Assessment Of The Effectiveness of Phentolamine
In Reducing The Duration Of Dental Local Anaesthesia

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ABSTRACT

Effective local anaesthesia is required to ensure pain-free dental procedures, but existing anaesthetics produce persistent soft tissue anaesthesia well beyond the average time for the painful treatment. This may result in lip and tongue biting in young children and the handicapped. The study reports the clinical attempts to usefully reduce this level of unwanted soft tissue anaesthesia by injecting into the anaesthetised tissues a potent vasodilating agent, phentolamine.

Lignocaine and adrenaline is the standard dental local anaesthetic combination, with the adrenaline included to prolong the duration and depth of anaesthesia in the highly vascular perioral tissues. The added catecholamine prevents the anaesthetic from being rapidly absorbed from the injection site by producing intense vasoconstriction.

An alternative dental local anaesthetic is the combination of prilocaine and felypressin. This also produces excessively long periods of soft tissue anaesthesia despite the vasoconstriction not being mediated through α-adrenoceptors. Investigation of the clinical effects of reversing residual soft tissue anaesthesia produced by prilocaine and felypressin were thought worthwhile to enable the clinical safety of a potentially inappropriate drug combination to be assessed. It also produced comparative data for interpreting the catecholamine responses to the routine lignocaine and adrenaline anaesthetics.

Phentolamine is a short-acting and potent α-adrenoceptor blocking agent. The investigations confirm that a 2mg/ml phentolamine reversal solution, when injected after a 30 minute delay into the perioral tissues in the vicinity of the local anaesthetic solution, quickly reverses the vasoconstrictor effects of adrenaline in vivo. Maxillary infiltration soft tissue anaesthesia was reduced from 138.1 to 16.9 minutes (95% CI) and pulp anaesthesia from 40.3 to 4.8 minutes (95% CI). Inferior dental nerve block reversals were reduced from 160.3 to 17.3 minutes (95% CI) and pulp anaesthesia from 59.5 to 11.1 minutes (95% CI).

Multiple injections of 6ml 2% lignocaine + 1:80,000 adrenaline were reversed with 3ml 2mg/ml phentolamine, and venous plasma local anaesthetic concentrations assayed using
High Performance Liquid Chromatography (HPLC). No statistically significant increase in plasma lignocaine concentration (95% CI) was observed.

Venous plasma lignocaine and catecholamine concentrations were measured after multiple perioral injections of 5.4ml 2% lignocaine with three differing adrenaline concentrations; 1:50,000, 1:80,000 and 1:100,000. No statistically significant difference in plasma lignocaine concentrations was noted between the groups. Plasma adrenaline concentrations were statistically raised in the 1:50,000 group (95% CI), but the noradrenaline concentrations showed no significant variation. The perioral injection of 3ml 2mg/ml phenolamine reversal agent after the 30 minute delay produced increases in plasma lignocaine concentrations, with the weakest 1:100,000 adrenaline anaesthetic the most statistically significant (95% CI).

The intense local vasodilatation was demonstrated to be effective against the non-adrenaline alternative anaesthetic prilocaine and felypressin, with a statistically significant rise in the venous plasma prilocaine concentration (95% CI). Plasma local anaesthetic concentrations failed to reach accepted levels of toxicity, and no adverse cardiovascular events were recorded.

Venous plasma adrenaline concentrations were measured before and after dental surgical extractions under lignocaine and adrenaline or prilocaine and felypressin local anaesthesia. Venous plasma adrenaline concentrations were significantly raised in only half the cases, but the prilocaine and felypressin group showed a marked reduction in data scatter.

Additional experiments attempting to reverse the soft tissue anaesthesia following injection of another dental local anaesthetic combination, mepivacaine and adrenaline, are presented as appendices. Plasma mepivacaine concentrations were not statistically raised compared with placebo.

Venous plasma catecholamine concentrations were measured following perioral injection of 6ml 2% mepivacaine + 1:100,000 adrenaline, and after a 30 minute delay, with 3ml 2mg/ml phenolamine or placebo. No statistical difference was noted between the groups for adrenaline or noradrenaline concentrations, but a trend for the noradrenaline
concentration to rise over the 2 hour test period was observed.

No adverse local reactions or systemic cardiovascular changes were observed in any of the studies, implying that the prolonged soft tissue effects of dental local anaesthesia may be safely and rapidly reversed by injection of small increments of phentolamine into the adjacent perioral tissues. Such a facility will be useful in clinical dental practice where needlessly prolonged anaesthesia may have significant consequences to patients, such as lip biting in the handicapped.
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ABBREVIATIONS

ADR .................................. Adrenaline
AUC .................................. Area Under Curve
CI  .................................. Confidence Interval
CNS  .................................. Central Nervous System
CSF .................................. Cerebrospinal Fluid
CVS .................................. Cardiovascular System
HPLC ................................. High Performance Liquid Chromatography
IV  ................................. Intravenous
Lig .................................. Lignocaine
Mep .................................. Mepivacaine
Mins .................................. Minutes
Nos  .................................. Numbers
pKa  ................................. Negative logarithm of the dissociation constant
Pril  .................................. Prilocaine
SC .................................. Subcutaneous
$t_{50}$  ............................... Time to reach 50% anaesthesia
UCH  ................................. University College Hospital
UK  ................................. United Kingdom
UV  ................................. Ultra violet
CHAPTER 2

INTRODUCTION AND CLINICAL OBJECTIVES

It was estimated in the early 1980's that 70 million dental local anaesthetics were administered annually in Britain. Their potentially significant complications were classified as cardiovascular reactions related to the vasoconstrictor, interactions between adrenaline and concurrent antidepressant medication, and hypersensitivity to lignocaine (Cawson et al., 1983).

The effectiveness of a dental local anaesthetic relies on the presence of the potentiating vasoconstrictor, and the length of effective pulp analgesia is much shorter than that of adjacent soft tissues, by as much as 75% (Roberts, Sowray, 1987). This is especially relevant in the residual soft tissue anaesthesia following inferior dental nerve blocks. Gilmour et al., (1984) reported on severe lip tissue loss as a result of self mutilation following such anaesthesia and commented:

"...every dentist is familiar with the complications of lip biting during the period of analgesia following an inferior dental nerve block."

Bedi et al., (1984) also commented on the excessive residual soft tissue anaesthesia, noting the special relevance to young children and the handicapped. Although the problem of inadvertent lip biting was not listed by Cawson et al., (1983) it occurs with sufficient frequency and is distressing enough to patients, parents and surgeons, to be taken seriously and merit investigation. Lignocaine has enjoyed an outstanding safety record in dental use, but is only reliably effective in the highly vascular perioral tissues when combined with a vasoconstrictor, usually adrenaline. Coplans and Curson, (1982) reported only ten deaths in the 1970-1979 period associated with the use of all types of dental local anaesthetics, and in many cases other drugs such as methohexitone, diazepam, nitrous oxide, oxygen and halothane had been concurrently administered.

Attempts by manufacturers to abbreviate the soft tissue effects whilst maintaining the pulp anaesthesia have only been partially successful, with lignocaine and adrenaline remaining the standard. Blair and Meechan, (1985) noted prilocaine and felypressin to be inferior to this standard in producing mandibular blocks, and recommended lignocaine and adrenaline to be used in preference, provided no contra-indications existed. In clinical use prilocaine with felypressin has a similar excessive duration of
soft tissue anaesthesia as the lignocaine and adrenaline combination (Jastak, Yagiela 1983; Roberts, Sowray 1987; Meecham, Rawlins 1988). Cowan (1968) and Newcombe and Waite, (1972) observed that prilocaine and felypressin anaesthesia failed to control blood loss during periodontal surgery, and recommended the routine use of the lignocaine and adrenaline mixture.

With this established safety record and there being no superior alternatives, it was postulated as to the ability to convert conventional lignocaine and adrenaline in vivo to plain lignocaine. As lignocaine plain solution has limited analgesic activity on both pulp and soft tissues (Mumford, Gray 1957; Berling 1958; Kabambe et al., 1982; Roberts, Sowray 1987; Knoll-Kohler, Fortsch 1992), this could prove clinically useful in providing an adequate working time of pulp analgesia without the unacceptably protracted soft tissue effects.

The adrenaline reversal agent chosen was phentolamine mesylate (Chapter 3), a potent, short acting, injectable α-adrenoceptor blocking agent with α<sub>2</sub> > α<sub>1</sub> selectivity (Doxey et al., 1977). With this property it was anticipated that the competitive antagonism of adrenaline for the vasoconstrictor α-adrenoceptors and the direct effect on the blood vessel smooth muscle, would produce a localised vasodilatation and increase the rate of lignocaine absorption from the injection site, so quickly reducing both soft tissue and pulp anaesthesia. Phentolamine was not anticipated to have a significant clinical effect on the duration of soft tissue anaesthesia produced by prilocaine and felypressin, as the vasoactivity of felypressin is not believed to be mediated via α-adrenoceptors (Lilienthal, Reynolds 1976; Fruhstorfer, Wagener 1993).

The objectives of this study are to outline the laboratory and clinical evaluation of this novel approach to dental anaesthesia. It involves knowledge of the pharmacokinetics of lignocaine, prilocaine, mepivacaine, adrenaline, felypressin and phentolamine, the mode of action of these drugs, the commercial preparations available, and the clinical risks of their use individually and collectively.
CHAPTER 3  

i. HISTORICAL ASPECTS OF LOCAL ANAESTHETICS

The coca leaf was believed to be a gift to the Inca people from Manco Capac, Son of the Sun God, bestowed as a token of esteem and sympathy for their suffering labours (Mortimer, 1974). In practice, coca leaves were bound into a ball (cocode) with guans or cornstarch and chewed with lime or alkaline ash to release the active alkaloid. This alkaloid was extracted by the French chemist, Francis Gaediscke in 1855. He called it erythroxylin following the botanical name for the plant, Erythroxylin Coca, ascribed by Lamark in 1786.

In 1860, Albert Nieman at the University of Gottingen isolated cocaine from Peruvian leaves of the coca shrub, given to him by a scientist colleague, Dr Scherzer (Koller, 1941). The cocaine crystals extracted from erythroxylin were noted in passing to produce anaesthesia of his tongue, but Nieman soon died and his research was continued by Wilhelm Lossen, who established the chemical formula as C_{17}H_{21}NO_{4}.

It was not until 1868 when a Peruvian army surgeon, Thomas Morenoy Maiz, published his experiences with cocaine acetate. He is credited with describing cocaine’s potential as a local anaesthetic and of the toxic effects such as fitting (von Oettingen, 1933).

European workers, Carl Koller and Sigmund Freud described in 1884 in Vienna, the corneal anaesthesia produced by cocaine solutions. Koller had been evaluating the effects of various drugs on the conjunctiva and cornea including chloral, morphine and bromides. With his assistant Joseph Gaerfner, Koller had produced anaesthesia of the cornea in frogs, rabbits and dogs using a 2% solution of cocaine hydrochlorate. Finally they produced similar effects on each others eyes, describing the lack of sensation and blink reflex to pressure from a needle.

The account of this discovery was presented by Joseph Brettaner, on behalf of Koller, on September 15, 1884 at the Congress of the German Society of Ophthalmologists, held in Heidelberg. (McAuley, 1985). Very quickly the use of cocaine for surgical pain control during ophthalmic procedures was established.
Injection techniques to obtain anaesthesia at other sites were attempted and the American, William Stewart Halsted, working in the Roosevelt Hospital, New York, produced an inferior alveolar nerve block. He was later to become one of America's leading surgeons, being Professor of Surgery at Johns Hopkins Hospital, Baltimore, for 33 years. However, the addictive properties of cocaine were becoming evident, with Halsted himself taking over two years to break the habit.

The first recorded evidence of cocaine injection in Britain appeared in the Journal of The British Dental Association in 1886, when William Alfred Hunt described perioral infiltration injections (Hunt, 1886).

It is probable that the drug combination of adrenaline and local anaesthetics is the most extensively used in clinical practice. It is safe to say that without adrenaline the mortality from local anaesthetics would have been much higher, but on the other hand, the prolonged soft tissue action may in some circumstances be disadvantageous.

John Jacob Abel, the first American Professor of Pharmacology at the Johns Hopkins Hospital Medical School was the first to isolate adrenaline in 1897 (Abel, Crawford, 1897). For all his expertise, Abel did not obtain the pure product, and it remained for an associate called Takamine to achieve this goal (Takamine, 1901).

The addition of adrenaline to increase the duration and potency of cocaine as a local anaesthetic was discussed in practical terms by Braun, (1903). Adrenaline, delaying the absorption of cocaine by its vasoconstrictor effect, prolonged the surgical working time. However, as both constituents were natural products they had great difficulty in obtaining uniform potency. By 1905 Dakin had prepared adrenaline in pure form and Lieberman and Giesch synthetically produced cocaine, and so a predictable local anaesthetic combination was achieved.

The quest for an ideal local anaesthetic without the toxic and addictive features of cocaine continued. In Sweden, benzoic acid esters were investigated by Einholm, (1899), and in 1904 he reported the synthesis of procaine. In 1905 the local anaesthetic procaine (Novocaine) was marketed being non-addictive and less irritant at the site of injection.
Modifications of the procaine ester structure resulted in other anaesthetic compounds, and in 1943 Nils Lofgren synthesized the aniline derivative diethylaminoaceto-2, 6-xylidine, which when tested by Lundquist on himself showed promising qualities of local anaesthesia. This new compound, lignocaine, was submitted to Goldberg at the Department of Pharmacology, Karolinska Institute, and it was there that the pharmacology and toxicology was established. In January 1948 lignocaine was released for general use in Sweden, and Björm and Huldt, (1947) conducted important experiments at the Department of Dental Surgery of the Royal School of Dentistry in Stockholm.

Increasing the potency of lignocaine solutions by the addition of adrenaline produced an effective local anaesthetic. Compared with procaine and adrenaline, the lignocaine mixture produced more consistent dental and soft tissue anaesthesia, with less paraesthesia and itching of the lips and nasal alae (Björm, Huldt, 1947). The commercial solutions currently available for dental use in sterile 1.8 - 2.2ml cartridges are:

- **2% lignocaine + 1:50,000 adrenaline**
- **2% lignocaine + 1:80,000 adrenaline**
- **2% lignocaine + 1:100,000 adrenaline**

Chemical compounds that demonstrate local anaesthetic activity usually possess an aromatic and an amine group separated by an intermediate chain (see pages 82,83). Clinically useful agents which possess an ester link between the aromatic portion and the intermediate chain include procaine and amethocaine. Local anaesthetics with an amide link between the aromatic end and the intermediate chain are referred to as amino amides, and include lignocaine, mepivacaine, prilocaine, bupivacaine and etidocaine.
The basic difference between the ester and amide compounds is in their chemical stability. Esters are relatively unstable in solution, and are readily hydrolysed in plasma by cholinesterase. Amides are more stable and are degraded in the liver. Unlike the esters, the amides are not degraded to para-aminobenzoic acid which is capable of inducing allergies in some patients. The properties of various local anaesthetics which are clinically relevant include potency, speed of onset and duration of action. The clinical profile of an individual local anaesthetic is essentially determined by the physico-chemical characteristics of the compound, which in turn is dependant on the chemical structure. The physico-chemical properties which influence anaesthetic activity are lipid solubility, protein binding and pKa. Minor changes in molecular structure have dramatic effects on these properties (Covino, 1986).
CHAPTER 3

ii. MODE OF ACTION AND VASOACTIVITY OF LOCAL ANAESTHETICS

Local anaesthetic solutions prevent impulse conduction along an axon in a reversible manner and without damage to the tissues. Block is by no means instantaneous, rather a progressive reduction of the action potential reducing the rate of its rise, elevating the firing threshold, slowing the speed of impulse conduction and lengthening the refractory period. The resting potential remains approximately the same as in the unblocked state, such that it retains its polarisation during the block with the prevention of depolarisation achieving the conduction block (deJong, 1977; Covino, 1986).

Local anaesthetics impede sodium ion access to the axon interior, directly or indirectly occluding the transmembrane sodium channels (Ritchie, 1975; Lee 1976). Lignocaine, procaine and cocaine reduce the transmembrane sodium ion movement in a dose-related fashion, with the greater the anaesthetic concentration the smaller the sodium eflux through the voltage-clamped axon (Hille et al., 1975).

The increase in permeability to sodium ions is believed to be the result of channels opening in the membrane permitting sodium ions to pass along the aqueous centre. Hille, (1977a) proposed a receptor model for local anaesthetic activity. The sodium channel has a constricted region near the outside which selectively discriminates between ions, and the inner axoplasmic side is normally in the resting state, closed by one or more gates.

The first of these gates, the $m$ gate, opens when the fibre is depolarised and is responsible for the influx of sodium. Closure of the second, $h$ gate, is responsible for inactivation or cessation of the sodium current. It is believed that in a myelinated nerve fibre at its resting potential approximately 30% of the channels have closed $h$ gates, the remainder permitting sodium permeation as soon as the $m$ gates are opened by depolarisation. Hyperpolarisation results in some of the closed $h$ gates to open, so more channels become available for sodium permeation subsequent to the $m$ gate opening.

The importance of pH relating to whether charged ionised or the uncharged non-ionised form of the anaesthetic is responsible for blockade has been the subject of contention.
for some years. The charged cationic form is capable of forming electrostatic bonds at the surface of the charged membranes, whilst the non-ionised form is not. Uncharged ions can readily penetrate the hydrophobic parts of the lipid molecules. Skou, (1961) noted this penetrating action produced a physical expansion of the membrane, and the resulting conformational changes could effectively hold the ion channels closed.

Subsequently it appeared that the two actions of surface electrostatic effect and lipid penetration had quite independent significance. Penetration of the membrane by the uncharged ion was considered merely a stage of the anaesthetic to its target, whilst the charged cationic form was responsible for actual blockade of the membrane through occupation of charged receptor sites on the surface.

Summarising, Ritchie, (1975) postulates that most surface blocking effect takes place at the inner surface of the axon membrane after passage of the uncharged form through the lipid phase.

Hille, (1977b) has been critical of experiments where the external pH has been altered to determine whether the ionised or non-ionised form is the more active. He suggests that such changes affect permeability and lipid solubility but the non-ionised form is required for penetration of nerve sheaths and access to the site of action, but does not consider the theory that only the ionised form interacts with a receptor in the sodium channel to be justified.

The effectiveness of local anaesthetics varies with the history of stimulation of the nerve. With the more hydrophilic, partly or fully ionised anaesthetics such as lignocaine, the level of conduction block is rapidly enhanced by brief repetitive depolarising pulses which favour open sodium channels (Hille, 1977a). This suggested that hydrophillic local anaesthetics gain access to sodium channels predominantly from the axoplasm when the activation m gates are open. Inactivating h gates could be expected to impede access of hydrophillic molecules into sodium channels and conditions which favour the opening of these gates before the depolarising pulses also enhance the onset and recovery of the stimulation dependent effects of local anaesthetics (Strichartz, 1973; Hille, 1977a).
Local anaesthetics modify the inactivation of sodium currents by modifying the fraction of $h$ gates that are closed. At any membrane potential where inactivation is not complete local anaesthetics increase the inactivation ie, shift the inactivation curve along the membrane potential axis in the direction of the repolarisation (Courtney, 1975). From these observations of the dependence of the effectiveness of local anaesthetics on the history of nerve stimulation and the enhancement of inactivation of sodium currents by non-ionised tertiary and quaternary amine anaesthetics, Hille, (1977a) concluded that the non-ionised as well as the ionised forms of local anaesthetic act at the same receptor in the interior of the sodium channel, or that the binding of the local anaesthetic to the receptor is stabilised by closure of the $h$ gate.

Local anaesthetics have been demonstrated to influence many membrane dependent responses to surface stimuli in a variety of cells. Among these effects, local anaesthetics inhibit phagocytosis of human leucocytes (Cullen, Haschke, 1974) and inhibit granulocyte adherence and delivery to inflammatory sites (MacGregor et al.,1980). Leucocyte adhesion and locomotion was shown to be inhibited by Schreiner and Hopen, (1979). Takagi et al., (1983) reported the inhibition of human natural killer cells by lignocaine in a dose-dependent manner. It was postulated that local anaesthetics may inhibit not only the recognition process but also the disruption of the microfilaments in the plasma membrane.

This lignocaine-membrane interaction has been demonstrated to inhibit membrane bound enzymes. Fowler et al., (1980) demonstrated that lignocaine inhibits the activity of membrane-bound monoamine oxidase. In a further study Haque and Poddar, (1983) showed that lignocaine increases the lipid fluidity of synaptosomal and erythrocyte membranes, which may result in inhibition of membrane-bound acetycholinesterase activity. The inhibitory effect of anaphylactic histamine release on mast cell membranes by lignocaine has been demonstrated by Kazimierczak et al., (1979). Johnson and Miller, (1979) used a rat mast cell model to assess the effect of lignocaine on the calcium permeability of the cell membrane function. They demonstrated that lignocaine inhibited calcium flux as well as histamine release, and at higher concentrations this was enhanced.
The majority of injectable local anaesthetics are weakly based tertiary amines with the exception of prilocaine and lexylcaaine, which are secondary amines. The basic structure of these amines comprises a lipophilic aromatic head derived from benzoic acid or aniline, and a hydrocarbon chain which is either an ester or amide in structure. The lack of the hydrophillic tail renders the anaesthetic relatively water insoluble, and so more suitable for topical mucosal application rather than injections (see pages 82,83). The local anaesthetic amine base is poorly soluble in water and unstable in air. For injectable use the hydrochloride salts are most often used in a vehicle of saline or water.

It has been demonstrated that raising the pH of the solution shortens the onset and enhances the effectiveness of anaesthetic block, presumably decreasing the ionisation (Rud, 1961). Alkalisation renders the solutions unstable with precipitation of the base so unsuitable for clinical use. Acidification of local anaesthetic solution prevents the oxidation of added adrenaline and may appear to be less effective clinically than their non-acidified counterparts (deJong, Cullen, 1963).

The relative concentrations of cation and base will vary with the hydrogen ion concentration of the solution. When the pH of the solution equals the local anaesthetics pKa (negative logarithm of the dissociation constant), equal amounts of cation and base co-exist. As the pKa of most local anaesthetics ranges between 7.5 and 9, the solution contains considerably more cation than base at tissue pH (deJong, 1977).

It has been postulated that it is the cation which produces the block, when present at the internal membrane surface. The binding site has to be at the sodium channel mouth within the protective shield of the channel gates, as prior depolarisation increases the degree of blockade (Courtney, 1975).

Clinically the anaesthetic must diffuse from the depot site to the nerve tissue and penetrate the lipid membranes to gain effect. The lipid-soluble base, being unionised, traverses the plasma membranes, and so alkalining a local anaesthetic solution should enhance drug penetration (deJong, 1977).

The pH of twenty three commercially available dental local anaesthetics was reported by Punnia-Moorthy et al., (1984), showing a range from 3.23 to 6.81. The adrenaline
containing local anaesthetics had low pH, as adrenaline in solution at pH 6 or greater and exposed to air becomes oxidised within a few hours to adrenochrome, a brownish oxidation product which is not vasoactive (De Jong, Cullen, 1963). To prevent this oxidative decomposition, an antioxidant, sodium metabisulphite, is added. This additive imparts marked acidity to the local anaesthetic solution, which may cause local irritation and pain at the injection site and the theoretical disadvantage of increasing the cationic form (Punnia-Moorthy et al., 1984).

Wennberg et al., (1982) investigated the possible tissue damage from commercially prepared lignocaine-adrenaline mixtures with a pH approximately 3.5. The addition of adrenaline could potentially reduce the local buffering capacity and induce a tissue hypoxia secondary to the vasoconstrictor effect and so further the risk of local tissue injury. Comparison was made with freshly prepared, sodium metabisulphite-free, lignocaine and adrenaline at a pH 6.5. They noted that the tissue pH was decreased for 90 minutes following the lignocaine and adrenaline mixtures, but with the exception of the first few minutes, no significant differences on tissue pH was noted between the two differing pH solutions. They concluded that despite the low pH and oxygen reducing properties of the commercially prepared lignocaine and adrenaline solution, rapid buffering occurs in the tissues.

Ischaemic ulceration has been reported by Jorgenson and Hayden, (1967) and Mitchell et al., (1969) following the rapid injections of excess anaesthetic solutions containing a vasoconstrictor into the unyielding palatal tissues. Carroll, (1980) described a necrotic sloughing ulcer following a painful periapical injection of lignocaine and adrenaline into the unreflected mucosa above a maxillary canine. This subperiosteal injection would produce direct cell damage, disruption of blood vessels and elevation of the periosteal tissues. Adrenaline vasoconstriction and the low pH would contribute to the tissue ischaemia sufficient to produce an area of infarction.

Punnia-Moorthy, (1988) investigated the buffering capacity of normal and inflamed tissues following the injection of local anaesthetic solutions. Contrary to the generally accepted view there was an increased buffering capacity in the inflamed tissues, offering no support to the hypothesis that tissue acidity is an important factor in the failure to obtain local anaesthesia in areas of tissue inflammation.
VASOACTIVITY

The rate of clearance from the site of injection is an important factor in the duration of action of a local anaesthetic. Vasoconstrictors enhance the duration of action, with blood flow at the site of injection an important factor. In the absence of vasoconstrictors some local anaesthetics have a vasodilator action and others vasoconstrictor activity, the degree varying with the individual anaesthetic, the concentration used and the method of assessment (Blair 1975; Aps, Reynolds 1976; Willats, Reynolds 1985; Covino 1986).

Skin is readily accessible for examination of the vasoactivity of local anaesthetics, with visual changes in colour (Aps, Reynolds 1976; Fairley, Reynolds 1981), thermal images (Lindorf 1979) and flowmetry using a laser Doppler (Cederholm et al., 1991) having been described. Results have been inconsistent and difficult to interpret as several problems exist when examining results. Even a minute injury such as needle insertion, or the injection of saline produces a rapid and significant vasodilation due to the local release of vasoactive peptides and histamine (Carpenter, Morell 1988; Cederholm et al., 1991).

Capillary skin perfusion at rest is usually so low that vasoconstrictor effects are very difficult to determine. (Jorfeldt et al., 1970). An attempt to determine the effect of lignocaine, mepivacaine and bupivacaine on capillary blood flow in man was described by Carpenter and Morell (1988). A laser Doppler capillary perfusion monitor was used to measure flow changes in the test abdominal skin. When compared with saline, capillary flow was increased by lignocaine concentrations of 0.5% and above, and by bupivacaine 0.25% and above. Blood flow was decreased by bupivacaine 0.25%, mepivacaine 0.5% and 0.05%. Clinically useful concentrations of lignocaine and bupivacaine increased blood flow while mepivacaine had minimal effect. At lower concentrations than these used clinically, local anaesthetics tend to diminish the increase in capillary blood flow that resulted from the needle prick injury. Consequently, the injection of such vasoactive agents produces both dilatation and constriction in unpredictable proportions.
Johns et al., (1985) used a rat cremaster muscle model and fourth order arterioles to measure the microvascular response to varying concentrations of lignocaine. Their results showed a bi-phasic dose-dependent response. At lesser concentrations, including those that occur in the plasma of patients during intravenous infusion or nerve blocks, dose related vasoconstriction occurred. Lignocaine $10^4 \mu g/ml$, a concentration similar to that occurring at the site of injection during infiltration, nerve block and epidural, produced vasodilatation.

The interpretation of two vasoactive drugs injected as a mixture such as lignocaine and adrenaline complicates the interpretation further. Covino (1986) suggested the localising vasoconstriction prevented the systemic uptake of the local anaesthetic. However, there have been indications that the vasoconstrictor effect of adrenaline may itself be countered by the local anaesthetic (Astrom 1964).

An in vivo investigation to determine the effects of intra-arterial lignocaine on adrenaline-induced vasoconstriction was reported by Pateromichelakis and Rood (1986). Using a rat model they showed lignocaine injected intra-arterially to the common carotid had no effect on arterial pressure and left the vascular resistance unchanged. Following the lignocaine infusion, the response of the vascular bed to adrenaline was significantly inhibited, with the effect persisting beyond the termination of the lignocaine infusion in a dose-related manner. The inhibition of adrenergic vasoconstriction could have been a non-specific anti-adrenaline effect or $\alpha$-adrenergic antagonism. A general effect mediated through calcium ions on vascular smooth muscle was thought more likely and Frühstorfer and Wagener (1993) designed a stable and reproducible vasodilated skin model to test his hypothesis. An intense vasodilatation was produced by the s.c. injection of 1% histamine into the subdermis of the forearm. This produced a neurogenic inflammation with cutaneous perfusion which was monitored by a two-channel laser Doppler flowmeter combined with an automatic scanning device. The vascular effects of small quantities of local anaesthetics were tested following s.c. injection into the artificially vasodilated skin bed, and three separate mechanisms postulated to explain the observed responses.

Firstly the initial mechanical trauma and chemical irritation stimulated nociceptors and produced additional vasodilatation. Secondly the local anaesthetic blocks sympathetic
axons and reduces the thermoregulatory vasoconstrictor tone and thirdly, the local anaesthetic has intrinsic effects on vascular smooth muscle, either direct or through mediators.

Lignocaine 0.5% and mepivacaine 0.25% demonstrated mild intrinsic vasoconstriction on the precapillary skin bed. Explanation related to the Doppler measurement of blood flowing through vertical skin capillary loops whereas skin colour changes were the result in changes of the deeper horizontal venous plexus. So if a drug has a differential effect on pre-capillary and post-capillary vessels, a large flux in a pale skin could result. In this study no pallor was observed with 0.5% lignocaine indicating the constrictor effect on the post-capillary vessels was less marked. A differential effect on the arterial and venous vascular beds has been reported when lignocaine was injected into the brachial artery of human volunteers (Jorfeldt et al., 1970). When added to 0.5% lignocaine, both adrenaline and omipressin caused a rapid decrease in flux to baseline values and produced significant pallor. In combination with 0.25% mepivacaine both vasoconstrictors produced a significantly less pallor. It was concluded that mepivacaine antagonises the action of omipressin mainly in the post-capillary bed. This antagonism between local anaesthetics and vasoconstrictors, including felypressin, has been observed by others (Altura et al., 1965; Pateromichelakis and Rood 1986; Pateromichelakis 1991;), and confirms the main site of vasoactivity of felypressin to post-capillary vessels (Lilienthal, Reynolds 1976; Lindorf 1979).

In summary lignocaine and mepivacaine at these concentrations had only a mild constrictive effect on pre-capillary vessels, and effective pre-capillary constriction which reduces capillary clearance of both local anaesthetics can be achieved only by addition of a vasoconstrictor. (Pateromichelakis, Rood 1986).
CHAPTER 3

iii. DENTAL AND SURGICAL IMPLICATIONS

The risk during administration of dental injections of lignocaine and adrenaline is related to unintended intravascular injection as a result of which drug boluses reach the central nervous system in high concentration (Scott, 1986). Bartlett, (1972) found 12% of inferior dental injections produced blood on aspiration, but very few from other routine dental sites. Rood, (1972) reported 12% positive aspirations during inferior dental injections and Bishop, (1983) noted this to be 20% in 7-12 year olds, but only 10% in 15-16 year olds.

The ability to aspirate prior to dental injection has been difficult to achieve due to the design of the dental syringe and cartridge. McCarthy, (1984) questioned the reliability of the ASTRA (Evers, 1971), self aspirating cartridge, and Williams, (1984) confirmed at that time no British Standard governed anaesthetic cartridge design. This variability was confirmed by Blair and Meechan, (1985) who reported a greater incidence of positive aspiration using the lignocaine than the prilocaine cartridge, and that prilocaine was inferior in producing mandibular blocks.

Meechan et al., (1986) and Petersen, (1987) used laboratory models to test these ASTRA self aspirating cartridges and found them effective, but so were cartridges with traditional bungs. The ability to aspirate was irrespective of pressure and needle diameter.

The literature contains much controversy as to the appropriate concentration of adrenaline to combine with lignocaine (Keesling, Hinds, 1963; Barclay, 1965; Boulton, 1967; Scott et al.,1972; Vreeland et al., 1989; Yerzingatsian, 1991; Knoll-Kohler, Fortsch 1992), and this has partly been due the variety of sites used and an inability to measure plasma catecholamines directly. Observing cardiovascular responses to dental injections was the conventional method of investigation, but was indirect and failed to apportion the endogenous catecholamine response to the stressful and painful experience. Pressures developed during dental infiltrations were measured by Maita and Horiuchi, (1984) who commented that inadvertent high forces could induce pain and force contents intravascularly.
A recent study by Knoll-Kohler and Fortsch (1992) compared 2% lignocaine with three differing adrenaline concentrations, and measured the effectiveness at producing dental pulp anaesthesia. They found 2% plain lignocaine unsatisfactory in producing adequate dental pain control, and the addition of 1:200,000 adrenaline was not advocated due to the high technical ability required to produce anaesthesia. No benefit was demonstrated in the anaesthetic profile of 1:50,000 adrenaline, and with a significantly increased length of satisfactory analgesia in the 1:100,000 group compared with 1:200,000, the authors recommended the 2% lignocaine + 1:100,000 adrenaline mixture as the most clinically effective.

Taggart et al., (1976) in an article "The Forgotten Vagus" documented the tachycardia minutes before and soon after dental procedures, but a bradycardia immediately prior and during actual treatment. Plasma adrenaline concentrations were raised during treatment, but noradrenaline and plasma lipid concentrations remained unchanged.

Vasovagal episodes during dental treatment were investigated by Edmondson et al., (1978) who defined the reaction as the "development of hypotension and bradycardia associated with the clinical manifestations of pallor, sweating and weakness". In this study 2% of young females and 3% of young males (20-24 year group) suffered an episode, the most significant factor being a shared strong dislike of dental treatment! The site of dental injection and the type of local anaesthetic used was not significant.

Fiset et al., (1985) investigated psychophysiological responses to dental injections and noted that the autonomic responses maybe caused by the exogenous adrenaline or by the endogenous response to fear. Clutter, (1980) infusing various adrenaline concentrations intravenously produced signs and symptoms mimicking these clinical observations. The ability to measure basal plasma catecholamine concentrations in conditions of virtually no physical or psychological stress has proved problematical, making interpretation of drug responses difficult. Buhler et al., (1978) used chronic indwelling catheters to overcome the discomfort of injections and obtained results lower than others previously reported.
Meyer, (1987) reported an increase in heart rate and a small rise in systolic and diastolic pressures during injection and tooth extraction, independent of the local anaesthetic used, and interpreted these findings as an endogenous catecholamine response to stress.

To overcome the problem of separating exogenous from endogenous catecholamine responses attempts were made to measure changes in circulating adrenaline concentrations following routine dental injections. Tolas et al., (1982) reported a 234% rise in arterial adrenaline levels following an 18 µg adrenaline and lignocaine injection in the pterygoid region. Chernow et al., (1983) injected a similar 18µg adrenaline dose as an inferior dental nerve block and measured serial venous plasma catecholamines. He reported a 248% rise in plasma adrenaline concentration. Despite these changes neither study reported clinically significant cardiovascular changes. Reviewing these data with extensive clinical experience, Cawson et al., (1983) concluded that the evidence suggests that anxiety alone can produce at least as severe an effect on the cardiovascular system as the adrenaline in local anaesthetics.

Despite the enormous volumes of dental local anaesthetic solutions used daily throughout the world, the dental literature had no information on the circulating plasma lignocaine concentrations achieved after routine dental injections until 1975. Cannell et al., (1975) compared 2% lignocaine with 2% + 1:80,000 adrenaline using standard dental 2ml cartridges, with doses ranging from 40mg to 160mg of lignocaine. This was a trial conducted in simulated clinical conditions, with a mean injection time of 34 seconds per 2ml cartridge, but no mention of the specific perioral sites of injections was made. Venous plasma assays were achieved using gas chromatography with a flame ionisation detector. Plain 2% lignocaine in doses from 40 mg - 160mg were rapidly absorbed and produced a peak circulating concentration 10 minutes after injection. One subject following 160mg lignocaine injections had a peak of 1.9µg/ml. Maximum concentrations without obvious peaks occurred 30 - 60 minutes following the perioral injection of 40 - 160mg with 1:80,000 adrenaline. The general increase in circulating concentration with dose was more marked after adrenaline solutions than after plain lignocaine. They suggested caution should be shown in cardiac compromised patients and in those on concurrent membrane stabilising drugs, such as phenytoin and propranolol.
Cannell and Beckett, (1975) reported further data on plasma lignocaine concentrations following dental injection of 40 - 80 mg 2% lignocaine or 2% lignocaine +1:80,000 adrenaline into detailed and specific sites. The presence of adrenaline did not have a significant effect on plasma concentrations of lignocaine up to 50 minutes, but the decay was delayed by its presence after this time. With repeated injections some 60 minutes after the first injection a detectable rise in plasma lignocaine was observed, but only after maxillary infiltration, not as inferior dental nerve blocks. They summarised their findings as achieving plasma concentrations less than from a comparable intravenous dose, but the concentrations achieved are more prolonged.

Cannell and Cannon, (1976) compared plasma lignocaine concentrations in healthy volunteers after 1ml 2% lignocaine and 1ml 2% lignocaine + 1:80,000 adrenaline as interosseous injections. They demonstrated the absorption from this intraosseous anterior maxillary site to be comparable to a direct intravenous injection, with the adrenaline not altering the plasma profile from this injection technique. As with their other paper some data were presented on whole blood concentrations, others on plasma. Whole blood concentrations are expected to be only 80% of plasma concentrations, suggesting that the levels could be increased by 20% for comparison with serum or plasma (Tucker, Mather, 1975).

Rood and Cannell, (1978) published a report of a comparative trial where the plasma lignocaine concentrations achieved after 5ml 1% lignocaine + 1:80,000 adrenaline were compared with those after 1ml 5% lignocaine + 1:80,000 adrenaline given as maxillary premolar infiltrations. Plasma concentrations after either of the 50mg injections were mainly between 0.1-0.4μg/ml. One subject felt dizzy after one hour and his plasma lignocaine concentration was 5.0μg/ml two hours following injection. Similar side-effects had been previously described at these plasma concentrations by Foldes et al., (1960) and Aps et al., (1975). The high plasma concentration was believed to be due to minor vascular anatomical differences which permitted the rapid absorption of a large depot of injected lignocaine once the vasoconstrictor effect of the adrenaline had worn off.

