STUDIES ON PROPOFOL IN SHEEP AND RATS

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Summary

The studies described in this thesis were carried out in two phases. Studies were performed in rat and sheep hepatic microsome preparations and in rat lung microsome preparations *in vitro*. A pilot study to choose an optimum incubation time for studies using rat hepatic microsomes indicated that propofol, 28μM, 56μM, 84μM and 140 μM, was rapidly degraded, depending on the initial propofol concentration. For all subsequent studies, an incubation time of 15 min was chosen. There were no significant differences in the rate of degradation of propofol by hepatic microsomes at any propofol concentration between rats and sheep. The mean maximal velocity (Vmax; μmoles/min.mg of protein) and the mean Michaelis constant values (Km; μM) were 0.43 ± 0.04 & 48.9 ± 2.2 for propofol in rat preparations and 0.43 ± 0.06 & 42.4 ± 7.1 for sheep tissues. However, the rate of degradation of propofol was significantly different between Sprague Dawley and Wistar rats ($n=3$; $p < 0.05$). Microsome preparations obtained from lung tissue showed a high capacity of degradation of propofol (5.6, 11, 28 & 56 μM). However, the amount of cytochrome P450 present in lung microsomes could not be determined. The Vmax and Km values for lung tissue were 0.93 ± 0.28 μmoles/min.mg of protein and 13.7 ± 4.8 μM , respectively.

1-aminobenzotriazole (12 mM), inhibited the degradation of propofol by rat hepatic microsomes by 75.4% - 29.5%, depending on the concentration of propofol assayed (28 - 140 μM), thus indicating that the degradation of propofol in this system is highly cytochrome P450-dependent.

Ketamine, 5 μg/ml, and alfentanil, 200 ng/ml, incubated with propofol (28μM, 56μM, 84μM & 140 μM) did not alter the rate of degradation of propofol in hepatic microsomes obtained from 6 Sprague Dawley rats. Mean (± sem) Vmax values (μmoles/min.mg protein) for propofol alone (P) and in the presence of ketamine (K) and alfentanil (A) were 0.52 ± 0.08 (P), 0.56 ± 0.06 (K); 0.43 ± 0.04 (P), 0.39 ± 0.03 (A). Mean (± sem) Km values (μM) for propofol alone (P) and in the presence of ketamine (K) and alfentanil (A) were 46.5 ± 2.5 (P), 53.2 ± 3.2 (K); 48.9 ± 2.2 (P), 48.7 ± 4.4 (A). However, aminopyrine, 5mM, impaired the degradation of propofol at all concentrations. The mean (± sem) Vmax and Km values for propofol alone (P) and for propofol in the presence of aminopyrine (Am) were 0.42 ± 0.04 (P), 0.43 ± 0.06 (Am) μmoles/min.mg protein and 43.6 ± 5.0, 66.2 ± 7.6 (Am) μM. The significant increase in Km value for propofol in the presence of aminopyrine ($p < 0.05$) indicates that the inhibition is competitive. Propofol (5.6μM, 56μM, 280μM, & 561μM) also inhibited dose dependently the formation of formaldehyde from the demethylation of aminopyrine. Propofol, 56μM, impaired the demethylation of aminopyrine by 34.9% (mean), which suggests that propofol has

the potential *in vivo* to inhibit the degradation of drugs that are metabolised in this way.

Propofol, alone (group 1 (G1), n=5) or in combination with ketamine (group 2 (G2), n=5) was used to anaesthetise 10 Scottish Blackface sheep undergoing superficial body surface surgery. All sheep were premedicated with acepromazine, 0.05 mg/kg and papaveretum, 0.4 mg/kg, i.m., 30 min before induction of anaesthesia with either propofol, 4 mg/kg (G1) or propofol, 3 mg/kg, and ketamine, 1 mg/kg, injected i.v. over 60 sec. Immediately after induction of anaesthesia, a variable infusion rate of either propofol alone (G1), 0.3 - 0.5 mg/kg/min or propofol 0.2 - 0.3 mg/kg/min and ketamine, 0.1 - 0.2 mg/kg/min (G2) was started, adjustments being made according to the clinical and cardiovascular and respiratory status of each animal. Blood samples for propofol analysis, carried out by high performance liquid chromatography, were obtained during the infusion period and for 24 h after the infusion was stopped. The mean infusion periods were 64.8 ± 3.1 min and 60 ± 0 min for G1 and G2 respectively. Induction and maintenance of anaesthesia was satisfactory for each group, although endotracheal intubation was not possible in one sheep in G1 after the induction dose of propofol. The inclusion of ketamine in the anaesthetic regime had a propofol - sparing effect. The total dose of propofol used was 801 ± 39 mg (G1) and 468 ± 32 mg (G2), while the total dose of ketamine used was 267 ± 15 mg. There were no significant differences in pulse rates or systolic arterial blood pressures recorded during anaesthesia between the 2 groups, however, the respiratory rates were consistently higher in G2 than in G1, although there was no evidence of respiratory depression in sheep in G1. No attempt was made to measure the 'depth' of anaesthesia in either group.

Recovery from anaesthesia was rapid for animals in both groups. The mean time to assuming sternal recumbency was 6.3 ± 1.2 min and 11.2 ± 1.7 min and to standing unaided was 10.9 ± 1.6 min and 15.1 ± 2.2 min for G1 and G2 respectively. The time to assume sternal recumbency was significantly longer for sheep in G2 compared with sheep in G1, however, the times to standing were not different.

The pharmacokinetics of propofol in sheep in G1 and G2 were described using non compartmental analsyses. The mean residence time, calculated for observed concentration values, was 0.71 ± 0.04 h and 0.71 ± 0.05 h, mean whole body clearance, 82 ± 11 ml/kg.min and 129 ± 16 ml/kg.min, and the mean volume of distribution at steady state, 3.4 ± 0.5 L/kg and 5.46 ± 0.72 L/kg for G1 and G2 respectively. There were no significant differences for these pharmacokinetic parameters between the 2 groups, although the group sizes were small (n=5). These data suggest that the concomitant administration of ketamine with propofol does not alter the pharmacokinetics of propofol in sheep.

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Dedication

Aos meus pais, ao Meninho e ao Manuel.

Declaration

I, Dacia Alzira de Augusto Correia, do hereby declare that the work carried out in this thesis is original, was carried out by myself or with due acknowledgement, and has not been presented for the award of a degree at any other University.

The following paper was presented as an oral communication at the 6th EAVPT International Congress, Edinburgh 1994:

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1. GENERAL INTRODUCTION

1 INTRODUCTION

General anaesthesia is a state of unconsciousness, produced by a process of controlled, reversible suppression of the central nervous system. General anaesthetics may then be defined as substances that cause a reversible loss of consciousness. They are used primarily to keep the patient unaware of, and unresponsive to, painful stimulation during surgical procedures. At the cellular level they inhibit the conduction of action potentials and the neurochemical transmission at synapses (Rang & Dale, 1991); but the mechanisms by which these effects occur are largely unknown (Miller, 1993). The occurrence of general anaesthesia involves three main neurophysiological changes, namely : unconsciousness; loss of response to painful stimulation (analgesia) and loss of motor reflexes (muscle relaxation).While the CNS changes induced by general anaesthetics are reversible, large doses of these agents can cause death due to loss of cardiovascular reflexes and respiratory paralysis. This is one of the reasons why a useful anaesthetic drug should permit rapid induction and recovery from anaesthesia and be easily controllable, thus making the level of anaesthesia easy to adjust during surgery.

General anaesthetic agents can be classified into two groups:
1. The inhalational anaesthetics: these may be either volatile liquids or gaseous agents which are delivered to the lungs.
2. The intravenous anaesthetics: a group of drugs which induce anaesthesia when administered intravenously, but can also be given by other parenteral routes.

1.1 INHALATION ANAESTHETICS

Since the introduction of nitrous oxide and diethyl ether, several other inhalation anaesthetics agents have been developed and used in men and animals. The first public clinical use of nitrous oxide was in 1845 for dental extraction, while ether anaesthesia was used publicly for removal of a tumour in 1846 by William Morton (Short, 1987). In 1847, chloroform was introduced into veterinary practice but, despite being a potent agent, it fell into disuse due to its toxicity (Hall & Clarke, 1991). It was only after the discovery of halothane that the modern era of inhalation anaesthesia began in veterinary medicine.

The pharmacokinetics of inhalational anaesthetic agents are related to their physicochemical properties, such as the blood gas solubility, oil water solubility, and to physiological variables such as the alveolar ventilation and cardiac output (Hull, 1991). In healthy patients, the most important determinant of the speed of onset of, and recovery from anaesthesia, is the solubility of the drug in blood. The

lower the blood solubility, the more rapid the induction of and recovery from anaesthesia. However, none of the inhalational agents can match the intravenous anaesthetics drugs for speed of induction of anaesthesia. Of all the inhalational anaesthetic agents, halothane, methoxyflurane and isoflurane are the most frequently used in veterinary practice.

Halothane (2-bromo-2 chloro-1,1,1-trifluorethane) is a halogenated anaesthetic discovered by Sweking in 1951 and introduced in veterinary anaesthesia in 1956 by Hall, becoming the most widely used inhalation anaesthetic (Short, 1987). It is a potent anaesthetic, providing a moderately fast induction of, and excitement-free recovery from anaesthesia (Adriani, 1970). If no other agents are administered, animals are able to walk without ataxia in 15-30 min, depending on the duration of anaesthesia (Hall & Clarke, 1991). Some 20-45% of the absorbed dose of halothane is biotransformed by oxidation, resulting in the formation of trifluoroacetic acid, chloride and bromide ions which are excreted in urine (Nunn *et al.*, 1989). Dose dependent depression of cardiac output, arterial blood pressure and respiration have been observed with the administration of halothane in animals (Hall & Clarke, 1991), although adaptation with time occurs for both cardiovascular and respiratory functions (Steffey *et al.*, 1987). Bradycardia and arrhythmias are common during halothane anaesthesia. Hepatotoxicity has been reported following repeated administration or long periods of halothane anaesthesia in humans (Rang & Dale, 1991), but, there are few similar reports in animals.

Methoxyflurane (2,2-dichloro-1,1-difluoroethyl methyl ether) is a halogenated ether, first introduced into veterinary practice in 1960 (Hall & Clarke, 1983). It is exceptionally potent, but due to its high blood solubility, induction and recovery from anaesthesia are slower than with halothane. Like halothane, it causes hypotension, but appears to produce better analgesia even at light surgical planes (Short, 1987). Methoxyflurane is extensively metabolised (50-75%) resulting in the formation of dichloroacetic acid, methoxy-difluoro acetic acid, oxalic acid and the release of fluoride and chloride ions as well as carbon dioxide (Nunn *et al.*, 1989). Nephrotoxicity associated with high fluoride production is a considerable risk factor and it is manifested as large amounts of urine eliminated and the inability to produce concentrated urine (Holaday *et al.*, 1970), consequently, its use has never become widespread.

Isoflurane (1-chloro-2,2,2- trifluoroethyl difluoromethyl ether), chemically related to enflurane, is the most recently introduced inhalant anaesthetic into veterinary practice, although as yet it is not commonly used in the UK. Isoflurane is highly volatile and has a low blood gas solubility coefficient, thus providing relatively

rapid induction of, and recovery from anaesthesia as well as easy control of the depth of anaesthesia. Only 0.2% of absorbed drug is metabolised by oxidation and the main metabolites are trifluoroacetic acid and inorganic fluoride (Nunn *et al.*, 1989). Isoflurane-induced cardiovascular and respiratory depressant effects are dose-dependent, but tachycardia and reduction in cardiac output are less than with halothane, although hypotension is more profound (Higgs & Carli, 1983). Arrhythmias have not been reported with isoflurane, nor have nephro- or hepato-toxicity (Engelking *et al.*, 1984; Gelman *et al.*, 1984; Hall & Clarke, 1991).

In an attempt to increase the speed of induction of anaesthesia and reduce recovery times associated with inhalational agents, newer agents are being developed with lower blood gas solubility coefficients than isoflurane. Agents such as sevoflurane and desflurane, may prove useful in the future and are undergoing clinical trials in humans.

Nitrous oxide is the only inorganic inhalation anaesthetic gas used widely in clinical practice. Nitrous oxide is particularly rapid in action and appears to possess analgesic properties. However it is a weak anaesthetic agent in animals and, even at concentrations of 80% in the inspired air, it does not produce anaesthesia in any domestic animal species and therefore, cannot be used as a sole anaesthetic agent (Finck, 1984).

1.1.1 Advantages of the inhalation anaesthetics agents

In comparison with anaesthetic agents developed before halothane, the new anaesthetic agents, halothane, methoxyflurane and isoflurane have the advantage of being non-flammable and non-explosive substances in the range of concentrations used in clinical practice. They are also stable, useful for use in closed re-breathing circuits during prolonged administration periods, and are also more potent drugs (Hall & Clarke, 1983). The inhalational agents can be removed rapidly from the body in the event of an anaesthetic overdose, by altering ventilation and the inspired concentration. For halothane and isoflurane, elimination of the drug from the body does not rely entirely on metabolism, and consequently, disease states which alter drug metabolism profiles should not affect the course of anaesthesia.

1.1.2 Disadvantages of inhalation anaesthesia

Although recovery from most inhalational anaesthetic agents occurs rapidly, a desirable property in many situations, particularly for day-case surgery, almost all inhalation anaesthetics cause cardiovascular depression by their effects on the

myocardium and blood vessels; while halothane in particular, sensitises the myocardium to adrenaline-induced arrhythmias (Johnston *et al.*, 1976). A major disadvantage of inhalation anaesthetic agents is related to their physical presentation. As gases or volatile substances, they rapidly spread in the ambient air of the operating and recovery rooms which can cause a potential contamination of the working environment and may be hazardous for the operating personnel (Paddleford, 1987). This exposure can result in long term ill health (Vaisman, 1967; Cohen *et al.*, 1980) and impaired psychomotor performance (Bruce *et al.*, 1974). It has been considered that anaesthetists may metabolise halothane more extensively than other people, since they exhibit more breakdown products in their urine (Cascorbi *et al.*, 1970) and a recurrent case of hepatitis in an anaesthetist due to halothane sensitisation has been reported (Klatskin, *et al.*, 1969). Nitrous oxide can interfere with DNA synthesis at concentrations below those required for the production of anaesthesia, (concentrations which the anaesthetist may inhale in the operating room) (Nunn, 1987). Inhalation anaesthetic agents have been implicated as one of the causes of increased incidence of abortion among operating staff (Vaisman, 1967; Cohen *et al.*, 1971). It has been agreed that most inhalation anaesthetics interfere with cell division (Sturrock & Nunn, 1975; Cullen, 1979), although this occurs at concentrations considerably higher than those required for anaesthesia. While scavenging systems employed in clinical practice limit it, total elimination of this hazard is considered impossible. Such a hazard does not occur with intravenous anaesthetic drugs.

1.2 INTRAVENOUS ANAESTHETIC AGENTS

Until recently, intravenous anaesthetic agents have not proved satisfactory for medium and long term maintenance of anaesthesia, but have been used almost exclusively for the induction of anaesthesia prior to maintenance with a gaseous agent or for anaesthesia of short duration. A major disadvantage of intravenous agents is the difficulty in controlling the depth of anaesthesia, because they cannot be recovered once injected. However, they have the advantage of acting rapidly, generally within 20 seconds, thus eliminating excitement before onset of anaesthesia. Intravenous anaesthetic drugs which have been used to maintain anaesthesia in veterinary practice include chloral hydrate, the barbiturates and alphaxalone/alphadolone combinations, but there are disadvantages associated with their use.

Chloral hydrate is a water soluble substance which has been used alone or as a mixture with magnesium sulphate or/and pentobarbitone (Hall & Clarke, 1983). It has serious disadvantages which have contributed to the decline in its use (Hall &

Clarke, 1991). After injection its effects are slow to appear, and it produces profound cardiovascular depression when surgical anaesthesia is achieved, while perivascular injection causes severe tissue reaction. In veterinary medicine, chloral hydrate was used mainly for anaesthesia in horses.

Pentobarbitone was the first barbiturate to be used as a general anaesthetic in veterinary medicine in 1931. Originally it was administered orally or by intraperitoneal injection; by the end of 1938, slow intravenous injection of pentobarbitone became the routine technique for dogs and cats after Wright had used the technique successfully in more than 2000 patients (Hall & Clarke, 1983). Methohexitone sodium is another barbiturate which is two to three times more potent than thiopentone, with a shorter duration of action and more rapid recovery. Thiopentone sodium is the most widely used drug for inducing anaesthesia in dogs and cats, and is licensed for use in most domestic species. The duration and depth of thiopentone anaesthesia depend on the amount of drug injected, the speed of injection, the rate of distribution of the drug in non fatty tissues and the rate of uptake by body fat (Hall & Clarke, 1991). Thiopentone crosses the blood-brain barrier rapidly due to its high lipid solubility, and consequently, unconsciousness occurs within 15-20 seconds of administration (Brodie *et al.*, 1952). The loss of consciousness is usually smooth, but the drug is unsatisfactory for producing surgical anaesthesia. Like other barbiturates it does not have analgesic properties (Briggs *et al.*, 1982b) and therefore, deep anaesthesia is necessary to obtund nociceptive reflexes. Barbiturates are metabolised by oxidation mainly in the liver, but also to a small extent in the brain and kidney (Short, 1987). Metabolites of these drugs appear in the urine in the form of alcohols, ketones, phenols or carboxylic acids (Nunn *et al.*, 1989). The clearance of thiopentone from the body is slow, 1.96 ml/min.kg in dogs (Baggot *et al.*, 1984) and the volume of distribution at steady state is large, 1.7 to 2.5 L/kg, due to extensive tissue uptake (Morgan *et al.*, 1981; Hudson *et al.*, 1983). The slow body clearance of thiopentone makes it unsuitable for use to maintain anaesthesia, as cumulation of the drug will rapidly occur (Hull, 1989). Barbiturates cause respiratory depression and apnoea is frequently observed after thiopentone injection (Bellman & Pleuvry, 1981). Rapid intravenous injection causes a fall in blood pressure and myocardial depression (Hall & Clarke, 1991). Perivascular injection of thiopentone causes local tissue necrosis and ulceration, sometimes with arterial spasm (Mazunder *et al.*, 1980; Short, 1987).

Alphaxalone is a steroid drug with hypnotic properties which is virtually insoluble in water but dissolves in cremophor EL. The mixture with alphadolone, another steroid drug with less hypnotic properties, increases its solubility and has been called saffan (Hall & Clarke, 1991). Saffan is licensed for use as an anaesthetic for

cats and primates. It causes mild hypotension but provides good muscle relaxation in the absence of severe respiratory depression. Saffan is not irritant if injected perivascularly. These properties make it a potentially useful agent for total intravenous anaesthesia, and indeed it was used for this purpose in humans (Sear & Prys-Roberts, 1979) although the potential to cause anaphylactic reactions in humans has led to its withdrawn in the U.K.. Anaphylactic reactions are commonly associated with this product in cats and potentially fatal hypotension induced by cremophor can occur in dogs due to massive histamine release (Hall, 1972; Eales *et al.*, 1974). It is metabolised very quickly and the major route of excretion is via the bile (Middleton *et al.*, 1982; Hall & Clarke, 1991).

The combination of the potent opioid agonist, etorphine and a phenothiazine tranquilliser, either methotrimeprazine (dogs) or acepromazine (horses) ('Immobilon') can be classified as a neuroleptanalgesic drug mixture and may be considered an intravenous anaesthetic. It produces many undesirable side effects, respiratory depression, tonic convulsions in voluntary muscles and cardiovascular stimulation in horses, while in dogs, Immobilon induces respiratory depression and hypotension (Lees & Hillidge, 1976). Because of these undesirable side effects, its use is declining.

Ketamine [2 - (chlorophenyl) - 2 - (methylamino) - cyclohexane hydrochloride] developed in 1963, is a dissociative anaesthetic related to phencyclidine (Wright, 1982). Its high therapeutic index, water solubility, compatibility with other drugs and lack of depressant effects on the CNS make it potentially useful in some circumstances (Short, 1987) but involuntary movements, sometimes even convulsions, during induction and recovery can occur, and during anaesthesia muscle tone is increased (Hall & Clarke, 1991). Ketamine causes increase in heart rate, cardiac output and arterial pressure, and produces only transient respiratory depression (Waterman & Livingston, 1978a; Short, 1987). Its major drawbacks are the observed hallucinations and delirium with irrational behaviour seen during recovery in humans, behavioural abnormalities have been also reported in dogs and horses (Nunn *et al.*, 1989; Hall & Clarke, 1991). However despite its disadvantages, ketamine remains popular in veterinary medicine, for example it has been used in combination with other drugs for total intravenous anaesthesia in horses (Muir *et al.*, 1978; Kaka *et al.*, 1979; Taylor *et al.*, 1992). Ketamine is metabolised in the liver by N-demethylation as the major pathway, resulting in the formation of nor-ketamine and dehydronorketamine, both active metabolites (Waterman & Livingston, 1978b).

Propofol has proved to be a valuable addition to the limited range of drugs currently available for maintenance of anaesthesia. Because propofol is rapidly metabolised permitting a better and more rapid control of the depth of anaesthesia, it can be used for total intravenous anaesthesia (TIVA.).

