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The Ontogeny of Opioid Analgesia

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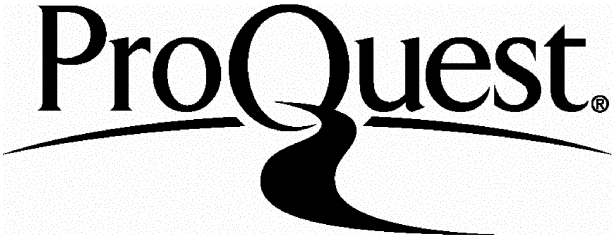

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Abstract

Opioids are increasingly used in infants to treat postoperative pain, despite our lack of knowledge of their analgesic efficacy. The aim of this study was to investigate the analgesic effects of opioid analgesics in neonatal rat pups and postoperative infant patients.

The contribution of individual opioid receptor subtypes in the spinal cord to analgesia at different developmental stages was investigated using epidural mu, delta and kappa opioid receptor agonists in neonatal rats aged P (postnatal day) 3, 10 and 21, in several different pain models. Thresholds for flexion withdrawal reflexes to low-intensity mechanical stimuli were determined in rats with carrageenan-induced inflammation and compared with controls. Neonatal rats have lower mechanical thresholds than older rats but carageenin still produces allodynia (increased sensitivity to touch) at P3 which increases with age. Heat thresholds were also lower in neonates but are sensitized by pretreatment of the skin with the C fibre stimulus, capsaicin.

The analgesic action of each opioid receptor agonist followed an individual developmental pattern with each pain model. However, all three opioid agonists are considerably more efficacious analgesics in younger animals and ED50s at P3 are always lower than at P21.

The clinical study examined the efficacy of morphine in infants after abdominal surgery. Using three different pain assessment tools, pain scores were correlated with plasma levels of morphine and its glucuronide metabolites. Morphine requirements were very low compared with adults, consistent with the rat studies, but varied widely reflecting changes in pharmacokinetics and opioid receptor development. There was

little correlation between plasma concentrations and pain scores highlighting the difficulty in pain assessment in pre-verbal children.

It is concluded that developmental changes in opioid receptor pharmacology lead to differing opioid requirements in neonates compared to older subjects. Further clinical studies are required to establish appropriate opioid analgesia in infants.

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1. Introduction

During the past ten years the explosion in research on opioids has added much to our understanding of opioid systems in adults (Guy and Abbott, 1992). Despite growing interest in the field of neonatal pain and analgesia, our knowledge of opioid systems in neonates and infants is still lacking.

Studies of laboratory animals have shown that pain pathways in neonates and infants are quite different to those of adults; we must therefore assume that analgesic mechanisms are also dissimilar (Fitzgerald, 1991). The pattern of distribution of the opioid receptor subtypes mu, delta and kappa at birth is different to the adult rat but develops an adult pattern by infancy (Kar and Quirion, 1995; Rahman, 1997). There is ample evidence that endogenous opioid systems contribute to analgesic mechanisms early in the post natal period. However, difficulty in behavioural testing in small animals has lead to discrepancies in results of studies of analgesic efficacy (Marsh et al. 1997). These suggest that opioid actions differ with age but the early literature is confused.

Evidence from studies of neonatal rats suggests that endogenous opioid systems may have other important roles such as the regulation of neuronal development (Hauser et al. 1989; Stiene-Martin and Hauser, 1990). Little is known of the long term effects of opioid administration in the neonatal period and early infancy on the developing CNS in humans.

There is now overwhelming evidence that neonates and infants are capable of nociception which has prompted an increase in the use of opioids in this age group after surgery (De-Lima et al. 1996). The advent of HPLC (high performance liquid

chromatography) has allowed sensitive and specific measurement of plasma opioid concentrations on small blood samples (Joel et al. 1988) allowing detailed pharmacokinetic studies (Hartley and Levene, 1995). However, difficulties in pain assessment in pre-verbal children have hampered pharmacodynamic studies and most research has concentrated on the adequacy of sedation of ventilated neonates in the intensive care unit (Hartley and Levene, 1995).

This thesis describes both laboratory based studies of neonatal rats and a clinical study. In the laboratory, I have investigated the analgesic efficacy of highly selective mu, delta and kappa agonists following epidural or systemic administration, in an attempt to elucidate the pattern of development of opioid mediated analgesia with age.

In our clinical study, we have investigated the analgesic efficacy of morphine in post-surgical neonates and infants using three different pain assessments. Blood samples were taken simultaneously in order to determine morphine and its glucuronide metabolites and their contribution to analgesia. We have then compared these blood levels with pain scores in an attempt to determine the role of morphine and its glucuronide metabolites in the production of analgesia.

2. Aims of laboratory investigations

In vitro autoradiography using selective opioid receptor ligands has allowed a visual and quantitative assessment of the distribution and ontogeny of mu, delta and kappa receptor binding sites in the spinal cord of the neonatal rat, and has shown that it is quite different to the adult (Kar and Quirion, 1995; Rahman, 1997). Because of practical difficulties in testing in this age group of animals, behavioural tests of the analgesic efficacy of exogenous opioids have been inconclusive and the early literature is confused (Marsh et al. 1997).

In these experiments I have aimed to investigate the ontogeny of opioid analgesia, using the neonatal rat as a model. I have systematically compared the analgesic effect of agonists to mu, delta and kappa opioid receptors using two different routes of administration and three different behavioural tests. Opioids were either applied spinally to the lumbar cord or applied systemically (subcutaneously) in neonatal rat pups and behavioural testing undertaken. Spinal application involved the use of a technique of 'single shot' epidural injections. This technique was developed especially for this project for use in very small rat pups and has formed the basis of the majority if all experiments. A background of its use and the reasons for its choice will be discussed below. A more detailed description is made in the methods section.

Epidurals

Epidurals were performed as a method of delivering opioids to the spinal cord, and one in which subsequent behavioural testing would be possible.

Epidural analgesia was first described in 1921 (Greiff and Cousins, 1994) but became more popular as a therapeutic option in the 1960's, and is now firmly established in adult and paediatric practice to provide analgesia per-operatively, during childbirth and in chronic pain states.

The epidural space is a potential space which lies between the spinal dura and the spinal periosteum. Gaining access to this space involves the insertion of a needle between the spinous processes, usually in the lumbar region, which is then guided through the interspinous ligament until the increased resistance of the ligamentum flavum is met. After a further few millimetres, a 'loss of resistance' is felt as the needle enters the epidural space. Local anaesthetics and analgesics may then be injected, which act locally on neurones as they enter and leave the spinal cord, and by diffusion through the meninges to the spinal cord.

The use of percutaneous single-shot epidural injections has not been described in rats, either for experimental or therapeutic use, but I have been successful in establishing a technique for doing so. Neonatal rats are extremely small, weighing between 10 and 40g until postnatal day 21 (P21), and a method for carrying out these epidural injections in such small animals was developed especially for these experiments.

It has been argued that the mode of action of morphine in the epidural space is by absorption into the systemic circulation and then delivery by the latter to the spinal cord by this route. To address this argument, I have compared dose-response curves

for morphine given both systemically and by the epidural route. At the same dose, and after pain testing, blood samples were taken to compare plasma morphine concentrations after epidural and systemic administration.

In order to investigate the exact distribution and destination of epidurally administered opioid agonists, autoradiographic studies were performed using tritiated morphine. This allowed me to map the pattern of spread of morphine through the spinal cord after epidural injection, and also enabled a quantitative comparison with spinal cords after an identical dose given systemically.

Opioid agonists

Three opioid agonists were chosen to study the pattern of development of analgesia and possible role of each opioid receptor in neonates. Morphine was chosen as the mu receptor agonist, DPDPE as the delta and U69593 the kappa receptor agonist.

Pain models

In order to investigate the analgesic efficacy of these epidurally administered opioids, three different behavioural tests were chosen:

a) Von Frey hairs were used on the hindpaw to measure the threshold required to elicit the flexion withdrawal reflex. In young pups, these thresholds are low and reflect mechanical cutaneous sensitivity.

b) Thermal noxious stimulation to the hindpaw was tested alone and following pre-treatment with topical capsaicin to provide a model of C-fibre mediated pain.

c) Intraplantar injections of carrageenan served as a model of experimental

inflammation. The inflammation results in pain and tenderness which can be measured as a fall in von Frey hair threshold.

3. Introduction to laboratory studies

3.1 Background

Sertürner isolated morphine, the active ingredient of opium in 1806 and named it after Morpheus, the God of Dreams, but it was not until 1973 that opioid receptors were first described (Simon et al. 1973; Terenius, 1973; Pert and Snyder, 1973). This was followed shortly afterwards by the identification of endogenous opioid peptides (Hughes et al. 1975). Despite its side effects morphine is still widely used in the treatment of acute and chronic pain, and the mechanisms of action of opioids in the spinal cord are now well understood (Pasternak, 1993; Dickenson, 1994; Kanjhan, 1995).

This introduction initially summarises our current knowledge of adult pain mechanisms and opioid systems, followed by a more detailed discussion of the development of pain pathways and opioid systems.

3.2 Adult pain mechanisms and opioid systems

Opioids act by binding with high affinity membrane receptors which comprise three major groups: mu, delta and kappa receptors, and each receptor can produce different effects. Further division into subtypes remains controversial although there is now evidence of differential effects of delta receptors in mice (Jiang et al. 1991). We still have little knowledge of the specific function of each receptor, but recent isolation and cloning of all three will significantly further research into their roles (Uhl et al. 1994; Reisine and Bell, 1993). Genetic techniques are now available to determine the

contribution of each receptor to opioid function in vivo (Iversen, 1996), and a recent study shows that mice lacking the mu opioid receptor do not show any of the normal responses to morphine, suggesting that the majority of the effects of this exogenous opioid are mediated through the mu opioid receptor (Matthes et al. 1996). In the absence of the mu opioid receptor, delta and kappa receptors do not appear to mediate any of the effects of morphine (Matthes et al. 1996). Hopefully the further understanding of opioid receptors will be aimed towards the development of clinically useful opioids with highly specific functions and minimal side effects.

The endogenous ligands for the opioid receptors are the opioid peptides which can be divided into three groups, enkephalins, endorphins and dynorphins. These endogenous opioids have some agonist activity at all three receptors but each receptor possesses distinct ligand selectivity (Kosterlitz, 1985).

Opioid effects are mediated by several mechanisms in all areas of the CNS, with the predominant effect of decreasing neuronal firing. By stimulation of opioid receptors situated pre-synaptically on the terminals of neurones they reduce the release of excitatory neurotransmitters involved in pain pathways (Hori et al. 1992). Stimulation of post-synaptic receptors inhibits neurotransmission by producing hyperpolarisation therefore reducing evoked activity (Lombard and Besson, 1989). A third possible mechanism involves the inhibition of interneurones which were preventing activity in other inhibitory neurones (Dickenson, 1994).

The spinal cord is a very important site of action of opioids, where they act to

modulate pain pathways. This is evident clinically in the profound analgesia produced by the administration of opioids in the epidural and subarachnoid spaces. Afferent nociceptor Aδ and C fibres synapse in laminae 1 and 2 (the substantia gelatinosa) of the dorsal horn (Besson and Chaouch, 1987). It is here that, around C fibre terminals, the majority of opioid receptors are located, although lower levels are found in the deeper layers of the dorsal horn (Dickenson, 1994). In the rat, mu receptors predominate in the spinal cord, with delta and kappa receptors comprising less than a third of the total, with 50-70% of these opioid receptors located pre-synaptically (Besse et al. 1990).

The recent proliferation of research into adult pain mechanisms has demonstrated that pain transmission in the spinal cord is not through a simple circuit, but rather the arrival of noxious information from the periphery via primary afferent fibres initiates a cascade of events in the dorsal horn allowing enhancement and modulation of the pain signal (Woolf, 1994; Dickenson, 1995). Thermal, mechanical and chemical noxious information from the periphery is transmitted to the spinal cord via thinly myelinated Aδ fibres which synapse in laminae 1 and 5 of the dorsal horn, and unmyelinated C fibres which synapse in laminae 1 and 2 (the substantia gelatinosa). They then activate large numbers of interneurones and projection neurones before projecting information to the thalamus and cortex, leading to the sensation of pain. Descending fibres projecting from brainstem nuclei modulate the incoming information via interneuronal networks. Thus the dorsal horn plays a major role in processing incoming noxious information.

The neurotransmitters involved in spinal nociceptive pathways have been the subject of intensive research (Levine et al. 1993; Dickenson, 1995). The excitatory amino acids glutamate and aspartate and peptides such as substance P (SP), CGRP (calcitonin gene-related peptide), somatostatin (SOM) and VIP (vasoactive intestinal peptide) are all found in afferent fibres and are released following noxious stimuli (Yaksh and Malmberg, 1994). SP is one of a large family of neurotransmitters known as the neurokinins, and is found in abundance in the dorsal horn of the spinal cord. At present, three types of neurokinin receptor have been identified: neurokinin-1 (NK1), NK2 and NK3, and SP is thought to act preferentially on the NK1 receptor. This group of receptors is located in laminae 1, 2 and 10 of the dorsal horn, and they are situated postsynaptically. SP has been shown to be involved in pain transmission and is released from primary afferents, interneurones and descending fibres (Dickenson, 1995).

The effects of the excitatory amino acids such as glutamate in particular have sparked considerable recent interest. Glutamate is abundant in the spinal cord and is released from many sources including myelinated and unmyelinated primary afferent neurones, interneurones and descending fibres. Its effects are mediated by NMDA (N-methyl-D-aspartate) receptors as well as non-NMDA receptors, and the former have been shown to play an important role in persistent pain states such as hyperalgesia and allodynia. The NMDA receptor requires the repeated or sustained stimulation by C fibres for its activation, and the release of glutamate from these fibres initiates a series of intracellular events, resulting the phosphorylation of intracellular proteins and the opening of an ion channel normally blocked by magnesium. The resultant increase in

intracellular calcium allows a greatly augmented postsynaptic response to glutamate; this is essentially an elaborate positive feedback mechanism, significantly increasing and prolonging the output of dorsal horn neurones (Dickenson, 1995). Thus the activated NMDA receptor plays a major role in establishing the many forms of central hyperalgesia. Following noxious stimulation, SP is also released from primary afferent fibres, and acts on NK1 receptors. The latter are also thought to play a role in the establishment of central hypersensitivity (Dickenson, 1995).

There is now growing evidence that NMDA receptors are involved in the mechanism of opioid tolerance (Mao et al. 1995). Animal studies in adult rats have shown that the production of hyperalgesia and opioid tolerance share some of the same common neurochemical pathways, such as activation of the NMDA receptor and subsequent intracellular activation of protein kinase C and nitric oxide; both hyperalgesia and opioid tolerance are reduced by NMDA antagonists and nitric oxide synthase inhibitors (Mao et al. 1995). This has important implications for the treatment of chronic pain states; the continued use of opioids to treat chronic pain reduces their efficacy, but this reduction may be in part be due to the pain state itself, and the use of opioids may actually exacerbate the pain (Basbaum, 1995). Opioid systems do not function in isolation and we need a better understanding of the pathways and networks which link the development of opioid tolerance and chronic pain states.

CCK (cholecystokinin) is a peptide neurotransmitter found in spinal neurones, which is known to affect opioid function. CCK appears to act as a natural 'break' to

endogenous opioid action both spinally and supraspinally. Application of CCK reduces the analgesic actions of morphine, which are enhanced by CCK_B receptor antagonists. CCK may also be involved in the development of opioid tolerance (Mao et al. 1995).

Supraspinal opioid systems contribute to analgesia by several mechanisms including decreasing nociceptive input to the brain by altering descending and ascending control systems, therefore reducing the central perception of pain. Intraventricular injection of opioids in humans in systemically inactive doses provides evidence for the role of supraspinal sites in the production of analgesia (Lazorthes et al. 1988). Intracerebral microinjection of opioid agonists has allowed the localisation of supraspinal receptors and their contribution to analgesia to be investigated (Yaksh et al. 1988). In rats, opioid receptors are widely distributed throughout the CNS, most densely in the brainstem, thalamus, amygdala, hippocampus and cortex. Mu receptors are found in the majority of these areas, whereas delta receptors are more restricted, found mostly in forebrain regions with poor binding in the midbrain and brainstem. Kappa receptors are widely distributed throughout the forebrain, midbrain and brainstem, comprising only 10% of the total number of receptors in the rat brain, but up to a third in humans (Mansour and Watson 1993). The periaqueductal grey and medulla are involved in the descending inhibitory control of dorsal horn neurones. Several supraspinal sites of receptors are situated close to the origin of these descending controls, and microinjection of opioids into these areas produce analgesia by their stimulation.

Opioids also act outside the CNS and possess 'local analgesic' effects. Animal studies have demonstrated the peripheral antinociceptive actions of opioid agonists in

inflammatory pain models (Stein et al. 1989). It is thought that inflammation initiates the axonal transport of opioid receptors to the periphery and increases their number on peripheral nerve terminals; inflammation disrupts the perineurium exposing neuronal opioid receptors (Stein, 1995). Endogenous peptides are produced by immune cells infiltrating the inflamed tissue (Stein, 1993). Although clinical evidence is inconclusive, the results of studies of intra-articular morphine for postoperative knee pain are encouraging (Dalsgaard et al. 1994; Heine et al. 1994). The local action of a topically applied, systemically inactive opioid lacking central side effects will have major benefits.

3.3 Developmental neurobiology

Successful management of pain in neonates and infants demands an understanding of the development and function of pain pathways. Animal studies have allowed us some insight and have shown essentially that they are quite different from adult pain mechanisms. Relatively little is still known, despite the recent explosion of research in the field of adult pain.

The sequence of events that takes place in the neurological development of the rat and human CNS are very similar but it is difficult to make exact comparisons. Generally speaking, the newborn rat CNS is approximately equivalent to a 24 week human foetus at birth, a full term neonate at seven days and a toddler at three weeks. Rat CNS maturation is extremely accelerated compared with the human and is mature by four weeks (Fitzgerald and Anand, 1993).

In the first part of this section I will discuss the current knowledge of the development of pain pathways; this will be followed by a more detailed discussion of the role and development of opioid systems.

Development of pain pathways

Anatomical

Large myelinated afferent fibres (A fibres) from cutaneous low-threshold mechanoreceptors enter the rat lumbar spinal cord well before birth at embryonic day 15 (E15, where E0 is conception and E21.5 is birth) and are some of the first afferent fibres to occupy the dorsal horn (Fitzgerald et al. 1994; Fitzgerald, 1995; Konstantinidou et al. 1995). They are present in the human cord at 14 weeks gestation. In the neonatal rat these fibres terminate in laminae 1 to 5 of the dorsal horn, gradually withdrawing to their final adult destination of laminae 3, 4 and 5. Lamina 2 (the substantia gelatinosa), is still densely populated with these A fibre terminals at P23 and there is evidence that they make synaptic connections here (Coggeshall et al. 1996) but by P30 A-fibre terminals have withdrawn and are restricted to laminae 4-5 (Fitzgerald et al. 1994).

In contrast, there is a delay in C-fibre appearance for several days and it has been shown that they grow into the lumbar cord at E19, and have begun to penetrate lamina 1 by E19.5. By E20, the terminals have increased in density and have reached lamina 2 and at birth (E21.5) exhibit the density and distribution seen in a young rat

(Fitzgerald, 1987). In the human foetus, C fibres are also slow to grow into the cord and are still not observed until at 19 weeks gestation (Konstantinidou et al. 1995). Although the arrival of C-fibre terminals in lamina 2 does not result in the immediate vacation of this area by A-fibres, they do eventually withdraw as described above. Destruction of C fibres at birth using capsaicin alters the subsequent development and organisation of A-fibre terminals and they remain in the SG into adulthood (Shortland et al. 1990).

Functional development

Natural cutaneous sensory input in the form of firm pressure and skin pinch can evoke action potentials in the rat lumbar dorsal horn at E19 in vivo (Fitzgerald, 1991). The delay between ingrowth and functional postsynaptic activity possibly indicates the maturation time for A fibre central synapses. The excitatory responses mediated by C-fibres develop more slowly, and C-fibres are unable to evoke spike activity until the second postnatal week (Fitzgerald, 1985; Fitzgerald, 1988). Polysynaptic activity evoked by small diameter A- and C-fibres in the rat dorsal horn is very immature in the first postnatal week (Fitzgerald, 1988).

During the first postnatal week, cutaneous reflexes are enhanced in rats and humans (Fitzgerald, 1995), and non-noxious low threshold stimuli are able to elicit a flexion withdrawal reflex (Fitzgerald et al. 1988). This excitability reduces to adult levels by P20 to P30 in the rat and by 30 weeks gestation in the premature human neonate (Fitzgerald, 1997). The considerable changes in A-fibre input over this period suggest an explanation (Jennings and Fitzgerald, 1996). Another explanation may

relate to the functional development of descending pathways. In the adult spinal cord, the output of the dorsal horn is significantly influenced by descending inhibition. Although in the neonatal rat it is anatomically intact, no functional descending inhibitory control from the dorsolateral funiculus exists until P10-12, reaching adult levels only at P22-24. This is possibly owing to a delay in the development of interneurones and to low level of transmitters in the neonatal period (Fitzgerald and Koltzenburg, 1986), and presumably underlies the lack of brainstem stimulus-produced analgesia in newborn rats until P21 (van Praag and Frenk, 1991). This reflects slow development of the control of afferent input, and would contribute to the neuronal excitability discussed above.

A further interesting feature of the neonatal dorsal horn cells is that their receptive fields are much larger at birth and gradually decrease in size to reach adult levels by 2 weeks of age in the rat. These receptive fields are well demarcated, but have a tendency to overlap, possibly due to the slow development of interneuronal connections (Fitzgerald, 1985).

Developmental neuropharmacology

The NMDA receptor is functionally different in the neonatal rat but its role in the development of pain pathways is unknown. Higher levels of NMDA receptors are found in the spinal cords of neonatal mice compared with the adult animal, and this binding is homogeneous in the dorsal horn until P10-12, after which time the highest density appears in the SG (Gonzalez et al. 1993). In the neonatal rat, NMDA induced

elevations in intracellular calcium are markedly raised, gradually falling to adult levels (Hori and Kanda, 1994). C fibre induced, NMDA mediated effects in the adult such as wind-up and central sensitisation are probably more marked in the neonate, and may contribute to the establishment of C fibre connections in the cord. Activation of these fine primary afferents may contribute to the developmental alterations in NMDA response of substantia gelatinosa neurones (Hori and Kanda, 1994).

Metabotropic glutamate receptors play an important role in pain transmission in the adult (Young et al. 1994), and it is likely that they play some part in the ontogeny of pain pathways. These receptors are differentially regulated in the spinal cord during development (Catallia et al. 1994).

Substance P (SP) and somatostatin appear in the foetal spinal cord before the advent of synaptic transmission and this suggests that they also have a role in spinal cord development, although their exact role is unknown (Fitzgerald, 1997). Levels of both these neurotransmitters increase until the second postnatal week, and SP and NK1 receptors in the SG do not reach adult levels until the second week of life (Kar and Quirion, 1995).

Gamma amino butyric acid (GABA) is an inhibitory neurotransmitter in adult pain transmission (Fitzgerald, 1997) but in the neonatal dorsal horn appears to have an excitatory role. GABA expression alters during development peaking during the first two postnatal weeks and declining to adult levels by the third (Schaffner et al. 1993). Again although GABA functions as a neurotransmitter in the adult, in the developing

neonate it is likely to also function as a modulator of neuronal development (Malcangio and Bowery, 1996; Leinekugel et al. 1997).

The ontogeny of opioid systems

Opioid receptors and ligands

Opioid receptors and endogenous opioid peptides appear very early in CNS development, and their ontogeny is non-uniform in the neonatal rat. At birth, opioid receptors are present in several areas of the brain, notably the neostriatum, olfactory tubercle, rostral midbrain and later in the thalamus and hypothalamus. The majority of receptors in the adult are mu and delta, but early in the postnatal period, on postnatal day 6 (P6), mu and kappa receptors predominate (Leslie et al. 1982). Delta receptors, however, have a very different ontogeny to mu and kappa receptors and are present in low densities in the brain at birth, progressing to maximum binding in the third post-natal week. A similar pattern of ontogenesis occurs in the spinal cord with the early appearance of mu and kappa receptors and delay in the development of delta receptors until the initial post-natal period (Attali et al. 1990). An autoradiographic study of the postnatal development of opioid receptors in the lumbar spinal cord using selective ligands allowed both a visual and quantitative representation of the distribution of opioid receptors in neonatal rat pups. It was found that mu receptor binding sites are present at P0, increasing in density until P7 then gradually decreasing to P21. Kappa receptor followed mu receptor binding closely, both being diffusely spread through the cord. Delta receptors were first observed at P7, and continued to increase in density even after P21 (Wahida Rahman, personal communication).

The pattern of development of mu receptors has been elegantly demonstrated in the rat by in vitro quantitative receptor autoradiography. Mu receptor binding sites are concentrated in the superficial laminae of the dorsal horn in the adult, but in contrast at P1, both the superficial and deeper laminae have relatively high densities. The density of mu opioid binding sites peaks at P4, then declines gradually to reach adult levels by the third postnatal week (Wahida Rahman, personal communication),(Kar and Quirion, 1995). Mu receptors are widely distributed throughout the neonatal spinal cord before becoming restricted to the substantia gelatinosa in the adult (Kar and Quirion, 1995).

Endogenous opioids appear in the mouse brain before opioid receptors on E11.5 (Rius et al. 1991), and enkephalin-like immunoreactivity detected in rat brain at E18 (Pickel et al. 1982). In human foetal spinal cord, enkephalin-like immunoreactive fibres have been detected as early as 10 weeks (Charnay et al. 1994). At embryonic day 16 (E16), endorphin levels are much greater than enkephalin levels with highest levels in the phylogenetically newer areas of the brain such as the diencephalon and telencephalon, with a rich distribution in the limbic system. These two peptide systems appear to develop differently, with a marked increase in enkephalin levels during the initial perinatal period. Between P6 and P25, both endorphin and enkephalin concentrations rise to approximately adult levels (Bayon et al. 1979).

Analgesic actions

There is ample evidence that these endogenous opioid systems contribute to functioning analgesic mechanisms in the early postnatal period, although the pattern

of development of effects is difficult to establish as nociceptive physiology itself changes markedly with age, and a comparable nociceptive stimulus at different ages may be impossible to achieve. Exogenous opioids and their analgesic efficacy have been investigated in the developing rat, but difficulties in behavioural testing of neonatal animals may in part explain apparent discrepancies in the results. Preliminary data from our own laboratory have shown major difficulties with thermal testing of neonatal rats, as limb withdrawal tests seem to rely on many variables. However, despite these problems of methodology, the effect of morphine was shown to increase with age in neonatal rat pups, progressing to a 40- fold analgesic potency at P14 compared to P3 when demonstrated by a limb withdrawal test of thermal analgesia (Giordano and Barr, 1987). Similar results were obtained in a further study of morphine analgesia in developing rats, in which it was shown that morphine was without any detectable effect to a tail-flick nociceptive test until P12, reaching analgesic potency comparable to adult levels at P14 (Barr et al. 1986). Comparing morphine, a potent mu agonist and ketocyclazocine, a kappa agonist in the developing rat revealed that morphine was more potent than ketocyclazocine in producing analgesia to limb withdrawal tests of thermal nociception, exhibiting analgesia in a dose dependent manner as early as three days in doses starting at 1-2mg/kg, with peak effects at 14 days. Ketocyclazocine was more effective at producing analgesia to a mechanical stimulus (a blunt probe applied with a force of 10-15g) in younger animals, with onset of effect at seven to ten days, this differential development in analgesic patterns confirming a non-uniform ontogeny of opioid receptors (Barr et al. 1986). However, unlike thermal and mechanical tests of pain, the response of neonatal rats to the tonic nociceptive effects using the formalin test revealed a similar

response to that seen in adults at P3. In this study it was revealed that this response was attenuated by morphine in a dose of 1-2mg/kg, in a manner similar to adults (McLaughlin et al. 1990).

Early studies have warned of the susceptibility of neonates to the CNS depressant effects of opioids, but a comparison of the sedative and analgesic effects of systemic morphine and pentobarbital in infant rats ranging in age from one to 20 days showed morphine 1mg/kg produced analgesia to an intra-plantar injection of formalin at all ages, which was qualitatively different from the sedative effects of pentobarbitone 10mg/kg (Abbott and Guy, 1995). Early studies indicated that neonates were more susceptible to the convulsant effects of morphine but an investigation of the effects of high dose morphine on EEG recordings and behavioural observations showed that although all doses of morphine used produced electrographic spikes in one, three and six day old neonatal rats, no behavioural convulsions were observed suggesting morphine is less toxic than originally thought (van Praag and Frenk, 1992).

Actions of opioids on neuronal growth and plasticity

There is now ample evidence to show that opioid systems play a significant part in the regulation of neuronal development in the neonate. The role of endogenous opioids in neural plasticity has been demonstrated by examining the effects of continuous receptor blockade on the developing neurones in the cerebral cortex, hippocampus and cerebellum in 10 day old rats. Neonatal rats were given daily injections of the potent opioid agonist naltrexone from P0, and after sacrifice on P10, it was demonstrated that the lengths of oblique dendrites of pyramidal cells in the cerebral cortex and basilar

dendrites of the hippocampus were significantly increased when compared with the control group. The dendritic lengths of spiny branches of cerebellar Purkinje cells were also increased. Endogenous opioids can therefore exert a marked influence on dendritic elaboration and spine formation (Hauser et al. 1987). In a similar study the effect of exogenous opioid blockade was examined at P10 and P21. Daily injections of naltrexone were given in a dose of 50mg/kg providing complete continuous blockade, and 1mg/kg producing only intermittent opioid receptor antagonism. Continuous blockade produced large increases in dendrite and spine elaboration, at P10, but these increases were only seen in the hippocampus at P21. With intermittent blockade, dendrite and spine growth were often subnormal (Hauser et al. 1989). The action of met-enkephalin on the growth of astrocytes in vitro mixed glial cultures was studied in 1 day old mouse cerebral hemisphere. Continuous treatment with either met-enkephalin, a met-enkephalin/naloxone combination or naloxone alone revealed that met-enkephalin caused a decrease in total cell numbers compared with naloxone treated cultures and controls. These results suggest that the endogenous opioid met-enkephalin suppresses astrocyte growth by a receptor mediated mechanism (Stiene-Martin and Hauser, 1990).

These results indicate that endogenous opioids act as inhibitory growth factors in neuronal development, probably by inhibition of DNA synthesis. After a single dose of either met-enkephalin or naloxone to 11 day old rats, DNA synthesis was shown to be increased in the naloxone treated group but decreased in the met-enkephalin group (Vértes et al. 1982). Comparing the effects of morphine on DNA synthesis in neonatal rat brain in vivo and in vitro, it has been shown that morphine inhibits DNA

synthesis in vivo in animals aged 1 to 4 days but not in older animals. The in vivo effects were blocked by pre-treatment with naloxone indicating a receptor mediated mechanism. Naloxone administered acutely or naltrexone chronically had no effect. Brain tissue incubated with morphine in vitro, however, showed no difference in DNA synthesis when compared with controls, pointing to an indirect mechanism in the control of cell proliferation (Kornblum et al. 1987).

Reduced latencies to tail-flick tests have been demonstrated on day 9, when β-endorphin (in doses of 1-50μg per pup) was administered daily from P1 to P7, suggesting hyperalgesia. In these animals, the number of opiate receptors in the brain was reduced when examined on P14 (Zadina and Kastin, 1986). Studies on neonatal rat pups exposed to morphine either in utero (pregnant rats given a s/c morphine pellet on E16) or postnatally (daily injections of morphine 5mg/kg) have demonstrated that chronic morphine exposure results in a significant decrease in brain mu receptor density until day 8, gradually increasing to control levels by day 14. These changes were accompanied by a tolerance to the analgesic action of morphine, though there was no significant change in delta and kappa receptor densities (Tempel et al. 1988). An autoradiographic study of neonatal rat brain compared the neuroanatomical pattern of opioid receptor changes in controls and animals treated chronically with morphine. In those receiving morphine from P1 to P4, mu receptor density on P5 was non-existent in parts of the striatum and decreased in other areas such as the amygdala. Longer treatment with morphine from P1 until P8 produced no further alterations in mu receptor density (Tempel, 1991).