Goebel et al., (1980) reported on a study comparing 1.8ml of 2% lignocaine with 2% mepivacaine following maxillary infiltration injections, with samples of venous plasma.
analysed by gas liquid chromatography. The 36mg lignocaine injection produced a peak concentration of 0.31μg/ml at 15 minutes with the values falling slowly through the two hour test period.

Cannell et al., (1975) concluded that the dose of local anaesthetic used did not significantly affect the blood concentrations, but that the adrenaline had a major effect on reducing blood concentrations. With these conclusions in mind, Perovic et al., (1980) designed a trial to assess the different absorption of the 2% lignocaine and 2% lignocaine + 1:80,000 adrenaline from the maxilla and from the mandible. Gas chromatography was used to analyse samples and results were reported as μg/ml of blood, not plasma. Maxillary infiltration of 80mg lignocaine produced concentrations 0.34 - 0.37μg/ml, and 0.21 - 0.29μg/ml after 80mg lignocaine and adrenaline. In the mandible for lignocaine plain 0.32 - 0.42μg/ml, and for lignocaine and adrenaline 0.32 - 0.31μg/ml. Analysis demonstrated a significant difference between the values obtained after lignocaine plain and lignocaine with adrenaline disregarding the injection site.

The cardiovascular responses to local anaesthetics containing adrenaline have been investigated by many authors. Aellig et al., (1970) noted a brief (<30 secs) precipitate heart rate rise of <40 beats/minute in 19 of 27 patients receiving 6ml 2% lignocaine + 1:80,000 adrenaline prior to third molar surgery. No such cardiovascular changes were recorded in a similar group using 6ml 3% prilocaine + 0.03iu/ml felypressin, each group being sedated with 20mg iv diazepam prior to the local anaesthetic injection. Lilenthal and Reynolds, (1975) measured the cardiovascular response to intraosseous and infiltration dental local anaesthetics containing adrenaline or noradrenaline. Irrespective of the catecholamine containing local anaesthetic, a fall in the heart rate immediately prior to the infiltration injection was recorded, followed by a rapid return to pre-injection levels. Intraosseous injections of 11.25μg adrenaline containing local anaesthetic was followed within a few seconds by a rapid increase in heart rate and blood pressure. This was similar to an intravascular injection, as the richly vascular cancellous bone rapidly absorbed the drug combination.

They found no significant systolic or diastolic pressure changes following an 18μg adrenaline dose. Hirota et al., (1986) used an echocardiograph to measure cardiovascular changes after 2% lignocaine + 1:100,000 adrenaline or 3% prilocaine + 0.03iu/ml felypressin in patients with known severe cardiovascular disease. The administration of lignocaine and adrenaline enhanced the cardiac output of all patients by increasing the heart rate in 4 cases and increasing the stroke volume in the other 5 patients. Prilocaine and felypressin produced less of an increase in cardiac function with only one patient showing a significant change in cardiac dynamics, and this during a period of painful dental treatment. The improvement may then have been due to the pain-induced endogenous catecholamine response.

Knoll-Kohler et al., (1989) measured the plasma adrenaline concentrations and cardiovascular changes in normotensive volunteers after maxillary buccal infiltration of three 2% lignocaine solutions, one plain, one with 1:100,000 adrenaline (20μg dose) and the third 1:25,000 adrenaline (80μg dose). The test period was limited to 15 minutes from injection, but they noted that the plain solution had no effect on venous plasma adrenaline concentration, heart rate or blood pressure. The 20μg adrenaline dose raised the venous plasma adrenaline concentration from 0.21 ± 1.0 pmol/ml to 0.42 pmol/ml at 2 minutes post-injection. The 80μg adrenaline mixture produced adrenaline concentrations of 2.23 pmol/ml at 15 minutes post-injection. The heart rate in both adrenaline groups fell, by 4 beats and 13 beats per minute and the diastolic pressure fell by 4mmHg in both groups, but the systolic pressure remained unchanged. The greater absorption from the 80μg group was considered to be due to the adrenergic β-adrenoceptor effects increasing the exogenous adrenaline’s absorption.

Knoll-Kohler et al., (1991) repeated the trial using articaine and 1:100,000 adrenaline (40μg dose) or 1:200,00 adrenaline (20μg dose) as local anaesthesia for third molar surgery. The results showed that the amount of adrenaline absorbed from the injected site predominantly determined the plasma venous adrenaline concentrations. The anaesthetic-induced increase in plasma adrenaline concentration did not correlate with observed changes in the cardiovascular system during the investigation. Serum potassium concentrations remained unchanged, despite observations to the contrary by others (Meechan, Rawlins 1987; Meechan, Rawlins 1988). Pain induced by tooth extraction produced significant increases in plasma noradrenaline concentrations,
confirming that poor local anaesthetic technique is more likely to produce a cardiovascular incident during surgery than the absorbed catecholamine.

Reduced concentrations of lignocaine and adrenaline have been advocated when treating children, with differences in plasma concentrations and pharmacokinetic variables being demonstrated in relation to age and injection site (Finholt et al., 1986; Freund et al., 1984; Scott et al., 1972). Michael et al., (1992) reported the plasma lignocaine concentrations achieved during cleft palate surgery under general anaesthesia. The 0.5% lignocaine and 1:200,000 adrenaline (0.5ml/kg) was injected into the tissues to reduce surgical blood loss and provide post-operative analgesia. The mean plasma lignocaine concentration was low at 1.3µg/ml with peaks occurring within 10 minutes of the injection, confirming the rapid absorption from intra-oral tissues.

Metabolic effects of dental local anaesthetics containing adrenaline have been intensely investigated (Struthers et al., 1983; Meechan, Rawlins, 1987; Meechan, Rawlins, 1988; Meechan et al., 1991). Struthers et al., (1983) infused adrenaline at 0.01 or 0.06µg/kg/min for 90 minutes to achieve plasma adrenaline concentrations (5.7 ± 1.7pmol/ml) similar to those seen after myocardial infarction. A significant hypokalaemia from 4.06mmol/L to 3.22mmol/L was detected and such potassium concentrations post-infarction are associated with an increased risk of ventricular dysrhythmias.

Meechan and Rawlins, (1987) reported on the cardiovascular response and changes in plasma potassium following maxillary infiltrations of 4.4ml 2% lignocaine + 1:80,000 adrenaline (55µg dose) or 4.4ml 3% prilocaine + 0.03iu/ml/ml felypressin. No significant differences in heart rate or systolic pressure were recorded, but the diastolic pressures at 0,10,20,30 minutes post-injection were significantly lower in the adrenaline group. Plasma potassium concentrations were significantly lower in the adrenaline group at 10,20,30 minutes post-injection, but the concentrations reported were less than noted by Struthers et al., (1983).

Meechan and Rawlins, (1988) found no significant difference in systolic or diastolic pressures in a similar follow-up clinical trial, designed to measure the effect of an adrenaline containing local anaesthetic compared with prilocaine and felypressin on
plasma potassium levels and cardiovascular changes. As reported earlier they confirmed the significant hypokalaemia immediately after the injection of adrenaline-containing local anaesthetic and sustained for 30 minutes. They concluded that their studies agreed with other reports (Boakes et al., 1973; Cawson et al., 1983) suggesting that cardiovascular responses to adrenaline injected during dental local anaesthesia are negligible, except perhaps in patients on concurrent tricyclic anti-depressant medication.

Repeating the study measuring the plasma potassium concentrations following dental local anaesthetic injections given during standard third molar surgery under general anaesthesia, Meechan et al., (1991) again showed the fall in plasma potassium in response to the lignocaine and adrenaline. The speed of injection of the local anaesthetic was thought to influence the biochemical effect as Lim et al., (1982) reported the hypokalaemic response to be dose dependant. However the changes in plasma potassium concentrations were small and not thought to be of clinical significance unless patients were severely depleted of potassium or on digitalis (Struthers et al., 1983).

Limited data from other related surgical disciplines exists on the clinical uses of lignocaine, and lignocaine and adrenaline solutions. Kanto et al., (1980) aimed to establish the plasma lignocaine concentrations in neurosurgical patients after cranial subcutaneous injection. Patients received lignocaine 0.8-3.7 mg/kg + 1:200,000 adrenaline and plasma was assayed by gas chromatography. They noted that the absorption of lignocaine is rapid producing peak values (0.6 - 1µg/ml) at between five and ten minutes. When lignocaine was given simultaneously both subcutaneously and intravenously, the plasma concentrations were additive.

Le Normand et al., (1989) attempted to determine the pharmacokinetics in 11 adult patients of a mixture of 40mg lignocaine (2%) and 20mg bupivacaine (0.5%) half as a retrobulbar block and half as a facial block. Serial venous plasma and cerebrospinal fluid (CSF) assays were measured using gas-liquid chromatography. Both drugs appeared rapidly in the plasma, the maximal lignocaine concentration being 0.73 ± 0.33µg/ml obtained 24.7 ± 23.0 minutes after injection. After the lignocaine peak, the plasma concentration declined biexponentially.
Yerzingatsian, (1991) also reported on high lignocaine doses associated with surgical procedures. He reported that low dose lignocaine solutions (0.2%, 0.4% and 0.5%) up to 500mg as plain solutions or 670mg with 1:400,000 adrenaline are satisfactorily for the infiltration technique described. For the 1200 general surgical procedures undertaken using this type of local anaesthesia there was no reported serious morbidity attributable to the lignocaine.

In summary, local anaesthetic analgesia has an outstanding safety record despite the potential toxicity of the drug combinations involved. The absorption of local anaesthetics and vasoconstrictors has been shown to depend upon the vascularity of the injection site, the dose injected, and the concentration of the vasoconstrictor present. Local anaesthetic toxicity is not closely related to plasma concentration in the clinical doses injected, and many "adverse reactions" are probably psychological responses to the injections or unintended intravascular injection.
CHAPTER 3

iv. LIGNOCAINE

Lofgren synthesised lignocaine (lidocaine, USP), the first of the amide local anaesthetic compounds in 1943. In January 1948 lignocaine was released for general use in Sweden and later in the same year it gained approval for clinical use in Washington, United States of America. In 1954 lignocaine officially entered the British Pharmaceutical Codex and since 1955, in the British Pharmacopoeia, despite being clinically available earlier.

Foldes et al., (1960) reported on the comparative intravenous toxicity of four local anaesthetics including for the first time lignocaine. The experiment was prompted by the differing metabolic pathways for the ester and amide local anaesthetics. Healthy male volunteers were infused with procaine, chloroprocaine, lignocaine or tetracaine on differing occasions at weekly intervals. Infusions were discontinued after 25 minutes or earlier if generalised twitching, convulsions, respiratory distress, loss of consciousness or total disorientation developed.

Plasma concentrations for the three ester and single amide (lignocaine) anaesthetic were serially measured. Lignocaine was infused at 0.5mg/kg/min, the duration of tolerated infusion being considerably shorter than with the three hydrolysable anaesthetics (12.8 ± 1.1 mins lignocaine, 19.2 ± 1.7 mins procaine). None of the ten subjects tolerated the lignocaine infusion for more than eighteen minutes. The lignocaine plasma concentrations at the termination of the infusion were 5.29 ± 0.55 μg/ml, and signs of toxicity occurred after 1.5 mg/kg body weight had been infused. The incidence of moderate elevation of blood pressure was greatest with lignocaine. The previously held belief that lignocaine was less likely to cause signs of central nervous system irritation than the ester types was not substantiated by this study. The rate of fasciculation was the highest after lignocaine, with one subject developing convulsions, and the incidence of euphoria was also highest after lignocaine.

Bromage and Robson, (1961) reported on the plasma concentrations of lignocaine after intravenous, intramuscular, epidural and endotracheal administration. Acute toxicity after intravenous infusion in patients already anaesthetised with thiopentone, nitrous
oxide and oxygen was observed with doses ranging between 9.7 - 16.3 mg/kg body weight, and was manifested by a falling blood pressure and diminished tidal volume. If the infusion was continued convulsions rapidly developed. The minimum concentration at which toxic effects occurred was approximately 10 µg/ml. In the epidural group the use of 1:200,000 adrenaline with the lignocaine produced significantly lower plasma lignocaine concentrations.

Regional anaesthesia was obtained using lignocaine as reported by Adams et al., (1964). Using the technique described by Bier, (1908), they injected 40 to 50 ml of 0.5% lignocaine intravenously into a previously exsanguinated arm, on which a tourniquet was applied. Anaesthesia was excellent in all cases, and less than 0.1 µg/ml lignocaine was detected five minutes after the tourniquet release. The absence of symptoms was explained by more complete absorption in the exsanguinated extremity, and the maintenance of the anaesthetic solution in the limb for usually more than one hour.

The literature was becoming clearer in the use of lignocaine as a safe local anaesthetic, but various recommendations were advocated for safe limits at different sites of injection. Barclay, (1965) made a plea for more general surgical procedures to be performed under local anaesthesia as he experienced no anaesthetic or post-anaesthetic mortality. He advocated a safe maximum dose of lignocaine being 400ml of 0.25% with 1:200,000 adrenaline, and 200ml of 0.25% plain lignocaine, and in patients with known coronary disease the adrenaline solution reduced to 1:400,000. This 1000mg lignocaine dose repeatedly produced no clinical signs or symptoms of toxicity.

Boulton, (1967) described some limitations to adequate surgical procedures performed under local anaesthesia. Fat absorbs anaesthetic solutions and obscures landmarks, peripheral vasoconstriction prevents the effective use of infiltration and infection at the proposed injection site may contraindicate the use of local anaesthesia. He advocated a maximum 500mg dose of lignocaine with 1:200,000 adrenaline and 200mg when used plain: ie approximately 7mg/kg with adrenaline and 3mg/kg plain.

The non-anaesthetic use of lignocaine in treating cardiac dysrhythmias has been extensively investigated with reports on effective plasma lignocaine concentrations being compared with those achieved following perioral dental injection (Cannell et al.,
Selective reports relating plasma lignocaine concentrations, toxicity and cardiovascular effects will be briefly discussed.

The value of intravenous lignocaine in the management of cardiac arrhythmias after acute myocardial infarction was assessed by Jewitt et al., (1968). After an intravenous injection of 1mg/kg, ventricular extrasystoles were completely abolished within 90 seconds. In patients requiring lignocaine infusions a dose of 1-2mg/minute was given, with no significant cardiovascular changes noted. Lignocaine concentrations after a single intravenous injection of 1mg/kg over 2 minutes produced a peak concentration of 10 µg/ml reached at the end of the injection. This falls rapidly such that after 30 minutes has reached 1-2 µg/ml. After a continuous infusion of 2mg/min the plasma lignocaine plateaued at 1.5 - 2.5 µg/ml after approximately 180 minutes. Suppression of frequent ventricular extrasystoles is achieved at this concentration. Apart from transient drowsiness, side effects were absent from a single bolus injection, but with continuous infusions they reported drowsiness, twitching, confusion and disorientation. Plasma concentrations in these patients were 2.7 µg/ml and 3.0 µg/ml. They conclude that there was no clear correlation between blood concentrations and the development of therapeutic effects or side effects.

Massive doses of lignocaine ranging from 880mg to 1860mg (11-20mg/kg) were injected to assess the circulatory effects of peridural block by Bonica et al., (1970). They demonstrated increases in heart rate and cardiac output as previously shown by Foldes et al., (1960). They suggested that the cardiac stimulating effects of lignocaine could be produced through central nervous system action or be a compensatory reflex response to vasomotor blockade. The plasma arterial concentrations obtained ranged from 4-7µg/ml and were associated with an increased cardiac output.

The cardiac responses to lignocaine boluses and infusions led Jebson, (1971) to investigate the use of an intramuscular injection of a high concentration (10%) lignocaine solution to avoid these problems. Volunteers were injected with 2ml of 10% lignocaine on one occasion and 10ml of 2% on another, as deep intramuscular injection. Venous plasma lignocaine concentrations were estimated by gas chromatography, and mean concentrations peaked in both groups 45 minutes after injection, the 2% 0.95 ±
0.60 μg/ml and 10% 0.61 ± 0.20 μg/ml. Five subjects produced plasma concentrations in excess of 1.5 μg/ml. Subjective signs of toxicity did not occur, but a significant fall in heart rate from control was noted in both groups. No other changes in cardiac rhythm, blood pressure or respiration were evident.

The effective therapeutic blood concentration of lignocaine for the treatment and prevention of ventricular arrhythmias was quoted as 1.2 - 5.5 μg/ml by Stenson et al., (1971) and was substantiated earlier by Jewitt et al., (1968). This concentration was achieved by an infusion rate of 20-50 μg/kg/min, but higher blood concentrations of lignocaine have been noted with similar infusion rates in patients with circulatory depression. Stenson et al., (1971) investigated the inter-relationships of hepatic blood flow, cardiac output and blood concentrations of lignocaine in patients undergoing cardiac catheterisation. Lignocaine was administered as a 50mg bolus followed by a constant infusion of 40μg/kg/min until steady state was achieved. With a low cardiac index of 1.9 ± 0.3 L/min/m² the arterial lignocaine concentration was 2.4μg/ml, while with a normal cardiac index of 3.3 ± 0.8 L/min/m² it was 1.5μg/ml. A linear relationship between estimated hepatic blood flow and cardiac index was observed, so an inverse relationship between arterial lignocaine concentrations and hepatic blood flow was noted. At steady state conditions the liver accounted for 70% of the metabolism or removal of the administered lignocaine. They concluded that lower doses of lignocaine would produce effective therapeutic concentrations in patients with low output states and with underlying liver disease.

Scott et al., (1972) investigated further factors affecting plasma concentrations of lignocaine comparing the same 400mg dose of the plain drug from four sites; intercostal, subcutaneous vaginal, subcutaneous abdominal and lumbar epidural.
Table 3:1

RELATIONSHIP OF SITE OF INJECTION TO MEAN MAXIMUM VENOUS PLASMA LIGNOCAINE CONCENTRATION FOLLOWING INJECTION OF 20ML 2% PLAIN LIGNOCAINE (SCOTT et al., 1972)

<table>
<thead>
<tr>
<th>Site</th>
<th>n</th>
<th>Mean Maximum Lignocaine Concentrations (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intercostal</td>
<td>11</td>
<td>6.48 ± 0.38</td>
</tr>
<tr>
<td>SC Vaginal</td>
<td>9</td>
<td>4.91 ± 0.43</td>
</tr>
<tr>
<td>Epidural</td>
<td>23</td>
<td>4.27 ± 0.24</td>
</tr>
<tr>
<td>SC Abdominal</td>
<td>10</td>
<td>1.95 ± 0.23</td>
</tr>
</tbody>
</table>

Plasma lignocaine concentrations were related to the vascularity of the injection site, highest concentrations being reached most quickly in the most vascular areas. The effect of adrenaline at the different sites was assessed, and the subcutaneous injection was most affected by adrenaline, regardless of tissue vascularity, with a 50% reduction in plasma concentration. Recommendations as to optional adrenaline concentrations have been argued for many years, ranging from 1:80,000 to 1:400,000. Scott's results showed that only rarely is 1:80,000 superior to 1:200,000, and that 1:400,000 cannot be distinguished from plain solutions.

As the newer longer acting local anaesthetics bupivacaine and etidocaine became available, data appeared on their relative toxicities compared with lignocaine. Scott, (1975) reported on studies where lignocaine and etidocaine were infused at 20µg/min to a maximum dose of 250mg. All subjects tolerated the lignocaine infusions but etidocaine was more toxic, the mean tolerated dose being only 161mg. Side effects of light headedness occurred in all subjects treated with lignocaine, but circumoral numbness and twitching was much less noticeable. Peak lignocaine plasma concentrations were achieved at 10 minutes with a mean concentration slightly greater than 2µg/ml, with etidocaine 1.55 more toxic than lignocaine. This data strongly suggested that until a steady state has been achieved, venous plasma concentrations of local anaesthetics may not be an accurate guide to toxicity, which is usually due to inadvertent rapid intravenous administration, overdosage or rapid absorption from the injection site.
The development of sophisticated methods of analysis lead to a greater understanding of the pharmacokinetics of local anaesthetics. Tucker and Mather, (1975) reported on such studies of lignocaine, mepivacaine, bupivacaine and etidocaine. They noted that plasma and whole blood drug concentrations are not interchangeable and for lignocaine the whole blood concentration is 0.8 to 0.9 of plasma. They noted reduced venous lignocaine concentrations compared with concurrent arterial samples, and felt that arterial concentrations might also be expected to be superior indicators of time profiles of the local anaesthetic agents in well perfused vital organs, especially when venous samples are taken from a limb with impaired perfusion.

Aps et al., (1975) in rationalising the approach to lignocaine therapy in suppressing ventricular dysrhythmias suggested the need to reach and maintain lignocaine concentrations without toxicity was essential. They reported a therapeutic range of 1.2 - 6μg/ml in whole blood and assuming 70% of the lignocaine is in plasma, a given packed cell volume of 45%, the equivalent plasma range would be 1.5-7.5μg/ml. As signs of toxicity have been reported within this range they recommended a plasma therapeutic range of 1.5 - 5μg/ml, but qualified this by suggesting lower infusion rates for patients with reduced cardiac output and hepatic dysfunction.

Increasing interest in spinal and extradural anaesthesia resulted in intensive investigation of the pharmacokinetics of the local anaesthetic agents used. Mather et al., (1976) noted the mean arterial plasma concentration of lignocaine following injection of plain solutions was significantly greater than those containing 1:200,000 adrenaline. The maximum plasma concentration occurred sooner with the plain solutions. Axelsson and Widman, (1981) also produced data showing the effectiveness of adrenaline at reducing blood lignocaine concentrations after spinal anaesthesia (0.14μg/ml with adrenaline, 0.28μg/ml plain). A case of acute lignocaine toxicity was reported by Brown and Sziendzielewski, (1980) when a 2g intravenous bolus was inappropriately administered and cardiopulmonary arrest resulted. Serum lignocaine concentrations 3 and 7 hours post-arrest were 10μg/ml and 5μg/ml, and at 6 hours post-arrest there was 3.9% methaemoglobin. Routledge et al., (1982) advocated plasma lignocaine monitoring when administering lignocaine infusions for prophylaxis of primary ventricular fibrillation, as no clinical end points, other than the dubious ones of ventricular fibrillation or CNS lignocaine toxicity, are available to the clinician.
Scott, (1986) reiterated the pharmacokinetics of an intravenous bolus effect, such that blood with a high drug concentration reaches the CNS followed by blood with a much lower concentration, and so in considering acute toxicity, it is the arterial blood concentration which is important. The lungs have a major role in determining the peak arterial concentration, in that all the injected drug must pass through the lungs before reaching other organs. There is evidence that the lung tissue takes up a considerable amount of the local anaesthetic reaching it, leading to a reduced arterial concentration compared with that of the pulmonary artery blood (Tucker, Boas, 1971).

It is a general principle that the free drug in serum is in equilibrium with the drug at receptor sites and is responsible for the pharmacological effect. In addition, changes in protein binding may effect the distributional and pharmacokinetic properties of a drug (Wilkinson, Shand, 1975). McNamara et al., (1981) and Routledge et al., (1980) had suggested that lignocaine binds to albumin and \( \alpha_1 \)-acid glycoprotein and that lignocaine free fraction is strongly correlated with serum \( \alpha_1 \)-acid glycoprotein. A decrease in pH was found to significantly increase the lignocaine free fraction, but Goolkasian et al., (1983) produced data that indicated binding interactions between lignocaine and various displacing drugs would not be clinically significant, with the exception of bupivacaine. The protein binding of lignocaine was shown to be increased in several clinical situations such as myocardial infarction, increasing age, chronic renal failure and trauma, believed to be due to a rise in plasma \( \alpha_1 \)-acid glycoprotein, (Routledge et al., 1982). The accumulation of active metabolites and individual differences in drug-protein binding was shown by Drayer et al., (1983) to negate the concept of a single value for the upper limit for the therapeutic concentration of lignocaine.

Haasio et al., (1988) investigated plasma arterial lignocaine concentrations in patients who had previously been premedicated with diazepam or morphine and hyoscine. A rapid intravenous infusion of lignocaine 1mg/kg was administered and the \( \alpha_1 \)-acid glycoprotein and plasma albumin fractions measured. They found no correlation between premedication and the CNS symptoms and no correlation between plasma lignocaine concentration and occurrence of CNS symptoms. There was no correlation between \( \alpha_1 \)-acid glycoprotein or albumin concentrations and plasma lignocaine concentration. From this data it would seem that it is the rapidity of exposure of CNS
tissues to local anaesthetics, rather than a defined concentration in the blood that produces CNS symptoms of toxicity.

The duration of anaesthesia is primarily related to the degree of protein binding. Conduction blockade is believed to occur following the interaction of the local anaesthetics with a protein receptor located within the sodium channel of the nerve membrane (Covino, 1986).

In vitro studies on isolated nerves infer a correlation between the partition coefficient of local anaesthetics and the minimum concentration required for blockade. (Gissen et al., 1980; Wildsmith et al., 1985). Mepivacaine and prilocaine are the least lipid soluble and weakest amide agents (0.8 and 0.9) compared with lignocaine (2.9) while etidocaine is the most lipophilic and most potent (141.0). A similar relationship between lipid solubility and potency exists among ester type drugs. (Wildsmith et al., 1985).

In vivo studies in man suggest that the correlation between lipid solubility and anaesthetic potency is not as precise as in isolated nerve preparations. Lignocaine is approximately twice as potent as prilocaine and mepivacaine in an isolated nerve preparation, but little difference is apparent clinically (Covino, Vassallo 1976). The difference between in vitro and in vivo results is thought to relate to the vasodilator or tissue redistribution properties of the anaesthetics. Lignocaine causes increased vasodilatation compared with either mepivacaine or prilocaine, resulting in a more rapid vascular absorption, so that less of the drug is available for neural block in vivo (Covino 1986).

Significant improvement in the efficacy of local anaesthetics has been demonstrated by the addition of vasoconstrictors (Keesling, Hinds 1963; Gangarosa, Halik 1967; Kabambe et al., 1982; Roberts, Sowray 1987; Knoll-Kohler 1992). An in vivo study conducted on a rat sciatic nerve preparation confirmed the increased time of onset, depth of anaesthesia and duration of block by the addition of adrenaline to lignocaine (Canuana et al., 1982). By reversing the activity of the adrenaline and enhancing the vasoactivity of the anaesthetic, it could be possible to usefully reduce the local anaesthetic effect.
Animal experiments and human studies have demonstrated an anti-thrombotic effect of local anaesthetics. Cooke et al., (1977) reported the use of intravenous lignocaine in prevention of deep venous thrombosis after elective hip surgery. In the hamster pouch, lignocaine and other similar local anaesthetics inhibit the formation of thrombi, and aid the restoration of the microcirculation following trauma induced by lasers (Luostarinen, 1981). The response is possibly related to the decreased leucocyte adherence noted in the presence of lignocaine (Giddon, Lindhe, 1979). This membrane stabilising effect may have some clinical use in other areas of inflammatory reaction such as in microvascular tissue transfers and high energy irradiation (Evers, 1988).
CHAPTER 3

v. PRILOCAINE

The emphasis of this thesis relates to the lignocaine and adrenaline local anaesthetic combination. However, prilocaine with felypressin is a routinely used alternative anaesthetic in clinical dentistry, and assessment of the potential to reduce the prolonged soft tissue anaesthesia is subsequently reported. Some background into the prilocaine and felypressin mixture is presented.

In 1959 Eriksson and Gordh reported on another amide local anaesthetic, prilocaine, having the same rapid onset and freedom of side effects as lignocaine, but a shorter duration of soft tissue analgesia. The frequency and depth of anaesthesia were similar to lignocaine, but demonstrated only 60% of its toxicity (Eriksson, 1961; Astrom, Persson, 1961).

In volunteers, the less toxic reaction to a 200mg prilocaine infusion compared with a 200mg lignocaine infusion was believed to be due to differences in tissue distribution and metabolism (Engelsson et al., 1962). Berling and Bjorn, (1960) showed 3% prilocaine + 1:300,000 adrenaline to be of equal effect to 2% lignocaine + 1:80,000 adrenaline. Goldman and Gray, (1963) reported on a dental clinical trial comparing 3% prilocaine + 1:300,000 adrenaline with 2% lignocaine + 1:80,000 adrenaline. There was a very high success rate of analgesia noted in all test solution groups and they found 3% prilocaine + 1:300,000 adrenaline to be comparable with 2% lignocaine + 1:80,000 adrenaline in effectiveness, though prilocaine had a reduced duration of soft tissue analgesia. Mandibular blocks demonstrated no difference in residual soft tissue analgesia periods.

Braid and Scott, (1965) reported on the systemic absorption of local anaesthetics, specifically estimating plasma concentrations of lignocaine and prilocaine during epidural and intercostal regional block anaesthesia. Discussion on toxicity was related to cardiovascular and central nervous systems and related the plasma concentrations achieved to the specific drug employed, the site and application of injection, presence of a vasoconstrictor, drug concentration, total dose administered, speed of injection and the rate of degradation. They noted considerably lower plasma prilocaine concentrations.
compared with lignocaine (400mg of 1% solution injected in each case). As Englesson et al., (1962) had found toxic effects with both drugs at the same plasma concentration, Braid and Scott, (1965) advocated the preferential use of prilocaine especially in doses of 400mg. They also noted a site association of plasma concentrations with significantly higher plasma concentrations following intercostal blocks compared with epidural, for both anaesthetic test solutions. It was postulated that this was due to the reduced absorption from epidural capillaries. The addition of 1:80,000 adrenaline reduced plasma lignocaine concentrations for intercostal and epidural blocks by 25%, but had no significant effect on prilocaine concentrations.

Many reports on the local analgesic action of prilocaine have substantiated the persistence of soft tissue anaesthesia comparable with lignocaine and procaine (Cowan 1968; Jastak, Yagiela 1983; Roberts, Sowray 1987, Meechan, Rawlins 1988). Padfield (1967) used an intradermal flexor forearm model as described by Mongar (1955), and as similarly reported in Chapter 5, 2% prilocaine was compared with similar concentrations of lignocaine and procaine and the percentage anaesthesia recorded against time. Prilocaine was more active than lignocaine (95% CI) and procaine (95% CI). An inherent mild vasoconstriction by prilocaine was noted which was less marked than that following 1:200,000 adrenaline. Cowan, (1968) postulated that this mild vasoconstriction of prilocaine compared with the vasodilatation of lignocaine could explain why it is so effective with little or no added adrenaline. Goldman and Evers, (1969) investigated a combination of 3% prilocaine and 0.03iu/ml felypressin comparing this with 3% prilocaine and 1:300,000 adrenaline using dental local anaesthesia models. A marginal increase in success rate of the prilocaine and felypressin combination was noted during procedures necessitating prolonged anaesthesia.

A similar clinical approach was reported by Epstein, (1969) when comparing 4% prilocaine plain with 4% prilocaine and 1:200,000 or 1:300,000 adrenaline. The duration of soft tissue anaesthesia was significantly reduced in the 4% prilocaine plain group.

The measurement of plasma concentrations of local anaesthetic drugs became increasingly popular because of the possible dose relationship between the systemic blood concentration and the toxicity of the drugs. Scott et al., (1972) suggested the
potential factors affecting plasma concentrations of local anaesthetics are:
(1) site of injection (2) drug used, (3) addition of a vasoconstrictor (4) concentration of the drug (5) speed of injection (6) total drug dosage (7) weight of patient (8) age of patient (9) rate of elimination. In a series of over 500 clinical cases they compared lignocaine and prilocaine solutions. Achieved plasma concentrations were related to the vascularity of the injection site, but prilocaine concentrations were always less than lignocaine. No head and neck or dental injections were used, the most comparable highly vascular site being the intercostal area. Subcutaneous injection, regardless of tissue vascularity or anaesthetic type, was most affected by adrenaline, which reduced plasma concentrations by 50%. In the highly vascular intercostal area both the addition of 1:200,000 and 1:80,000 adrenaline to the 2% prilocaine produced significant reductions in plasma concentrations, but in the less vascular epidural model where fewer capillaries are present, there was no significant advantage in the addition of the higher adrenaline concentration. However, there was a suggestion that the optimal concentration of 1:200,000 adrenaline at these specific test sites is more effective in combination with lignocaine than prilocaine. The dosage of drug was tested by comparison with and without 1:200,000 adrenaline at the epidural site. The relationship, as to be expected, was linear in the dose range from 200-600mg with regression lines 0.76 and 0.57 confirming the lower plasma anaesthetic concentrations attained in the adrenaline group. The weight and age of all patients (adult females) was unrelated to maximum plasma concentrations.

The effectiveness of felypressin in reducing plasma prilocaine concentrations had been assessed by Cannell and Whelpton, (1986). Clinically, the effectiveness of a vasoconstrictor in the surgical environment had long been questioned, and Newcombe and Waite, (1972) confirmed that over four times as much bleeding occurred with prilocaine and felypressin used as the local anaesthetic, as compared with lignocaine and 1:80,000 adrenaline. A 4% prilocaine + 1:200,000 adrenaline solution was reported by Greenfield and Karpinski, (1975) as having an adequately rapid onset, duration of effect and provided good haemostasis in the dental surgical test model. They commented on methaemoglobin concentrations achieved using this 4% prilocaine solution and concluded that the possibility of clinically significant methaemoglobinemia as extremely remote.
As prilocaine and felypressin became more routinely available in dental and oral surgery practice, the cardiovascular responses were reported, usually in comparison with the more commonly used vasoconstrictor adrenaline. Lilienthal, (1976) investigated a comparison of 0.9ml of 4% prilocaine with 0.9ml of 3% prilocaine + 0.03iu/ml felypressin and 0.9ml 4% prilocaine + 1:200,000 adrenaline as intraosseous mandibular injections. Prilocaine plain solution had no effect on systolic and diastolic pressures, nor heart rate. The felypressin combination slightly dropped the heart rate in 6 of the 9 subjects, but returned to pre-injection rates within five minutes. Adrenaline injection was associated with a rise in systolic pressure and a corresponding slight decrease in diastolic pressure, but did not indicate major effects. The heart rate increased in a range of 11 to 30 beats per minute within 10 seconds of completing the injection. All blood pressure and heart rate changes had returned to pre-injection concentrations within 3 minutes.

Aellig et al., (1970) reported the cardiovascular changes in patients undergoing third molar surgery under diazepam sedation. Patients were randomised to receive 6ml 2% lignocaine + 1:80,000 adrenaline or 3% prilocaine + 0.03iu/ml felypressin. A "precipitate" rise in heart rate of 40 beats per minute (lasting <30 seconds) was noted in 19 of 27 patients in the adrenaline group during or immediately after the injection, irrespective of the volume injected. A more "gradual" rise in heart rate after the adrenaline injection (31 beats per minute maximum) was also noted. No significant changes in heart rate were observed in the prilocaine group, and neither group had significant blood pressure or cardiograph changes. The "precipitate" rise in heart rate was assessed as some of the adrenaline entering small veins, whereas adrenaline slowly absorbed from the injection site would produce the more "gradual" heart beat changes. The method of monitoring heart rate was with a beat-to-beat heart ratemeter from the electrocardiogram, and the reliability of this type of recording device was not discussed.

Dentoalveolar surgery has been developed as a test model for local anaesthetics enabling assessment of the efficiency of the anaesthetic and the ability of vasoconstrictors to reduce blood loss. Lindorf, (1979) investigated the vascular effects using infrared thermography to measure the effects of vasoconstrictors and local anaesthetics. Prilocaine 4% did not produce vasodilatation compared with the positive response seen with lignocaine 2%, and mepivacaine produced only a mild vasoconstriction.
Vasoconstriction after a dental injection containing adrenaline was noted immediately, but felypressin produced an infrared effect only after 10 minutes. The reduction in vasoconstriction of both adrenaline and felypressin anaesthetics began after 60 minutes, but there was a marked reactive hyperaemia 3 hours later in the adrenaline group.

Investigation of the effects of lignocaine, adrenaline, prilocaine and felypressin alone or in combination were investigated on dental pulp function by Olgart and Gazelius, (1977) using a cat molar tooth model. Adrenaline either alone or with lignocaine caused almost complete inhibition of pulp blood flow within a few minutes. This was followed by a total inhibition of sensory nerve activity taking 3 hours to recover. Felypressin, prilocaine and lignocaine had no inhibitory effects on pulp blood flow. As both adrenaline and felypressin delay the absorption of local anaesthetics, then at the injection site, adrenaline and felypressin have similar effects on the pre-capillary vasculature, so reducing the numbers of open capillaries. Olgart and Gazelius, (1977) postulated that the diffusion limits for the large polypeptide felypressin may be greater than for adrenaline and so penetrate interosseously to a much reduced capacity. Blair and Meechan, (1985) in a comparative study of the dental use of lignocaine and adrenaline with prilocaine and felypressin showed successful anaesthesia significantly more likely with the lignocaine and adrenaline anaesthetic combination.

Little data on plasma prilocaine concentrations achieved following perioral injections exist, and Cannell and Whelpton, (1986) reported on concentrations achieved after only 2ml of 3% prilocaine (60mg) and 2ml of 3% prilocaine and 0.03iu/ml felypressin. On one occasion two subjects were injected into the maxillary premolar region with 80mg of 4% prilocaine. Venous plasma samples were assayed for prilocaine using gas chromatography and a nitrogen-phosphorus detector. Peak concentrations after 3% prilocaine occurred at between 10 and 20 minutes after maxillary premolar injection. Following this the loss from the circulation was rapid and even, such that at 120 minutes less than 0.1µg/ml plasma was detected. Peak concentrations in the prilocaine and felypressin group were in some cases earlier at 10 minutes or attained after a more gradual rise by 30 minutes. In both test subjects greater amounts of prilocaine were found in the plasma, and positioned for longer in the felypressin group. As might be anticipated infiltration of 4% prilocaine produced higher peak concentrations than the 3% solution. Explanation of these findings was that the rapid absorption from this
maxillary premolar site was such as to render the localising effect of the felypressin ineffective, and as such the addition of a vasoactive substance to a formulation did not necessarily limit circulating concentrations of the local anaesthetic drug.

Maclean et al., (1988) reported on plasma prilocaine concentrations achieved after brachial plexus blocks achieved via three different techniques. 35ml of 1.5% prilocaine was injected as axillary, perivascular subclavian or interscalene blocks and plasma samples assayed using HPLC over a 60 minute test period. The mean areas under the curves were not significantly different in the three groups studied. The range of results was wide and one patient in the interscalene group developed a peak concentration of 10.94mg/L, but the timing of the peak did not suggest an intravenous injection. It has been suggested that the threshold for toxicity of prilocaine is 6.0mg/L by Scott and Cousins, (1980) and Maclean et al., (1988) stressed the need for vigilance when even using the 52.5mg dose described.