1.2.1 PROPOFOL

1.2.1.1 CHEMISTRY, PRESENTATION AND USES

Propofol, 2,6 diisopropylphenol, is a relatively new intravenous anaesthetic agent, introduced into clinical practice for humans in 1980. It is one of a series of sterically-hindered alkyl phenols (James & Glen, 1980; Simons *et al.*, 1988) that have anaesthetic properties in animals (Glen & Hunter, 1984). It is highly lipophilic, poorly soluble in water and virtually non-ionised at physiological pH (Simons *et al.*, 1988). Due to its insolubility in water, a solubilising agent is necessary to produce a preparation of the drug suitable for intravenous injection. It was first presented as a 1% active solution in 16% cremophor EL (Langley *et al.*, 1988; Simons *et al.*, 1988; Kanto, 1988), but this formulation was associated with hypersensitivity reactions and pain around the injection site. Propofol is now formulated in Soya bean emulsion, 1% w/v aqueous emulsion in 10% soya bean oil, 1.2% egg phosphatide and 2.25% glycerol (Glen & Hunter, 1984; Langley *et al.*, 1988; Kanto, 1988). It is widely used in human medicine, both for induction of anaesthesia prior to maintenance with inhalation anaesthetic agents and for total intravenous anaesthesia (for review see Langley *et al.*, 1988). In veterinary medicine, propofol is licensed for use in the cat and the dog, but its use in other species either as a induction agent or for total intravenous anaesthesia, has been documented (Nolan *et al.*, 1985; Waterman, 1988; Reid *et al.*, 1993).

1.2.1.2 PHARMACODYNAMICS

The mechanism of action of propofol is not totally understood, however like the benzodiazepine and barbiturate groups of drugs, it appears to be linked to a site of action at the gamma amino butiric acid A receptor ($GABA_A$) (Concas *et al.*, 1990; Hales *et al.*, 1991 & Jewett *et al.*, 1992).

When propofol is administered as a single bolus, the induction of anaesthesia depends on both the dose and the speed of injection. Rolly *et al.* (1985) found that the mean induction time in humans was 50.5 seconds when 2 mg/kg was given over 60 seconds, but this was reduced to 21.5 seconds when the period was reduced to 5 seconds. Induction of anaesthesia with propofol is rapid, quiet, smooth and without excitement. Premedication with other drugs appears to decrease the amount of propofol necessary for induction of anaesthesia. Watkins *et al.* (1987) found that

premedication with acepromazine between 0.02 and 0.04 mg/kg reduced the overall induction dose of propofol from 5.95 to 3.81 mg/kg in dogs, a finding supported by Weaver & Raptopoulos (1990). The sensitivity to propofol seems to increase with age, and it has been suggested that this may be due to a decrease in hepatic enzymatic activity. It was reported that doses of at least 2.25 mg/kg were necessary for successful induction of anaesthesia in younger patients (less than 60 years) while in the elderly, doses between 1.25 - 1.75 mg/kg were adequate (Langley *et al.*, 1988). Recent work in dogs in Glasgow Veterinary School, indicated that in dogs over 8 years of age the dose of propofol required to induce anaesthesia was less than in dogs less than 5 years old (5 mg/kg cf 6.5 mg/kg) (Reid, personal communication).

Propofol administration may be associated with pain at the injection site. Grood *et al.* (1985) reported that 6 patients out of 30 complained of pain during propofol injection, and Kirkpatrick *et al.* (1988) observed pain which varied from mild to moderate in 7 patients out of 24 during propofol injection. Weaver & Raptopoulos (1990) observed involuntary movements during induction of anaesthesia with propofol in 1 out of 89 dogs which they suggested might have been a sign of pain.

Patients recover very quickly from propofol anaesthesia and do not usually suffer hangover effects. Grood *et al.* (1985) reported that the mean time to eye opening in humans after infusion was 10.5 minutes, while the time to orientation and adequate answers to question was 11.5 minutes. Watkins *et al.* (1987) also reported rapid recovery times after maintenance with intermittent injection in dogs, 18 min in 68 unpremedicated dogs and 22 min in 65 premedicated animals after the administration of the last dose, with one particular feature of the recovery being the absence of any residual ataxia. Following a 60 min zero order infusion of propofol in 7 dogs undergoing surgery, Nolan and Reid (1993) reported a rapid return of consciousness and ability to stand unaided. The high volume of distribution and rapid clearance are the main factors which determine the decline in propofol concentrations necessary for recovery of consciousness (Cockshott *et al.*, 1990; Nolan *et al.*, 1993; Reid *et al.*, 1993).

Studies in animals have demonstrated that propofol has effects on the cardiovascular system. Glen & Hunter (1984) reported that single intravenous doses of propofol, 3.75 mg/kg, administered to mini-pigs, increased the heart rate and cardiac output, and decreased the mean arterial blood pressure and total peripheral resistance in the early minutes of administration. In humans, decreases in systolic and diastolic blood pressures have been observed also during the early stages of anaesthesia. Kirkpatrick *et al.* (1988) reported hypotension following induction in 4 out of 24

patients. Goodchild *et al.* (1989) suggested that in humans cardiac output and arterial pressure are well maintained at normal anaesthetic blood propofol concentrations if the preload is maintained; a suggestion also made by Kirvela *et al.* (1992). The concomitant administration of propofol, 2.5 mg/kg and alfentanil, 3 μg/kg, caused a greater decrease in the mean arterial blood pressure than when propofol was administered alone (24 vs 45 mm Hg) (Van Aken *et al.*, 1986). The initial hypotensive effects of propofol may be advantageous in humans during the intubation period, because it has been observed that propofol does not increase the mean peak of systolic arterial blood pressure post intubation, and in some cases it appears to lower it (Monk *et al.*, 1987). This contrasts with other anaesthetic drugs which usually increase the arterial blood pressure following intubation. In dogs, hypotension was recorded during propofol infusion, with a maximum effect (20% decrease) 5 min after induction of anaesthesia (Nolan & Reid, 1993).

Propofol causes apnoea post induction in both humans and animals. Grood *et al.* (1985) observed apnoea in all patients (19 women and 11 men) who had total intravenous anaesthesia with propofol combined with fentanyl and vecuronium. Morgan *et al.* (1989) recorded apnoea in 5 out of 207 cats and 6 out of 290 dogs, and Nolan *et al.* (1993) noted an incidence of 25% after induction of anaesthesia in dogs. Although this apnoea is usually of short duration, about 60 seconds or less, it can in some cases last considerably longer and opioid drugs may enhance this respiratory depression (Goodman *et al.*, 1987; Nolan & Reid, 1993). The incidence and occurrence of apnoea may be related to the speed of drug injection. Watkins *et al.* (1987), suggested that when propofol is administered slowly (over 10 to 40 seconds) apnoea is less evident.

Nolan & Reid (1993) observed respiratory depression throughout anaesthesia in spontaneously breathing dogs which received propofol infusions for anaesthesia. The dogs had end tidal pressure of carbon dioxide values in excess of 5.3 kPa throughout the infusion period, however, this did not persist into the recovery period. Hall & Chambers (1987) also observed respiratory depression, in dogs anaesthetised with propofol infusions, with $PaCO_2$ values ranged from 4.9 to 8.1 kPa during anaesthesia. Ponies premedicated with xylazine (0.5 mg/kg iv) in which anaesthesia was induced with propofol (2.0 mg/kg iv) and maintained with two infusion rates of propofol (0.15 mg/kg/min and 0.2 mg/kg/min), showed respiratory depression which was characterised by a decrease in rate and an increase in arterial carbon dioxide tension ($PaCO_2$ above 41 mm Hg throughout infusion) (Nolan & Hall, 1985). Despite the respiratory depression associated with propofol administration, its use is not precluded in spontaneously breathing patients where there is not pre-existing respiratory dysfunction.

The analgesic properties of propofol are controversial, but it may be better in this respect than thiopentone. Briggs *et al.* (1982b) suggested that administration of subanaesthetic doses of propofol had an analgesic effect in 35%, anti-analgesic effect in 5%, and no effect in 60% of patients, while thiopentone was anti-analgesic in 60%, had no effect in 30% and was analgesic in only 10% of patients. It has also been reported that the incidence of nausea and vomiting with propofol is low, with suggestions that it might have some anti-emetic properties (McCollum *et al.*, 1987), however, Hall & Chambers (1987) observed that 16% of dogs vomited following anaesthesia with propofol infusions.

1.2.1.3 PHARMACOKINETICS

Pharmacokinetics describe the relationship between the administration, distribution and the magnitude of the concentration, in different regions of the body, of a given drug (Pratt, 1990). Pharmacokinetic parameters represent the mathematical description of changes in drug concentration within the body (Baggott, 1977). The pharmacokinetic characteristics of a drug determine its access to the site of action.

The disposition of a drug in the body is described by four phases namely, absorption, distribution, metabolic alteration and excretion. In order to have an effect, a drug must first enter the blood, unless it acts directly at the site of administration. Subsequently it reaches the tissues at a rate determined by the blood flow through each specific organ (Pratt, 1990). During this process some of the drug molecules might be bound to plasma proteins, which may influence its distribution and metabolism. Pharmacokinetic studies can be done using compartmental or non compartmental analyses. The compartmental model, assumes the body as a system of distribution compartments (one, two or three) with mathematical, but not physiological meaning (Baggot, 1977). It assumes that a drug once absorbed, distributes quickly and homogeneously into the central compartment, which consists of the blood and well perfused tissues. Distribution then takes place more slowly to the peripheral compartments. It is assumed that elimination takes place from the central compartment only (Baggot, 1977; Hull, 1991).

The non compartmental model is based on the theory of statistical moments, which represent the times taken for individual drug molecules to pass through the body before elimination. This movement of drug molecules is assumed to be proportional to its concentration gradient in the distribution and elimination compartments (Hull, 1991). For the optimal use of an intravenous anaesthetic agent, particularly where an infusion regime is to be established, detailed pharmacokinetic evaluation is critical (Ghoneim *et al.*, 1977; Kanto *et al.*, 1988) because it might be influenced by

both premedication and inhalation anaesthetics (Langley *et al.*, 1988) and also by other drugs that may be used during anaesthesia.

Many pharmacokinetic studies of propofol have been carried out in humans, and it appears that propofol has many advantageous kinetic properties when compared to other intravenous anaesthetics agents, which explain its usefulness by bolus for induction of anaesthesia or as a continuous infusion for maintenance of anaesthesia and sedation in intensive care units (Mendoza *et al.*, 1992). Its pharmacokinetic parameters do not depend on the speed of injection, dose, sex or body weight (Adam *et al.*, 1983; Kay *et al.*, 1986; Kanto *et al.*, 1988). The pharmacokinetic profile of propofol makes it suitable for administration by a combination bolus and a variable-rate infusion technique, which permits the anaesthetist to titrate the propofol dose to the desired clinical effect (Shafer *et al.*, 1988).

After a single bolus injection or infusion for maintenance of anaesthesia, propofol distributes extensively to the central nervous system (CNS) and other tissues such as muscle and fat (Langley *et al.*, 1988; Kanto *et al.*, 1988). In most studies, the blood concentration *versus* time data is best described by a three compartment model with the data from some individuals fitting a 2 compartment model. Gepts *et al.* (1987), studying the behaviour of a constant rate infusion at 3, 6, and 9 mg/kg/h in humans with a mean body weight of 75kg, 65.5kg and 61.6kg respectively, found that for all three rates the pharmacokinetic profile of propofol was best described by a three compartment open model with central elimination. Simons *et al.* (1991b) using rat, dog and rabbit as experimental models, given a radioactive bolus dose of propofol (7-10 mg/kg) intravenously (rat, dog, rabbit) or a continuous infusion (0.47 mg/kg/min) in the dog, reported that the plasma concentration-time relationship was best described by a three compartment open model in the rat and dog, but by a two compartment model in rabbits. Reid *et al.* (1993) also reported that the blood propofol concentration-time data for goats was best described by a two compartment model.

Propofol has a very large volume of distribution reflecting extensive tissue distribution, which is probably related to its high lipophilicity. In humans, mean volumes of distribution at steady state (Vdss) of 287 ± 213 L and up to 1666 ± 756 L have been reported (Gepts *et al.*, 1987; Albanese *et al.*, 1990). Simons *et al.* (1988) described extensive distribution, after a single dose of propofol. In dogs Vdss values around 6.51 L/kg have been reported which are higher than reported by some authors in humans (Nolan & Reid, 1993). Reid *et al.* (1993) similarly reported widespread distribution and a large Vdss (mean 2.56 L/kg) in goats. Large variations have been reported for the half life of propofol which has been suggested to be

related to the length of the sampling period (Campbell *et al.*, 1988). In studies where 2 compartment model was used, the elimination half life values ranged from 92 to 106 min (Schüttler *et al.*, 1985, 1986; Simons *et al.*, 1988), when a three compartment model was used the elimination half life was observed to be biphasic, the first stage was rapid with a half life of 25 to 56 min while the terminal phase was longer with a half life of 184 to 309 min. (Cockshott *et al.*, 1987; Gepts *et al.*, 1987) and indeed half-lives of up to 44-47 h have been reported (Morgan *et al.*, 1990). It has been suggested that prolonged infusion periods (Albanese *et al.*, 1990; Morgan *et al.*, 1990), length of the sampling period (Campbell *et al.*, 1988 ; Albanese *et al.*, 1990), and the severity of the surgery (eg: abdominal surgery) (Shafer *et al.*, 1988) can prolong the elimination half-life of propofol.

Propofol is rapidly cleared from the body, with clearance values in humans varying from 1.7 L/min to 2.2 L/min (Cockshott *et al.*, 1985; Gepts *et al.*, 1987; Simons *et al.*, 1988; Albanese *et al.* 1990). Nolan & Reid (1993) reported that in dogs, body clearance (50.1 ml/kg.min) was similarly high, while Reid *et al.* (1993) in goats, reported that clearance (275 ml/min.kg) was vastly in excess of liver blood flow. Due to the high body clearance values observed it has been suggested that an extra hepatic site of metabolism cannot be excluded (Simons *et al.*, 1991a). Gray *et al.* (1992) proposed an extra hepatic site of metabolism of propofol, after their findings that only 42%-89% of blood propofol radioactivity was unchanged propofol during the anhepatic phase of anaesthesia in six patients undergoing hepatic transplantation given a bolus injection of ^{14}C propofol. Two patients showed evidence of propofol glucuronide and 4-quinol sulphate during the same phase indicating that metabolism of propofol was occurring elsewhere. Veroli *et al.* (1992) also reported the appearance of a propofol metabolite and did not detect unchanged propofol in the urine of 10 patients, during the anhepatic phase of orthotopic liver transplantation, further evidence of extra hepatic metabolism of the drug. Simons *et al.* (1988) and Shafer *et al.* (1988) also proposed extrahepatic metabolism of propofol after recording clearance values of 2.2 L/min and 2.09 ± 0.65 L/min respectively in humans, values in excess of liver blood flow. It was suggested by Nolan & Reid (1993) and by Reid *et al.* (1993) that extrahepatic clearance might occur in dogs and goats since total body clearance values recorded in these species were similarly in excess of liver blood flow values for these species.

Studies with propofol in humans and experimental animals have shown that individual variation in blood concentrations of propofol may occur (Schuttler *et al.*, 1988; Puttick *et al.*, 1992). Despite an identical zero-order infusion rate of propofol in 7 dogs undergoing surgery, considerable between animal variation in blood propofol levels were observed and in blood concentrations required to maintain

anaesthesia for surgery (Nolan & Reid, 1993). In humans, there seems to be also a wide variation in blood propofol levels. (Gepts *et al.*, 1987) administered propofol to patients by continuous infusion at a maximum rate of 0.15 mg/kg and found that there was an initial increase in blood concentration over 10 min, followed by a slower rate of increase over the infusion period. Recovery from anaesthesia has been observed at propofol concentrations of 0.98±0.04 to 1.96±0.18 μg/ml in humans and 1.7 to 2.7 μg/ml at head lift in dogs (Cockshott *et al.*, 1990; Nolan & Reid, 1993). In some patients a small secondary peak in blood propofol concentration has been observed at the time of regaining consciousness (Cockshott *et al.*, 1987; Kay *et al.*, 1986; Schüttler *et al.*, 1985).

Other drugs are frequently administered during anaesthesia in humans and animals which may influence the distribution and metabolism of the anaesthetic agent propofol. Cockshott *et al.* (1987) reported that the opioid analgesic fentanyl reduced the volume of the central compartment (Vc) by 46% and increased the half life ($t_{1/2\beta}$) of propofol by 24%. In contrast, Nolan *et al.* (1993) reported that maintenance of anaesthesia with halothane and nitrous oxide in dogs had no effect on the pharmacokinetics of propofol administered as an intravenous bolus. They reported clearance values of 58.6 and 56.3 ml/kg.min and Vdss of 4889 and 4863 ml/kg for propofol alone and propofol with halothane and nitrous oxide respectively. It has also been suggested that in humans premedication and other drugs do not affect the rapid recovery from (Morgan *et al.*, 1989), and the good induction of (Briggs *et al.*, 1982a; Morgan *et al.*, 1989) anaesthesia with propofol.

Pathology may influence the pharmacokinetics of propofol. Audibert *et al.* (1993) suggested that inflammatory reaction *in vivo* prolonged the elimination half-life of propofol, but did not change its clearance or volume of distribution, while hypoxia decreased propofol clearance and as a consequence, the elimination half-life was increased.

The pharmacokinetics of propofol given by infusion, are not markedly affected by moderate cirrhosis and recovery, measured as the time of eye opening, occurs at similar blood propofol concentration in healthy patients and in those with cirrhosis (Kanto *et al.*, 1988; Servin *et al.*, 1988). In a later report, Servin *et al.* (1990) reported a reduction although not significant in total body clearance of propofol in patients with cirrhosis (1.56±0.48 L/min in cirrhotics and 1.75±0.32 L/min in non cirrhotics) and a significantly greater Vdss (202±82 L) than in non cirrhotic group (121±49 L), but the terminal half life was not altered.

Metabolism of propofol

After absorption into the body, many drugs undergo chemical reactions which change their physical properties and biologic effects. Drug metabolism is normally divided into two phases, phase I (or functionalisation reactions) and phase II (or conjugative reactions). Phase I metabolism, includes oxidation, reduction, hydrolysis and hydration reactions; in most cases the final product contains a reactive group, prepared for the phase II reactions (Gibson & Skett, 1994). Metabolic reactions usually affect the polarity of the compound, altering the way in which it is distributed and excreted from the body while in some cases, this metabolism is required for therapeutic effect.

Propofol is rapidly metabolised, mainly by the liver, and the urine is the major route of excretion in most species including humans. According to Massey *et al.* (1990) 88% of propofol administered to humans is metabolised in this way. Following an intravenous bolus dose of radioactive ^{14}C propofol to men, 88% of the radioactivity was recovered in the urine in five days and about 2% in faeces, with less than 0.3% excreted unchanged (Simons *et al.*, 1988). Simons *et al.* (1991b) reported that only 52-85% of radioactive ^{14}C propofol was found in the circulation 2 min after bolus i.v. administration to dogs, rats, and rabbits. Radioactivity was detected in the urine 60-95%, but 13-31% was detected in faeces of rats and dogs. Simons *et al.* (1991a) suggested that only 2% of the propofol dose administered to female rats remained in the body tissues 24 hours after dosing. Simons *et al.* (1988) and Simons *et al.* (1992) described glucuronic acid conjugate of propofol (propofol glucuronide) and glucuronic acid and sulphate conjugates of its hydroxylate derivative, 2,6-diisopropyl-1,4-quinol (1 & 4-quinol glucuronide, 4-quinol sulphate) to be the major metabolites in man, with the glucuronide accounting for about 53% of the urinary radioactivity. These metabolites have been also reported to be present in rat, dog and rabbit by Simons *et al.* (1991b), while in the same study trace amounts of propofol sulphate were detected in rabbit urine.

1.2.2 TOTAL INTRAVENOUS ANAESTHESIA (TIVA)

In order to eliminate the potential hazards of environmental pollution, the use of intravenous anaesthetic agents for the maintenance of anaesthesia is becoming a popular technique in humans. In addition the technique is easy to perform and greater control of the depth of anaesthesia can be afforded using closed loop circuits (Schüttler *et al.*, 1988). Since there is more scope for the development of new

intravenous drugs compared with those given by inhalation, it is likely that research will concentrate on the development of such agents with better pharmacological characteristics and potential for use in a total intravenous technique.

Recovery from anaesthesia is determined by the kinetic profile of the drugs used. If the drug is metabolised rapidly in the body, recovery from anaesthesia can be quicker than that seen with inhalation anaesthetics and consequently, such a drug would be useful for total intravenous anaesthesia. Older intravenous agents such as thiopentone are unsuitable for TIVA, because they are slowly metabolised in the body, and recovery from anaesthesia is slow after repeat administration or long infusions (Hull, 1989). In contrast propofol, has been shown to be rapidly metabolised and cleared from the body, which affords a good control of the depth of anaesthesia during the course of surgery and rapid recovery once drug administration is stopped.

An ideal intravenous anaesthetic should produce a rapid onset and short duration of action and inactivation by metabolism. It should possess no cumulative properties, minimal cardiorespiratory effects, analgesic properties and be stable in solution as well as soluble in water. It should not cause histamine release after injection, no tissue damage if injected perivascularly or venous damage (Dundee, 1979). Although propofol does not fulfill all the properties of an ideal intravenous anaesthetic, since it depresses the cardiovascular and respiratory systems, has minimal analgesic properties, is not stable in solution and is not soluble in water, if used properly these disadvantages can be overcome and propofol is the agent most suitable for TIVA to date.

However, there are disadvantages associated with a total intravenous technique. It requires specialised equipment such as an infusion pump and good venous access. In addition the influence of drugs given concurrently can alter the rate of metabolism of the anaesthetic agent giving rise to prolonged recovery (Reid & Nolan, 1992).

1.3 *IN VITRO* DRUG METABOLISM STUDIES

For better understanding of drug metabolism, drug interactions and other aspects related to drug administration, a good knowledge of the enzymatic processes and organs involved is important. These studies are not always possible to carry out *in vivo*, for ethical, economic and scientific reasons. Studies carried out *in vitro*, permit the investigation of a specific biotransformation pathway under strictly controlled conditions, allow the use of very high drug concentrations (Klooster, 1992), and

provide useful background information on how to approach and what to expect during *in vivo* studies.