A recent study has shown that mice lacking the mu opioid receptor gene do not respond to the biological effects of morphine, as these effects are not mediated by delta or kappa receptors. These homozygous mutant mice showed no morphological abnormalities and did not differ from their littermates in health and growth (Matthes et al. 1996). These results suggest that if mu receptors play a role in neuronal development, delta and kappa receptors, and possibly other neurotransmitter systems, will compensate in the absence of mu receptors to produce a phenotypically normal animal.

Little is known of the long term effects of opioid administration in the neonatal period on the developing CNS in humans.

3.4 In summary

The results from animal experiments suggest that opioid actions differ with age in the neonate, but the literature is rather confused. Autoradiographic studies in neonatal rats using highly specific receptor ligands have shown that mu, delta and kappa receptor have different patterns of development in the lumbar spinal cord (Whaheda Rahman, personal communication) but results of behavioural studies are confused. I have therefore conducted a series of experiments to study the relative role and importance of each receptor subtype in providing analgesia during development in the neonatal rat. By administering these opioid agonists via a lumbar epidural injection, I have been able to study their effects within the spinal cord, avoiding all the problems that may arise from systemic administration. The pattern of development of these opioid receptor in other areas of the central and peripheral nervous system has yet to

be investigated.

With the recent increase in the use of opioids in human neonates (De-Lima et al. 1996; Purcell-Jones et al. 1988), there is clearly a need to increase our understanding of the development of opioid systems and the factors modulating this development.

4. Methods of laboratory studies

All experiments involved comparisons of Sprague Dawley rat pups aged P3 (postnatal day 3, mean weight 10g), P10 (mean weight 20g) and P21 (mean weight 40g). Both sexes of rat pups were studied and were obtained from the animal house at University College, London.

4.1 Injection techniques

In the majority of experiments, opioids were administered via the epidural route. Following anaesthesia with 2-4% halothane in oxygen, a 1ml syringe and 26 gauge needle and a 'loss of resistance' to the injectate technique was used to find the epidural space. Although the exact level of injection was difficult to determine because of the size of the animals, most were made in the lower lumbar or upper sacral region. Methylene blue was added to the injectate, and in the hairless animals aged P3 and P10, this allowed correct placement of the injectate to be seen as a blue line beneath the skin. The volumes observed for optimum cephalad spread were 0.05mls for P3, 0.1mls for P10 and 0.2mls for P21; these volumes were therefore used in all experiments. Recovery time from anaesthesia was for a minimum of 30 minutes in all experiments. At the end of experimentation, all animals were sacrificed, and the cords removed. The use of methylene blue as a marker allowed determination of the final location of the epidural injection, and at the end of experimentation all spinal cords were removed and examined. If methylene blue was detected by the naked eye on the surface of the exposed spinal cord, we felt this signified confirmation of epidural injection; if not, the data was excluded. The overall successful injection rate for P3

animals was 87.3%, for P10 84.8% and for P21 75.3%. Occasionally in the P3 and P10 animals, methylene blue travelled very rapidly upwards, probably in the cerebrospinal fluid (CSF), as methylene blue then became visible in the cerebral ventricles. These animals all became apnoeic almost immediately and were unresuscitatable.

To allow comparison, in several experiments morphine was administered via the systemic route consisting of a subcutaneous injection during anaesthesia, using the same volumes as for epidural injection described above.

4.2 Opioid agonists studied

An agonist to each opioid receptor was studied. Morphine sulphate (University College Hospital Pharmacy) was chosen as the mu agonist; morphine sulphate 1mg/ml was diluted in sterile normal saline to achieve the required concentrations for experimentation. DPDPE ([D-Pen^{2,5}]-Enkephalin, Peninsula Laboratories, Inc.) the delta agonist, was dissolved initially in 1% DMSO (dimethylsulfoxide), and then frozen in aliquots at -70^{o}C. The latter were subsequently diluted in normal saline. U69593 (Upjohn), a kappa agonist was buffered with trisodium citrate 150μM to pH 5.5 and then diluted with normal saline.

For epidural injections, methylene blue was added to the normal saline dilutant (in a volume of 10% of the total dilutant) to act as a marker as described above.

4.3 Studies with von Frey hairs

The cutaneous flexion withdrawal reflex was used to determine the reaction to a low-intensity mechanical stimulus to the foot, the threshold of which was elicited using von Frey hairs (vFh). Von Frey hairs are single nylon monofilaments of graded diameter (0.08-1.0 mm) attached at right angles to a perspex handle, and are made according a logarithmic scale of the weight that they apply. Each is calibrated using a balance accurate to 1mg, a value being given to the weight produced on pressing the hair onto the balance, mimicking the action used during experimentation.

Table 1 shows the weights and $\log_{10}$ values of the numbered von Frey hairs (vfh) when calibrated in our laboratory. As discussed above, the absolute values for their weights lie on an exponential scale, and since all three age groups of animal demonstrate a different baseline vFh threshold, comparison of threshold changes between age groups was extremely difficult. To overcome these difficulties in dealing with a $\log_{10}$ based scale of stimulus intensities I have allocated each consecutive vFh a simple numerical value from one to 20 and this converted the thresholds to a linear scale, which I have called the vFh number. For example: change of absolute threshold from 0.04g to 0.06g is a change of one vFh number, as is a change of threshold from 0.86g to 0.97g. In both cases the change or vFh 'index' is one.

The cutaneous flexion withdrawal reflex gives a very clear reaction to stimulus with a von Frey hair even in these young experimental animals (Fitzgerald et al. 1988); it therefore seemed an ideal method for testing the effects of opioids. A slightly different method of handling was required in each age of animal to allow testing: P3

animals were simply allowed to settle on the testing bench, P10 animals required minimal handling before testing to calm and reassure them, but P21 animals needed considerably more reassurance and comforting before they could be reliably tested. A small hair was pressed onto the dorsal surface, mid-paw of the right hindlimb toe three times at one second intervals. If this did not produce a flexion withdrawal of that limb, then the same process was repeated using the next 'size' of von Frey hair. The threshold was obtained when a hair produced a flexion withdrawal of the limb, and the weight of that hair gave the threshold value. A dose response curve was therefore obtained using von Frey hairs as a mechanical sensory stimulus for all opioids administered by the epidural route and for morphine given systemically.

During experimentation, a vFh threshold was obtained in all animals at t_0. An epidural was performed under anaesthesia and following a 30 minute period to allow recovery from anaesthesia, the vFh threshold was again measured. Two control groups were injected with epidural or subcutaneous saline.

A control group was studied initially following epidural saline, then a dose response curve was obtained for each animal using the following doses of epidural opioids:

Morphine - 1, 10 and 20μg/kg

DPDPE - 2, 5 and 10μg/kg

U69593 - 0.3, 3 and 6mg/kg

4.4 Comparison of epidural and systemic morphine

As discussed earlier, it has been argued that epidural morphine acts by absorption into the systemic circulation, and delivery to the spinal cord via this route leads to its

action, rather than a more direct route to the cord through the meninges. We have therefore compared the analgesic effect of 10μg/kg of morphine administered either epidurally or systemically, using von Frey hairs (vFh) as described above. At the end of the experiment, all animals were sacrificed and then exsanguinated. The blood samples were immediately centrifuged and the plasma frozen at -70 °C until analysis. Plasma morphine concentrations were measured using high-performance liquid chromatography (HPLC) using ultraviolet (UV) detection (Joel et al. 1988).

4.5 Studies of carrageenan-induced inflammation

An inflammatory process was induced by the intraplantar injection of carrageenan. This has been shown to be a reliable method of producing experimental inflammation in numerous studies (Di Rosa et al. 1971; Kayser and Guilbaud, 1987). Before general anaesthesia, the vFh threshold was measured in the right hindpaw. Under anaesthesia, the epidural was performed initially as described above, followed immediately by injection of 1% carrageenan subcutaneously into the dorsal surface of the right hindpaw. The volume of the latter was varied with age: 0.05mls for P3 rats, 0.1mls for P10 an 0.2mls for P21. The vFh threshold was then again measured in the right hindpaw one hour after injection (and cessation of anaesthesia), and hourly thereafter concluding with a reading at 4 hours. A control group was studied initially following a saline epidural.

The effect of a single, low dose of all three opioids given via the epidural route was studied. The doses were as follows:

Morphine - 1μg/kg

DPDPE - 5μg/kg

U69593 - 300μg/kg

4.6 Studies with capsaicin

Initially, high temperature was used to provide a selective C fibre mediated noxious stimulus. Limb withdrawal from a hot plate, and a waterbath at different temperatures were tested, but consistent results were very difficult to obtain in these small, immature animals even under control conditions, and it was felt that neither of these methods were sufficiently reliable to allow testing of the analgesic efficacy of opioids. Capsaicin cream was therefore applied to the hindpaw to act as an additional C-fibre nociceptor stimulus by activating nociceptors in these pups (McMahon et al. 1991). When capsaicin neurotoxin was applied to a hindpaw under anaesthesia, thermal nociceptor testing using a waterbath one hour later gave reliable results. This was used as the method of choice and is described below.

The effects of low-dose opioid epidurals on a capsaicin-induced noxious stimulus were studied. Under anaesthesia, an epidural was performed. Capsaicin cream 0.75% (prepared by pharmacy department, University College Hospital) was then applied to the entire surface of the right hindpaw, and rubbed into the skin for 30 seconds using a cotton bud. After a one hour recovery from anaesthesia, reaction times for withdrawal of both hindlimbs from a heated waterbath were measured. Therefore each animal acted as its own control. The temperature of the waterbath was varied following a pilot study to determine the optimum for each age of animal: 40°C for P3 and P10, and 45°C for P21. To determine withdrawal times, measured using a stopwatch, each hindpaw was dipped into the waterbath three times. An average of the three withdrawal times was then noted for each limb.

Again, a control group was studied initially following epidural saline, then a dose

response curve was obtained for each animal using the following doses of epidural opioids:

Morphine - 1, 10 and 20μg/kg

DPDPE - 2, 5 and 10μg/kg

U69593 - 0.3, 3 and 6mg/kg

4.7 In vivo autoradiography

In vivo autoradiography of [^{3}H] morphine distribution was conducted to determine the degree of spread of opioid in the spinal cord following epidural or systemic injection. This technique also allowed us to make some quantitative comparisons of spinal cord opioid after subcutaneous and epidural injection.

[^{3}H]morphine was used with specific activity of 50Ci/mmol (DuPont). Morphine sulphate and saline were then added to the [^{3}H]morphine solution to achieve a final concentration of 10μg/kg for all ages in both the systemic and epidural group.

Subcutaneous or epidural injection of the radiolabelled morphine was performed under anaesthesia in exactly the same way as for all other experiments. After a recovery of 30 minutes the animals were sacrificed and the spinal cords removed. The cords were then embedded in O.C.T. compound and frozen in liquid nitrogen before being divided into four segments, from cervical to lumbar cord. Longitudinal sections of 50μm thickness were then cut on a cryostat at -25^{o}C and thaw-mounted on gelatinised microscope slides. In the systemic group, only a 'sample' area (the lumbar cord) was cut into sections and mounted; I assumed that spread throughout the cord would be uniform in this group, and wished simply to compare the uptake of

[^{3}H]morphine in the lumbar cord in the systemic and epidural groups.

The slide mounted tissue was apposed to [^{3}H] hyper-film (Amersham) in x-ray cassettes and stored at 4°C for 2 to 4 weeks. After exposure, hyper-film was developed in undiluted D19 (Kodak) for 5 minutes at room temperature, rinsed with tap water and fixed in Hypam (Ilford), diluted 1:4 with tap water for 5 minutes at room temperature. The film was then washed for at least 20 minutes in running tap water and dried.

The autoradiographs were analysed using Seescan software that measures optical density. The system was calibrated from films exposed to known concentrations of tritium, and the optical density of individual autoradiographs of spinal cord sections were then measured to give the concentrations of [^{3}H] morphine in dorsal and ventral horn sections.

4.8 Analysis of results

The effect of age on analgesic response to vFh stimulus was compared at each dose and to controls, for each agonist, using one-way ANOVA (analysis of variance). Plasma morphine concentrations were compared after systemic and epidural morphine using ANOVA.

Carrageenan results are expressed as the mean fall in vFh index with time, and the effect of age on this fall analysed using one-way ANOVA. Capsaicin data is expressed as percentages of control values.

5. Results

In all experiments, four animals of each age were studied for each dose of opioid agonist used.

5.1 Effect of opioid agonists on mechanical sensory thresholds

Increase in baseline vFh threshold

Figure 1 shows the baseline cutaneous hindpaw vFh thresholds required to elicit the flexion withdrawal reflex of the hindlimb in each age group. It demonstrates the basic observation that this vFh threshold is lower in P3 animals, and increases with age.

Epidural morphine

Figure 2 shows the effect on vFh threshold of an increasing dose of epidural morphine with age. Results are expressed as the changes in vFh threshold i.e. the vFh index. P3 animals demonstrate a much greater analgesic response to morphine at all doses, with maximum effect at 5μg/kg. ANOVA comparing all age groups at each dose revealed a statistically significant difference between P3 animals and the two older age groups at all doses, although there was no difference between P10 and P21.

Systemic morphine

Figure 3 shows the analgesic effect of increasing doses of systemic morphine is shown in figure 3. Again, there is a decrease in efficacy of morphine with age, with the greatest effect in P3 animals at all doses. One-way ANOVA revealed a statistically significant difference in effect of morphine between P3 animals and P10 and P21, at doses above 0.5mg/kg.

Epidural DPDPE

Figure 4 shows the dose response curve for epidural DPDPE in the three age groups. It appears that there is a slightly increased effect of DPDPE in the P3 animals at all doses. DPDPE is not an effective analgesic at these doses in P10 and P21 animals. One-way ANOVA was performed on results at all doses between each age group, and statistically significant results ($p<0.05$) are illustrated.

Epidural U69593

Figure 5 shows the dose response curve for epidural U69593. It demonstrates the reduced effect of this kappa agonist at P21 compared with P3 and P10 at doses above 2mg/kg. ANOVA was performed on all data, and again statistically significant results are demonstrated in the figure.

Comparison of effects of morphine ,DPDPE and U69593 within each age group

In order that the effects of the three different agonists can be compared within each age group, these results have been presented for each age in figure 6. Figure 6A shows the dose response curves for all three agonists in P3 animals. At this age, morphine has the greatest effect at the doses studied compared with DPDPE and U69593. Figure 6B shows similar results at P10, although morphine and U69593 both appear to have a greater effect than DPDPE, which is not an effective analgesic in these doses at this age Figure 6C illustrates a similar effect of all three agonists at P21, with morphine and U69593 being more potent than DPDPE which is ineffective.

Comparison of ED_{50} for each drug

The potency of a drug may be expressed in terms of the median value of the individual doses, known as the ED_{50} (the dose required to produce an effect in 50% of subjects tested) (Rang et al. 1995). An approximate ED_{50} (half the dose required to

produce maximum analgesia) was calculated for all three agonists in each age group, and these are illustrated in table1. These results demonstrate several points. Firstly, there is a much lower ED_{50} following epidural morphine compared with systemic morphine, demonstrating the efficiency of local application. Secondly, there is a difference in ED_{50} between all agonists, which increases with age. This was especially marked following epidural U69593, which increases over three fold. The increase in ED_{50} of morphine and DPDPE was similar at 1.5 to 2 fold. The increase in ED50 for DPDPE was complete by P10, but the others increased to P21.

5.2 Effects of opioid agonists on carrageenan induced inflammation

Controls (saline epidurals)

Following a saline epidural under anaesthesia, 1% carrageenan was injected subcutaneously into the right hindpaw, and following a 30 minute recovery period, the vFh threshold measured hourly for five hours.

Von Frey hair threshold increases with age between P3, P10 and P21 animals as discussed earlier and illustrated in Figure 1. Table 3 illustrates this showing the mean absolute vFh threshold at each time point. Because of this, all graphical results are expressed as the mean change in vFh threshold (i.e. index) from the initial, pre-carrageenan value within each age group. Figure 7 compares the effect of carrageenan-induced inflammation at each age and shows a parallel fall in von Frey hair index in P3 and P10 animals, with maximum effect at 4 hours. P21 animals appear to have a lower fall in vFh threshold. However, at each age there is still a clear drop in threshold in the five hours following carrageenan injection. This represents a hypersensitivity to mechanical stimulation or 'allodynia'. One-way ANOVA revealed

a statistically significant difference in the effect of carrageenan at 3 and 4 hours between P21 animals and the two other ages studied.

Epidural morphine and carrageenan

Figure 8 illustrates the effects of low dose epidural morphine (1μg/kg) on the carrageenan induced 'allodynia'. Morphine reverses the allodynia created by carrageenan leading to reduced sensitivity and increased thresholds. There is a much greater effect of morphine in the P3 animals, but little if any effect in the P10 and P21 animals using the same dose. One-way ANOVA shows a statistically significant difference between P3 animals and P10 and P21 at several time points.

Epidural DPDPE and carrageenan

Results following low dose epidural DPDPE (5μg/kg) are shown in figure 9. Again the carrageenan induced allodynia is reversed. There is less difference in the effect of DPDPE with age compared with morphine, although DPDPE is more effective at P3 and P10 than at P21. ANOVA revealed a statistically significant difference between effects at P21 and other ages at 1 and 3 hours.

Epidural U69593 and carrageenan

Figure 10 illustrates the changes in carrageenan induced allodynia following low-dose epidural U69593 (300μg/kg). This kappa agonist produces its greatest analgesic effect at P3, where it reverses carrageenan induced allodynia, but is relatively ineffective at P10 and P21. ANOVA revealed a statistically significant difference in effect in P3 animals at all time points.

Comparison of the effects of morphine, DPDPE and U69593 on carrageenan induced allodynia at each age group

Figure 11A compares the effects of each agonist at P3. It shows that all three agonists

reverse carrageenan induced allodynia, with morphine and U69593 having a greater effect than the delta agonist DPDPE.

Figure 11B compares the effects at P10, and shows that the allodynia is reversed most effectively by morphine and DPDPE. At this age, the kappa agonist U69593 is antanalgesic to this inflammatory stimulus.

At P21, again all three agonists are effective analgesics (except U69593 at one hour), and this is demonstrated in figure 11C. Morphine is the most potent, followed by DPDPE, with U69593 being the least efficacious.

5.3 Effects of opioid agonists on heat and capsaicin

For this set of experiments, I wished to look at the change threshold to a nociceptive, C fibre stimulus with age, and the effect of opioid agonists on this noxious stimulus. I initially undertook this at the very start of all my investigations, using simply a waterbath as a heat stimulus, and measuring the latency to hindpaw withdrawal. I found this experiment difficult to perform, which was reflected in the extremely variable nature of the results. After finishing the studies with von Frey hairs and with carrageenan, I returned to complete these studies and decided to use capsaicin cream as a stimulus. To investigate the possible sensitizing effects of capsaicin on nociceptive thresholds, I compared the noxious heat threshold following capsaicin, with the heat threshold in the contralateral untreated hindlimb. This involved returning to heat testing with a waterbath and at this stage it gave much clearer results with less variability, and I could only conclude that I had now gained much more experience in handling neonatal animals.

Capsaicin was applied under anaesthesia, and after a thirty minute recovery period, analgesia was tested by immersion of the hindpaw in a heated waterbath. Following an initial pilot study, it was found that the optimum temperature of the waterbath was 40°C for P3 and P10, and 45°C for P21 animals. The latency to withdrawal was tested both in the treated and in the untreated hindlimb of the same animal; each animal therefore acted as its own control. Each of these results are compared in all graphs.

Effect of capsaicin on latency to hindlimb withdrawal (controls)

Figure 12 compares the latency to hindlimb withdrawal from a heated waterbath in capsaicin treated hindlimbs and untreated hindlimbs of the same animal at different ages. Firstly it shows that nociceptive heat thresholds increase with age as latency increases, and secondly that capsaicin reduces this latency at all ages, although this value only reached statistical significance at P3 and P10. In other words, capsaicin sensitized the response to noxious heat i.e. it resulted in an induced hyperalgesia.

P3 animals

The effect of the three epidural opioid agonists is demonstrated in figure 13A. It can be seen that all three agonists increase the latency to heat withdrawal in a dose-dependant manner, with U69593 possibly having the greatest effect. Furthermore, they all reduce the difference between the latencies in the untreated and the capsaicin-treated limbs, and therefore reverse the 'hyperalgesia' produced by capsaicin.

P10 animals

The results for P10 animals are shown in figure 13B. Again all the epidural opioids provide analgesia to heating, particularly U69593. At this age, however, they were less effective, compared with P3, at reducing the difference in latencies between capsaicin treated and untreated limbs. In other words, the hyperalgesic response was

less effective.

P21 animals

Figure 13C shows the results for P21 animals. Epidural morphine appears to have the greatest analgesic efficacy on the heat response of all three opioid agonists, with DPDPE showing the least. Again, all three agonists have less effect in the capsaicin-treated limb, so that capsaicin induced hyperalgesia is virtually unaffected.

5.4 Autoradiography

Four animals of each age were studied in the epidural group and two of each in the systemic group. The calibration curve for the radiolabelled ligand is shown in figure 14 (see methods).

Epidural group

Figures 15 and 16 schematically represent the density of [^{3}H]morphine binding in the lumbar spinal cords of P3, P10 and P21 animals following epidural injection. The approximate site of morphine administration (10μg/kg) at L4-L5 is marked with a star. They demonstrate quite clearly that there is no systematic change in [^{3}H]morphine activity with age. Levels at P3 were similar compared with P10 and P21.

Systemic group

After two weeks exposure, there was no detectable activity in the spinal cords of animals of all ages following systemic administration of 10μg/kg of [^{3}H]morphine. Exposure times of six weeks revealed barely detectable activity, which was the same in all ages.

6. Discussion of laboratory investigations

6.1 Injection technique and autoradiographic studies

In clinical practice, opioids may be applied to the spinal cord using both the epidural and subarachnoid injection routes. Both these methods can provide excellent analgesia using a fraction of the systemic dose, whilst reducing potential side effects such as nausea, vomiting and sedation (Greiff and Cousins, 1994; Abuzaid et al. 1993). In these experiments, the 'loss of resistance' method was used to identify the epidural space. A 26 gauge needle was employed for the procedure in neonatal rats, and combined with a probable low cerebrospinal fluid (CSF) pressure did not allow the detection of an inadvertent dural puncture, which would lead to injection of opioid directly into the CSF. In a proportion of the P3 animals, this obviously occurred; the blue injectate could be seen through the skin travelling up the spinal canal in the CSF and staining the cerebral ventricles. Even at low dose, all such animals became immediately apnoeic, and the data were excluded. Although the appearance of the marker was not visible through the skin in the older animals, occasionally an animal would become apnoeic immediately after injection, and was assumed to have had a subarachnoid injection.

The use of methylene blue as a marker gave information about the anatomical location of the injectate macroscopically; it gave no confirmation that opioid was delivered to the spinal cord, and the degree of spread within the cord. The autoradiographic studies confirmed this injection technique. In all ages of animal, tritiated morphine was detected with maximal activity in the lumbar and upper sacral regions, becoming less

abundant in the thoracic and lower sacral regions of the cord.

One theory of the mechanism of action of opioids administered epidurally is that they are first absorbed into the systemic circulation, and are then delivered to the spinal cord and brain. For morphine, the autoradiographic studies refute this theory. Following an identical dose of [H^3]morphine, no activity was seen in the spinal cords of the animals in the systemic group after two and six weeks exposure, whereas the spinal cords of animals in the epidural group showed very high activity after only two weeks exposure, indicating a high absorption of morphine into the spinal cord after epidural injection.

6.2 General properties of reflexes

Von Frey hairs

Noxious cutaneous input in the adult sufficient to cause pain can evoke a motor response which is called the nociceptor flexor reflex. This noxious information is carried to the dorsal horn of the spinal cord by small diameter Aδ and C fibres, leads to activation of flexor motor neurones in the ventral horn, and results in a withdrawal reflex. Aδ fibres terminate in laminae 1 and 5, and C fibres in laminae 1 and 2 of the dorsal horn, both of which are remote from the ventral horn. No direct link exists between the input and output limbs of this reflex arc; it is likely that interneurones serve this function, and certainly neurones are present in the substantia gelatinosa with ventrally directed axons. The flexor reflex in the adult rat can be evoked by noxious stimuli such as heat, cold and high threshold mechanical stimuli, but not by innocuous

input. Although electrical stimulation has shown that a component of the adult reflex has low-threshold Aβ fibre input, this does not appear to have a physiological function, as the reflex is not evoked by low intensity natural stimulation in the adult (Woolf and Swett, 1984).

In both neonatal humans, rats and also in cats, this reflex can be elicited by a low intensity, non-noxious mechanical stimuli (Quiding et al. 1986), for example using von Frey hairs. The force of vFh required to elicit this reflex increases with age (Andrews and Fitzgerald, 1994; Fitzgerald et al. 1988; Fitzgerald et al. 1988) and this is confirmed by the differences in initial vFh threshold seen in these experiments. Lowered thresholds are unlikely to be due to peripheral sensory receptors whose thresholds in rats do not change postnatally (Fitzgerald et al. 1987), but may reflect the increased excitability of dorsal horn neurones during the first three postnatal weeks as a result of lack of descending and local inhibition (Fitzgerald and Koltzenburg, 1986).

In the adult this reflex is mediated by Aδ and C but not Aβ fibres. In neonates, Aβ fibre stimulation may play a significant role, which may in part explain the low threshold required to elicit the reflex. C-fos is an 'immediate early gene' which has been used extensively to study spinal nociceptive pathways in the adult, and is produced in the adult spinal cord by high threshold Aδ and C fibre afferent stimulation, but not by Aβ fibre afferents. In the neonatal rat, C-fos induction in the dorsal horn can be produced by low-threshold, short latency stimulation evoking Aβ action potentials (Jennings and Fitzgerald, 1996). This production is age dependant

and is marked at P3, decreasing at P10 and absent by P21. These large diameter A fibres terminate in the deeper laminae of the dorsal horn in the adult rat, but in the neonate they make synaptic connections much more superficially in laminae 1 and 2 (Coggeshall et al. 1996; Fitzgerald et al. 1994). This suggests that Aβ fibres are able to activate the same neuronal pathways as Aδ and C fibres in the early neonatal period, and this difference in afferent connectivity may contribute to the lowered threshold required to elicit the flexion withdrawal reflex at this age.

Heat

The latency to hindlimb withdrawal from a heated waterbath was shortest at P3, increased to a maximum at P21, with a higher temperature needed to elicit this reflex in at this age. These results are consistent with a previous study of the changes in thermal nociceptive responses seen with development in neonatal rats, in which the authors found that neonatal rats (five to ten days old) withdrew their tails more rapidly to a conductive heat source (at moderate temperatures i.e. 40-45°C) than older animals. They concluded that these differences could be due to a number of factors: developmental changes in skin thickness or texture, differences in the amount of subcutaneous fat, relatively deeper positioning of thermal nociceptors in the older animals which themselves may be different, or developmental changes in afferent fibre function and their central connections (Conway et al. 1997). In the adult rat, most noxious heat information is transmitted by unmyelinated fibres, but a small proportion is probably A fibre mediated (Lynn, 1975). Presumably since C fibre central connections are immature in the neonatal rat, heat responses are Aδ fibre mediated. The lower threshold of the heat response is most probably due to the

increased level of excitability of the neonatal spinal cord discussed above.

Capsaicin and heat

Capsaicin is the active ingredient of chilli peppers. Its initial effect is of irritation and algesia produced by the selective activation of C fibres. Prolonged or repeated applications in animals and man leads to desensitisation by blocking C fibre, and eventually to cell death (Winter et al. 1995). Capsaicin has therefore been used extensively as a research tool (Fitzgerald, 1983) and has provided much information on the role and function of C fibres, but is also thought to have an effect on thinly myelinated Aδ primary sensory neurons (Winter et al. 1995; Lynn, 1990). Systemic capsaicin treatment in neonatal rats results in complete loss of unmyelinated and a smaller loss of myelinated fibres from dorsal roots (Nagy et al. 1983). Because of its selectivity as a neurotoxic agent, it has also been used clinically to treat chronic pain states such as postherpetic neuralgia (Lynn, 1990; Winter et al. 1995).

It is thought that capsaicin stimulates a membrane 'receptor' which results in an influx of sodium and calcium ions, and an efflux of potassium. Depolarisation and excitation then occurs, causing pain. Functional desensitisation can inhibit the effects of a wide range of noxious stimuli and this is reversible; the mechanism of this sensitisation may rely on the calcium dependant phosphorylation of intracellular proteins such as enzymes and ion channels. Irreversible neurotoxicity occurs at high doses as a result of activation of calcium dependant proteases, and of osmotic lysis (Winter et al. 1995). During my experiments, capsaicin was applied topically and therefore is acting on C fibre terminals in the skin.

In the skin, polymodal nociceptors are sensitised by capsaicin, and peptide release from peripheral nerves leads to neurogenic inflammation and peripheral sensitisation (Winter et al. 1995). Centrally, activation of C fibres leads to the release of neurotransmitters such as substance P (SP) and glutamate, and results in a longer term increase in central excitability. A study of isolated rat spinal cords has shown that application of capsaicin results in the calcium-dependant release of SP. It is still unclear whether this substance P release is solely from small diameter fibres in the dorsal horn of the spinal cord, or if capsaicin may cause its release from central SP containing neurones (Theriault et al. 1979).

On rat, rabbit and primate skin, local application of capsaicin selectively excites C polymodal nociceptors. Following this initial excitation, some polymodal nociceptors enhance their sensitivity to heat (Lynn et al. 1992). I have shown that in all age groups studied, topical application of capsaicin cream clearly sensitises the skin to heat responses. This is interesting, as no mustard oil reflexes can be evoked until P10, which are purely C fibre mediated (Fitzgerald and Gibson, 1984). As the relatively uncommon Aδ mechano-heat nociceptors in rat skin are excited by capsaicin (Purcell-Jones et al. 1988), it suggests that the effects of capsaicin are primarily Aδ fibre mediated in the early neonatal period. It is also possible that although direct C fibre responses cannot be evoked in the early neonatal period, stimulation of C fibres by capsaicin may produce long lasting subthreshold depolarisation in the dorsal horn leading to sensitisation to subsequent noxious stimuli such as heat (Fitzgerald, 1995).

Carrageenan

The polysaccharide carrageenan was first described by Winter et al in 1962. Following intra-plantar injection, a sequence of inflammatory mediators are released which can be divided into three phases: an initial phase (at 0 to 90 minutes) of histamine release, an intermediate phase of kinin release and third phase after two hours when prostaglandins are produced. The complement system appears to act as a trigger for the acute inflammatory process (Di Rosa et al. 1971; Neil et al. 1987).

The inflammatory process stimulates peripheral nerve fibres such as Aδ and C fibres; it also results in increases in local blood flow and vascular permeability, and changes in release of growth and trophic factors (Dray, 1995). The excitation of peripheral nerve endings is primarily mediated by activation of membrane receptors coupled to regulatory intermediate proteins and secondary messengers which alter ion channels and membrane excitability (Dray, 1995). During the inflammatory process, the neurokinins substance P and neurokinin A are released from sensory nerve endings and exert efferent effects on target tissues (Dray, 1995). This neurogenic inflammation contributes to the inflammatory process and to the resultant peripheral and central hyperalgesia. In rats, there is evidence that the inflammatory process is immature at birth but undergoes considerable development during the first two postnatal weeks (Fitzgerald, 1995). Using the chemically noxious stimulus mustard oil (a C fibre irritant), it has been shown that C fibres are unable to produce the neurogenic oedema seen in adult rats until P11 (Fitzgerald and Gibson, 1984). Part of the reason may be due to the low levels of substance P and other neuropeptides, but inflammatory mediators injected peripherally are unable to produce oedema until P3,

suggesting receptor immaturity (Gonzalez et al. 1993).

Mechanical hypersensitivity is very prominent in inflammation; one reason is that it is elicited by low-threshold Aβ fibres which under most circumstances mediate innocuous stimuli (Woolf, 1995). During inflammation, the excitability of the spinal cord becomes enhanced by C fibre stimulation, and therefore even non-noxious information from the periphery becomes amplified. It has been shown that Aβ fibres can also increase the excitability of dorsal neurones in inflammation, as a result of a phenotypic switch in these neurones, which become able to express SP (Neumann et al. 1996). However, the resultant mechanical hypersensitivity should be termed allodynia, as it is evoked by a non-noxious stimulus.