Prilocaine has the unusual effect of causing methaemoglobinaemia in a dose-related fashion. Daly et al., (1964) defined methaemoglobinaemia as a condition in which cyanosis in the absence of cardiac or respiratory disease, is due to the presence of excessive amounts of methaemoglobin in the blood. Neonatal methaemoglobinaemia was reported by Harley and Celermajer, (1970) in an eight hour old female infant whose mother had received 20ml of 1% prilocaine as a paracervical block.

Warren et al., (1974) reported on methaemoglobinaemia induced by 12ml of 4% prilocaine +1:200,000 adrenaline and by 15ml of 4% prilocaine + 1:200,000 adrenaline. They noted that 1.5g/100ml of altered haemoglobin will produce clinical signs and symptoms of methaemoglobinaemia. The treatment for the condition was discovered by Wendel in 1937, who found that 1% methylene blue acts as an electron carrier in the hexose monophosphate shunt and would accelerate the reduction of ferric to ferrous ions (Cohen, Bovasso, 1971). The advocated dose is 1 to 2mg/kg body weight administered slowly intravenously.

Weiss et al., (1987) reported results of methaemoglobin concentrations assayed before and after bolus and maintenance infusions of lignocaine used to stabilise cardiac patients. There were statistically significant elevations of methaemoglobin con-
centrations in all patients but the highest concentrations obtained was only 1.2% and this was noted to be clinically insignificant.

More recently a case has been reported of neonatal methaemoglobinemia following the subcutaneous injection of 0.7mls 3% prilocaine and 0.03iu felypressin in a 3.5kg baby that required a facial laceration suturing. Cyanosis developed after one hour with a methaemoglobin concentration in excess of 20%. Neonates are more susceptible to methaemoglobin due to the reduced methaemoglobin reductase concentrations in the neonatal erythocyte (Lloyd 1992).

In this study methaemoglobin concentrations were assayed (Chapter 10) to exclude this possibility following multiple prilocaine dental injections which had been reversed with phentolamine.
Felypressin is partly responsible for the extensive soft tissue anaesthesia of the orofacial tissues following routine dental injections of the prilocaine and felypressin mixture (Cowans 1968; Jastak, Yagiela 1983; Roberts, Sowray 1987; Meechan, Rawlins 1988). By neutralising the effect of the vasoconstrictor in vivo may help to usefully reduce the period of soft tissue anaesthesia. Some relevant background details of felypressin are presented to substantiate this hypothesis.

The initial synthesis of the posterior hypophyseal hormones by du Vigneaud, (1969) subsequently led to the development of structural analogues of the compounds. Ribot et al., (1963) reported on the haemodynamic effects of one of the compounds, phenylalanyl-2-lysyl-8vasopressin (PVL-2), known subsequently as felypressin. They noted a primary vascular effect supported by the increase in systemic arterial pressure despite a decrease in cardiac output.

Altura et al., (1965) reported on the effects of felypressin (PVL-2) on the microvascular smooth muscle in rats and isolated muscle strip preparations. Topical application of 0.05iu felypressin was the lowest concentration to produce significant arteriolar, capillary and venular constriction. A 0.01iu concentration affected venules only. They concluded that felypressin exhibits paradoxical selective microvascular effects as the gradient of activity is from the venule towards the arteriole in contrast to the arteriolar to venule gradient seen with the catecholamines adrenaline and noradrenaline. They also noted that felypressin in low doses may inhibit adrenaline activity, whilst higher doses potentiate vasoconstriction. This is similar to serotonin which has been shown to have constrictor or dilator effect depending on the vessel tone present.

Early combinations of adrenaline with prilocaine were reported by Cowan, (1968) comparison made with a 2% lignocaine + 1:100,000 adrenaline solution. The 3% prilocaine + 1:300,000 adrenaline produced dental anaesthesia as effective as the lignocaine and adrenaline combination, but with a shorter duration of activity. Epstein,
(1969) presented data from a double blind study comparing the effectiveness of 4% prilocaine, 4% prilocaine and 1:200,000 adrenaline, 4% prilocaine and 1:300,000 adrenaline and 2% lignocaine with 1:200,000 adrenaline. The 4% prilocaine had a significant shorter duration of action and was equally as effective with or without adrenaline as the lignocaine and adrenaline mixture for both nerve blocks and infiltrations.

The actions and effects of felypressin are different in several respects from the catecholamines. Felypressin reduces local blood flow by directly causing vascular smooth muscle to contract. Although intravenous infusions of large doses can increase systemic and pulmonary vascular resistance, decrease in heart rate and cardiac output (Maxwell, 1965), the primary effect when used in similar doses typically found in local anaesthetics is of vasoconstriction of the venous side of the circulation with no major cardiovascular effects (Lilienthal, Reynolds, 1976; Lindorf 1979; Fruhstorfer, Wagener 1993). Felypressin seems to lack any dysrhythmic potential and has been advocated in patients with cardiac disease or in conjunction with anaesthesia, as it lacks the cardiac chronotropic and inotropic α and β effects of catecholamines. (Katz, 1965).

Although useful in localising the local anaesthetic so increasing potency and duration, it provides an inadequate control of haemorrhage during surgery due to the poor ability to constrict arterioles (Cowan 1968; Newcomb, Waite, 1972).

The determination of optimal concentrations of both local analgesic agent and the vasoconstrictor is critical. As most local anaesthetics apart from cocaine have an intensive vasodilatory effect which increases with concentration, this must be countered by increasing the concentration of the added vasoconstrictor.

The mixtures produced have been analysed by Goldman et al., (1967) in vivo using a rabbit’s ear preparation, assessing vasoconstriction by transillumination. Goldman and Evers, (1969) reported on the prilocaine-felypressin combination being compared with a prilocaine-adrenaline mixture. The combination of 3% prilocaine and 0.03iu/ml felypressin gave marginally superior results, particularly when dental procedures lasted over half an hour. They reported that the vasoactive nature of felypressin in conjunction with prilocaine was not effective when coupled with lignocaine.
Previously, Berling, (1966) had tested the overall effectiveness of lignocaine combined with different felypressin concentrations. He found no combination of lignocaine and felypressin to be as effective as 2% lignocaine and 1:80,000 adrenaline. The optimal concentration of felypressin in the 3% prilocaine solution was found to be very low at 0.03iu/ml. Lower and higher concentrations of felypressin were both found to decrease the duration of pulp analgesia.

Goldman and Evers, (1969) reported in the dental literature a new local anaesthetic combination of prilocaine and felypressin. They noted that prilocaine plain solution had initial pain blocking efficacy, but the short duration of activity after infiltration was inconvenient during prolonged dental procedures. They presented data on over 1500 dental injections using 3% prilocaine + 0.03iu/ml felypressin and 3% prilocaine + 1:300,000 adrenaline. However, this was a particularly low concentration of adrenaline to use in dental anaesthetic practice. They reported that 3% prilocaine and felypressin 0.03iu/ml produced marginally more effective anaesthesia compared with the prilocaine and adrenaline combination, especially when the procedure lasted over thirty minutes.

Fisher et al., (1965) reported on animal and human studies and confirmed that felypressin is of low toxicity with few systemic effects. Lilenthal, (1976) reported on the cardiovascular responses to intraosseous injections of prilocaine and prilocaine with felypressin. The prilocaine and felypressin combination dropped the heart rate slightly in 6 out of 9 subjects, but returned to normal within 5 minutes.

Lindorf, (1979) used infrared thermography to make comparative assessment of the vascular effects of dental local anaesthetics. Felypressin had a delayed effect compared to the immediate vasoconstrictor cooling produced by 1:100,000 adrenaline. The delay was in the order of 10 minutes and after 20 minutes significant vasoconstriction was achieved. The decrease in vasoconstriction of both agents began after 60 minutes. No rebound vasodilation was seen in the felypressin groups as compared to that seen at 3 hours in the adrenaline group.

In a review article Jastak and Yagiela, (1983) described the incorporation of vasoconstrictors in local anaesthetics; for enhancement of anaesthesia, reduction in peak plasma concentration of the anaesthetic, and control of bleeding. Although intravenous
infusions of felypressin can increase systemic and pulmonary vascular resistance and decrease heart rate and cardiac output (Maxwell, 1965; Ribot et al., 1963), when used in the small amounts typically found in routine local anaesthetics it is the venular side of the circulation that is responsive for the activity of vasoconstriction and no other significant cardiovascular responses were reported (Altura et al., 1965).

Cannell and Whelpton, (1986) measured plasma concentrations after perioral injection of 3ml of 3% prilocaine with and without felypressin 0.03iu/ml. Their results demonstrated early peak plasma prilocaine concentrations soon after injection and were almost independent of the inclusion of felypressin. They suggested that the response of blood vessels adjacent to the injection site was a variable one with smaller diameter vessels constricted by felypressin whilst those >6 μm were less affected, or not at all. Matthews, (1986) states felypressin is a vasoconstrictor but suggests at 0.03iu/ml (1:1,850,000) could not have any direct effect upon vascular smooth muscle. His comments seem to be without foundation. Munchow and Denson, (1964) clearly demonstrated a blanching vasoconstrictor effect of felypressin with a relative potency to adrenaline of 0.7. In view of Newcomb and Waite’s, (1972) observation that prilocaine and felypressin failed to control blood loss during periodontal surgery it would seem that Jastak and Yagiela’s, (1983) reasons for incorporating felypressin with prilocaine are restricted only to the enhancement of anaesthesia.
vii. MEPIVACAINE

Mepivacaine and adrenaline is an alternative local anaesthetic used in mainland Europe. The length of residual soft tissue anaesthesia is comparable to standard lignocaine and adrenaline (Cowan 1965; Aberg, Sydnes 1978; Aberg 1980). Data are presented (appendix ii, iii) on attempts at reducing the soft tissue residual anaesthesia to enable comparisons to be made with the standard lignocaine and adrenaline anaesthetics. Some relevant background information on mepivacaine is presented.

Mepivacaine, dl-N-methylpipecolic acid-2,6-dimethylanilide hydrochloride, is an amide local anaesthetic, similar to lignocaine in terms of anaesthetic profile, producing a profound depth of anaesthesia with a relatively rapid onset and moderate duration of action. It may be used successfully for infiltration, peripheral nerve blocks and extradural anaesthesia in concentrations ranging from 0.5% to 2%. Mepivacaine is not effective as a topical agent and metabolism is prolonged in the foetus and newborn, so it is not used in obstetrics. In adults, however, it seems less toxic than lignocaine and has a measurable inherent vasoconstrictor effect compared with the vasodilatation of lignocaine (Willatts, Reynolds, 1985).

Cowan, (1965) compared prilocaine and mepivacaine over a range of concentrations, as dental anaesthetics. Little difference in onset time and duration was noted for 4% prilocaine and 3% mepivacaine solutions. A success rate of dental anaesthesia of 94% with prilocaine and 82% with mepivacaine was reported by Aberg and Sydnes, (1978).

Dhuner et al., (1965) measured mepivacaine concentrations in venous whole blood samples from patients undergoing brachial plexus blocks or infiltration hernia repair. Injection groups were divided into 0.5% mepivacaine ± 1:200,000 adrenaline for infiltration or 2.0% mepivacaine ± 1:200,000 adrenaline for the nerve blocks. In the hernia group after a mean injection of 360mg mepivacaine plain produced blood concentrations which peaked at 30-45 minutes with a mean concentration of 1.25µg/ml.
The addition of adrenaline delayed the peak to 120 minutes and reduced the mean blood concentration to 0.72μg/ml. Brachial plexus blocks with 2% mepivacaine ± 1:200,000 adrenaline demonstrated no statistical influence by the vasoconstrictor in reducing venous blood mepivacaine concentrations, and peak values were achieved in both groups between 30 and 45 minutes after injection (1.24μg/ml and 1.25μg/ml). Tucker et al., (1972) measured arterial mepivacaine concentrations following loading doses of 500mg as 1% or 2% solutions, with or without adrenaline 1:200,000. Epidural, caudal intercostal nerve, brachial plexus and sciatic-femoral nerve blocks were used to evaluate the mepivacaine absorption. The highest concentrations achieved were observed in the intercostal nerve block using 2% plain solution, rising into the 6-10μg/ml range associated with toxicity, reported by Jorfeldt et al., (1968). The addition of adrenaline to the intercostal nerve block group reduced the peak concentrations achieved in both the 1% and 2% solutions to similar concentrations achieved by plain solutions in brachial plexus and sciatic-femoral blocks. They concluded that the quantitative effect of adrenaline is dependant upon the route of injection and the concentration of local anaesthetic used. Intravenous infusions of mepivacaine and lignocaine were undertaken by Foldes et al., (1975) using a 0.5mg/kg/min body weight dose. Mepivacaine produced the longer time interval prior to toxic symptoms appearing at 10.3 ± 0.5 minutes, with lignocaine at 2.8 ± 0.3 minutes. Mepivacaine produced a consistent increase in blood pressure (greater in systolic) and pulse rate in all subjects, but they concluded mepivacaine was less toxic than lignocaine. Aberg and Sydnes, (1978) infiltrated various volumes and concentrations of plain mepivacaine above maxillary lateral incisors. They observed a linear increase in both tooth and soft tissue anaesthesia with increasing mepivacaine concentration, but a non-linear relationship with varying volumes. They concluded that a small volume of high concentration gave a better anaesthetic effect than a larger volume of a low concentration.

Goebel et al., (1980) reported data achieved following maxillary infiltration above the second premolars of either 1.8ml 2% mepivacaine plain or 2% lignocaine plain. Peripheral venous samples were analysed for the anaesthetic using gas liquid chromatography. Mepivacaine produced significantly higher peak serum concentrations throughout the test period of two hours (at 15 minutes for lignocaine at 0.32μg/ml, and 30 minutes for mepivacaine at 0.40μg/ml). The also reported on a comparison of the venous plasma lignocaine and mepivacaine concentrations after perioral injection of
36mg of the anaesthetics as 2% solutions with vasoconstrictors. The mepivacaine was combined with 1:200,000 levo-noradrenaline and the lignocaine with 1:100,000 adrenaline. They demonstrated a delayed peak for lignocaine and adrenaline from 15 minutes to 30 minutes, (0.22µg/ml and 0.31µg/ml), but reported as not statistically significant. Statistical methods were not described, but only five subjects were in each test group. Mepivacaine plain and with levo-noradrenaline peaked at 30 minutes, then declined slowly at similar rates over the two hour test period. The peak was 0.40µg/ml for plain mepivacaine and 0.37µg/ml with the vasoconstrictor.
The pressor effect of extracts from the adrenal gland was described independently by Polish workers Szymonowicz, (1895) and Cybulski, (1895) and at University College London by Oliver and Schafer, (1895). Abel at Johns Hopkins termed the vasoactive agent from the suprarenal extract epinephrine, but Takamine, (1901) suggested that Abel's substance was contaminated with impurities and his crystalline extract from the adrenal gland he called adrenaline. Dakin, (1905) also synthesized crystalline adrenaline and their effects were noted by Elliott, (1904) to be similar to the sympathetic activity observed during stimulation of nerve endings.

At the Wellcome Institute, Barger and Dale, (1910) described the sympathomimetic effect of many aliphatic and aromatic amines on the arterial pressure of decerebrate cats. Similar indirect measurements of sympathetic activity in humans of quantifying the urinary metabolic products of catecholamines were, until modern assays were developed, the only means of inference on catecholamine activity.

By 1968 a radioenzymic plasma assay of greater sensitivity than the previous fluorometric technique used to measure urinary metabolism was described. Engleman et al., (1968) used a double radioisotope method involving the enzymic conversion of catecholamines in the presence of catechol-o-methyltransferase (COMT) and radio-labelled $^{14}$C-S-adrenosylmethionine to their respective 3, o-methyl derivatives ie, $^{14}$C-normetadrenaline and metadrenaline. The second isotope used was $^{3}$H* catecholamine, used to assess the extent of recovery from plasma samples. This method was sensitive but required pre-extraction and concentration of catecholamines, requiring large plasma samples.

Modifications of the double isotope assay resulted in single isotope methods which are reported to possess assay coefficients of variation of less than 5% for noradrenaline and less than 10% for adrenaline within physiological ranges (Passon, Peuler, 1973). Radioenzymic techniques which are used currently are usually modifications of more recent methods described by Da Prada and Zurcher, (1976) and Peuler and Johnson, (1977).
An assay using High Performance Liquid Chromatography (HPLC) to measure plasma catecholamines was described by Hallman et al., (1978). Comparison between the sensitivities of the two assay methods has been made by Hjelmdahl et al., (1979) and by Goldstein et al., (1981). Both groups concluded that although the radioenzymic assay was of slightly greater sensitivity it was more costly and time consuming, and recently automated HPLC machines permit rapid analysis of many samples.

The stability of blood catecholamines has been investigated by Falconer et al., (1982), who demonstrated that blood catecholamines do not degrade if stored at room temperature for less than one hour prior to separation, and plasma samples were stable in cold storage at -20°C for up to six months.

There has been controversy in the literature as to the anticipation and apprehension of venepuncture affecting the plasma catecholamine concentrations. Carruthers et al., (1970) attempted to sample venous catecholamine concentrations from an already indwelling central cannula, assumed without anxiety, and simultaneously via a fresh venepuncture. The significantly raised concentrations achieved with venepuncture were thought to be unsubstantiated by Derbyshire and Smith, (1984) who showed little change in catecholamine concentrations associated with immediate sampling on cannula insertion and in the subsequent two hour period. They conclude that the more sensitive HPLC assay was superior to Caruthers' fluorometric assay, and their data was similar to values obtained in unstressed healthy anaesthetised patients, and venepuncture in healthy volunteers must be regarded as a very mild stimulus of anxiety.

Catecholamines have short biological and plasma half-lives, and catabolism may therefore significantly effect the values in blood samples from differing sites. Ginn and Vane, (1968) demonstrated that noradrenaline undergoes significant breakdown in the lungs, showing in vitro that 7-30% of noradrenaline was taken up in a single passage through the lungs. This has been confirmed by Derbyshire et al., (1983) with significantly lower concentrations of noradrenaline but not adrenaline in arterial samples, as compared with central venous samples, in response to induction of anaesthesia and tracheal intubation.
Extreme anxiety and stress related catecholamine concentrations have been investigated by Taggart and Carruthers, (1971; 1972) and Carruthers, (1975). Results showed rises in plasma adrenaline concentrations but not noradrenaline when under extreme stress and anxiety such as during dental treatment (Taggart et al., 1976).
CHAPTER 3

ix. EXOGENOUSLY INJECTED ADRENALINE

The improvement in surgical vision with reduced bleeding following the local infiltration of adrenaline containing local anaesthetics is commonly seen during dental, ear nose and throat, plastic, thyroid, ophthalmic and pelvic surgery. (Cowan 1968; Scott et al., 1972; Newcomb, Waite 1972; Lew et al., 1988; Michael et al., 1992). The adrenaline concentration in these local anaesthetic solutions varies from 1:400,000 (2.5μg/ml) to 1:80,000 (12.5μg/ml), the higher dose being widely used in dental procedures where the associated tissues are extremely vascular. To date few studies have examined the changes in plasma adrenaline concentrations following the perioral injection of such solutions. Tolas et al., (1982) measured plasma catecholamine concentrations following injection of a relatively small dose of dental local anaesthetic containing 18μg adrenaline into the maxillary tuberosity region in patients who had already had a dental extraction in that area one hour earlier. The site of the second injection was already anaesthetised, so painless. The control group received a lignocaine plain injection. Arterial samples were assayed for catecholamines and results showed the adrenaline injected groups had significantly raised arterial plasma adrenaline concentrations at 3 minutes (0.54pmol/ml) and 5 minutes (1.26pmol/ml) after injection. The mean arterial pressure fell slightly at 3 minutes and significantly so at 5 minutes. This fall was associated with a drop in diastolic pressure from 62±5 to 56±4 mmHg. The heart rate was slightly raised at these time intervals.

Taylor et al., (1984) reported on 19 patients, allocated randomly to receive submucosal infiltration with 4ml of either 0.5% lignocaine and 1:200,000 adrenaline (20μg) or prilocaine 0.5% with octapressin 0.03iu/ml. Venous samples were assayed with plasma adrenaline increasing from 0.35 to 1.72pmol/ml at 2 minutes and little change in noradrenaline concentrations. No peaks were noted in the prilocaine group. This 20μg adrenaline dose produced a 390% increase in plasma adrenaline concentration, but was only just outside the upper range of normal (0.1 - 1.2pmol/ml).

Low et al., (1984) measured the changes in venous plasma catecholamines in six women undergoing cone biopsies under halothane general anaesthesia and with a local infiltration of the cervix with 15ml of 0.5% bupivacaine + 1:200,000 adrenaline (75μg).
Plasma adrenaline concentrations rose to peak 2 minutes post-injection, rising from 1.01 ± 0.23pmol/ml to 18.6pmol/ml. No adverse cardiovascular changes were recorded despite the high plasma adrenaline concentrations.

Cotton et al., (1986) investigated the plasma catecholamine concentrations following larger loading doses of adrenaline at two differing anatomical sites, firstly the nose prior to rhinoplasty and secondly the axilla prior to brachial plexus blockade. The rhinoplasty group sustained a 566% rise in plasma adrenaline 2 minutes after completion of a 105μg adrenaline injection. The brachial plexus group produced a 112% rise in plasma adrenaline, but this peak was delayed at 10 minutes post-injection after the 200μg adrenaline and local anaesthetic nerve block. There was no change in plasma noradrenaline concentrations in either group.

Lew et al., (1988) measured plasma catecholamine concentrations in 12 patients undergoing bat ear surgery, using 4ml 2% lignocaine + 1:100,000 adrenaline (40μg dose) local anaesthetic. Venous plasma adrenaline concentrations increased from 0.8pmol/ml to 2.2pmol/ml at 2 minute post-infiltration, a 175% increase. Heart rates increased from 78 to 97 beats per minute immediately post-injection and in common with this trend the systolic pressure rose from 120 to 130mmHg immediately post-injection. Diastolic pressures remained unaltered.

Ueda et al., (1988) reported on the interaction of pH and lignocaine on adrenaline absorption using elective craniotomy patients. Patients were divided into five groups and adrenaline solutions of differing pH and lignocaine concentrations were compared with two plain adrenaline solutions. The results showed that lignocaine increased the absorption of adrenaline and the lower the pH the greater the plasma adrenaline concentrations achieved. Vasodilation by lignocaine would be expected at the concentrations used in the study, but the actual mechanism is unknown (Johns et al.,1985). Commercial preparations of lignocaine and adrenaline have a pH less than 5 (Punnia-Moorthy et al.,1984) and at such concentrations the acidity will increase the plasma adrenaline concentration and reduce the anaesthetic action (Bokesh et al.,1987).

Recent studies by Di Fazio et al., (1986) have shown that alkalinising lignocaine solutions is an effective way of shortening the time of onset and increasing the duration
of anaesthesia. The mechanism involved is believed to be due to increasing the
demonstrated that it was possible to alkalise lignocaine and adrenaline solutions using
8.4% or 1.4% sodium bicarbonate solution to values closer to physiological pH, and that
the solutions were stable for at least six hours.

From these studies it is evident that the plasma catecholamine concentrations achieved
following injection is dependant upon dose, pH and site of injection. The so called safe
dose of adrenaline (1.0μg/kg) during halothane anaesthesia is meaningless unless the
dose and site of administration is specified.
CHAPTER 3
X. ALPHA ADRENOCEPTORS IN CIRCULATORY REGULATION

The observation that catecholamine responses were of different types was first made as early as 1906, when Dale showed that certain effects of catecholamines were inhibited by ergot alkaloids while others were not. The concept of discrete binding sites, "receptors", for drugs and hormones on the surface of cells was postulated by Clarke, (1933). By 1948 Ahlquist had extended Dale's concept and proposed that adrenoceptors could be divided into two types, α and β, based on the relative potency of the natural catecholamines and some of their related compounds, as well as on the type of response observed. Some cells were noted to respond to noradrenaline released into the synapse as a direct result of stimulation of noradrenergic neurones, while others respond to circulating catecholamines. With this discovery it was necessary to distinguish between the inhibitory receptor on noradrenergic nerve terminals and the classical α-adrenoceptor which mediates the constriction of vascular smooth muscle.

The terminology adopted was an anatomical one, with the neuronal α-adrenoceptor designated pre-synaptic, and that on the effector cell, post-synaptic (Langer, 1974). As research developed the properties and function of the pre-synaptic α-adrenoceptor were noted to differ from post-synaptic ones, with respect to the relative activity and affinity of agonists and antagonists. Berthelsun and Pettinger, (1977) and Starke, (1977) labelled the post-junctional adrenoceptors α₁, and the pre-junctional adrenoceptor α₂. The first evidence that the α-adrenoceptors found post-synaptically in vascular smooth muscle were not purely α₂ in type, came from Bently et al., (1977). They demonstrated in both rat and cat, a subpopulation of α-adrenoceptors mediating vasoconstriction that was not blocked by the selective α-adrenoceptor antagonist, prazosin. Moulds and Jauernig, (1977) also observed similar findings with a human artery preparation.

An alternative method of estimating receptor affinities for adrenergic agents apart from the physiological responses of relevant agonists and antagonists, was described by Wood et al., (1979). This measures directly the competition for receptor binding for radiolabelled adrenergic ligands. This method, too, confirmed the presence of at least two subclasses of post-synaptic α-adrenoceptors.
Drugs have been developed with selective agonist and antagonist effect on the receptors. Classical $\alpha$-adrenoceptor antagonists such as phentolamine and phenoxybenzamine, and agonists such as noradrenaline and adrenaline, have not proved useful in clinical practice. The pharmacological basis for $\alpha$ and $\beta$ adrenoceptors proposed by Ahlquist, was related to activities of noradrenaline and related compounds. For the $\alpha$-adrenoceptor the relative potency is: noradrenaline > adrenaline > phenylephrine > isoprotenerol. For the $\beta$-adrenoceptor the order is: isoprotenerol > adrenaline > noradrenaline > phenylephrine. This classification is based on the ligand specificity of receptors. The subclassification of $\alpha$-adrenoceptors is related to the radiolabelled ligand receptor binding studies, but receptor populations located pre and post-synaptically are not homogeneous, eg $\alpha_2$-adrenoceptors are also found post-synaptically.

The distribution of alpha-adrenoceptors in vascular smooth muscle was investigated by Langer et al., (1980), with prazosin used as a selective $\alpha$-adrenoceptor antagonist. The pressor effects of phenylephrine were blocked by prazosin, but paradoxically, the injection of exogenous noradrenaline into the same vascular bed produced a pressor response which was resistant to prazosin blockade. This series of experiments demonstrated that the responses to exogenous noradrenaline became susceptible to prazosin blockade provided the neuronal catecholamine uptake mechanism was inhibited with drugs such as cocaine. These results were interpreted by the suggestion that the distribution of post-synaptic $\alpha_1$ and $\alpha_2$-adrenoceptors was not homogeneous.

It was postulated that the $\alpha_1$-adrenoceptors were concentrated at the neuroeffector junction and the $\alpha_2$-adrenoceptors located mainly extra-junctionally. This would allow the $\alpha_1$-adrenoceptors to be protected from exogenous or circulating noradrenaline by the neuronal uptake mechanism.

The anticipated position of post-synaptic $\alpha$-adrenoceptors within blood vessels predicts the innervated $\alpha_1$-adrenoceptor to be distant from the lumen of the border of adventitia and medial layer, where the predominant innervation is localised (Van Zwieten, 1988; Maze, Tranquilli 1991). Exogenous noradrenaline or circulating catecholamines would therefore have to traverse the media to reach the neuroeffector junction. It is postulated that the $\alpha_2$-adrenoceptors are predominantly located close to the intima and the vasoconstriction response to noradrenaline mediated through these intimal $\alpha_2$.
-adrenoceptors. The effect of prazosin would be via the $\alpha_1$-adrenoceptors distant from the lumen, within the media. However, this is probably an oversimplification, as the density and distribution of noradrenergic innervation of blood vessels varies between differing vessel types. It may be that the ratio of $\alpha_1$ and $\alpha_2$-adrenoceptors is a function of vessel type, eg large capacitance, small resistance, but further evidence is not conclusive.

In summary, the distribution of post-synaptic adrenoceptor subtypes may be explained by the $\alpha_2$-adrenoceptor being sensitive to the circulating hormonal vasoconstrictor adrenaline ($\alpha_2 > \alpha_1$) and being sited close to the lumen, with the remote $\alpha_1$-adrenoceptor mediating neuronal noradrenaline ($\alpha_1 > \alpha_2$).
Following the introduction in the late 1940's of ganglion blocking agents in the treatment of hypertension, drugs were sought which would allow more selective blockade of the vascular effects of sympathetic stimulation.

Phentolamine, an imidazoline hydrochloride is an α-adrenoceptor blocking drug first developed by Marxer and Miescher in 1947, and its pharmacological properties reported by Meier et al., (1949) as being adrenolytic (action opposed to that of adrenaline) in small doses and sympatholytic (action antagonistic to the activity produced by stimulation of the sympathetic system) in higher concentrations, the latter being reported as greater than that of priscoline, a progenitor in the imidazoline series. With these properties it was first investigated as an adjuvant to treatment of peripheral vascular disease, and as a potential hypotensive agent in managing essential hypertension. Green and Grimsley, (1953) suggested oral phentolamine to be useful in treating peripheral vascular disease with the atherosclerotic group obtaining greater relief than the Raynaud’s group.

Attempts at reducing raised blood pressure by oral and parental routes was investigated by Moyer and Caplovitz, (1953), but the limited antihypertensive effect due to pre and post-synaptic α-adrenoceptor blockade (Richards et al., 1978), and severely intolerable gastrointestinal side effects, restricted the oral use of phentolamine for many years. However with the established propensity in blocking the effects of circulating catecholamines (Meier et al.,1949; Freis et al.,1951), the drug rapidly achieved eminence as a diagnostic test for phaeochromocytoma (Helps et al.,1955).

Taylor et al., (1965d) reported on the circulatory effects following the rapid intravenous injection of 5mg phentolamine in a 2ml volume completed within 2 seconds. Their observations in both normotensive and hypertensive subjects was an abrupt reduction in the general systemic vascular resistance, resulting in a rapid fall in systemic blood pressure in spite of an increase in cardiac output. A similar vasodilating effect on the pulmonary circulation was noted as well as a rapid but transient shift of blood volume from the systemic to the pulmonary vasculature (Taylor et al., 1965a).
These studies would suggest that phentolamine has potent vasodilator properties and with the rapid reduction in systolic pressure occurring in both normotensives and hypertensives would suggest a primary direct vasodilating effect on resistance vessels in the systemic circulation. Other evidence to support the theory that phentolamine has only a weak sympathetic blocking activity was shown by Walker et al., (1951) when the effect was insufficient to affect the vasoconstriction of the upper limbs during supine leg exercises or to block the valsalva response (Taylor et al.,1965c; Taylor et al.,1965b). The weak sympathetic blocking activity is combined with a greater effect which antagonises the haemodynamic changes of circulating catecholamines. This was demonstrated by Richards et al., (1978) who infused noradrenaline intravenously at a rate of 4µg/minute for 4 minutes, then doubling the dose to 8µg/minute and 16µg/minute over the same interval. After a 15 minute delay during which cardiovascular changes normalised, phentolamine 0.1mg/kg was infused over 1 minute, and 20 minutes later the noradrenaline infusion was repeated at 1, 16, 32µg/minute each for 4 minutes. The results showed a shift of the log-dose response curve to the right in a parallel manner, suggesting a competitive antagonism at vascular α-adrenoceptor sites. It is known that pre-synaptic α-adrenoceptors take part in a negative feedback system which particularly under conditions of high sympathetic activity reduces the output of noradrenaline from these nerve terminals (Langer, 1981). Doxey et al., (1977) demonstrated that phentolamine blocked pre-synaptic α-adrenoceptors more readily than the post-synaptic α-adrenoceptors that produce pressor responses, and the effective phentolamine antagonism is assumed to be via these same post-synaptic adrenoceptors. Richards et al., (1978) concluded that phentolamine had an immediate and short acting effect reducing diastolic pressure, increasing heart rate and cardiac output due to a non-specific vasodilator effect, and in addition a longer α-adrenoceptor blocking activity of weaker effect. By blocking both pre and post-synaptic α-adrenoceptors this could explain why phentolamine has only a week anti-hypertensive effect.

However, a β-adrenoreceptor agonist action of phentolamine may contribute to the peripheral vasodilatation. This follows the observation that the fall in blood pressure and the increase in cardiac rate produced by 5mg of phentolamine can be significantly blocked by the prior administration of propranolol (Zahir, Gould, 1971).
The possible inotropic and chronotropic action of phentolamine may be explained by experiments on rats which were infused with phentolamine and then at the height of the alpha blockade a tracer dose of C^14 tyrosine administered. A threefold increase of tracer noradrenaline in adrenal, heart and brain tissues inferred that the receptor blockade led to increased synthesis and release of noradrenaline in these tissues (Dairman et al., 1968).

The use of phentolamine in various cardiac conditions was reported with the positive inotropism and reduction in afterload shown to be the mechanism that led to the cardiac improvement in treating heart failure (Majid et al., 1971). The use of oral phentolamine in treating chronic heart failure was confirmed producing a significant decrease in the pre-ejection period index, as well as the ratio of the pre-ejection period over the left ventricular ejection time (Gould, Reddy, 1979).

Haemodynamic improvement following a single oral 150mg dose of phentolamine was observed in patients with severe heart failure. Duration of effects, increased cardiac index and heart rate and significant reduction in mean pulmonary, wedge and right atrial pressures, lasted for at least four hours and suggested that intermittent oral therapy maybe useful in the management of such patients (Schreiber et al., 1979).

The haemodynamic effects of phentolamine infused at a dose of 0.75-1.5mg/min was investigated in patients with left ventricular failure and hypertension following acute myocardial infarction. Significant improvement in Cardiac Index and reduction in left ventricular filling pressure was present ten minutes after the infusion ceased (Kelly et al., 1973). The main action of the phentolamine seemed to be arteriolar dilatation, with relatively less effect on the venous system in comparison to nitrates and nitroprusside. The effect seems to be due to a combination of α-adrenoceptor blockade and the direct relaxing action of vascular smooth muscle, with the possibility of the beta effect contributing to the vasodilatation (Gould, Reddy, 1976). The increase in heart rate during phentolamine infusions (Majid et al., 1971; Richards et al., 1978), or following oral administration (Gould et al., 1973; Schreiber et al., 1979), might be of clinical importance when the drug is used in patients with recent myocardial infarction, but Gould et al., (1975a) showed the myocardial clearance of rubidium obtained by praeordial counting after intravenous injection of Rb^86Cl was significantly higher in the
phentolamine group. It appears that phentolamine increases coronary blood flow to the heart while it has probably little effect on overall myocardial oxygen requirements. The increase in arterial pressure of the systemic circulation during myocardial infarctions may be due to increased release of catecholamines. Phentolamine in such cases reduced the left ventricular filling pressure and increased the cardiac output in such patients with recent myocardial infarctions. The positive inotropic effect of phentolamine is indirect and dependent on the release of endogenous noradrenaline (Gould et al., 1974).

Recently the effectiveness of phentolamine therapy for the prevention of cardiac arrhythmias was determined in a double blind study of 39 patients with uncomplicated acute myocardial infarction. Oral phentolamine or placebo was administered for five days and a significant protection from ventricular premature beats and supraventricular premature beats was noted in the phentolamine group. However the drug did not suppress ventricular arrhythmias until the third day of therapy, and this long delay will severely limit its usefulness in this clinical situation (Gould et al., 1975b). Liquid chromatography has been used to measure the plasma concentrations of phentolamine following oral ingestion and it has been demonstrated that after an oral dose of 40mg phentolamine the maximum blood concentration is attained in 30 minutes, with none detectable at 90 minutes. This may explain the poor early cardiac protection from ventricular arrhythmias (Imhof, 1975).

Phentolamine has been administered at 0.6mg/minute to 20 patients in various types of clinical shock. In the seven cardiogenic cases, phentolamine infusion of 0.6mg/minute increased cardiac output and the velocity of blood flow and reduced the peripheral resistance. This was associated with an increase in oxygen to the tissues and reversal of anoxic metabolism. However, the reduction in mean arterial pressure from 65 to 55mmHg would limit its clinical application (DaLuz et al., 1973).

The abrupt intravenous injection of 5mg phentolamine in a 2ml volume, completed within two seconds was investigated with respect of the pulmonary circulation (Taylor et al., 1965a). Pulmonary arterial, wedge and right atrial pressures were recorded through a triple lumen catheter and transducers. The acute injection was followed by an abrupt fall in systemic arterial pressure, the maximum fall occurring within one
minute in all subjects. The average reduction in mean arterial pressure in normotensives was 15mmHg (range 13-20). Subsequently, the systemic blood pressure gradually returned towards pre-injection control values in all subjects. The maximum increase in cardiac output occurred within one minute in normotensives. Changes in the mean pulmonary arterial and wedge pressures in the normal subjects were within normal limits prior to injection (except one case), but in all six cases the injection produced a rapid decrease in pulmonary arterial and wedge pressures.
CHAPTER 4

MATERIALS AND METHODS

i. LOCAL ANAESTHESIA IN THE MAXILLA

Maxillary infiltration of dental local anaesthetics produces adequate anaesthesia in the adjacent hard and soft tissues as the amount of bone overlying the teeth is quite small. It was anticipated that similar infiltration of the phentolamine reversal agent would adequately permeate the soft and hard tissues to affect its action so reducing the length of anaesthesia.

Sensory nerve endings are present in the dental pulp, periodontal ligament, alveolar bone, periosteum and mucous membrane. The posterior superior dental nerve block deposits local anaesthetic adjacent to the nerve distal and apical to the roots of the last maxillary molar around the maxillary buttress. From such a dental infiltration the upper third molar, second molar and disto-buccal and palatal roots of the first molar are anaesthetised, along with the supporting hard and soft tissues.

The upper incisor and canine teeth are innervated by fibres which form the anterior superior dental nerve. This nerve ascends in a fine bony canal to join the infra-orbital nerve about 0.5cm inside the infra-orbital canal. The central and lateral incisors and canines may be anaesthetised by infiltrating local anaesthetic solution above the apex of the tooth concerned.