In vitro studies of drug metabolism have limitations since many factors which influence the response *in vivo* are absent. However, such techniques are still very useful and important. Much fundamental knowledge regarding the molecular mechanisms affecting drug disposition, has been obtained using liver tissue *in vitro* since it is the main site of drug metabolism (phase I and phase II reactions) and detoxification.

Studies of drug metabolism *in vitro* can be carried out using techniques and tissues such as: organ perfusion, tissue slices, separated cell suspensions, tissue homogenates, tissue cultures (e.g. hepatocellular cultures), subcellular fractions (microsomal and cytosolic cell fraction preparations), solubilisation and purification of enzymes. The most commonly used tissues are the microsomal fraction preparations. The first description of metabolism of a foreign compound by hepatic microsomes was made by Mueller and Miller in 1953, and since this time, hepatic microsomes have been used widely.

Microsomal preparations are small bodies of homogenised endoplasmic reticulum of any group of cells, but mainly of the liver, kidney, lung and intestine. The main enzymatic system is the mixed-function oxidase system, cytochrome P450, which exists as multiple forms or isoenzymes, and to date, more than 200 different cytochrome P450s have been identified (for review see Soucek & Gut, 1992; Gibson & Skett, 1994). They are subdivided into gene families and gene subfamilies according to their similarity. The cytochrome P450 enzyme system performs a great variety of phase I reactions involving many structurally diverse drugs and chemicals, whose only common feature is a reasonable degree of lipophilicity (Mannering, 1971; Gibson & Skett, 1994). All reactions occur in the presence of molecular oxygen and NADPH. The activity of the microsomal enzymatic system can be affected by factors such as sex, nutrition, diurnal variation, species, breed, age, liver pathology, animal bedding, stress, insecticides, ambient temperature and cage crowding (Gibson & Skett, 1994).

The use of microsomal preparations has many advantages, firstly, the methodology is relatively simple and inexpensive; material can be preserved for long periods of time with little loss in enzyme activity; the method facilitates the study of specific biotransformation steps and finally, the preparation costs less than that of hepatocytes, especially for interspecies studies. The disadvantages of the use of microsomal preparations are mainly because they do not take into account the uptake of drugs by the cells; there is a loss of some biotransformation enzyme

systems (example the cytosolic); it needs a continuous supply of NADPH generating system and in general, the incubation cannot be prolonged for more than one hour.(Mannering, 1971).

1.4 AIMS OF THIS STUDY

The pharmacokinetic properties of propofol (Cockshott *et al.*, 1985), are such that it has potential advantages, especially for day case surgery in humans and animals where a rapid recovery from anaesthesia is desirable. This latter feature is a particular requirement for ruminant species in order to minimise the risk of regurgitation and tympanism (Waterman, 1988), and for brachycephalic breeds of dogs and all animals where a rapid return of consciousness is desirable. Total intravenous anaesthesia (TIVA) offers many advantages over inhalational anaesthesia and to this end, propofol appears to be the intravenous anaesthetic most suitable. However, propofol has some undesirable side effects and consequently, supplementation with other drugs during propofol anaesthesia is necessary in order to improve the quality for surgery. Drugs commonly used concomitantly with propofol in humans include alfentanil, a μ opioid receptor analgesic which produces excellent suppression of responses to surgical stimuli and has little effect on the cardiovascular system (Schüttler *et al.*, 1988). These workers reported that computer controlled infusions of alfentanil and propofol given to 20 patients undergoing surgery, produced satisfactory conditions for both minor and major surgical stimulation and that recovery from anaesthesia was rapid. This combination has been infused in dogs undergoing thoracic surgery (Flecknell *et al.*, 1990) although bradycardia was a considerable problem. However the major disadvantage of alfentanil is its depressant effect on the respiratory system and consequently, its use by infusion is not recommended in spontaneously breathing patients. Since most surgical procedures in animals are performed in spontaneously breathing animals it was considered appropriate to investigate drugs which would complement propofol infusions, without depressing the respiratory systems. Ketamine apperead to be a drug worthy of study for several reasons. Firstly, it does not depress the cardiovascular or respiratory systems in animals, indeed it may cause cardiovascular stimulation (Waterman & Livingston, 1978a). Secondly, it is considered to be a good analgesic both in humans and in many animal species (Sparks *et al.*, 1973) and thirdly, the pharmacokinetics of ketamine reported in animals (Heaver & Bloedow, 1979; Kaka *et al.*, 1979; Waterman & Livingston, 1978a) indicates that is rapidly cleared from the body and suggest that it would be suitable for infusion regimens. Disadvantages of ketamine use include the possibility that it might alter the metabolism of propofol, since ketamine has been shown to induce liver enzyme

activity in rats (Livingston & Waterman, 1978) and if used inappropriately might cause convulsions and delirium as has been reported in dogs (Hall & Clarke, 1991).

The aims of this thesis were firstly, to study the degradation of propofol by hepatic microsomal enzymatic systems in two species, rat and sheep, particularly the cytochrome P450 system; with this information it was then considered of interest to study potential interactions *in vitro* between propofol and other drugs metabolised by the same enzymatic system, in particular ketamine and alfentanil, drugs which might be coadministered with propofol during anaesthesia. Since it had been suggested that propofol might undergo extrahepatic degradation it was considered appropriate to study other potential sites of degradation. Finally, it was decided to study the use of propofol/ketamine infusions using sheep as a model.

2. MATERIALS AND METHODS

2.1 Degradation and interaction of propofol with other drugs *in vitro*

2.1.1 Animals

Adult healthy Sprague Dawley or Wistar rats, weighing between 400 and 450 grams were used for the preparation of hepatic or lung microsomal specimens. The animals were housed in groups of six, on sawdust bedding, at a constant temperature of 23°C and on a twelve hour light : dark cycle. They were allowed free access to food and water.

Sheep hepatic microsomes were prepared from livers of healthy adult Scottish Blackface sheep obtained from a local abattoir.

All studies were carried out with rat microsomes, unless otherwise stated. Each experiment was carried out in replicates of six rats and each concentration was done in duplicate. The exceptions to this were for the study of propofol degradation by lung microsomes where five rats were used, and the time course pilot study where two determinations from one animal only was used at each time point.

2.1.2 Preparation of hepatic microsomal specimens

All chemicals were purchased from Sigma Chemicals Co., Dorset, England, unless stated otherwise.

Sodium chloride (Analar, BDH Chemicals Ltd, Poole) was used as 0.9% solution in distilled water. Potassium chloride (Analar BDH Chemicals Ltd, Poole) was used as a 1.15% solution in distilled water. Tris buffer was prepared from the mixture of trizma hydrochloride and trizma base, as a 0.1M solution, pH 7.4, containing 20% (v:v) glycerol. Before the preparation of microsomes, all tubes, the homogeniser, the solutions, the centrifuge rotors, and other material used for the preparation of microsomes were cooled at -20°C for approximately 45 min.

Hepatic microsomes were prepared according to the method of Rutten *et al.* (1987) with some minor modifications. Briefly, the rats were killed by cervical dislocation, the abdomen was incised with a scalpel blade and a catheter was inserted into the hepatic portal vein. The liver was then washed *in situ* with approximately 300 ml of ice cold sodium chloride, 0.9%, injected through the catheter to remove excess blood. After washing, the liver was excised, freed of connective tissue, weighed, placed in 3 volumes of ice cold potassium chloride, 1.15%, finely sliced and homogenised, using a Potter-Elvehjem glass Teflon homogeniser (5 complete passes with the pestle). Once the tissue was collected, all subsequent procedures were

carried out at 0-4°C. The liver homogenate was centrifuged at 9000 g for 20 min in an ultra centrifuge (Beckman Instruments, INC., California) at 2°C, in order to remove cell debris, nuclei and mitochondria. The floating fat layer was carefully removed with a spatula and the underlying supernatant fraction was decanted into ultra-clear centrifuge tubes and recentrifuged at 105000 g for 75 min, at 2°C. The cytosolic fraction (supernatant) was discarded and the precipitate (microsomal pellets) was resuspended carefully using an ultra Turrax (Janke & Kunkel GmbH & Co, Germany) in 12 ml of Tris buffer (0.1M, pH 7.4), containing 20% glycerol. The resuspended microsomes were divided into aliquots (1 ml), frozen immediately in liquid nitrogen and stored at -80°C until used within 15 days of preparation. Three hundred µl of the microsomal suspension was used for protein and cytochrome P450 measurements, which were carried out on the same day the microsomes were prepared.

For sheep liver microsomes, the caudate lobe was collected into ice cold sodium chloride, 0.9%, within 15 min of death and placed in a bucket with ice. The liver was then washed with approximately 300 ml of ice cold sodium chloride, 0.9%, through a catheter which had been placed into the central vein of the lobe. After washing, microsomes were prepared as described for rats (*vide supra*).

Protein measurement

Microsomal protein concentrations were measured using a commercially available kit (Sigma Chemical Co., Dorset England) which is based on the method reported by Lowry and colleagues in 1951. Briefly, 0.125, 0.250, 0.500, 0.750 and 1.000 ml of a protein standard solution (bovine serum albumin, fraction V) were diluted (where appropriate), to 1 ml with water to yield protein concentrations of 50, 100, 200, 300 and 400 µg/ml respectively. Microsomal suspensions (10 µl) were diluted similarly to 1 ml with water and a blank tube was prepared with 1 ml of water, corrected to the same glycerol concentration present in the sample tubes. One ml of Lowry reagent solution was added to all tubes, the tubes vortexed and allowed to stand for 20 min at room temperature (approximately 20°C); 0.5 ml of Folin & Ciocalteu`s phenol reagent were then added to each tube and the tubes vortexed again. Colour was allowed to develop at room temperature for 30 min. The tube contents were subsequently transferred to 3 ml quartz cuvettes and the absorbance of the solution at 750 nm read using an ultraviolet/visible (UV/Vis) spectrophotometer (Pye Unicam Ltd, Cambridge). All standards and samples were assayed in duplicate.

A calibration curve was prepared by plotting the absorbance of the standards against their respective protein concentrations. The protein concentration of the samples

was determined from the calibration curve and the result was adjusted according to the dilution factor in order to calculate the protein concentration in the original sample.

Cytochrome P450 determination

The cytochrome P450 (cyto P450) concentration in the microsome preparations, was determined using the method developed by Omura and Sato (1964) and described by Gibson and Skett (1994). This method is based on the ability of cyto P450, a haemoprotein, to bind to carbon monoxide (CO) which yields a characteristic absorption spectrum at 450 nm when the haem iron is reduced. A molar extinction coefficient of 91 $mM^{-1}cm^{-1}$ (for the wavelength couple 450-490 nm) and a cuvette path length of 1 cm were used to convert absorbance units to nmoles of cyto P450 as follows: (absorbance difference (450-490 nm) * cuvette path length)/extinction coefficient = nmoles of cyto P450/ml of the diluted sample. Once the concentration of protein in the original microsomal suspension is known the concentration of cyto P450 per mg of protein can be calculated as follows: (cyto P450 in the diluted sample * dilution factor)/protein content in the original sample = nmoles cyto P450/mg of protein. Briefly, 200 µl of microsomal protein were diluted 20 times (up to 4 ml), with Tris buffer, 0.1M, pH 7.4 containing 20% (v:v) glycerol. Two ml of the sample were transferred to both matched sample and reference cuvettes and the absorbance baseline was recorded between 400 nm and 500 nm using an UV/Vis spectrophotometer. The sample and reference cuvette solutions were reduced with a few grains of sodium dithionite, and the sample cuvette only was bubbled gently with 100% carbon monoxide (BOC, Polmadie, Scotland) for approximately 1 min. The spectrum was then re-scanned from 400 nm to 500 nm and the absorbance at 490 and 450 nm recorded.

2.1.3 Incubation of propofol with hepatic microsome preparations

All chemicals used for incubations were purchased from Sigma Chemical Co., Dorset, England unless stated otherwise. All solutions were stored at 0-4°C.

Since the activity of cyto P450 relies on the presence of NADPH, a continuing supply of this system, must be present throughout the incubation period (Gibson & Skett., 1994). The NADPH generating system used consisted of $NADP^+$, 1 mM, isocitrate dehydrogenase (0.2 units/ml), trisodium isocitrate, 15 mM, nicotinamide, 0.5M and $MgCl_2$, 0.15M, made up to a final volume of 10 ml in Tris buffer, 0.1M, pH 7.4. $MgCl_2$ and nicotinamide were prepared in water, from hexahydrate (Analar, Hopkin & Williams, Chadwell Heath, Essex) and niacinamide respectively. This

NADPH generating system was prepared immediately before incubation and 1 ml was used in each tube.

Microsomes were diluted to a working protein concentration of 12 mg/ml in Tris buffer, 0.1M, pH 7.4 containing 20% glycerol (v:v), unless stated otherwise. In order to assess the degree of extra microsomal drug degradation and drug stability during the incubation process, control microsomes (inactivated) were prepared by heating aliquots (70-100°C) for 10 min.

2.1.3.1 Degradation of propofol by hepatic microsome preparations (rat & sheep)

Propofol was prepared from pure compound, (ICI Pharmaceuticals Co.), in dimethyl sulfoxide (DMSO) to a standard concentration of 500 μg/ml (2805 μM).

A pilot experiment was carried out to approximate the time course of propofol degradation by rat hepatic microsomes in order to select an appropriate incubation time for other studies. The incubation procedure was a modification of methods described previously by Fargetton *et al.* (1986); Galtier *et al.* (1986), for benzimidazole studies and by Baker *et al.* (1993), for propofol. All incubations were conducted aerobically at 37°C, in a shaking water bath (Grant Instruments Ltd, Cambridge) with a shaking speed of 120 cycles/min. The final incubation volume was 2 ml. Firstly, propofol, 28, 56, 84 and 140 μM in DMSO or DMSO (range 20-100 μl) and NADPH generating system (1 ml) were added to glass test tubes and made up to a final volume of 2 ml with appropriate volumes of Tris buffer (range 400-480 μl). This mixture was incubated for 2 min and subsequently 500 μl of active or inactive microsomal protein was added and re incubated for 5, 10, 15, 20, 30, 45 or 60 min. Microsomal activity was stopped by placing the test tubes in ice for 5 min. The contents of the test tubes were transferred to 5 ml neutral tubes (Sarstedt, Ltd. Leicester) and stored at -20°C until chromatography was performed, usually within one week of incubation. Seven rats (Sprague Dawley) were used in this study. Tissue from one animal was used for each incubation time. All tubes were prepared in duplicate.

Based on the results of this pilot experiment, the degradation of propofol by sheep and rat microsome preparations was studied using a 15 min incubation period.

2.1.3.2 Interaction of propofol with cytochrome P450 and the influence of other drugs on propofol degradation

The effect of propofol on cyto P450 activity

The effect of propofol on cytochrome P450 activity was assayed by studying the rate of formation of formaldehyde from aminopyrine, a cytochrome P450 dependent N-demethylation process, in the presence and absence of various concentrations of propofol. A standard solution of aminopyrine, 25 mM, was prepared in water from 4-dimethyl aminoantipyrine and the solution was stored in an amber bottle. Propofol standard solutions were prepared in DMSO at concentrations of 280 μM, 2.8 mM and 28 mM. The NADPH generating system was modified to include semicarbazide ($MgCl_2$, 0.15M was replaced by $MgCl_2$, 0.15M/semicarbazide, 0.1M), (see 2.1.3). Microsomal protein was diluted to a working concentration of 10 mg/ml in Tris buffer, 0.1M, pH 7.4 containing 20% glycerol (v:v).

The method used was described by Gibson and Skett (1994). Four hundred μl of the standard aminopyrine solution was placed into glass test tubes, (blank samples were prepared by replacing aminopyrine with 400 μl of distilled water). Propofol, 5.6, 56, 280 or 561 μM (40 μl of the different standard solutions), DMSO (40 μl) or Tris buffer (40 μl) were then added to each respective tube. Subsequently, NADPH generating system (1 ml) and Tris buffer (60 μl) were added to give a total volume of 2 ml in each tube. The mixture was pre incubated for 2 min as described previously. The reaction was started by the addition of active or inactive microsomes (500 μl) and the mixture was then incubated for 15 min. Enzyme activity was stopped by placing the tubes on ice for 5 min and with the addition of zinc sulphate, 25% (500 μl). Protein was precipitated by the addition of a saturated solution of barium hydroxide (500 μl) and the tubes were maintained on ice for a further 5 min. The mixture was then centrifuged at 3000 g for 5 min. Subsequently, 1 ml of the clear supernatant was removed into glass test tubes, mixed with 2 ml of Nash reagent, covered to prevent water loss and incubated at 60°C for 30 min to permit maximum colour development. The tubes were then cooled by placing them into ice for 5 min and the absorbance of each solution was read at 415 nm in a UV/Vis spectrophotometer. The concentration of formaldehyde in the sample tubes was calculated with the use of a standard curve.

The standard curve for the assay of formaldehyde concentration was prepared by diluting 0, 0.2, 0.4, 0.6, 0.8 and 1.0 ml of a freshly prepared solution of formaldehyde, 0.7 mM, to 1 ml with distilled water. Nash reagent (2 ml) was added to each tube, and the tubes incubated and assayed as described above. Absorbance values were plotted against known concentrations of formaldehyde and a standard curve was constructed.

Extra cyto P450 degradation of propofol

In order to determine whether propofol was metabolised by hepatic enzymatic systems other than cyto P450, the activity of this enzymatic system was inhibited with 1-aminobenzotriazole (ABT; 12 mM), a suicide substrate for the cyto P450 enzymatic system (Mugford *et al.*, 1992). A standard solution of propofol (500 µg/ml) was prepared as described previously (see 2.1.3.1) and a solution of ABT, 240 mM, was prepared freshly in Tris buffer, 0.1M, pH 7.4.

Propofol, 28, 56, 84 or 140 µM in DMSO or DMSO (range 20-100 µl) was placed into glass test tubes. ABT, 12 mM (100 µl of 240 mM) was allocated to appropriate tubes. NADPH generating system (1 ml) and Tris buffer, to yield a final tube volume of 2 ml (range 300-480 µl), were then added to each tube and pre incubated for 2 min. Five hundred µl of active or inactive microsomes was allocated into appropriate tubes and the mixture incubated for a further 15 min as described in 2.1.3.1

The effect of other drugs on the degradation of propofol

Standard solutions of ketamine (200 µg/ml) and alfentanil (Janssen Pharmaceuticals Ltd), (100 µg/ml) were prepared in Tris buffer and standard solution of aminopyrine (25 mM) was prepared in water from 4-dimethyl aminoantipyrine.

For each drug-propofol interaction study, 28 tubes were used. These included propofol alone, propofol & DMSO, propofol & drug and propofol & inactive microsomes. Propofol 28, 56, 84 or 140 µM in DMSO or DMSO (range 20-100 µl) were placed into glass test tubes. Ketamine, 5 µg/ml (50 µl of 200 µg/ml), alfentanil, 200 ng/ml (4 µl of 100 µg/ml) or aminopyrine, 5 mM (400 µl of 25 mM) was placed in appropriate tubes. NADPH generating system (1 ml) and Tris buffer, to yield a final volume of 2 ml (range 0-480 µl) were then added to each tube and pre incubated for 2 min. Five hundred µl of active or inactive microsomes were allocated into appropriate tubes and the mixture incubated for 15 min as described in 2.1.3.1.

2.1.4 Lung microsomes

Preparation of lung microsomal specimens

Lung microsomes were prepared according to the method of Miles and colleagues, (1993). Rats were killed by cervical dislocation, the thoracic and abdominal cavities opened and the lungs collected into ice cold Tris buffer, 0.1M, pH 7.4. Once the lungs were collected, all procedures were carried out at 0-4°C. The lungs were freed of connective tissue, blotted dry, weighed, placed into 4 volumes of ice cold Tris buffer, and finely sliced before homogenisation using a Teflon-glass Potter-Elvejhem homogeniser (6 complete passes with the pestle). The tissue homogenate was then subjected to differential centrifugations. Cell nuclei and debris were removed by centrifugation at 1000 g for 10 min. The mitochondrial fraction was slowly sedimented by two sequential centrifugations for 20 min each, at 10000 g and 15000 g and subsequently, the microsomal fraction was sedimented by centrifugation at 105000 g for 75 min. The cytosolic fraction was discarded and the microsomal pellet resuspended in 2 ml of Tris buffer, containing 20% glycerol (v:v), divided into aliquots (1 ml), immediately frozen in liquid nitrogen and stored at -80°C until used (within one week). Twenty µl of the resuspended microsomes was used for measurement of the protein concentration in the microsomal preparation. This was carried out as described previously (see 2.1.2).

Because of the high spectral interference of oxy- and deoxy-haemoglobin during the spectral measurement of cytochrome P450, the content of this enzymatic system in the lung microsomal preparations could not be measured.

The incubation of propofol with lung microsomes was performed as described previously, but, because of the small concentration of protein obtained from lung preparations, the concentration of protein in the incubation mixture was decreased to 400 µg. Tubes without microsomes were used to assess for extra microsomal degradation of propofol and drug stability during incubation. Propofol was used at tube concentrations of 5.6, 11, 28 and 56 µM. Briefly, propofol in DMSO or DMSO (range 4-40 µl), Tris buffer , to yield a final volume of 2 ml (range 760-796 µl) and NADPH generating system (1 ml) were pre incubated in glass test tubes for 2 min. Two hundred µl of active microsomes or Tris buffer, were then added to appropriate tubes and the incubation performed for 15 min as described previously (see 2.1.3.1).