In the spinal cord, glutamate release from C fibre terminals following repeated or sustained stimulation activates NMDA receptors initiating a series of intracellular events, resulting in the phosphorylation of intracellular proteins and the opening of an ion channel normally blocked by magnesium. The resultant increase in intracellular calcium allows a greatly augmented response of the receptor to glutamate; this is essentially an elaborate positive feedback mechanism, significantly increasing and prolonging the output of dorsal horn neurones (Dickenson, 1995). Thus the activated NMDA receptor plays a major role in establishing central excitation following an inflammatory stimulus.

These processes undergo considerable development postnatally. In the neonatal rat, NMDA induced elevations in intracellular calcium are markedly raised, gradually

falling to adult levels (Hori and Kanda, 1994). NMDA mediated effects in the adult such as wind-up and central sensitisation may be more marked in the neonate, and may contribute to the establishment of afferent connections in the cord, but we do not have any information on this at present.

I have compared the effects of carrageenan between age groups; all ages of animal appear to develop a clear inflammatory response within two hours, manifested by redness and oedema. All animals developed 'allodynia' sensitivity to a low-threshold non-noxious mechanical stimuli of von Frey hairs. The maximal effect of this inflammatory stimulus was between four and five hours, and lacks, as reported elsewhere, the biphasic response which appears in response to formalin at P15 in the neonatal rat (Guy and Abbott, 1992).

A significant difference in effect is observed with age. At all time points it appears that there is a greater effect at P21, this difference reaching statistical significance at two time points. It is likely that age differences in the inflammatory process and development of neurogenic oedema partly explain these results, although alterations or differences in the central excitability generated by a prolonged inflammatory stimulus probably contributes. Although the neonatal spinal cord is already excitable in the early neonatal period and A fibre stimulation is able to evoke withdrawal reflexes, an inflammatory stimulus is still able to produce 'tenderness' or allodynia. This agrees with human findings (Fitzgerald et al. 1989).

6.3 Effects of opioid agonists on sensory thresholds

The increase in baseline von Frey hair threshold

Morphine

Morphine appears to increase the vFh threshold required to elicit the flexion withdrawal reflex in all ages of animal, with greatest effect at P3. This effect is unlikely to be analgesia; I have already discussed whether this reflex itself is nociceptive. However, morphine has been shown in behavioural studies of neonatal rats to have an analgesic effect which is qualitatively and quantitatively different to that of a sedative (Abbott and Guy, 1995), therefore it is unlikely that sedation can explain the observed alterations. It is possible that mu receptors may be located on A fibres in the neonate, or that their distribution is more widespread.

High doses of morphine abolish C fibre evoked reflex discharges in flexor motorneurones in the adult decerebrate-spinal rat (Woolf and Wall, 1986). There are several components to the flexion reflex: a fast or short latency component, a slow component (which is probably NMDA receptor mediated) and a very long lasting component (probably peptide mediated) (Sivilotti et al. 1995). Electrophysiological studies of spinal cord preparations in neonatal rats (aged P8 to P10) have shown that even low concentrations of morphine depress C fibre evoked ventral root potentials (VRPs) in spinal cord neurones with maximal effect on the slowest component of this reflex. Morphine even at high doses had no effect on the response to Aβ fibre strength stimuli at this age (Sivilotti et al. 1995). This was confirmed in a study of

isolated spinal cord preparations of neonatal rats (P8 to P15) in which flexion reflexes were initiated by stimulation of myelinated and unmyelinated afferents. Reflex discharges secondary to myelinated fibre stimulation were exaggerated compared with those in adults. At P11, morphine had a depressant effect on the late, C fibre component of the flexion reflex, with the A fibre short latency component relatively unaffected (Hori and Watanabe, 1987). The above studies have reported effects in neonates aged P8 and above; it is possible that at P3, morphine may have an effect on the A fibre component of this reflex.

My results show in all tests that morphine is more effective at a younger age. This is in contrast to a behavioural study which showed that the analgesic effect of morphine to a thermal noxious stimulus increased with age in neonatal rats aged P3 to P14 (Giordano and Barr, 1987); a further study showed no detectable analgesic effect of morphine to a tail-flick test until P12 (Barr et al. 1986). However, it is in agreement with my study of the analgesic efficacy of morphine in postsurgical infants, in which I have found that this age group appear to have low morphine requirements in the immediate postoperative period.

DPDPE

DPDPE, or [2-D-Penicillamine, 5-D-Penicillamine], is an enkephalin analogue with a cyclical structure, developed to aid determination of the physiological role of delta agonists. It is a peptide, therefore highly lipid soluble and shows marked delta receptor selectivity (Mosberg et al. 1983). Activation of the delta receptor results in cellular effects mediated by G proteins, as described above. DPDPE has been shown

to modulate nociceptive transmission in the spinal cord of the adult rat by inhibition of C fibre evoked neuronal activity, with little effect on A fibre function (Dickenson et al. 1987).

Our results show that at high dose, DPDPE increases the von Frey hair threshold needed to elicit the flexion withdrawal reflex in all ages, but only to a small degree compared with controls. Its effect is minimal at P10 and P21, but greater at all doses at P3. In comparison with other agonists studied, DPDPE is less potent at P3, but has a similar depressant effect on these reflexes at P10 and P21.

The autoradiographic study discussed (Waheda Rahman, personal communication) has shown that there is no specific binding of [H^3]DPDPE at P3, which is surprising in view of our findings of a delta receptor mediated depression of the flexion withdrawal reflex occurs at P3. There are many possible explanations for this discrepancy; delta receptors may contain a different amino acid sequence in the early neonatal period, altering the ligand-receptor interaction. These differences may still result in the production of analgesia, but not allow the detection of [H^3]DPDPE binding in autoradiographic studies. Again, the exact location of these receptors has not been determined in the neonatal animal; delta receptors may be distributed widely and more peripherally.

U69593

U69593 is a highly selective agonist and exerts its effects by interaction with kappa opioid receptors (Lahti et al. 1985). At high doses in the adult rat, it has been shown

to inhibit C fibre and to a lesser extent Aβ responses to innocuous and noxious peripheral stimuli (Sullivan and Dickenson, 1991).

U69593 given by the epidural route appears less effective in reducing the flexion withdrawal reflex at P21 than at P3 or P10. This pattern of development of kappa mediated effect might be expected from autoradiographic studies, with the relative proportion of these receptors at P3 being 28%, at P7 26%, falling to 18% at P21 (Waheda Rahman, personal communication). These results also demonstrate that this highly selective kappa agonist has the ability to reduce cutaneous reflexes in the early neonatal period when applied to the spinal cord, but its efficacy does decline with development.

The blood brain barrier

It could be argued that differences in maturity and function of the blood-brain barrier (BBB) could explain the increased analgesic effect of morphine in P3 animals after epidural injection. The BBB acts to control the solute content of the cerebral ECF (extracellular fluid) by preventing the movement of solutes from the systemic circulation to the cerebral ECF. The barrier is maintained by the presence of tight intercellular junctions between capillary endothelial cells. In 1963, Kupferburg and Way studied the effect of morphine in neonatal and adult rats and concluded that the increased sensitivity of neonates to morphine was due to the greater permeability of the BBB (Kupferberg and Way, 1963). However, since then, considerable work has been conducted on this subject, and has shown that the BBB is intact both histologically and physiologically at birth in the neonatal rat (Butt et al. 1990). After

subcutaneous administration of morphine-6-glucuronide (M6G), a potent analgesic, to neonatal ginea pigs, the proportion of M6G 'transferred' into the brain did not alter with either dose or age, suggesting a functionally intact BBB (Murphey and Olsen, 1994).

This is supported by our study using ^{3}H morphine. Although quantitative analysis demonstrated a wide range in the amount of tritiated morphine binding in the spinal cord within each age group, there was no difference between ages. This would lead to a conclusion that a developmental alteration in BBB function is unlikely to explain the differences in the effects of opioids seen with age seen in the majority of experiments. Also, all three agonists have very different molecular structures and yet all three are more potent at P3.

Opioids acting systemically

It is thought that opioids administered epidurally act via the systemic circulation. Improved delivery may then explain the increased analgesic effects of opioids at P3. However again, our autoradiographic studies refute this theory. Following an identical dose of [H^{3}]morphine given subcutaneously or epidurally, no activity was seen one hour after injection in the spinal cords of the animals in the systemic group after two and six weeks exposure to x ray film, whereas the spinal cords of animals in the epidural group (one hour after injection) showed very high activity after only two weeks exposure, indicating a direct effect on the cord.

Widespread receptors

Autoradiographic studies have shown a higher proportion of mu receptor binding in the early neonatal period which offers explanation for the greater effectiveness of morphine at P3 (Waheda Rahman, personal communication). Quantitative studies have shown that although mu receptor binding sites are concentrated in the superficial laminae of the dorsal horn in the adult, at P1 the deeper layers share this relatively high density, with a marked change in distribution occurring with development (Kar and Quirion, 1995). In the adult, mu receptors are situated both pre-synaptically (primarily on C fibres) and post-synaptically (on nociceptive specific interneurones) (Lombard and Besson, 1989). We have no information on the location of these receptors in the neonatal animal; it is possible that, post-synaptic effects play a much greater role in the analgesic action of morphine. Further investigation is required to explain this age related difference.

Receptor pharmacology

Morphine is a naturally occurring alkaloid derived from the unripe seed pod of the opium poppy (Boerner et al. 1975), and exerts its effects by interacting with high affinity mu opioid receptors which mediate its effects in the CNS (Reisine and Bell, 1993). Mu, delta and kappa opioid receptors are members of the superfamily of G-protein (guanine nucleotide-binding protein)-coupled receptors. Each contains seven sequences of peptides which take the form of membrane-spanning α helices. Above and below the membrane are peptide loops, the second and third cytoplasmic loops providing the G-protein coupling site (Pleuvry, 1997). Cloning of mu, delta and kappa receptors has shown that they share a high degree of their amino acid

sequences, with approximately half of the residues being identical (Reisine and Bell, 1993).

All three opioid receptors are linked to G proteins, but differ in the subsequent cellular effects. Mu and delta receptors (and probably kappa) both inhibit adenyl cyclase by activation of Gi (inhibitory) proteins which reduces the production of cyclic AMP (cAMP). This then leads to inhibition of calcium entry through voltage-sensitive channels and stimulates potassium efflux from the cell, resulting in hyperpolarisation and modulation of neurotransmission (Greiff and Cousins, 1994). Ion channels may also be affected directly.

The structure of these receptors (i.e. the amino acid sequence) is unknown in neonates and may differ; this would alter ligand binding and subsequent cellular effects. Other receptor systems such as the NMDA are functionally different in neonates, and indeed their affinity for NMDA decreases with postnatal age (Fitzgerald, 1997). In the substantia gelatinosa of the neonatal rat, NMDA-induced elevations in intracellular calcium are markedly increased, gradually falling to adult levels (Hori and Kanda, 1994).

Effects of opioid agonists on heat and capsaicin

The withdrawal of a hindlimb from a heated waterbath involves a purely nociceptive reflex; the addition of capsaicin introduces a preceding stimulus and it is likely that

this establishes a degree of central sensitisation, as discussed above. Therefore these experiments not only test the effect of opioids on this simple nociceptive reflex, but also on their ability to block the sensitising or hyperalgesic effect of capsaicin.

The most striking observation when comparing the effects of mu delta and kappa agonists at P3, is that there is little difference in the analgesia afforded by these agonists to a heat stimulus alone or heat with the addition of capsaicin. It appears that the effect of capsaicin is negated by even the smallest dose of each agonist. The suggestion is that whilst capsaicin can provide a noxious stimulus at P3 as seen in the control animals, its effect appears to be more readily blocked at this age, and may reflect a difference in the degree of central sensitisation. It is interesting to note also that whilst DPDPE produces minimal depression of reflexes compared with the mu and kappa agonists, it does seem to have the ability to block the effect of capsaicin. The kappa agonist U69593 appears to be the most potent opioid in these experiments; this may suggest, for example, that this receptor subtype is exclusively located on C fibre terminals in the dorsal horn at this age, with little postsynaptic effect.

At P10, mu, delta and kappa agonists all appear less able to block the central sensitizing effects of capsaicin. Again DPDPE has little effect but does provide some analgesia to heat stimulus alone. It surprising that again the kappa agonist U69593 appears to be the most potent at this age group.

At P21, capsaicin decreases the latency to hindlimb withdrawal from a heated waterbath as at P10 and P3 in control animals. Using opioid agonists as analgesics to

these noxious stimuli, it appears that the algesic effect of capsaicin is much more difficult to block at this age and may reflect the full development of C fibre function, possibly a greater degree of central sensitisation, or alternatively a greater sensitivity of the system in younger animals. Morphine and DPDPE seem to have very little effect on capsaicin at the doses studied; U69593 at highest doses again seemed the most potent.

Effects of opioid agonists on carrageenan induced inflammation

Carrageenan provides a prolonged inflammatory stimulus which resulted in the reduction of mechanical thresholds, and there appears to be an age related difference in the degree of allodynia generated.

Morphine again showed the greatest effect at P3 compared with P10 and P21. This mu agonist had a profound effect on the allodynia or the von Frey hair threshold needed to elicit the flexion withdrawal reflex in carrageenan animals. It is likely that this reflex is Aβ fibre mediated, and suggests that either morphine has some action on these fibres in the early postnatal period, or that at this age, C fibres play a significant role in the development and maintenance of central sensitisation, but that it can be readily blocked by morphine.

DPDPE again readily blocks the allodynia generated by carrageenan, but there is little difference with age.

U69593 markedly raises the von Frey hair threshold required to elicit the flexion reflex at P3 and P21, but surprisingly appears to be antanalgesic at P10. This is difficult to explain, as kappa receptors are abundant in the spinal cord at this age.

It is clear that far more research is required into the developmental pharmacology of these receptors.

7. Aims of Clinical study

The aim of this study was to investigate the analgesic effects of morphine in neonates and infants undergoing major surgery. Morphine is commonly used at Great Ormond Street Hospital in this age group of patients. We wished to study a group of babies who required opioid analgesia postoperatively but who were otherwise well, and would therefore not require postoperative ventilation and sedation. We hoped to provide an insight into requirements of morphine in this clinical setting, in comparison with adults and older children, and to determine the analgesic efficacy of morphine and its glucuronide metabolites in addition to any side effects. It was envisaged that the data could be compared to laboratory findings in rat pups.

Plasma morphine and its glucuronide metabolites were measured during the study period in order to observe the ratio of morphine-6-glucuronide to morphine-3-glucuronide produced, and to correlate blood levels with analgesic effect.

Pain scoring is extremely difficult in this age group of patients; we aimed to compare three different methods of assessing pain in neonates and to determine if the use of all three might be a more clinically useful tool.

8. Introduction to clinical studies

There is now overwhelming evidence both clinically and neurobiologically that neonates and infants are capable of nociception, although the extent of their perception of pain in comparison to adults is as yet unknown. This knowledge has prompted a widespread increase in the use of opioids for the treatment of postoperative pain (de Lima et al. 1996), although we are still very much ignorant of their effects, both short and long term. Despite the difficulties of clinical studies in this age group, there is now growing evidence that the pharmacokinetics of opioids are altered, but we still have very limited information on their pharmacodynamics. This clinical study therefore aimed to investigate the analgesic efficacy of morphine in post-surgical neonates and infants. In this introduction, our current knowledge of the developmental pharmacology of morphine will be reviewed, in addition to a discussion of the assessment of pain in pre-verbal children.

8.1 Developmental pharmacology of morphine

Among paediatric anaesthetists, morphine is the most popular opioid for intra- and post- operative use (de Lima et al. 1996). It is a naturally occurring alkaloid derived from the unripe seed pod of the opium poppy (Boerner et al. 1975).

Morphine is metabolised predominantly in the liver to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G). Glucuronidation is facilitated in the liver by the action of uridine diphosphate glucuronosyltransferases (UDPGTs), a family of isoenzymes, of which 11 different forms have been identified, and full function of

these enzymes appears to develop at different rates postnatally (Hartley and Levene, 1995). M6G possesses significant pharmacological properties (Paul et al. 1989) and has been shown to be a potent μ agonist and respiratory depressant both in humans and in experimental animals (Hand et al. 1987; Hanna et al. 1990; Gong et al. 1991), and morphine is probably in part dependant on the production of M6G for its activity. The effects of morphine are therefore dependant on the proportion of M6G produced, and this may be altered in neonates because of the differential maturation of UDPGTSs (Lawrence et al. 1992). M3G, the predominant metabolite, was thought until recently to be antagonistic to the analgesic and respiratory depressant effects of morphine and M6G; it has since been shown to have no activity at the mu opioid receptor (Smith et al. 1990). There is now evidence from clinical studies to suggest that M3G is devoid of significant activity and does not appear to antagonise M6G or morphine (Penson et al. 1997).

Premature and young term neonates have been shown to have an impaired ability to glucuronidate morphine and this has been the subject of much investigation. The plasma ratio of M3G:morphine has been used as a measure of the capacity of liver glucuronidation in the presence of normal renal function. When morphine metabolism was compared in premature neonates and children, it was found that the ratio of M3G to morphine was significantly higher in the children than the neonatal group, demonstrating impaired glucuronidation in the latter (Choonara et al. 1989). A study of term neonates (ages three to 15 days) and infants (one month to one year) on continuous morphine infusions showed well developed glucuronidation in these neonates, with the ability to produce both M3G and M6G, measured in a single blood

sample taken at 24 or 48 hours. No significant difference was observed in the M3G:morphine ratios between the two groups (Choonara et al. 1992), but mean values were higher than those in premature babies and lower than those in children (Choonara et al. 1992; Choonara et al. 1989). In 31 premature neonates on a continuous morphine infusion, M3G and M6G were detectable in plasma in all babies at 24 hours, demonstrating the ability of these immature neonates to produce both these metabolites. M3G:morphine and M6G:morphine ratios correlated positively with birth weight (probably reflecting an increased liver weight) but not with age (Hartley et al. 1994).

Most studies have confirmed altered pharmacokinetics of morphine in premature and full term neonates. Investigating 10 term infants (postnatal age less than 10 weeks), those one to four days of age had significantly reduced plasma clearance of morphine compared to the older infants. Elimination half life was increased in the neonatal group compared with the infant group, and these data suggest that clearance of morphine reaches adult levels by one month of age (Lynn and Slattery, 1987). A larger difference in pharmacokinetics was observed in a study of 12 children (ages one to 12 years) and 9 premature neonates on continuous fixed rate infusions of morphine. Mean plasma clearance of morphine was significantly reduced in the premature neonatal group compared with the older children (Choonara et al. 1989). To determine how early in childhood morphine clearance reaches adult levels, 49 children were studied aged 1 day to 2 1/2 years on constant rate morphine infusions; it was found that clearance values reach adult levels between the ages of 6 months and 2 1/2 years (McRorie et al. 1992).

A recent emergence of more sensitive and specific measurements of metabolites using High Performance Liquid Chromatography has allowed more detailed investigation of the pharmacokinetics of opioids in neonates, but the difficulties of assessing pain in these pre-verbal patients has left us with little knowledge of the analgesic efficacy of the parent drugs and perhaps, more importantly, their metabolites.

Kupferburg and Way, in 1963 (Kupferberg and Way, 1963), deduced that the increased sensitivity of neonatal rats to morphine was due to the greater permeability of the blood-brain barrier, but this argument has been refuted in the discussion section of my laboratory investigations. A study by Way and Way in 1965 (Way et al. 1965) compared carbon dioxide response curves after morphine and pethidine in a total of eight spontaneously breathing neonates before surgery. The authors found 0.05mg/kg of morphine shifted the CO_2 response curve to the right to a greater degree than 0.5mg/kg of pethidine. The doses were chosen on the assumption that they were equi-analgesic, and no consideration was given to the consequences of maternal pethidine administration (some had pethidine prior to delivery) in the immediate postnatal period. Although they concluded that morphine was a more powerful respiratory depressant than pethidine, the statistical and clinical significance of these results was not discussed. Early studies such as these have probably done much to enhance the perception that opioids, in particular morphine, are potent respiratory depressants in the neonate, and should only be used with extreme caution.

Subsequent studies, however, do not necessarily agree. A retrospective study of the

use of opioids in neonates found that 13% of spontaneously breathing neonates developed apnoea or respiratory depression thought to be due to opioids (Purcell-Jones et al. 1987). 19% of these babies received morphine and none developed respiratory complications, which were all attributable to papaveretum or pethidine. In comparing the respiratory effects of morphine infusions in neonates and children after cardiac surgery, no age related differences were found, and it was concluded that neonates have the same ventilatory response to morphine as infants and children at the same plasma concentration (Lynn et al. 1993).

Studies of morphine use in neonates have not reported any significant cardiovascular side effects such as bradycardia and hypotension (Koren et al. 1985; Hartley et al. 1993).

What is the analgesic efficacy of morphine in the neonate? Most research has concentrated on the adequacy of sedation of ventilated neonates on the intensive care unit. In one such study, comfort scores incorporating both behavioural and physiological parameters were used to assess the adequacy of sedation in premature and term neonates requiring continuous morphine infusions during mechanical ventilation for respiratory distress syndrome. There was no significant difference in the correlation between plasma morphine and analgesic effect in these two age groups. Adequate sedation occurred in 50% of patients with a plasma morphine level of 125 ng/ml (with a 95% confidence interval of 116-135 ng/ml) (Chay et al. 1992).

As we now have much more knowledge of morphine pharmacokinetics and the

activity of its metabolites, it is possible that if equi-analgesic rather than 'scaled-down' adult doses are given, the risk of serious respiratory depression would be no greater. The wide variation in half life among this age group demands a careful dosing regimen for morphine, especially if repeated doses are given. Although we have no information of the efficacy of morphine in post surgical neonates and infants, it would be anticipated that this wide variation would be mirrored. Studies of the analgesic effects of morphine and its metabolites in post-surgical, spontaneously breathing neonates are urgently required before we can adopt a more informed approach to the use of this opioid. For this reason, the clinical study has aimed to link the analgesic effectiveness of morphine with plasma concentrations of morphine and its glucuronide metabolites.

8.2 Assessment of pain in neonates and infants

A major problem in the clinical management of pain in neonates and infants is its assessment and measurement. Reliable and accurate assessment is essential to allow early detection and adequate treatment of pain, to investigate the efficacy of analgesics and treatment regimens and to determine the safety and effects of newer drugs. Difficulty in assessment and measurement of pain is probably the most important factor which has lead to its undertreatment in this age group for many years. To date, we have relied on rather non-specific indices of pain; it is not surprising, therefore, that so few pharmacodynamic studies of analgesics have been conducted in this age group.

Most of the recent research has been aimed at developing a multidimensional

approach in children, for example using behavioural, physiological, cognitive, affective and sociocultural dimensions (Hester, 1995). Unfortunately, in the pre-verbal child, we are limited to the measurement of behavioural and physiological responses to pain.

Behavioural assessment

Neonates and infants are limited in their means of communication, and behaviour is really the only way in which they can express pain. Much research has therefore been conducted into behavioural patterns thought to be associated with pain; the most frequently studied are facial expression, body movement, behavioural state and cry (Porter, 1993), and these will each be discussed below.

Many emotions can be demonstrated by facial expressions, and a number of facial actions have been found to be associated with pain in adults, resulting in the development of a pain score which has been named the Facial Action Coding System (Prkachin, 1992). Neonates and infants undergo considerable neurological development within the first year of life, including a change in their facial expressions. Coding systems have therefore been developed to examine facial activity in neonates in response to a noxious stimulus (Grunau and Craig, 1987). In one study which examined the response of infants to routine immunisation, heart rate, cry and body movements were observed; using a system which scored three segments of the face separately, the authors concluded that facial expression was the most consistent across-infant indicator of pain (Johnston and Strada, 1986). Further studies have examined multiple variations in facial expression in neonates and infants in response

to pain, for example brow bulge, eye squeeze, open lips and mouth stretch, and have shown that there can be a marked variability in the display pattern of these expressions (Grunau and Craig, 1987). For clinical measurement of pain in infants, it is essential that a facial scoring system is quick and easy to perform whilst retaining its accuracy and reliability.

Body movements of neonates and infants have been studied to determine their response to painful stimuli and it has been shown that all neonates have a well developed flexion withdrawal reflex in response to a low grade mechanical stimulus elicited using von Frey hairs (vFh) (Fitzgerald et al. 1988). The threshold of stimulus required to produce this reflex has been shown to parallel perceived pain in adults (Woolf and Swett, 1984), but can be elicited using noxious and non-noxious stimuli in neonatal rats and humans (Fitzgerald et al. 1988). Although it has been shown to be a useful research tool in the study of the response of neonates to noxious input, its use has yet to be studied in the clinical setting as a measure of pain and in the assessment of analgesic efficacy.

The behavioural state of neonates and infants is also an important method of responding to pain. A change from a quiet, sleeping state to crying may be associated with pain. However, there is a big inter-individual variation in babies reaction to pain, for example following circumcision a huge variation in response was found (Wiliamson and Williamson, 1983). Severe withdrawal states have been described in response to painful stimuli, and this was labelled as a 'sleeping fit' in one infant with acute glaucoma (Burton and Derbyshire, 1958). In a study of the response of neonates

to a heel lance, it was found that awake and alert babies responded (with facial activity and a shorter latency to cry) to a greater degree compared with infants in quiet sleep (Grunau and Craig, 1987). The state of arousal is therefore an important determinant of pain expression, and some measure of behavioural state should be included in pain assessment.

Cry seems the most obvious way for a baby to communicate pain, and it is likely that neonates do not have a single cry with a single meaning but that they develop a specific cry which is recognised by their mothers; detecting this difference is not easy in the clinical situation. Research aimed at differentiating the crying associated with various painful stimuli has shown that adults are not always able to recognise these differences (Porter, 1993). Limitations of its use include babies who cannot cry normally because of intubation and mechanical ventilation, or in premature neonates who have a different cry. It has also been suggested that a small number of infants do not cry at all in response to pain (Porter, 1993).

Physiological assessment

The physiological responses to painful stimuli (or we assume are in response to painful stimuli) are a much more objective way of measuring the pain response, and are certainly easier to quantify than behavioural responses. Many parameters can be used, some which are easy to measure in the clinical situation and others which are more useful as research tools.

Cardiorespiratory parameters

In adults, pain leads to an increase in the activity of the autonomic nervous system, resulting in an increase in heart rate, blood pressure and respiratory rate (Möltner et al. 1990). In neonates, painful stimuli such as heel lance have been shown to significantly increase heart rate compared with tactile stimulation (Owens and Todt, 1984), and the authors felt that changes in heart rate were a useful method of measuring pain clinically. Other studies have shown a big variation in the response of the heart rate to painful stimuli (Johnston and Strada, 1986). It is possible that increases in heart rate are mediated through a change in parasympathetic control. During respiratory sinus arrhythmia, heart rate changes are controlled by the vagus, and a painful stimulus decreases the amplitude of this sinus arrhythmia (Porter, 1993). This has potential as an index of pain and research has been directed at finding a clinically useful method of measuring these changes (Porter, 1993), although it seems unlikely to emerge as a rapidly obtainable, and accurate bed-side tool.

Other cardiorespiratory parameters have been investigated, including measurement of transcutaneous $P0_2$ ($tcP0_2$). In ten healthy term neonates undergoing elective circumcision without anaesthesia, the $tcP0_2$ appeared be reduced during painful parts of the procedure, and increased again after the circumcision had been performed (Rawlings et al. 1980). Although $tcP0_2$ has now been largely replaced $Sa0_2$ as a measure of oxygenation, there is no published information of the effect of noxious stimulation on $Sa0_2$ in neonates and infants. Palmar sweating has also been shown to be increased in term neonates following a heel prick for routine blood sampling (Harpin and Rutter, 1983).

Metabolic responses

Studies have shown that during anaesthesia, administration of opioid analgesics can reduce the metabolic stress response to surgery i.e. the production of catecholamines, growth hormone, cortisol and other corticosterioids (Anand et al. 1987) , and it was deduced that this effect was due to a reduction in pain. However, there is no evidence to allow correlation of the level of this stress response to pain (Wolf, 1993). Difficulties of multiple blood testing in small children and delay in obtaining results suggest that this is not a practical bedside measurement of postoperative pain.

Pain scales

To allow successful measurement and therefore treatment of pain in pre-verbal children, pain scales have been developed incorporating several of the different parameters discussed above. Ideally a pain scale should be easy to remember, quick to perform, be validated and incorporate both physiological and behavioural measurements. The development of these scales has aimed at allowing nurses to objectively assess pain behaviour at the bedside, and therefore to treat it successfully. The evidence that nurses are reliable in their assessment of pain is conflicting, and surveys have shown a lack of consistency in attitudes and practice (Lawrence et al. 1993).

CHEOPS (Children's Hospital of Eastern Ontario Pain Scale) is a multi-item scale of several behavioural factors. It resulted from a survey of neonatal nurses who identified particular patterns of behaviour they thought were associated with varying levels of pain, and reached a high level of agreement (McGrath et al. 1987). This

scale was designed for use from the age of one to seven years; it relies on factors such as facial expression, which changes during the neonatal period, and verbal expression which cannot be used in this age group. The same group therefore developed a similar scale for use in neonates, the NIPS (Neonatal Infant Pain Scale) (Lawrence et al. 1993). Again, it incorporates six behavioural items such as facial expression, cry, breathing pattern, posture of arms and legs and the state of arousal. Each has two possible scores of 0 or 1, except cry which may be scored 0, 1 or 2. This scale was specifically designed for use in neonates under going painful procedures in the intensive care unit. Invasive needle procedures in many infants scored at the high end of the scale, allowing little room to differentiate this from pain caused by tissue trauma following surgery, which is likely to be much greater. It was concluded that NIPS provided an objective and replicable tool for the assessment of pain in infants on the intensive care unit.

CRIES is a behavioural and physiological scale which has been specifically designed for the measurement of postoperative pain in neonates and infants (Krechel and Bildner, 1995). CRIES is an acronym of five physiological and behavioural variables which have been shown to be associated with neonatal pain. These variables are: C-Crying; R-Requires increased oxygen administration; I-Increased vital signs; S-Sleeplessness. The system uses a 10 point scale similar to the APGAR score. The requirement for oxygen is based on the SaO_2, and although there is some evidence that $tcPO_2$ falls during painful procedures (Rawlings et al. 1980), as already stated, the effect on SaO_2 has never been formally studied. Measurement of blood pressure was excluded as it was felt that this would often upset an infant. CRIES was found to be

valid, reliable and well accepted by the nursing staff for measurement of pain in postsurgical neonates and infants.

At the time of commencement of the clinical study, NIPS was the most appropriate scoring system available for neonates and infants, and was therefore included as a method of pain measurement. CRIES has been designed specifically for postsurgical infants, but had not been published before the start of the study.

Summary

Our knowledge of the pharmacokinetics of opioids in neonates and infants has improved enormously in the last ten years, but we still remain ignorant of their pharmacodynamics, and in particular, their analgesic efficacy.

In a recent survey of paediatric anaesthetists in the United Kingdom (De-Lima et al. 1996), morphine was the most commonly used opioid in this age group of patients. It is surprising, therefore, that we know so little of its effects, both short and long term.

At Great Ormond Street Hospital, morphine is administered to post surgical neonates and infants to treat postoperative pain via a Nurse Controlled Analgesia (NCA) infusion. My clinical study aimed to investigate its use in these patients, and in particular, I hoped to be able to determine the analgesic efficacy of morphine.

9. Methods

Approval for the study was obtained from the local research ethics committee.

We planned to divide the population to be studied into two groups of 10 patients each: infants (one month to one year of age) and mature neonates (defined as any baby born at or after 37 weeks gestation and within the first 28 days of life). All babies were undergoing major abdominal surgery, and we aimed to choose those who would, by virtue of their type of surgery and skin incision, have a similar pain stimulus after their operation. Only those babies who were returned to the ward breathing spontaneously after surgery were studied, in order to allow a full pharmacodynamic assessment of morphine. Those with pre-existing hepatic, renal or neurological impairment were excluded, as were those taking drugs known to affect morphine metabolism.

Patients were premedicated with atropine 10-20μg/kg orally or intramuscularly at the discretion of the anaesthetist in charge of the case, but no sedative pre-medicant was given. There was no change in the preoperative preparation for surgery or conduct of anaesthesia in this age group of patients as practised in our hospital. After a gaseous induction with either halothane or sevoflurane, intravenous access was established to allow administration of drugs during surgery, and this was used for withdrawal of blood samples for the study. It is normal practice to insert a second intravenous cannula for administration of analgesia. Tracheal intubation was facilitated with

atracurium given intravenously, the lungs were mechanically ventilated and anaesthesia was maintained with nitrous oxide in oxygen and halothane or isoflurane. Intraoperative analgesia was given at the discretion of the anaesthetist in charge of the case, and consisted of local anaesthesia by wound infiltration with bupivacaine 0.25% (up to 0.75 mls/kg), or if opioids were required intraoperatively, morphine (20 μg/kg per dose) intravenously.