A disposable 27 gauge needle is applied to an ASTRA aspirating metal syringe cartridge holder and a standard self-aspirating dental anaesthetic glass cartridge inserted. The needle is inserted in the conventional manner just above the reflection of the oral mucosa. Aspiration is made before slowly depositing the local anaesthetic solution. Should a positive aspiration be made, the needle tip is repositioned and the procedure repeated, thus avoiding the intravascular injection of the local anaesthetic.
ii. LOCAL ANAESTHESIA IN THE MANDIBLE

Due to the density of the buccal plate of bone, infiltration techniques of anaesthesia are of limited value in the mandible, and regional block anaesthesia is most frequently employed. The technique aims to deposit the anaesthetic solution adjacent to the inferior dental and lingual nerves within the pterygo-mandibular space. The anaesthesia achieved is from third molar to canine and includes mandibular and lingual tissues. A disposable 27 gauge needle is applied to an ASTRA aspirating metal syringe cartridge holder and a standard self-aspirating dental anaesthetic glass cartridge inserted. The "direct" technique is employed avoiding excessive flexion of the needle (Rood, 1972). With the barrel of the syringe held parallel to the mandibular occlusal plane and above the premolar teeth of the opposite side, the tip of the needle is slowly advanced towards the lingula which lies half way towards the posterior border of the mandible on the medial side. Once light contact with bone is made, the needle is slightly withdrawn, aspiration attempted, and if negative the anaesthetic deposited. Should blood be aspirated the needle is repositioned and the aspiration repeated.

iii. EXPERIMENTAL METHOD

Ethical Committee approval was obtained prior to the commencement of all trials. Healthy, non-pregnant volunteers from the University student population (18-25 years) were invited to participate, and the procedure explained and written consent obtained.

Cardiovascular monitoring using conventional 3 lead ECG to an oscilloscope and pen recorder (Cardiorater Type CR9) to assess rate and rhythm, and blood pressures using an upper arm inflatable cuff (Dinamap Vital Signs Monitor Model 8) was used throughout the clinical studies. The Ethics Committee, University College Hospital, recommending that all dental local anaesthetic clinical trials should meet such criteria and be supervised by an anaesthetist with resuscitation equipment readily available. Recordings were obtained throughout the monitoring period of the clinical experiments. Venous access in the antecubital fossa was obtained using a 20 gauge cannula and three way tap. Patency was achieved using repeated heparinised saline flushes. Venous samples were drawn from the tap into a 5ml disposable syringe, the first 2ml being
discarded as being contaminated with heparinised saline. The blood sample was transferred to EDTA bottles and centrifuged at 3000 revs for 10 minutes (Hearuens-Christ Megafuge), immediately prior to freezing the plasma at -20°C.

In the experiments reported in Chapters 7, 10, 11, 12, appendix ii and iii each subject was injected with the local anaesthetic as described (p. 76, 77) using standard dental sterile cartridges (p. 80, 81). The contents of one dental cartridge was infiltrated using an aspirating technique into the following sites:

(i) posterior maxillary alveolar region,
(ii) above the maxillary canine,
(iii) as a mandibular inferior alveolar nerve block.

An Analytic Technology Pulp Tester, Model 200; (Analytic Technology, Redmond, Washington, USA) was used to assess sensory thresholds of the teeth being anaesthetised. The selected tooth’s buccal surface was dried and a small amount of conducting gel placed on it. The probe is applied to the tooth surface through the gel and the operators finger placed onto the patients adjacent teeth. The contact automatically increases the current as demonstrated on the dial. The probe tip was placed on the labial or buccal surface midway between the gingival margin and the occlusal or incisal edge. Confirmation of a perceivable threshold of sensitivity, ie, a "twinge" from the tooth was recorded. A mean of three recordings was made for each tooth (Vreeland et al., 1989). Recovery of the tooth pulp from the local anaesthetic was adjudged when the pulp tissue responded to the pre-anaesthetic thresholds for the individual tooth.

An adapted nerve stimulator was used to test the sensitivity of the lip vermilion tissues. The volunteer grasped the hand electrode and with the other electrode touched the moistened lip to be tested. By increasing the scale setting the current increased and the individual adjusted the dial to produce a comfortable, reproducible level of sensitivity. The setting was not adjusted further. To assess the resolution of threshold sensitivity the electrode was again swept along the moistened vermilion border. An audible sensor indicated to the volunteer when adequate electrical contact was made with the lip tissues. The time was recorded for the pre-anaesthetic vermilion threshold of stimulation to be re-established.
Phentolamine and matching placebo solutions were prepared by the University College Hospital Pharmacy, Sterile Supplies Unit. Solutions were supplied in standard 2.0ml ASTRA dental glass cartridges and identified by number. The pharmacist kept the codes enabling double-blind, cross-over trials to be conducted. **The technique for injecting the phentolamine or placebo was the same as described for the local anaesthetic injections.** A disposable 27 gauge needle was attached to an ASTRA aspirating metal syringe cartridge holder and the needle point was injected through the same needle puncture site in the oral mucosa. Aspiration prior to slow injection prevented intravascular deposition of the phentolamine.  

A delay of 30 minutes elapsed after the initial local anaesthetic injections before the phentolamine was injected. This was to simulate the time taken for a potentially painful routine dental procedure to be completed.

The placebo matched trials were conducted on the same subjects one week apart, in a double blind, cross-over fashion, randomising the side of the jaw being anaesthetised.

**Results are presented as the time interval (minutes) from the second injection of phentolamine or placebo.** It is appropriate in this context as firstly, controls confirm the lengthy soft tissue and pulp anaesthesia well beyond the 30 minute latency so premature return of sensation was extremely unlikely, and secondly, in clinical dentistry the reversal agent would only be injected after completion of the painful procedure, and 30 minutes was selected as an appropriate interval. Proposed future experiments injecting the phentolamine earlier and later than the existing 30 minute delay will enable dental surgeons to manipulate the injection time to optimise resolution of soft tissues to the benefit of their patients.
MATERIALS

iv. LOCAL ANAESTHETIC FORMULATIONS

Listed below are the local anaesthetic formulations used in the investigations.

a: Lignocaine

i. Lignocaine 2%
   Lignocaine hydrochloride anhydrous 20mg/ml
   2.0ml dental cartridges
   ASTRA PHARMACEUTICAL
   Product Licence Numbers  0017/5034R, 0017/1220R

ii. Lignocaine 2% + 1:80,000 adrenaline
    Lignocaine hydrochloride anhydrous 20mg/ml + Adrenaline BP 12.5μg/ml
    2.0ml dental cartridge
    Product Licence Number  0017/5027

iii. Lignocaine 2% + 1:100,000 adrenaline
    Lignocaine hydrochloride anhydrous 20mg/ml + Adrenaline BP 10.0μg/ml
    1.8ml dental cartridges
    Product Licence Number  0017/5027

iv. Lignocaine 2% + 1:50,000 adrenaline
    Lignocaine hydrochloride anhydrous 20mg/ml + Adrenaline BP 2.0μg/ml
    1.8ml dental cartridges
    Product Licence Number  0017/5027

v. Lignocaine 0.2% + 1:200,000 adrenaline
   Lignocaine 0.2% + 1:200,000 adrenaline + 1μg phentolamine
   Lignocaine 0.2% + 1:200,000 adrenaline + 10μg phentolamine
   Lignocaine 0.2% + 1:200,000 adrenaline + 50μg phentolamine
   Lignocaine 0.2% + 10μg phentolamine
   Product Licence Number  0017/5027
   UNIVERSITY COLLEGE HOSPITAL, Sterile Supplies Unit
b: **Mepivacaine**

Mepivacaine 2% + 1:100,000 adrenaline
Mepivacaine hydrochloride anhydrous 20mg/ml + Adrenaline BP 20μg/ml
2.2ml dental cartridges
SPECIALITIES SEPTODONT
58 Rue du Pont de Creteil, 94100 Saint Mew, France

c: **Prilocaine**

Prilocaine 3% + Felypressin 0.03iu/ml
Prilocaine hydrochloride anhydrous 30mg/ml + Felypressin 0.03iu/ml
2.2ml dental cartridges
ASTRA PHARMACEUTICALS
Product Licence Number 0017/5003
v. MOLECULAR STRUCTURES

Phentolamine

Lignocaine

Mepivacaine
Prilocaine

Noradrenaline
vi. PHENTOLAMINE SOLUTIONS

Sterile solutions supplied by University College Hospital Pharmacy, Sterile Supplies Unit.

Solutions:  1.0mg/ml  
           1.4mg/ml  
           2.0mg/ml  
           3.0mg/ml  

Phentolamine mesylate solution 10mg/ml injection dilutions made with 5% dextrose solutions.

Solutions supplied in sterile ASTRA dental cartridges, (0.2μm filter).

Placebo solutions, 5% Dextrose, supplied in identical sterile ASTRA dental cartridges.

All solutions supplied to be used within 24 hours.

pH phentolamine 4.9-5.0.

vii. PLACEBO SOLUTIONS

5% dextrose was used as matching placebo as the phentolamine solutions were prepared using this solution for dilution.

pH placebo 5.0-5.1.

All solutions used within 24 hours.
1. Local Anaesthetic Assays

Plasma assays of lignocaine, prilocaine and mepivacaine were undertaken at the Poisons Unit, Guys Hospital by Dr. D. W. Holt. Unmodified silica columns used with non-aqueous, methanolic eluents containing an ionic modifier provided a simple HPLC analysis for these specified local anaesthetics. Spectrophotometry using specific UV (ultra-violet) filters were used to detect the local anaesthetic agents in these samples. The method was developed "in-house" at the Poisons Unit, Guy’s Hospital, by Miss Angela Hayler BSc, under the direction of Dr David W Holt, BSc, PhD, MRCPath. It was based on a published method for the measurement of the cardiac drug tocainide (Holt, Flanagan, 1985). Reference substances were provided by the appropriate manufacturers for the preparation of standards, using drug-free human plasma. Standards and internal quality control samples were prepared from independent stock solutions of each drug. A six point calibration curve and two quality control samples were assayed with each batch of samples. Duplicate analyses were performed and the mean reported.

The reproducibility (cv%), both within and between-assay, for each assay was better than 7% over the range of concentrations assayed.

There was no interference in these assays from commonly prescribed drugs, and all three compounds could be resolved from each other on the chromatographic system used. Analysis and analytic-free samples showed no interferences from endogenous compounds.
CONDITIONS FOR ASSAYING THE TEST LOCAL ANAESTHETICS

LIGNOCAINE

Mobile Phase: 3 mM camphor sulphonic acid in 500ml methanol.

Extraction: 200μl sample plasma
20μl 2M tris
40μl 15 mg/L butriptyline
200μl methyl-tert-butyl ether
Mix and centrifuge
Inject 100μl

Column: 250 x 5mm id Spherisorb S5 SCX

Injection: 100μl of methyl-t-butyl ether extract (pH 11) of sample (100μl)

Eluent: acetonitrile : methanol (4:1)
containing ammonium perchlorate (15ml)
adjusted to pH 5.7 using methanolic sodium hydroxide (0.1M)

Flow: Flow rate set at 2ml/min

Detection: Spectrophotometric UV 220nm filter

Plasma Standards: at 1.0, 5.0, 10.0 mg/L extracted in singlicate
ORTHO 1 (3.2 mg/L) or ORTHO 2 (8.7 mg/L) extracted in duplicate
for quality control.

MEPIVACAINE

Mobile Phase: 3.4 mM camphor sulphonic acid in 500ml methanol.

Extraction: 200μl samples
20μl tris
20μl 4mg/L aqueous amitriptyline
200μl methyl-tert-butyl ether

Injection: 100μl of methyl-t-butyl ether extract (pH 11) of sample (100μl)

Flow: 2ml/min

Column: 250 x 5mm id Spherisorb S5 SCX.

Detection: Spectrophotometric UV 215nm filter

Eluent: acetonitrile: methanol (4:1) containing ammonium perchlorate (15mm)
adjusted to pH 5.7 using methanolic sodium hydroxide (0.1M)
PRILOCAINE

Mobile Phase: 3mM camphor sulphonic acid in 500ml methanol

Extraction: 200μl plasma sample
20μl 2M tris
20μl 15mg/L butriptyline
200μl methyl-tert-butyl ether
Mix and centrifuge
Inject 100μl

Column: 250 x 5mm id Spherisorb S5 SCX

Injection: 200μl methyl-tert-butyl ether extract (pH11) of sample

Flow: 2ml/min

Eluent: acetonitrile: methanol (4:1) containing ammonium perchlorate (15mM)
adjusted to pH 5.7 using methanolic sodium hydroxide (0.1M)

Detection: Spectrophotometric UV 215nm filter
2. **Catecholamine Assays**

Many procedures for measuring catecholamines in human plasma have been described, principally radioenzymic assays (Peuler, Johnson, 1977) and high performance liquid chromatography (HPLC) with electrochemical detection (Hallman *et al*., 1978; Causon *et al*., 1983).

The assays in this study were conducted under the guidance of and by Dr C. C. T. Smith of the Cardiovascular Research Laboratory, Rayne Institute, University College Hospital.

Venous blood samples were collected with EDTA as anticoagulant and centrifuged immediately to obtain platelet-poor plasma which was frozen at -20°C. Catecholamine extraction was by adding 20mg of alumina and 20μL Tris buffer (1mol/L ph 8.6) to 2ml of plasma. The internal standard used was 100pmol of 3,4-dihydroxybenzylamine (DHBA). Catecholamines absorbed onto the alumina were eluted with 0.5mol/L phosphoric acid, to provide samples for injection into the chromatograph.

The HPLC system consisted of a model 300/02 pump (Applied Chromatography Systems, Luton, Bedfordshire), a SPECAC 34,000 injection valve fitted with a 100μL loop (Analytical Accessories Ltd, Orpington, Kent), and a 4.6 x 150mm Altex column of ultrasphere OD5 5μm particles (Anachem Ltd, Luton, Bedfordshire). For separation a solvent system consisting of an acetate-citrate buffer (5.75g of citric acid, 6.80g of solution acetate 3H₂O, 1.05 mL of glacial acetic acid, and 2.04 g of sodium hydroxide diluted to 1L, ph 5.2), containing, per litre 1.25g of sodium octane sulfonate, 3mmol of EDTA and 140ml of methanol (Keller *et al*., 1976). The flow rate was 1ml/min and the effluent was monitored for catecholamines with an electrochemical detector (EDT Research, London), held at 0.42 volts (Smith, Betteridge, 1984).
**ix. ESTIMATION OF METHAEMOGLOBIN**

Make 1/50 dilution of whole blood in water (0.2ml blood into 10ml H$_2$O). Mix to lyse red cells, and spin at 2,000 rpm for 5 minutes. Set spectrophotometer to scan between 700 - 400 nm, and scan the haemolysate against a water blank. Mark 632nm on chart. A reference curette of sodium dithionite is scanned in the same way starting at 700nm. The percentage of Methaemoglobin is calculated from the difference in optical density between the scan for methaemoglobin and a similar one for total haemoglobin.

\[
\% \text{ Methaemoglobin} = \frac{\text{Observed Optical Density at 632nm}}{(\text{Optical Density at 570nm})} \times 0.0023
\]

Methaemoglobin concentrations greater than 3% are clinically significant.

NB: The blood must be fresh as methaemoglobin concentrations rise in stored blood.

**x. MONITORING EQUIPMENT**

a. **CARDIOVASCULAR**

Blood Pressure Monitor
Dinamap
Adult Vital Signs Monitor Model 845
Critikon Inc
Tampa, Florida, USA

Cardiac Monitor
Cardiorater Type CR9
Cardiac Recordings Ltd
London

b. **TEETH**

Pulp Tester
Analytic Technology Pulp Tester
Model 2001
Analytic Technology
Redmond, Washington, USA
c. LIP TISSUES

**Soft Tissue Tester (Modified Nerve Stimulator)**

<table>
<thead>
<tr>
<th>Dial Setting</th>
<th>Infinite Resistance (Volts)</th>
<th>10K Load (Volts)</th>
<th>10K Load (mA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>2.</td>
<td>0.80</td>
<td>2.00</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>3.80</td>
<td>5.00</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>6.20</td>
<td>7.50</td>
<td></td>
</tr>
<tr>
<td>5.</td>
<td>9.00</td>
<td>12.50</td>
<td>0.50 (5mA)</td>
</tr>
<tr>
<td>6.</td>
<td>11.00</td>
<td>15.00</td>
<td></td>
</tr>
<tr>
<td>7.</td>
<td>15.00</td>
<td>20.00</td>
<td></td>
</tr>
<tr>
<td>8.</td>
<td>18.00</td>
<td>24.00</td>
<td></td>
</tr>
<tr>
<td>9.</td>
<td>23.00</td>
<td>27.00</td>
<td></td>
</tr>
<tr>
<td>10.</td>
<td>27.00</td>
<td>30.00</td>
<td></td>
</tr>
<tr>
<td>11.</td>
<td>30.00</td>
<td>32.00</td>
<td>12.50 (125mA)</td>
</tr>
</tbody>
</table>

xi. BLOOD PRESSURES

According to Master et al., (1958), the clinical "normal range" of the systolic or diastolic blood pressure in adults is given by the mean ± 1.282 sd. Graphical representation of the blood pressures is made using data ranging from 137 - 104mmHg (systolic) and 87 - 63mmHg (diastolic). These are shown as stippled areas on the relevant Figures.
The optimal design for this series of experiments would be an "explanatory" design with each trial having either a cross-over or repeated measures design as appropriate. Unfortunately due to the financial constraints it was necessary to use a "pragmatic" design. Although this type of trial was not as efficient, it closely relates to the more realistic clinical situation (Schwartz, Lellouch, 1967).

**Experimental Measures**

In general the variables measured in this project can be classified as "serial measures", ie. a drug concentration measured as a function of time for a series of subjects. The commonly accepted way to analyse this type of data is to compare the groups at a series of times, normally the sampling times, by t-tests or analysis of variance. However, this type of statistical analysis is invalid and can easily lead to incorrect conclusions being made (Matthews et al., 1990). There are several reasons for this:

1. the analysis does not allow for the fact that measurements at different times are on the same subject.
2. successive observations on the same subject will be correlated.
3. the data for the groups are presented as a composite graph through the mean response value at each time with associated error bars. The consequences of this include the following:
   a. There is no information on individual patient response (it implies a homogeneous set of patients with identical responses, the only variation being due to experimental error).
   b. The error bars only describe variation between patients at a given time and as such have little meaning.

To obviate these problems and provide a valid statistical analysis we have used summary measures to describe the data. In general the concentration-time response curves are peaked and the general null hypothesis was:

\[ H_0: \text{is the overall effect the same in all groups.} \]

The summary measure chosen was the standardised area under the concentration-time curve, AUC. This is the area under the curve divided by the duration of the trial. Where appropriate the time to peak concentration was used, but this is of limited value due to the discrete sampling times used.
Data Presentation

It is common practice to quote the results of a test as either significant or non-significant at the arbitrary probability level of \( p=0.05 \). Although this may be acceptable in a physical study in large number of repeats, it is generally inadequate for describing a clinical trial.

It is an unfortunate fact that many clinical trials, often for pragmatic reasons described earlier have a small sample size with a high inherent scatter of the results. This often results in a "non-significant" probability occurring. This does not mean that a clinical difference between results may not occur. For this reason Confidence Intervals rather than hypothesis testing has been used in the present work (Gardener, Altman, 1986; Simon, 1986).

The Confidence Interval is given by:

\[
CI = \frac{t_{n-1, \frac{1-\alpha}{2}} \times sd}{\sqrt{n}} \quad \alpha = \text{probability level}
\]

\[
\text{sd} = \text{standard deviation}
\]

from which it can be seen that it depends on both the standard deviation, a descriptive measure of the variability between individual subjects, and the sample size.

In the above the use of summary statistics and Confidence Intervals has been justified. However, to enable comparison of the present work with other published data, some mean composite data has been presented. It is unfortunate that the authors who have used incorrect statistical methods do not, in general, present their raw data to enable the appropriate analysis to be carried out. One can only speculate on how many incorrect conclusions have been reached (Gore, Altman, 1982).
Data Analysis

All data was analysed using SAS PC/Version 6.04. Where appropriate AUC was calculated using the trapezoid rule:

\[
AUC = \frac{1}{2\Delta t} \sum_{i=0}^{n-1} (y_{i+1} + y_i) (t_{i+1} - t_i)
\]

where \(y_i\) is the concentration at time \(t_i\), and \(\Delta t\) is the absolute time period. All data was tested for normality using the Shapiro-Wilk test prior to analysis. Depending on the outcome on this test the data was analysed using parametric or nonparametric tests listed below.

Data Analysis Sequence

Summary Statistics

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<table>
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Test for Normality

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<th>Non-Normal</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>t-test (1 or 2 sample)</td>
<td>Wilcoxon/Mann-Whitney</td>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>Analysis of Variance</td>
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CHAPTER 5

INTRADERMAL WHEAL ANAESTHESIA.

The design of a test for comparing the activity of a local anaesthetic is a compromise between accuracy and relevance to the conditions of use. The isolated nerve preparation which measures depression of conduction is probably the most precise method and the simplest to interpret (Caruana et al., 1982; Johns et al., 1985; Johns et al., 1986; Covino 1986), but results do not relate to practical clinical conditions of diffusion and buffering capacity (Covino 1986). In vivo tests on the intact animal are less accurate, but do approximate to clinical conditions. Mongar, (1955) described a simple method of assessing local anaesthetic potency by raising intradermal wheals on the flexor forearms of volunteers. After injecting (0.2ml) the local anaesthetic solutions in varying concentrations into the dermis assessment of the depth of anaesthesia was measured by recording the response to simple pin prick stimulation.

OBJECTIVE

A trial, based on Mongar’s observation, was conducted to clinically assess the potential of phentolamine to significantly reduce the length of soft tissue anaesthesia produced by conventional local anaesthetic agents.

METHOD

Test Solutions

(A) 0.2% lignocaine + 1:200,000 adrenaline
(B) 0.2% lignocaine + 1:200,000 adrenaline + 10μg phentolamine
(C) 0.2% lignocaine + 1:200,000 adrenaline + 50μg phentolamine
(D) 0.2% lignocaine + 10μg phentolamine
(E) 0.2% lignocaine + 1:200,000 adrenaline + 1μg phentolamine
(F) 0.2% lignocaine

Each subject was injected on the flexor forearm with the solutions as 0.2ml intradermal wheals. The test solutions were arranged in random order along the forearm to allow for difference in skin surface sensitivity, and the trial conducted in a double blind fashion. Each wheal was tested for anaesthesia by pricking ten times within the marked border of the wheal in different places, using enough stimulus to elicit pain on normal skin adjacent to the wheal. The number of pricks not felt was recorded as a measure
of anaesthesia, and the testing was repeated at 5 minute intervals for 60 minutes or until the normal sensation in all of the test area was achieved.

RESULTS

A logistic analysis (SAS Probit) of data produced \( t_{50} \) concentrations, the time to reach 50% anaesthesia, enabling comparisons to be made between the test solutions.

<table>
<thead>
<tr>
<th>Table 5:1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>INFLUENCE OF PHENTOLAMINE ON INTRADERMAL WHEAL ANAESTHESIA</strong></td>
</tr>
<tr>
<td>(A) Lignocaine + Adrenaline</td>
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<tr>
<td>No analysis possible</td>
</tr>
<tr>
<td>(B) Lignocaine + Adrenaline + 10( \mu )g Phentolamine</td>
</tr>
<tr>
<td>(C) Lignocaine + Adrenaline + 50( \mu )g Phentolamine</td>
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<tr>
<td>(D) Lignocaine + 10( \mu )g Phentolamine</td>
</tr>
<tr>
<td>(E) Lignocaine + Adrenaline + 1( \mu )g Phentolamine</td>
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<tr>
<td>(F) Lignocaine</td>
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<td>Due to data scatter median values were used.</td>
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The simplicity of the study proved very effective in demonstrating the efficacy of adrenaline to prolong lignocaine anaesthesia. Phentolamine reduced the duration of anaesthesia of the plain lignocaine and adrenaline solutions. Increasing the dose of phentolamine reduced the duration of anaesthesia of the lignocaine and adrenaline mixture, in a dose-response manner, as demonstrated in Table 5:1 and Figure 5:1.

With reference to Figure 5:1

1. Adrenaline prolongs the anaesthesia of lignocaine
2. Phentolamine reduces the duration of lignocaine anaesthesia
3. Increasing the dose of phentolamine reduces the duration of anaesthesia in the lignocaine and adrenaline mixture
CONCLUSIONS

Mongar, (1955) described an effective and simple method for assessing local anaesthetic potency, and in this experiment the flexor forearm model proved sensitive in determining the efficacy of phentolamine at reversing the effects of adrenaline in local anaesthetics. The effectiveness of the lignocaine and adrenaline mixture at producing almost 100% soft tissue anaesthesia over the 60 minute test period compared with the lignocaine plain solution, confirms the efficacy of adrenaline to enhance the anaesthetic effect of lignocaine (Keesling, Hinds, 1963; Barclay, 1965; Boulton, 1967; Scott et al., 1972; Yerzingatsian, 1991; Knoll-Kohler, Fortsch 1992). The inherent vasoactivity of the lignocaine anaesthetic was not investigated in this trial, but at the 0.2% concentrations injected the likelihood was of a mild pre-capillary vasoconstriction (Blair 1975; Aps, Reynolds 1976; Willats, Reynolds 1981; Carpenter, Morell 1988; Cederholm et al., 1991; Fruhstorfer, Wagener 1993). This weak vascular effect was overwhelmed by the pre and post-capillary effect of the vasoconstrictor adrenaline. Increasing the concentration of phentolamine in the mixture produced a dose response such that the curves took on the profile of the lignocaine plain solution. The rapid direct, non-specific effect of phentolamine on the pre and post-capillary vessels coupled with the catecholamine antagonism produced sufficient vasodilatation to overcome the effects of adrenaline (Gould, Reddy 1976; Doxey et al., 1977; Richards et al., 1978; Van Zwieten 1988).

The surprisingly effective response of phentolamine on plain lignocaine may be explained by any inherent pre-capillary vasoconstrictor effect of the lignocaine being countered by the more intense direct smooth muscle pre and post-capillary vasodilatation. The hypothesis that the addition of phentolamine to the lignocaine and adrenaline mixture would effectively alter this to the characteristics of a plain lignocaine solution in vivo has been substantiated.
PHENTOLAMINE EFFECTS ON WHEAL ANAESTHESIA.

A: Lignocaine + Adrenaline  
B: Lignocaine + Adrenaline + 10ug Phentolamine  
C: Lignocaine + Adrenaline + 50ug Phentolamine  
D: Lignocaine + 10ug Phentolamine  
E: Lignocaine + Adrenaline + 1ug Phentolamine  
F: Lignocaine

Figure 5:1
Demonstration of the potentially useful effects of phentolamine in the human flexor forearm model (Chapter 5) enabled a successful application to the U.C.H. Ethics Committee to conduct a clinical trial to investigate the potential usefulness in general dentistry. A small pilot study of three injections infiltrated into the maxilla of the author suggested that 0.5ml of a 2mg/ml phentolamine solution may effectively reduce the soft tissue anaesthesia produced by a 1ml maxillary infiltration of 2% lignocaine + 1:80,000 adrenaline.

OBJECTIVE
The aim was to establish whether phentolamine reversed soft tissue and pulp anaesthesia produced by routine maxillary infiltration dental anaesthesia. A clinical investigation into the use of phentolamine as an antagonist to the potentiating adrenaline effects of dental local anaesthetics was designed. The area of anaesthesia to be tested was the upper lip in student volunteers and the trial design was double blind, with the phentolamine solutions compared with a matching placebo, 5% dextrose solution. Standard dental local anaesthetic cartridges (Chapter 4) were to be used for the anaesthesia, and the phentolamine solutions were inserted into similar vials by the Sterile Supplies Department at University College Hospital. Phentolamine concentrations of 2mg/ml were anticipated to be of clinical usefulness by extrapolating data from the flexor forearm study, and by three clinical examples in a small pilot study conducted on the author.

METHOD
Following the protocol described in Chapter 4, 10 subjects from the student population were randomly assigned to receive the following drug combinations (Table 6:1), as maxillary infiltrations, 30 minutes after the standard 1ml of 2% lignocaine + 1:80,000 adrenaline maxillary infiltration.
Table 6:1

REVERSAL OF MAXILLARY INFILTRATION ANAESTHESIA

- TRIAL DESIGN

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<tr>
<th>Code</th>
<th>Phentolamine Test Solutions</th>
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<tr>
<td>2</td>
<td>1mg v 2mg</td>
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<tr>
<td>3</td>
<td>1mg v 1.4mg</td>
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<td>4</td>
<td>2mg v 1.4mg</td>
</tr>
<tr>
<td>5</td>
<td>3mg v 2mg</td>
</tr>
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</table>

The pH of each test solution was measured using the method described by Punnia-Moorthy et al., (1984).

The subjects received subsequent drug combinations at weekly intervals. Threshold concentrations of electrical stimulation of the maxillary canine teeth, using an average of three readings from the Analytic Technology Pulp Tester, were recorded (Chapter 4).

Blood pressure was monitored periodically throughout the procedure using a Dinamap Adult Vital Signs Monitor (Chapter 4) and the cardiac rhythm using an oscilloscope and standard chest leads. An anaesthetist was in constant attendance, at the insistence of the Ethics Committee.

A standard dental maxillary infiltration of 1ml of 2% lignocaine + 1:80,000 adrenaline (Chapter 4) was injected above each maxillary canine, into the reflected mucosa, using an aspirating technique. Repeated electric pulp and vermilion testing confirmed tooth and soft tissue anaesthesia. The 0.5ml phentolamine solution or matching placebo was injected through the same puncture, 30 minutes after the dental anaesthetic injection, using the identical aspirating technique. The times were recorded for resolution of pulp and soft tissues (see Chapter 4), as adjudged by the restoration to previous electrical thresholds of stimulation.
RESULTS

The range for the placebo was from pH 5.0 - 5.1, and the phentolamine solutions from pH 4.9 - 5.0.

Results are presented as the time interval (minutes) from the second injection of phentolamine or placebo. This is appropriate in this context as firstly, controls confirm the lengthy soft tissue and pulp anaesthesia well beyond the 30 minute latency so premature return of sensation was extremely unlikely, and secondly, in clinical dentistry the reversal agent would only be injected after completion of the painful procedure, and 30 minute was selected as an appropriate interval. Further proposed studies are aimed to optimise the timing of the phentolamine injection to produce maximal soft tissue effect and comparing with the existing 30 minute data will allow direct comparison.

The pronounced soft tissue anaesthesia from the 1ml of 2% lignocaine and 1:80,000 adrenaline is demonstrated in the control group, the mean interval being 138.1 minutes from the time of the delayed second injection.

Pulp analgesia was noticeably shorter compared with the soft tissue anaesthesia, with a mean of 40.3 minutes. All phentolamine solutions reduced the time of the soft tissue and pulp anaesthesia, but statistically the 2mg/ml solution was the most effective. The 2mg/ml solution was effective in reducing the soft tissue anaesthesia from 138 to 18.2 minutes (95% CI) and the pulp anaesthesia from 40.3 to 8.7 minutes (95% CI). There was no significant change in any of the cardiovascular parameters measured, including cardiac rhythm. Figures 6:5 and 6:6 presents the individual cardiovascular data from the group receiving a total phentolamine dose of 2.5mg. Despite this significant dose of phentolamine no adverse events were noted during the monitoring period.
Table 6:2

INFLUENCE OF PHENTOLAMINE ON THE DURATION OF SOFT TISSUE AND PULP ANAESTHESIA

<table>
<thead>
<tr>
<th>SOFT TISSUE</th>
<th>PULP TISSUES</th>
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</thead>
<tbody>
<tr>
<td>Mean Anaesthesia Times From Second Injection (Minutes)</td>
<td>Mean Anaesthesia Times From Second Injection (Minutes)</td>
</tr>
</tbody>
</table>

| Placebo | 138.1 | Placebo | 40.3 |
| 1.0mg/ml | 23.95 | 1.0mg/ml | 5.95 |
| 1.4mg/ml | 22.05 | 1.4mg/ml | 5.6 |
| 2.0mg/ml | 16.90 | 2.0mg/ml | 4.8 |
| 3.0mg/ml | 14.60 | 3.0mg/ml | 3.9 |

Graphical representation of the data is demonstrated in the Figures 6:1, 6:2, 6:3, 6:4, confirming the effectiveness of the 2mg/ml phentolamine solution.

Table 6:3

PAIRED t TEST SOFT TISSUE ANALYSIS

<table>
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<tr>
<th>Code</th>
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<th>Mean of difference</th>
<th>sd of difference</th>
<th>95% CI</th>
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<td>8.017</td>
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</table>

Analysis of the soft tissue data confirms the 2mg/ml phentolamine solution to be the most effective concentration.
Table 6:4

**PAIRED t TEST PULP ANALYSIS**

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<th>Mean of difference</th>
<th>sd of difference</th>
<th>95% CI</th>
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<td>-46.8 to -16.4</td>
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<td>-3.1</td>
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<td>-6.27 to 0.07</td>
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<td>5</td>
<td>-0.19</td>
<td>0.850</td>
<td>-0.2</td>
<td>3.259</td>
<td>-2.53 to 2.13</td>
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</tbody>
</table>

If t is negative then the dose/control is greater than a dose of 2mg/ml.
The 2mg/ml solution was statistically the most significant concentration in reducing soft tissue and pulp anaesthesia.

**CONCLUSIONS**

The aim of the trial was to determine the clinical effectiveness of phentolamine at reversing soft tissue and pulp anaesthesia produced by routine maxillary infiltration dental anaesthesia. The phentolamine test solutions ranged from 1.0μg/ml to 3.0μg/ml and were infiltrated after a 30 minute delay into the maxillary soft tissues above the canine teeth. Electrical sensory thresholds were used to determine the resolution of anaesthesia, so avoiding subjective bias. The Analytic Pulp Tester and Soft Tissue Tester (Chapter 4) proved effective in this respect with resolution of anaesthesia adjudged when pre-anaesthetic thresholds had been re-established. The phentolamine injection was reduced in volume to 0.5ml, as in the small pilot study it was found that the smaller the volume, the less post-injection discomfort. However, some of the discomfort may have been due to the low pH (5.0-5.1) of the agents as solutions neutralised by the addition of sodium bicarbonate have been reported to not "sting" (Korbon et al., 1987). No difficulty was experienced in re-injecting the 0.5ml phentolamine solutions through the same puncture hole as the local anaesthetic as optical loupe magnification was used. This avoided the phentolamine being lost into the mouth if injected through an additional puncture in the mucosa.
Analysis of the paired data confirmed the impression of the pilot study that the 2mg/ml phentolamine solution was statistically the most effective concentration for reversing the soft tissue and pulp effects of the dental local anaesthetic. No major change in pulse rate, diastolic or systolic pressures was observed, (Figures 6:5, 6:6) and no local soft tissue damage observed or reported. This would infer that the phentolamine did not enter the systemic circulation as a bolus (Taylor et al., 1965 a,d; Richards et al., 1978).
MAXILLARY INFILTRATION SOFT TISSUE REVERSAL

0.5ml. 2mg/ml Phentolamine Or Placebo Injected 30 Min After 1ml 2% Lignocaine 1:80,000 Adrenaline. n=10. Bars 95% Cl.

0.5ml. Phentolamine Injected 30 Minutes After 1ml 2% Lignocaine + 1:80,000 ADR n=10. Bars 95% Cl. Figure 6:1
MAXILLARY INFILTRATION SOFT TISSUE REVERSAL

0.5ml. Phentolamine Injected 30 Minutes
After 1ml 2% Lignocaine + 1:80,000 ADR
n=10. Bars 95% CI.

Figure 6:2
MAXILLARY INFILTRATION PULP REVERSALS

0.5ml. 2mg/ml Phentolamine Or Placebo Injected 30 Min After 1ml 2% Lignocaine 1:80,000 Adrenaline. n=10. Bars 95% Cl.

0.5ml. Phentolamine Injected 30 Minutes After 1ml 2% Lignocaine + 1:80,000 ADR n=10. Bars 95% Cl. Figure 6:3
MAXILLARY INFILTRATION PULP REVERSALS

0.5ml. Phentolamine Injected 30 Minutes
After 1ml 2% Lignocaine + 1:80,000 ADR
n=10. Bars 95% CI.

0.5ml. Phentolamine Injected 30 Minutes
After 1ml 2% Lignocaine + 1:80,000 ADR
n=10. Bars 95% CI.

0.5ml. Phentolamine Injected 30 Minutes
After 1ml 2% Lignocaine + 1:80,000 ADR
n=10. Bars 95% CI.

Figure 6:4
MAXILLARY INFILTRATION 3mg v 2mg
[ Nos 1-5 ]

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Standard Pressure Range

Figure 6:5
MAXILLARY INFILTRATION 3mg v 2mg
[ Nos 6-10 ]

PULSE RATES

Beats/min

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SYSTOLIC PRESSURES

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DIASTOLIC PRESSURES

mmHg

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<td>80</td>
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<td>70</td>
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<tr>
<td>60</td>
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<tr>
<td>50</td>
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<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
<td></td>
<td></td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Standard Pressure Range

Figure 6:6
CHAPTER 7

REVERSING MANDIBULAR NERVE BLOCK ANAESTHESIA.

Anaesthesia of the lower jaw and teeth usually requires a nerve block of the inferior dental nerve before it enters the bony canal on the medial side of the ascending mandibular ramus. It is normal dental practice to achieve this block by injecting 2ml of 2% lignocaine + 1:80,000 adrenaline in the vicinity of the inferior dental nerve. By utilising a greater volume of local anaesthetic the chance of achieving adequate surgical anaesthesia is improved, but this prolongs the period of residual soft tissue anaesthesia of the lower lip, oral mucosa and the ipsilateral anterior two thirds of the tongue.

Having achieved considerable success at reversing infiltration soft tissue effects in the maxilla, a further Ethics Committee approval was sought to investigate the efficacy of phentolamine reversing the effects of the inferior dental nerve block.

OBJECTIVE

The aim of the study was to investigate the clinical potential of reversing the mandibular soft tissue and pulpal tissues following routine inferior dental nerve block using a 2mg/ml phentolamine solution. Assuming a fixed ratio of phentolamine to 2% lignocaine + 1:80,000 adrenaline, 1ml of the phentolamine solution was anticipated to reverse 2ml of the local anaesthetic.