2.1.5 Propofol analysis

Propofol was assayed by high performance liquid chromatography (HPLC) with fluorescence detection

Standards and chemicals

All solutions were stored at 4°C. A stock solution of propofol (200 μg/ml), was prepared from pure compound (ICI Pharmaceuticals), using methanol (HPLC grade, Rathburn Chemicals Limited, Walkerburn) as solvent. This solution was used for all subsequent dilutions. Thymol was used as the internal standard. A stock solution (100 μg/ml) was prepared in methanol, from pure compound (Sigma Chemical Co. Ltd, Dorset England) and was further diluted to a working solution of 2 μg/ml. Tetramethylammonium hydroxide (TMAH, Sigma Chemical Co. Ltd, Dorset England) was used as a 7.5% solution in propan-2-ol (Analar, Rathburn Chemicals Ltd Walkerburn), which was prepared immediately prior to use. Phosphate buffer, 0.1M, was prepared in distilled water from sodium dihydrogen orthophosphate (Analar, BDH Chemical Ltd, Poole England). The HPLC mobile phase consisted of a mixture of methanol:water:trifluoroacetic acid (TFA, Sigma Chemical Co Ltd, Dorset England) in a 70:30:0.1 (v:v:v:) ratio, respectively. The mobile phase was degassed for twenty minutes before use. A 2 μg/ml solution of propofol prepared from the stock solution in the HPLC mobile phase was used as an external standard for HPLC injection.

2.1.5.1 Extraction of propofol from spikes and samples

Spiked samples (hereafter referred to as spikes) were used to assess the percent recovery of propofol from microsomes for each assay, and to prepare a calibration curve in order to calculate the concentration of propofol in the sample tubes. Spikes were prepared as follows: microsomes (125 μl), Tris buffer (375 μl), were placed into six tubes (s_1-s_6) into which propofol or methanol only (25 μl), were added to yield final concentrations of 0, 0.5, 1.0, 2.0, 5.0 and 10 μg/ml. The spikes were then incubated between 5 and 60 min depending on the study, to parallel the same conditions as the samples.

Once the spikes were prepared, 0.5 ml of the sample was pipetted into appropriate tubes. Spikes and samples were then treated identically. 0.5 ml of phosphate buffer, 0.1M, was pipetted into each tube, followed by 10 μl of thymol (2 μg/ml). The tubes were vortexed for 30 sec and cyclohexane (Analar, Rathburn Chemicals Ltd), (5 ml) was added. All tubes were then mixed for 30 min on a slow rotary mixer. Four ml of cyclohexane were removed from each tube, placed into fresh tubes containing TMAH, 50 μl, and evaporated to dryness under a stream of nitrogen, in a

dri-block (Techne, Cambridge) at 50°C. When the samples were dried, the sides of the tube were washed down with cyclohexane (approximately 0.5 ml) and allowed to evaporate to dryness once again. Spikes and samples were reconstituted in at least 200 µl of mobile phase, vortexed and sonicated for approximately 1 min and 200 µl of the reconstituted sample were used for chromatography.

2.1.5.2 Chromatography of propofol

The HPLC system (Gilson, Scotlab CoatBridge, Scotland) comprised an isocratic pump with a manometric module, connected to a sample injector. The samples were chromatographed on a 5 µ ODS hypersil column, 120 mm x 6 mm (Shandon Scientific Ltd, Cheshire), connected to the sample injector and to a variable wavelength fluorescence spectrophotometer (Perkin Elmer Ltd., Beaconsfield, Buckinghamshire), The detector output was connected to a chart recorder (Rikadenki, Mitsui Electronics Ltd, Surrey).

The mobile phase was pumped through the column for at least 45 min at a flow rate of 1 ml/min before any injections were made. The excitation wavelength of the detector was set at 276 nm and the emission wavelength fixed at 310 nm. The chart recorder was set to 10 mV with a paper speed of 5 mm/min. When the baseline was stable, three or four external standards were injected before the injection of spikes and samples. The smallest peak height considered for calculations was twice the background noise and this was assumed to be the limit of quantification of the assay. One external standard was injected after the injection of two spikes or samples. The injection volume was 15 µl, in a 20 µl loop, and the analysis time was 6 min. The chromatography always started and finished with the injection of an external standard.

2.1.5.3 Recovery of propofol from microsomes spikes and samples

The recovery of propofol from the spikes and samples was calculated by reference to the peak heights resulting from the injection of external standard solutions. The recoveries were then used to obtain a calibration curve, from which the concentration of the samples was obtained.

2.1.5.4 Intra- and inter-assay coefficient of variation

The reproducibility of the assays was assessed by determination of the coefficient of variation in replicated microsome spikes, which were analysed in a single day to obtain the intra-assay (within assay) coefficient of variation. The inter-assay or (between assay) coefficient of variation was obtained by reference to the assays performed on different days.

2.1.5.5 Sensitivity of propofol chromatographic assay

The limit of quantification of the assay, corresponding to a peak height of one unit of chart paper (2.5 mm) was 20 ng/ml, and was twice the background noise of the baseline.

2.1.6 Result calculation and presentation

The overall velocity of propofol degradation was calculated as follows: concentration of propofol degraded (μmoles)/[time of incubation (min)* total protein in 2 ml (mg)]. The concentration of propofol degraded was obtained by subtracting the final propofol concentration (in 2 ml) from the initial propofol concentration (in 2 ml). This is considered to be overall velocity of the reaction because a large extent of substrate was used during the course of the assay (Segel, 1975).

When a substantial fraction of the substrate is used during the assay (>5%), the Km and Vmax values obtained from the linear plots will be in error, if the initial substrate concentration is used. For these cases a linear plot can be used (even for 90% use of the substrate), provided the arithmetic mean substrate ½([So] + [St]) (where t is the time of incubation), is used for the horizontal axis as shown by Lee & Wilson (1971). The substrate concentration used to obtain the graphs of the velocity plotted against substrate concentration and for the reciprocal of the velocity plotted against the reciprocal of the substrate (Lineweaver Burk plot) throughout the thesis were obtained by this way. The best fitting curve for the Lineweaver Burk plot was obtained by regression analysis (Cricket Graph, Computer associates Ltd. Manchester). The equation of the best-fitting regression curve was then used to obtain the maximal velocity (Vmax) and the Michaelis constant (Km).

2.1.7 Statistical analyses of the results

Results are presented as mean ± standard error of the mean (±sem). Stastitical analyses were performed using MINITAB stastitical software (CLE COM Ltd, Birmingham). The statistical tests used for each specific study are detailed below:

Analysis of the effect of propofol on cyto P450 activity: a paired two sample t-test.

Study of extra cyto P450 degradation of propofol: a two way analysis of variance was used intially, followed by a post hoc paired two sample t-test.

Effect of other drugs on the degradation of propofol: a two way analysis of variance was initially used, and a post *hoc* paired two sample t-test was carried out for the effect of aminopyrine on propofol degradation.

Comparison of the degradation of propofol by sheep microsome preparations with degradation by rat microsome preparations: a two way analysis of variance was used.

Comparisons of Vmax & Km values for propofol in the presence or absence of other drugs: a two sample paired t-test was used.

For all tests, a $P < 0.05$ was considered significant.

2.2 Pharmacokinetics and pharmacodynamics of propofol in sheep

2.2.1 Animals and husbandry

Ten Scottish Blackface healthy female sheep, weighing between 21 and 29.5 kg and aged between 6 and 7 months were used in this study. They were housed in stables, and received hay and water *ad libitum* and concentrate pellets twice a day. All sheep underwent superficial surgery for the implantation of subcutaneous tissue cages. Two polypropylene tissue cages were implanted in the lumbar fossa of each side. The sheep were in lateral recumbency and were turned once during the surgery. The animals were divided randomly into 2 groups of five animals; Group 1 (G1) was anaesthetised with propofol (sheep numbers 01 to 05), while group 2 (G2) received propofol and ketamine (sheep numbers 06 to 10). All animals were fasted for 24 hours before each experiment, but left with access to water until just before the induction of anaesthesia.

2.2.2 Drug administration

Animals were premedicated intramuscularly with acepromazine maleate (ACP; C-Vet Ltd, Lancashire), 0.05 mg/kg and papaveretum (Ounopon, Roche, Hertfordshire), 0.4 mg/kg, mixed in the same syringe, 30 min before induction of anaesthesia. An intravenous catheter (Intraflon; Vygon) was placed in both the right and left jugular veins for animals in G1. Two intravenous catheters were placed in the right jugular vein and one in the left jugular vein in all animals in G2. The catheters in the right jugular veins were used for drug administration while the one in the left jugular vein was used for drug sampling.

In G1, anaesthesia was induced with propofol (Rapinovet; Mallinckrodt Veterinary, Cheshire), 4 mg/kg, i.v., and maintained with a variable infusion rate of propofol (0.3-0.5 mg/kg.min). In G2, anaesthesia was induced with propofol 3 mg/kg and ketamine (Vetalar; Parke Davis, Gwent), 1 mg/kg mixed together and given i.v.

Anaesthesia was maintained in this group with infusion rates of propofol (0.2-0.3 mg/kg.min) and ketamine (0.1-0.2 mg/kg.min). The decision to lower the infusion rate was based on the cardiovascular status of each individual animal and on clinical parameters such as the absence of palpebral reflexes, absence of skin twitches and movements to surgical manipulations. The induction drugs were injected over 60 sec and the infusion started immediately after induction.

2.2.3 Sample collection

A control blood sample was taken before induction of anaesthesia. During the infusion period, samples were taken 2, 5, 10, 15, 30, 45 and 60 min after the beginning of the infusion. An extra sample was taken at the end of the infusion in animals in which the infusion lasted for more than 60 min. After the infusion was switched off, blood samples were collected 2, 5, 10, 15, 30, 45, 60, 90, 120, 180, 240, 360, 540, 1320 and 1440 min. Samples (2 ml) were collected into glucose fluoride tubes (Sarstedt Ltd, Leicester, England), mixed well and kept at 4^{o}C until drug assay, which was carried out within three weeks of sample collection.

2.2.4 Anaesthesia Monitoring

Pulse and respiratory rates were measured just before the induction of anaesthesia and were monitored throughout anaesthesia at 5 min intervals. Systolic arterial blood pressure was measured indirectly using a non invasive monitor (DINAMAP, Critikon Canada Inc.) also at 5 min intervals. The cuff for recording blood pressure was placed on a foreleg, just above the carpus. The time to recovery from anaesthesia was recorded from the time the infusion was switched off and was recorded as the time to endotracheal extubation, the time to sternal recumbency and the time to standing unaided.

2.2.5 Analysis of propofol in blood samples

Standards and chemicals

All standards and chemicals were prepared as described previously for *in vitro* studies (see 2.1.5). The external standard solution for HPLC injection containing both propofol (300 ng/ml) and thymol (100 ng/ml) was prepared in the HPLC mobile phase.

2.2.5.1 Extraction of propofol from spikes and samples

The extraction procedure was similar in all aspects to that described for microsomes except that blood (0.5 ml) was used (see 2.1.5.1).

2.2.5.2 Chromatography of propofol

The chromatography was performed as described previously for microsomes (See 2.1.5.2.).

2.2.5.3 Recovery of propofol from blood spikes and samples

Recovery of propofol from blood was calculated the same way as for microsomes (See 2.1.5.3).

2.2.5.6 Intra- and inter-assay coefficient of variation

The reproducibility of the assays was assessed by determination of the coefficient of variation in replicated blood spikes, which were analysed in a single day to obtain the intra-assay (within assay) coefficient of variation. The inter-assay or (between assay) coefficient of variation was obtained by reference to the assays performed on different days.

2.2.5.7 Sensitivity of propofol chromatographic assay

The limit of quantification of propofol in blood samples, corresponding to a peak height of one unit of chart paper (2.5 mm), was 10 ng/ml.

2.2.6 Pharmacokinetic analysis

The data used for pharmacokinetic analysis was obtained by analysing the changes in blood propofol concentration versus time, after a variable infusion rate of the drug which lasted for up to 75 min. All pharmacokinetic parameters were described using non-compartmental analyses of the data.

The mean residence time [MRT_{obs} (h)] is a quantitative estimate of the persistence of a drug in the body, or the time for 63.2% of a drug to be eliminated. It is calculated from $AUMC_{obs}/AUC_{obs}$, where $AUMC_{obs}$ ($\mu g.h^2/ml$) is the total area under the drug concentration time *versus* time or first moment curve, from time zero to last measured concentration, and AUC_{obs} ($\mu g.h/ml$) is the total area under the drug concentration *versus* time or zero moment curve, from time zero to the last measured concentration. The AUC_{obs} was calculated using the trapezoidal rule. The total body clearance, CLb, (ml/kg.min) is the sum of all clearance processes and is calculated from $Dose/AUC_{obs}$. The apparent volume of distribution at steady state concentration, Vdss, (L/kg) is calculated from Dose I.V. * ($AUMC_{obs}/AUC_{obs}$) (Gibaldi & Perrier, 1975).

2.2.7 Statistical analyses

Statistical analyses were carried out using a Mann-Whitney test for pharmacokinetic data, an unpaired t-test for anaesthetic recovery times and a two way analysis of variance with replication for pulse rate, arterial blood pressure and respiratory rates. A post hoc unpaired t-test was carried out for respiratory rates. $P<0.05$ was considered significant. Results are expressed as means ± standard error of the mean (±sem).

3. RESULTS

3.1 Degradation and interaction of propofol with other drugs *in vitro*

The protein and cytochrome P450 (cyto P450) concentrations of rat and sheep hepatic microsome preparations used in all studies are reported in appendices A1 to A5. The protein content of lung microsome preparations is reported in appendix A6. Due to spectrophotometric interference of haemoglobin during cyto P450, determination in microsomes prepared from lung tissue, it was not possible to measure the concentration of this enzymatic system in this tissue.

The mean percentage recovery of propofol microsome standards (range 0.5-10 μg/ml) was 83.70 ± 0.82%. Four sets of standards (range 0.5-10 μg/ml) analysed during the same day showed a within a day coefficient of variation of 6.58 % and when the standards were analysed on different days the between assays coefficient of variation was 9.54 %.

3.1.1 Degradation of propofol by hepatic microsome preparations

When propofol was incubated with inactive microsomes there was no evidence that extra enzymatic degradation of the drug or drug instability during incubation accounted for any of the disappearance of propofol, since concentrations of propofol in boiled microsomes incubated under the same conditions as with active microsomes were in accordance with the initial concentrations used. No peaks were observed when hepatic microsomes were incubated with the vehicle (DMSO) alone.

Propofol was rapidly metabolised by hepatic microsomes with 36% or more metabolised after 5 min of incubation. The extent of substrate degradation was variable depending on the initial concentration of the drug; 86.1, 70.5, 56.9 and 36.6% of propofol was degraded after 5 min of incubation for initial propofol concentrations of 28, 56, 84 and 140 μM respectively (table 3.1.1). The velocity of degradation of propofol varied with different concentrations and decreased, for the same initial concentration, as the time of incubation, increased from 5 to 30 min (table 3.1.2). After 45 min of incubation the concentration of propofol remaining was variable, possibly due to enzyme instability or product inhibition (table 3.1.1). Consequently, data from these incubation times (45 & 60 min), were not used to estimate Vmax and Km. The graphical representation of the velocity (V) plotted against the substrate concentration (S) and the reciprocal of this plot (Lineweaver Burk plot) for 15 min incubation time is shown in figs 3.1.1 & 3.1.2 (substrate concentrations used to obtain these plots are shown in appendix A7).

I.C.	Incubation time (min)						
	5	10	15	20	30	45	60
28	3.9	2.4	1.7	0.8	0.2	0.3	0.1
56	16.5	15.4	10.4	7.2	2.2	4.3	3.3
84	36.2	29.7	26.9	17.6	8.2	12.6	12.1
140	88.8	73.7	65.4	57.8	32.7	36.7	44.4

Table 3.1.1 - Initial propofol concentrations [(I.C) (μM)] and concentrations (μM), remaining after 5, 10, 15, 20, 30, 45 and 60 min of propofol incubation with rat hepatic microsomes. Each result is the mean of 2 determinations from one animal.

prop.	Incubation time				
	5 min	10 min	15min	20 min	30 min
28	0.29	0.15	0.10	0.08	0.06
56	0.47	0.24	0.18	0.15	0.11
84	0.57	0.32	0.23	0.20	0.15
140	0.61	0.40	0.30	0.24	0.21

Table 3.1.2 - The velocity (enzyme activity; μmoles/min.mg of protein) of the degradation of different concentrations of propofol (prop.; μM), when incubated for different times with hepatic microsome preparations. Each result is the mean of two determinations from one animal.

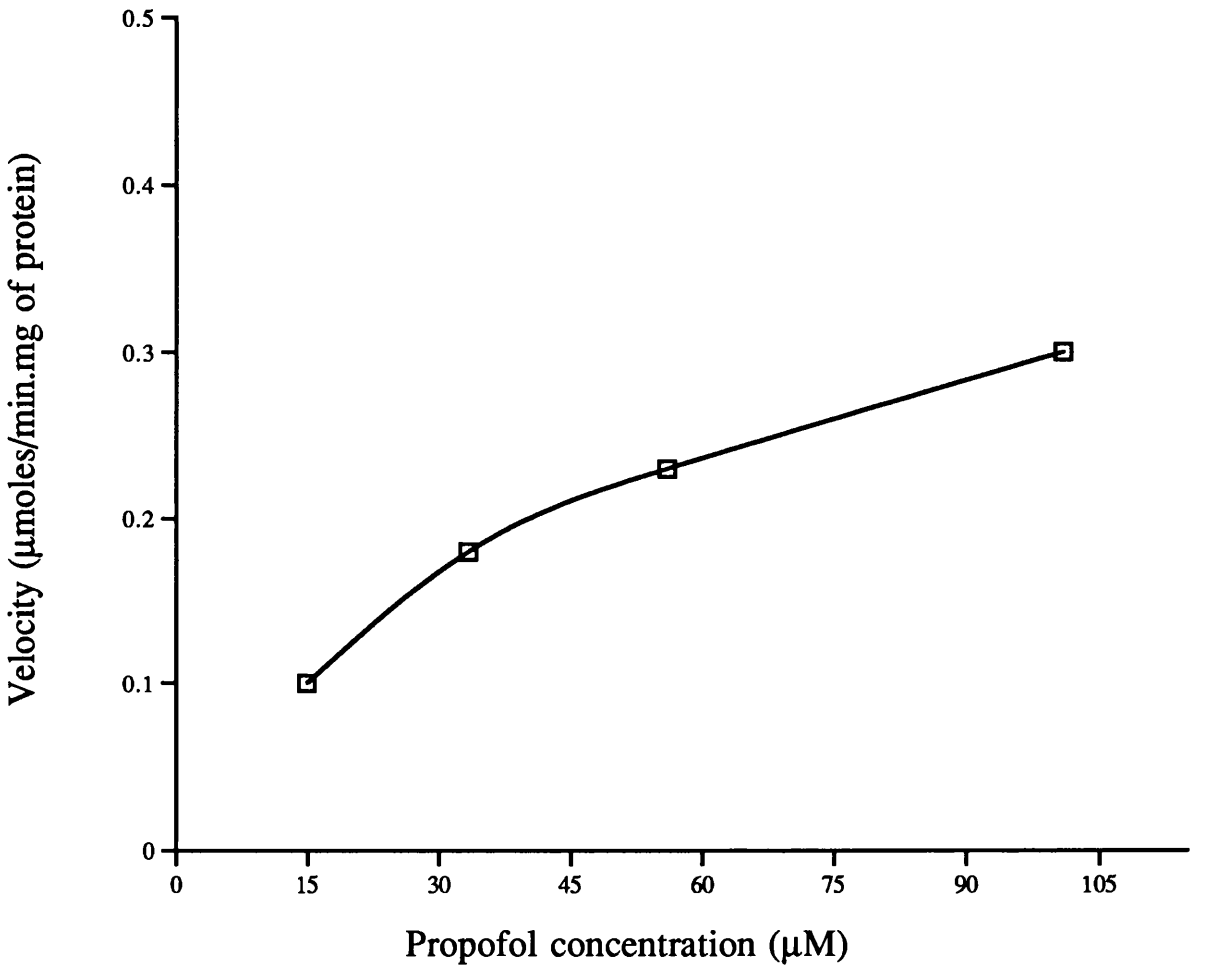

Fig 3.1.1 - Velocity of propofol degradation plotted against propofol concentration for rat liver microsomes incubated for 15 min. Each point is the mean of two determinations from one rat.