On arrival in the recovery area, a Nurse Controlled Analgesia (NCA) intravenous infusion was started, according to a standard regimen consisting of no background infusion, intermittent doses of 20μg/kg with a lockout period of 20 minutes. After recovery from anaesthesia, patients were transferred to either the surgical ward or the intensive care unit, depending on their individual requirements.

We assessed pain by three different methods (at each time point). The first of these methods was a physiological assessment; heart rate, blood pressure, respiratory rate and the oxygen saturation of blood were recorded at all time points. Secondly, a behavioural assessment of pain was performed using the validated behavioural pain scoring system NIPS (Neonatal Infant Pain Score), and thirdly sensory testing which measured cutaneous sensitivity. Each test was performed rapidly and within 30 seconds of each other. Sensory testing involved the use of calibrated Von Frey Hairs (vFh) in two areas. Firstly, the vFh threshold required to elicit the flexion withdrawal reflex in the lower limb was measured, using the same method described in the laboratory section to test neonatal rat pups. Von Frey hairs are single nylon monofilaments of graded diameter (0.08-1.0 mm) attached at right angles to a perspex

handle, and are made according a logarithmic scale of the weight that they apply. Each is calibrated using a balance accurate to 1mg, a value being given to the weight produced on pressing the hair onto the balance, mimicking the action used during experimentation. A small hair was pressed onto the plantar surface of the right foot three times at one second intervals. If this did not produce a flexion withdrawal of that limb, then the same process was repeated using the next 'size' of von Frey hair. The threshold was obtained when a vFh produced a flexion withdrawal of the limb, and the weight of that hair gave the threshold value. In a similar manner, a small hair was pressed around the wound, in order to observe any reflex movement of the abdominal muscles indicating allodynia, and again the threshold required to elicit this reflex was measured. The limb thresholds represent general mechanical sensory thresholds, possibly measuring the general analgesic effect of morphine while those around the wound represent local allodynia, and the effect of morphine on this.

When, using clinical criteria, the primary nurse judged the patient to be in pain and requiring analgesia, either in recovery or on the ward, an initial dose of 20μg/kg of morphine was given. Before this dose of analgesia, pain was assessed by the investigator using the three methods described above, and a blood sample taken for measurement of morphine and its metabolites. Samples were immediately centrifuged and the plasma stored at -70°C. If further analgesia was required during the study period as described above, additional doses of morphine (20μg/kg) were given via the NCA, and the exact timing of these doses was recorded.

Blood was sampled from the intravenous line sited by the anaesthetist in theatre, and a

maximum of 0.4ml was taken for each sample. Samples and pain assessment occurred simultaneously at the following times:

- t_0 after induction of anaesthesia (sample only) and before any opioids had been given
- t_1 immediately before first dose of morphine post-op
- t_2 5 mins post-dose
- t_3 15 mins post dose
- t_4 30 mins post-dose
- t_5 2 hours post-dose
- t_6 4 hours post dose
- t_8 A final sample was taken at 8 hours post dose

All samples were analysed at the Barry Reid oncology unit, St. Bartholomew's hospital, under the direction of Dr. Simon Joel. Morphine, M3G and M6G concentrations were determined simultaneously, by solid phase extraction followed by reverse-phase ion-paired chromatography with electrochemical and fluorescence detection as described by Joel et al (Joel et al. 1988). Samples were purified by Aspec using 10mg C8 Varian cartridges which were conditioned with methanol, 10mM sodium dihydrogenphosphate (pH 2.1) containing 10% acetonitrile and water. A 1ml volume of plasma was buffered and applied to the cartridge. The extract was then buffered and a 1ml volume of the eluent injected into the HPLC column. A microprocessor controlled extraction device allowed simultaneous extraction of numerous samples. Morphine and M6G were measured using electrochemical

detection, but M3G was quantified with the use of fluorescence with M6G as a reference standard, as it is not electrochemically active. The limits of detection for this method are 1 ng/ml for morphine-6-glucuronide and morphine and 5 ng/ml for morphine-3-glucuronide (Joel et al. 1988).

A plasma standard curve was prepared covering the range 2.6 - 263.0 nmol/l for morphine, 2.0 - 201.0 nmol/l for morphine-6-glucuronide, and 20.0 - 2010.0 nmol/l for morphine-3-glucuronide. Upper and lower limits of quantitation were taken as the highest and lowest standard values for each compound.

Mean extraction efficiency for morphine at concentrations of 26.3 nmol/l, 131.5 nmol/l and 263.0 nmol/l, M6G at concentrations of 20.0 nmol/l, 100.5 nmol/l and 201.0 nmol/l, and M3G at levels of 201.0 nmol/l, 1005.0 nmol/l and 2010.0 nmol/l was > 80%, with a reproducibility of < 5% in each case.

Within-run reproducibility (CV) for a plasma sample at 131.5 nmol/l morphine, 100.5 nmol/l M6G, and 1005 nmol/l M3G extracted 48 times in the same assay (over a 12 hour period) was 5.1% for M3G, 3.9% for M6G and 5.1 for morphine.

Between-run reproducibility for quality control samples at low (26.3 nmol/l morphine, 20.1 nmol/l M6G, 201 nmol/l M3G), medium (98.6.5 nmol/l morphine, 75.4 nmol/l M6G, 754.0 nmol/l M3G) and high (210.4 nmol/l morphine, 160.8 nmol/l M6G, 1608 nmol/l M3G) concentrations was < 9% in each case. Accuracy (measured vs nominal QC values) was < 8.5% for M3G , < 7.0% for M6G and < 4.0% for morphine (Simon Joel, personal communication).

This was planned as a pilot study aimed at investigating the relationship between morphine and its metabolite concentrations and pain scores.

10. Results

This clinical research was planned as a pilot study. The study period lasted for a year and during this time I recruited a total of 15 babies. Despite initial plans to study a group of neonates, very few babies of this age group who fitted all criteria presented for surgery at Great Ormond Street Hospital. I was therefore only able to study one neonate. Results for this neonate are considered separately.

10.1 Measurement of morphine and its glucuronide metabolites

The maximum volume of plasma samples was 200µl, and to enable measurement of all metabolites, all samples were diluted by a factor of five. As the original plasma concentrations were very low initially, dilution resulted in a significant number falling below the limits of detection for the measurement technique; it was felt important to include these data in the calculation of correlation coefficients, and each of these values has been taken as zero. However, these data have not been used in the calculation of M6G:morphine ratios. Details of plasma concentrations and pain scores for each patient at each time point are tabulated in appendix 1. As plasma concentrations were at the lowest end of the assay detection sensitivity, the results have to be treated with caution.

10.2 Patient data

Patient ages, weights, type of surgery and its duration, are summarised in table 4.. The group had a median age of six months (range three to nine and a half), and the median weight of these babies was 7.7kg (range 5.9 to 11.1). Each patient underwent

abdominal surgery, and might be expected to experience comparable degrees of pain postoperatively.

10.3 Morphine administration

A summary of the pattern of analgesia administration to all infants is illustrated in table 5.

From table 5, it can be seen that the range of morphine dose given in theatre was from 20 to 160μg/kg, with a median value of 120μg/kg. The time to first postoperative dose of morphine after surgery varied greatly, from 55 to 665 minutes, with a median value of 240; one infant did not require any morphine in the postoperative study period. The length of this delay is related to the total dose of morphine given in theatre; patients given at least 100μg/kg of morphine did not require morphine postoperatively for at least 3 hours. Three of the four patients who were given 80μg/kg or less of morphine intraoperatively required further analgesia within 65 minutes. None of the infants who received local anaesthesia infiltration of their wound site required morphine for at least 4 hours postoperatively, although they all received at least 100μg/kg of morphine in theatre. Again, the total amount of morphine given in the postoperative study period varied greatly, with a range of 0 to 220μg/kg, and a median value of 80μg/kg.

10.4 Plasma levels

Plasma M6G:morphine ratios were calculated in all samples in which the plasma level

was detectable, and the results for each patients in which this calculation was possible are illustrated in table 6. These values, with a collective mean value of 1.50 and range of 0.60 to 3.21, demonstrate a large variation in M6G production measured in this age group. Mean plasma morphine and M6G concentrations are also shown in this table.

From each individual patient table in appendix 1, it can be seen that morphine and M6G concentrations fall rapidly following each dose of morphine administered.

10.5 Local anaesthesia

In four infants, local anaesthesia was used to infiltrate around the wound site at the end of surgery. Although all these babies received at least 100 μg/kg of morphine in theatre, it resulted in an apparent lengthening of the time to first postoperative dose of morphine to at least four hours, but total morphine requirements in the postoperative study period did not appear to differ. The effect of this supplementation on von Frey hair threshold around the wound site is demonstrated graphically in figure 17. This threshold was measured in two patients who did not receive local analgesia, and four infants who were given 0.5 mls/kg of bupivacaine; it appears from these results that local anaesthesia reduces allodynia around the wound site in the initial postoperative period. The von Frey hair threshold around the wound site was not measured in all patients; in some it was inappropriate for the type of surgery, and others received dressings to their wounds. In several patients who had not received local anaesthesia, touch with even a small von Frey hair precipitated crying in previously sleeping babies, indicating a marked degree of allodynia; plasma morphine concentrations were therefore not high enough to block this.

10.6 Plasma analgesia concentrations vs pain scores

The relationship between plasma morphine concentration and each pain score in the infant group is shown in figure 18, together with the correlation coefficient. Morphine concentrations below the limit of detection, for which no actual value was given, are included in these data, and these values have been plotted and used in calculations as zero. There is little correlation between plasma morphine concentration and NIPS score, von Frey Hair limb threshold, heart rate, respiratory rate or SaO_2, with r values ranging between -0.057 and +0.245. The relationship between plasma M6G concentration and pain scores is illustrated in figure 19; again there is little correlation between these plasma levels and each pain score, with correlation coefficients ranging from -0.133 to +0.123.

10.7 Neonatal data

One term neonate, aged 3 days and weight 2.5 kg, was studied following a laparotomy. Morphine was not given intraoperatively, although this baby received local anaesthesia to its wound at the end of surgery. A total of 40 g/kg of morphine was given during the study period. The time to first postoperative dose of morphine was relatively short in comparison with the infant group, at 130 minutes, but this baby required minimal postoperative morphine.

At most time points, plasma morphine and M6G concentrations fell below the limit of detection and correlation with pain scores was not calculated; this also precluded the calculation of M6G:morphine ratios. NIPS scores, however remained low for the

duration of the study period, suggesting adequate analgesia.

11. Discussion

The majority of research into the use of morphine in neonates and infants has been directed towards the study of ventilated neonates on the intensive care unit requiring morphine for sedation, and there is little information on the use of morphine as a postoperative analgesic in infants and in particular neonates. I have studied the effects of morphine NCA (nurse controlled analgesia) both on clinical assessment of analgesia and by blood levels in this age group of patients, who have all undergone abdominal surgery. I was able to recruit only one neonate, but this research was planned as a pilot study, and will continue with the aim of studying a larger group of term neonates.

This discussion will focus on results from the infant group.

11.1 Morphine metabolism

Morphine is metabolised predominantly in the liver to M3G and M6G. It has been suggested that hepatic clearance of morphine reaches adult levels by one month of age (Lynn and Slattery, 1987), but a further study shows that clearance reaches adult values only between the ages of six months and 21/2 years (McRorie et al. 1992). This highlights the difficulty in studies in this age group, and confirms the wide variation in pharmacokinetics. M6G is a potent mu agonist, possessing potent analgesic and respiratory depressant properties (Gong et al. 1991; Gong et al. 1992), but M3G has been shown to be devoid of pharmacological activity in human volunteer studies (Penson et al. 1997). Premature neonates glucuronidate morphine in

a highly variable manner due to immaturity of hepatic enzyme systems, but term neonates and infants have been shown to possess well-developed glucuronidation pathways with the ability to produce both M3G and M6G (Choonara et al. 1992). This is reflected in plasma M3G:morphine ratios greater than those of premature babies, but less than those of older children (Choonara et al. 1989). In summary, the amount of M6G produced can be highly variable in neonates and infants, and may have a profound effect on the pharmacodynamics of morphine.

This study has confirmed the wide variability in metabolite production after morphine administration, reflected in the large range in M3G:M6G ratios. Plasma levels of morphine and M6G were very low in all patients during the study period, often below the limit of detection; this suggests that despite a decreased clearance in this age group, morphine requirements are low. Pharmacokinetic differences such as a larger volume of distribution compared with adults may result in lower plasma levels following the same dose (Hartley and Levene, 1995).

11.2 Time to first postoperative dose

Despite similar surgery in the infant group, the time from last intraoperative dose to first postoperative dose of morphine is very variable and appears, as would be expected, to be related, in part, to the dose of morphine given in theatre. However, the administration of a large dose (i.e. greater than 80μg/kg) of morphine in theatre had no effect on the total dose of morphine given in the postoperative study period. These results suggest that morphine given early during surgery may have some pre-emptive effect resulting in a degree of opioid sparing in the early postoperative period.

11.3 Morphine requirements

Perhaps the most striking feature of these results is that infants, even after major surgery, appear to have minimal morphine requirements. NIPS scores appear to remain low for up to eight hours after the first postoperative dose (see tables in appendix) demonstrating clinically adequate analgesia according to the most validated scoring system available, despite low plasma concentrations of morphine and M6G. The variability in requirements, ranging from none at all to 220 μg/kg, may be explained by possible variations in morphine metabolism and M6G formation, as has been reported in children of this age (Choonara et al. 1989). The total doses given are considerably less than the average requirement in older children and adults after surgery (Woodhouse et al. 1996; Hansen et al. 1996), and possible reasons are discussed below.

Low morphine requirements in these infants may reflect a greater proportion of M6G produced from morphine, itself a potent analgesic, and this is certainly reflected in the relatively high and variable M6G:morphine ratios seen in comparison with those previously reported in children (Hartley and Levene, 1995). It has also been argued that a relatively immature blood brain barrier results in its increased permeability to morphine in rats (and possibly M6G) (Kupferberg and Way, 1963), but subsequent research has demonstrated that this barrier is intact from birth (Butt et al. 1990). In a study of subcutaneous administration of M6G to neonatal guinea pigs, the permeability of the BBB to M6G did not significantly alter with dose or increasing age (Murphey and Olsen, 1994).

Studies of neonatal laboratory rats have shown that the proportion of mu opioid receptors in the spinal cord is greatest at birth, gradually reducing to adult levels by three weeks of age (Waheda Rahman, personal communication). These receptors are also more widely distributed in the spinal cord in the early neonatal period (Kar and Quirion, 1995). No similar data are available for the brain, but it seems likely that that here also there is an increased proportion of mu receptors. Although only a pilot clinical study, these results offer some explanation for the increased sensitivity of infants to morphine. They also suggests that human opioid systems may also undergo considerable development postnatally. Morphine also appears to be much more efficacious in neonatal rats, as I have shown in my laboratory investigations.

Autoradiographic studies of neonatal rats have shown that delta opioid receptors are absent from the spinal cord at P3 (postnatal day 3) (Wahida Rahman, personal communication) but my laboratory studies have shown that a delta agonist can still provide analgesia at this age. One explanation may be that opioid receptors have a different distribution in the early neonatal period and may be abundant outside the spinal cord, for example in the dorsal root ganglion. It is also possible that the structure of opioid receptors may be different at birth and in infancy, resulting in an altered (although still specific) agonist-receptor interaction, and therefore effect.

Following the initial postoperative dose of morphine, the amount administered to infants during the study period was dependant on the primary nurse in charge of their care. At Great Ormond Street Hospital, no formal scoring system is used to measure neonatal and infant pain by the nursing staff on the surgical wards, and therefore each

nurse relies on her own clinical judgement and experience to determine a patient's level of pain. This has been shown to be unreliable (Craig et al. 1993). Although I used NIPS scores for up to eight hours (at the predetermined study times) following the first postoperative dose, these scores were low and agreed with the low morphine requirements, it is still a possibility that inaccuracies in pain assessment are reflected in apparently low morphine doses administered.

11.4 Local anaesthesia

The use of local anaesthesia to infiltrate the wound at the end of surgery was not formally randomised, and was used at the discretion of the anaesthetists and surgeon; I was not blinded to its use. Despite these deficiencies, I will comment on the observations made.

Clinically, the use of local anaesthesia to infiltrate the wound site seemed to make an overwhelming difference to hyperalgesia around the wound site. If no local anaesthesia was used, stimulation with even a very small von Frey hair could precipitate screaming in a previously sleeping baby; with its use, this allodynia did not seem to occur in any of the patients studied, appearing to raise the von Frey hair threshold around the wound site for the duration of the study period. Presumably any handling of a baby with such marked allodynia would result in intense pain, and the doses of morphine used were not adequate to reduce this. However, from these results, there was no difference in morphine requirements between these two groups, and this may reflect the lack of effect of wound local anaesthesia on muscle and

visceral pain. Certainly clinical observation and the use of von Frey hairs suggest that its use reduces cutaneous hyperalgesia, and this warrants further investigation in this age group with a randomised controlled trial, with a blinded investigator.

11.5 Pain assessment and measurement

The problems of assessment of pain in pre-verbal children are well documented and have been discussed in the introductory section (Porter, 1993). Measuring pain once assessed poses further difficulties, and this study aimed to compare the effects of three different methods of measurement.

Studies have shown that heart rate increases in this age group following painful stimuli (Owens and Todt, 1984),but this response is variable (Johnston and Strada, 1986). $TcPO_2$ may also fall with pain, but no data are available for the effect of pain on SaO_2 (Rawlings et al. 1980). I have found that there is no correlation between plasma morphine or M6G concentrations and either heart rate, respiratory rate or SaO2, but none of the babies studied suffered from respiratory or cardiovascular depression during the study period. Although these physiological parameters may not provide an accurate measurement of pain, they are essential to detect the side effects of analgesia early.

The NIPS scoring is easy to learn and quick to perform; it involves a seven point scoring system which accounts for five variables, and has been designed for use in babies undergoing procedures on the neonatal intensive care unit (Lawrence et al.

1993). However, from this study, there appears to be little correlation between NIPS score and morphine and M6G levels. The score measured appeared to be highly dependant on environmental factors in many of the babies studied; for example, a busy recovery area in theatre would wake a baby and increase its score. One baby sleeping on the ward with a NIPS score of 0 was awoken by a crying baby, and the score immediately rose to 7. Another baby had a NIPS score of 7, and was about to receive analgesia when the mother arrived thinking it hungry, and it settled and slept following a breast feed. It appears NIPS may not be suitable for neonates and infants who have undergone surgery and require postoperative analgesia; a system such as CRIES, published since commencing this study and specifically designed for use in postsurgical neonates and infants, may be more appropriate (Krechel and Bildner, 1995).

The flexion withdrawal reflex may be elicited in the neonate by a low-threshold mechanical stimulus such as a von Frey hair (Fitzgerald et al. 1988). In the adult, this reflex is evoked only by noxious stimuli but in the neonate, innocuous stimuli may also do so (Shanahan et al. 1983). I have shown in my laboratory investigations that epidural opioids depress this reflex, measured by a rise in the limb von Frey hair threshold. The use of von Frey hairs has also been reported in human neonates (Fitzgerald et al. 1988), and in this study, I have shown that the lower limb von Frey hair threshold bore no correlation to plasma morphine or M6G concentrations, but appeared to be dependant on the wakeful state, with a higher threshold in a sleeping baby.

Measurement of the von Frey hair threshold around the wound site gave a clear indication of the degree of hyperalgesia, with even the smallest von Frey hair markedly irritating a baby who had not received local anaesthesia infiltration of their surgical wound. The use of von Frey hairs may therefore have a role in the study of wound hyperalgesia and its treatment, but as it can cause marked distress in some babies its routine clinical use seems hard to justify.

These results highlight the difficulties in assessment and measurement of pain in pre-verbal children. I have found no correlation between plasma morphine or M6G and any of the pain scores recorded. One possible explanation is that these methods of measuring pain are insensitive, but this is not supported by previous reports (Krechel and Bildner, 1995; Lawrence et al. 1993), and it is surprising that not one of these scores relates to analgesic levels. It is also possible that plasma morphine and M6G concentrations at a certain time point do not indicate the degree of analgesia that they provide at that time, i.e. the plasma level does not reflect tissue (brain and spinal cord) concentration. It is also be possible that mu receptors are structurally different at birth and in infancy, and that this structure changes with development; the interaction of morphine and M6G with the mu receptor could then be altered, possible resulting in a longer half life of these agonists at the receptor, and leading to their prolonged effect despite falling plasma concentrations. However, no data are available at present to support this hypothesis.

11.6 Neonatal data

It is difficult to comment on or draw any conclusions from the data form the single

neonate. As with the infant group, this baby required minimal morphine analgesia postoperatively, but again pain scores did not correlate with plasma morphine concentration perhaps for reasons similar to those discussed above.

In conclusion

This study has shown that morphine requirements are very variable after abdominal surgery in infants and are low in comparison with adults. This may reflect pharmacokinetic differences such as the variation in M6G:morphine ratios, a difference in the proportion and distribution of mu receptors at this age or a change in structure and or function of opioid receptors with development.

Plasma morphine and M6G concentrations do not appear to correlate with pain scores; the former may not reflect actual tissue levels, and hence the degree of analgesia. We have not been able to measure, therefore, the contribution of morphine and M6G to analgesia.

Pain assessment and measurement is extremely difficult in this age group of patients and has hampered its treatment. Scoring systems incorporating the use of behavioural and physiological parameters designed for use in postsurgical babies are probably the best method that we have at present, although they are far from ideal.

Overall, 'Nurse Controlled' administration of morphine to postsurgical infants appears to be safe and effective.

12. Overall conclusions

This thesis has aimed to investigate the pattern of development of opioid analgesia, and has shown that opioid systems undergo considerable changes in the postnatal period, both in neonatal rats and humans.

Results from laboratory investigations have shown that the analgesia provided by mu, delta and kappa opioid receptor agonists to each pain model studied undergoes considerable change in the early postnatal period in laboratory animals, and in particular analgesic potency is much gretaer in neonatal animals.

The clinical study has shown that, despite difficulties in pain assessment in pre-verbal children, morphine requirements are considerably lower after major surgery than those in older children and adults.

This developmental pattern of opioid analgesia may be explained by changes in the number and distribution of opioid receptors, and differences in receptor pharmacology, and probably reflects a change in the role of opioid receptor in providing endogenous analgesia in the early postnatal period.

Studies of the anatomical distribution, pharmacology and moleculr biology of opioid receptors, both within and outside the CNS, are urgently needed to allow us to use opioid analgesics more effectively in neonates and infants.

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14. Figures and tables

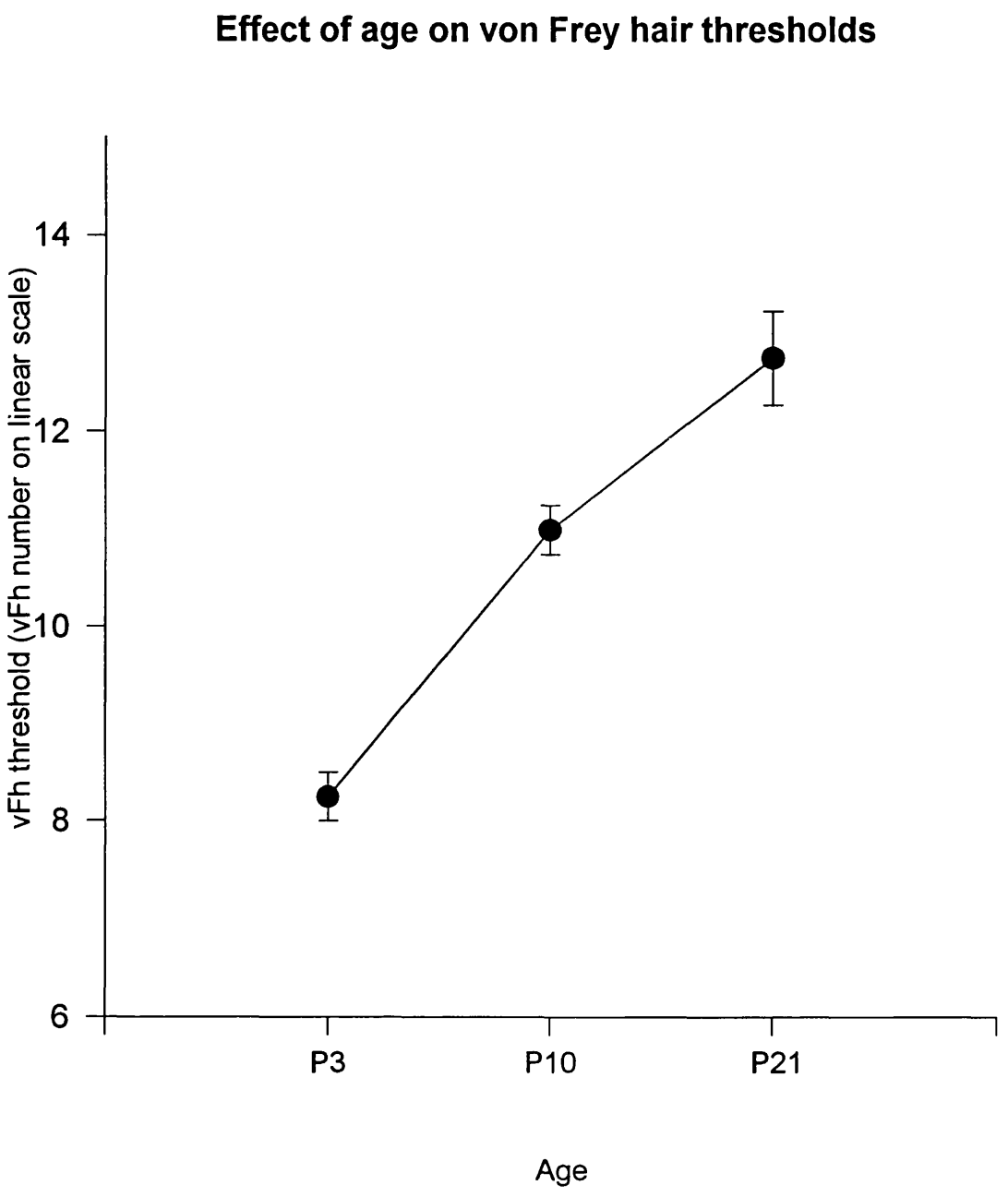


Figure 1

A comparison of the effect of age on the mean (SEM) baseline von Frey hair threshold required to elicit the flexion withdrawal reflex.

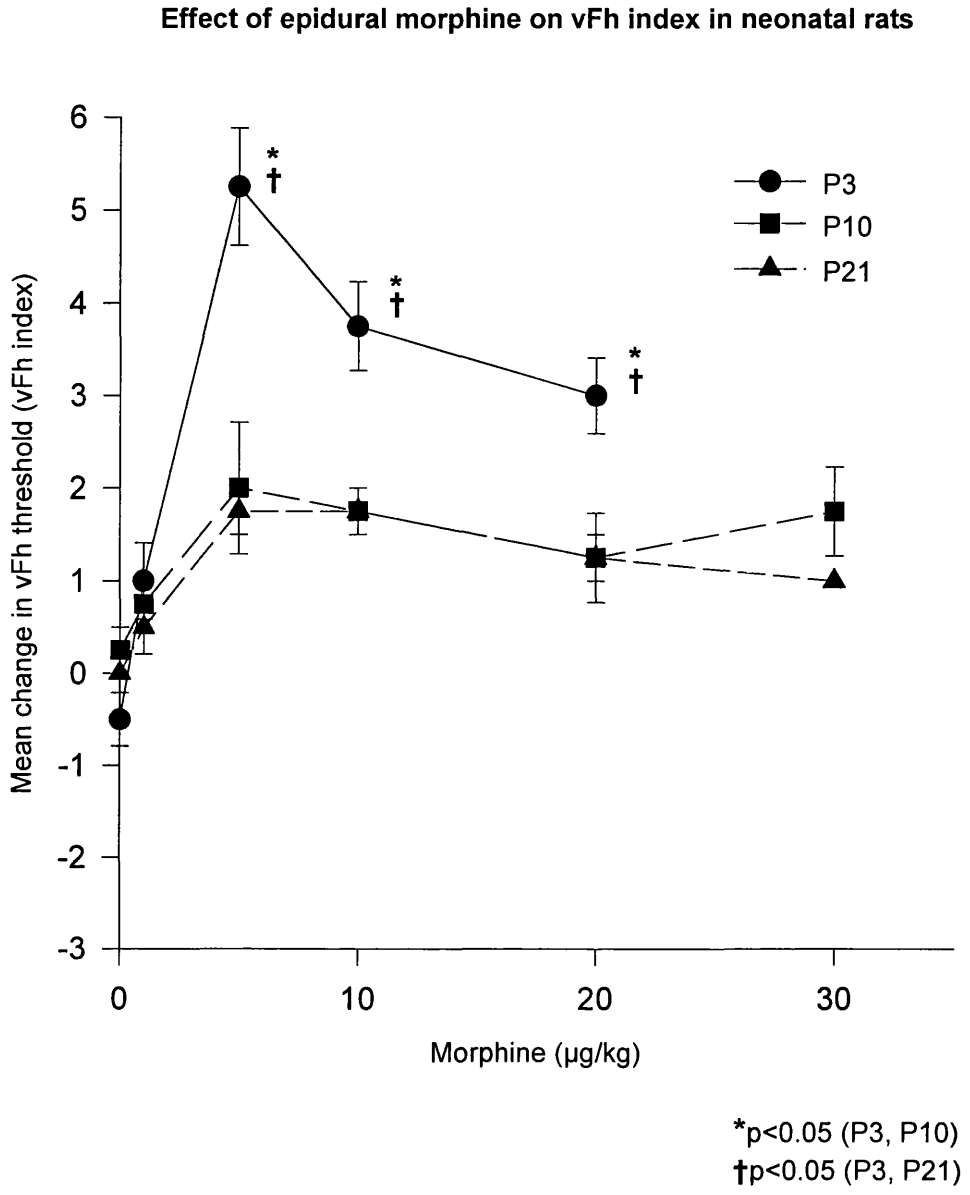


Figure 2

A comparison of the dose response curve of epidural morphine with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex. Statistically significant differences in values between ages are shown.

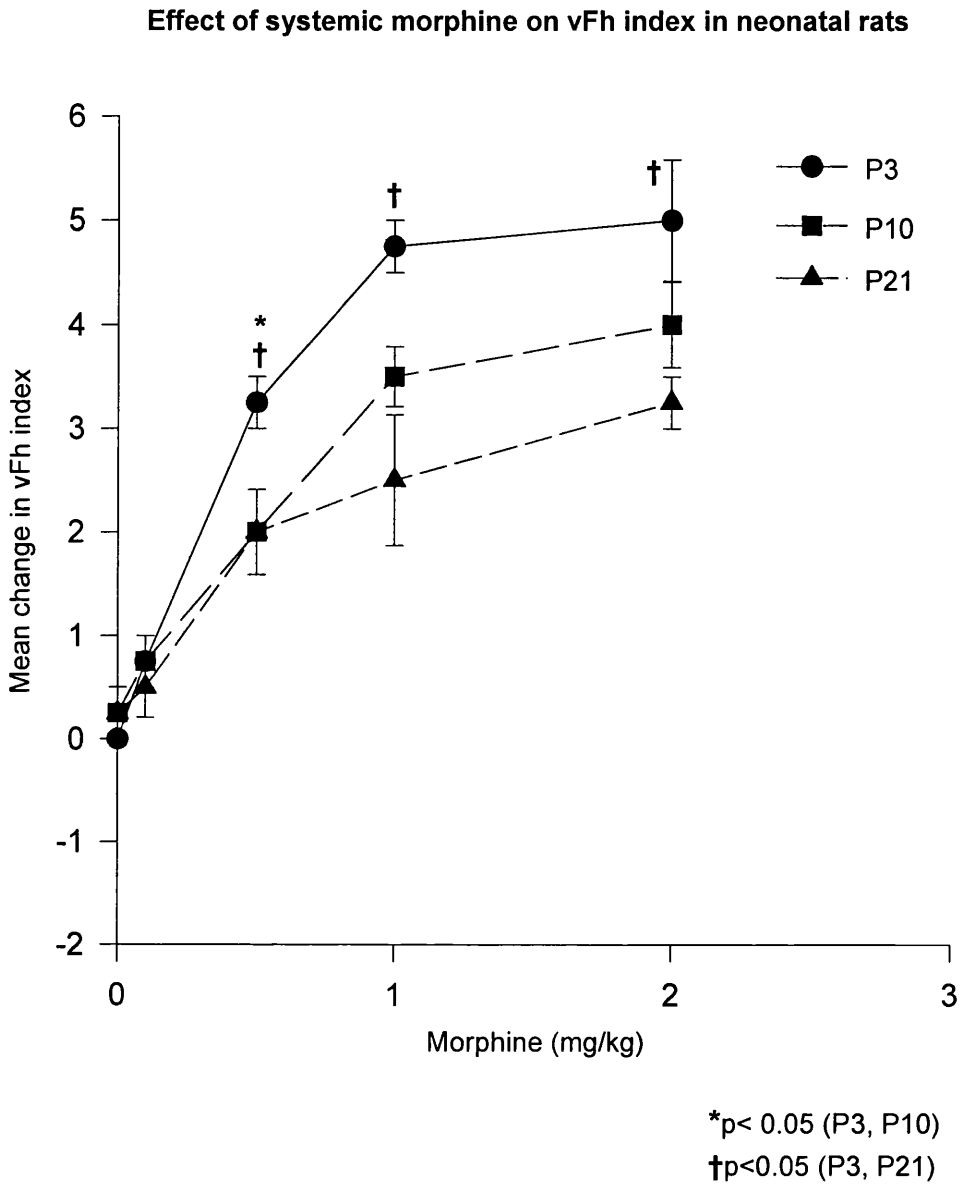


Figure 3

A comparison of the dose response curve of systemic morphine with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex. Statistically significant differences in values between ages are shown.