METHOD

Following the protocol described in Chapter 4, ten subjects from the student population were randomly assigned to receive the phentolamine or placebo injection following the conventional inferior dental nerve block. It was thought inappropriate to compare the drugs simultaneously as the degree of soft tissue anaesthesia would be substantial, consequently the drugs were injected on separate occasions, one week apart, as a cross-over trial.

Levels of soft tissue thresholds of stimulation of both the lower lip vermilion tissue and the mandibular canines were established as previously described (Chapter 4). The local anaesthetic was injected using an aspirating technique, to complete the inferior dental nerve block on the selected side. Standard 2ml of 2% lignocaine + 1:80,000 adrenaline...
adrenaline was injected using the direct method of placement (Rood, 1972).

Confirmation of anaesthesia of both soft tissues and mandibular canine pulp was established prior to the second injection 30 minutes after the local anaesthetic injection. The 1ml placebo or phentolamine injection was made using the same aspirating technique and the time recorded for resolution of soft tissue and pulp sensation as judged by the re-establishment of electrical perception at the pre-anaesthetic thresholds.

Blood pressure was monitored periodically throughout the procedure using a Dinamap Adult Vital Signs Monitor (Chapter 4) and the cardiac rhythm using an oscilloscope and standard chest leads. An anaesthetist was in constant attention.

The trial was repeated one week later with the same ten subjects, using the same anaesthetic technique described, but injecting the alternative placebo or phentolamine solution. Codes for the double blind trial were produced and kept by the Sterile Supplies Department at University College Hospital Pharmacy.

RESULTS
The 2ml injection of 2% lignocaine + 1:80,000 adrenaline produced a significant soft tissue and pulpal anaesthesia far beyond 30 minutes adjudged for a routine dental procedure. As described in Chapter 6, results are presented as a time interval (minutes) from the second injection of phentolamine or placebo (ie, following the 30 minute delay). The phentolamine group had reduced anaesthesia of the lip soft tissue from 160.3 to 17.3 minutes and the pulps from 39.5 to 11.1 minutes. Analysis of the paired data confirms the clinical impression that the phentolamine injection usefully reduced the soft tissue and pulp anaesthesia following the mandibular nerve block (Tables 7:1, 7:2). There was no evidence of significant blood pressure or cardiovascular rhythm changes observed during the procedures. Graphical representation of the data is demonstrated by Figure 7:1. The lack of cardiovascular changes during the test period is confirmed in Figures 7:2, 7:3, 7:4, 7:5, where the individual volunteers responses are presented graphically.
### Table 7:1

**INFERIOR ALVEOLAR REVERSALS - DURATION OF SOFT TISSUE ANAESTHESIA.**

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>PLACEBO (mins)</th>
<th>2mg PHENTOLAMINE (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>200</td>
<td>25</td>
</tr>
<tr>
<td>2</td>
<td>135</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>165</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>151</td>
<td>16</td>
</tr>
<tr>
<td>5</td>
<td>142</td>
<td>60</td>
</tr>
<tr>
<td>6</td>
<td>180</td>
<td>10</td>
</tr>
<tr>
<td>7</td>
<td>180</td>
<td>12</td>
</tr>
<tr>
<td>8</td>
<td>170</td>
<td>13</td>
</tr>
<tr>
<td>9</td>
<td>165</td>
<td>10</td>
</tr>
<tr>
<td>10</td>
<td>115</td>
<td>16</td>
</tr>
</tbody>
</table>

Means  = 160.3 and 17.3  
Mean of differences  = 143  
SD of differences  = 31.4  
SEM  = 9.92  
T  = 14.41  
P  = <0.001  
CI 95%  = 121 to 165

### Table 7:2

**INFERIOR ALVEOLAR REVERSALS - DURATION OF PULP ANAESTHESIA.**

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>PLACEBO (mins)</th>
<th>2mg PHENTOLAMINE (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>7</td>
</tr>
<tr>
<td>2</td>
<td>64</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>41</td>
<td>7</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>55</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>70</td>
<td>12</td>
</tr>
<tr>
<td>7</td>
<td>41</td>
<td>14</td>
</tr>
<tr>
<td>8</td>
<td>53</td>
<td>10</td>
</tr>
<tr>
<td>9</td>
<td>66</td>
<td>16</td>
</tr>
<tr>
<td>10</td>
<td>60</td>
<td>11</td>
</tr>
</tbody>
</table>

Means  = 59.5 and 11.1  
Mean of differences  = 48.4  
SD of differences  = 12.8  
SEM  = 4.05  
T  = 11.95  
P  = <0.001  
CI 95%  = 39.2 to 57.6
CONCLUSIONS

Chapter 7 reports the statistically significant reduction of pulp and soft tissue anaesthesia produced by a standard 2ml 2% lignocaine + 1:80,000 adrenaline inferior dental nerve block, by 1ml of the 2mgl/ml phentolamine reversal agent. The Analytic Pulp Tester and Soft Tissue Tester (Chapter 4) again proved effective at determining time of restoration of sensation to pre-anaesthetic concentrations. No difficulty was experienced in producing adequate block anaesthesia, and no major change in pulse rate, diastolic or systolic pressures observed. It is inferred that the 2mg phentolamine was not injected intravenously as no substantial cardiovascular changes were noted (Taylor et al., 1965a,d; Richards et al., 1978). No local soft tissue damage was observed or reported.
INFERIOR ALVEOLAR NERVE
SOFT TISSUE REVERSAL.

1ml 2mg/ml Phentolamine Injected 30 min
After 2ml 2% Lignocaine + 1:80,000 ADR.
n=10. Bars 95% CI.

PULP REVERSAL

1ml 2mg/ml Phentolamine Injected 30 min
After 2ml 2% Lignocaine + 1:80,000 ADR.
n=10. Bars 95% CI.

Figure 7:1
INFERIOR ALVEOLAR PHENTOLAMINE REVERSALS [Nos 1-5]

PULSE RATES

Beats/min

-5 0 5 10 15 20 25 30

Minutes

SYSTOLIC PRESSURES

mmHg

-5 0 5 10 15 20 25 30

Minutes

DIASTOLIC PRESSURES

mmHg

-5 0 5 10 15 20 25 30

Minutes

Standard Pressure Range

Figure 7:2
INFERIOR ALVEOLAR PHENTOLAMINE REVERSALS [Nos 6-10]

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Figure 7:3
INFERIOR ALVEOLAR PLACEBO REVERSAL
[ Nos 1-5 ]

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Figure 7:4
INFERIOR ALVEOLAR PLACEBO REVERSAL
[ Nos 6-10 ]

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 7:5
CHAPTER 8

THE EFFECT OF PHENTOLAMINE ON VENOUS PLASMA LIGNOCAINE CONCENTRATIONS.

INTRODUCTION
The phentolamine 2mg/ml solution was shown to produce a clinically useful reduction in the duration of soft tissue and pulp anaesthesia following single routine dental infiltration and nerve block injections. It is not uncommon during modern dental practise to inject multiple dental cartridges during extensive procedures, resulting in substantial areas of persistent residual soft tissue anaesthesia.

It is reasonable to propose that as the phentolamine selectively competes with the adrenaline at the α-adrenoceptor, the resulting vasodilatation increases absorption of lignocaine into the plasma. The kinetic profile of the lignocaine and adrenaline mixture is converted in vivo to that of a lignocaine plain solution.

Limited data exist on the venous plasma concentrations achieved after multiple dental injections, and Scott et al., (1972) have demonstrated the necessity for specifying the concentration and site of local anaesthetic injection as the achieved plasma lignocaine concentrations vary greatly in areas of differing vascularity. It was thought essential to establish the kinetic profiles of the lignocaine plain and lignocaine with adrenaline anaesthetic solutions before attempting the phentolamine reversals. Local Ethics Committee approval for the project was gained.

OBJECTIVE
(i) To determine baseline plasma kinetic profiles following the perioral injection of 6ml of 2% lignocaine + 1:80,000 adrenaline, and 6ml 2% plain lignocaine.

(ii) To assess the efficiency of phentolamine at reversing the potentiating adrenaline as demonstrated by a significant rise in venous plasma lignocaine concentration.
METHOD (i): Measuring The Venous Plasma Lignocaine Concentration Of
The Test Anaesthetics.

Fifteen volunteer students were randomly assigned to two groups, five to be injected
with 6ml of 2% lignocaine plain and the remaining ten with 6ml of 2% lignocaine +
1:80,000 adrenaline. Plasma concentrations of lignocaine were determined over a 120
minute test period as previously described in Chapter 4.

RESULTS

Peak plasma concentrations tended to occur earlier and were of greater magnitude in the
lignocaine plain group as demonstrated by the Plasma - Time profiles for the individual
subjects shown in Figures 8:1,8:2, and the distribution of the time interval to maximum
concentration in Figure 8:2. As previously described the area under the curve (AUC)
was used as the summary statistic and the data presented in Table 8:1.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Lignocaine AUC</th>
<th>Lignocaine + 1:80,000 ADR AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>0.571</td>
<td>0.423</td>
</tr>
<tr>
<td>2.</td>
<td>0.464</td>
<td>0.573</td>
</tr>
<tr>
<td>3.</td>
<td>0.623</td>
<td>0.513</td>
</tr>
<tr>
<td>4.</td>
<td>0.515</td>
<td>0.403</td>
</tr>
<tr>
<td>5.</td>
<td>0.521</td>
<td>0.517</td>
</tr>
<tr>
<td>6.</td>
<td></td>
<td>0.433</td>
</tr>
<tr>
<td>7.</td>
<td></td>
<td>0.453</td>
</tr>
<tr>
<td>8.</td>
<td></td>
<td>0.401</td>
</tr>
<tr>
<td>9.</td>
<td></td>
<td>0.543</td>
</tr>
<tr>
<td>10.</td>
<td></td>
<td>0.471</td>
</tr>
<tr>
<td>means</td>
<td>0.538</td>
<td>0.473</td>
</tr>
<tr>
<td>mean of differences = 0.0658</td>
<td></td>
<td></td>
</tr>
<tr>
<td>sem    = 0.0332</td>
<td></td>
<td></td>
</tr>
<tr>
<td>t      = 1.99</td>
<td></td>
<td></td>
</tr>
<tr>
<td>p      = 0.0687</td>
<td></td>
<td></td>
</tr>
<tr>
<td>95% CI  = -0.00582 to 0.137</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>


PLASMA LIGNOCAINEN CONCENTRATIONS WITH ADRENALINE

6ml 2% Lignocaine + 1:80,000 Adrenaline

Figure 8:1
PLASMA LIGNOCAIN CONCENTRATIONS WITHOUT ADRENALINE

---

**Lignocaine mg/L**

-- Subject 1
-- Subject 4
-- Subject 3
-- Subject 4
-- Subject 5

**Minutes**

6ml 2% Lignocaine

---

**PEAK PLASMA LIGNOCAIN CONCENTRATIONS**

**Lignocaine mg/L**

-- Lig
-- Lig + 1:80,000 ADR

**Minutes**

6ml 2% Lignocaine
6ml 2% Lignocaine + 1:80,000 ADR

Figure 8:2
6ml 2% LIGNOCAINE PLAIN

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 8:3
6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE
[ Nos 1-5 ]

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Figure 8:4
6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE [Nos 6-10]

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Figure 8:5

Standard Pressure Range
It can be seen from these results that the added adrenaline produces a reduction in both AUC and the peak plasma concentration compared to lignocaine, but analysis failed to demonstrate a significance at the 95% Confidence Interval. Figures 8:3, 8:4, 8:5 confirm the lack of significant cardiovascular changes of the individual volunteers.

METHOD (ii): Assessing The Effectiveness Of Phentolamine At Reversing The Effects Of Adrenaline As An Increase In Plasma Lignocaine Concentrations.

Having established the kinetic profiles of the lignocaine and lignocaine with adrenaline anaesthetics from specific perioral injection sites, the objective of the second part of the study was to measure the effectiveness of phentolamine in reversing the potentiating adrenaline. The significant doses of lignocaine and adrenaline with phentolamine could produce deleterious cardiovascular changes, and the aim was to carefully monitor the cardiovascular parameters and quantify the vasodilatory effect of phentolamine as increased plasma lignocaine concentrations.

Following the protocol described in Chapter 4, ten subjects were randomly assigned to two groups, each receiving the phentolamine reversing agent and the matching placebo one week apart, as a cross-over trial. Each was injected with 6ml of 2% lignocaine + 1:80,000 adrenaline 30 minutes prior to the second injection of 3ml 2mg/ml phentolamine or matching placebo. Plasma concentrations of lignocaine were determined over a 30 minute period in the first five subjects and over a 120 minute period in second five subjects. This alteration in time scale was made following analysis of the first group, where the extended time period seemed appropriate to observe the drug elimination.

RESULTS
Plasma-Time profiles for the individual subjects are shown in Figures 8:6, 8:9, and as previously described the AUC used as the summary statistic for the data listed in Table 8:2. Analysis of the two distinct time periods are presented. Subjects were the same in each test group, and the AUC of subject 3/8 in both placebo test periods were significantly higher, to an extent that the results were noticeably distorted.

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Table 8:2

**PLASMA-TIME (AUC) LIGNOCAINE PROFILES FOLLOWING 6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE REVERSED BY 3ml OF 2mg/ml PHENTOLAMINE OR PLACEBO. SUBJECTS 1-5, 30 MINUTE TIME COURSE.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Phentolamine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.7867</td>
<td>0.5433</td>
</tr>
<tr>
<td>2</td>
<td>0.7092</td>
<td>0.4708</td>
</tr>
<tr>
<td>3</td>
<td>0.6542</td>
<td>0.8491*</td>
</tr>
<tr>
<td>4</td>
<td>0.5983</td>
<td>0.6150</td>
</tr>
<tr>
<td>5</td>
<td>0.6150</td>
<td>0.6108</td>
</tr>
</tbody>
</table>

mean of differences = 0.0549  
sd of differences = 0.187  
sem = 0.0835  
t = 0.658  
P = 0.5467  
95% CI = -0.177 to 0.287

Table 8:3

**PLASMA-TIME (AUC) LIGNOCAINE PROFILES FOLLOWING 6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE REVERSED BY 3ml OF 2mg/ml PHENTOLAMINE OR PLACEBO. SUBJECTS 6-10, 120 MINUTES TIME COURSE.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Phentolamine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.5990</td>
<td>0.4213</td>
</tr>
<tr>
<td>7</td>
<td>0.5877</td>
<td>0.4692</td>
</tr>
<tr>
<td>8</td>
<td>0.4150</td>
<td>0.6192*</td>
</tr>
<tr>
<td>9</td>
<td>0.5440</td>
<td>0.3840</td>
</tr>
<tr>
<td>10</td>
<td>0.4304</td>
<td>0.4000</td>
</tr>
</tbody>
</table>

mean of differences = 0.0565  
sd of differences = 0.156  
sem = 0.0700  
t = 0.807  
P = 0.4648  
95% CI = -0.138 to 0.251
CONCLUSIONS

Chapter 8 reports the plasma lignocaine concentrations achieved following multiple dental injections of 2% lignocaine + 1:80,000 adrenaline and concentrations achieved after reversing with phentolamine (2mg/ml).

Table 8:1 presents the Plasma-Time profiles of the individual subjects following the injection of 6ml 2% lignocaine plain or 2% lignocaine + 1:80,000 adrenaline. There was no statistical difference at the 95% Confidence Interval but this was marginal with a probability of 0.068. Figure 8:2 confirmed the peak plasma concentrations of lignocaine plain appeared earlier at 10 minutes and were higher (0.92mg/L, range 0.71-1.24) than the lignocaine and adrenaline mixture (0.59mg/L, range 0.45-0.71). These figures are comparable with those reported by Cannell et al., 1975 and Perovic et al., 1980, although they used lower loading doses of anaesthetics. The adrenaline had an effect of reducing the plasma kinetic profile but not by a statistically significant degree. The cardiovascular responses to the plain and adrenaline anaesthetics remained stable with no significant differences between the groups (Figures 8:3, 8:4, 8:5), as noted by other investigators (Piperno, Kaim 1981; Meechan, Rawlins 1988; Knoll-Kohler et al., 1989).

Reversing the soft tissue anaesthesia produced by perioral injection of 6ml 2% lignocaine + 1:80,000 adrenaline, was achieved using a total phentolamine dose of 6mg. Plasma-Time profiles are presented in Table 8:2 with the two groups being isolated due to the different time courses. No statistical difference between phentolamine and placebo for the 30 minute test period is noted. However, the data is significantly distorted by one subject, identified as nos. 3 and 8, where the placebo injection results in much higher plasma lignocaine concentrations. In their paper reporting the HPLC technique used in this study, Holt and Flanagan (1985) emphasized the high risk of interference from other basic drugs during the assaying, as the lignocaine has no usable u.v. absorption above 215mm and electrochemical oxidation or fluorescence detection cannot be used directly. It appears that some plasma contaminant may have produced these spurious results, especially in the light of many further data presented in Chapters 9,10,11. It would seem that despite the accuracy of the HPLC assays the plasma of subject 3/8 contained some contaminant that was not distinguishable from lignocaine.
by this form of analysis. The peak lignocaine concentrations were achieved 15 minutes after the phentolamine injection (0.704mg/L, range 0.62-0.82) compared with placebo at 0 minutes (0.657mg/L, range 0.48-0.83). Trials with small numbers of participants are likely to produce data distorted by such variations in results. The measurable clinical reduction is residual soft tissue anaesthesia (Chapters 6, 7) produced by the phentolamine injection is not associated with a significant rise in plasma lignocaine concentration. It is possible that the plasma volume diluted the released lignocaine to an extent that the venous sampling was not sensitive enough to detect the changes in plasma concentration.

In summary the local effects of the injected phentolamine did not produce a statistically significant increase in venous plasma lignocaine concentrations compared with placebo, and the plasma concentrations achieved were below previously reported toxic concentrations. No major changes in pulse rate, diastolic or systolic pressures were noted, and no local soft tissue damage observed or reported. The significant 6mg phentolamine dose did not induce the cardiovascular changes observed following an intravenous 5mg injection (Taylor et al., 1965a,d; Richards et al., 1978). This suggests that the perioral injection remains confined to the depot tissue for some time, being dissipated into the circulation at a slow rate.
PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PHENTOLAMINE REVERSALS.

Lignocaine mg/L

![Graph showing lignocaine concentrations over time for different subjects.](image)

Minutes

6ml 2% Lignocaine + 1:80,000 Adrenaline Reversed After 30 Minutes With 6mg Phentolamine.

Lignocaine mg/L

![Graph showing lignocaine concentrations over time for different subjects.](image)

Minutes

6ml 2% Lignocaine + 1:80,000 Adrenaline Reversed After 30 Minutes With 6mg Phentolamine.
6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE & [Nos 1-5] 6mg PHENTOLAMINE

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 8:7
6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE &
[ Nos 6-10 ] 6mg PHENTOLAMINE

PULSE RATES

Pulse Rates

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 8:8

132
PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PLACEBO REVERSALS.

Lignocaine mg/L

Minutes

6ml 2% Lignocaine + 1:80,000 Adrenaline
Reversed After 30 Minutes With Matching Placebo.

Lignocaine mg/L

Minutes

6ml 2% Lignocaine + 1:80,000 Adrenaline
Reversed After 30 Minutes With Matching Placebo.
6ml 2% LIGNOCaine + 1:80,000 ADRENALINE & [ Nos 1-5 ] PLACEBO

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Standard Pressure Range

Figure 8:10
6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE + [ Nos 6-10 ] PLACEBO

PULSE RATES

Beats/min

Minutes

SYSTOLIC PRESSURES

mmHg

Minutes

DIASTOLIC PRESSURES

mmHg

Minutes

Standard Pressure Range

Figure 8:11
CHAPTER 9
THE EFFECT OF PHENTOLAMINE ON VENOUS PLASMA LIGNOCAINE
CONCENTRATIONS AFTER MULTIPLE DENTAL INJECTIONS
CONTAINING VARIOUS ADRENALINE CONCENTRATIONS.

Dental local anaesthetic manufacturers in the United States of America and on the
European mainland formulate local anaesthetics differently from the United Kingdom
varying the volume of local anaesthetic and the concentration of adrenaline. There is
much confusion in the literature as to the recommended safe dose of adrenaline, partly
as it has been clearly demonstrated that the contributions from exogenous adrenaline to
the total venous plasma concentrations of adrenaline varies with the degree of
vasularity of the injection site (Scott et al., 1972; Cotton et al., 1986; Yerzingatsian
1991; Knoll-Kohler, Fortsch 1992). The higher the concentration of adrenaline in the
dental anaesthetic solution, the less lignocaine should be absorbed and detected in the
plasma. Phentolamine reversals would be anticipated to be more effective against the
weakest adrenaline concentration producing higher peak plasma local anaesthetic
concentrations.

OBJECTIVE

(i) To determine baseline plasma lignocaine profiles following the dental injection
of:
5.4ml 2% lignocaine + 1:50,000 adrenaline
5.4ml 2% lignocaine + 1:80,000 adrenaline
5.4ml 2% lignocaine + 1:100,000 adrenaline
and assess the justification for the high 1:50,000 adrenaline solutions as a
significant Plasma-Time (AUC) difference from the other test anaesthetics.

(ii) To assess the efficiency of phentolamine at reversing the three differing
adrenaline local anaesthetic solutions as demonstrated by a significant rise in
venous plasma lignocaine concentration.

136
METHOD (i): To Determine The Plasma Lignocaine Profiles Of The Test Anaesthetics.

Following the protocol described in Chapter 4, ten volunteer students were assigned to be injected with the test local anaesthetics on three different occasions, one week apart, the order being randomised. Venous plasma concentrations of lignocaine were determined over a 120 minute test period as previously described (Chapter 4), enabling the individual baseline plasma profiles to be determined.

RESULTS

Plasma-Time profiles for the individual subjects are shown in Figures 9:1, 9:3, 9:6, and as previously described the area under the curve (AUC) used as the summary statistic for the data, listed in Table 9:1.

<table>
<thead>
<tr>
<th></th>
<th>Lig + 1:50,000</th>
<th>Lig + 1:100,000</th>
<th>Lig + 1:80,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.3685</td>
<td>0.2681</td>
<td>0.2321</td>
</tr>
<tr>
<td>2</td>
<td>0.4300</td>
<td>0.3029</td>
<td>0.2840</td>
</tr>
<tr>
<td>3</td>
<td>0.4323</td>
<td>0.3996</td>
<td>0.2406</td>
</tr>
<tr>
<td>4</td>
<td>0.3281</td>
<td>0.3452</td>
<td>0.3294</td>
</tr>
<tr>
<td>5</td>
<td>0.3550</td>
<td>0.3263</td>
<td>0.2915</td>
</tr>
<tr>
<td>6</td>
<td>0.3946</td>
<td>0.2844</td>
<td>0.1835</td>
</tr>
<tr>
<td>7</td>
<td>0.2827</td>
<td>0.2079</td>
<td>0.3606</td>
</tr>
<tr>
<td>8</td>
<td>0.2238</td>
<td>0.3398</td>
<td>0.1879</td>
</tr>
<tr>
<td>9</td>
<td>0.2517</td>
<td>0.4825</td>
<td>0.2198</td>
</tr>
<tr>
<td>10</td>
<td>0.2358</td>
<td>0.2629</td>
<td>0.2533</td>
</tr>
</tbody>
</table>

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>x</td>
<td>0.3303</td>
<td>0.3220</td>
<td>0.2583</td>
</tr>
<tr>
<td>sd</td>
<td>0.0784</td>
<td>0.0774</td>
<td>0.0581</td>
</tr>
<tr>
<td>sem</td>
<td>0.0248</td>
<td>0.0245</td>
<td>0.0184</td>
</tr>
<tr>
<td>95%CI</td>
<td>0.274 to 0.386</td>
<td>0.267 to 0.377</td>
<td>0.217 to 0.300</td>
</tr>
</tbody>
</table>
Table 9:2

ANALYSIS OF VARIABLES

Bonferroni (Dunn) T tests for variables : AUC

<table>
<thead>
<tr>
<th>Bon Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.3384</td>
<td>10</td>
<td>1:50,000 ADR</td>
</tr>
<tr>
<td>A</td>
<td>0.3303</td>
<td>10</td>
<td>1:100,000 ADR</td>
</tr>
<tr>
<td>A</td>
<td>0.2726</td>
<td>10</td>
<td>1:80,000 ADR</td>
</tr>
</tbody>
</table>

Means with the same letters are not statistically different.

Table 9:3

PEAK PLASMA LIGNOCAINE CONCENTRATIONS

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Time After Injection (Minutes)</th>
<th>Plasma Concentration + Range (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lig + 1:50,000 ADR</td>
<td>30</td>
<td>0.424 (0.60-0.24)</td>
</tr>
<tr>
<td>Lig + 1:80,000 ADR</td>
<td>30</td>
<td>0.356 (0.53-0.20)</td>
</tr>
<tr>
<td>Lig + 1:100,000 ADR</td>
<td>20</td>
<td>0.433 (0.60-0.18)</td>
</tr>
</tbody>
</table>

No statistically significant difference is demonstrated between the test solutions despite the significant variation in adrenaline concentration. Figures 9:2, 9:4, 9:5, 9:7 confirm the clinical impression that no clinically important cardiovascular changes occurred during the test period.
PLASMA LIGNOCaina CONCENTRATIONS

5.4ml 2% Lignocaine + 1:50,000 Adrenaline

5.4ml 2% Lignocaine + 1:50,000 Adrenaline Figure 9:1
5.4ml 2% LIGNOCAINE + 1:50,000 ADRENALINE

PULSE RATES

Beats/min

Minutes

SYSTOLIC PRESSURES

mmHg

Minutes.

DIASTOLIC PRESSURES

mmHg

Minutes.

Standard Pressure Range

Figure 9:2
PLASMA LIGNOCAINE CONCENTRATIONS

5.4ml 2% Lignocaine + 1:80,000 Adrenaline

Figure 9:3
5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE [Nos 1-5]

PULSE RATES

Beats/min

Minutes

SYSTOLIC PRESSURES

mmHg

Minutes.

DIASTOLIC PRESSURES

mmHg

Minutes.

Standard Pressure Range

Figure 9:4
5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE [Nos 6-10]

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 9:5
PLASMA LIGNOCAINE CONCENTRATIONS

Lignocaine mg/L

Minutes

Subject 1
Subject 2
Subject 3
Subject 4
Subject 5

5.4ml 2% Lignocaine + 1:100,000 Adrenaline

Lignocaine mg/L

Minutes

Subject 6
Subject 7
Subject 8
Subject 9
Subject 10

5.4ml 2% Lignocaine + 1:100,000 Adrenaline

Figure 9:6
5.4ml 2% LIGNOCAINE + 1:100,000 ADRENALINE

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range  Figure 9:7
METHOD (ii): To Determine The Effect Of Phentolamine On The Plasma Lignocaine Concentrations.

Having established the baseline plasma lignocaine profiles for the three differing adrenaline containing local anaesthetic solutions, the effectiveness of the 2mg/ml phentolamine solution at reversing the potentiating adrenaline was studied, measuring the changes in venous plasma lignocaine concentrations.

Following the protocol described in Chapter 4, the same five subjects who participated in the first part of the trial were assigned to receive either the phentolamine reversing agent or matching placebo on separate occasions, one week apart. Each was injected on three separate occasions with 5.4ml of one of the test local anaesthetic solutions as described in Chapter 4, followed after 30 minutes by the phentolamine or matching placebo. Venous plasma lignocaine concentrations were obtained over a 120 minute test period as previously described. The protocol for the 1:100,000 adrenaline group measured serial venous plasma lignocaine concentrations over a reduced time scale of 30 minutes following the phentolamine or placebo injection. This was expected to include any statistically significant change in plasma lignocaine profiles but after the analysis of the raw data the time scales for the 1:50,000 and 1:80,000 groups were expanded to 120 minutes.

RESULTS

Plasma-Time profiles for the individual subjects are shown in Figures 9:8, 9:11 and 9:14, and as previously described the area under the curve (AUC) used as the summary statistic, listed in Table 9:4.
Table 9:4

PLASMA-TIME (AUC) LIGNOCAINE PROFILES FOLLOWING 5.4ml 2% LIGNOCAINE
+ 1:50,000, 1:80,000 AND 1:100,000 ADRENALINE REVERSED BY 3ml 2mg/ml
PHENTOLAMINE OR PLACEBO

<table>
<thead>
<tr>
<th></th>
<th>Lig + 1:50,000 ADR + Phentolamine</th>
<th>Lig + 1:50,000 ADR + Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.4742</td>
<td>0.4777</td>
</tr>
<tr>
<td></td>
<td>0.4458</td>
<td>0.2871</td>
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<tr>
<td></td>
<td>0.5167</td>
<td>0.4146</td>
</tr>
<tr>
<td></td>
<td>0.3377</td>
<td>0.2752</td>
</tr>
<tr>
<td></td>
<td>0.4004</td>
<td>0.3735</td>
</tr>
<tr>
<td>mean of differences</td>
<td>0.0693</td>
<td>0.0637</td>
</tr>
<tr>
<td>sd of difference</td>
<td>0.0637</td>
<td>0.0637</td>
</tr>
<tr>
<td>sem of difference</td>
<td>0.0285</td>
<td>0.0285</td>
</tr>
<tr>
<td>t</td>
<td>2.236</td>
<td>2.236</td>
</tr>
<tr>
<td>p</td>
<td>0.0716</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.00971 to 0.148</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Lig + 1:80,000 ADR + Phentolamine</th>
<th>Lig + 1:80,000 ADR + Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.3796</td>
<td>0.3477</td>
</tr>
<tr>
<td></td>
<td>0.5323</td>
<td>0.3950</td>
</tr>
<tr>
<td></td>
<td>0.3750</td>
<td>0.3683</td>
</tr>
<tr>
<td></td>
<td>0.5208</td>
<td>0.2973</td>
</tr>
<tr>
<td></td>
<td>0.4002</td>
<td>0.2808</td>
</tr>
<tr>
<td>mean of differences</td>
<td>0.104</td>
<td></td>
</tr>
<tr>
<td>sd of difference</td>
<td>0.0870</td>
<td></td>
</tr>
<tr>
<td>sem of difference</td>
<td>0.0389</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>2.666</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.0560</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>-0.00429 to 0.212</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Lig + 1:100,000 ADR + Phentolamine</th>
<th>Lig + 1:100,000 ADR + Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.491</td>
<td>0.057</td>
</tr>
<tr>
<td></td>
<td>0.546</td>
<td>0.080</td>
</tr>
<tr>
<td></td>
<td>0.535</td>
<td>0.109</td>
</tr>
<tr>
<td></td>
<td>0.418</td>
<td>0.141</td>
</tr>
<tr>
<td></td>
<td>0.519</td>
<td>0.116</td>
</tr>
<tr>
<td>mean of differences</td>
<td>0.401</td>
<td></td>
</tr>
<tr>
<td>sd of differences</td>
<td>0.0730</td>
<td></td>
</tr>
<tr>
<td>sem of difference</td>
<td>0.0326</td>
<td></td>
</tr>
<tr>
<td>t</td>
<td>12.288</td>
<td></td>
</tr>
<tr>
<td>p</td>
<td>0.0003</td>
<td></td>
</tr>
<tr>
<td>95% CI</td>
<td>0.311 to 0.492</td>
<td></td>
</tr>
</tbody>
</table>
Table 9:5

PHENTOLAMINE :: PLACEBO RATIO

<table>
<thead>
<tr>
<th>Ratio</th>
<th>1:50,000</th>
<th>1:80,000</th>
<th>1:100,000</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.092</td>
<td>1.554</td>
<td>8.614</td>
<td></td>
</tr>
<tr>
<td>1.347</td>
<td>1.246</td>
<td>6.825</td>
<td></td>
</tr>
<tr>
<td>1.019</td>
<td>1.229</td>
<td>4.908</td>
<td></td>
</tr>
<tr>
<td>1.754</td>
<td>1.070</td>
<td>2.965</td>
<td></td>
</tr>
<tr>
<td>1.429</td>
<td></td>
<td>4.474</td>
<td></td>
</tr>
<tr>
<td>Mean</td>
<td>1.33</td>
<td>1.22</td>
<td>5.56</td>
</tr>
<tr>
<td>SD</td>
<td>0.293</td>
<td>0.216</td>
<td>2.19</td>
</tr>
<tr>
<td>SEM</td>
<td>0.121</td>
<td>0.0966</td>
<td>0.981</td>
</tr>
<tr>
<td>95% CI</td>
<td>0.964 to 1.69</td>
<td>0.95 to 1.49</td>
<td>2.83 to 8.28</td>
</tr>
</tbody>
</table>

Table 9.5 lists the ratio of phentolamine :: placebo AUC of lignocaine data. With no differences between the groups the ratio would be 1. Phentolamine is confirmed as being most effective at increasing the plasma lignocaine AUC of the 1:100,000 adrenaline solution.

Table 9:6

PEAK PLASMA LIGNOCAINE CONCENTRATIONS FOLLOWING 6ml AND 5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE

<table>
<thead>
<tr>
<th>Test Anaesthetic</th>
<th>Peak Plasma Concentration (Range) mg/L</th>
<th>Time Of Peak Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>6ml 2% lig + 1:80,000</td>
<td>0.593 (0.45-0.71)</td>
<td>30</td>
</tr>
<tr>
<td>5.4ml 2% lig + 1:80,000</td>
<td>0.356 (0.20-0.53)</td>
<td>30</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The first part of the trial describes the attempt to demonstrate the plasma lignocaine concentrations achieved following multiple perioral injections of various adrenaline containing local anaesthetics. Table 9:1 summarises the AUC for the individual subjects with Table 9:2 demonstrating that there was no statistical difference between the test solutions. Peak plasma lignocaine concentrations were achieved after 30 minutes in the
1:50,000 and 1:80,000 groups, and earlier at 20 minutes for the 1:100,000 adrenaline containing anaesthetic (Table 9:3). The volume injected in this series was reduced at 5.4ml (108mg) compared with the 6ml (120mg) described in Chapter 8 and this produced the expected reduced peak plasma concentrations (Table 9:6).

These figures are similar to those produced by Cannell et al., (1975) and Rood and Cannell, (1978), with the plasma peaks occurring after the same interval (30 minutes) following the perioral injections. The weakest 1:100,000 adrenaline containing anaesthetic produced an earlier peak mean concentration at 20 minutes post-injection. This was most likely due to the lower adrenaline loading dose, as lignocaine plain solutions are seen to have much earlier peak concentrations (Table 9:1).

With the clinical absence of any additional benefit from using the 1:50,000 adrenaline anaesthetic, and it having no significant effect on reducing plasma lignocaine concentrations, it would be inappropriate to recommend its use in clinical dentistry. This conclusion has also been made by Knoll-Kohler and Fortsch (1992) but the 1:50,000 adrenaline containing local anaesthetic was believed to be advantageous for enhanced haemostasis during periodontal surgery (Jastak, Yagiela, 1983).

The second part of the trial records the effect of phentolamine on the three adrenaline solutions, and the analysis of the effect of phentolamine in producing a significant rise in the AUC of the individual Plasma-Time profiles is shown in Table 9:4. There was no statistically significant difference between phentolamine and placebo in the 1:50,000 (p = 0.071) and the 1:80,000 (p = 0.056) test groups, although an obvious trend was evident. A significant difference (95% CI) was demonstrated in the weakest 1:100,000 (p = 0.0003) adrenaline test group with a significant increase in plasma lignocaine concentration, (Table 9:4, Figure 9:14).

Table 9:5 lists the ratio of phentolamine:placebo AUC data and with no difference between the two groups the ratio would be 1. The table data confirms the impression that the phentolamine reversal agent is statistically more significant in increasing the AUC of the Plasma-Time profiles in the weaker 1:100,000 adrenaline local anaesthetic.
Figures 9:9, 9:10, 9:12, 9:13, 9:15, 9:16 record the cardiovascular parameters measured during the test periods. No significant differences in the pulse rates, systolic or diastolic pressures are noted in any of the phentolamine test groups. Again this infers that the absorption of the 6mg phentolamine from the perioral injection sites does not mimic an intravenous injection (Taylor 1965 a,d; Richards et al., 1978).

<table>
<thead>
<tr>
<th>Table 9:7</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>EFFECT OF PHENTOLAMINE REVERSALS ON PEAK PLASMA ANAESTHETIC CONCENTRATIONS.</strong></td>
</tr>
<tr>
<td>Test Anaesthetic</td>
</tr>
<tr>
<td>------------------</td>
</tr>
<tr>
<td>6ml lig + 1:80,000</td>
</tr>
<tr>
<td>6ml mep + 1:100,000</td>
</tr>
<tr>
<td>6ml pril + 0.03iu/ml fely</td>
</tr>
<tr>
<td>5.4ml lig + 1:50,000</td>
</tr>
<tr>
<td>5.4ml lig + 1:80,000</td>
</tr>
<tr>
<td>5.4ml lig + 1:100,000</td>
</tr>
</tbody>
</table>

The summary Table 9:7 lists the peak plasma concentrations achieved following the phentolamine reversals and the time interval to achieve them (Chapters 8, 9, 10 and appendix ii). The trend is of the higher the adrenaline concentration in the anaesthetic solution the longer the delay to achieve peak plasma anaesthetic concentrations. With a fixed-dose phentolamine reversal solution (2mg/ml) this is to be anticipated, and is clearly demonstrated with the 5.4ml lignocaine + 1:50,000 adrenaline and lignocaine + 1:100,000 adrenaline where doubling the adrenaline concentration extended the interval to achieve the plasma peak concentration from 10 to 30 minutes. The non-adrenaline anaesthetic, prilocaine and felypressin produced peak plasma concentrations 5 minutes following the phentolamine injection.
PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PHENTOLAMINE REVERSALS.

5.4ml 2% Lignocaine + 1:50,000 Adrenaline Reversed After 30 Minutes With 6mg Phentolamine.

PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PLACEBO REVERSALS.