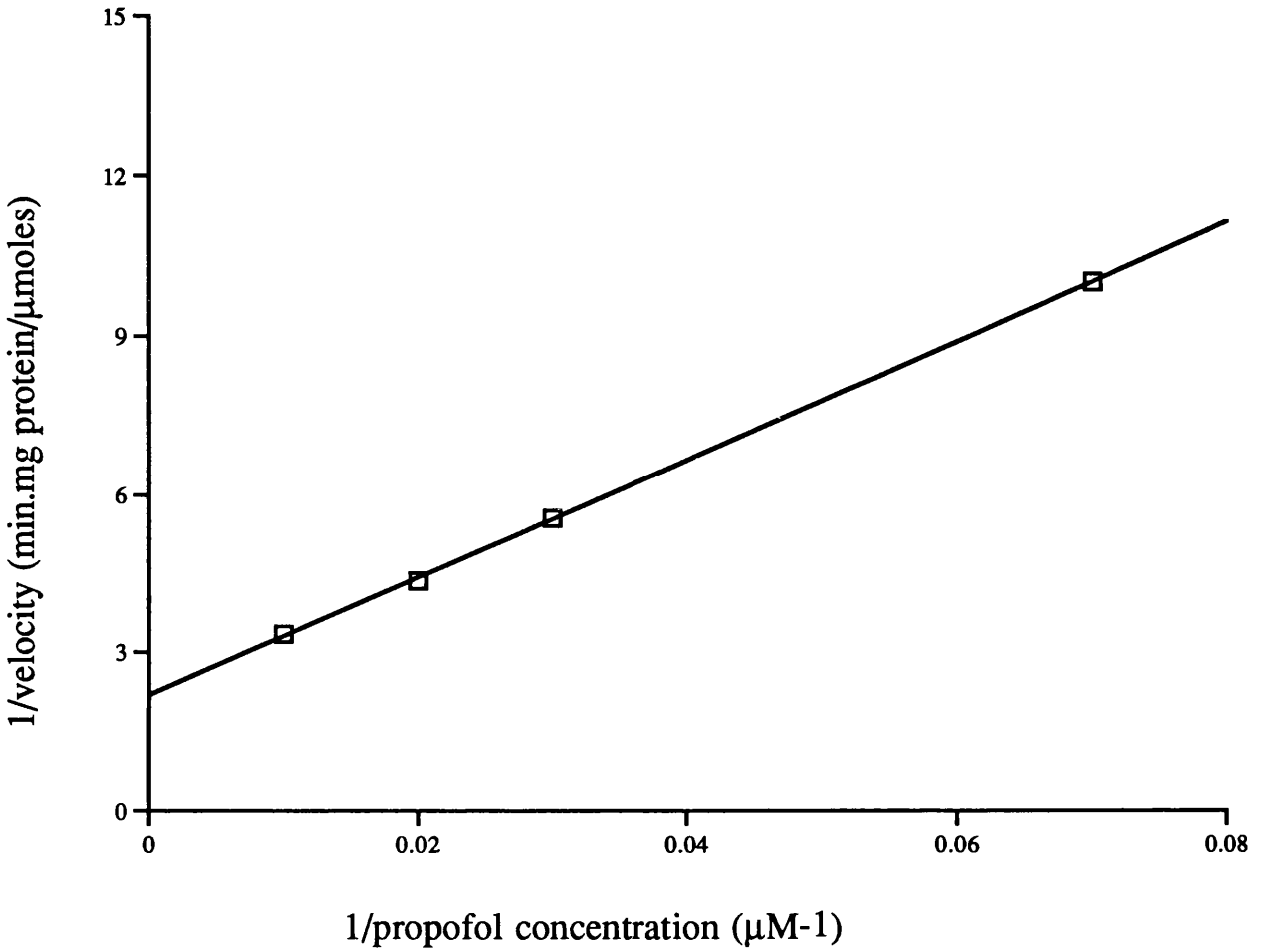

Fig 3.1.2- Lineweaver Burk plot for propofol incubated for 15 min with rat hepatic microsomes. Each point is the mean of two determinations from one rat.

The maximal velocity (Vmax; μmoles/min/mg of protein) and the Michaelis constant (Km; μM) obtained were, 0.6/34.5; 0.53/37.6; 0.46/52.6; 0.41/58.8 and 0.36/71.4 for 5, 10, 15. 20 and 30 min of incubation respectively. They were obtained by extrapolation from the Lineweaver Burk plots (Segel, 1975).

For all subsequent studies, the time of incubation was set at 15 min. This time was chosen in order to study drug interactions accurately although, the proportion of propofol degraded at this was relatively large (see discussion).

time

3.1.2 Effect of propofol on cytochrome P450 activity

The production of formaldehyde from the oxidative N-demethylation of aminopyrine was inhibited dose-dependently by propofol. There was no detectable production of formaldehyde when inactivated microsomes were substituted for active microsomes in the incubation. The concentration of formaldehyde produced in the presence of DMSO (vehicle), in the presence of increasing concentrations of propofol and in the absence of both is shown in table 3.1.3 and illustrated in Fig 3.1.3. The presence of the vehicle alone reduced the production of formaldehyde by 18.5 ± 2.2% (n=6), but this difference was not statistically significant ($P>0.05$). Propofol decreased the mean production of formaldehyde by 21 ± 2.7 to 60.6 ± 3.1%, dependent on the concentration of propofol present (table 3.1.4). The individual velocities of the production of formaldehyde are shown in table 3.1.5, the means varying from 0.09 ± 0.01 to 0.04 ± 0.01 μmoles/min.mg of protein, dependent on the concentration of propofol present. The concentration of formaldehyde produced in the presence of different concentrations of propofol were assessed statistically against the production of formaldehyde in the presence of DMSO only. Concentrations of propofol as small as 5.61 μM did not significantly alter the production of formaldehyde ($P>0.05$), but concentrations equal to or greater than 56 μM significantly affected the reaction, with $P<0.0001$ for 56 μM; $P<0.001$ for 280 μM and $P<0.0005$ for 560 μM. These data indicated that propofol has potential to inhibit the activity of cyto P450 enzymatic system.

	R1	R2	R3	R4	R5	R6	mean	±sem
control	0.14	0.27	0.34	0.15	0.23	0.23	0.23	0.03
dmso	0.11	0.22	0.3	0.11	0.20	0.19	0.19	0.03
5.6	0.11	0.23	0.28	0.10	0.19	0.18	0.18	0.03
56	0.08	0.18	0.25	0.07	0.15	0.14	0.15	0.03
280	0.06	0.15	0.18	0.06	0.10	0.08	0.11	0.02
561	0.05	0.12	0.17	0.05	0.10	0.07	0.09	0.02

Table 3.1.3 - Concentration of formaldehyde (mM) produced from the N-demethylation of aminopyrine by rat hepatic microsomes incubated in the absence (control) and in the presence of 5.6, 56, 280 and 561 μM of propofol in dmso (40 μl) and of dimethylsulfoxide alone (dmso; 40 μl). R1 and R4 were Wistar rats while R2, R3, R5 and R6 were Sprague Dawley rats. Each result is the mean of two determinations from one rat.

	R1	R2	R3	R4	R5	R6	mean	±sem
dmso	21.4	18.5	11.8	26.7	13.1	17.4	18.5	2.2
5.6	21.4	14.8	17.7	33.3	17.4	21.7	21.0	2.7
56	42.9	33.3	26.5	33.3	34.8	39.1	34.9*	2.2
280	57.2	44.5	47.1	60.0	56.5	65.2	55.1•	3.2
561	64.3	55.6	50.0	66.7	56.5	69.6	60.4[a]	3.1

Table 3.1.4 - Percentages inhibition of the production of formaldehyde from the N-demethylation of aminopyrine by rat hepatic microsomes, in the presence of dimethylsulfoxide, (dmso, 40 μl) and of increasing concentrations (5.6, 56, 280 and 561 μM) of propofol (40 μl) dissolved in dmso. Each result is the mean of two determinations from one rat. (*$P<0.0001$; •$P<0.001$; [a]$P<0.0005$, compared with dmso alone).

	R1	R2	R3	R4	R5	R6	mean	±sem
control	0.06	0.11	0.14	0.06	0.09	0.09	0.09	0.01
dmso	0.04	0.09	0.12	0.04	0.08	0.08	0.08	0.01
5.6	0.04	0.09	0.11	0.04	0.08	0.07	0.07	0.01
56	0.03	0.07	0.10	0.03	0.06	0.06	0.06	0.01
280	0.02	0.06	0.07	0.02	0.04	0.03	0.04	0.01
561	0.02	0.05	0.07	0.02	0.04	0.03	0.04	0.01

Table 3.1.5 - The velocity (μmoles/min.mg of protein) of the production of formaldehyde from the N-demethylation of aminopyrine by rat hepatic microsomes, incubated for 15 min, in the presence of dimethylsulfoxide (dmso) alone, of increasing concentrations of propofol in dmso (5.6, 56, 280 and 561 μM) and in the absence of both (control). Each result is the mean of two determinations from one rat.

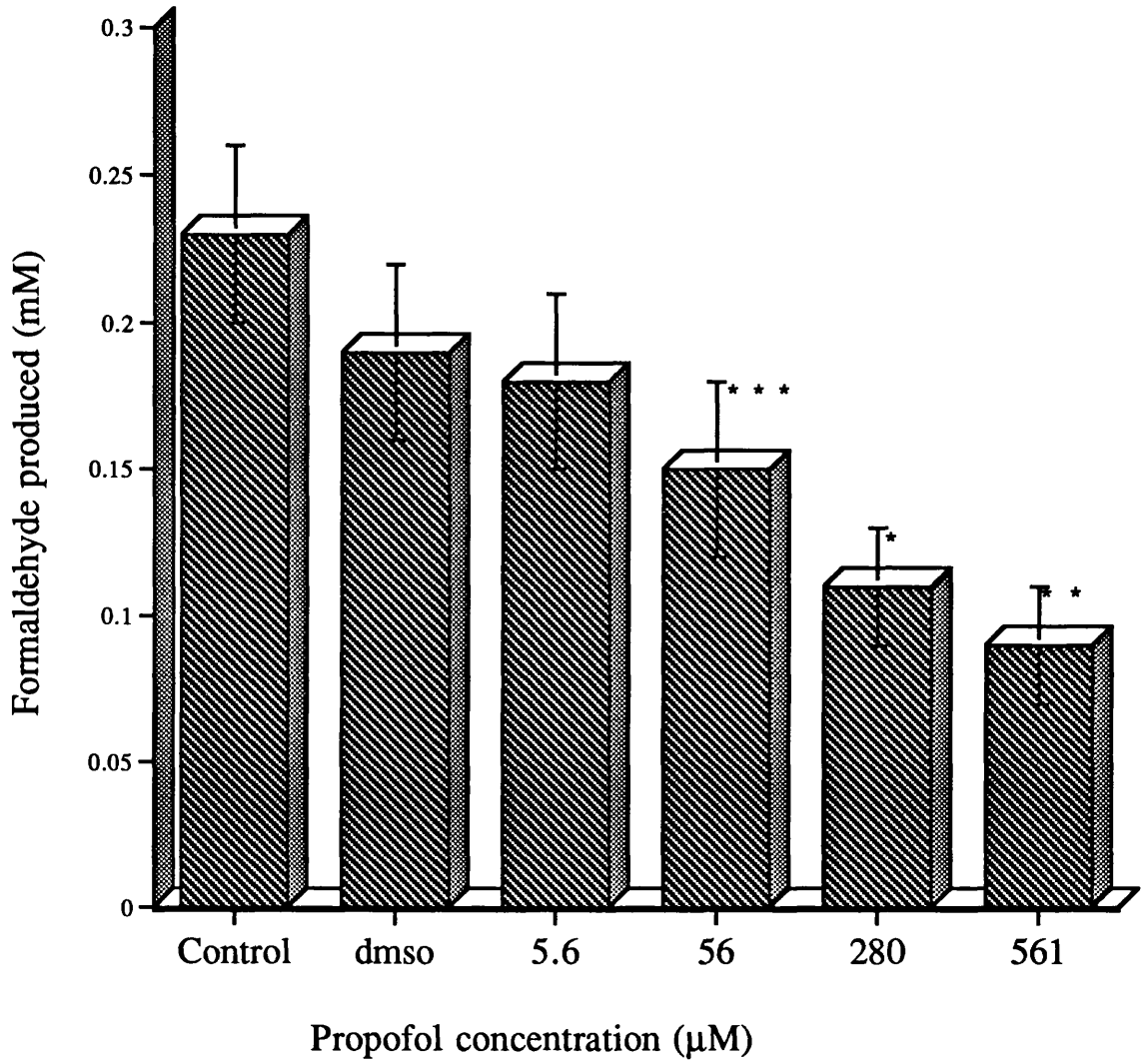

Fig 3.1.3 -The concentration of formaldehyde produced from the N-demethylation of aminopyrine by rat hepatic microsomes in the presence of dimethylsulfoxide (dmso); 5.6, 56, 280 and 561 μM of propofol in dmso or in the absence of both (Control). Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate. *P<0.001; **P<0.0005; ***P<0.0001(compared with dmso).

3.1.3. Extra cytochrome P450 degradation of propofol

Inhibition of cyto P450 with 1-aminobenzontriazole (ABT) caused a significant ($P<0.0001$) decrease in propofol degradation. The mean percentages inhibition of propofol degradation were 75.4, 57.2, 47 and 29.5% which were recorded for initial propofol concentrations of 28, 56, 84 and 140 μM respectively. Table 3.1.6 presents the concentration of propofol remaining after 15 min incubation, for the three different treatments (propofol alone, propofol with ABT and propofol with inactive microsomes). The effect of cyto P450 inhibition with ABT and the presence of inactive microsomes on propofol degradation is demonstrated in fig 3.1.4. The velocity of degradation of propofol was also significantly decreased in the presence of ABT ($p<0.001$). The mean velocities (μmoles/min.mg of protein) were 0.09 ± 0.00; 0.13 ± 0.01; 0.16 ± 0.02 and 0.18 ± 0.02 in the absence of ABT and 0.02 ± 0.00; 0.02 ± 0.01; 0.04 ± 0.00 and 0.04 ± 0.01 in the presence of ABT for propofol concentrations of 28, 56, 84 and 140 μM, respectively, ($n=6$) (table 3.1.7).The velocity of propofol degradation plotted against substrate concentration for this inhibition is illustrated in fig 3.1.5. (concentrations of substrate used for this plot are in appendix A8).

During this assay, two different strains of rat were used, 3 Wistar rats (R1, R3 and R4) and 3 Sprague Dawley rats (R2, R5 and R6). Microsomes from Sprague Dawley rats degraded propofol significantly ($P<0.05$) faster than those obtained from Wistar rats (table 3.1.6 & 3.1.7).

These data indicate that there is no extra enzymatic degradation of propofol or evidence of propofol instability when incubated with inactive microsomes. The lack of difference between initial concentrations of propofol and concentrations remaining after incubation with ABT indicates that propofol does not undergo extra cyto P450 metabolism.

3.1.4 The effect of other drugs on the degradation of propofol by rat hepatic microsomes (Sprague Dawley)

The concentration of propofol remaining after 15 min incubation with and without ketamine, alfentanil or aminopyrine are shown in tables 3.1.8, 3.1.9 & 3.1.10 and illustrated in figs 3.1.6, 3.1.7 & 3.1.8 respectively.

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	8.2	3.2	8.3	7.1	1.9	3.5	5.4	1.1
56	26.5	17.9	64.6	28.9	13.3	17.8	28.2	7.7
84	50.4	39.2	57.4	50.8	31.1	36.3	44.2	4.1
140	98.2	88.6	114.4	96.8	80.1	85.3	93.9	5.0
28•	21.5	21.2	26.2	25.7	26.9	23.6	24.2	1.0
56•	47.1	48.2	56.8	52.7	50.7	45.9	50.2	1.6
84•	71.4	75.7	75.6	75.2	78.5	71.0	74.6	1.2
140•	133.6	130.8	141.7	131.7	126.5	120.7	130.8	2.9
28*	29	31.8	27.2	28.7	27.2	27.1	28.5	0.7
56*	58.2	62.5	60.8	57.4	58.5	53	58.4	1.3
84*	83.4	90.8	90.6	82.9	85.1	82.6	85.9	1.6
140*	136.4	143.4	139.9	139.8	146.9	140.7	141.4	2.6

Table 3.1.6 - Initial propofol concentrations (I.C.; μM) and concentrations (μM) remaining after incubation of rat hepatic microsomes for 15 min, with propofol only, propofol and 1-aminobenzontriazole (•) 12 mM, or propofol with inactivated microsomes (*). R1, R3 and R4 were Wistar rats, while R2, R5 and R6 were Sprague Dawley rats. Each result is the mean of two determinations from one rat.

prop	R1	R2	R3	R4	R5	R6	mean	±sem
28	0.08	0.1	0.08	0.08	0.10	0.10	0.09	0.00
56	0.12	0.15	0.08	0.11	0.17	0.15	0.13	0.01
84	0.13	0.18	0.11	0.13	0.21	0.19	0.16	0.02
140	0.17	0.20	0.10	0.17	0.24	0.22	0.18	0.02
28•	0.03	0.03	0.01	0.01	0.001	0.02	0.02*	0.00
56•	0.04	0.03	0.00	0.01	0.02	0.04	0.02*	0.01
84•	0.05	0.03	0.03	0.04	0.02	0.05	0.04*	0.00
140•	0.03	0.04	0.00	0.03	0.05	0.09	0.04*	0.01

Table 3.1.7 - The velocity (enzyme activity; μmoles/min.mg of protein) of the degradation of propofol (prop. 28, 56, 84 and 140 μM), incubated with hepatic microsome preparations for 15 min, in the absence and in presence (•) of 1-aminobenzotriazole (ABT, 12 mM). Each result is the mean of two determinations from one rat. (*P<0.001).

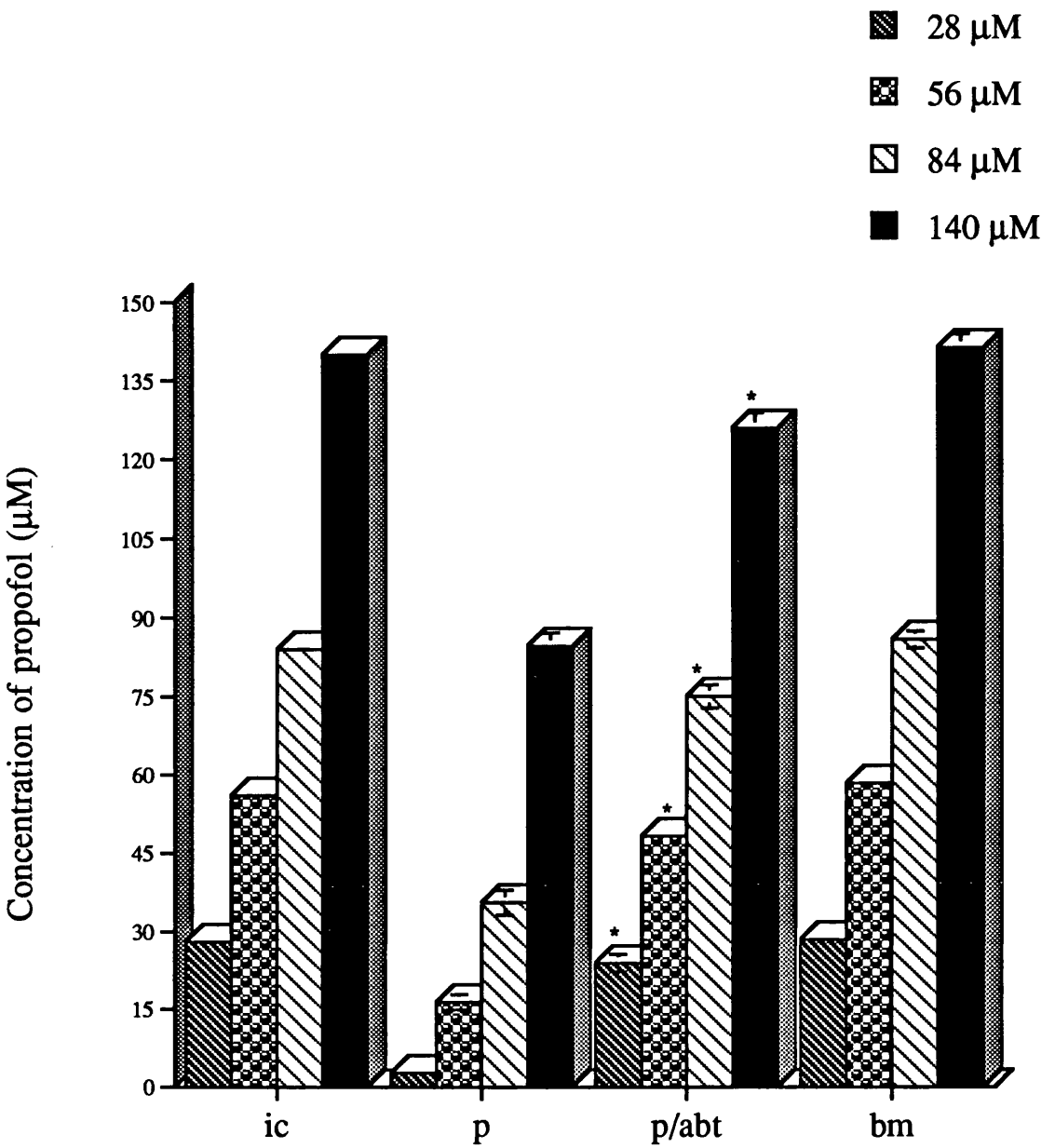

Fig. 3.1.4- Initial propofol concetrations (ic) and propofol concentrations remaining after incubation of rat hepatic microsome preparations with variable concentrations of propofol (p), propofol and 1-aminobenzotriazole (p/abt) and propofol in the presence of inactivated microsomes (bm). The incubation time was 15 min. The data is shown as the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate. *P<0.0001(p compared to p/abt).

