence of this is an increased probability of repetitive firing of neurones. In addition, if the spinal mechanisms mirror those in the periphery it can be imagined that the increased levels of spinal prostaglandins, which have been observed after a peripheral injection of formalin, enhance the excitatory effects of spinal bradykinin. The role of spinal prostaglandins after U.V.A irradiation of the hindpaw was not investigated but future studies may find that spinal prostaglandins are also contributors to the increased excitability of the dorsal horn neurones after U.V.A irradiation of the hindpaw. This is extremely probable since spinal bradykinin, which has been demonstrated to have a role in this model of prolonged nociception, is known to stimulate the production of prostaglandins via phospholipase A2 activation (Rang et al. 1991).

Overall there are a number of possible positive feedback systems which could maintain the spinal generation of prostaglandins, bradykinin and NO and facilitate the release of primary afferent transmitters. Importantly the second phase of the response of the dorsal horn neurones to a peripheral injection of formalin requires a peripheral input. Therefore the induction and maintenance of the events described above are dependent on afferent activity and this may be, in part, due to the rapid inactivation of prostaglandins, bradykinin and NO so that continual production, driven by the afferents is needed. It is possible that inhibiting one of the links in this cascade of events which contribute to the spinal processing of prolonged nociceptive transmission may effectively break this chain. In view of my findings combination therapy either with opiates and NMDA receptor antagonists or as recently suggested opiates and NSAIDs (Malmberg and Yaksh 1993) may effectively reduce prolonged inflammatory pain.

It is well accepted that numerous peripheral mediators activate C-fibre nociceptors. This thesis and current literature illustrates that this drive induces many excitatory and inhibitory systems at the spinal level during inflammatory pain. A better understanding of these events could aid the clinical management of inflammatory pain.
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APPENDIX
The response of a single dorsal horn neurone to a peripheral injection of formalin

The effect of intrathecal pre-administration of 2% lignocaine on the response of a single dorsal horn neurone to a peripheral injection of formalin

The effect of intrathecal pre-administration of 50µg CNQX on the response of a single dorsal horn neurone to a peripheral injection of formalin

The effect of intrathecal pre-administration of 1µg of 7CK on the response of a single dorsal horn neurone to a peripheral injection of formalin

The response of a single dorsal horn neurone to a peripheral injection of formalin

The effect of intrathecal post-administration of 2% lignocaine on the response of a single dorsal horn neurones to a peripheral injection of formalin

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The effect of intrathecal post-administration of 1µg of 7CK on the response of a single dorsal horn neurone to the peripheral injection of formalin

Appendix I
Example responses of single dorsal horn neurones to a peripheral injection of formalin in the presence of different drug treatments. The spikes per second are plotted on the vertical axis and the full horizontal scale represents one hour.
The response of a single dorsal horn neurone to a peripheral injection of formalin

The effect of intrathecal administration of 500\mu g of HOE 140 on the response of a single dorsal horn neurone to a peripheral injection of formalin

The effect of intrathecal administration of 10\mu g of RP67580 on the response of a single dorsal horn neurone to the peripheral injection of formalin

The effect of intrathecal administration of 250\mu g of indomethacin on the response of a single dorsal horn neurone to a peripheral injection of formalin

The effect of intrathecal administration of 20\mu g of sandostatin on the response of a single dorsal horn neurone to the peripheral injection of formalin

Appendix II
Example responses of single dorsal horn neurones to a peripheral injection of formalin in the presence of different drug treatments. The spikes per second are plotted on the vertical axis and the full horizontal scale represents one hour.
PUBLICATIONS