5.4ml 2% Lignocaine + 1:50,000 Adrenaline Reversed After 30 Minutes With Matching Placebo.
5.4ml 2% LIGNOCAINE + 1:50,000 ADRENALINE & 6mg PHENTOLAMINE

**PULSE RATES**

<table>
<thead>
<tr>
<th>Beats/min</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
</tr>
<tr>
<td>80</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>30 50 70 90</td>
</tr>
<tr>
<td>1 1 0</td>
</tr>
<tr>
<td>130 150</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 50 70 90 110 130 150</td>
</tr>
</tbody>
</table>

**SYSTOLIC PRESSURES**

<table>
<thead>
<tr>
<th>mmHg</th>
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</thead>
<tbody>
<tr>
<td>150</td>
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<tr>
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<tr>
<td>110</td>
</tr>
<tr>
<td>90</td>
</tr>
<tr>
<td>90</td>
</tr>
<tr>
<td>110</td>
</tr>
<tr>
<td>130 150</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 50 70 90 110 130 150</td>
</tr>
</tbody>
</table>

**DIASTOLIC PRESSURES**

<table>
<thead>
<tr>
<th>mmHg</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
</tr>
<tr>
<td>80</td>
</tr>
<tr>
<td>60</td>
</tr>
<tr>
<td>40</td>
</tr>
<tr>
<td>30 50 70 90</td>
</tr>
<tr>
<td>1 1 0</td>
</tr>
<tr>
<td>130 150</td>
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</tbody>
</table>

<table>
<thead>
<tr>
<th>Minutes</th>
</tr>
</thead>
<tbody>
<tr>
<td>30 50 70 90 110 130 150</td>
</tr>
</tbody>
</table>

[Standard Pressure Range]

**Figure 9:9**
5.4ml 2% LIGNOCAINE + 1:50,000 ADRENALINE & PLACEBO

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 9:10
PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PHENTOLAMINE REVERSALS.

5.4ml 2% Lignocaine + 1:80,000 Adrenaline Reversed After 30 Minutes With 6mg Phentolamine.

PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PLACEBO REVERSALS.

5.4ml 2% Lignocaine + 1:80,000 Adrenaline Reversed After 30 Minutes With Matching Placebo.

Figure 9:11
5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE & 6mg PHENTOLAMINE

**PULSE RATES**

Beats/min

**SYSTOLIC PRESSURES**

mmHg

**DIASTOLIC PRESSURES**

mmHg

Figure 9:12

Standard Pressure Range
5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE & PLACEBO

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 9:13
PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PHENTOLAMINE REVERSALS.

Lignocaine mg/L

Subject 1
Subject 2
Subject 3
Subject 4
Subject 5

Minutes
30 40 50 60

5.4ml 2% Lignocaine + 1:100,000 Adrenaline Reversed after 30 Minutes With 6mg Phentolamine.

PLASMA LIGNOCAINE CONCENTRATIONS AFTER MULTIPLE PLACEBO REVERSALS.

Lignocaine mg/L

Subject 1
Subject 2
Subject 3
Subject 4
Subject 5

Minutes
30 40 50 60

5.4ml 2% Lignocaine + 1:100,000 Adrenaline Reversed after 30 Minutes With Matching Placebo.
5.4ml 2% LIGNOCAINE + 1:100,000 ADRENALINE &
6mg PHENTOLAMINE

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 9:15
5.4ml 2% LIGNOCAINE + 1:100,000 ADRENALINE & PLACEBO

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Standard Pressure Range

Figure 9:16
CHAPTER 10

THE EFFECT OF PHENTOLAMINE ON VENOUS PLASMA PRILOCaine CONCENTRATIONS.

INTRODUCTION

The phentolamine 2mg/ml solution has been demonstrated to produce a clinically useful reduction in the duration of soft tissue and pulp anaesthesia, and with multiple reversals producing peak venous plasma lignocaine concentrations statistically significantly higher than after placebo reversal injection. The competitive antagonism by phentolamine at the α-adrenoceptors and the direct vasodilating effect on vascular smooth muscle produces maximal local vasodilatation rapidly decreasing the anaesthetic concentration within the perioral tissues and restoring sensation (Gould, Reddy 1976; Doxey et al., 1977; Richards et al., 1978; Van Zwieten 1991).

The most frequently used alternative to 2% lignocaine + 1:80,000 adrenaline for dental local anaesthesia is 3% prilocaine and 0.03iu/ml felypressin. The mechanism of action of felypressin is not entirely clear (Matthews, 1986) but may act as a catalyst for prilocaine absorption and retention into nerve membrane. Others have suggested that constriction of post-capillary capacitance vessels alone is responsible (Cannell, Whelpton 1986; Fruhstorfer, Wagener 1993) with a potency at the skin surface being 0.7 that of adrenaline (Olgart, Gazelius, 1977).

The length of soft tissue and pulp anaesthesia following routine dental injection with 3% prilocaine + 0.03iu/ml felypressin is similar in duration to 2% lignocaine + 1:80,000 adrenaline (Padfield 1967; Goldman, Evers 1969; Meechan, Rawlins, 1988), and so the possibility for reducing the excessive duration of anaesthesia warrants investigation. Data from these experiments will also be used for comparative studies in Chapters 11, 12, where prilocaine and felypressin anaesthesia is used as controls in assessing standard adrenaline containing dental local anaesthetics. The effects of felypressin are different in several respects from those of sympathomimetic amines. The potentiating effect of felypressin is not mediated via α-adrenoceptors, so the ability of phentolamine to reverse soft tissue and pulpal effects would not be anticipated to be clinically useful. However, if the local vasodilatation produced by phentolamine is of sufficient potency, some increase in venous plasma prilocaine concentration may be detected on attempting reversal.
OBJECTIVE
(i) To determine baseline plasma profiles following the perioral injection of 6ml of 3% prilocaine + 0.03iu/ml felypressin.
(ii) To assess the efficiency of phentolamine at reversing the potentiating felypressin as demonstrated by a significant rise in venous plasma prilocaine concentration.

METHOD (i): Measuring The Venous Plasma Prilocaine Concentrations.
Following the protocol described in Chapter 4, five subjects from the student population were assigned to be injected with the test local anaesthetic, 6ml of 3% prilocaine + 0.03iu/ml felypressin. Venous plasma concentrations of prilocaine were determined over a 120 minute test period as previously described, enabling the individual baseline plasma profiles to be determined, and graphically presented in Figure 10:1.

RESULTS
Plasma-Time profiles for the individual subjects are shown in Figure 10.1 and as previously described the area under the curve (AUC) used as the summary statistic for the data listed in Table 10:1. Peak plasma prilocaine concentrations were achieved at 10 minutes following injection with a mean concentration of 0.874mg/L (range 0.41 - 1.38). There were no significant cardiovascular changes identified during the test period, and results are shown in Figure 10:2.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma-Time AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.467</td>
</tr>
<tr>
<td>2</td>
<td>0.482</td>
</tr>
<tr>
<td>3</td>
<td>0.513</td>
</tr>
<tr>
<td>4</td>
<td>0.397</td>
</tr>
<tr>
<td>5</td>
<td>0.376</td>
</tr>
</tbody>
</table>

mean of difference = 0.447
sd of difference = 0.0581
sem of difference = 0.0260
95% CI = 0.375 to 0.519
PLASMA PRILOCAINE CONCENTRATIONS

Prilocaine mg/L

Minutes

Subject 1  Subject 2  Subject 3

Subject 4  Subject 5

6ml 3% Prilocaine + 0.03iu/ml Felypressin

Figure 10:1
6ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN

**PULSE RATES**

![Graph showing pulse rates over time]

**SYSTOLIC PRESSURES**

![Graph showing systolic pressures over time]

**DIASTOLIC PRESSURES**

![Graph showing diastolic pressures over time]

Standard Pressure Range

**Figure 10:2**

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METHOD (ii): To Determine The Effect Of Phentolamine On The Venous Plasma Prilocaine Concentrations.

Having established the baseline prilocaine profiles, the effectiveness of phentolamine at reversing the potentiating felypressin was studied.

Following the protocol described in Chapter 4, the same five subjects who participated in the plasma prilocaine baseline trial were assigned to two groups, receiving the phentolamine reversing agent or matching placebo. Each was injected with 6ml of 3% prilocaine + 0.03iu/ml felypressin as described in Chapter 4, followed after 30 minutes by 3ml of 2mg/ml phentolamine or matching placebo. The second test solution was repeated in identical fashion one week later, as a cross-over trial.

Venous plasma prilocaine concentrations were determined over a 120 minute test period as previously described. Samples taken at 30 and 150 minutes after the prilocaine and felypressin injection were assayed for methaemoglobin (Chapter 4) and data presented in Table 10:2. Plasma prilocaine concentrations are plotted in Figure 10:3.

RESULTS

Plasma-Time profiles for the individual subjects are shown in figures, and as previously described the area under the curve (AUC) used as the summary statistic for the data, listed in Table 10:3.

Table 10:2

<table>
<thead>
<tr>
<th>Subject</th>
<th>% Methaemoglobin 30 mins</th>
<th>150 mins</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>none detected</td>
<td>0.2%</td>
</tr>
<tr>
<td>2</td>
<td>none detected</td>
<td>0.2%</td>
</tr>
<tr>
<td>3</td>
<td>0.1%</td>
<td>0.8%</td>
</tr>
<tr>
<td>4</td>
<td>0.2%</td>
<td>0.7%</td>
</tr>
<tr>
<td>5</td>
<td>0.8%</td>
<td>1.2%</td>
</tr>
</tbody>
</table>
Table 10:3

PLASMA-TIME (AUC) PRILOCAINE PROFILES FOLLOWING 6ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN REVERSED BY 3ml 2mg/ml PHENTOLAMINE OR PLACEBO.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma-Time AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Phenolamine</td>
</tr>
<tr>
<td>1</td>
<td>0.294</td>
</tr>
<tr>
<td>2</td>
<td>0.395</td>
</tr>
<tr>
<td>3</td>
<td>0.438</td>
</tr>
<tr>
<td>4</td>
<td>0.283</td>
</tr>
<tr>
<td>5</td>
<td>0.335</td>
</tr>
</tbody>
</table>

|            |               |            |            |
|            | mean of difference | 0.0542    |            |
|            | sd of difference | 0.0266     |            |
|            | sem of difference | 0.0119     |            |
|            | t               | 4.564      |            |
|            | p               | 0.0103     |            |
|            | 95% CI          | 0.0212 to 0.0872 |

CONCLUSIONS

The plasma prilocaine concentrations resulting from the perioral injection of 6ml 3% prilocaine + 0.03iu/ml felypressin are recorded in the first part of the trial.

Table 10:1 presents the summary data as areas under curves for the five test subjects. Peak plasma concentrations were achieved after 10 minutes (0.874mg/L, range 0.41-1.38), and no changes in pulse rate, diastolic or systolic pressures observed. These concentrations are comparable with those reported by Cannell and Whelpton (1986), although they used a reduced loading dose and injected only as a maxillary premolar infiltration. The stability of the cardiovascular system parameters confirms similar observations by others (Scott et al., 1972; Lilienthal 1976; and Maclean et al., 1988).
In the second part of the prilocaine trial the same five subjects were subsequently reinjected with a similar dose of prilocaine and felypressin and after the 30 minute delay received phentolamine or placebo. Results of the areas under the curves are tabulated in Table 10:3. A significant increase in plasma prilocaine concentrations of the phentolamine group is demonstrated at the 95% Confidence Interval. Although the felypressin has a non-adrenergic vasoconstrictor effect on the venular side of the vasculature, the strong direct vasodilating effect on vascular smooth muscle by the phentolamine apparently countered this weak vasoconstriction, allowing the prilocaine to be removed from the injection site, (Gould, Reddy 1976; Doxey et al., 1977; Richards et al., 1978; Van Zwieten 1988). This is represented in Figure 10:3, and no cardiovascular abnormalities were detected throughout the test period (Figures 10:4, 10:5). Prilocaine has the unusual effect of causing methaemoglobinaemia in a dose-related fashion, (Daly et al., 1964; Weiss et al., 1987; Lloyd 1992). To exclude the possibility of phentolamine inducing a significant prilocaine plasma concentration sufficient to induce methaemoglobinaemia, plasma concentrations were sampled for this altered haemoglobin just prior to and at completion of the phentolamine test period.

Methaemoglobin concentrations presented as a percentage of the total haemoglobin are listed in Table 10:2. Concentrations in excess of 3% are clinically significant, but the 6ml of 3% prilocaine + 0.03iu/ml felypressin produced insignificant concentrations following the phentolamine reversals.

The 95% Confidence Intervals seen in Table 10:3 confirm that the action of phentolamine produces a significant rise in plasma prilocaine concentrations. The subject numbers are small due to financial constraints restricting the extent of the trial. No clinically significant cardiovascular changes were recorded during the test period when comparing the active drug with matching placebo (Figure 10:4, 10:5). Concentrations of methaemoglobin remain low but did rise during the test period.
PLASMA PRILOCAINE CONCENTRATIONS AFTER MULTIPLE PHENTOLAMINE REVERSALS.

![Graph](image)

6ml 3% Prilocaine + 0.03iu Felypressin Reversed After 30 Minutes With 6mg Phentolamine

PLASMA PRILOCAINE CONCENTRATIONS AFTER MULTIPLE PLACEBO REVERSALS.

![Graph](image)

6ml 3% Prilocaine + 0.03iu Felypressin Reversed After 30 Minutes With Matching Placebo

Figure 10:3
6ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN &
6mg PHENTOLAMINE

PULSE RATES

Beats/min

Minutes

SYSTOLIC PRESSURES

mmHg

Minutes.

DIASTOLIC PRESSURES

mmHg

Minutes.

Standard Pressure Range

Figure 10:4
6ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN & PLACEBO

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Standard Pressure Range

Figure 10:5
CHAPTER 11

VENOUS PLASMA CATECHOLAMINE CONCENTRATIONS AFTER
MULTIPLE DENTAL LOCAL ANAESTHETIC INJECTIONS.

The absorption of adrenaline from local anaesthetic mixtures has been demonstrated to vary with the anatomical site and the concentration injected (Tolas et al., 1982; Low et al., 1984; Taylor et al., 1984; Cotton et al., 1986; Lew et al., 1988). Little data exist on venous catecholamine concentrations achieved after multiple perioral injections of routine dental local anaesthetic agents. The venous plasma lignocaine concentrations measured in Chapter 9 demonstrated that the 1:50,000 adrenaline solution failed to significantly reduce the lignocaine concentration below those achieved by the 1:80,000 and 1:100,000 adrenaline containing local anaesthetics. This may have been due to an excessive absorption of the 1:50,000 adrenaline from the depot site, and if so, would produce significantly higher venous plasma adrenaline concentrations compared with the other two local anaesthetic test solutions.

OBJECTIVE

(i) To measure venous plasma catecholamine concentrations after multiple perioral injections of various local anaesthetic solutions, and correlate the plasma concentrations achieved with the injected adrenaline concentrations in human volunteers.

(ii) To compare the plasma catecholamine concentrations achieved in (i) with those reported from other anatomical sites.

METHOD Measuring Venous Plasma Catecholamine Concentrations After Injection Of The Test Solution.

The following test local anaesthetic solutions were injected (Chapter 4)

a. 5.4ml 2% lignocaine + 1:50,000 adrenaline (108μg ADR)
b. 5.4ml 2% lignocaine + 1:80,000 adrenaline (67.5μg ADR)
c. 5.4ml 2% lignocaine + 1:100,000 adrenaline (54μg ADR)
d. 5.4ml 3% prilocaine + 0.03iu/ml felypressin (0μg ADR)
Following the established protocol described in Chapter 4, five volunteer students were assigned to be injected with a test local anaesthetic on four different occasions, one week apart, the order being randomised. Venous catecholamine concentrations were determined over the 120 minute test period as previously described in Chapter 4. A further five volunteers received the 1:80,000 adrenaline anaesthetic, and the venous catecholamines measured over the same time course.

RESULTS

Adrenaline and noradrenaline Plasma-Time (AUC) profiles for the individual subjects are shown in Figures 11:1, 11:3, 11:4, 11:7, 11:9 and as previously described, the area under the curve (AUC) used as the summary statistic for the data, and listed in Tables 11:1 and 11:2.

<table>
<thead>
<tr>
<th>Table 11:1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PLASMA-TIME (AUC) ADRENALINE PROFILES FOLLOWING 5.4ml 2% LIGNOCAINE + 1:50,000, 1:80,000 AND 1:100,000 ADRENALINE AND 5.4ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN</strong></td>
</tr>
<tr>
<td>Subject</td>
</tr>
<tr>
<td>---</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
<tr>
<td>5</td>
</tr>
<tr>
<td>6</td>
</tr>
<tr>
<td>7</td>
</tr>
<tr>
<td>8</td>
</tr>
<tr>
<td>mean</td>
</tr>
<tr>
<td>sd</td>
</tr>
<tr>
<td>sem</td>
</tr>
<tr>
<td>95% CI</td>
</tr>
</tbody>
</table>
Table 11:2

**ANALYSIS OF VARIABLES: ADRENALINE**

Analysis of Variance Procedure

Bonferroni (Dunn) T tests for variable : AUC

<table>
<thead>
<tr>
<th>Bon</th>
<th>Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>1.203</td>
<td>5</td>
<td>1:50,000</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.848</td>
<td>5</td>
<td>1:100,000</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.730</td>
<td>5</td>
<td>1:80,000</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.295</td>
<td>5</td>
<td>Prilocaine</td>
<td></td>
</tr>
</tbody>
</table>

Means with the same letter are not statistically different.

Table 11:3

**PLASMA-TIME (AUC) NORADRENALINE PROFILES FOLLOWING 5.4ml 2% LIGNOCAINE + 1:50,000, 1:80,000 AND 1:100,000 ADRENALINE AND 5.4ml 3% PRILOCAIN + 0.03iu/ml FELYPRESSIN**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Lig + 1:50,000</th>
<th>Lig + 1:80,000</th>
<th>Lig + 1:100,000</th>
<th>Pril + 0.03 Fely</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.2389</td>
<td>3.12</td>
<td>3.1833</td>
<td>1.9048</td>
</tr>
<tr>
<td>2</td>
<td>2.4152</td>
<td>5.9008</td>
<td>3.2492</td>
<td>1.7602</td>
</tr>
<tr>
<td>3</td>
<td>3.5513</td>
<td>2.0723</td>
<td>2.3118</td>
<td>1.4123</td>
</tr>
<tr>
<td>4</td>
<td>1.8140</td>
<td>3.0602</td>
<td>1.2629</td>
<td>1.7758</td>
</tr>
<tr>
<td>5</td>
<td>2.2613</td>
<td>3.0094</td>
<td>3.3844</td>
<td>1.7758</td>
</tr>
<tr>
<td>6</td>
<td>0.947</td>
<td>0.353</td>
<td>0.308</td>
<td>0.138</td>
</tr>
<tr>
<td>7</td>
<td>1.424</td>
<td>0.438</td>
<td>0.158</td>
<td>0.138</td>
</tr>
<tr>
<td>9</td>
<td>1.84</td>
<td>1.63 to 3.62</td>
<td>2.70 to 3.58</td>
<td>1.05 to 2.41</td>
</tr>
</tbody>
</table>

Mean 3.02 2.63 3.14 2.03
sd 0.947 1.39 0.353 0.308
sem 0.424 0.438 0.158 0.138
95% CI 1.84 to 4.19 1.63 to 3.62 2.70 to 3.58 1.05 to 2.41
Table 11:4

ANALYSIS OF VARIABLES: NORADRENALINE

Analysis of Variance Procedure

Bonferroni (Dunn) T tests for variable : AUC

<table>
<thead>
<tr>
<th>Bon</th>
<th>Grouping</th>
<th>Mean</th>
<th>N</th>
<th>Code</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>3.169</td>
<td>5</td>
<td>1:80,000</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>3.142</td>
<td>5</td>
<td>1:100,000</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>3.016</td>
<td>5</td>
<td>1:50,000</td>
</tr>
<tr>
<td>A</td>
<td></td>
<td>2.110</td>
<td>5</td>
<td>Prilocaine</td>
</tr>
</tbody>
</table>

Means with the same letter are not statistically different.

The Analysis of Variance confirmed the significant adrenaline absorption from the 1:50,000 adrenaline solution, as increased Plasma-Time profiles of the venous plasma adrenaline concentrations.

Table 11:5

PEAK PLASMA ADRENALINE CONCENTRATIONS FOLLOWING 5.4ml 2% LIGNOCaine + 1:50.000, 1:80.000, 1:100.000 ADRENALINE AND 5.4ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Adrenaline Dose µg</th>
<th>Peak Adrenaline Range (pmol/ml)</th>
<th>Time of Peak (mins)</th>
<th>Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50,000</td>
<td>108µg</td>
<td>1.86 (3.31-0.36)</td>
<td>15</td>
<td>169.6%</td>
</tr>
<tr>
<td>1:80,000</td>
<td>67.5µg</td>
<td>1.37 (5.27-0.55)</td>
<td>15</td>
<td>104.5%</td>
</tr>
<tr>
<td>1:100,000</td>
<td>54µg</td>
<td>1.54 (1.92-0.87)</td>
<td>15</td>
<td>363.6%</td>
</tr>
<tr>
<td>Felypressin</td>
<td>0µg</td>
<td>0.37 (0.5-0.28)</td>
<td>120</td>
<td>19.4%</td>
</tr>
</tbody>
</table>

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Mean plasma catecholamine concentrations are shown in Tables 11:5, 11:6 to allow comparison with previous similar reports (Tolas et al., 1982; Taylor et al., 1984; Low et al., 1984; Cotton et al., 1986; Salnen et al., 1988; Knoll-Kohler et al., 1989). Peak plasma concentrations for the three adrenaline containing local anaesthetics occurred some 15 minutes after injection. There was no peak identifiable in the felypressin group, but the highest plasma adrenaline concentration was achieved 120 minutes post injection.

Table 11:6

<table>
<thead>
<tr>
<th>Anaesthetic</th>
<th>Peak Noradrenaline + Range pmol/ml</th>
<th>Time of Peak (Mins)</th>
<th>Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:50,000</td>
<td>3.248 (4.62-1.45)</td>
<td>15</td>
<td>19.93%</td>
</tr>
<tr>
<td>1:80,000</td>
<td>3.104 (5.8-1.13)</td>
<td>40</td>
<td>50.49%</td>
</tr>
<tr>
<td>1:100,000</td>
<td>3.27 (4.33-2.61)</td>
<td>15</td>
<td>16.37%</td>
</tr>
<tr>
<td>Felypressin</td>
<td>2.59 (3.45-1.93)</td>
<td>120</td>
<td>42.31%</td>
</tr>
</tbody>
</table>

Mean peak adrenaline and noradrenaline concentrations were coincidental at 15 minutes post injection for two of the three adrenaline containing local anaesthetics. The highest noradrenaline concentration in the prilocaine and felypressin groups occurred 120 minutes after injection, again coincidental with the mean peak adrenaline concentration. Figures 11:2, 11:5, 11:6, 11:8, 11:10 demonstrate the cardiovascular changes to be within normal ranges with no significant trends identifiable.
Table 11:7

COMPARATIVE PEAK PLASMA ADRENALINE CONCENTRATIONS FOLLOWING VARIOUS DOSES OF ADRENALINE CONTAINING LOCAL ANAESTHETICS AT DIFFERING ANATOMICAL SITES

<table>
<thead>
<tr>
<th>Source</th>
<th>Adrenaline Concentration</th>
<th>Dose Adrenaline (µg)</th>
<th>Peak Plasma Adrenaline (pmol/L + range)</th>
<th>Peak (min)</th>
<th>Increase %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chapter 11 (Maxilla &amp; Mandible)</td>
<td>1:50,000</td>
<td>108µg</td>
<td>1.86 (3.31-0.36)</td>
<td>15</td>
<td>170%</td>
</tr>
<tr>
<td></td>
<td>1:80,000</td>
<td>67.5µg</td>
<td>1.37 (5.27-0.55)</td>
<td>15</td>
<td>105%</td>
</tr>
<tr>
<td></td>
<td>1:100,000</td>
<td>54.0µg</td>
<td>1.54 (3.92-0.87)</td>
<td>15</td>
<td>364%</td>
</tr>
<tr>
<td></td>
<td>Felypressin</td>
<td>0µg</td>
<td>0.37 (0.5-0.28)</td>
<td>120</td>
<td>19%</td>
</tr>
<tr>
<td>TOLAS (1982) (Maxilla)</td>
<td>1:100,000</td>
<td>18µg</td>
<td>-</td>
<td>5</td>
<td>144%</td>
</tr>
<tr>
<td>LEW et al. (1988) (Bat Ears)</td>
<td>1:100,000</td>
<td>40µg</td>
<td>2.2</td>
<td>2</td>
<td>175%</td>
</tr>
<tr>
<td>TAYLOR (1984) (Nasal Submucosa)</td>
<td>1:200,000</td>
<td>20µg</td>
<td>1.72</td>
<td>2</td>
<td>390%</td>
</tr>
<tr>
<td>COTTON et al. (1986) (Rhinoplasty)</td>
<td>1:200,000</td>
<td>105µg</td>
<td>4.1</td>
<td>2</td>
<td>566%</td>
</tr>
<tr>
<td></td>
<td>(Axillary Block)</td>
<td>1:200,000</td>
<td>100µg</td>
<td>10</td>
<td>112%</td>
</tr>
<tr>
<td>LOW et al. (1984) (Cervix)</td>
<td>1:200,000</td>
<td>75µg</td>
<td>18.6</td>
<td>2</td>
<td>1741%</td>
</tr>
<tr>
<td>SALONEN et al. (1988) (Mandible)</td>
<td>1:80,000</td>
<td>44µg</td>
<td>1.0 (0.7-1.3)</td>
<td>5</td>
<td>400%</td>
</tr>
<tr>
<td>KNOLL-KOHLER et al (1989) (Maxilla)</td>
<td>1:100,000</td>
<td>20µg</td>
<td>0.42 (0.22-0.62)</td>
<td>2</td>
<td>100%</td>
</tr>
<tr>
<td></td>
<td>1:25,000</td>
<td>80µg</td>
<td>2.23 (1.54-2.92)</td>
<td>15</td>
<td>962%</td>
</tr>
</tbody>
</table>

CONCLUSIONS

The first aim of the trial was to measure the plasma venous catecholamine concentrations following multiple dental injections of four different dental local anaesthetics.

Table 11:1 confirms the 1:50,000 adrenaline containing local anaesthetic produced statistically significantly higher plasma adrenaline profiles compared with the two other less concentrated adrenaline containing local anaesthetics, and the non-adrenaline containing prilocaine and felypressin mixture, (95% CI).
Similarly, the prilocaine and felypressin anaesthetic produced statistically significantly lower plasma venous adrenaline profiles compared with the adrenaline containing local anaesthetics (95% CI). There was no statistical significance between the test anaesthetics in analysis of the noradrenaline profiles (Table 11:4). Peak adrenaline absorption of the adrenaline mixtures was at 15 minutes following injection, and the highest adrenaline concentration achieved in the prilocaine and felypressin group was at 120 minutes post-injection, but with no obvious peak identifiable (Figure 11:9).

The second aim of the trial was to compare the plasma catecholamine concentrations with those reported from other anatomical sites. Table 11:7 lists the relevant literature reports on the plasma adrenaline concentrations achieved following local anaesthetic injections of adrenaline containing solutions and the time of the peaks and the percentage increase over baseline. The results presented confirm the variation in plasma venous concentrations achieved following injection to areas of differing vascularity (Scott et al., 1972). These data are used for comparison in Appendix ii where 1:100,000 adrenaline containing local anaesthetic is reversed by phentolamine. High concentrations of adrenaline injected into very vascular tissues produced the highest plasma concentrations, as demonstrated by others (Knoll-Kohler et al., 1989).

Throughout the trials no adverse cardiovascular events were detected confirming the extensive safety record of these local anaesthetic drugs (Coplans, Curson 1982; Cawson et al., 1983; Covino 1986; Knoll-Kohler et al., 1991).
PLASMA ADRENALINE CONCENTRATIONS AFTER 5.4ml 2% LIGNOCAINE + 1:50,000 ADRENALINE.

pmol/ml ADR [0.1-1.2]

Minutes.

Subject 1 — Subject 2 — Subject 3 — Subject 4 — Subject 5

NORMAL ADR

PLASMA NORADRENALINE CONCENTRATION AFTER 5.4ml 2% LIGNOCAINE + 1:50,000 ADRENALINE.

pmol/ml NOR [0.5-3.5]

Minutes.

Subject 1 — Subject 2 — Subject 3 — Subject 4 — Subject 5

NORMAL NOR

Figure 11:1
5.4ml 2% LIGNOCAINE + 1:50,000 ADRENALINE

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 11:2
PLASMA ADRENALINE CONCENTRATIONS
AFTER 5.4ml 2% LIGNOCAINE + 1:80,000
ADRENALINE.

pmol/ml ADR [0.1-1.2]

Minutes.

Subject 1  Subject 2  Subject 3
Subject 4  Subject 5

pmol/ml ADR [0.1-1.2]

Minutes.

Subject 6  Subject 7  Subject 8
Subject 9  Subject 10

NORMAL ADR

Figure 11:3
PLASMA NORADRENALINE CONCENTRATION AFTER 5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE.

pmol/ml NOR [0.5-3.5]

Minutes.

Subject 1 — Subject 2 — Subject 3
Subject 4 — NORMAL NOR — Subject 5

pmol/ml NOR [0.5-3.5]

Minutes.

Subject 6 — Subject 7 — Subject 8
Subject 9 — NORMAL NOR — Subject 10

Figure 11:4
5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE
[ Nos 1-5 ]

PULSE RATES

Beats/min

Minutes

SYSTOLIC PRESSURES

mmHg

Minutes.

DIASTOLIC PRESSURES

mmHg

Minutes.

Standard Pressure Range

Figure 11:5
5.4ml 2% LIGNOCAINE + 1:80,000 ADRENALINE
[ Nos 6-10 ]

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Standard Pressure Range

Figure 11:6
PLASMA ADRENALINE CONCENTRATIONS AFTER 5.4ml 2% LIGNOCAINE + 1:100,000 ADRENALINE.

pmol/ml ADR [0.1-1.2]

PLASMA NORADRENALINE CONCENTRATION AFTER 5.4ml LIGNOCAINE + 1:100,000 ADRENALINE.

pmol/ml NOR [0.5-3.5]

Figure 11:7
5.4ml 2% LIGNOCAINE + 1:100,000 ADRENALINE

PULSE RATES

Beats/min

Minutes

SYSTOLIC PRESSURES

mmHg

Minutes.

DIASTOLIC PRESSURES

mmHg

Minutes.

Standard Pressure Range

Figure 11:8

184
PLASMA ADRENALINE CONCENTRATIONS AFTER 5.4ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN.

pmol/ml ADR [0.1-1.2]

PLASMA NORADRENALINE CONCENTRATION AFTER 5.4ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN.

pmol/ml NOR [0.5-3.5]

Figure 11.9
5.4 ml 3% PRILOCAINE + 0.03iu/ml FELYPRESSIN

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 11:10
CHAPTER 12
PLASMA CATECHOLAMINES DURING DENTAL EXTRACTIONS UNDER LOCAL ANAESTHESIA.

INTRODUCTION
Having demonstrated the catecholamine absorption from routine dental local anaesthetic solutions it was proposed to test the hypothesis that the endogenous response to stress would significantly elevate the plasma adrenaline concentrations further, to potentially significant concentrations. Taggart et al., (1976) reported higher plasma adrenaline concentrations during routine dental conservation treatment in a group of potentially emotionally labile young nurses. Raw data of plasma adrenaline concentrations was not presented, and it was stated that the noradrenaline concentrations remained unaffected. Knoll-Kohler et al., (1991) used the wisdom tooth extraction model as a significant stress-inducing stimulus to measure catecholamine responses to surgery with a local anaesthetic (articaine) with 1:100,000 or 1:200,000 adrenaline. Only 20μg or 40μg of adrenaline was injected, and this is less than would be expected in the UK using the standard 2% lignocaine + 1:80,000 adrenaline solution.

OBJECTIVE
The trial was designed to determine the plasma catecholamine concentrations achieved during routine dental extractions under local anaesthesia, investigating the contribution endogenous adrenaline contributes during stress to the total venous plasma adrenaline concentrations. Local Ethical Committee approval for the trial was obtained, and to exclude operator variables, the author completed all the surgical procedures.

METHOD
Routine minor oral surgery cases were selected for bilateral surgical removal of impacted lower third molars. A randomised cross-over trial enabled each patient to receive 6ml of 2% lignocaine + 1:80,000 adrenaline or 6ml 3% prilocaine + 0.03iu/ml felypressin. The second surgical extraction was undertaken 4 weeks after the first.

Local anaesthesia was obtained in the conventional fashion described in Chapter 4, but an extra 2ml of the anaesthetic was injected buccally. Venous access was obtained using a cannula and 3-way tap in the antecubital fossa, and kept patent with frequent
heparinised saline flushes. Following the local anaesthetic injection plasma samples were drawn and transferred to EDTA bottles (Chapter 4). Surgery began 10 minutes after the local anaesthetic had been injected, and the mean surgical time was 7 minutes. Samples were taken on the completion of surgery and for a further 20 minutes. Data are presented in Table 12:1, and graphical presentation in Figure 12:1.

RESULTS

<table>
<thead>
<tr>
<th>Table 12:1</th>
</tr>
</thead>
</table>

**PLASMA ADRENALINE CONCENTRATIONS DURING DENTAL EXTRCTIONS USING 6ml 2% LIGNOCAINE + 1:80,000 ADRENALINE OR 6ml 3% PRILocaINE + 0.03iu/ml FELYPRESSIN**

(i) Lignocaine + 1:80,000 Adrenaline (pmol/ml Adrenaline)

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
<th>Case 6</th>
</tr>
</thead>
<tbody>
<tr>
<td>-10</td>
<td>0.53</td>
<td>1.4</td>
<td>0.36</td>
<td>0.23</td>
<td>1.43</td>
<td>0.12</td>
</tr>
<tr>
<td>-5</td>
<td>0.34</td>
<td>1.92</td>
<td>0.47</td>
<td>0.33</td>
<td>3.24</td>
<td>0.11</td>
</tr>
<tr>
<td>0</td>
<td>0.34</td>
<td>0.4</td>
<td>0.53</td>
<td>0.49</td>
<td>1.22</td>
<td>1.39</td>
</tr>
<tr>
<td>5</td>
<td>1.55</td>
<td>1.05</td>
<td>0.57</td>
<td>0.83</td>
<td>0.72</td>
<td>1.35</td>
</tr>
<tr>
<td>10</td>
<td>0.93</td>
<td>0.61</td>
<td>0.80</td>
<td>0.72</td>
<td>0.54</td>
<td>2.51</td>
</tr>
<tr>
<td>20</td>
<td>0.86</td>
<td>0.18</td>
<td>0.43</td>
<td>2.03</td>
<td>0.72</td>
<td>0.36</td>
</tr>
</tbody>
</table>

(ii) Prilocaine + 0.03iu/ml Felypressin (pmol/ml Adrenaline)

<table>
<thead>
<tr>
<th>Minutes</th>
<th>Case 1</th>
<th>Case 2</th>
<th>Case 3</th>
<th>Case 4</th>
<th>Case 5</th>
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</thead>
<tbody>
<tr>
<td>-10</td>
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<td>0.22</td>
<td>0.45</td>
<td>0.23</td>
<td>0.18</td>
<td>0.27</td>
</tr>
<tr>
<td>-5</td>
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<td>0.58</td>
<td>0.26</td>
<td>0.23</td>
<td>0.16</td>
<td>0.21</td>
</tr>
<tr>
<td>0</td>
<td>0.41</td>
<td>0.25</td>
<td>0.14</td>
<td>0.19</td>
<td>0.48</td>
<td>0.43</td>
</tr>
<tr>
<td>5</td>
<td>0.53</td>
<td>0.28</td>
<td>0.44</td>
<td>0.48</td>
<td>0.23</td>
<td>0.32</td>
</tr>
<tr>
<td>10</td>
<td>0.66</td>
<td>0.15</td>
<td>0.22</td>
<td>0.27</td>
<td>0.41</td>
<td>0.41</td>
</tr>
<tr>
<td>20</td>
<td>0.40</td>
<td>0.18</td>
<td>0.21</td>
<td>0.41</td>
<td>0.11</td>
<td>0.58</td>
</tr>
</tbody>
</table>
Table 12:2

PAIRED t TEST ANALYSIS

<table>
<thead>
<tr>
<th>Case</th>
<th>Mean</th>
<th>sd</th>
<th>t</th>
<th>p</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.268</td>
<td>0.429</td>
<td>1.53</td>
<td>0.1865</td>
<td>-0.18 to 0.72</td>
</tr>
<tr>
<td>2</td>
<td>0.650</td>
<td>0.544</td>
<td>2.926</td>
<td>0.0328</td>
<td>0.79 to 1.22</td>
</tr>
<tr>
<td>3</td>
<td>0.240</td>
<td>0.228</td>
<td>2.576</td>
<td>0.0497</td>
<td>0.0005 to 0.48</td>
</tr>
<tr>
<td>4</td>
<td>0.470</td>
<td>0.587</td>
<td>1.961</td>
<td>0.107</td>
<td>-0.15 to 1.09</td>
</tr>
<tr>
<td>5</td>
<td>1.050</td>
<td>1.059</td>
<td>2.428</td>
<td>0.059</td>
<td>-0.06 to 2.16</td>
</tr>
<tr>
<td>6</td>
<td>0.603</td>
<td>0.926</td>
<td>1.559</td>
<td>0.1715</td>
<td>-0.37 to 1.50</td>
</tr>
</tbody>
</table>

Analysis demonstrated significantly raised venous plasma adrenaline concentrations in the adrenaline group in 50% of the cases studied. The variable scatter of the data produced large standard deviations resulting in the low probability levels. Figure 12:1 demonstrates the large data scatter in the lignocaine and adrenaline group with individual points above the upper limit of normal (1.2pmol/ml). This is in sharp contrast to the prilocaine and felypressin group. Comparison with data presented in Table 11:1 and Figure 11:9 confirms the plasma adrenaline concentrations remained relatively unchanged despite the surgical trauma.