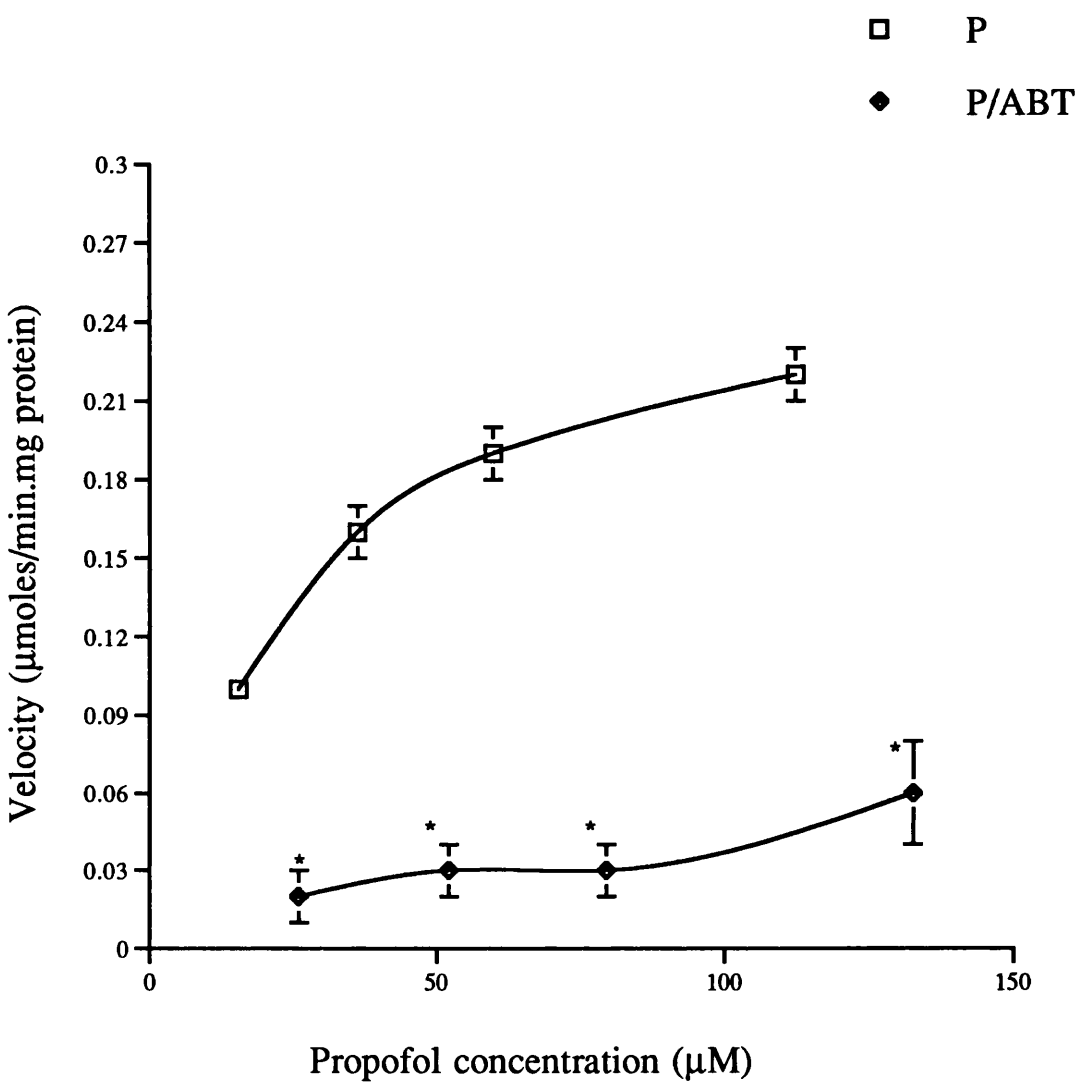

Fig 3.1.5- The velocity of propofol degradation plotted against propofol concentration for rat hepatic microsomes incubated with propofol alone (P) or with propofol and 1-aminobenzotriazole (P/ABT), for 15 min. Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate. *P<0.001(P compared to P/ABT).

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	0.3	1.5	1.5	1.7	1.5	1.3	1.3	0.2
56	1.8	8	9.8	12.4	8.4	8.3	8.1	1.4
84	8.6	19.8	26.9	24.9	24.1	20.5	20.8	2.7
140	32.0	55.2	66.9	69.8	62.1	59.2	57.5	5.5
28*	0.5	1.7	1.7	1.9	2.2	1.8	1.6	0.2
56*	3.5	8.4	11.2	13.6	10.38	9.5	9.4	1.4
84*	10.1	20.9	26.3	28.2	25.2	22.2	22.1	2.6
140*	33.7	52.8	66.4	70.8	60.0	55.7	56.6	5.3

Table 3.1.8 - Initial propofol concentration (I.C.; μM) and propofol concentrations (μM) remaining after incubation of rat hepatic microsome preparations for 15 min, with propofol only or propofol and ketamine (*). Each result is the mean of two determinations from one rat.

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	2.4	3.8	4.8	1.2	1.9	3.3	2.7	0.5
56	12.8	15.8	18.3	7.8	10.4	16.2	13.5	1.6
84	34.6	39.1	35.7	17.1	25.6	32.3	30.7	3.3
140	89.4	109.9	81.7	46.9	65.4	75.6	78.1	8.7
28*	2.4	3.2	5.0	1.3	2.2	3.5	2.9	0.5
56*	13.2	15.4	16.2	7.9	10.8	13.5	12.8	1.2
84*	33.8	40.2	34.2	17.1	22.9	30.5	29.8	3.4
140*	94.61	107.4	80.1	41.8	65.0	76.8	77.6	9.3

Table 3.1.9 - Initial propofol concentration (I.C.; μM) and propofol concentrations (μM) remaining after incubation of rat hepatic microsome preparations for 15 min, with propofol only or propofol and alfentanil (*). Each result is the mean of two determinations from one rat.

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	1.6	1.6	1.6	2.0	1.5	1.1	1.6	0.1
56	11.3	10.9	9.8	12.7	10.1	7.4	10.4	0.7
84	25.9	34.6	21.7	27.9	25.8	16.9	25.5	2.4
140	68.2	81.6	57.6	83.7	64.6	45.3	66.8	5.9
28*	6.7	7.3	6.1	8.8	5.8	5.7	6.7	0.5
56*	19.6	22.7	18.2	25.4	15.1	14.7	19.3	1.7
84*	37.0	43.9	32.6	40	34.8	27.3	35.9	2.4
140*	78.1	89.9	78.8	88.1	74.0	58.1	77.8	4.7

Table 3.1.10 - Initial propofol concentration (I.C.; μM) and propofol concentrations (μM) remaining after incubation of rat hepatic microsome preparations for 15 min, with propofol only or propofol and aminopyrine (*). Each result is the mean of two determinations from one rat.

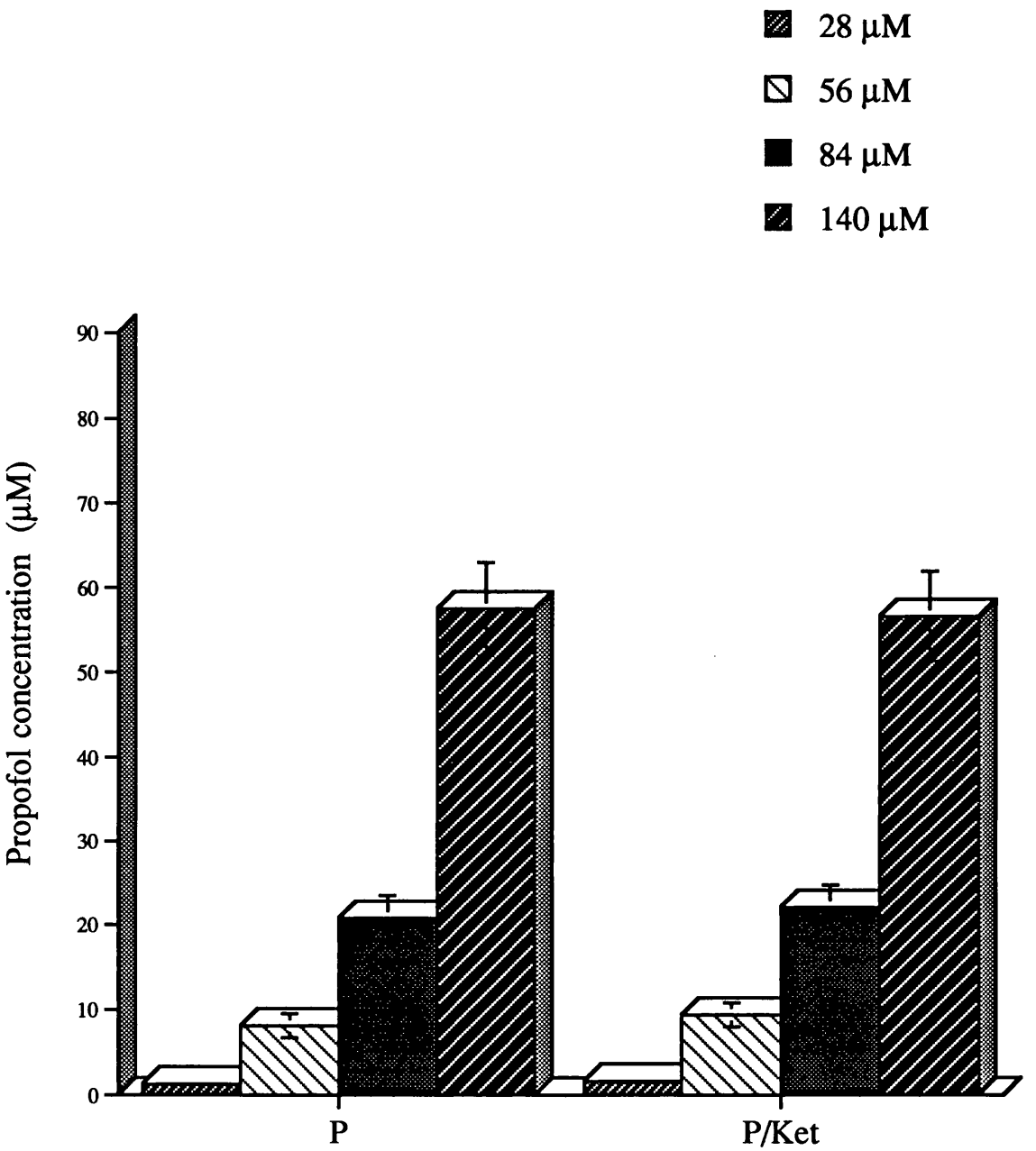

Fig 3.1.6 - The concentration of propofol remaining after 15 min incubation of rat hepatic microsomes with propofol (28, 56, 84 and 140 µM) alone (P) or in the presence of ketamine, 5 µg/ml (P/Ket). Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate.

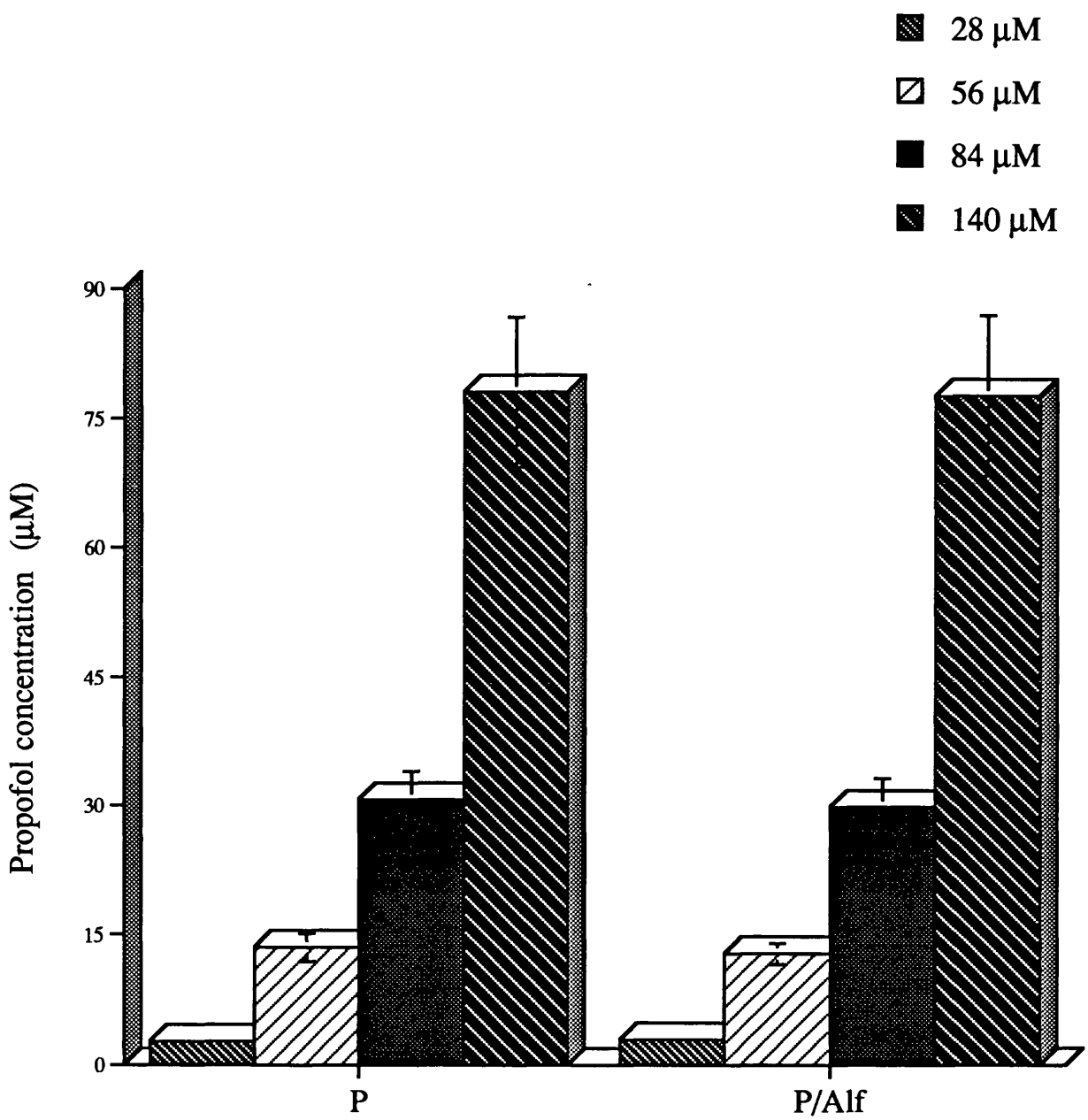

Fig 3.1.7 - The concentration of propofol remaining after 15 min incubation of rat hepatic microsomes with propofol (28, 56, 84 and 140 µM) alone (P) or in the presence of alfentanil, 200 ng/ml (P/Alf). Each point is the mean (+/-sem) of microsome preparations from six rats and each rat was assayed in duplicate.

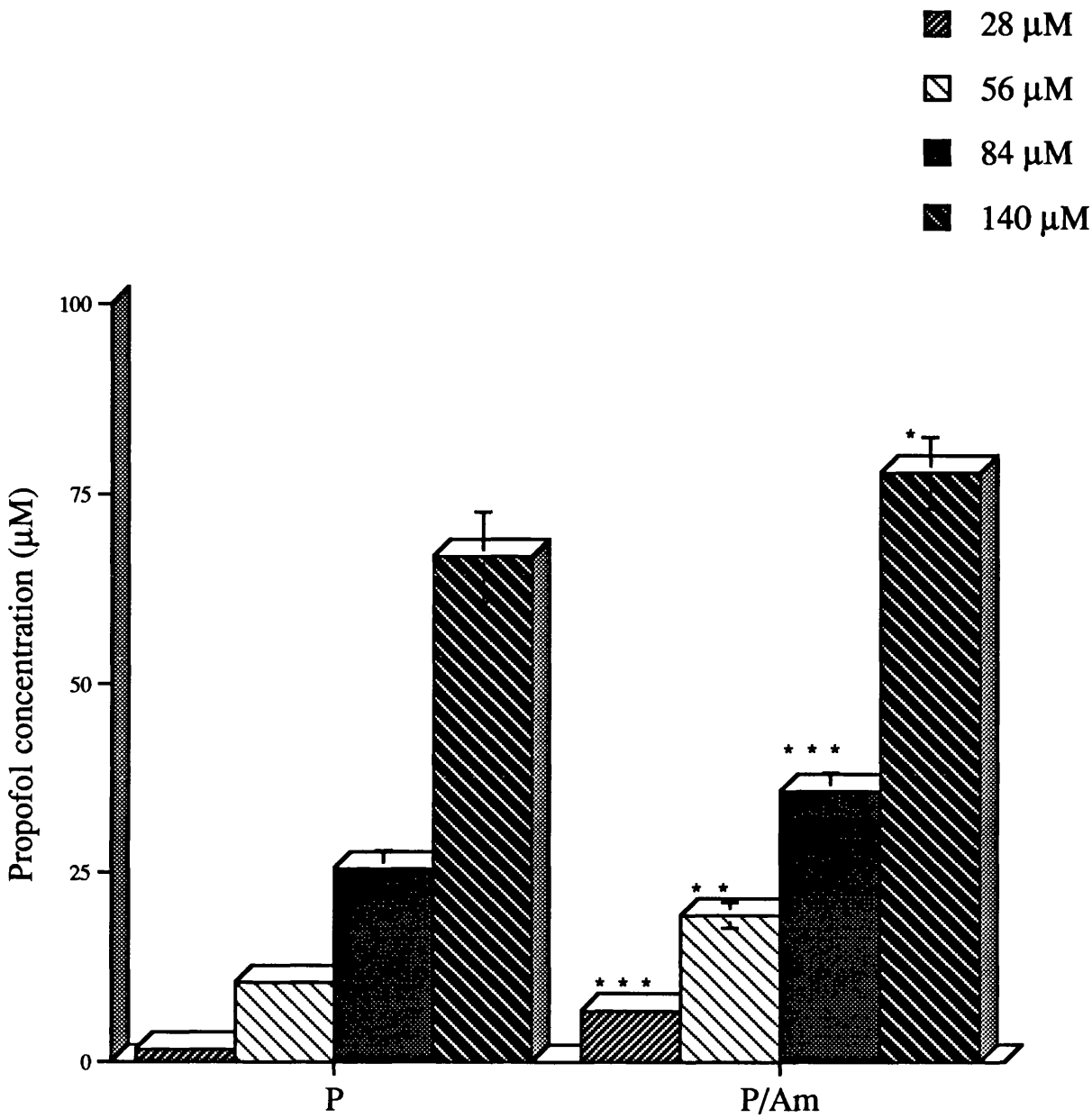

Fig 3.1.8 - The concentration of propofol remaining after 15 min incubation of rat hepatic microsomes with propofol (28, 56, 84 and 140 µM) alone (P), or in the presence of aminopyrine, 5 mM (P/Am). Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate. *P<0.005; **P<0.001; ***P<0.0001(comparison made between P and P/Am data at each concentration.

Ketamine (5 μg/ml) and alfentanil (200 ng/ml) did not significantly alter the cyto P450 rate of degradation of propofol ($P>0.05$), but the rate of propofol (28, 56, 84 & 140 μM), degradation was significantly decreased by aminopyrine, 5 mM ($P<0.001$). The mean velocities decreased from a range 0.11 ± 0.00 & 0.29 ± 0.02 to a range 0.09 ± 0.00 & 0.25 ± 0.02. Post hoc "t" test analysis showed significance values of $P<0.0001$ for 28 μM, $P<0.001$ for 56 μM, $P<0.0001$ for 84 μM and $P<0.005$ for 140 μM of propofol. The mean velocities (μmoles/min.mg of protein) of propofol degradation for concentrations of 28, 56, 84 and 140 μM respectively were 0.11 ± 0.01, 0.19 ± 0.01, 0.25 ± 0.01 and 0.33 ± 0.02 in the absence of, and 0.10 ± 0.00, 0.19 ± 0.01, 0.25 ± 0.01 and 0.33 ± 0.02 μmoles/min/mg protein in the presence of ketamine (table 3.1.11). The mean velocities of propofol degradation in the absence and presence of alfentanil were respectively 0.10 ± 0.00, 0.17 ± 0.01, 0.21 ± 0.01, 0.25 ± 0.03 and 0.10 ± 0.00, 0.17 ± 0.00, 0.20 ± 0.01, 0.22 ± 0.02 μmoles/min/mg protein (table 3.1.12). The mean velocities of propofol degradation in absence and presence of aminopyrine were 0.11 ± 0.00, 0.18 ± 0.01, 0.23 ± 0.01, 0.29 ± 0.02 and 0.09 ± 0.00, 0.14 ± 0.01, 0.20 ± 0.01, 0.25 ± 0.02 μmoles/min/mg protein. (table 3.1.13). Figs 3.1.9 to 3.1.11 show the velocities of propofol degradation plotted against substrate concentrations. The Lineweaver Burk plots for the three drugs are shown in figs 3.1.12 to 3.1.14 (substrate concentrations used for these plots are presented in appendix A9, A10 and A11).The mean maximal velocities (Vmax, μmoles/min.mig protein) were respectively, 0.52 ± 0.08 & 0.56 ± 0.06 for propofol and propofol/ketamine; 0.43 ± 0.04 & 0.39 ± 0.03 for propofol and propofol/alfentanil and 0.42 ± 0.04 & 0.43 ± 0.06 for propofol and propofol/aminopyrine. The mean Michaelis constants (Km, μM) were respectively 46.5 ± 2.5 & 53.2 ± 3.2 for propofol and propofol/ketamine (table 3.1.14); 48.9 ± 2.2 & 48.7 ± 4.4 for propofol and propofol/alfentanil (table 3.1.15) and 43.6 ± 5.0 & 66.2 ± 7.6 for propofol and propofol/aminopyrine (table 3.1.16). The Vmax for propofol incubated alone was not significantly altered from the Vmax obtained for incubations of propofol with the three different drugs ($P>0.05$), however, the Km was significantly increased from 43.6 μM to 66.2 μM when propofol was incubated without and with aminopyrine respectively ($P<0.05$). These data indicate that aminopyrine is acting as a competitive inhibitor of propofol metabolism.

prop.	R1	R2	R3	R4	R5	R6	mean	±sem
28	0.11	0.11	0.11	0.10	0.11	0.11	0.11	0.01
56	0.22	0.19	0.18	0.17	0.19	0.19	0.19	0.01
84	0.30	0.25	0.23	0.23	0.24	0.25	0.25	0.01
140	0.43	0.34	0.29	0.28	0.31	0.32	0.33	0.02
28*	0.11	0.10	0.10	0.10	0.10	0.10	0.10	0.00
56*	0.21	0.19	0.18	0.17	0.19	0.18	0.19	0.01
84*	0.29	0.25	0.23	0.22	0.23	0.25	0.25	0.01
140*	0.42	0.35	0.29	0.28	0.32	0.33	0.33	0.02