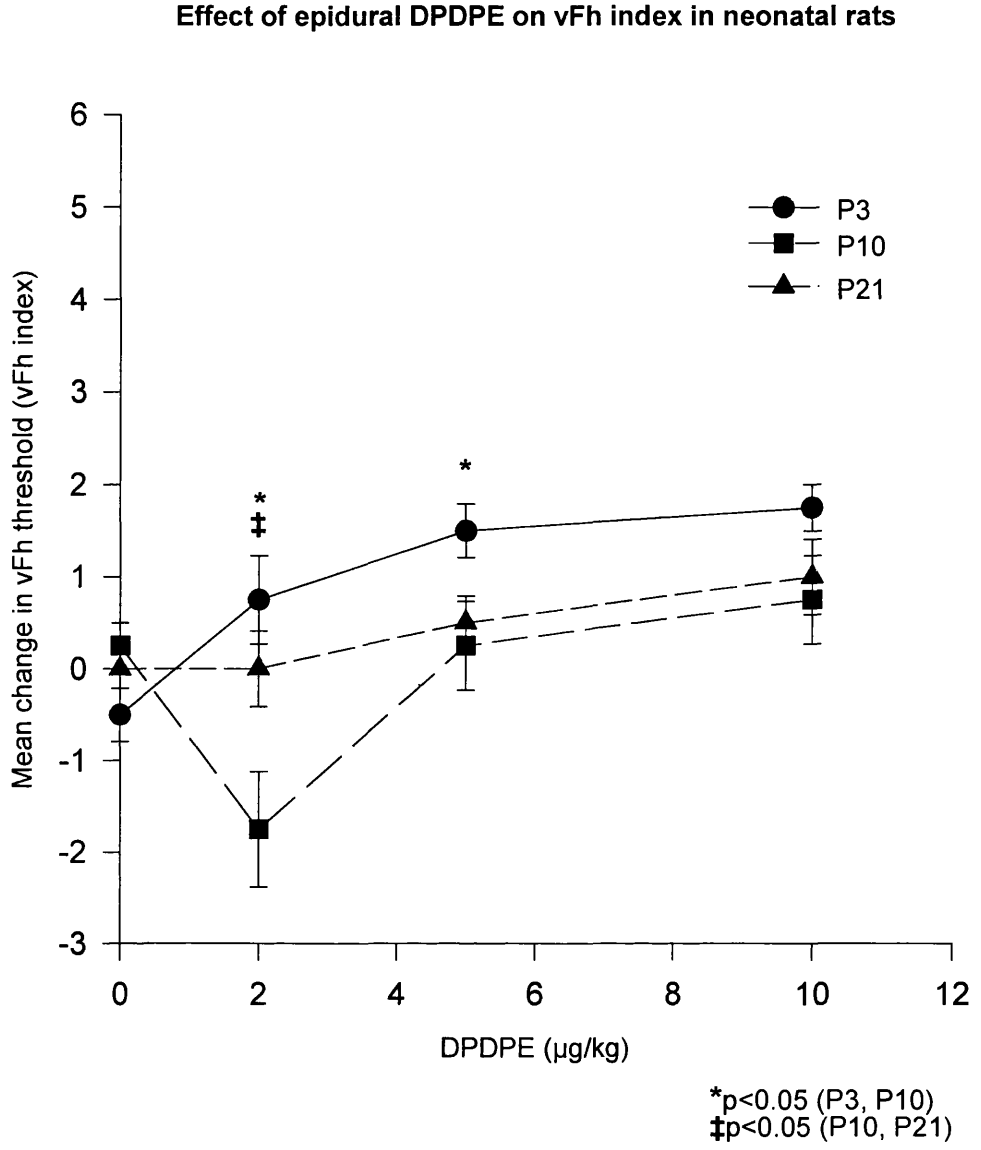


Figure 4

A comparison of the dose response of epidural DPDPE with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex. Statistically significant differences in values between ages are shown.

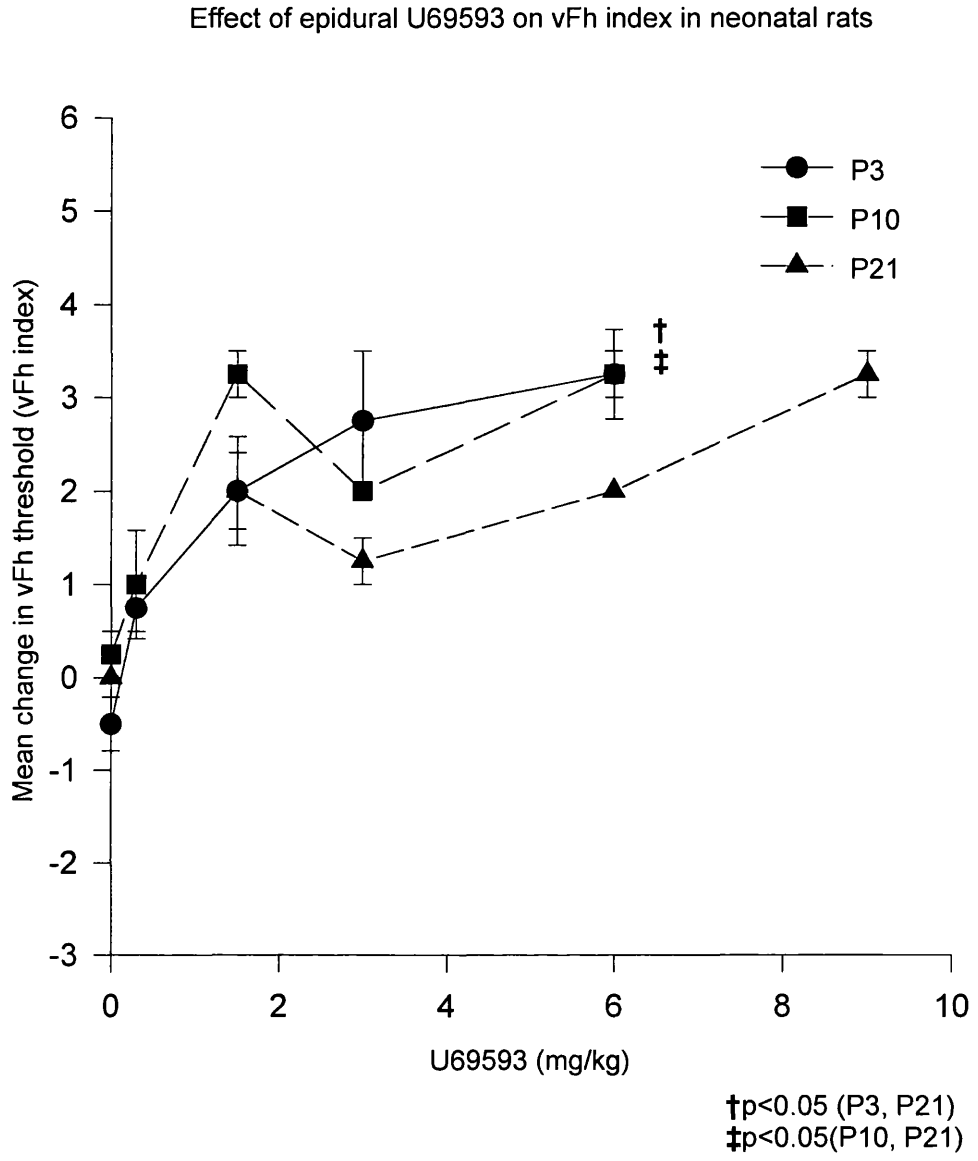
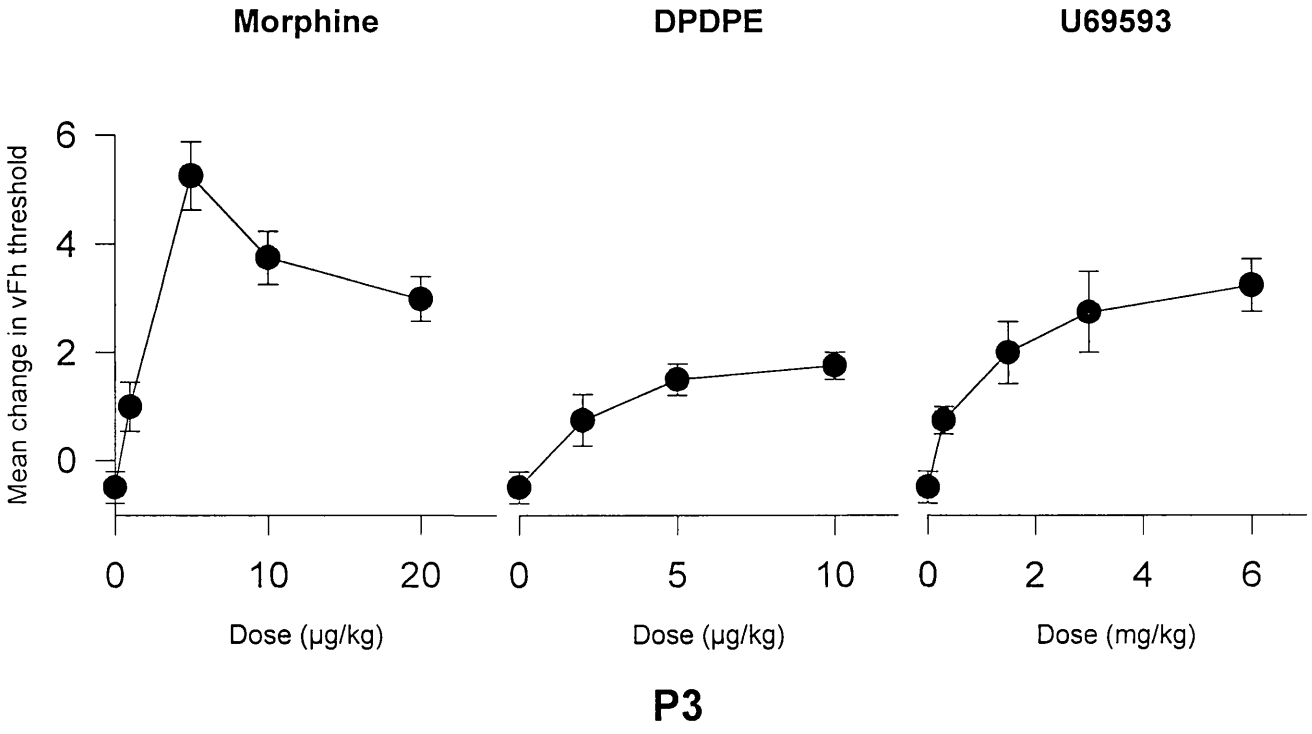


Figure 5

A comparison of the dose response curve of epidural U69593 with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex. Statistically significant differences in values between ages are shown.



Figures 6A A comparison of the dose response curves of each epidural agonist at P3, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex.

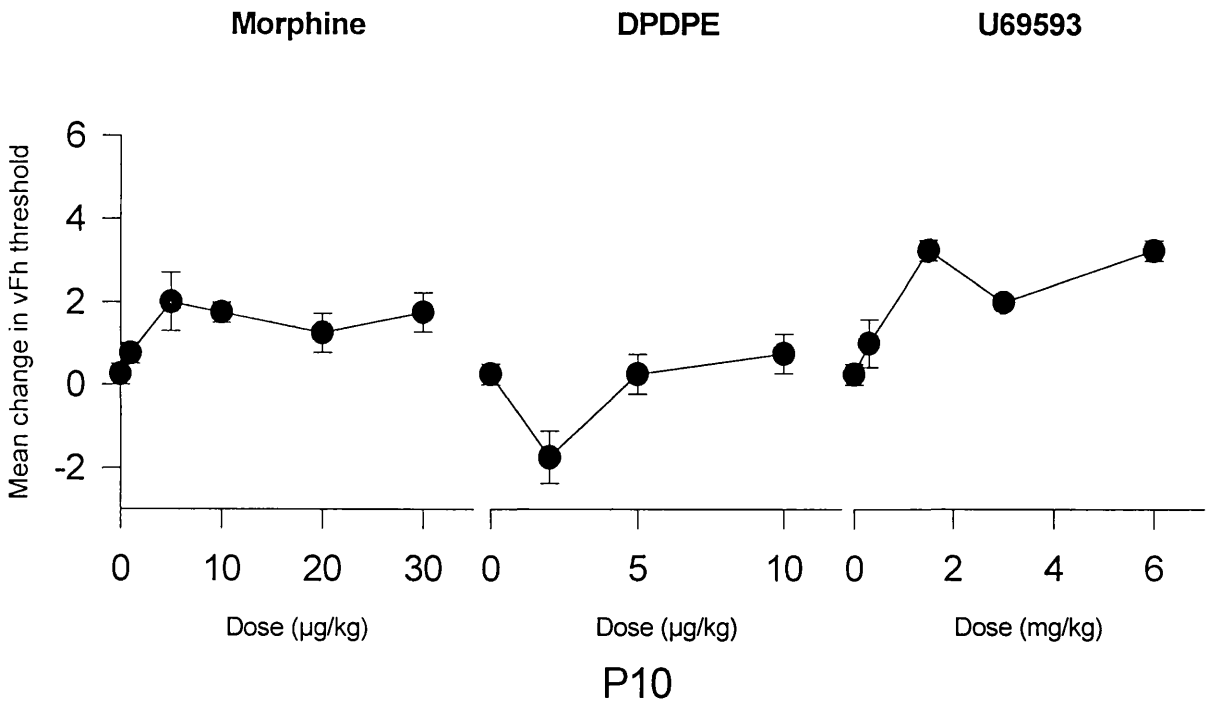


Figure 6B A comparison of the dose response curves of each epidural agonist at P10, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex.

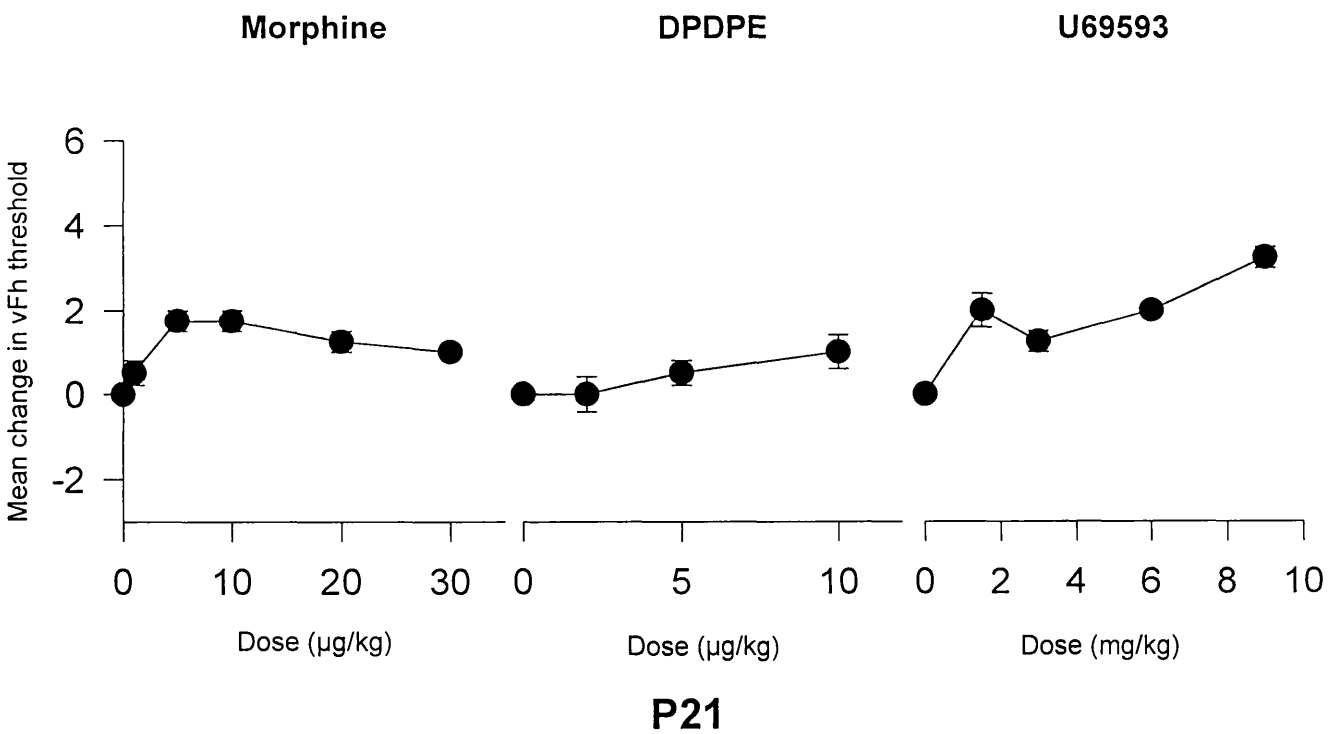


Figure 6C A comparison of the dose response curves of each epidural agonist at P21, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex.

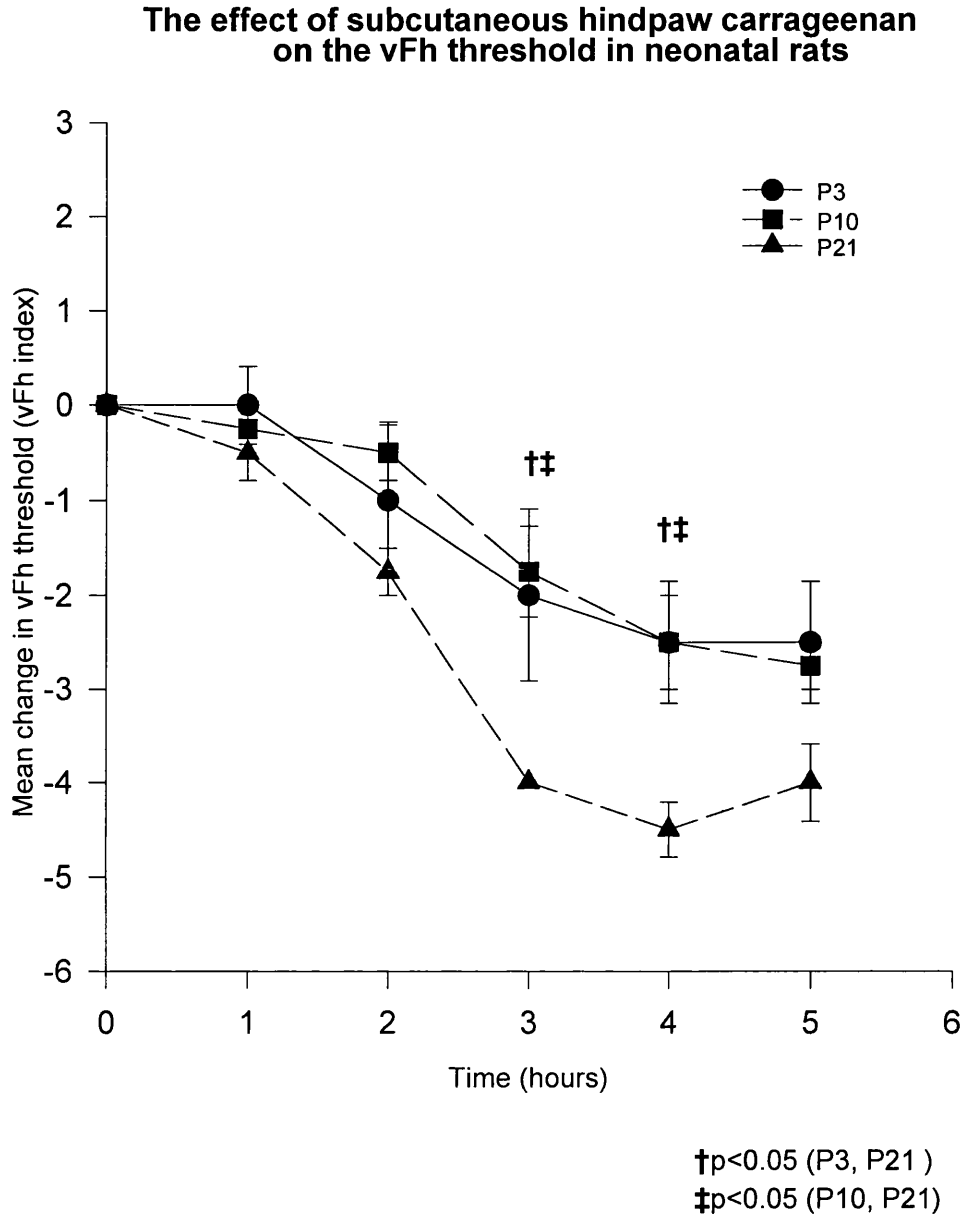


Figure 7

A comparison of the effect of carrageenan induced inflammation with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex, measured hourly for five hours. Statistically significant differences in values between ages are shown.

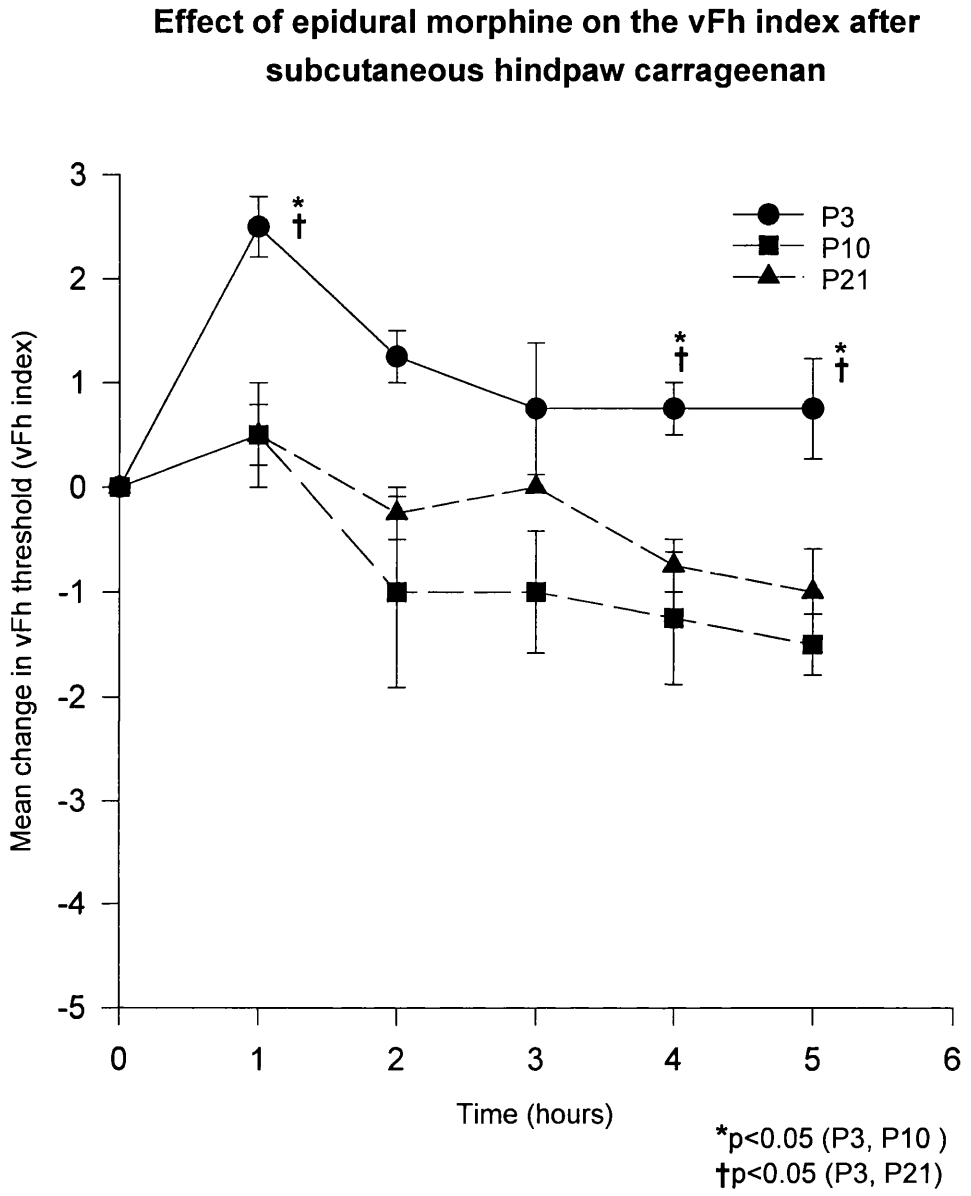


Figure 8

A comparison of the effect of low dose epidural morphine on carrageenan induced allodynia with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex, measured hourly for five hours. Statistically significant differences in values between ages are shown.

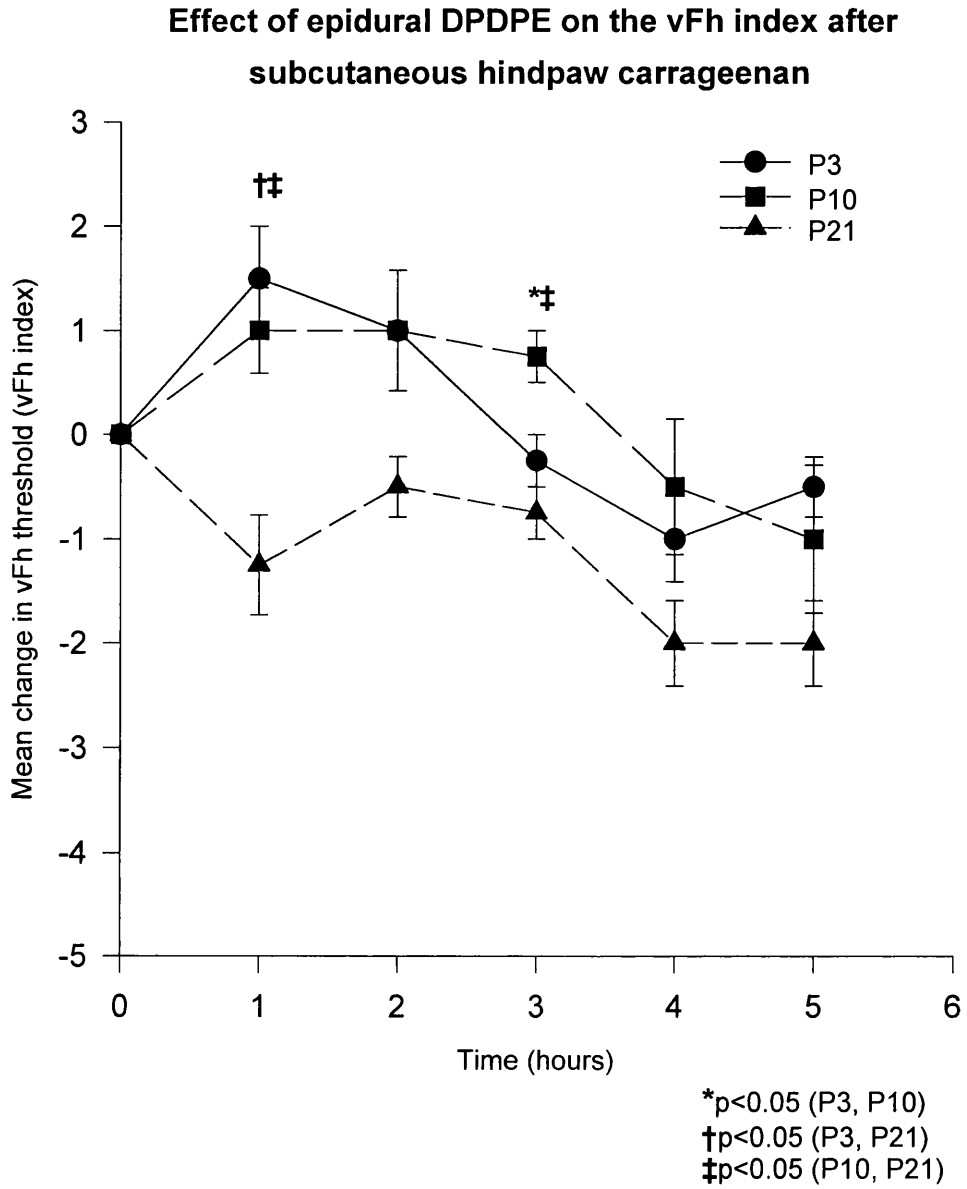


Figure 9

A comparison of the effect of low dose epidural DPDPE on carrageenan induced allodynia with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex, measured hourly for five hours. Statistically significant differences in values between ages are shown.

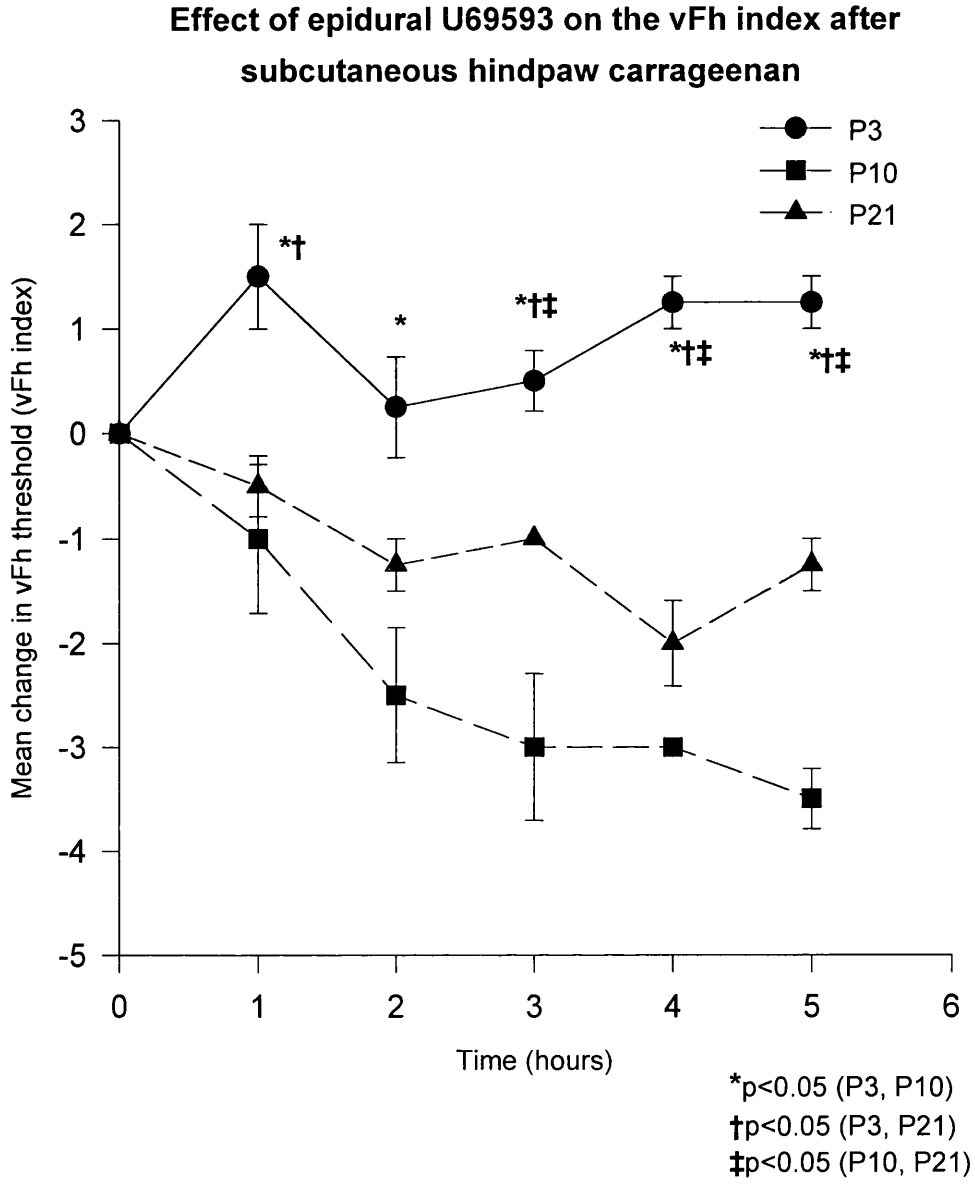


Figure 10

A comparison of the effect of low dose epidural U69593 on carrageenan induced allodynia with age, represented by the mean (SEM) change in vFh threshold required the elicit the flexion withdrawal reflex, measured hourly for five hours. Statistically significant differences in values between ages are shown.