CONCLUSIONS

The prilocaine and felypressin group produced less data scatter with no individual peaks beyond the upper limit of normal plasma adrenaline concentration. Surgical stress of a third molar extraction did not contribute significantly to venous plasma adrenaline concentrations, but the chance of absorbing statistically significant amounts of exogenous adrenaline was observed in 50% of patients. No difficulty was experienced in obtaining satisfactory surgical anaesthesia in the prilocaine and felypressin group, despite the previous report to the contrary (Blair, Meechan, 1985).
PLASMA ADRENALINE CONCENTRATIONS DURING DENTAL EXTRACTIONS UNDER LIGNOCAINE & ADRENALINE LOCAL ANAESTHESIA.

pmol/ml ADR [0.2-1.2]

Minutes

Subject 1 Subject 2 Subject 3
Subject 4 Subject 5 Subject 6

PLASMA ADRENALINE CONCENTRATIONS DURING DENTAL EXTRACTIONS UNDER PRIOCAINE & FELYPRESSIN LOCAL ANAESTHESIA.

pmol/ml ADR [0.2-1.2]

Minutes

Subject 1 Subject 2 Subject 3
Subject 4 Subject 5 Subject 6

Standard Adrenaline Range Figure 12:1
CHAPTER 13  DISCUSSION

The effectiveness of a dental local anaesthetic relies on the presence of the potentiating vasoconstrictor to produce effective pulp analgesia but results in prolonged anaesthesia in the adjacent soft tissues of the lips and tongue (Roberts, Sowray, 1987). Concern has been expressed over this soft tissue anaesthesia as self-inflicted damage has been reported in young children and the handicapped (Bedi et al., 1984; Gilmour et al., 1984).

Studies have shown plain lignocaine solutions are unreliable as dental local anaesthetics and Kabambe et al., (1982) reported that adequate analgesia was achieved in only 43% of cases. This was associated with a reduction in the duration of soft tissue anaesthesia, so that the possibility of blocking the action of adrenaline was considered as a clinical option in reducing the prolonged soft tissue anaesthesia.

The selected reversing agent, phentolamine mesylate (Chapter 3) is a potent, short-acting, injectable α-adrenoceptor antagonist with activity (Doxey et al., 1977). It has three vasodilating effects, a weak sympathetic blocking activity, (Walker et al., 1950), a more significant effect mediated by antagonising the effects of circulating catecholamines, and a powerful direct non-specific but short-acting vasodilatation effect (Richards et al., 1978).

The effects of adrenaline on lignocaine nerve block anaesthesia were investigated by Caruana et al., (1982) using a rat sciatic nerve model. Adrenaline was shown to improve duration, depth and speed of onset of lignocaine nerve block anaesthesia, but although substantiating the clinical findings of others (Cowan, 1974; Kabambe et al., 1982) they do not relate to the practical clinical conditions of diffusion and buffering. To avoid these artificial conditions Mongar’s, (1955) flexor forearm model was used to test the hypothesis that phentolamine could usefully reduce soft tissue anaesthesia produced by lignocaine and adrenaline.

Mongar (1965) described an effective and simple method of assessing local anaesthetic potency, and this flexor forearm model proved sensitive in determining the efficacy of
Increasing the concentration of phentolamine in the mixture produced a dose-response such that the curves took on the profile of lignocaine plain solution. The rapid direct non-specific effect on the pre and post-capillary beds coupled with the catecholamine antagonism produced sufficient local vasodilatation to overcome the localising effect of adrenaline (Gould, Reddy 1976; Doxey et al., 1977; Richards et al., 1978; Van Zwieten 1988). Adding 10μg phentolamine to the plain lignocaine solution produced a \( t_{50} \) (time to reach 50% anaesthesia) of 6.97 minutes compared with \( t_{50} \) of 11.5 minutes for plain lignocaine. At the low (0.2%) lignocaine concentration used throughout this experiment the slight vasoconstriction likely to have been induced by the lignocaine was efficiently countered by the more powerful vasodilatoratory effects of phentolamine. It is postulated that any pre-capillary vasoconstriction was readily overcome by the powerful direct smooth muscle effect of phentolamine and coupled with the additional post-capillary effect, contributed to the speedy restoration of normal skin sensation. The effect of phentolamine on plain lignocaine was much quicker (\( t_{50} \) 11.5 minutes) than (\( t_{50} \) 16.6 minutes) for the highest (50μg) phentolamine concentration, used to reverse the lignocaine and adrenaline combination. It is postulated that to overcome the adrenaline induced vasoconstriction, the combination effects of smooth muscle vasodilatation plus
the anti-adrenaline activity is required. As a consequence the time for restoring skin sensation with the lignocaine and adrenaline was prolonged compared with the plain lignocaine group. The hypothesis that the addition of phentolamine to the lignocaine and adrenaline combination would effectively alter this mixture to the characteristics of a lignocaine plain solution in vivo has been substantiated. Adding phentolamine to the plain lignocaine solution substantially reduced the anaesthesia further, inferring that the anaesthetic effectiveness of the lignocaine plain would be reduced, further shortening the duration of residual soft tissue anaesthesia.

Due to their limited success rate of anaesthesia Kabambe et al., (1982) suggested plain lignocaine solutions were inadequate for producing analgesia for minor oral surgery procedures. If the phentolamine was injected as a mixture with the lignocaine and adrenaline as in the flexor forearm study, the depth of anaesthesia and working time would be insufficient. The clinical efficacy of phentolamine at reducing the effects of standard dental local anaesthesia was tested by infiltrating the reversal agent through the same puncture hole, in identical fashion as producing maxillary dental anaesthesia (Chapter 4). Although this would appear to be clinically difficult, the use of loupe magnification overcame the anticipated difficulty. A 30 minute delay was chosen before the phentolamine was infiltrated to simulate the time taken for an average painful dental procedure to be completed. The results throughout the relevant experiments are tabulated as time for resolution of sensation from the second injection. This is open to some criticism but was chosen as a realistic time interval by consultant dental clinicians as a reasonable period to lapse simulating a painful dental procedure. Delaying the second (phenolamine) injection beyond the 30 minutes may be expected to have an increasingly profound effect at reducing residual soft tissue anaesthesia as a greater proportion of the local anaesthetic will have been lost from the depot site. Extrapolating the effective phenolamine concentration from the flexor forearm model, concentrations in the 1mg to 2mg/ml range were anticipated to be clinically effective at reversing a 1ml 2% lignocaine + 1:80,000 adrenaline maxillary infiltration. A small pilot study of three injections conducted on the author confirmed that 0.5ml of a 2mg/ml phenolamine solution would clinically reduce the duration of soft tissue anaesthesia (Chapter 6).
The method of injecting phentolamine into the soft tissues was considered at length. Dental injections are routinely administered using a metal cartridge holder and sterile glass anaesthetic cartridge and screw on needle (Chapter 4). It was decided to use this similar technique, inserting the phentolamine solution into sterile glass ASTRA cartridges enabling aspiration to be made before injection, thus avoiding inappropriate intravascular injection (Scott, 1986).

Chapter 6 describes the trial substantiating the clinical effectiveness of the 2mg phentolamine solution at reducing pulp and soft tissue anaesthesia. Pronounced soft tissue analgesia of 138.1 minutes following placebo injection was of similar duration to that reported by Walton and Thompson (1970), and Roberts and Sowray (1987).

The importance of pH and its influence on dissociation has been implicated in the effectiveness and duration of local anaesthetics (Rud, 1961; deJong, Cullen, 1963; deJong, 1977; Di Fazio et al., 1986; Bokesh et al., 1987; Bonhomme et al., 1988). To exclude any local influence by pH differences between placebo and phentolamine the pH of each group of test solutions was measured. The placebo ranged from pH 5.0-5.1, and the phentolamine solution pH 4.9-5.0. It was thought unlikely that any reduction in analgesia would be due to this small pH difference in the solutions (Punnia-Moorthy, 1988). The 0.5ml 2.0mg/ml phentolamine injection reduced the soft tissue anaesthesia from 138.1 to 16.90 minutes and the pulp anaesthesia from 40.3 minutes to 4.8 minutes. Repeating the trial in identical fashion with phentolamine solutions ranging from 1.0mg/ml to 3.0mg/ml confirmed that the 2.0mg/ml phentolamine concentration was the most effective test solution (Figures 6:1, 6:2, 6:3, 6:4), with no statistical benefit seen by increasing the concentration to 3.0mg/ml (Table 6:3, 6:4). No soft tissue damage was noted at the time of the trial, or one week later when subjects presented for clinical examination.

The cardiovascular responses to dental local anaesthetics containing lignocaine and adrenaline have been investigated by many authors (Aellig et al., 1970; Lilienthal, Reynolds, 1975; Pipemo, Kaim, 1981; Hirota et al., 1986; Meechan, Rawlins, 1988; Knoll-Kohler et al., 1989). Meechan and Rawlins, (1988) concluded that their study agreed with the consensus of others that cardiovascular responses to adrenaline injected during dental local anaesthesia are negligible, and with the doses of local anaesthetics
used in these trials no major cardiovascular events were anticipated. Figures 6:5, 6:6 are the raw data plots of the 3mg and 2mg test group of 10 subjects receiving a total dose of 2.5mg of phentolamine. Richards et al., (1978) concluded that intravenous phentolamine had an immediate short acting effect reducing diastolic pressure, increasing heart rate and cardiac output due to a non-specific vasodilator effect, and in addition a longer but weaker $\alpha$-adrenoceptor blocking activity. The presented data shows no significant rise in heart rate and the systolic pressures staying within the normal range (Master et al., 1958). There was a tendency for the diastolic pressure to be low in this 3mg and 2mg group, but not statistically different from the other groups. These were fit and healthy young adults from the student population, many of them athletes, and the trend for low diastolic pressure was not thought to be of significance. In summary, the phentolamine reversal solutions effectively reduced the soft tissue and pulp residual anaesthesia in a dose related manner, with the cardiovascular effects being negligible compared to the intravenous injection reported by Taylor et al., 1965 a.d; Richards et al., (1978). The 2mg/ml phentolamine solution was statistically the most effective dose tested.

Adequate dental and surgical anaesthesia of the mandible and teeth requires a proximal nerve block of the inferior alveolar nerve (Roberts and Sowray 1987). This produces profound hard and soft tissue anaesthesia which involves the ipsilateral lower lip and tongue. It is such extensive anaesthesia that has been criticised as potentially hazardous, especially in children and the mentally and physically handicapped. (Gilmour et al., 1984; Bedi et al., 1984). Chapter 7 describes the results of the progression to testing the hypothesis that the 2mg/ml phentolamine solution could reverse the duration of soft tissue and pulp anaesthesia in routine dental nerve blocks. The model chosen was the inferior dental nerve block, which is achieved with 2ml of the standard 2% lignocaine + 1:80,000 adrenaline local anaesthetic. Assuming a fixed ratio of phentolamine to local anaesthetic, 1ml of the phentolamine (2mg/ml) solution was chosen as the reversing agent. The results confirmed a significant reduction in soft tissue anaesthesia from 160.3 to 17.3 minutes, and for the pulp from 59.5 to 11.1 minutes (Figure 7:1). These reductions were significant at the 95% Confidence Interval. The subjects were the same in each group, and the cardiovascular monitoring failed to reveal any significant differences between the groups (Figures 7:2, 7:3, 7:4, 7:5). The length of pulp and soft tissue anaesthesia in the placebo group was similar to that
reported by Walton and Thompson (1970) and by Roberts and Sowray (1987). No significant cardiovascular changes were noted and no local soft tissue damage observed or reported.

The results confirmed the longevity of soft tissue residual anaesthesia following mandibular nerve block injection, and that this may be significantly reduced by the profound vasodilatation produced by the phentolamine. It is postulated that the non-specific vasodilatory effect on the pre and post-capillary vessels, coupled with the catecholamine antagonist effect, neutralised the effectiveness of the adrenaline and converted the local anaesthetic mixture to plain lignocaine. The effectiveness of plain lignocaine was reduced by the presence of phentolamine (Chapter 5) and this may well have influenced further the rapid reduction in soft tissue and pulp residual anaesthesia. The assumption of using 1ml of the 2mg/ml phentolamine solution to reduce the effects of 2mls of 2% lignocaine and 1:80,000 adrenaline was vindicated and suggests a fixed ratio of phentolamine to local anaesthetic at these concentrations. The hypothesis that lignocaine and adrenaline is converted in vivo to plain lignocaine by the introduction of phentolamine is an attractive theory in light of the clinical effectiveness noted in Chapters 5, 6, 7. It was postulated that such profound vasodilatation would result in increased plasma absorption of both constituents, ie lignocaine and adrenaline.

To test this hypothesis Chapter 8 reports on the plasma lignocaine concentrations achieved after multiple dental injections of plain lignocaine and the standard lignocaine with adrenaline. Larger doses of local anaesthetics were chosen as the injection of such amounts (6mls 2% lignocaine solutions) is not uncommon during routine dental treatment, and the literature had little data on the kinetics of such quantities (Cannell et al., 1975; Cannel; and Beckett 1975; Cannell and Cannon 1976; Rood and Cannell 1978; Goebel et al., 1980; Knoll-Kohler 1989). Venous plasma lignocaine concentrations were assayed using HPLC and the results reported in the first part of Chapter 8. There was no statistically significant difference at the 95% Confidence Interval in the Plasma-Time profiles of the two test local anaesthetics. The peak plasma concentrations of the plain lignocaine anaesthetic was reached earlier at 10 minutes post-injection and were higher than the lignocaine and adrenaline mixture (Figure 8:2). These concentrations were outside the therapeutic range described by Aps and Reynolds, (1976), but comparable with those reported by Cannell et al., (1975) and Rood and
Cannell, (1978). The adrenaline effectively delayed peak plasma lignocaine concentrations, potentiating the depth and duration of the anaesthetic, and the timing of the peak concentration was similar to that noted by Cannell et al., (1975). Figures 8:3, 8:4, 8:5 confirm that cardiovascular stability of the parameters measured during the experiments.

Reversal of 6ml 2% lignocaine + 1:80,000 adrenaline using 3ml 2mg/ml phentolamine was initially conducted over a 30 minute time course as this early period was anticipated to demonstrate any rise in plasma lignocaine concentration as the soft tissue and pulp anaesthesia would have been expected to have already resolved (Chapters 6 and 7). The injection of phentolamine into the already anaesthetised tissues following the 30 minute delay was painless, however, there was no statistically significant difference in plasma lignocaine concentrations over either the 30 minute or extended 120 minute time courses. None of the previously reported clinical or haemodynamic effects of lignocaine toxicity were observed during the trial (Routledge et al., 1982; Scott 1986; Haasio et al., 1988).

The data were skewed by one volunteer which produced paradoxical results. In light of the results in Chapters 9, 10, 11, Appendix ii, iii, it is postulated that there was some interference from another drug during the assaying (Holt, Flangan 1985). However, it does confirm the benefit of presenting data as individual AUC, rather than mean values, as this would not have been identified. The financial constraints were such that small numbers were inevitable in the trial, but there would be merit in repeating the experiment over the extended time course using greater numbers of volunteers. Clinically, the phentolamine had reduced the length of soft tissue anaesthesia over both time courses, and significantly, peak plasma lignocaine concentrations were reached 15 minutes after the phentolamine injection, compared to an absence of a peak in the control group, confirming the effectiveness of the local vasodilatation. The plasma lignocaine peak concentrations were not in the therapeutic range (Jewitt et al., 1968; Stenson et al., 1971; Aps, Reynolds, 1976), and Figures 8:7, 8:8, 8:10, 8:11 confirm the stability of the cardiovascular parameters measured during the test periods. It is possible that the plasma volume diluted the released lignocaine to an extent that the venous sampling was not sensitive enough to elicit the changes in plasma lignocaine concentration.
The lack of overall statistical significance in the phentolamine groups raises the possibility that a reduced concentration of adrenaline in the anaesthetic mixture may improve the clinical effectiveness of the fixed dose phentolamine reversing agent. To test this hypothesis a trial was designed using three differing concentrations of adrenaline in standard dental cartridges.

The first part of Chapter 9 aimed to assess the effectiveness of three differing adrenaline containing anaesthetics on the venous plasma lignocaine concentrations. Tables 9:1, 9:2 demonstrate no significant statistical difference between the test groups despite the two-fold range in adrenaline concentrations. The literature contains much controversy over the appropriate concentration of adrenaline to combine with lignocaine (Keesling, Hinds, 1963; Barclay, 1965; Boulton, 1967; Scott et al., 1972; Yerzingatsian, 1991) but in the dental environment relatively high concentrations of adrenaline are traditionally used in conjunction with the local anaesthetic to counter the highly vascular tissues of the area, so intensifying the anaesthetic potency. In the light of these results the 1:50,000 adrenaline anaesthetic combination cannot be recommended, as no further reduction in plasma lignocaine concentration is achieved (Knoll-Kohler et al., 1989).

Peak venous plasma lignocaine concentrations were achieved after 30 minutes in the 1:50,000 and 1:80,000 groups, and earlier at 20 minutes in the 1:100,000 test group (Figures 9:1, 9:3, 9:6). It is concluded that this weakest 1:100,000 adrenaline concentration failed to localise the lignocaine to the injection site as efficiently as the other test solutions, despite the effective period of clinical anaesthesia. Table 9:6 confirms the reduction in plasma lignocaine concentration following the 108mg loading dose compared with the 120mg dose results from Chapter 8, but were well below the described therapeutic range (Jewitt et al., 1968; Stenson et al., 1971; Aps, Reynolds, 1976) and similar to those produced by Cannell et al., (1975), and Rood and Cannell, (1978). Peak plasma concentrations in both series were coincidental at 30 minutes post-injection. Figures 9:2, 9:4, 9:5, 9:7 confirm the stability of the cardiovascular parameters measured throughout the trials.

The second part of Chapter 9 records the venous plasma lignocaine concentrations achieved following the 3ml 2mg/ml phentolamine reversals, and results listed in Table 9:4. The phentolamine reversal produced statistically significant increases in venous
plasma lignocaine concentrations in the 1:100,000 group (95% CI). Table 9:5 lists the ratios of phentolamine::placebo AUC data, and with no difference between the groups the ratio would be 1. This confirms the impression that the fixed dose phentolamine reversal agent was most effective at raising plasma lignocaine concentrations with the weakest 1:100,000 adrenaline local anaesthetic combination. Table 9:7 confirms that the weaker the adrenaline concentration in the local anaesthetic the quicker the peak plasma concentration is achieved after the phentolamine injection. Figures 9:9, 9:10, 9:12, 9:13, 9:15, 9:16 confirm the cardiovascular stability during the test periods, and in conclusion the most effective combination with the 2mg/ml phentolamine reversal agent is confirmed as the 1:100,000 adrenaline solution. The peak plasma lignocaine concentrations attained (Table 9:6) suggest that the 120mg lignocaine perioral loading dose is unlikely to produce plasma lignocaine concentrations within the therapeutic range of 1.5-5.0mg/L (Jewitt et al., 1968; Stenson et al., 1971; Aps, Reynolds, 1976).

It has been strongly suggested that single peak plasma concentrations of lignocaine are not ideal indicators of toxicity, and in the absence of steady state kinetics such values do not correlate with clinical findings (Scott, 1975). This would suggest that with the Plasma-Time profiles presented in Chapters 8, 9, 10, Appendix iii, this technique of using phentolamine to reverse the soft tissue anaesthesia following dental perioral injections has been validated as clinically useful and safe, provided intravascular injection is avoided.

The most frequently used alternative to 2% lignocaine + 1:80,000 adrenaline for dental local anaesthesia is 3% prilocaine + 0.03iu/ml felypressin. The potential for the phentolamine reversal agent to be effective against this non-catecholamine containing local anaesthetic was investigated as the length of soft tissue and pulp anaesthesia following routine dental injection is similar in duration to 2% lignocaine + 1:80,000 adrenaline (Padfield 1967; Goldman, Evers 1969; Meechan, Rawlins 1988), and as it was to be used as a control in Chapters 11, 12. Felypressin reduces local blood flow by directly contracting vascular smooth muscle with vasoconstriction of the venular side of the circulation and with no significant cardiovascular effects (Lilienthal, Reynolds, 1976; Lindorf, 1979). Very little data exists on plasma prilocaine concentrations following multiple dental injections, but Cannell and Whelpton, (1986) reported on concentrations after only 2ml of 3% prilocaine and 3% prilocaine + 0.03iu/ml felypressin.
Figure 10:1 and Table 10:1 presents the summary data as areas under curves for the five test volunteers. Peak plasma prilocaine concentrations were achieved at 10 minutes (0.874mg/L, range 0.41-1.38) which confirms conclusions made by Cannell and Whelpton, (1986) although their original data were not presented, a graph suggested plasma concentrations between 0.2 and 0.5mg/L following a single dental injection of 2ml 3% prilocaine + 0.03iu/ml felypressin. The plasma prilocaine concentrations remained relatively low and it has been suggested that the threshold for toxicity of prilocaine is 6.0mg/L (Scott, Cousins, 1980). Maclean et al., (1988) in a comparative study of brachial plexus blockade, produced plasma prilocaine concentrations in excess of 2.5mg/L without toxic effect.

In the second part of the prilocaine trial the same five subjects were reinjected with a similar dose of prilocaine and felypressin and after the 30 minute delay received phentolamine or placebo. Table 10:3 records the statistically significant increase in plasma prilocaine concentrations following the phentolamine injections (95% CI). The intense local vasodilatation overcame the weak venular vasoconstriction of felypressin, and in the absence of any exogenous adrenaline, the prilocaine was quickly dispersed into the circulation. Peak plasma prilocaine concentrations were reached 5 minutes following the phentolamine injection (0.602mg/L, range 0.49-0.71), with no such peak in the control group. This slight increase in plasma prilocaine concentration did not produce significant concentrations of methaemoglobin (Table 10:2) and no adverse cardiovascular events were detected (Figures 10:4, 10:5). These results confirm that the immediate non-specific vasodilator effect of phentolamine is more powerful than the α-adrenoceptor blocking activity, as previously demonstrated by Richards et al., (1978). The results infer that phentolamine may be clinically useful in reducing the prolonged soft tissue anaesthesia produced by prilocaine and felypressin.

The other drug in the local anaesthetic combination is the catecholamine adrenaline. As described earlier, there has been great controversy as to the optimal adrenaline concentration for combination with local anaesthetics (Keesling, Hinds, 1963; Boulton, 1967; Scott et al., 1972; Yerzingatsian, 1991; Knoll-Kohler et al., 1989; Knoll-Kohler, Fortsch 1992), and this has been partly due to the variety of sites used in testing the anaesthetics and an inability to measure plasma catecholamines directly. Although Chapters 8, 9, 10, failed to demonstrate any adverse cardiac reaction to the local
anaesthetics and phentolamine, it was postulated that the phentolamine could adversely effect plasma catecholamine concentrations. Very limited data exists on the absorption profiles of adrenaline injected periorally in dental local anaesthetics, and the majority of the reported trials were of low dose, single dental cartridge injections (Tolas et al., 1982; Taylor et al., 1984; Knoll-Kohler et al., 1989; Knoll-Kohler et al., 1991).

Chapter 11 aimed to measure the absorption of adrenaline from multiple injections of various local anaesthetic mixtures injected periorally. Previous reports have clearly demonstrated that adrenaline absorption varies with the anatomical site and the concentration injected (Tolas et al., 1982; Taylor et al., 1984; Cotton et al., 1986; Roberts, Sowray, 1987; Lew et al., 1988). Indirect measurements of the activity of adrenaline, such as the lignocaine absorption kinetics described in Chapter 9, have produced conflicting reports. Data from Chapter 9 show no statistical difference in the AUC of the individual curves for the lignocaine absorption despite the considerable variations in adrenaline concentrations. However, direct measurements of adrenaline absorption demonstrated in Table 11:2 confirmed the absorption from the most concentrated (1:50,000) solution produced statistically significant plasma concentrations (95% CI), with the peak absorption at 15 minutes, coincidental with the other solutions (Table 11:5). Knoll-Kohler et al., (1989) also demonstrated higher plasma adrenaline concentrations following larger doses of periorally injected adrenaline, greater than the depot dose would have anticipated, and it was suggested that the increased absorption rate was the result of β-effects, increasing the perfusion rate from the site of injection (Zahir, Gould 1979). It may have been, however, that local vascular anomalies or sudden absorption from previously constricted vessels produced the effects in a similar manner as described by Rood and Cannell, (1978). As anticipated the non-adrenaline prilocaine and felypressin local anaesthetic produced statistically lower venous plasma adrenaline concentrations (95% CI). The summary Table 11:7 displays the experimental venous plasma adrenaline concentrations with other comparable historical data. The wide variety of sites and enormous variation of increases is difficult to interpret, but the trend appears to be the greater the loading dose the higher the plasma concentration achieved, and the more vascular the site of injection, the greater the absorption. Expressing the data as percentage changes and peak plasma concentrations gives little detail to the individual kinetics over the complete test period and is far less sensitive than measurement of AUC for each subject.
The noradrenaline concentrations listed in Tables 11:3, 11:4 demonstrate no statistically significant differences between the test local anaesthetic solutions, despite the wide variation in exogenous adrenaline concentrations. This confirms observations made by Hjemdahl et al., (1979) that venous noradrenaline concentrations are dependent on local noradrenaline release within the tissues from which the plasma is sampled. Only significant sympathetic activity in other parts of the body would contribute to raising noradrenaline concentrations. Similar results were reported by Knoll-Kohler et al., (1991) where significant increases in plasma noradrenaline concentrations were observed only when local anaesthesia was insufficient to abolish pain during third molar surgery. The increase in noradrenaline concentrations (Table 11:6) remained low, although individual peak concentrations were outside the upper range of normal, and would be due to the discomfort of the dental injection and the slight rise in plasma adrenaline concentrations. Figures 11:2, 11:5, 11:6, 11:8, 11:10 confirm the cardiovascular stability during the test periods.

The objective of the trial reported in Chapter 12 was to assess changes in venous plasma adrenaline concentrations related to the stressful procedure of third molar extractions using adrenaline and non-adrenaline containing local anaesthetics. Previous reports by Knoll-Kohler et al., (1991) suggested that the amount of adrenaline absorbed from the intraoral injection site predominantly determined the total plasma adrenaline concentration. However, they used quite low doses of adrenaline (20µg and 40µg) with the anaesthetic (articaine) compared with common surgical practise in the United Kingdom, when 75µg adrenaline is more usual. Plasma venous adrenaline concentrations before and after the surgical extraction of third molars are presented. The prilocaine and felypressin group produce less data scatter with no individual peaks beyond the upper limits of the normal range. Surgical stress did not contribute to venous plasma adrenaline concentrations by a statistically significant amount, but there was a 50% chance of absorbing statistically significant quantities of exogenous adrenaline demonstrated in the lignocaine and adrenaline group (Table 12:1). Although the more stable plasma adrenaline concentration in the prilocaine and felypressin group may be desirable biochemically, the lack of intense vasoconstriction impairs surgical vision, making the operation more difficult. No difficulty in achieving adequate surgical anaesthesia was noted in either group, despite the observations made by Blair and Meechan, (1985) that prilocaine and felypressin produced poor quality block anaesthesia.
These results confirm observations made by others that it is the absorption of adrenaline from the injection site rather than the endogenous release of adrenaline that affects plasma adrenaline concentrations, provided adequate analgesia is achieved (Knoll-Kohler et al., 1991). Tolas et al., (1982) reported no significant cardiovascular changes following injection of a single 1.8ml cartridge of anaesthetic containing 18μg of adrenaline, despite a doubling of the plasma adrenaline concentration. After 54μg of adrenaline Dionne et al., (1984) noted a threelfold increase in plasma adrenaline concentrations among unsedated patients and eightfold increase in sedated patients. Troullos et al., (1987) observed a 27.5 times elevation of plasma adrenaline concentration following the administration of eight dental cartridges of 2% lignocaine and 1:100,000 adrenaline (160μg adrenaline).

The outstanding safety record of the lignocaine and adrenaline anaesthetic in routine dentistry confirms these findings and as suggested by Knoll-Kohler et al., (1991), it is the stress of pain during a procedure that induces greater cardiovascular changes rather than the absorption of adrenaline from the anaesthetic site.

As both lignocaine and adrenaline, and the prilocaine and felypressin produced adequate surgical anaesthesia, the final choice of the anaesthetic can be safely left with the surgeon.
CHAPTER 14

CONCLUSIONS

Increasingly, patients expect dental procedures to be not only painless but also that there should be no uncomfortable after effects. The standard local anaesthetic solution of 2% lignocaine + 1:80,000 adrenaline if competently administered, abolishes pain reliably and completely, and is remarkably effective even in the hands of inexperienced students. This preparation also has an outstanding safety record as confirmed by its use on a vast scale over nearly half a century. Its main drawback is the deep and prolonged soft tissue anaesthesia. An effective inferior dental nerve block can leave parts of the face so numb that children and the mentally handicapped particularly, can cause deep wounds by biting the anaesthetised lip or cheek. That aside, the soft tissue anaesthesia can be so dense that it can give a subjective impression that the side of the face is partially paralysed. This is obviously embarrassing for persons on social occasions or attending important business meetings.

There are therefore cogent reasons for considering how the abolition of pain can be maintained for the duration of any dental treatment, but abbreviate the post-operative soft tissue anaesthesia. Experiments have been carried out (and form the body of this thesis) to test the effectiveness and safety of the α-blocker, phentolamine, for this purpose.

Another consideration is that despite the extreme paucity of clinical evidence of significant toxic effects of local anaesthetics containing catecholamine vasoconstrictors, anxiety continues to be expressed about them and many previous workers have attempted by various means to determine whether such fears are justified. The opportunity has therefore been taken to measure plasma concentrations of both components under clinical conditions and after the administration of phentolamine. Further, to establish the clinical relevance of these findings, detailed cardiovascular monitoring has been carried out to determine the systemic effects of phentolamine, vasoconstrictors and local anaesthetic agents both under routine conditions and when their release into the circulation has been accelerated by the α-blocker. In addition by testing the effect of phentolamine on the non-catecholamine vasoconstrictor, felypressin, additional light may have been thrown on the mechanisms of its vasodilator activity.
The human flexor-forearm intradermal wheal remains a simple and effective model for assessing and comparing local anaesthetics, and clearly demonstrates the effect of phentolamine.

Maxillary soft tissue anaesthesia following 1ml of 2% lignocaine + 1:80,000 adrenaline lasts 168 minutes, and after 2ml as an inferior dental nerve block, mandibular soft tissue anaesthesia persists for 190 minutes. This is excessive in relation to an average 30 minute dental appointment.

0.5ml of 2mg/ml phentolamine solution injected 30 minutes after a maxillary infiltration of 1ml 2% lignocaine + 1:80,000 adrenaline reduced soft tissue anaesthesia to 16.9 minutes, and pulp anaesthesia to 4.8 minutes.

1.0ml of 2mg/ml phentolamine solution injected 30 minutes after an inferior dental nerve block of 2ml of 2% lignocaine + 1:80,000 adrenaline reduced mandibular soft tissue anaesthesia to 17.3 minutes, and pulp anaesthesia to 11.1 minutes.

The addition of 1:80,000 adrenaline effectively reduces and delays peak plasma lignocaine concentrations after perioral injections of 6ml of 2% lignocaine.

3ml of 2mg/ml phentolamine injected locally into the perioral tissues 30 minutes after 6ml of 2% lignocaine + 1:80,000 adrenaline produced a significant increase in peak plasma lignocaine concentrations, indicating the lignocaine and adrenaline had been effectively reduced in vivo to lignocaine plain.

Perioral injection of 6ml of 3% prilocaine + 0.03iu/ml felypressin produces plasma prilocaine concentrations well below reported toxic concentrations.

3ml of 2mg/ml phentolamine injected locally into the perioral tissues 30 minutes after 6ml of 3% prilocaine + 0.03iu/ml felypressin produced significant increases in plasma prilocaine concentrations, suggesting the phentolamine vasodilatation to be significantly greater than the vasoconstrictor powers of felypressin.
6ml of 3% prilocaine + 0.03iu/ml felypressin injected periorally does not produce significant concentrations of methaemoglobin.

Plasma lignocaine concentrations following perioral injection of 5.4ml of 2% lignocaine and 1:100,000, 1:80,000, or 1:50,000 adrenaline differed statistically, at the 95% Confidence Interval. There is no pharmacological or clinical indication for the use of the lignocaine and 1:50,000 adrenaline combination in dental practice.

3 dental cartridges (6ml) of 2% lignocaine and 1:80,000 adrenaline injected periorally are well within accepted limits with respect to plasma adrenaline and lignocaine concentrations.

3ml of 2mg/ml phentolamine injected locally into the perioral tissues 30 minutes after 5.4ml of 2% lignocaine and 1:100,000, 1:80,000 or 1:50,000 adrenaline produced raised plasma lignocaine concentrations. The phentolamine solution was significantly effective (95% CI) against the weakest 1:100,000 adrenaline containing local anaesthetic.

Venous plasma adrenaline concentrations following perioral injection of 5.4ml of 2% lignocaine and 1:100,000, 1:80,000 and 1:50,000 adrenaline and 5.4ml of 3% prilocaine + 0.03iu/ml felypressin were significantly raised (95% CI) in the 1:50,000 group and reduced in the felypressin group. Venous plasma noradrenaline concentrations did not differ significantly.

6ml of 2% lignocaine and 1:80,000 adrenaline produced raised venous plasma adrenaline concentrations during dental extractions in 50% of the patients, compared with 6ml of 3% prilocaine + 0.03iu/ml felypressin. Surgical stress did not contribute to venous plasma adrenaline concentrations in a statistically significant manner. However, the improved surgical vision and potent depth of

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anaesthesia obtained from the lignocaine and adrenaline combination made the small biochemical difference insignificant as no adverse cardiovascular changes were monitored.

* The "safe dose" of adrenaline or local anaesthetic is meaningless unless the dosage, site and method of injection are specified, as the great variability in plasma peaks and percentage increases are demonstrated to be anatomically dependant.

* 2mg/ml phentolamine successfully reverses the prolonged soft tissue and pulp anaesthesia of dental local anaesthetics. No significant cardiovascular changes were associated with the biochemical alterations produced by the poly-pharmacy.

* 6ml of 2% mepivacaine + 1:100,000 adrenaline injected periorally produces plasma mepivacaine concentrations well below reported toxic concentrations.

* 3ml of 2mg/ml phentolamine injected locally into the perioral tissues 30 minutes after 6ml of 2% mepivacaine + 1:100,000 adrenaline did not produce significant increases in plasma mepivacaine concentrations.

* 3ml of 2mg/ml phentolamine injected locally into the perioral tissues 30 minutes following 6.0ml of 2% mepivacaine and 1:100,000 adrenaline did not significantly effect venous plasma adrenaline concentrations. Venous plasma noradrenaline concentrations gradually rose over the test period suggesting the phentolamine induced vasodilatation, secondary to the α-adrenoceptor blockade preventing reuptake of noradrenaline at the synaptic cleft, persisted longer than the direct vasodilating effect on vascular smooth muscle.

* The data presented confirms the clinical efficacy and safety of using a 2mg/ml phentolamine solution to reduce the lengthy soft tissue and pulp anaesthesia produced by routine dental local anaesthesia, and most effectively when reversing the effects of 2% lignocaine + 1:100,000 adrenaline solutions.
CHAPTER 15

FURTHER INVESTIGATIONS

* The primary aim of the investigation was to establish a safe and effective method of reducing soft tissue dental anaesthesia. Having established this in an adult group of volunteers it would be essential to test the same hypothesis in a younger age group, as it is these children who tend to bite the anaesthetised tissues of the lips and tongue.

* No data were obtained on soft tissue and pulp anaesthesia times for mepivacaine and prilocaine in conjunction with the phentolamine reversal agent. In retrospect this should have been documented at the time of the plasma estimations and would be worthwhile repeating.

* The 2mg/ml phentolamine and 2% lignocaine + 1:100,000 adrenaline was the most effective combination at reducing the duration of soft tissue anaesthesia. As this local anaesthetic is infrequently used by dental surgeons in the United Kingdom, it would seem appropriate to obtain more information relating to the plasma lignocaine and catecholamine concentrations achieved following multiple injections, and measure the duration of soft tissue and pulp anaesthesia following the phentolamine reversal injections.

* The arbitrary choice of delaying the phentolamine injection 30 minutes after the local anaesthetic is open to criticism. In clinical use any potential reversal of soft tissue anaesthesia would be made at the completion of the painful procedure. This may be shorter or longer, and as a consequence it would be appropriate to investigate the effects by injecting earlier and later. It may be anticipated that delaying the phentolamine injection allows more dissipation of the anaesthetic and an even quicker resolution of soft tissue sensation.

* The hypokalaemia induced by the lignocaine and adrenaline local anaesthetic has been implied to be potentially hazardous. It could be that the phentolamine corrects this kalaemic imbalance and the possibility would be worth examining.
ACKNOWLEDGEMENTS

* This thesis is the result of many fragmented periods of investigation over a seven year period of clinical surgical training. I am indebted to the many Consultants who allowed me to indulge in this personal goal.

* I am indebted to Professor D R Laurence for his enthusiasm and inspiration in supporting this work, and to Professors R A Cawson and D Grahame-Smith for their advice in preparation of the text.

* To the British Association of Oral & Maxillofacial Surgeons who provided funding for part of the project.

* To Dr C C T Smith and Dr D W Holt for their patience and considerable assistance in the laboratory analysis of the many plasma samples. Approximately 50% of the plasma catecholamine assays were undertaken by Dr C C T Smith and local anaesthetic assays by Miss A Hayler, as the author found great difficulty in maintaining a satisfactory degree of accuracy over the many years involved in preparing these data whilst completing his concurrent surgical training. This should not convey the impression that others did all the work as significant time was spent in the laboratories being supervised in the preparation of samples for HPLC and their subsequent analysis.

* To Sue Ellison, Dr M Sherriff and Mr H Laing for their assistance and preparation of the statistical analyses and text.

* To my wife and family whose many evenings, weekends and holidays have been ruined by this thesis, but without their patience and understanding it would never have been completed.


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APPENDICES

Appendix i

RAW DATA

Experimental raw data from all the trials are presented in microfiche form - see inside back cover.
Appendix ii

VENOUS PLASMA MEPIVACAINE CONCENTRATIONS AFTER MULTIPLE DENTAL INJECTIONS AND PHENTOLAMINE REVERSALS.

The success of phentolamine usefully reducing the length of soft tissue anaesthesia following dental injections has been clinically demonstrated (Chapters 6, 7). The proposed trial was designed to assess the efficacy of the phentolamine reversing agent on another short acting dental local anaesthetic, mepivacaine. This anaesthetic is combined with 1:100,000 adrenaline, and it was predicted that should the phentolamine activity be independent of the vasoactivity of the local anaesthetic, then increased concentrations of mepivacaine should be detected with the introduction of the phentolamine injection. However, no data exists on the venous plasma mepivacaine concentrations achieved after multiple period injections of 6ml 2% mepivacaine +1:100,000 adrenaline. Ethics Committee approval was gained to establish such a baseline.