Table 3.1.11 - The velocity (μmoles/min.mg of protein) of the degradation of propofol (prop 28, 56, 84 and 140 μM), incubated with rat hepatic microsome preparations for 15 min, in the absence and in the presence (*) of ketamine (5 μg/ml). Each result is the mean of two determinations from one rat.

prop.	R1	R2	R3	R4	R5	R6	mean	±sem
28	0.10	0.10	0.09	0.11	0.10	0.10	0.10	0.00
56	0.17	0.16	0.15	0.19	0.18	0.16	0.17	0.01
84	0.20	0.18	0.19	0.27	0.23	0.21	0.21	0.01
140	0.20	0.12	0.23	0.37	0.30	0.26	0.25	0.03
28*	0.10	0.10	0.09	0.11	0.10	0.25	0.10	0.00
56*	0.17	0.16	0.16	0.19	0.18	0.21	0.17	0.00
84*	0.20	0.17	0.20	0.20	0.24	0.17	0.20	0.01
140*	0.18	0.13	0.24	0.24	0.30	0.10	0.22	0.02

Table 3.1.12 - The velocity (μmoles/min.mg of protein) of the degradation of propofol (prop 28, 56, 84 and 140 μM), incubated with rat hepatic microsome preparations for 15 min, in the absence and in the presence (*) alfentanil (200 ng/ml). Each result is the mean of two determinations from one rat.

prop.	R1	R2	R3	R4	R5	R6	mean	±sem
28	0.10	0.10	0.11	0.10	0.11	0.11	0.11	0.00
56	0.18	0.18	0.18	0.17	0.18	0.19	0.18	0.00
84	0.23	0.20	0.25	0.20	0.23	0.27	0.23	0.01
140	0.29	0.23	0.33	0.22	0.30	0.38	0.29	0.02
28*	0.08	0.08	0.09	0.08	0.09	0.09	0.09	0.00
56*	0.14	0.13	0.15	0.12	0.16	0.16	0.14	0.01
84*	0.19	0.16	0.20	0.18	0.20	0.24	0.20	0.01
140*	0.25	0.20	0.24	0.21	0.26	0.33	0.25	0.02

Table 3.1.13 - The velocity (µmoles/min.mg of protein) of the degradation of propofol (prop. 28, 56, 84 and 140 µM), incubated with rat hepatic microsome preparations for 15 min, in the absence and in the presence (*) of aminopyrine (5 mM). Each result is the mean of two determinations from one rat.

	propofol		propofol/ketamine	
	Vmax	Km	Vmax	Km
R1	0.89	55.0	0.78	65
R2	0.50	50.9	0.61	50
R3	0.39	36.9	0.44	49
R4	0.41	45.0	0.40	43
R5	0.44	43.6	0.51	56
R6	0.47	47.4	0.63	56
mean	0.52	46.5	0.56	53.2
±sem	0.08	2.5	0.06	3.2

Table 3.1.14- Maximal velocity (Vmax; µmoles/min.mg protein) and Michaelis constant (Km; µM) for the degradation of propofol by rat hepatic microsome preparations incubated for 15 min with and without ketamine. Each result is the mean of two determinations from one rat.

	propofol		propofol/alfentanil	
	Vmax	Km	Vmax	Km
R1	0.44	45	0.44	56.3
R2	0.32	40	0.29	33.3
R3	0.37	51	0.42	60.8
R4	0.58	53	0.31	38.0
R5	0.46	51	0.48	53.8
R6	0.41	51	0.41	49.9
mean	0.43	48.9	0.39	48.7
±sem	0.04	2.2	0.03	4.4

Table 3.1.15- Maximal velocity (Vmax; μmoles/min.mg protein) and Michaelis constant (Km; μM) for the degradation of propofol by rat hepatic microsome preparations incubated for 15 min with and without alfentanil. Each result is the mean of two determinations from one rat.

	propofol		propofol/aminopyrine	
	Vmax	Km	Vmax	Km
R1	0.44	49.1	0.49	85.2
R2	0.32	31.4	0.30	45.9
R3	0.47	47.0	0.41	58.2
R4	0.30	32.0	0.27	48.8
R5	0.40	38.5	0.47	69.1
R6	0.59	63.7	0.65	90
mean	0.42	43.6	0.43	66.2*
±sem	0.04	5.0	0.06	7.6

Table 3.1.16- Maximal velocity (Vmax; μmoles/min.mg protein) and Michaelis constant (Km; μM) for the degradation of propofol by rat hepatic microsome preparations incubated for 15 min with and without aminopyrine. (*$P<0.05$). Each result is the mean of two determinations from one rat.

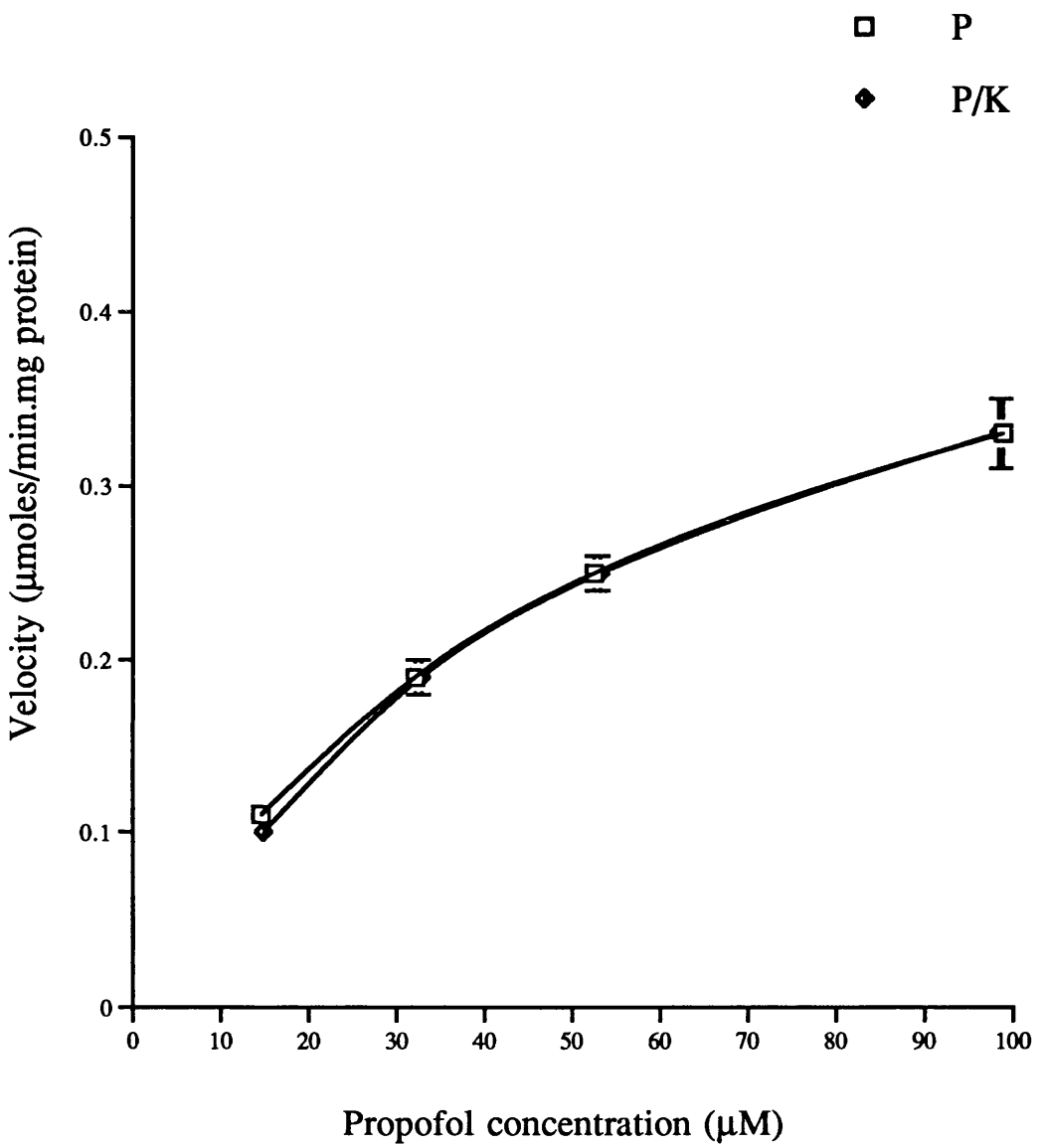

Fig 3.1.9 -The velocity of propofol degradation plotted against propofol concentrations for rat hepatic microsomes incubated for 15 min with propofol alone (P) or with propofol and ketamine, 5 µg/ml, (P/K). Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate.

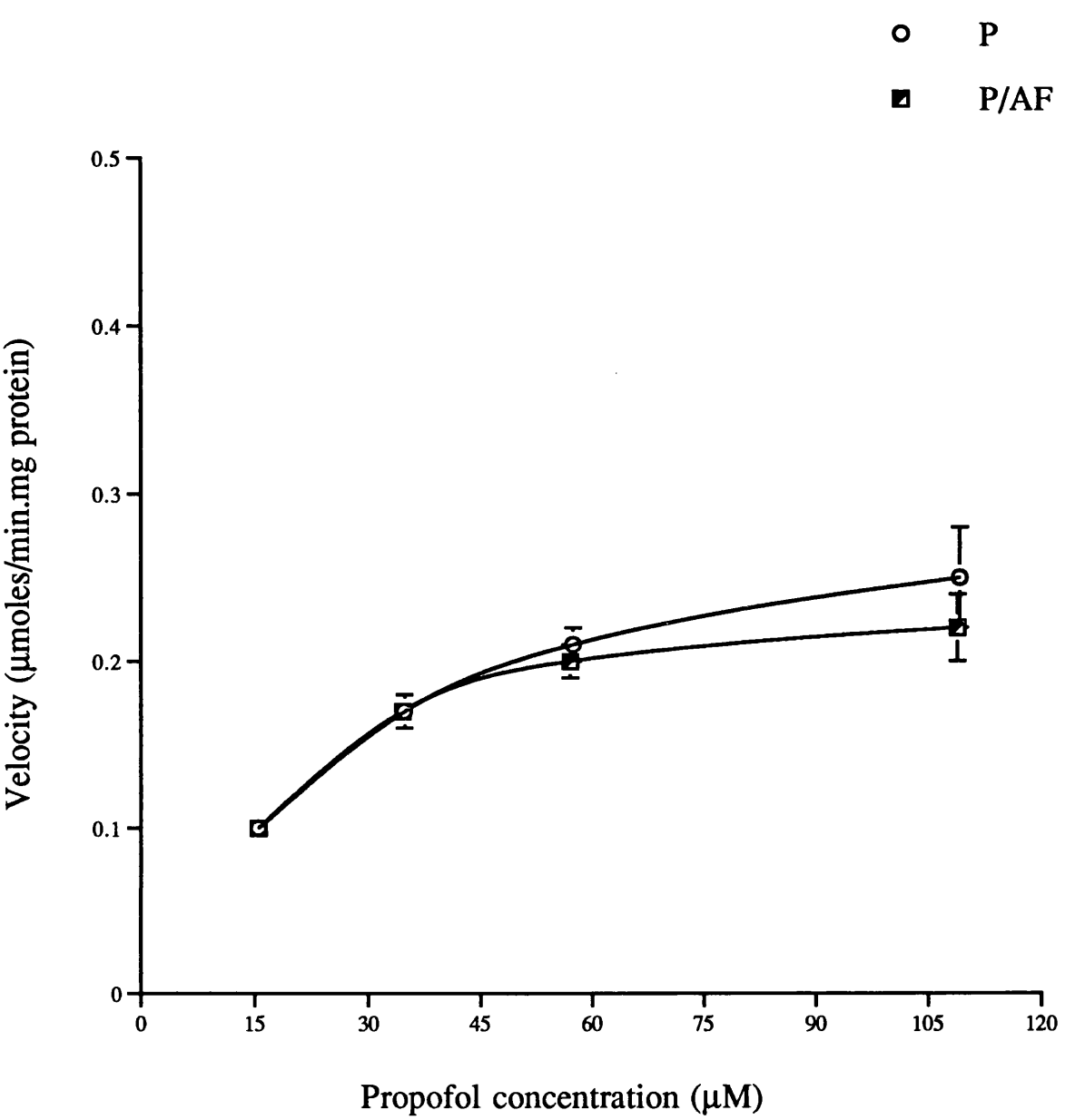

Fig 3.1.10 -The velocity of propofol degradation plotted against propofol concentrations for rat hepatic microsomes incubated for 15 min with propofol alone (P) or with propofol and alfentanil, 200 ng/ml (P/AF). Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate.

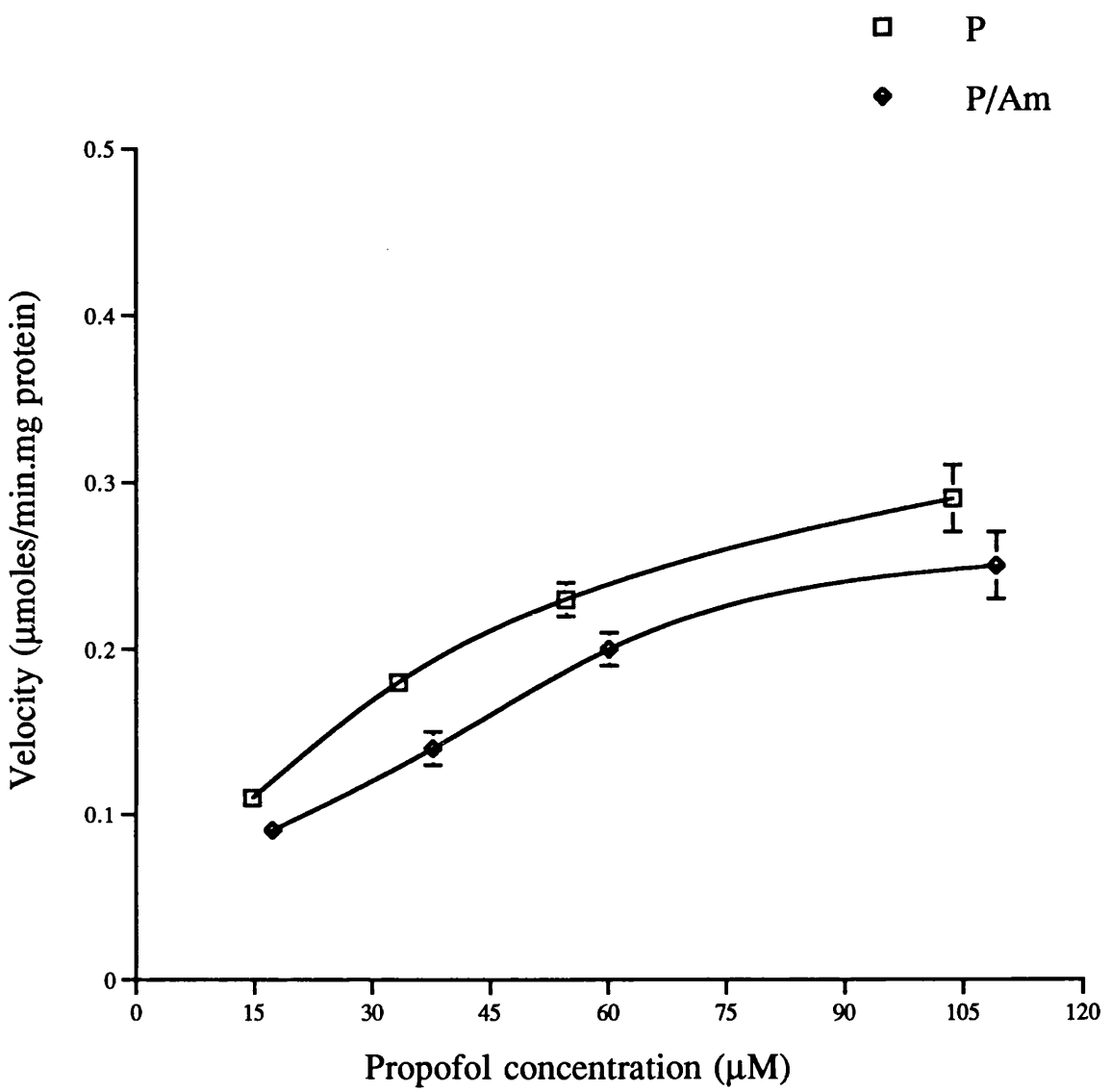

Fig 3.1.11 -The velocity of propofol degradation plotted against propofol concentrations for rat hepatic microsomes incubated for 15 min with propofol alone (P) or with propofol and aminopyrine, 12 mM, (P/Am). Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate.

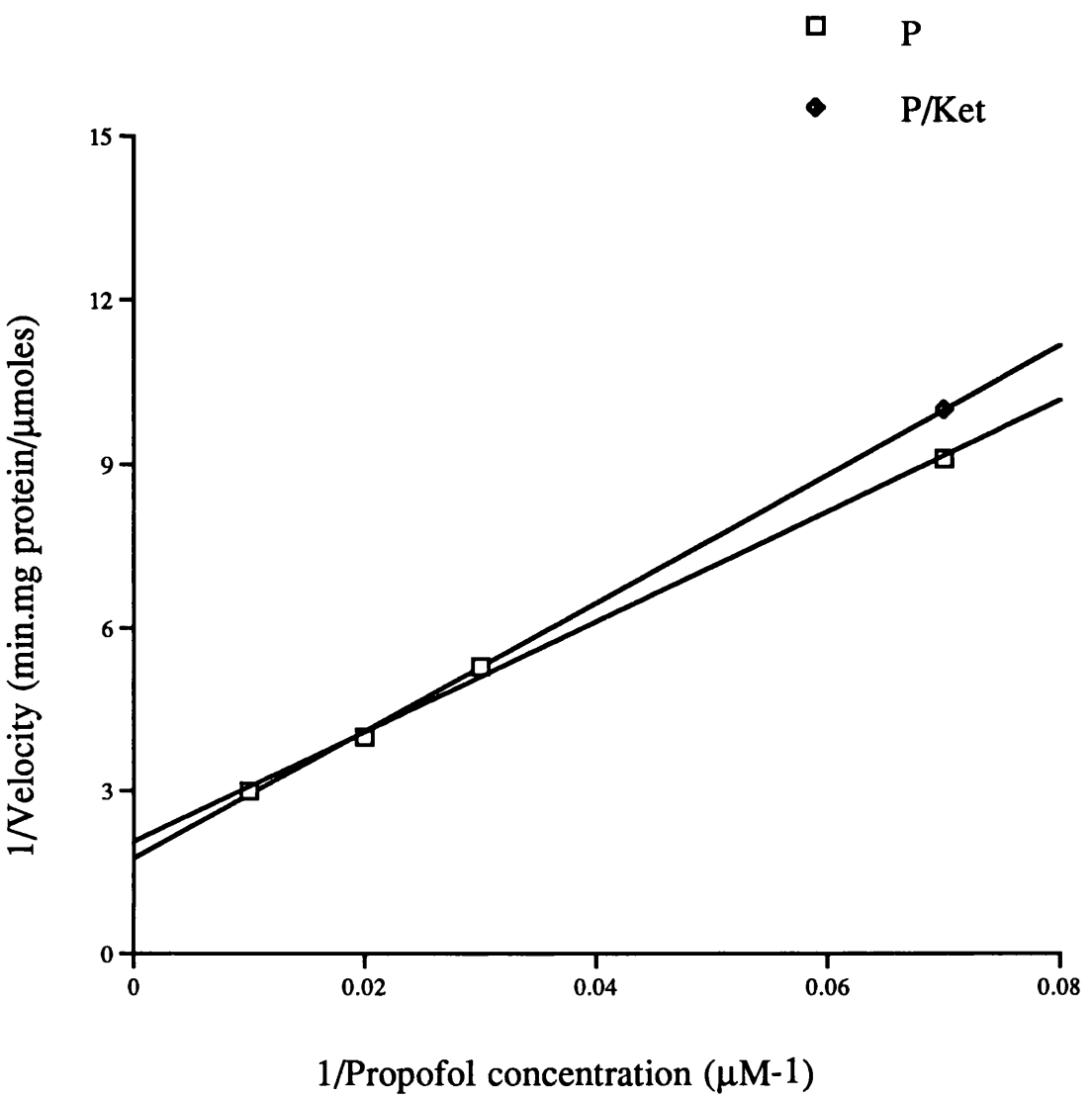

Fig 3.1.12 -Lineweaver Burk plot for propofol incubated for 15 min with rat hepatic microsomes in the absence (P) or in the presence of ketamine (P/Ket). Each point is the mean of microsome preparations from six rats and each preparation was assayed in duplicate.