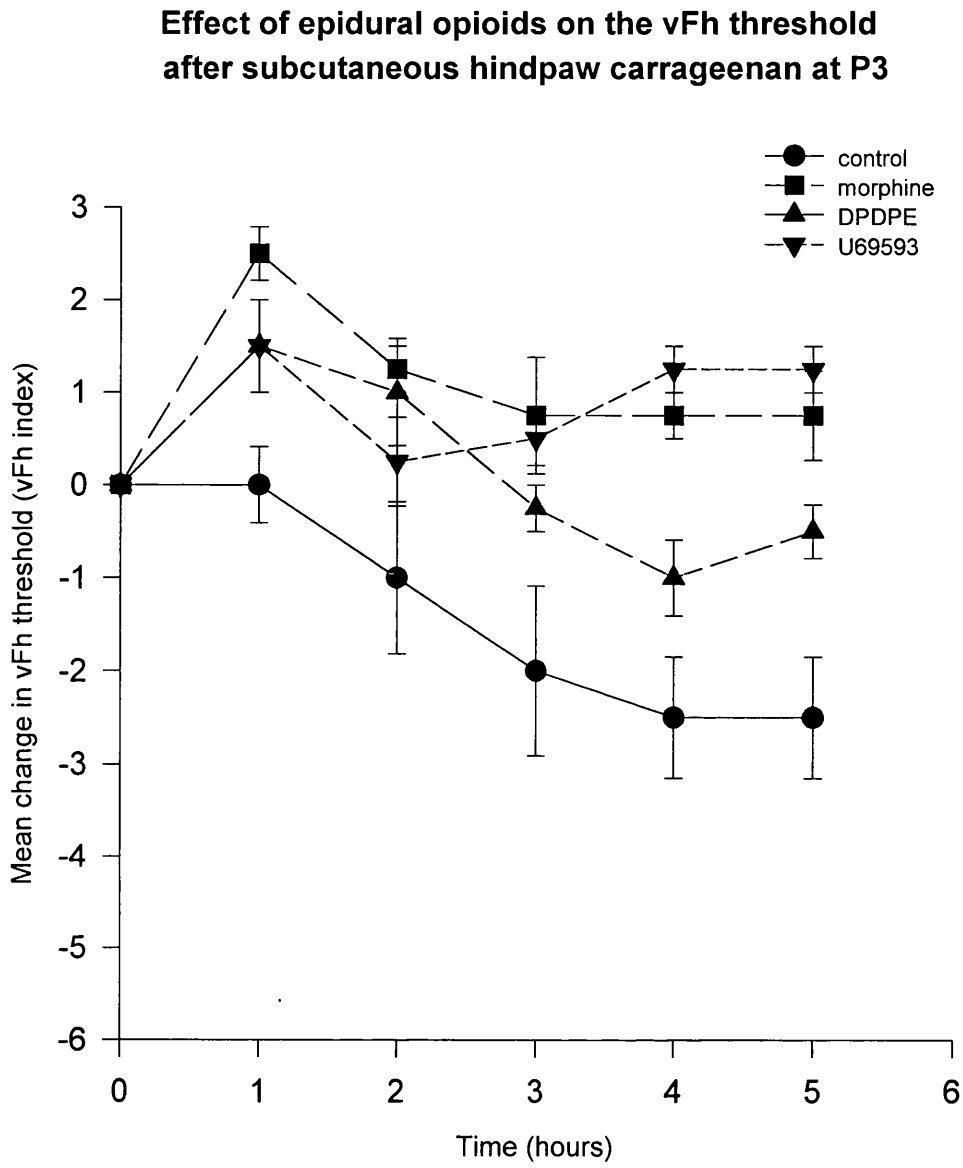


Figure 11A

A comparison of the effect low dose epidural morphine, DPDPE and U69593 with control animals on carrageenan induced allodynia at P3. Each agonist and control group is represented by a different symbol and line, joining mean (SEM) values.

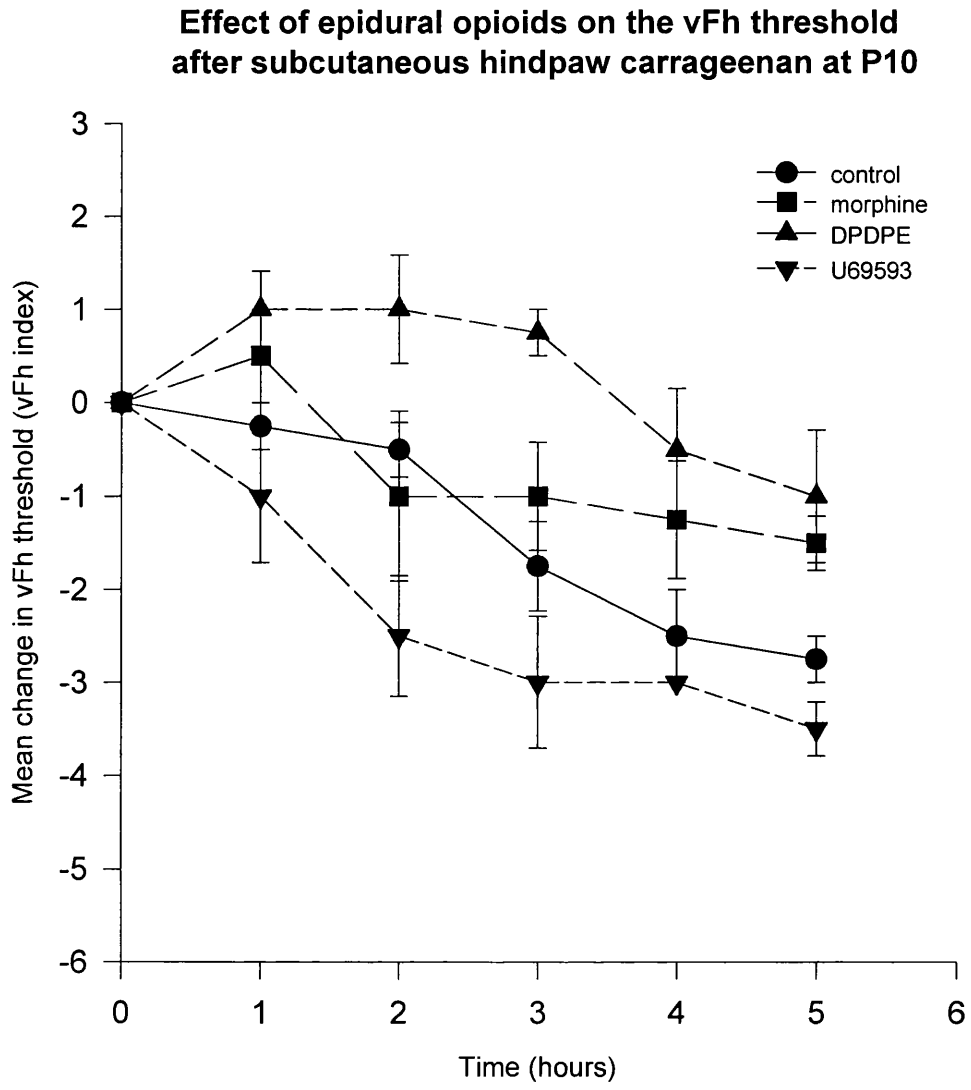


Figure 11B

A comparison of the effect low dose epidural morphine, DPDPE and U69593 with control animals on carrageenan induced allodynia at P10. Each agonist and control group is represented by a different symbol and line, joining mean (SEM) values.

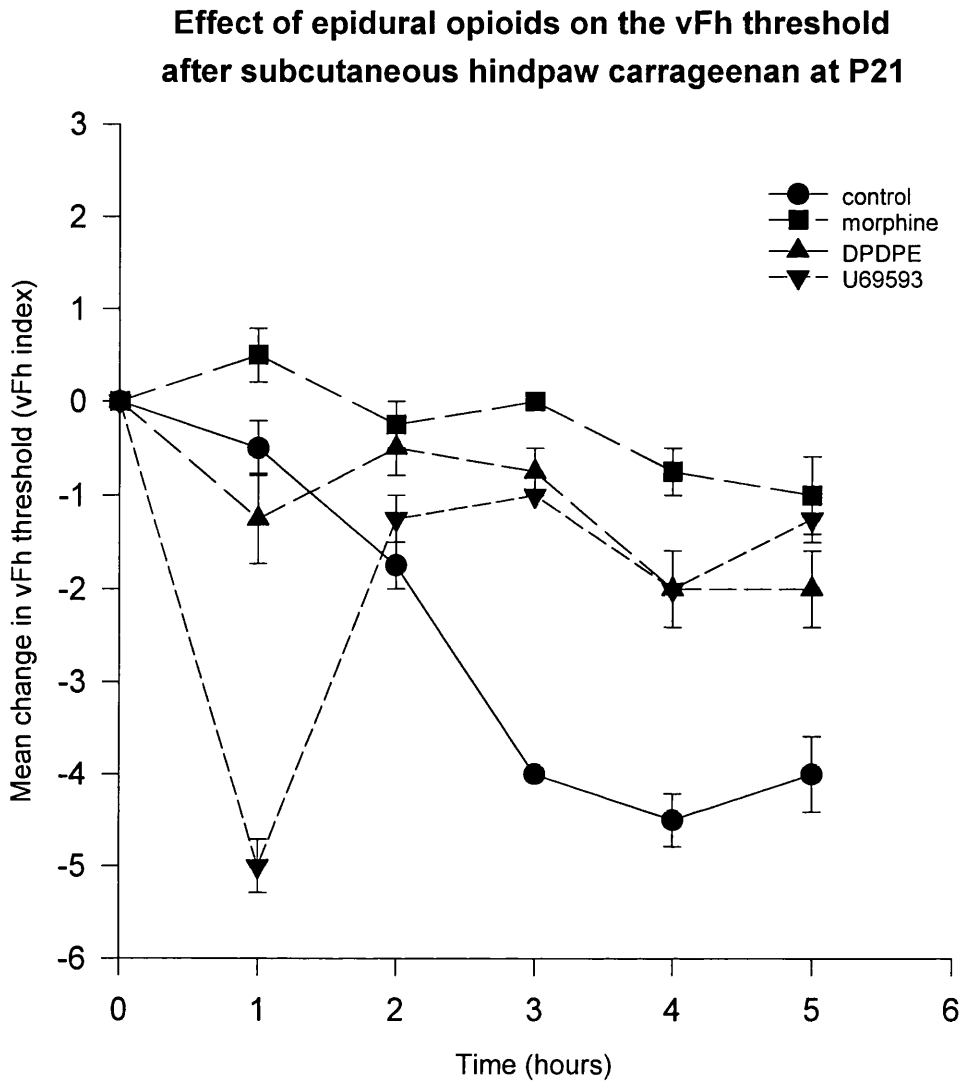


Figure 11C

A comparison of the effect low dose epidural morphine, DPDPE and U69593 with control animals on carrageenan induced allodynia at P10. Each agonist and control group is represented by a different symbol and line, joining mean (SEM) values.

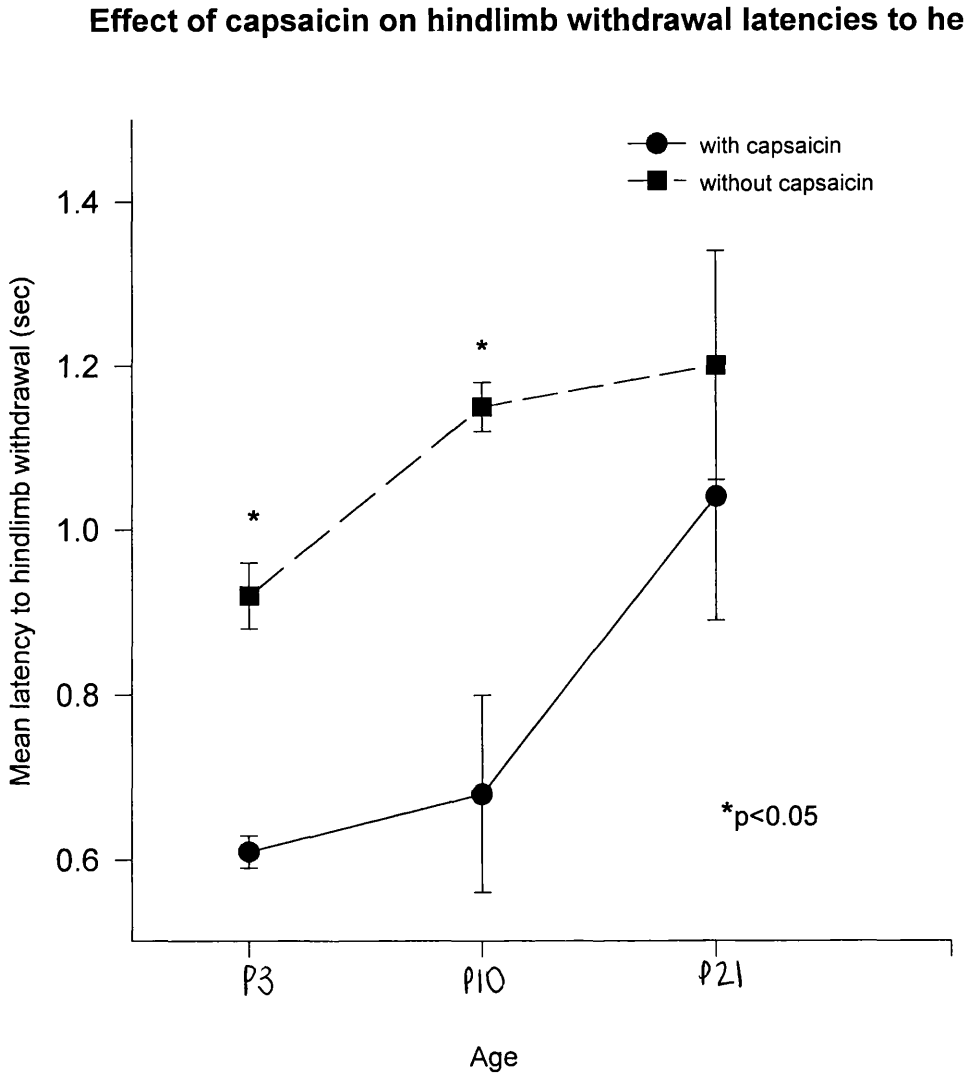


Figure 12

A comparison of the effects of age on the latency to hindlimb withdrawal from a heated waterbath in capsaicin treated and untreated limbs of each animal. The solid line joins mean (SEM) values for treated hindlimbs, and the dotted line for untreated hindlimbs. Statistically significant differences in values between ages are denoted by symbols.

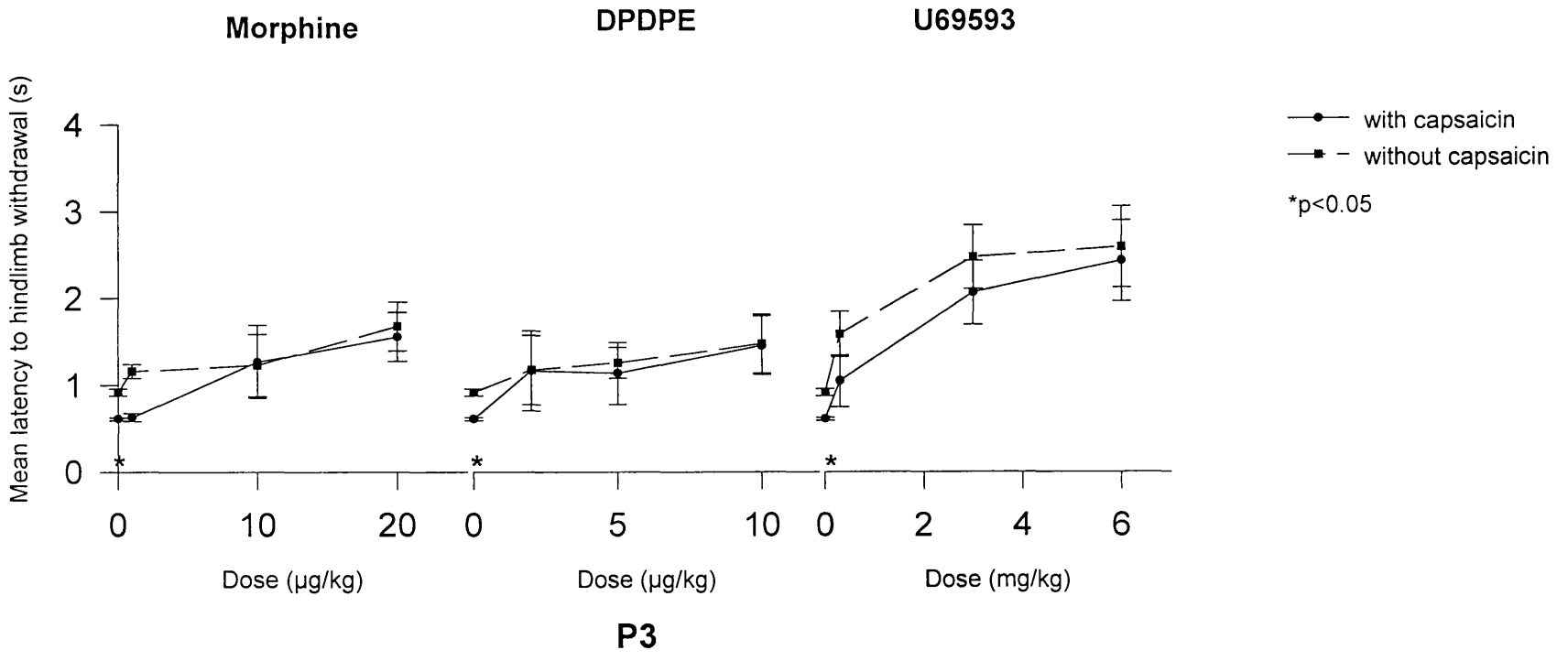


Figure 13A A comparison of the effects of morphine, DPDPE and U69593 on the latency to hindlimb withdrawal from a heated waterbath in capsaicin treated and untreated limbs of each animal at P3. The solid line joins mean (SEM) values for treated hindlimbs, and the dotted line for untreated hindlimbs. Statistically significant differences in values are denoted by symbols.

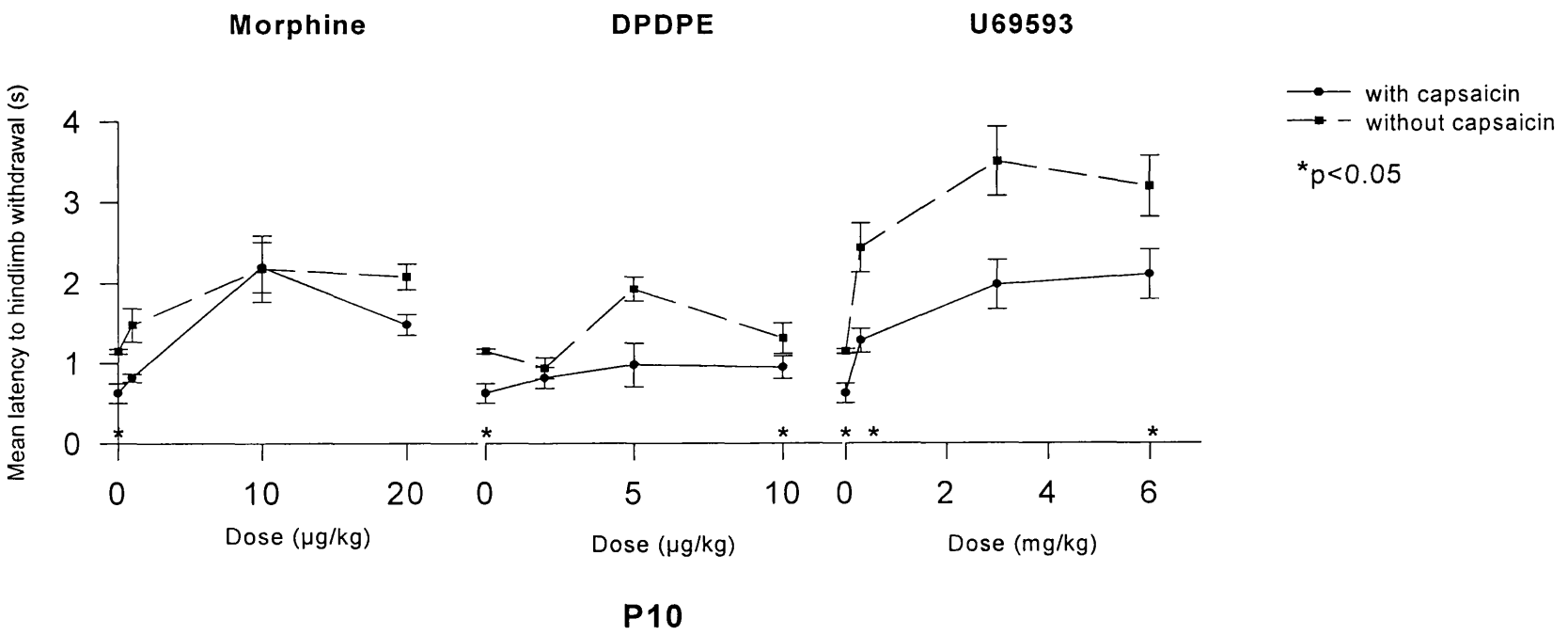


Figure 13B A comparison of the effects of morphine, DPDPE and U69593 on the latency to hindlimb withdrawal from a heated waterbath in capsaicin treated and untreated limbs of each animal at P10. The solid line joins mean (SEM) values for treated hindlimbs, and the dotted line for untreated hindlimbs. Statistically significant differences in values are denoted by symbols.

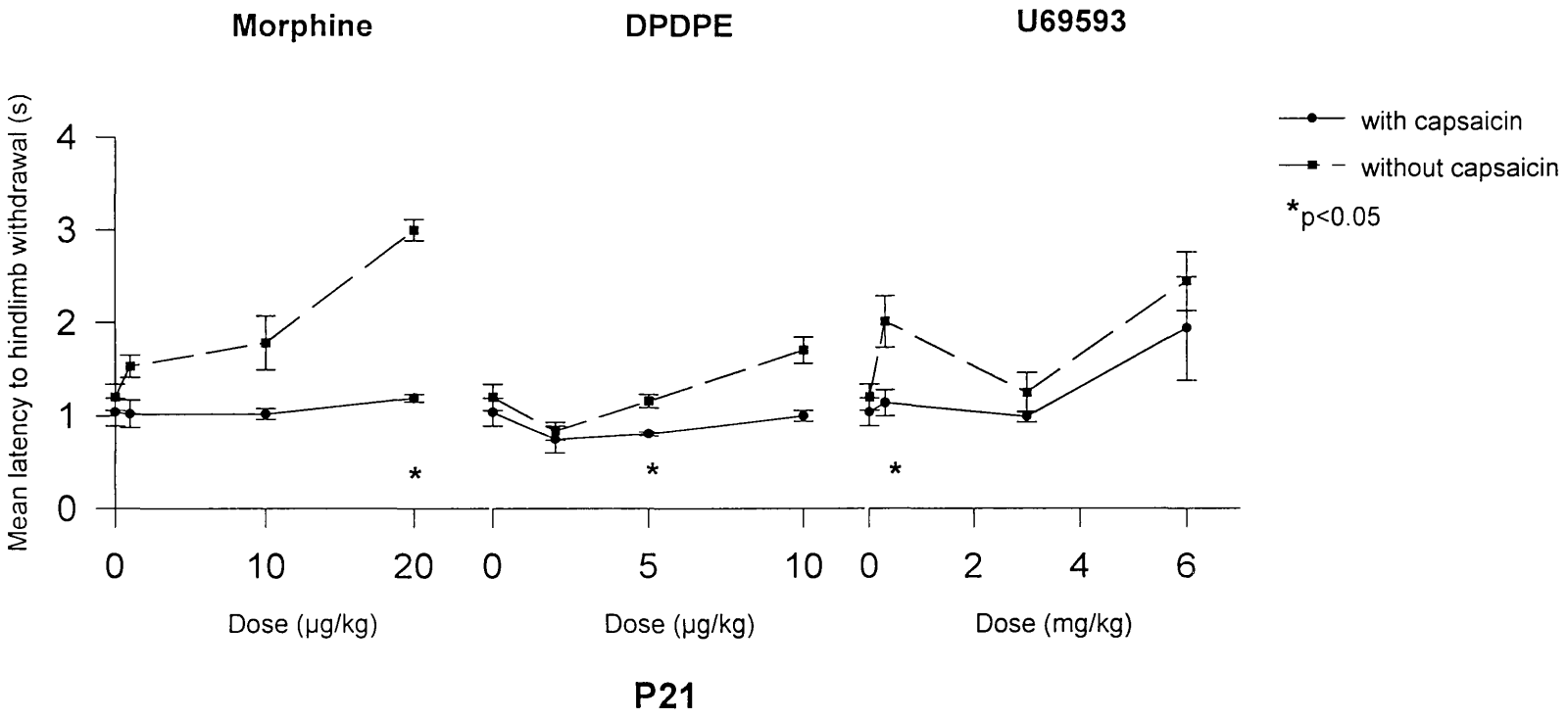


Figure 13C A comparison of the effects of morphine, DPDPE and U69593 on the latency to hindlimb withdrawal from a heated waterbath in capsaicin treated and untreated limbs of each animal at P21. The solid line joins mean (SEM) values for treated hindlimbs, and the dotted line for untreated hindlimbs. Statistically significant differences in values are denoted by symbols.

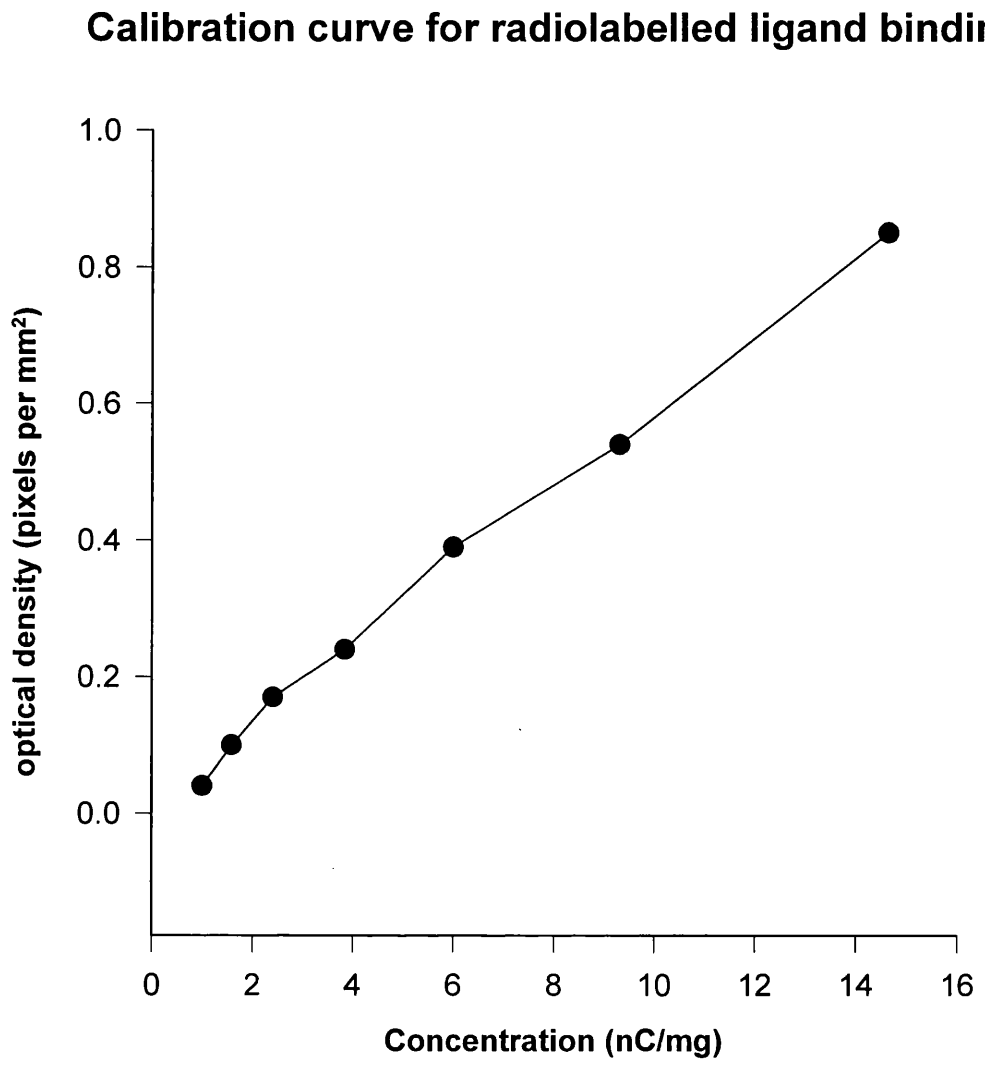


Figure 14

The calibration curve for radiolabelled ligand showing the relationship between radioactive concentration and optical density.

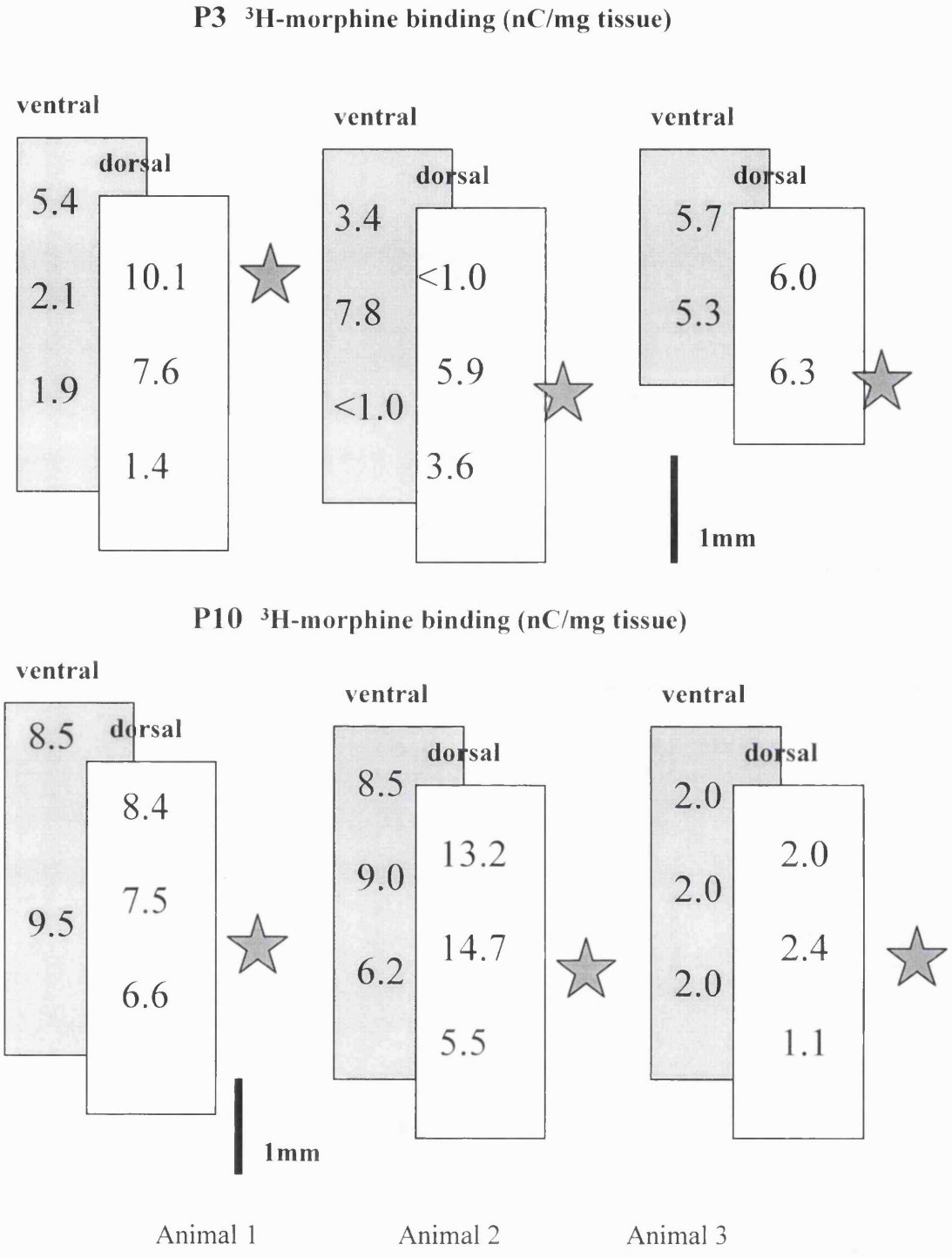


Figure 15

Represents the density of [3H]morphine binding in the lumbar spinal cord of P3 and P10 animals following epidural injection, with amounts in both dorsal and ventral areas shown. The stars denote the probable site of injection.