OBJECTIVE
(i) To determine baseline plasma profiles following the perioral injection of 6ml of 2% mepivacaine + 1:100,000 adrenaline.

(ii) To assess the efficacy of phentolamine at reversing the potentiating 1:100,000 adrenaline as demonstrated by a significant rise in venous plasma mepivacaine concentration.

METHOD (i) Measuring Venous Plasma Mepivacaine Concentrations Of The Test Anaesthetic.

Following the protocol described in Chapter 4, five subjects from the student population were assigned to be injected with the test local anaesthetic, 6ml of 2% mepivacaine + 1:100,000 adrenaline. Venous plasma concentrations of mepivacaine were determined over a 120 minute test period as previously described (Chapter 4).
RESULTS

Plasma-Time profiles for the individual subjects are shown in Figure 19:1, and as previously described the area under the curve (AUC) used as the summary statistic for the data, and listed in Table 19:1.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma-Time AUC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.540</td>
</tr>
<tr>
<td>2</td>
<td>0.549</td>
</tr>
<tr>
<td>3</td>
<td>0.818</td>
</tr>
<tr>
<td>4</td>
<td>0.626</td>
</tr>
<tr>
<td>5</td>
<td>0.747</td>
</tr>
</tbody>
</table>

mean of difference = 0.656
sd of difference  = 0.123
sem of difference = 0.0549
95% CI            = 0.504 to 0.808

The plasma profiles of the mepivacaine test local anaesthetic are shown in Figure 19:1 with peak plasma mepivacaine concentrations achieved 20 minutes following injection with a mean concentration of 0.812 mg/L (range 0.63-1.03). No significant cardiovascular changes were observed during the test period, as displayed in Figure 19:2.
PLASMA MEPIVACAINE CONCENTRATIONS.

Mepivacaine mg/L

Minutes

0 20 40 60 80 100 120

Subject 1 Subject 2 Subject 3
Subject 4 Subject 5

6ml 2% Mepivacaine + 1:100,000 Adrenaline

Figure 19:1
6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Standard Pressure Range

Figure 19:2
METHOD (ii) To Determine The Effect Of Phentolamine On The Venous Plasma Mepivacaine Concentrations.

Having established the baseline mepivacaine profiles, the effectiveness of phentolamine at reversing the potentiating adrenaline was studied. It was anticipated that the phentolamine would significantly increase the Plasma-Time AUC profiles of the mepivacaine compared with placebo.

Following the protocol described in Chapter 4, ten volunteer students from the student population were assigned to two groups, receiving the phentolamine reversing agent or matching placebo. Each was injected with 6ml of 2% mepivacaine + 1:100,000 adrenaline as described in Chapter 4, following after thirty minutes by the phentolamine or matching placebo. The second test injection was repeated in identical fashion one week later. Venous plasma mepivacaine concentrations were determined over a 120 minute test period as previously described.

RESULTS

Plasma-Time profiles for the individual subjects are shown in Figures 19:3, 19:5, and as previously described the area under the curve (AUC) used as the summary statistic for the data, listed in Table 19:2.
### Table 19:2

**PLASMA-TIME (AUC) MEPIVACAINE PROFILES FOLLOWING 6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE REVERSED BY 3ml OF 2mg/ml PHENTOLAMINE OR PLACEBO.**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Plasma-Time AUC</th>
<th>Phentolamine</th>
<th>Placebo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.887</td>
<td>0.828</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>0.676</td>
<td>0.546</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.785</td>
<td>0.933</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.659</td>
<td>0.611</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>0.773</td>
<td>0.816</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>0.680</td>
<td>0.604</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.515</td>
<td>0.453</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.037</td>
<td>0.598</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>0.766</td>
<td>0.466</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>0.594</td>
<td>0.481</td>
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</tbody>
</table>

- mean of difference = 0.107
- sd of difference = 0.167
- sem of difference = 0.0528
- t = 1.47
- p = 0.157
- 95% CI = -0.0121 to 0.227

### CONCLUSIONS

The success of phentolamine at reversing both the lignocaine and adrenaline and prilocaine and felypressin anaesthetic combinations has been presented in Chapters 6, 7, 8 and 9. A third drug combination, mepivacaine 2% and adrenaline 1:100,000 is used as a dental local anaesthetic, especially in Europe. Originally introduced as a shorter acting local anaesthetic, as an alternative to prilocaine, it found little favour with the British dental profession. Chapter 19 describes the plasma mepivacaine concentrations achieved in five subjects following the standardised perioral injection of 6ml 2% mepivacaine + 1:100,000 adrenaline, during the 120 minute test period. Peak
plasma concentrations (Figure 19:1) were achieved 20 minutes after injection with a mean concentration of 0.812mg/L (range 0.63-1.03). This was slightly earlier than the 30 minute peak recorded by Goebal et al., (1980) but the vasoconstrictor in that study was levo-nordefrin, and not adrenaline. The plasma peaks of 0.37mg/L reported by Goebal et al., (1980) were after a 36mg perioral injection, and the 0.812mg/L concentration following the 120mg mepivacaine dose described in Chapter 19 is much less than the 6-10mg/L reported by Tucker et al., (1972) following intercostal nerve blocks.

The second part reports the results of the trial comparing the plasma mepivacaine concentrations achieved following the phentolamine or placebo reversals. It was anticipated that should the phentolamine be clinically effective, then a significant rise in plasma mepivacaine concentrations was to be predicted. Table 19.2 lists the AUC for the two groups with the 95% Confidence Intervals. Although not statistically significant (95% CI) the data confirms the clinical impression of efficiency at reversing the soft tissue anaesthesia. The inherent vasoconstriction of mepivacaine may have been sufficient to delay the vasodilating effect of phentolamine (Willatts, Reynolds, 1983), but the peak plasma mepivacaine concentrations were achieved 15 minutes following the phentolamine injection, coincidental with the 15 minute peak following the lignocaine and adrenaline reversals (Chapter 8). Significantly, no such peak was identified in the placebo group. No adverse cardiovascular effects were noted during the test period (Figures 19:4, 19:6) and no local soft tissue damage observed or reported. This is in contrast to the increase in systolic pressures observed by Foldes et al., (1975) following an intravenous mepivacaine dose of 0.5mg/Kg/min body weight.
PLASMA MEPIVACAINE CONCENTRATIONS AFTER MULTIPLE PHENTOLAMINE REVERSALS.

6ml 2% Mepivacaine + 1:100,000 Adrenaline Reversed After 30 Minutes With 6mg Phentolamine.

Figure 19:3
6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE & 6mg PHENTOLAMINE

PULSE RATES

Beats/min

SYSTOLIC PRESSURES

mmHg

DIASTOLIC PRESSURES

mmHg

Standard Pressure Range

Figure 19:4
PLASMA MEPIVACAINE CONCENTRATIONS AFTER MULTIPLE PLACEBO REVERSALS.

6ml 2% Mepivacaine + 1:100,000 Adrenaline Reversed After 30 Minutes With Matching Placebo.

Figure 19:5
6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE & PLACEBO

PULSE RATES

Beats/min

Minutes

SYSTOLIC PRESSURES

mmHg

Minutes

DIASTOLIC PRESSURES

mmHg

Minutes

Standard Pressure Range

Figure 19:6
Appendix iii

VENOUS PLASMA CATECHOLAMINE CONCENTRATIONS AFTER MULTIPLE DENTAL LOCAL ANAESTHETIC INJECTIONS AND PHENTOLAMINE REVERSALS.

The contribution of exogenous adrenaline in dental local anaesthetics to the total venous plasma pool is demonstrated in Chapter 11. Locally injected phentolamine would competitively inhibit $\alpha_2 > \alpha_1$ adrenoceptors, and by inhibiting the reuptake of noradrenaline at the pre-synaptic $\alpha_2$-adrenoreceptors, an anticipated rise in plasma noradrenaline concentration would be anticipated. Peak plasma adrenaline concentrations occurred 15 minutes after the local anaesthetic had been injected (Chapter 11), but despite the delay of 30 minutes prior to the phentolamine injection sufficient adrenaline may be at the injection site to be displaced from the adrenoceptors by phentolamine to be measurable in the venous plasma.

OBJECTIVE

To assess the effect locally injected phentolamine has on venous plasma catecholamine concentrations following the multiple perioral injection of a routine dental local anaesthetic in human volunteers.

METHOD

The local anaesthetic chosen for this trial was 6ml 2% Mepivacaine + 1:100,000 adrenaline (Scandonest Special) and the reversal agent 3.0ml 2mg/ml phentolamine or 3.0ml matching placebo.

Following the established protocol described in Chapter 4, five students were assigned to be injected with the test local anaesthetic on two different occasions, one week apart, with the second injection of phentolamine or placebo being administered after a 30 minute delay. The order of the reversal injections was randomised, and venous catecholamine concentrations were determined over the full 150 minute test period, as previously described in Chapter 4.
RESULTS

Table 20:1 lists the areas under the curves for the venous plasma adrenaline concentrations following the placebo and phentolamine injections. No statistical difference at the 95% CI is noted between the two groups.

Results tabulated in Table 20:2 for the noradrenaline concentrations also demonstrate no statistical difference between the two groups.

Figure 20:1 confirms that the venous plasma adrenaline in all but one reading in each group stayed within the normal range for plasma adrenaline concentrations (0.1-1.2pmol/ml). However, in Figure 20:4 there is a suggestion that plasma noradrenaline concentrations rise slightly following the phentolamine injection. Figure 20:5 infers that such a trend may exist, when the data is normalised to the -5 minute concentration, with a tendency toward a rise in the plasma noradrenaline over the whole of the test period. Figures 20:2, 20:3 confirm that there was no significant cardiovascular changes monitored during the test period in either group.

Table 20:1

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<tr>
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mean = 0.0507
sd = 0.165
sem = 0.0738
t = 0.687
p = 0.530
PLASMA-TIME (AUC) NORADRENALINE PROFILES FOLLOWING 6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE REVERSED BY 3ml 2mg/ml PHENTOLAMINE OR PLACEBO

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mean = 1.06  
sd = 1.37  
sem = 0.614  
t = 1.735  
p = 0.158  
95%CI = -0.639 to 2.77

CONCLUSIONS

Chapter 20 reports the venous plasma catecholamine response of the local anaesthetic 2% mepivacaine + 1:100,000 adrenaline to the reversal agent phentolamine. Plasma-Time profiles were produced from the venous plasma adrenaline and noradrenaline concentrations for the individual subjects and the AUC analysed. There was no statistical increase in plasma adrenaline or noradrenaline concentrations at the 95% Confidence Interval for the phentolamine solution (Table 20:1). This despite the data presented in Chapter 19 suggesting that the phentolamine was increasing the rate of mepivacaine absorption from the depot site of injection. It would appear that the local antagonism at the α-adrenoceptor for this 1:100,000 adrenaline concentration is sufficient to permit mepivacaine absorption including antagonising the inherent vasoconstriction of the local anaesthetic, but that the residual adrenaline left at the injection site is not significant to affect plasma adrenaline concentrations. Figure 20:4 infers that plasma noradrenaline concentrations may rise slightly throughout the course
of the test period in the phentolamine group. This would not be unexpected as phentolamine has three specific vasodilatory effects, and by preventing noradrenaline reuptake at the synaptic cleft would result in a spill-over of noradrenaline into the plasma. This is a greater vasodilatory effect than the weak sympathetic blocking activity shown by Walker et al., (1951). It is known that presynaptic $\alpha$-adrenoceptors take part in a negative feedback system controlling the release of noradrenaline (Langer, 1981), and Doxey et al., (1977) demonstrated that phentolamine blocked presynaptic $\alpha$-adrenoceptors producing the pressor changes. Normalising the data and representing it as Figure 20:5, confirms the impression that the noradrenaline concentrations seem to be rising towards the end of the test period, suggesting that the $\alpha$-adrenoceptor antagonism vasodilatory effect lasts longer than the more powerful direct but nonspecific effect noted by Richards et al., (1982), which is mediated directly through vascular smooth muscle.

In summary, the data from Chapter 20 demonstrates a rise in plasma adrenaline concentrations following the injection of 6ml of mepivacaine + 1:100,000 adrenaline, producing few individual peaks outside the upper range of normal (>1.2pmol/ml) but without significant cardiovascular changes. Injection of the phentolamine after 30 minutes enabled the soft tissue reversal to be effective. It was seen as a rise in plasma mepivacaine concentrations (Chapter 19), but with no significant rise in peak plasma adrenaline concentrations. Plasma noradrenaline concentrations were unaffected by the local anaesthetic injection, a similar finding to Taggart et al., (1976) and Knoll-Kohler et al., (1991), but concentrations tended to rise slightly over the test period as a result of the phentolamine preventing reuptake of noradrenaline at the synaptic cleft.
PLASMA ADRENALINE CONCENTRATIONS
AFTER 6ml 2% MEPIVACAINE + 1:100,000
ADRENALINE & 6mg PHENTOLAMINE.

pmol/ml ADR [0.1-1.2]

Subject 1  Subject 2  Subject 3
Subject 4  NORMAL ADR  Subject 5

PLASMA ADRENALINE CONCENTRATIONS
AFTER 6ml 2% MEPIVACAINE + 1:100,000
ADRENALINE & PLACEBO.

pmol/ml ADR [0.1-1.2]

Subject 1  Subject 2  Subject 3
Subject 4  NORMAL ADR  Subject 5

Figure 20:1
6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE & 6mg PHENTOLAMINE

**PULSE RATES**

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**SYSTOLIC PRESSURES**

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Standard Pressure Range

Figure 20:2
6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE & PLACEBO

PULSE RATES

SYSTOLIC PRESSURES

DIASTOLIC PRESSURES

Standard Pressure Range

Figure 20:3
PLASMA NORADRENALINE CONCENTRATION
AFTER 6ml 2% MEPIVACAINE + 1:100,000
ADRENALINE & 6mg PHENTOLAMINE.

pmol/ml NOR [0.5-3.5]

Minutes.

Subject 1
Subject 2
Subject 3
Subject 4
NORMAL NOR
Subject 5

PLASMA NORADRENALINE CONCENTRATION
AFTER 6ml 2% MEPIVACAINE + 1:100,000
ADRENALINE & PLACEBO.

pmol/ml NOR [0.5-3.5]

Minutes.

Subject 1
Subject 2
Subject 3
Subject 4
NORMAL NOR
Subject 5

Figure 20:4
PLASMA NORADRENALINE RATIO
AFTER 6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE & 6mg PHENTOLAMINE

Noradrenaline Ratio

Minutes

Subject 1
Subject 2
Subject 3
Subject 4
Subject 5

PLASMA NORADRENALINE RATIO
AFTER 6ml 2% MEPIVACAINE + 1:100,000 ADRENALINE & PLACEBO

Noradrenaline Ratio

Minutes

Subject 1
Subject 2
Subject 3
Subject 4
Subject 5

Figure 20:5
CELLULAR INTERACTIONS
BETWEEN LYMPHOCYTES AND RETINAL
ENDOTHELIAL CELLS

YUFEI WANG
(September 1994)

Addendum to be incorporated into Thesis
CONTENT

A. Anatomy of the eye ................................................................. A3-5
B. The blood-ocular barriers .................................................... A5-6
C. Uveitogenic antigens ............................................................ A7-7
D. Movement of molecules (antigens) out of the eye .................. A7-8
E. The entry of inflammatory cells into the eye ......................... A8-9
F. Molecules controlling inflammatory cell entry into the eye ........ A9-10
G. Antigen presenting cells in the eye ....................................... A10-12
H. Ocular inflammatory disorders ............................................. A12-14
I. Animal models of human ocular inflammation ....................... A14-16

ABBREVIATIONS:

APC, antigen presenting cell; BRB, blood-retinal barrier; CNS, central nervous system; EAU, experimental autoimmune uveitis; EC, endothelial cell; ICAM-1, intercellular adhesion molecule 1; IFN-γ, interferon gamma; IL-2, interleukin 2; IRBP, interphotoreceptor binding protein; LFA-1, lymphocyte function-associated antigen 1; LIU, lens-induced uveitis; LPS, lipopolysaccharide; MHC, major histocompatibility complex; RPE, retinal pigment epithelium; S-Ag, retinal soluble antigen; VKH, Vogt-Koyangi-Harada disease.
A. ANATOMY OF THE EYE

The eyeball consists essentially of three separate concentric layers that are modified anteriorly to admit and regulate the passage of light. The outermost of these layers, the sclera, is purely protective; the innermost, the retina, is a light-sensitive recorder of images; and the intervening uveal layer consists principally of the choroid, a nutrient vascular bed for the retina. The anterior rim of this uveal layer is continued forwards to the ciliary body and the iris which contain the intra-ocular muscles that govern the accommodative and pupillary movements (Figure 1).

The uvea or uveal track: The vasculature of the eye is known as the uvea or uveal track, and consists of the choroid, ciliary body and iris. The former two are adherent to the sclera, but the iris is separated from the cornea by the anterior chamber. Between the iris and choroid lies the ciliary body. The layers of the iris and ciliary body are essentially comparable, consisting of a stroma which contains ciliary muscle and vessels, and an epithelium of varying junctional tightness. A double layer of cells is continuous with the two epithelial layers lining the back of the iris.

Figure 1. Diagrammatic horizontal section of the eye.
The choroid vascular sheet provides nutrients for the outer layers of the retina and separates the sclera from the retina. Three vascular layers are conventionally described, the size of the vessels decreasing as the retina is approached: the large vessel, small vessel and choriocapillaris. The choriocapillaris is permeable to macromolecules and other blood borne-substances.

On the inner surface of the choroid lies a thin structureless lamella, Bruch's membrane, which adheres to the retinal pigment epithelium (RPE). It contains the basement membrane of the choroicapillaris, a collagen fibre layer and the basement membrane of the RPE.

*The retina:* The retina is a light sensitive organ, transparent in life. It lines the whole inner surface of the eye behind the ora serrata, except where it is pierced by the optic nerve head (the 'optic disc'). The retina can be divided into two major parts: neural retinal layer and RPE layer. Histologically, ten layers are apparent, composed of five cell-families and of three cell relays: an outer relay whose processes form the receptive rods and cones and whose packed nuclei form the thick outer nuclear layers (photoreceptors), an intermediate relay of bipolar cell whose nuclei are aggregated as the inner nuclear layer, and an inner relay of sparse ganglion cells whose axons pass backwards to form the optical nerve. Between each nuclear layer is a plexiform in which their dendrites arborize. The outer wall of the neural retina is a single cell layer formed by RPE (Figure 2).

The human retina is vascularized in its inner layers. The choriocapillaris of the choroid is mostly responsible for nourishing the photoreceptor layer. The majority of retinal vessels are arterioles, venules and capillaries, which are lined by non-fenestrated EC that are closely associated with Müller cells, astroglia and numerous pericytes.

*The canal of Schlemm and trabecular meshwork:* The canal of Schlemm is a circular sinus, which lies among the deep fibres of the sclera at the limbus (Fig.1). It has a firm wall lined by fenestrated EC on a basement membrane. The trabecular meshwork lies circumferentially between the canal of Schlemm and the anterior chamber. It is formed by a porous meshwork of elastic and collagen fibrils lined by EC.
Fig. 2. Schematic drawing of the retinal layers and the retinal vascular EC (a) and RPE (b) which form the BRB.
The aqueous humour: Aqueous humour which is produced by the ciliary body bathes and supports internal structures of the eye. The composition of this liquid is comparable to the cerebro-spinal fluid. The aqueous humour is believed to be secreted continuously by the cells of the ciliary epithelium into the posterior chamber of the eye; outflows into the anterior chamber (Fig. 1), and drains into the canal of Schlemm via the trabecular meshwork, and then to the veins of the superficial and deep scleral plexus.

B. THE BLOOD-OCULAR BARRIERS

In the eye, barriers to circulating macromolecules are localized either at vascular EC or at epithelia. EC barriers are present in the vessels of the retina, optic nerve, ciliary body and iris. Epithelial barriers are present in the pigment epithelium of the retina, the non-pigmented layer of the ciliary epithelium and the posterior layer of the epithelium of the iris.

The blood-retinal barriers: Under normal conditions the retina is isolated from the constituents of the blood by the presence of selective cellular barriers. At the inner retina, this blood-tissue interface is comprised of the vascular EC which lacks both paracellular and intercellular pathways through which polar molecules or cells can readily traverse. At the outer layer of the retina, the barrier is formed by a sheet of RPE which overlies the highly permeable choroidal circulation. In this respect the RPE is similar to the epithelium of the choroid plexus that forms the blood-CSF barrier. Within the retina, however, these two anatomically distinct blood-tissue interface are known collectively as the blood-retinal barrier (BRB, Figure 2.a and b).

The blood-aqueous barrier: This barrier also has two components. One is an epithelial barrier localized in the ciliary and iridal epithelium which protects the posterior chamber from the circulating macromolecules by the presence of tight junctions. The other is an EC barrier which prevents movement of macromolecules from the lumen of the vessels ciliary body and iris into the iridal stroma and thence into the anterior chamber. It should be noted that in contrast to the vessels the retina, the EC lining of the iridal and ciliary vessels lacks the structure of tight junctions between the cells and thus is a more permeable barrier.
C. UVEITOGENIC ANTIGENS

The concept that uveitogenic antigens in the eye are capable of inducing disease was proposed almost a century ago. Currently several antigens, mostly of retinal origin, have been identified, which in animals are capable of inducing ocular inflammatory diseases, similar in many respects to that seen in man.

Retinal soluble antigen (S-Ag) was one of the first uveitogens to be purified and characterized. This intracellular protein (48 KD) is a major component of rod outer segments, as well as being found in the pineal gland, and is believed to participate in light-signal transduction (Pfister et al., 1985). S-Ag is a potent uveitogen in all the animal models so far studied, with the possible exception of the mouse (Caspi et al., 1988).

Interphotoreceptor binding protein (IRBP) is a 140 KD glycoprotein whose physiological role in the retina is in the transport of vitamin A derivatives between the RPE and the photoreceptors (Lai et al., 1982). Similar to S-Ag, IRBP induces EAU in rats, rabbits and primates. In contrast to S-Ag, IRBP is poorly pathogenic in guinea pigs, but in mice it was found to be a more potent immunopathogen than S-Ag (Caspi et al., 1988).

The uveitogenic potential of rhodopsin, and its light activated form opsin has been shown in both guinea pig and rats (Marak et al., 1980). The pathogenicity of this molecule appears to be conformation-dependent since opsin was shown to be considerably less pathogenic than rhodopsin (Schalken et al., 1988).

D. MOVEMENT OF MOLECULES (ANTIGENS) OUT OF THE EYE

The eye lacks a normal lymphatic drainage system. Thus, protein molecules of the retina are unlikely to directly reach the local lymph tissues where they can be processed and recognized by immune competent cells. However, there are several possible routes through which retinal antigens can enter the circulation: 1). transcytosis across retinal vascular EC; 2). phagocytosis and release into the choroid circulation by RPE; and 3). passage into the aqueous humour and then via the trabecular meshwork to the circulation.

Although retinal EC contain very few cytoplasmic vesicles, evidence shows
that EC of the central nervous system (CNS) can selectively transport molecules from the blood into the CNS (Vass et al., 1984). Thus, retinal antigens may be taken by retinal EC and released into the circulation by reverse transcytosis. Another potential route of antigen movement is through the RPE. This cell is situated at a crucial interface between the choroidal blood supply and the photoreceptor cell layer of the neural retina. One of its many functions includes phagocytosis of photoreceptor outer segments where known uveitogenic antigen lies. Once phagocytosis has occurred the debris is broken down by enzymes and thought to be released into the choroid circulation. It is possible therefore that uveitogenic antigens in the photoreceptors may enter the circulation via this route.

It has been known that injection of antigen into the anterior chamber can cause systemic immune responses. This has lead to the suggestion that antigen in the aqueous humour can drain into the deep scleral capillary plexuses through the spongy mass of trabecular tissue in the angle of the anterior chamber, and then be trapped in the spleen via the blood circulation (Streilein et al., 1992).

Finally, it should be pointed out that when the retinal EC and RPE become damaged during injury and disease, transfer of retinal antigens across the BRB into the circulation would be dramatically increased, leading to the immune system being exposed to these antigens.

E. THE ENTRY OF INFLAMMATORY CELLS INTO THE EYE

Inflammatory cells have been recorded infiltrating the neuroretina via the RPE and the EC of the retinal vascular bed (Lightman and Greenwood 1992; Dua et al., 1991; Greenwood et al., 1994). As with BRB leakage, the evidence suggests that in the vascularized retina the initial site of infiltration is across the retina EC (Lightman and Greenwood 1992). Morphological observations also show that the majority of infiltrated cells are often located around retinal vascular vessels forming what is called as a leucocyte cuff.

It is obvious that due to the anatomical location, only EC lining the vascular wall are able to recruit circulating leucocytes into the tissues. Although RPE were found to be capable of expressing adhesion molecules, they can only interact with
lymphocytes which have already migrated across the choroidal vascular EC wall. Despite the choroidal vasculature being highly permeable to molecules it still represents a barrier to migrating cells and must therefore possess the requisite adhesion molecules for lymphocyte attachment. Once inflammatory cells have adhered to and migrated across the choroidal EC, adhesion to the basal membrane of the RPE will be further hindered by the presence of Bruch's membrane which will mask the RPE adhesion molecules especially as these extend only a small distance from the cell surface (Springer 1990). When damage to the retina occurs inflammatory cells undoubtedly do traffic out of the choroidal circulation and across the RPE, although this is only seen at later stages of disease in the animal models. However, in some species of animals, such as guinea-pig, whose retina is non-vascularized, inflammatory cell infiltration can only occur via the RPE route (Forrester et al., 1985).

In addition, it has been observed that there is leucocyte infiltration in the choroid, ciliary body and iris in human uveitis and EAU rats. The inflammatory cells within the ciliary body and iris may also further infiltrate into the aqueous humour and accumulate in the posterior and anterior chamber. However, whether they can reach the retina and then migrate into the retinal parenchyma is awaited for elucidation.

F. MOLECULES CONTROLLING INFLAMMATORY CELL ENTRY INTO THE EYE

Currently it is poorly understood how autoimmune T cells migrate across the BRB into the retina from the circulation. Studies with large vessel EC show that a number of adhesion molecules expressed on EC control lymphocyte movement across the EC wall. Although large vessels differ considerably from retinal and brain vascular EC, these studies have provided a starting point to examine the adhesion molecules which might be expressed by retinal EC and control cell traffic.

Some initial observations indicate that ICAM-1 can be expressed on retinal vascular EC, RPE and choroidal vessels in ocular inflammatory diseases, such as human posterior uveitis and EAU rats, but not on normal retinal tissues (Wakefield
et al., 1992, 1993a). In EAU rats, this expression appeared to be involved in the initial stages of inflammatory cell infiltration as ICAM-1 induction was prior to histological evidence of ocular inflammation (1993a), and administration of the antibody to ICAM-1 could prevent the onset of inflammation (Whitcup et al., 1993a; Uchio et al., 1994).

LFA-1 and Mac-1 have been found on infiltrating cells in ocular inflammatory tissues, and these molecules have also been suggested to play an important role in the disease since the antibodies to LFA-1 and Mac-1 can significantly inhibit IRBP induced EAU and LPS-induced uveitis (Whitcup et al., 1993a, 1993b). In LPS induced uveitis, the expression of E-selectin was also found on cells of the ciliary body and iris rapidly after the injection of endotoxin. This E-selectin expression may be important in the disease in promoting polymorphonuclear cell accumulation in the anterior segment of the eye (Whitcup et al., 1992).

Aberrant expression of MHC class II molecules on retinal vascular EC, may also contribute to lymphocyte adhesion to the EC by a CD4-dependent mechanism. However, most studies with brain EC have been unable to block lymphocyte adhesion to EC with the anti-CD4 antibodies (Male et al., 1990), indicating that MHC class II molecules on the EC are unlikely to be important in lymphocyte recruitment into the retina.

G. ANTIGEN PRESENTING CELLS IN THE EYE

A number of studies have addressed the question of how antigens and particularly retinal antigens can be presented within the eye. Immunohistochemical studies have been directly toward identified professional APC in the intraocular compartments, particularly the uvea and the retina. Indeed, MHC class II positive cells have been detected in various compartments of the normal eye, including the cornea, iris, ciliary body and choroid. In contrast, there is no detectable MHC class II positive cells in the retina (Wang et al., 1987). A recent study has demonstrated that the MHC class II bearing cells in the uvea are dendritic cells (Forrester et al., 1994), which indicates that antigen presentation to CD4+ T cells can preferentially occur within this tissue.
Although the retina normally lacks professional APC, clinical and experimental evidence indicates that autoimmune reactions can develop within this organ. It has been suggested that retinal resident cells, such as Müller cells, RPE and retinal vascular EC may play a role as APC in autoimmune diseases, since in these situations these retinal resident cells are capable of expressing MHC class II molecules.

Müller cells belong to the group of accessory glia cells of the retina. Their analogue in the brain is probably astrocytes. In *in vitro* experiments it has been shown that Müller cells are able to express MHC class II molecules following IFN-γ induction, and that these MHC class II induced cells can present antigen to T cells resulting in T cell proliferation (Roberge et al., 1988).

In addition to forming the outer BRB, RPE may also be potential APC of the retina since these cells are found to be capable of expressing MHC class II molecules under pathogenic conditions. In *in vitro* studies it has also been demonstrated that RPE can stimulate CD4+ T cell proliferation in an antigen-dependent manner (Percopo et al., 1990). Physiologically, RPE continuously phagocytose the outer segment of photoreceptors, and this property of the RPE is similar in many aspects to the phagocytic capabilities of macrophages. Thus, retinal antigens may be processed by RPE and re-expressed on the cell surface in a form associated with MHC class II, which can be recognized by CD4+ T cells. It may also be important that RPE transfer retinal antigens into the stroma of the choroid where dendritic cells can present these antigens to infiltrated T cells, which would result in a more significant immune response in this tissue.

Microglia, which are proposed to be the most potential APC in the brain (Matsumoto et al., 1992), could also be important APC within the retina. However, there is no evidence to indicate the antigen presenting abilities of retinal microglia, and this may be due to the difficulty in isolating these bone-marrow derived cells from normal tissues of the retina (Fine and Yanoff 1984).

The potential role of retinal vascular EC in the antigen presentation has also been proposed since these cells are found to aberrantly express MHC class II molecules under many autoimmune conditions (Lightman 1988). It has also been
suggested that the appearance of MHC class II molecules on retinal EC, possibly in conjunction with antigens released from damaged photoreceptor tissue, may constitute a signal for retaining infiltrated lymphocytes (Lightman et al., 1987). However, whether retinal EC are capable of presenting antigen to T cells resulting in antigen specific T cell activation remains to be elucidated.

Finally, it should be noted that circulating APC may be recruited into the retina, and thereafter serve as APC within this tissue. Although under normal conditions trafficking of circulating cells, such as monocytes, into the retina are very low, this can be markedly increased when inflammatory reactions develops in this organ. These recruited circulating APC, however are likely to play a secondary, rather than primary role in the interactions with antigen specific T cells within the retina.

**H. OCULAR INFLAMMATORY DISORDERS**

Ocular inflammation can occur in patients with systemic disorders such as sarcoidosis and Behçet's disease or can be localized to the eye as in sympathetic ophthalmia and pars planitis. Many of these disorders have characteristic features that are recognizable clinically but whose cause are unknown. The patient often requires immunosuppressive therapy to control the inflammatory response so as to limit the damage to the ocular tissue. Autoimmune mechanisms are thought to be involved in the initiation and/or perpetuation of the inflammatory response, although the exact role in the pathogenesis is unknown. With the advent of immunohistochemical staining with monoclonal antibodies to cell surface markers, much more is now known about the cell types infiltrating the inflamed eyes under these conditions, although these studies give no information on the aetiology of these inflammatory disorders.

**H1. Sympathetic ophthalmia**

Sympathetic ophthalmia is defined as a bilateral inflammation of the entire uveal track that follows perforating injury to one eye, either in the form of trauma from a penetrating injury or following intraocular surgery. Immunohistopathological
studies of sympathetic ophthalmia demonstrate that the choroid is infiltrated mainly by T cells with a small number of B cells (Chan et al., 1986). In the eyes enucleated early in the disease process, the T cells were mainly CD4+ (Müller-Hermelink et al., 1984) with CD8+ cells appearing in later stages. In most studies, there was no alteration of the T cell populations in the patient's blood. The T cell infiltration suggests that it is the cellular rather than the humoral arm of the immune response that is predominately activated.

H2. Vogt-Koyangi-Harada (VKH) syndrome

VKH syndrome is a chronic bilateral exudative uveitis associated with whitening of hair and eye lashes, vitiligo, and meningeal irritation. Ocular involvement is usually bilateral, although one eye may present first, and is associated with a panuveitis, optic nerve swelling, serous detachment of the retina and infiltration of both the choroid and RPE. Histological examination demonstrates many features of sympathetic ophthalmia but also obliteration of the choroidal capillaries, focal active chorioretinitis and marked involvement of the RPE. In a patient with long-standing disease, T cell infiltration was seen in the uvea and retina, but in contrast to sympathetic ophthalmia, foci of aggregated B cells were also seen (Chan et al., 1988). Limbal biopsy in patients with VKH demonstrated that an increase in CD4+ T cells in early stages of the disease and an increase in CD8+ cells at a later stage. Antibodies to ganglion, outer segment of photoreceptors and Müller cells have all been detected, as has evidence for cell-mediated immunity to basic myelin protein and melanin (Lightman and Chan 1989).

H3. Sarcoidosis

Sarcoidosis is a systemic granulomatous disorder of unknown aetiology. Hilar lymphadenopathy and pulmonary infiltration are common findings, but many organ systems can become involved, including skin, joints, liver and central nervous system. In the eye there is usually an anterior uveitis, classically with mutton-fat keratic precipitates and retinal vasculitis. Immunohistopathological examination of severe intraocular inflammation of the sarcoidosis patient found that within the
granulomas in the retina and uvea, 90% of T cells were CD4+ (Chan et al., 1987). Fewer than 10% of the total cells were CD8+ and these were confined to the lymphocyte cuff and were not within the granulomas. Lymphocytes both within and around the granuloma had demonstrable IL-2 receptors and class II MHC antigens were diffusely distributed over the granuloma. Very few B cells were seen and those were at the periphery of the granulomas. These finding suggest that the T cells seen in the granulomas are activated and secreting both IL-2 and IFN-γ.

H4. Behçet’s disease

This disease is characterised by an occlusive vasculitis and can affect many different organ systems. The ocular disease is characterized by a panuveitis and occlusive retinal vasculitis. Early lesions of the skin are characterized by large numbers of infiltrating lymphocytes and macrophages, particularly in the perivascular areas; 60-80% of the T cells being of the CD4+ type with 20-40% of the T cells being of the CD8+. Natural killer cells were found in the infiltrate in 50% of biopsies examined but only constituted 5% of total cellular infiltrate, whereas B cells were not present (Poulter et al., 1985).

I. ANIMAL MODELS OF HUMAN OCULAR INFLAMMATION

In order to study the immunopathogenic mechanisms that might underlie immune-mediated ocular inflammatory diseases in man, it is necessary to employ experimental models of uveitis in laboratory animals. The best studied immunological-mediated ocular inflammation is experimental autoimmune uveoretinitis (EAU), of which several models have been developed in various rodent species as well as in primates. EAU serves as a model for a variety of posterior uveitis conditions in man, among them sympathetic ophthalmia, Behçet’s disease and VKH syndrome.

II. EAU

EAU is a CD4+ T cell mediated ocular inflammatory disease in laboratory animals. This disease is characterized by destruction of the photoreceptor cells of the retina, where the eliciting antigen(s) are located, and is usually accompanied by
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autoimmune inflammation of the pineal gland (third eye), which shares many ocular-specific antigens with the retina. Although the fine details of EAU pathology and clinical manifestations vary from species to species, EAU can be classified as primarily a posterior segment inflammation, developing into panuveitis in its more acute forms. Lesions such as photoreceptor damage, serous retinal detachment, vitritis, retinitis, choroiditis, vasculitis, perivasculitis and anterior chamber infiltration of varying intensity are commonly observed.

Rodent models of EAU have been developed in the guinea pig, rat, rabbit and mouse species. Differences have been noted in the uveitogenic responses of the various species. The reasons for these differences are not well understood, however, some of them might be due to fairly straightforward anatomical differences, such as the lack of retinal vessels in guinea pigs. Although none of the animal models of EAU reproduce the full spectrum of human uveitis, each model offers some unique properties, making it suitable for study of specific aspects of ocular inflammatory disease.

II. Other (non-EAU) models of ocular inflammation

**Endotoxin (LPS)-induced uveitis:** LPS uveitis is elicited by a single intravitreal or systemic injection of endotoxin. Although the mechanisms of induction of ocular symptoms by systemically administered LPS are not understood, the mediator directly implicated in the pathogenesis of this disease was demonstrated to include arachidonic acid metabolites, as well as macrophage- and neutrophil-chemotactic activities (Fleisher 1988). It is believed that the mode of action of LPS in the eye may involve its well-documented ability to stimulate production of cytokines such as IL-1 and TNF, as these cytokines can induce uveitis when they are applied locally (Rosenbaum et al., 1988).

**Immunogenic uveitis (albumin-induced uveitis):** Ocular inflammation is induced by hyperimmunizing animals with xenogenic serum albumin, followed by an intravitreal challenge with the immunizing antigen at the peak of antibody production (Lightman et al., 1986). It can also be induced by injection of albumin into the vitreous of unprimed animals, resulting in a delayed induction of uveitis, coinciding
with humoral antibodies. Immune complexes are present in the aqueous humour during clinically evident inflammation, indicating involvement of complement activation (Howes et al., 1982). This model of uveitis is considered primarily as a model of antibody-mediated disease, although T-cells may also participate in the pathogenesis of this model.

**Lens-induced uveitis (LIU):** LIU in rodents serves as a model for human lens-induced uveitis and is considered to be an immune-complex mediated, Arthus-type reaction (Marak et al., 1982). Immunity is induced by priming with xenogenic lens proteins, and uveitis is subsequently precipitated by mechanical trauma to the lens. As in other forms of acute uveitis accompanied by massive accumulation of inflammatory leucocytes, activated oxygen products and hydroxyl radicals appear to play an important role in tissue damage.
REFERENCES