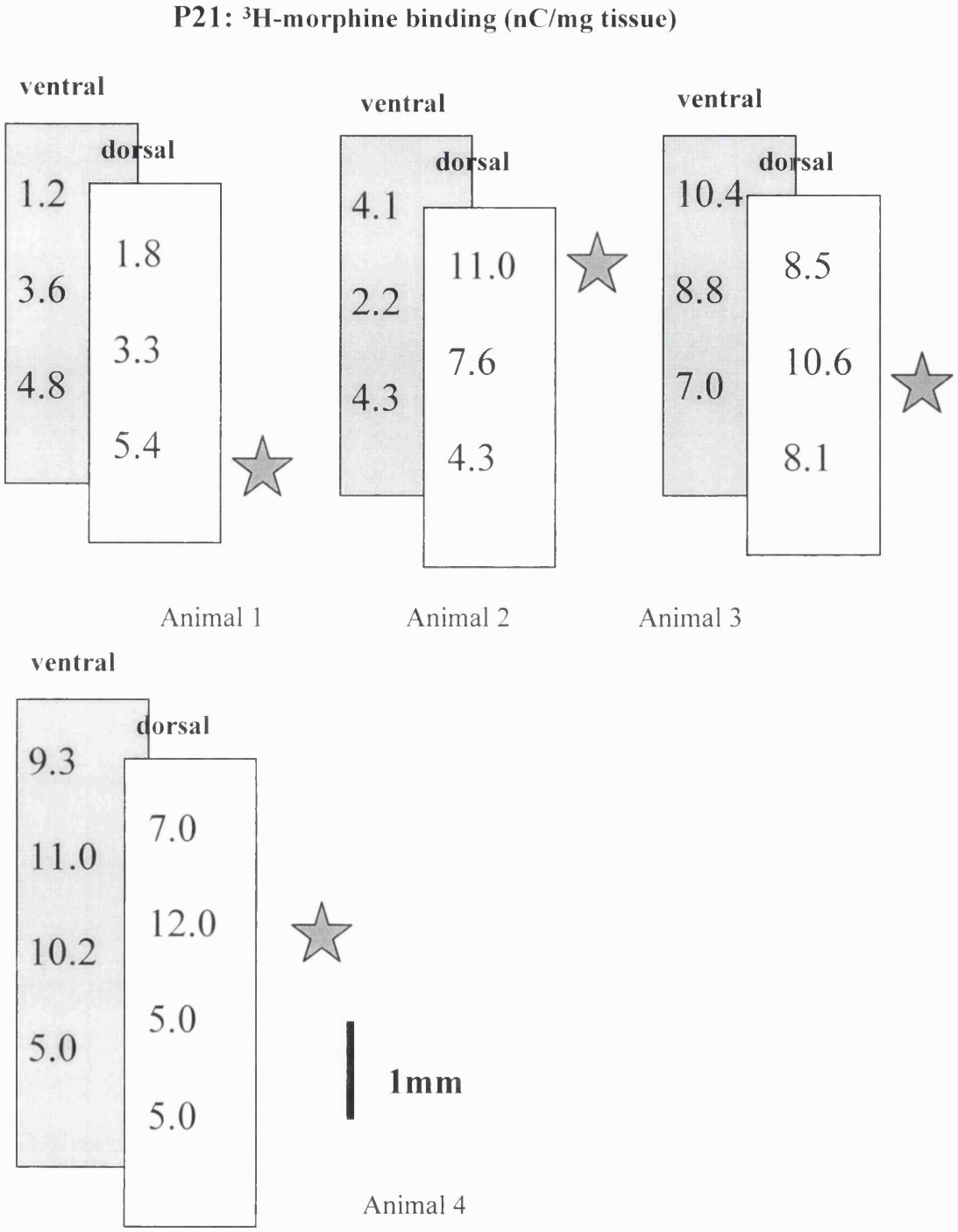


Figure 16

Represents the density of [3H]morphine binding in the lumbar spinal cord of P21 animals following epidural injection, with amounts in both dorsal and ventral areas shown. The stars denote the probable site of injection.

Von Frey threshold around wound during the postoperative study period

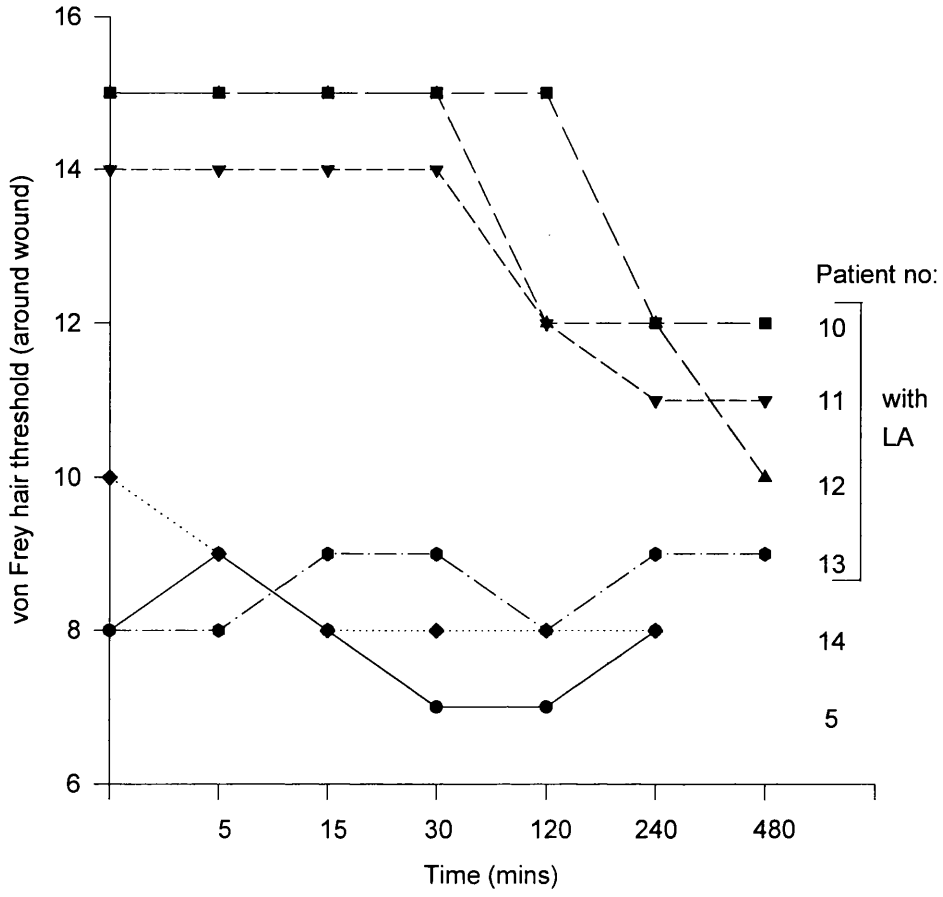


Figure 17

Change in von Fey hair threshold around the wound with time following the first postoperative dose of morphine, in patients with or without local anaesthetic wound infiltration.

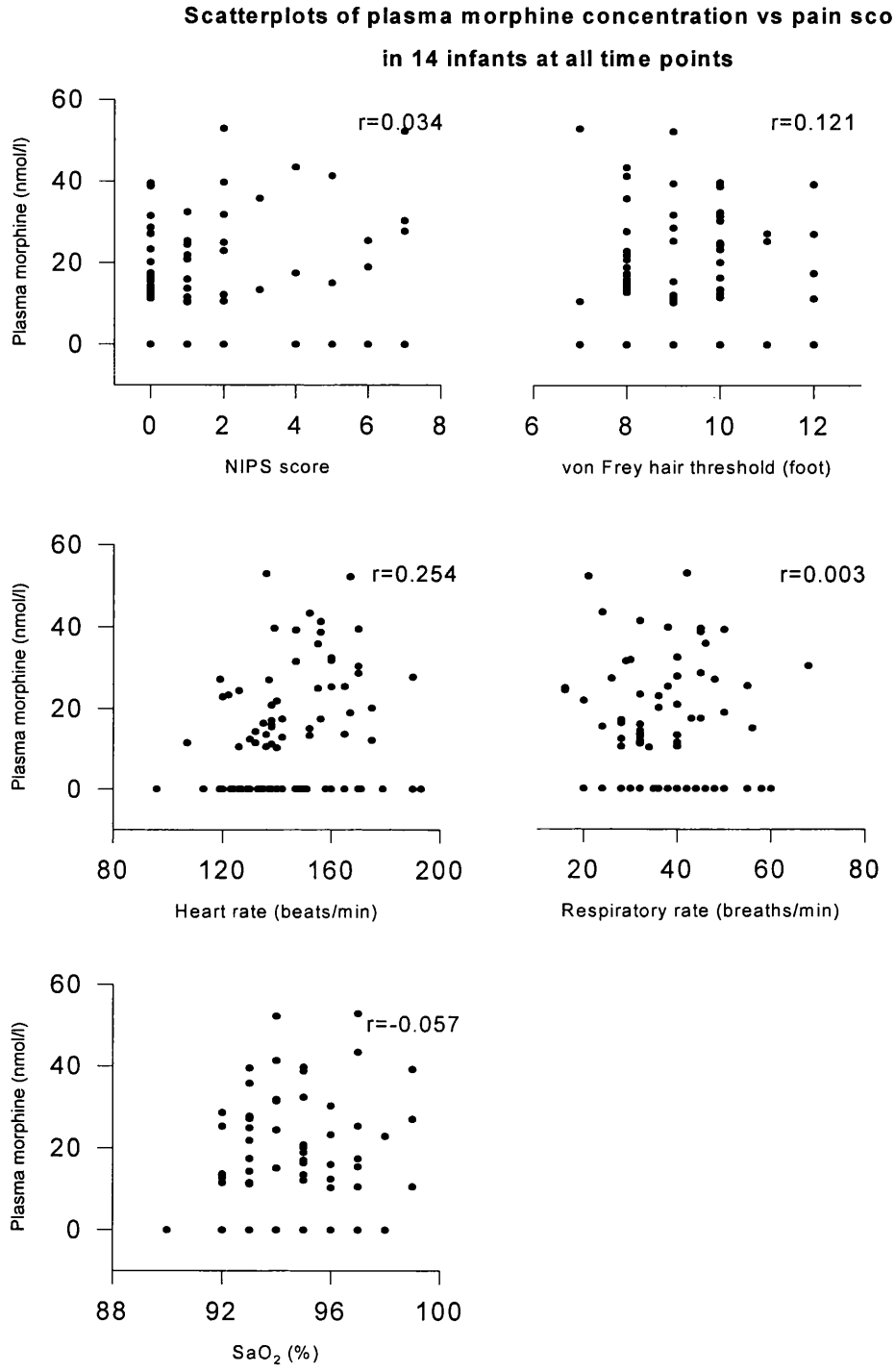


Figure 18 Relationship between plasma morphine concentrations and NIPS score, von Frey hair threshold, heart rate, respiratory rate and SaO_2 in infant group at all time points. Correlation coefficients are denoted by r.

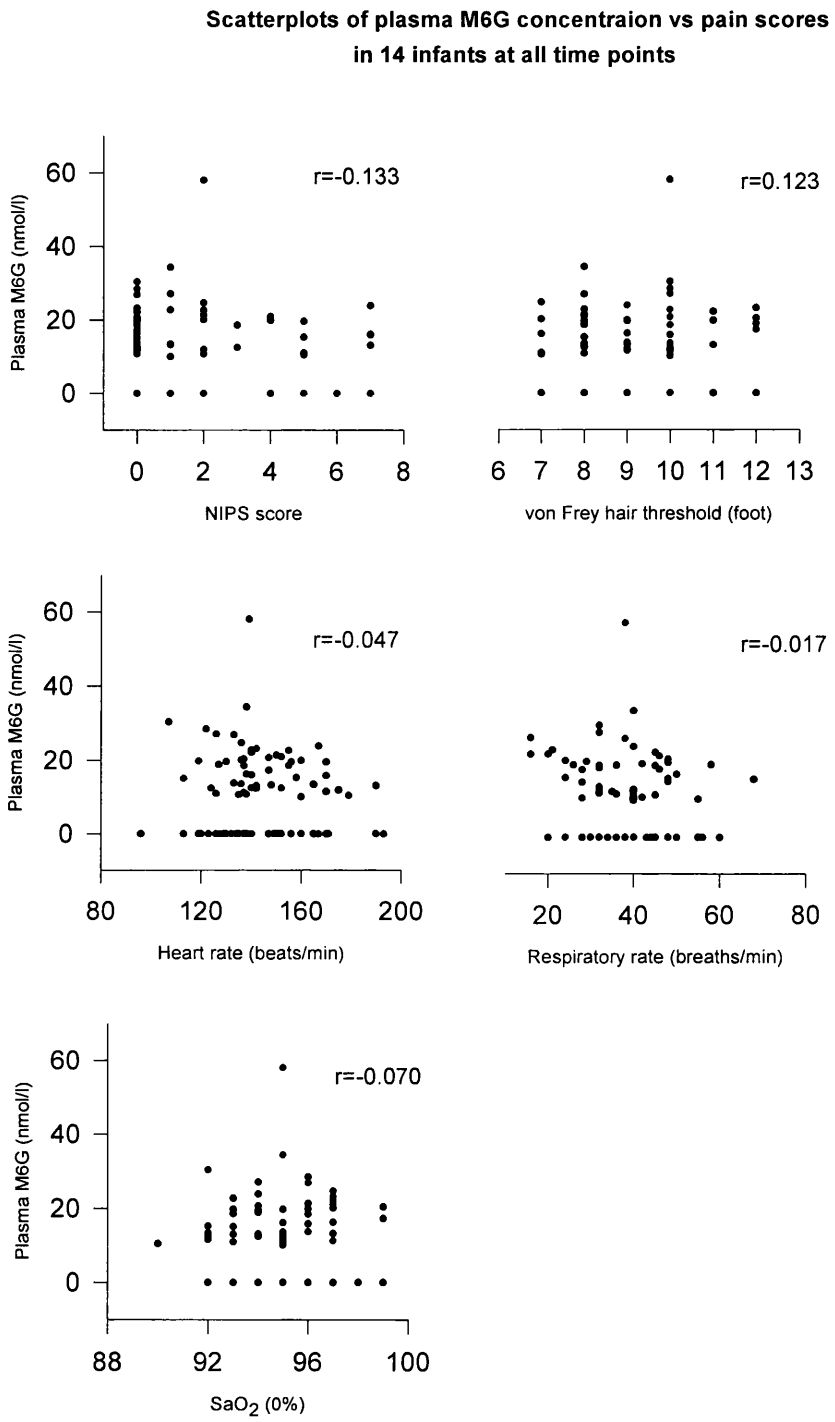


Figure 19 Relationship between plasma M6G concentrations and NIPS score, von Frey hair threshold, heart rate, respiratory rate and SaO_2 in infant group at all time points. Correlation coefficients are denoted by r.

Tables

vFh number (vFh index)	weight (gms)	log_{10}
1	0.02	-1.699
2	0.04	-1.398
3	0.06	-1.222
4	0.12	-0.921
5	0.16	-0.796
6	0.29	-0.538
7	0.31	-0.509
8	0.86	-0.066
9	0.97	-0.013
10	2.1	0.322
11	3.19	0.504
12	6.19	0.839
13	10.93	1.039
14	13.41	1.127
15	23.2	1.365
16	29.34	1.467
17	48	1.681
18	53	1.724
19	82	1.914
20	110	2.042

Table 1

Weights and log_{10} weights of each numbered von Frey hair when applied to a calibrated balance.

	epidural morphine µg/kg	systemic morphine µg/kg	epidural DPDPE µg/kg	epidural U69593 mg/kg
P3	2.24	312	2.4	1.5
P10	3.08	456	4.6	3.0
P21	3.64	528	4.0	4.75

Table 2

A comparison of the ED_{50}s of opioid agonists in P3, P10 and P21 animals.

	P3	P10	P21
Mean vFh threshold at time 0	8.5	10.75	13.55
at 1 hour	8.5	10.5	12.75
2 hours	7.5	10.25	11.5
3 hours	6.5	9	9.25
4 hours	6	8.25	8.75
5 hours	6	8	9.25

Table 3

Mean (±SEM) vFh thresholds following subcutaneous hindpaw carrageenan and saline epidurals at each age.

Patient no.	Age (months)	Weight (kg)	Surgery	Duration of surgery (mins)
1	9.5	7.7	Anal transposition	95
2	5	6.3	Duhamel pull-through	90
3	3	6.2	Nephrectomy	60
4	7.5	7.7	Duhamel pull-through	150
5	4	6.9	pyeloplasty	90
6	6	7.7	colostomy	90
7	6	7.5	PSARP	110
8	6.5	8.7	nephrectomy	45
9	3.5	5.9	Duhamel pull-through	120
10	6.5	8.4	pyeloplasty	90
11	5	6.6	pyeloplasty	90
12	5	8.7	nephrectomy	105
13	7	8.2	nephrectomy	70
14	9	11.1	pyeloplasty	120

Table 4

Patient data and surgical details.

Patient no.	Total morphine in theatre (µg/kg)	Local anaesthesia to wound?	Time from last intraop. to first postop. dose (mins)	Total morphine during post op study period (µg/kg)
1	80	No	63	80
2	100	Yes	295	160
3	80	No	55	100
4	140	No	235	220
5	140	No	575	80
6	30	No	250	40
7	160	No	407	60
8	20	No	55	40
9	100	No	N/A	nil given
10	160	Yes	240	100
11	160	Yes	255	60
12	120	Yes	355	140
13	120	No	240	200
14	120	Yes	665	40

Table 5

Details of morphine administration and local anaesthesia to wound.

Patient no.	Mean plasma morphine (nmol/l)	Mean plasma M6G (nmol/l)	Mean M6G:morphine ratio	Range of ratios
1 (n=6)	30.35	22.3	1.36	0.90-2.18
2 (n=2)	31.42	13.9	2.25	2.02-2.47
3 (n=4)	28.48	18.89	1.56	0.75-2.27
4 (n=6)	27.43	15.68	1.82	1.01-3.21
6 (n=1)	13.9	14.2	0.99	0.99
10 (n=4)	17.78	12.45	1.43	1.01-2.11
12 (n=4)	30.97	28.81	1.09	0.60-2.63
Overall mean	**25.76**	**18.03**	**1.5**	**0.60-3.21**

Table 6

Mean plasma morphine and M6G concentrations and their ratios, in patients in which this calculation was possible.

15. Appendix

N\R = not recorded
* = not recordable

Patient No. 1.
Age; 9.5 months.
Weight: 7.7kg.
Surgery; anal transposition.

Study time	NIPS score	vFh index	HR/min	RR/min	SaO_2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	N/R	N/R
Before 1st dose	7	9	96	20	96	*	*	131
5 mins post dose	7	9	167	21	94	52.2	23.9	*
15 mins	2	10	155	16	93	24.9	22.7	357
30mins	1	10	126	16	94	24.4	27.1	*
2 hours	1	8	140	20	93	21.8	22.8	*
4 hours	4	8	152	24	97	43.4	21.0	*
8 hours	0	9	138	24	97	15.4	16.3	*

Total dose of morphine in theatre: 80μg/kg, with no local anaesthesia supplementation. Time to first postoperative dose: 5 minutes.
Further postoperative doses (20μg/kg) given at: 3 hours, 3.5 hours and 5 hours.

Patient No. 2.
Age: 5 months
Weight: 6.3kg
Surgery: Duhamel pull-through.

Study time	NIPS score	vFh index	HR/min	RR/min	SaO_2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	*	*	*
Before 1st dose	6	7	190	60	95	*	*	*
5 mins post dose	6	8	167	50	95	18.9	*	161
15 mins	0	10	156	45	95	38.7	*	280
30 mins	4	7	165	50	94	*	*	215
2 hours	0	9	170	45	92	28.6	11.6	*
4 hours	0	9	170	45	93	39.5	19.6	177
8 hours	5	7	179	55	90	*	10.5	*

Total dose of morphine in theatre: 100 μg/kg, with local anaesthesia supplementation.
Time to first postoperative dose: 6 hours.
Further postoperative doses (20μg/kg) given at: 35 minutes, 1 hr 50 mins, 3 hours, 3 hrs 30 mins, 3 hrs 55 mins, 4 hrs 30 mins, 7 hours.

Patient No. 3.
Age: 3.5 months.
Weight: 6.2 kg.
Surgery: nephrectomy.

Study time	NIPS score	vFh index	HR/min	RR/min	SaO_2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	*	*	*
Pre-dose	7	10	170	68	96	30.3	15.9	*
5 mins post dose	0	12	147	50	99	39.2	17.3	*
15 mins	0	12	137	48	99	27	20.4	*
30 mins	0	12	142	45	97	17.4	23.2	*
2 hours	0	11	140	46	97	*	22.2	126
4 hours	4	9	160	58	N/R	*	19.9	121
8 hours	1	9	148	32	N/R	*	13.3	105

Total dose of morphine in theatre: 80μg/kg, with no local anaesthesia supplementation.
Time to first postoperative dose: 30 minutes.
Further postoperative doses (20μg/kg) given at: 2 hrs 5 mins 4 hrs 15 mins
2 hrs 35 mins 6 hrs 45 mins.

Patient No. 4.
Age: 7.5 months.
Weight: 7.73kg.
Surgery: Duhamel pull-through.

Study time	NIPS score	vFh index (foot)	HR/min	RR/min	SaO_2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	*	*	176.0
Pre-dose	7	8	193	28	95	*	*	*
5 mins post dose	1	10	160	40	95	32.4	10.1	*
15 mins	0	10	175	36	95	20.1	11.9	101.1
30 mins	2	9	175	32	95	12.1	12.0	*
2 hours	5	8	156	32	94	41.3	19.6	221.6
4 hours	0	10	147	29	94	31.5	20.7	178.1
8 hours	0	11	119	26	93	27.2	19.8	*

Total dose of morphine in theatre: 140μg/kg, with no local anaesthesia supplementation.
Time to first postoperative dose: 3 hours.
Further postoperative doses (20μg/kg) given at: 45 mins 2 hrs 45 mins
1 hr 5mins 5 hrs 20 mins
1 hr 30 mins 6 hrs 15 mins
1 hr 50 mins 7 hrs 20 mins
2 hrs

Patient No. 5.
Age: 4 months.
Weight 6.9 kg.
Surgery: pyeloplasty.

Study time	NIPS score	vFh index (foot)	vFh index (wound)	HR /min	RR /min	SaO2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	*	*	124.0
Pre-dose	5	7	8	138	44	97	*	*	*
5mins	1	8	9	138	32	96	15.9	*	203.7
15mins	1	9	8	132	40	93	11.5	*	*
30mins	5	7	N/R	126	42	93	*	11.0	*
2hrs	1	7	7	151	40	97	*	*	294.8
4hrs	0	11	8	142	40	94	*	13.1	111.5
8hrs	0	10	N/R	137	40	97	*	11.3	*

Total dose morphine in theatre: 140μg/kg, with no local anaesthesia supplementation.
Time to first dose: 8.5 hours.
Further doses (20μg/kg) given at: 32 mins, 3 hrs 20 mins and 5 hrs and 15 mins.

Patient No. 6.
Age: 6 months.
Weight: 7.7kg.
Surgery: colostomy.

Study time	NIPS score	vFh index (foot)	HR/min	RR/min	SaO_2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	0.0	*	0.0
Pre-dose	5	7	130	40	94	0.0	*	0.0
5 mins post dose	0	8	132	32	93	14.3	*	*
15 mins	0	10	140	35	95	*	12.6	*
30 mins	0	10	124	32	94	*	12.5	0.0
2 hours	0	10	136	32	95	13.5	13.6	*
4 hours	0	10	137	28	96	*	18.5	102.9
8 hours	0	9	133	32	96	*	13.8	*

Total dose of morphine in theatre: 60μg/kg, with no local anaesthesia to wound.
Time to first dose: 2 hours.
Further doses (20μg/kg) given at: 2 hrs 25 mins.

Patient No. 7.
Age: 6 months.
Weight: 7.5 kg.
Surgery: PSARP.

Study time	NIPS score	vFh index (foot)	HR/min	RR/min	SaO_2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	0.0	*	0.0
Pre-dose	6	10	160	48	94	*		0.0
5 mins post dose	1	11	160	38	92	25.3	*	*
15 mins	0	12	138	32	93	11.2	*	*
30 mins	0	11	127	28	94	*	*	*

2 hours	0	12	127	32	94	0.0	18.9	0.0
4 hours	2	11	120	32	96	*	*	0.0
8 hours	0	11	126	30	94	*	*	*

Total dose of morphine in theatre: 160μk/kg, with no local anaesthesia.
Time to first dose: 6.5 hours.
Further doses (20μg/kg) given at: 2 hrs 7 mins and 5 hrs 32 mins.

Patient No. 8.
Age: 6.5 months.
Weight: 8.7kg.
Surgery: nephrectomy.

Study time	**NIPS score**	**vFh index (foot)**	**HR/min**	**RR/min**	**SaO_2 (%)**	**Morphine (nmol/L)**	**M6G (nmol/L)**	**M3G (nmol/L)**
Baseline	N/R	N/R	N/R	N/R	N/R	0.0	*	0.0
Pre-dose	6	8	149	60	97	0.0	*	0.0
5 mins post dose	6	9	165	55	97	25.4	*	0.0
15 mins	5	8	152	56	94	15.0	*	0.0
30 mins	4	8	156	43	93	17.4	*	0.0
2 hours	0	8	130	36	95	*	19.7	*
4 hours	2	7	135	40	95	*	10.7	0.0
8 hours	0	8	138	38	95	*	*	0.0

Total morphine given in theatre: 20μk/kg, with no locla anaesthesia to wound.
Time to first dose of morphine: 10 minutes.
Further morphine (20μg/kg) given at 27 mins.

Patient No. 9.
Age: 3.5 months.
Weight: 5.9 kg.
Surgery: Duhamel pull-through.

Study time	**NIPS score**	**vFh index (foot)**	**HR/min**	**RR/min**	**SaO_2 (%)**	**Morphine (nmol/L)**	**M6G (nmol/L)**	**M3G (nmol/L)**
Baseline	N/R	N/R	N/R	N/R	N/R	*	*	0.0
at 4 hours post surgery	0	11	113	20	97	*	*	*
at 6 hours post surgery	0	8	151	32	93	*	*	0.0

Total morphine in theatre: 100μk/kg, with no local anaesthesia.
No further morphine given during the study period.

Patient No. 10.
Age: 6.5 months.
Weight: 8.4 kg.
Surgery: pyeloplasty.

Study time	**NIPS score**	**vFh index (foot)**	**vFh index (wound)**	**HR /min**	**RR /min**	**SaO2 (%)**	**Morphine (nmol/L)**	**M6G (nmol/L)**	**M3G (nmol/L)**
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	*	*	0.0
Pre-dose	7	8	>14	150	40	94	*	*	101.2
5mins	7	8	>14	190	40	93	27.7	13.1	*
15mins	1	8	N/R	165	32	92	13.6	13.5	107.1

30mins	0	8	N/R	142	32	92	12.8	12.4	*
2hrs	0	8	N/R	138	28	95	17.0	10.8	*
4hrs	0	8	12	119	32	97	*	*	*
8hrs	0	9	13	n/r	32	95	0.0	*	102.8

Total morphine in theatre: 160 μg/kg, with local anaesthesia.
Time to first postoperative dose: 3 hrs.
Further doses (20μg/kg) of morphine given at: 3 hrs 35 mins 3 hrs 40 mins
5 hrs 10 mins 6 hrs 10 mins

Patient No. 11.
Age: 5 months.
Weight: 6.6 kg.
Surgery: pyeloplasty.

Study time	**NIPS score**	**vFh index (foot)**	**vFh index (wound)**	**HR /min**	**RR /min**	**SaO2 (%)**	**Morphine (nmol/L)**	**M6G (nmol/L)**	**M3G (nmol/L)**
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	0.0	*	0.0
Pre-dose	4	9	15	135	30	98	0.0	*	*
5mins	2	8	N/R	120	36	98	22.9	*	*
15mins	1	9	N/R	126	28	99	10.5	*	*
30mins	0	10	N/R	134	28	98	*	*	*
2hrs	0	9	12	171	28	98	*	*	*
4hrs	0	10	12	130	28	96	12.4	*	*
8hrs	1	9	10	140	34	96	10.3	*	0.0

Total dose morphine in theatre: 160μk/kg, with local anaesthesia.
Time to first dose: 3 hrs, 45 mins.
Further doses (20μg/kg) given at: 3 hrs 45 mins and 6 hours 50 mins.

Patient No. 12.
Age: 5 months.
Weight: 8.7 kg.
Surgery: nephrectomy.

Study time	**NIPS score**	**vFh index (foot)**	**vFh index (wound)**	**HR /min**	**RR /min**	**SaO2 (%)**	**Morphine (nmol/L)**	**M6G (nmol/L)**	**M3G (nmol/L)**
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	0.0	*	*
Pre-dose	7	7	14	140	48	95	0.0	16.1	*
5mins	2	7	N/R	136	42	97	52.9	20.1	*
15mins	2	7	N/R	136	40	97	10.5	24.7	166.6
30mins	0	8	N/R	133	38	96	*	26.9	136.1
2hrs	2	8	12	150	48	96	*	21.4	115.4
4hrs	1	8	11	138	40	95	20.8	34.4	198.8
8hrs	2	10	11	139	38	95	39.7	58.1	339.7

Total dose morphine in theatre: 120μg/kg, with local anaesthesia.
Time to first dose morphine: 4 hrs 15 mins.
Further doses givan at: 1 hr 35mins 7 hrs 5 mins
3 hrs 55 mins 8 hrs
5 hrs 5 mins

Patient No. 13.
Age: 7 months.
Weight: 8.2 kg.
Surgery: nephrectomy.

Study time	NIPS score	vFh index (foot)	vFh index (wound)	HR /min	RR /min	SaO2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	*	0.0	*
Pre-dose	5	8	10	158	48	92	*	15.3	*
5mins	3	8	9	155	46	93	35.8	18.6	*
15mins	3	8	8	152	40	92	13.3	12.5	*
30mins	0	8	8	113	28	93	*	15.1	*
2hrs	0	10	8	107	32	92	11.5	30.4	145.5
4hrs	0	10	8	122	32	96	23.3	28.5	162.9
8hrs	N/R	N/R	N/R	N/R	N/R	N/R	133.2	32.7	175.6

Total morphine in theatre: 120µg/kg, with local anaesthesia.
Time to first dse: 3 hours.
Further doses given at:

30 mins	3 hrs 30 mins
55 mins	3 hrs 50 mins
1 hr 18 mins	4 hrs 30 mins
2 hrs 30 mins	4 hrs 50 mins
2 hrs 50 mins	5 hrs 10 mins

Patient No. 14.
Age: 9 months.
Weight: 11.1kg.
Surgery: pyeloplasty.

Study time	NIPS score	vFh index (foot)	vFh index (wound)	HR /min	RR /min	SaO2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	*	0.0	0.0
Pre-dose	6	8	8	170	32	95	0.0	0.0	0.0
5mins	2	9	8	160	30	94	31.8	0.0	*
15mins	0	10	9	135	28	95	16.3	*	*
30mins	0	10	9	129	24	96	*	*	0.0
2hrs	0	10	8	123	24	97	*	*	0.0
4hrs	0	8	9	147	36	93	*	*	*
8hrs	1	9	9	137	38	94	0.0	0.0	*

Total morphine in theatre: 120 µg/kg, with local anaesthesia.
Time to first dose: 9 hours.
Further doses given at: 2 hours 55 mins.

Patient No. 15. Neonatal data
Age: 3 days.
Weight: 2.5kg.
Surgery: laparotomy.

Study time	NIPS score	vFh index (foot)	vFh index (wound)	HR /min	RR /min	SaO2 (%)	Morphine (nmol/L)	M6G (nmol/L)	M3G (nmol/L)
Baseline	N/R	N/R	N/R	N/R	N/R	N/R	0.0	0.0	0.0
Pre-dose	5	8	8	165	56	99	0.0	0.0	0.0
5mins	3	8	9	161	52	99	0.0	0.0	0.0
15mins	3	8	9	164	49	99	0.0	0.0	0.0
30mins	3	11	9	155	47	99	0.0	0.0	0.0
2hrs	0	9	8	143	33	99	11.2	0.0	0.0
4hrs	0	9	8	137	38	99	*	0.0	0.0
8hrs	0	8	7	124	32	98	10.4	*	0.0

Total morphne in theatre: nil, but with local anaesthesia supplementation.
Time to first dose: 2 hrs 30 mns.
Further doses (20μg/kg) given at: 1hr 22 mins.

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Opioid Systems and the Newborn

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Opioid Systems and the Newborn

The neonatal central nervous system (CNS) is both structurally and functionally immature, and significant changes in opioid analgesic mechanisms occur both before and after birth. Recent research has shown that neonatal pain pathways are quite different from those of older children and adults and it is thought that analgesic mechanisms are therefore also likely to differ significantly [18]. However, our understanding of the neurobiology and development of pain pathways is still limited.

In this review we shall discuss our current understanding of the developmental neurobiology of opioids and the action of opioids in the human neonate. We will briefly review background studies of opioid actions in adults and then focus on actions in neonates. The discussion will include both basic laboratory studies and clinical pharmacology, issues which are usually considered separately in the literature. However, if we are to advance our knowledge and improve our management of neonatal pain it is important that they are discussed in relation to one another.

Background

Whilst Sertürner isolated morphine, the active ingredient of opium, in 1806, naming it after Morpheus, the God of Dreams, it was not until 1973 that opioid receptors were first described [58][69][77], shortly before the identification of endogenous opioid peptides [33]. Despite its side effects, morphine is still widely used in the treatment of acute and chronic pain and our knowledge of opioid systems in adults has been the

subject of several detailed recent reviews [15][37][56]. Current thinking about adult opioid mechanisms will therefore only be briefly summarised.

Opioids act by binding with high affinity membrane receptors which comprise three major groups: the mu, delta and kappa receptors; and each receptor can produce different effects. Further division into subtypes remains controversial although there is now evidence of differential effects of delta receptors in mice [36]. We still have little knowledge of the specific function of each receptor, but the recent isolation and cloning of all three will significantly advance research into their roles [65][78] hopefully assisting the search for clinically useful opioids with highly specific functions and minimal side effects. The endogenous ligands for the opioid receptors are the opioid peptides which can be divided into three groups: enkephalins, endorphins and dynorphins. These endogenous opioids have some agonist activity at all three receptors but each receptor possesses distinct ligand selectivity [41].

Opioid effects are mediated by several mechanisms in all areas of the CNS, with the predominant effect of decreasing neuronal firing. By stimulation of opioid receptors situated pre-synaptically on the terminals of neurones opioids reduce the release of excitatory neurotransmitters involved in pain pathways [32]. Stimulation of post-synaptic receptors inhibits neurotransmission by producing hyperpolarisation thus reducing evoked activity [47]. A third possible mechanism involves the inhibition of one neurone which lifts the inhibition on the second, allowing it to become active in its own inhibitory capacity [15].

The spinal cord is a very important site of action of opioids, where they act to modulate pain pathways. This is evident clinically in the profound analgesia produced by the administration of opioids in the epidural and subarachnoid spaces. Afferent nociceptor Aδ and C fibres synapse in laminae 1 and 2 (the substantia gelatinosa) of the dorsal horn [8] and it is here that the majority of spinal cord opioid receptors are located, although a smaller percentage are found in the deeper layers of the dorsal horn [15]. In the rat, mu receptors predominate in the spinal cord, with delta and kappa receptors comprising less than a third of the total. More than half of these opioid receptors are located pre-synaptically [7].

The recent proliferation of research in adult pain mechanisms has demonstrated that pain transmission in the spinal cord is not through a simple circuit, but rather the arrival of noxious stimuli from the periphery via primary afferent fibres initiates a cascade of events in the dorsal horn and the complexity of these reactions allows enhancement or modulation of the pain signal. Neurotransmitters are released from afferent fibres, interneurones, projection and descending neurones and there is considerable interaction between these fibres. The effects of excitatory amino acids such as glutamate in particular have sparked considerable recent interest. Glutamate is abundant in the spinal cord, and is released from both myelinated and unmyelinated fibres. Its effects are mediated by NMDA (N-methyl-D-aspartate) receptors as well as non-NMDA receptors, and the former have been shown to play an important role in persistent pain states such as hyperalgesia and allodynia [84]. The NMDA receptor requires repeated or sustained stimulation by C-fibres for its activation, and release of glutamate from these fibres stimulates the receptor and initiates a series of

intracellular events, resulting in the phosphorylation of intracellular proteins and the opening of an ion channel normally blocked by magnesium. The resultant increase in intracellular calcium allows a greatly augmented response of the receptor to glutamate; this is essentially an elaborate positive feed-back mechanism. The activated NMDA receptor leads to many forms of central hyperalgesia [14]. There is now growing evidence that NMDA receptors are involved in the mechanism of opioid tolerance [51]. Mu receptor stimulation by exogenous opioids seems to lead to the same intracellular events which result in 'wind-up' of the NMDA receptor and these same mechanisms may also uncouple G-proteins from the mu receptor. This in turn results both in a decreased response of the mu receptor to opioids, and activation of the NMDA receptor [5][51]. It is now becoming apparent that opioid receptors do not function in isolation but rely in part on other receptors such as the NMDA to elicit their effects.

Supraspinal opioid systems contribute to analgesia by several mechanisms including decreasing nociceptive input to the brain by altering descending and ascending control systems, therefore reducing the central perception of pain. Intraventricular injection of opioids in humans is clinically effective in systemically inactive doses, providing evidence for the role of supraspinal sites in the production of analgesia [44]. Intracerebral microinjection of opioid agonists in several species has allowed the localisation of supraspinal receptors and their contribution to analgesia to be investigated [85]. In rats, opioid receptors are widely distributed throughout the CNS, most densely in the brainstem, thalamus, amygdala, hippocampus and cortex. Mu receptors are found in the majority of these areas, whereas delta receptors are more

restricted, found mostly in forebrain regions with poor binding in the midbrain and brainstem. Kappa receptors are widely distributed throughout the forebrain, midbrain and brainstem, comprising only 10% of the total number of receptors in the rat brain, but up to a third in humans [50]. The periaqueductal grey matter and medulla are involved in the descending inhibitory control of dorsal horn neurones. Several supraspinal sites of receptors are found close to the origin of these descending controls, and microinjection of opioids into these areas produces analgesia by their stimulation.

Opioids also act outside the CNS and possess 'local analgesic' effects. Laboratory rat studies have demonstrated the peripheral antinociceptive actions of opioid agonists in inflammatory pain models [73]. It is thought that inflammation initiates the axonal transport of opioid receptors to the periphery and increases their number on peripheral nerve terminals; inflammation disrupts the perineurium exposing neuronal opioid receptors [72]. Endogenous peptides are produced by immune cells infiltrating the inflamed tissue [71]. Although clinical evidence is inconclusive, the results of studies of intra-articular morphine for postoperative knee pain are encouraging [12][31]. The local action of a topically applied, systemically inactive opioid lacking central side effects should have major benefits.

The Developmental Neurobiology of Opioid Systems

A lack of data in humans has lead to increasing reliance on basic studies of laboratory animals in the understanding of the development and function of opioid systems in the

human neonate, and the rat has become the acceptable model. The sequence of events that take place in the neurological development of the rat and human CNS are very similar but it is difficult to make exact comparisons. Generally speaking, the newborn rat CNS is approximately equivalent to a 24 week human foetus at birth, a full term neonate at seven days and a toddler at three weeks. Rat CNS maturation is extremely accelerated compared with the human and is mature by three to four weeks [19].

The ontogeny of opioid receptors and ligands

Opioid receptors and endogenous opioid peptides appear very early in CNS development, and their ontogeny is non-uniform in the neonatal rat. The majority of receptors in the adult are mu and delta, but early in the postnatal period, on postnatal day 6 (P6), mu and kappa receptors predominate [45]. At birth, opioid receptors are present in several areas, notably the neostriatum, olfactory tubercle, rostral midbrain and later in the other areas such as the thalamus and hypothalamus. Delta receptors, however, have a very different ontogeny to mu and kappa receptors and are present in low densities in the brain at birth, progressing to maximum binding in the third post-natal week. A similar pattern of ontogenesis occurs in the spinal cord with the early appearance of mu and kappa receptors and delay in the development of delta receptors until the initial post-natal period [3].

The pattern of development of mu receptors has been elegantly demonstrated in the rat by in vitro quantitative receptor autoradiography. Mu receptor binding sites are concentrated in the superficial laminae of the dorsal horn in the adult, but in contrast

at P1, both the superficial and deeper laminae have relatively high densities. The density of mu opioid binding sites peaks at P4, then declines gradually to reach adult levels by the third postnatal week [38]. The change in distribution of mu opioid binding sites in neonatal and adult rat spinal cord is shown in figure 1 below.

(FIGURE 1 near here)

Endogenous opioids appear in the mouse brain before opioid receptors on E11.5 [67], and enkephalin-like immunoreactivity detected in rat brain at E18 [59]. In human foetal spinal cord, enkephalin-like immunoreactive fibres have been detected as early as 10 weeks [10]. At embryonic day 16 (E16), endorphin levels are much greater than enkephalin levels with highest levels in the phylogenetically newer areas of the brain such as the diencephalon and telencephalon, with a rich distribution in the limbic system. These two peptide systems appear to develop differently, with a marked increase in enkephalin levels during the initial perinatal period. Between P6 and P25, both endorphin and enkephalin concentrations rise to approximately adult levels [6].

Development of spinal pain pathways related to opioid function

As described, opioids play a role in pain transmission in adults by initiating descending inhibitory controls from the brainstem to the spinal cord, and by reducing the resultant activity primarily of C fibres but also Aδ fibres in the substantia gelatinosa.

In the neonatal rat, considerable development of these pathways occurs postnatally. Although anatomically intact at birth, there is no functional descending inhibitory control until P10-12, reaching adult levels only at P22-24. This is possibly owing to a delay in the development of interneurones and to low levels of neurotransmitters in the postnatal period [21], and presumably underlies the lack of brainstem stimulus produced analgesia in newborn rats until P21 [79]. C fibre and small diameter A fibres grow into the spinal cord a few days before birth, C fibres developing a considerable number of connections in the early post-natal period. However, C fibres are unable to evoke spike activity in the dorsal horn until the second post natal week. A fibre terminals are more widespread in the newborn terminating superficially in lamina 2 as well as deeper laminae [20]. There is evidence that A fibre input is enhanced during this time [34][35].

In the substantia gelatinosa of the neonatal rat, NMDA induced elevations in intracellular calcium are markedly raised, gradually falling to adult levels. C fibre induced, NMDA mediated effects in the adult such as wind-up and central sensitisation are probably more marked in the neonate, and may contribute to the establishment of C fibre connections in the cord. As in the adult, opioid receptors possibly have close links with the NMDA receptor, and their function in this respect is still unknown [17].

Analgesic actions

There is ample evidence that these endogenous opioid systems contribute to functioning analgesic mechanisms in the early postnatal period, although the pattern of development of effects is difficult to establish as nociceptive physiology itself changes markedly with age, and a comparable nociceptive stimulus at different ages may be impossible to achieve. Exogenous opioids and their analgesic efficacy have been investigated in the developing rat, but difficulties in behavioural testing of neonatal animals may in part explain apparent discrepancies in the results. Preliminary data from our own laboratory have shown major difficulties with thermal testing of neonatal rats, as limb withdrawal tests seem to rely on many variables. However, despite these problems of methodology, the effect of morphine was shown to increase with age in neonatal rat pups, progressing to a 40- fold analgesic potency at P14 compared to P3 when demonstrated by a limb withdrawal test of thermal analgesia [22]. Similar results were obtained in a further study of morphine analgesia in developing rats, in which it was shown that morphine was without any detectable effect to a tail-flick nociceptive test until P12, reaching analgesic potency comparable to adult levels at P14 [4]. Comparing morphine, a potent mu agonist and ketocyclazocine, a kappa agonist in the developing rat revealed that morphine was more potent than ketocyclazocine in producing analgesia to limb withdrawal tests of thermal nociception, exhibiting analgesia in a dose dependant manner as early as three days in doses starting of 1-2mg/kg, with peak effects at 14 days. Ketocyclazocine was more effective at producing analgesia to a mechanical stimulus (a blunt probe applied with a force of 10-15g) in younger animals, with onset of effect at seven to ten days, this differential development in analgesic patterns confirming a non-uniform ontogeny of opioid receptors [4]. However, unlike thermal and mechanical tests of pain, the

response of neonatal rats to the tonic nociceptive effects using the formalin test revealed a similar response to that seen in adults at P3. In this study it was revealed that this response was attenuated by morphine in a dose of 1-2mg/kg, in a manner similar to adults [55].

Early studies have warned of the susceptibility of neonates to the CNS depressant effects of opioids, but a comparison of the sedative and analgesic effects of systemic morphine and pentobarbital in infant rats ranging in age from one to 20 days showed morphine 1mg/kg produced analgesia to an intra-plantar injection of formalin at all ages, which was qualitatively different from the sedative effects of pentobarbitone 10mg/kg [1]. Early studies indicated that neonates were more susceptible to the convulsant effects of morphine but an investigation of the effects of high dose morphine on EEG recordings and behavioural observations showed that although all doses of morphine used produced electrographic spikes in one, three and six day old neonatal rats, no behavioural convulsions were observed suggesting morphine is less toxic than originally thought [80].

Actions of opioids at a cellular level

There is now ample evidence to show that opioid systems play a significant part in the regulation of neuronal development in the neonate. The role of endogenous opioids in neural plasticity has been demonstrated by examining the effects of continuous receptor blockade on the developing neurones in the cerebral cortex, hippocampus and cerebellum in 10 day old rats. Neonatal rats were given daily injections of the potent opioid agonist naltrexone from P0, and after sacrifice on P10, it was demonstrated that

the lengths of oblique dendrites of pyramidal cells in the cerebral cortex and basilar dendrites of the hippocampus were significantly increased when compared with the control group. The dendritic lengths of spiny branches of cerebellar Purkinje cells were also increased. Endogenous opioids can therefore exert a marked influence on dendritic elaboration and spine formation [29]. In a similar study the effect of exogenous opioid blockade was examined at P10 and P21. Daily injections of naltrexone were given in a dose of 50mg/kg providing complete continuous blockade, and 1mg/kg producing only intermittent opioid receptor antagonism. Continuous blockade produced large increases in dendrite and spine elaboration, at P10, but these increases were only seen in the hippocampus at P21. With intermittent blockade, dendrite and spine growth were often subnormal [30]. The action of met-enkephalin on the growth of astrocytes in vitro mixed glial cultures were studied in 1 day old mouse cerebral hemisphere. Continuous treatment with either met-enkephalin, a met-enkephalin/naloxone combination or naloxone alone revealed that met-enkephalin caused a decrease in total cell numbers compared with naloxone treated cultures and controls. These results suggest that the endogenous opioid met-enkephalin suppresses astrocyte growth by a receptor mediated mechanism [74].

These results indicate that endogenous opioids act as inhibitory growth factors in neuronal development, probably by inhibition of DNA synthesis. After a single dose of either met-enkephalin or naloxone to 11 day old rats, DNA synthesis was shown to be increased in the naloxone treated group but decreased in the met-enkephalin group [81]. Comparing the effects of morphine on DNA synthesis in neonatal rat brain in vivo and in vitro, it has been shown that morphine inhibits DNA synthesis in vivo in

animals aged 1 to 4 days but not in older animals. The in vivo effects were blocked by pre-treatment with naloxone indicating a receptor mediated mechanism. Naloxone administered acutely or naltrexone chronically had no effect. Brain tissue incubated with morphine in vitro, however, showed no difference in DNA synthesis when compared with controls, pointing to an indirect mechanism in the control of cell proliferation [40].

Reduced latencies to tail-flick tests have been demonstrated on day 9 in neonatal rat pups from P1 to P7 when β-endorphin (in doses of 1-50μg per pup) was administered daily, suggesting hyperalgesia. In these animals, the number of opiate receptors in the brain was reduced when examined on P14 [86]. Studies on neonatal rat pups exposed to morphine either in utero (pregnant rats given a s/c morphine pellet on E16) or postnatally (daily injections of morphine 5mg/kg) have demonstrated that chronic morphine exposure results in a significant decrease in brain mu receptor density until day 8, gradually increasing to control levels by day 14. These changes were accompanied by a tolerance to the analgesic action of morphine, though there was no significant change in delta and kappa receptor densities [76]. An autoradiographic study of neonatal rat brain compared the neuroanatomical pattern of opioid receptor changes in controls and animals treated chronically with morphine. In those receiving morphine from P1 to P4, mu receptor density on P5 was non-existent in parts of the striatum and decreased in other areas such as the amygdala. Longer treatment with morphine from P1 until P8 produced no further alterations in mu receptor density [75].

Little is known of the long term effects of opioid administration in the neonatal period on the developing CNS in humans.

Implications of basic studies

The results from animal experiments suggest that opioid actions differ with age in the neonate, but the early literature is confused, and more studies are required in this area. Prolonged exposure of the newborn rat to morphine has been shown to have an effect on neuronal development and alterations in receptor expression demonstrate the marked plasticity of opioid systems in the rat neonate. However, pain itself in the neonatal period may also adversely affect this development. With the recent increase in the use of opioids in human neonates [13][62], there is clearly a need to increase our understanding of the development of opioid systems and the factors modulating this development.

Neonatal Opioid Pharmacology

The pharmacology of opioids in the newborn has recently been reviewed in an excellent article by Hartley and Levene [28]. This article, therefore, will examine the current knowledge we have of the effects of opioid administration in human neonates.

Morphine and Semi-Synthetic Opioids

Morphine, and its semi-synthetic derivatives codeine and diamorphine, all rely in part on the production of metabolites for activity. Owing to a large variation in pharmacokinetics of these drugs in neonates, metabolite production can be unpredictable [28]. A recent emergence of more sensitive and specific measurements of metabolites using High Performance Liquid Chromatography has allowed more detailed investigation of the pharmacokinetics of opioids in neonates, but the difficulties of assessing pain in these pre-verbal patients has left us with little knowledge of the analgesic efficacy of the parent drugs and perhaps, more importantly, their metabolites.

Morphine

Morphine is a naturally occurring alkaloid derived from the unripe seed pod of the opium poppy [9]. Among paediatric anaesthetists, it is the most commonly used opioid both intra- and post- operatively [13].

Morphine is metabolised predominantly in the liver to morphine-3-glucuronide (M3G) and morphine-6-glucuronide (M6G) [9]. Glucuronidation is facilitated by the uridine diphosphate glucuronosyltransferases (UDPGTs), a family of isoenzymes of which 11 different forms have been identified [49]. Both M3G and M6G possess significant pharmacological properties [24]. M6G is a potent analgesic and respiratory depressant both in humans and in experimental animals [25][57], but M3G, the predominant metabolite, is antagonistic to the analgesic and respiratory depressant effects of morphine and M6G [70].

As these two glucuronide metabolites of morphine have opposing actions, the effects of morphine are therefore dependant on the ratio of M6G:M3G produced [43], and differential maturation of UDPGT enzymes in the premature and term neonate may produce an evolving ratio of M6G:M3G following morphine administration. This may in part explain a large variation in effects of morphine in neonates. In premature neonates, the M6G:M3G ratio is inversely related to birth weight, and decreases significantly with increasing birth weight and gestational age [27].

What is the analgesic efficacy of morphine in the neonate? Most research has concentrated on the adequacy of sedation of ventilated neonates on the intensive care unit. In one such study, comfort scores incorporating both behavioural and physiological parameters were used to assess the adequacy of sedation in premature and term neonates requiring continuous morphine infusions during mechanical ventilation for respiratory distress syndrome. There was no significant difference in the correlation between plasma morphine and analgesic effect in these two age groups. Adequate sedation occurred in 50% of patients with a plasma morphine level of 125 ng/ml (with a 95% confidence interval of 116-135 ng/ml) [11]. Studies of the analgesic effects of morphine and its metabolites in post-surgical, spontaneously breathing neonates are urgently required before we can adopt a more informed approach to the use of this opioid.

Kupferburg and Way, in 1960 [42], deduced that the increased sensitivity of neonatal rats to morphine was due to the greater permeability of the blood-brain barrier. A study by Way and Way in 1965 [83] compared carbon dioxide response curves after

morphine and pethidine in a total of eight spontaneously breathing neonates before surgery. The authors found that 0.05mg/kg of morphine the CO_2 response curve shifted to the right to a greater degree than 0.5mg/kg of pethidine. The doses were chosen on the assumption that they were equi-analgesic, and no consideration was given to the consequences of maternal pethidine administration (some had pethidine prior to delivery) in the immediate postnatal period. Although they concluded that morphine was a more powerful respiratory depressant than pethidine, the statistical and clinical significance of these results was not discussed. Early studies such as these have probably done much to enhance the perception that opioids, in particular morphine, are potent respiratory depressants in the neonate, and should only be used with extreme caution.

Subsequent studies, however, do not necessarily agree. A retrospective study of the use of opioids in neonates found that 13% of spontaneously breathing neonates developed apnoea or respiratory depression thought to be due to opioids [61]. 19% of these babies received morphine and none developed respiratory complications, which were all attributable to papaveretum or pethidine. In comparing the respiratory effects of morphine infusions in neonates and children after cardiac surgery, no age related differences were found, and it was concluded that neonates have the same ventilatory response to morphine as infants and children at the same plasma concentration [48].

We now have much more knowledge of morphine pharmacokinetics and the activity of its metabolites, and it is possible that if equi-analgesic rather than 'scaled-down' adult doses are given, the risk of serious respiratory depression would be no greater.

Studies of morphine use in neonates have not reported any significant cardiovascular side effects such as bradycardia and hypotension [26][39].

Codeine

Codeine, or methylmorphine, is a semi-synthetic opioid produced by the substitution of a methyl group for the hydroxy group on carbon 3 of morphine. Ten percent of codeine is demethylated in the liver to form morphine, which is likely to be the main contributor to its analgesic effects [63]. The remainder is demethylated to the inactive norcodeine which is either conjugated or excreted unchanged in the urine.

Despite a marked increase in its use amongst paediatric anaesthetists since 1988 [13][64], data on its kinetics is lacking and we have minimal information on the pharmacodynamics of codeine in neonates. Case reports of adverse reactions such as vasodilatation, severe hypotension and apnoea in infants and children following intravenous administration have precluded its use by this route in all age groups [68]. In the retrospective study of opioid use discussed above, respiratory depression and apnoea occurred in 2 out of 26 spontaneously breathing babies who received intramuscular codeine phosphate (1mg/kg) post-operatively, although the number of doses was not recorded [61].

Although no data are available for neonates, it is likely that immature hepatic metabolic pathways may result in prolonged half lives of both codeine and

subsequently morphine, resulting in an unpredictable quality of analgesia. More research is required if we are to use this opioid effectively.

Diamorphine

Diamorphine is used less commonly that morphine as a sedative for neonates on the intensive care unit, and it is rarely used for postoperative analgesia. Diamorphine is a semi-synthetic opioid produced by the acetylation of morphine. In adults it is rapidly deacetylated in plasma following systemic administration to 6-monoacetyl morphine (6-MAM) by the action of the enzyme plasma cholinesterase, with a half life of approximately 3 minutes [46]. 6 MAM is relatively fat soluble and enters the CNS readily where it is probably further de-acetylated to morphine. It is thought that these two active metabolites contribute greatly to the analgesic effect of diamorphine, which is considered to act as a pro-drug [82].

The effects of diamorphine were studied in 34 premature neonates (gestational age 26-40 weeks) requiring sedation for mechanical ventilation, after a loading dose of 50μg/kg, followed by an infusion of 15 μg/kg/hr. A transient fall in mean arterial blood pressure was noted at 30 minutes, coinciding with a statistically significant fall in heart rate. These effects were not considered clinically significant. However, a significant fall in respiratory rate at 30 and 60 minutes was felt to be beneficial in allowing synchrony with the ventilator. This dosage regimen provided a mean steady state morphine concentration of 62.6 ± 20.8 ng/ml and resulted in adequate sedation in

all neonates [16]. It was concluded that diamorphine can provide safe sedation for ventilated premature neonates on the intensive care unit.

Synthetic Opioids

Fentanyl, alfentanil and sufentanil are all synthetic opioids derived form pethidine. The parent compounds are potent analgesics and their metabolites are either weak analgesics or inactive [54]. Since they do not rely on metabolism for activity, they would seem to be a more logical choice of opioid to treat neonatal pain. However, their pharmacokinetics are still very variable in this age group [28]. A low therapeutic index and fears of respiratory depression have precluded their use as postoperative analgesics in spontaneously breathing neonates but they are commonly used during anaesthesia and as sedatives for patients requiring intensive care.

Fentanyl

Fentanyl is a synthetic opioid agonist derived from the phenylpiperidines with an analgesic potency 50-100 times that of morphine. This may be explained by its high lipid solubility and therefore ease of access to the CNS, and after intravenous injection it is widely distributed to the tissues and viscera. In the adult, metabolism is hepatic with less than 6% of the unchanged drug excreted in the urine. This is primarily by de-alkylation to norfentanyl but also by hydroxylation of fentanyl and norfentanyl by the isoenzyme system cytochrome P450 [54]. A short duration of action and more predictable pharmacokinetics are reasons for its popularity as an analgesic during major surgery and as a sedative on the intensive care unit. A narrow

therapeutic index and side effects such as respiratory depression have hampered its use as a post-operative analgesic, both in adults and children.

In order to determine a dosage regimen for fentanyl use on neonates ventilated for respiratory distress syndrome, its effects were compared in 20 newborn and premature neonates with a control group who did not receive fentanyl. The mean dose required to produce adequate sedation was 0.64 μg/kg in neonates of less than 34 weeks gestational age, rising to a mean of 0.75μg/kg in those 34 weeks or above. Mechanical ventilation was well tolerated in the fentanyl group compared with controls, without the need for additional sedation or analgesia. There were no significant cardiovascular side effects in the fentanyl group, and no reports of rigidity of the thorax [66]. Interestingly, a significant change in the pharmacodynamic response with time was found in eight neonates requiring sedation during extracorporeal membrane oxygenation for respiratory failure in the first few days of life. The mean fentanyl infusion rate to achieve adequate sedation rose steadily during the six day infusion period, with a parallel rise in mean plasma fentanyl concentration. These data suggest that a marked tolerance to the sedative effects of fentanyl occurs in the early neonatal period, and probably reflect opioid receptor desensitisation rather than an alteration in pharmacodynamics as a consequence of maturation [2]. Fentanyl may be a beneficial agent for use as a short term sedative or analgesic in ventilated neonates, but again we need a clearer understanding of its pharmacodynamics.

Alfentanil

Alfentanil is an analogue of fentanyl with a fifth to a tenth of its potency and a shorter duration of action [54]. Alfentanil is seldom used as an adjunct to anaesthesia in neonates but, because of its favourable pharmacokinetic properties, it is used as a sedative and analgesic in neonatal intensive care.

All data on the pharmacodynamics of alfentanil has arisen from the study of premature neonates ventilated on the intensive care unit. 20 premature neonates (gestation 25 to 36 weeks, ages less than 24 hours), ventilated for respiratory distress, received a dose of 20 μg/kg of alfentanil intravenously over 2 minutes which produced a rapid and significant fall in heat rate and blood pressure and an accompanied fall in PaO_2 [52]. The same dose, in a matched age group, was noted to result in a 'transient' fall in blood pressure, heart rate and arterial oxygen tension [53].

Observing the use of alfentanil before treatment procedures in 20 critically ill mechanically ventilated neonates, a mean dose of 11.7μg/kg (range 9-15) produced severe muscle rigidity and jerking in four babies which impaired ventilation. EEG recordings of three of these babies showed no increase in seizure activity. Nine babies had mild to moderate rigidity which did not interfere with mechanical ventilation. It was concluded that alfentanil should not be used in neonates without muscle relaxation because of the danger of rigidity [60].

Sufentanil

Sufentanil is a further synthetic opioid, a thienyl analogue of fentanyl. It possesses five to ten times the potency of fentanyl due to a greater affinity for opioid receptors [54]. Despite widespread use as an adjunct to anaesthesia in the States, it is not as yet, licensed for use in the UK. Little has been published on its use in neonates.

Some pharmacodynamic deductions were made in a comparative study of sufentanil in 28 paediatric patients. Neonates were compared with infants, children and adolescents during elective cardiac surgery. After induction of anaesthesia, a single dose of intravenous sufentanil (10-15μg/kg) was given rapidly. Anaesthesia with sufentanil was supplemented with nitrous oxide or another narcotic if heart rate or mean blood pressure rose to greater than 20% of baseline values. This occurred at significantly higher plasma sufentanil concentrations in the neonatal group compared with all other groups. It was deduced that neonates may have a decreased sensitivity to the anaesthetic effects of sufentanil, or that an acute tolerance developed. However, patients over nine months all received pethidine, diazepam and pentobarbital orally as a premedicant 75 minutes before surgery, and it is likely that this had a marked effect on the analgesic efficacy of sufentanil [23].

Conclusions

Although care must be taken in extrapolating data from animals to humans, our knowledge of opioid system development in human neonates has been enhanced by information from animal experimentation. Analgesic pathways are anatomically and functionally different in the neonatal as compared to the adult rat, and display a

marked plasticity. Although opioid systems appear very early in foetal life they do not reach adult levels of function for some time. In addition to a contribution to analgesic mechanisms, they probably have other significant roles such as regulation of neuronal development within the CNS.

There is now overwhelming clinical evidence that neonates react adversely to a noxious stimulus, although the extent of their perception of pain in comparison with adults is as yet unknown. This information has prompted a widespread increase in the use of opioids for sedation and treatment of postoperative pain, although our knowledge of their effects in this age group is still far from complete. In particular, too few studies have actually looked at the analgesic potency of opioids in the newborn, and we are largely ignorant of the longer term consequences of early opioid administration on the ontogeny of opioid systems and subsequent neurological development in humans. Information from animal experiments allows us an insight into possible mechanisms in humans, but the degree to which they can be compared can only be established by a careful analysis of the developmental regulation of opioid function in human infants.

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Legend for figure 1.

Photomicrographs illustrating μ receptor binding site distribution at P1, P7, P14 and P21 and at different levels of the adult rat spinal cord. (Modified from Kar and Quirion [38]).

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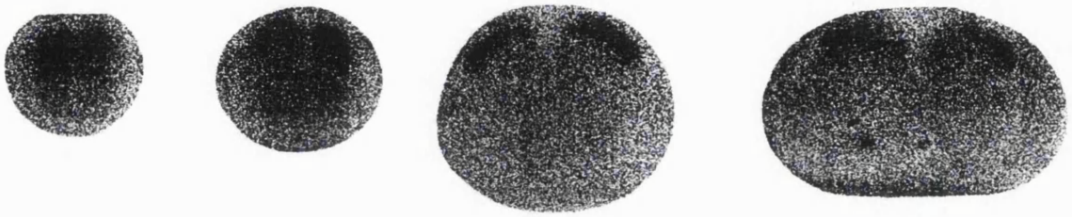
A : Development



P1 P7 P14 P28

B : Adult

Cervical Thoracic Lumbar