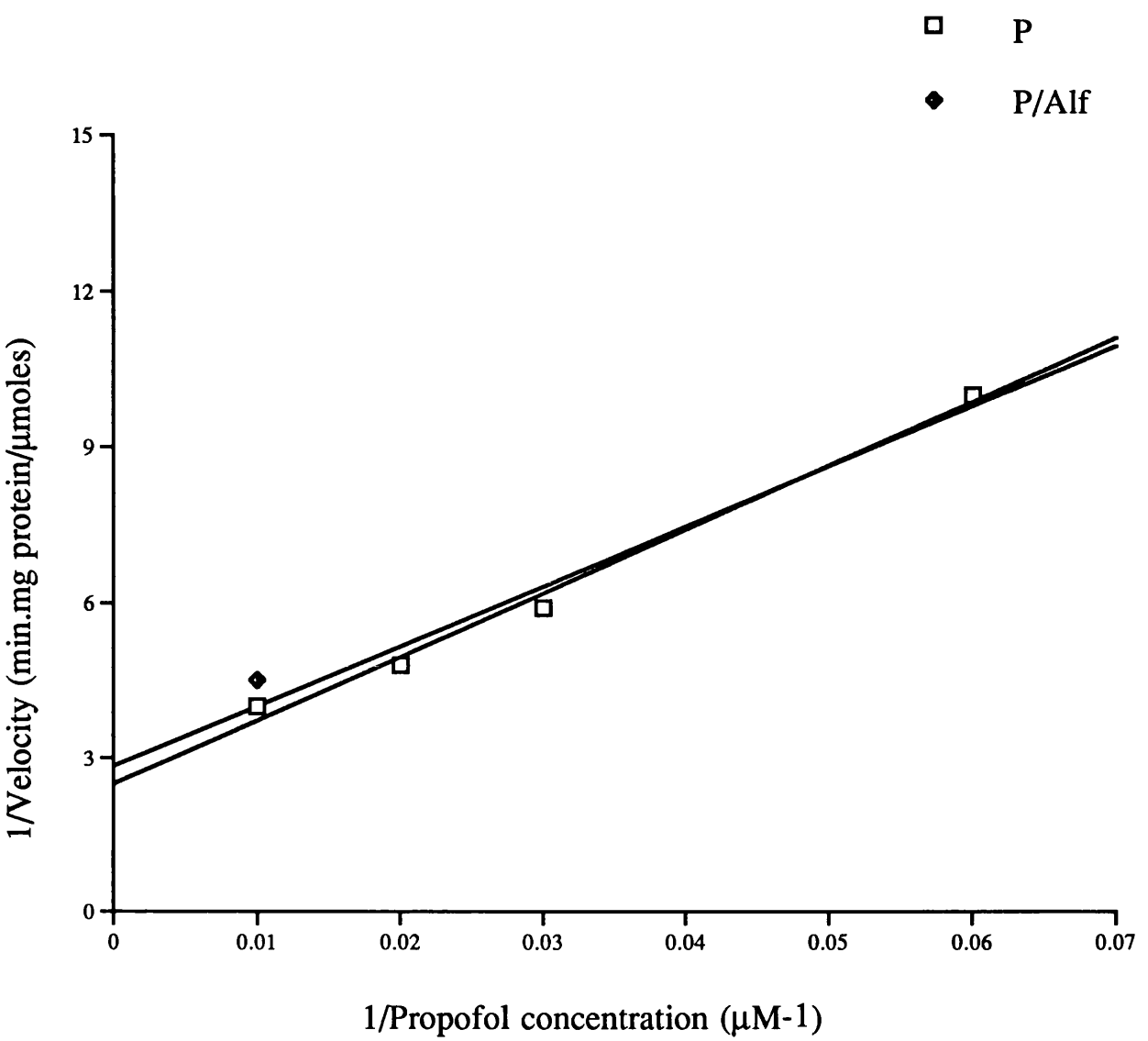

Fig 3.1.13 -Lineweaver Burk plot for propofol incubated for 15 min with rat hepatic microsomes in the absence (P) or in the presence of alfentanil (P/Alf). Each point is the mean of microsome preparations from six rats and each preparation was assayed in duplicate.

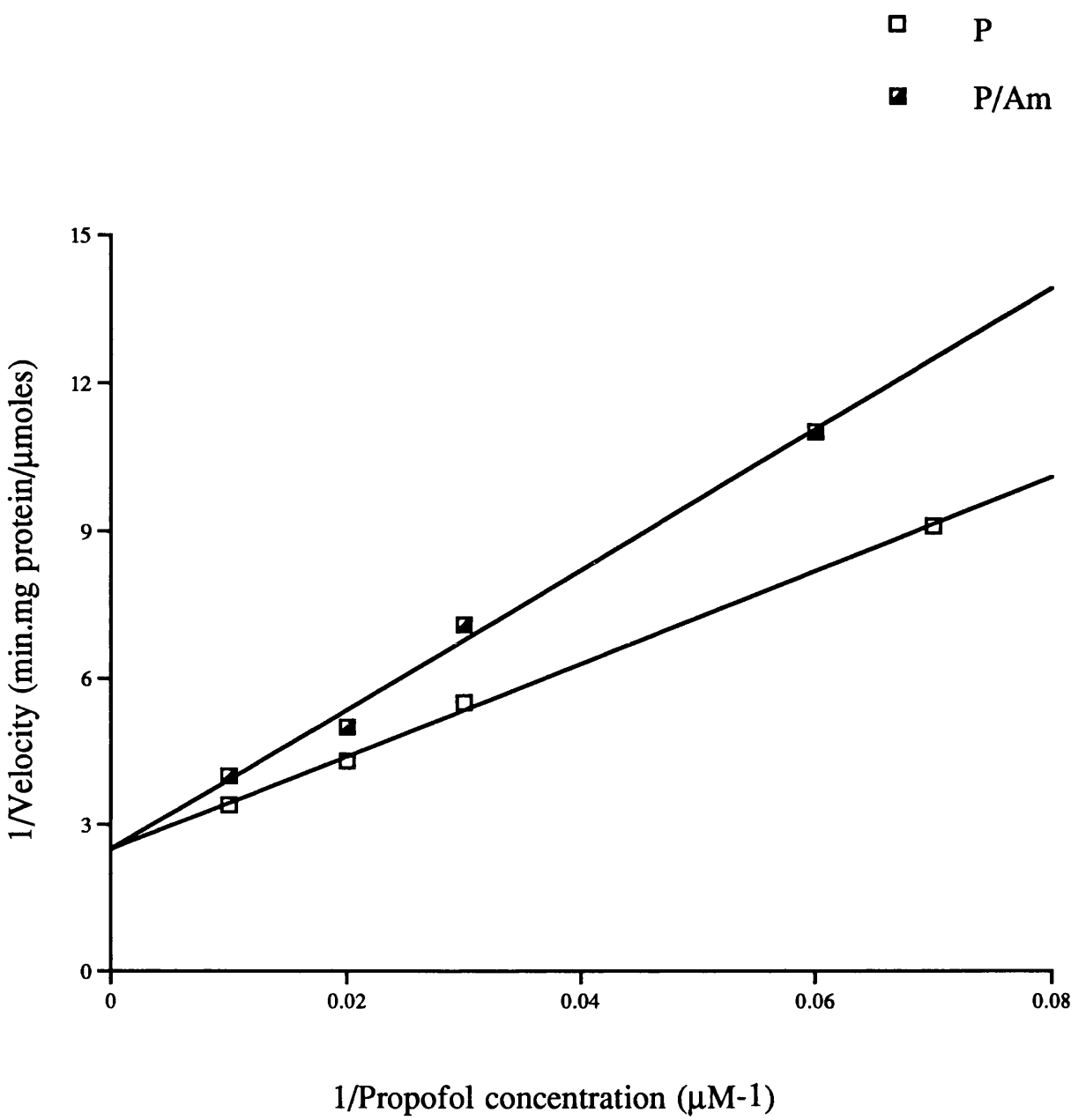

Fig 3.1.14 -Lineweaver Burk plot for propofol incubated for 15 min with rat hepatic microsomes in the absence (P) or in the presence of aminopyrine (P/Am). Each point is the mean of microsome preparations from six rats and each preparation was assayed in duplicate.

3.1.5 Degradation of propofol by sheep hepatic microsome preparations (a comparison with rat)

There were no statistically significant differences for the degradation of propofol by sheep hepatic microsomes when compared to rat hepatic microsomes (Sprague Dawley) at any drug concentration ($P>0.05$). Table 3.1.17 shows the concentrations of propofol remaining after incubation for 15 min, for both species. The mean velocities of propofol degradation for concentrations of 28, 56, 84 and 140 μM were 0.11 ± 0.00, 0.18 ± 0.01, 0.23 ± 0.02 and 0.27 ± 0.03 μmoles/min.mg protein for sheep and 0.10 ± 0.00, 0.17 ± 0.01, 0.21 ± 0.01 and 0.25 ± 0.03 μmoles/min.mg protein for rat, respectively (table 3.1.18). The graphical representation of the velocity of propofol degradation plotted against substrate concentration and the Lineweaver Burk plot are illustrated in figs 3.1.15 and 3.1.16 respectively, (substrate concentrations used for these graphical representations are in appendix A12). The mean maximal velocities (Vmax; μmoles/min.mg protein) were 0.43 ± 0.06 for sheep and 0.43 ± 0.04 for rat, and the mean Michaelis constant (Km; μM) 42.0 ± 7.1 for sheep and 48.9 ± 2.2 for rat (table 3.1.19). There were no statistically significant differences in the Vmax and Km between the two species.

3.1.6 Degradation of propofol by lung microsome preparations

The degradation of propofol (5.6, 11, 28 & 56 μM) by lung microsome preparations (400 μg of protein), obtained from Sprague Dawley rat was evident after 15 min incubation of these preparations with propofol. The mean velocities were 0.23 ± 0.01, 0.36 ± 0.02, 0.48 ± 0.08 and 0.56 ± 0.17 μmoles/min.mg of protein for propofol concentrations of 5.6, 11, 28 and 56 μM respectively (table 3.1.20). The concentration of propofol remaining after 15 min incubation with and without microsome preparations are presented in table 3.1.21. There was no evidence of drug instability or extra enzymatic degradation when propofol was incubated without microsome preparations. The graphical representation of the velocity plotted against substrate concentration and the Lineweaver Burk plot are illustrated in figs 3.1.17 and 3.1.18 respectively (substrate concentration used for these plots are in appendix A13). The mean maximal velocity (Vmax) and the mean Michaelis constant (Km) obtained by extrapolation from the Lineweaver Burk plot were 0.93 ± 0.28 μmoles/min/mg of protein and 13.7 ± 4.8 μM respectively (table 3.1.22). These data indicate that lung tissue can degrade propofol *in vitro*.

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	4.8	0.6	1.1	0.4	0.1	ND	1.4	0.9
56	27.0	10.3	9.8	7.5	3.3	0.8	9.8	3.8
84	52.0	25.3	26.7	22.4	14.9	11.7	25.5	5.8
140	109.0	72.4	78.0	63.2	58.0	57.2	73.0	7.9
28*	2.4	3.8	4.8	1.2	1.9	3.3	2.7	0.5
56*	12.8	15.8	18.3	7.8	10.4	16.2	13.5	1.6
84*	34.6	39.1	35.7	17.1	25.6	32.3	30.7	3.3
140*	89.4	109.9	81.7	46.9	65.4	75.6	78.1	8.7

Table 3.1.17 - Initial propofol concentration (I.C.; μM) and propofol concentrations (μM) remaining after incubation of sheep and rat (*) hepatic microsome preparations for 15 min with propofol. Each result is the mean of two determinations from one animal.

prop.	R1	R2	R3	R4	R5	R6	mean	±sem
28	0.09	0.11	0.11	0.11	0.11	0.11	0.11	0.00
56	0.12	0.18	0.18	0.19	0.21	0.22	0.18	0.01
84	0.13	0.23	0.23	0.24	0.27	0.29	0.23	0.02
140	0.12	0.27	0.25	0.31	0.33	0.33	0.27	0.03
28*	0.10	0.10	0.09	0.11	0.10	0.10	0.10	0.00
56*	0.17	0.16	0.15	0.19	0.18	0.16	0.17	0.01
84*	0.20	0.18	0.19	0.27	0.23	0.21	0.21	0.01
140*	0.20	0.12	0.23	0.37	0.30	0.26	0.25	0.03

Table 3.1.18 - The velocity (μmoles/min.mg of protein) of the degradation of propofol (prop. 28, 56, 84 and 140 μM), incubated with sheep and rat (*) hepatic microsome preparations for 15 min. Each result is the mean of two determinations from one animal.

	Sheep		Rat	
	Vmax	Km	Vmax	Km
A1	0.26	20	0.44	45
A2	0.37	33.8	0.32	40
A3	0.35	30.6	0.37	51
A4	0.44	43.7	0.58	53
A5	0.56	58.2	0.46	51
A6	0.62	65.8	0.41	51
mean	0.43	42.0	0.43	48.9
±sem	0.06	7.1	0.04	2.2

Table 3.1.19 - Maximal velocity (Vmax; μmoles/min.mg protein) and Michaelis constant (Km; μM) for the degradation of propofol by sheep and rat hepatic microsome preparations incubated for 15 min. Each result is the mean of two determinations from one animal.

prop.	R1	R2	R3	R4	R5	mean	±sem
5.6	0.23	0.19	0.21	0.27	0.26	0.23	0.01
11	0.34	0.30	0.34	0.44	0.39	0.36	0.02
28	0.30	0.36	0.43	0.71	0.60	0.48	0.08
56	0.09	0.46	0.37	1.07	0.82	0.56	0.17

Table 3.1.20 - The velocity (μmoles/min.mg of protein) of the degradation of propofol (prop. 5.6, 11, 28 and 56 μM), incubated with rat lung microsome preparations for 15 min. Each result is the mean of two determinations from one rat.

I.C.	R1	R2	R3	R4	R5	mean	±sem
5.6	1.7	2.4	2.1	1.0	1.2	1.7	0.3
11	5.4	6.2	5.6	3.9	4.6	5.1	0.4
28	22.9	22.0	20.9	16.0	17.9	19.9	1.3
56	54.6	48.3	49.9	38.1	42.3	46.6	2.9
5.6*	5.2	6.4	5.4	4.9	5.4	5.5	0.2
11*	10.0	12.0	10.9	12.5	10.0	11.1	0.5
28*	30.0	29.9	27.0	27.8	28.6	28.7	0.6
56*	56.8	54.2	57.4	55.4	57.0	56.2	0.6

Table 3.1.21 - Initial propofol concentration (I.C.; μM) and propofol concentrations (μM) remaining after incubation of propofol with and without (*) rat lung microsome preparations for 15 min. Each result is the mean of two determinations from one rat.

	R1	R2	R3	R4	R5	mean	±sem
Vmax	0.39	0.63	0.53	1.90	1.18	0.93	0.28
Km	2.9	11.2	6.3	30.2	17.9	13.7	4.8

Table 3.1.22 - Maximal velocity (Vmax; μmoles/min.mg protein) and Michaelis constant (Km; μM) for the degradation of propofol by rat lung microsome preparations incubated for 15 min. Each result is the mean of two determinations from one rat.

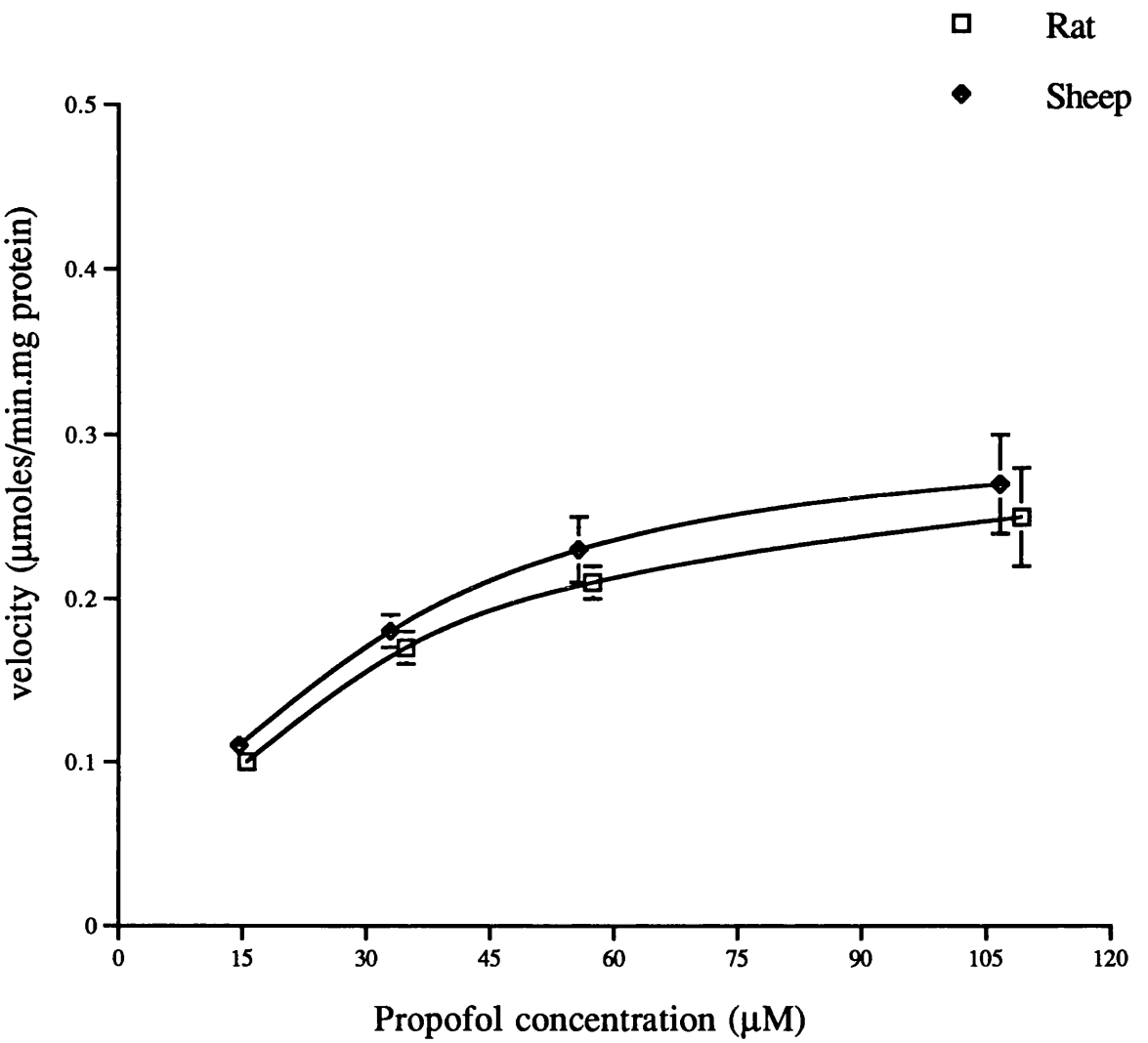

Fig 3.1.15 -The velocity of propofol degradation plotted against propofol concentrations for sheep and rat hepatic microsomes incubated for 15 min. Each point is the mean (+/-sem) of microsome preparations from six animals and each preparation was assayed in duplicate.

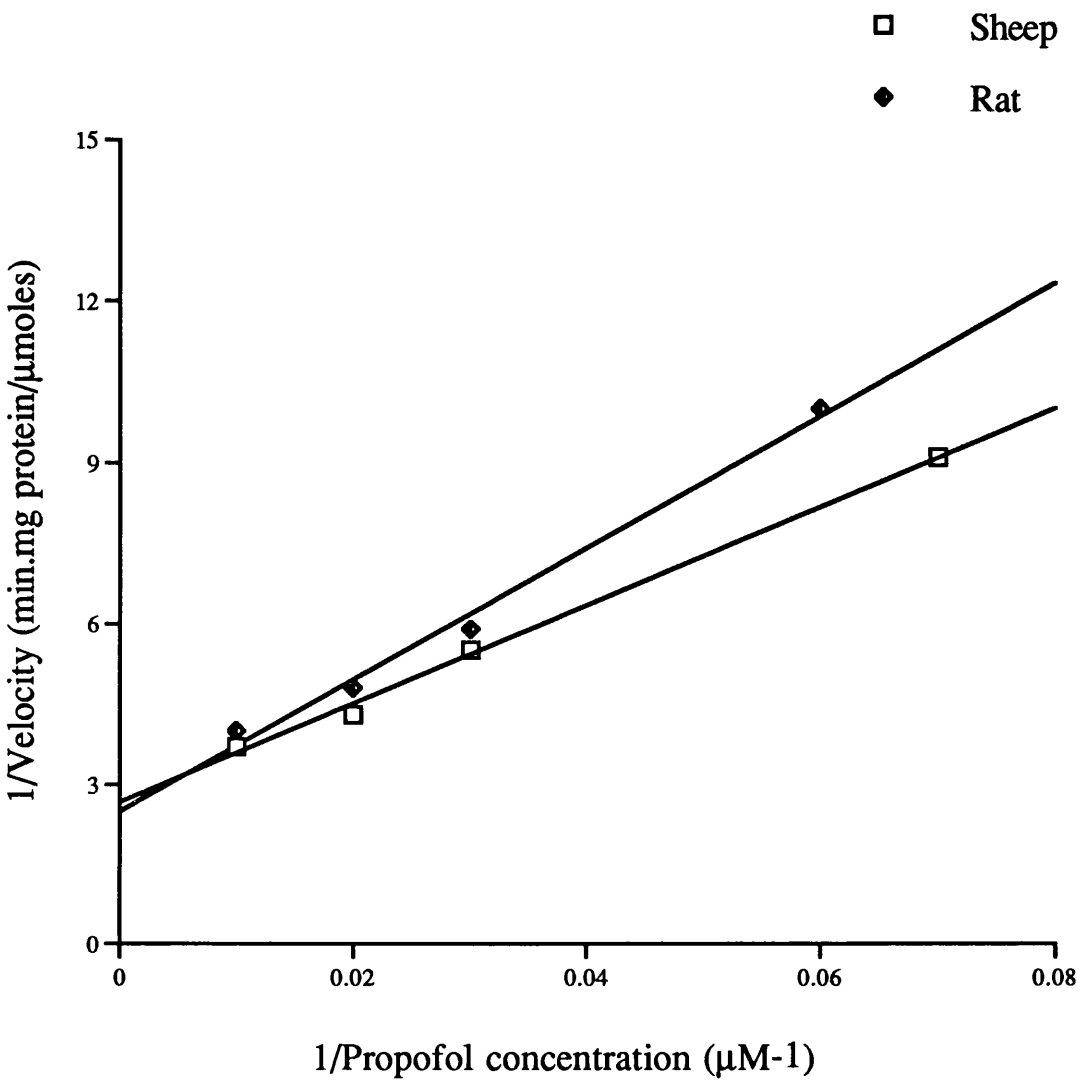

Fig 3.1.16 -Lineweaver Burk plot for propofol incubated for 15 min with sheep and rat hepatic microsomes. Each point is the mean (+/-sem) of microsome preparations from six rats and each preparation was assayed in duplicate.

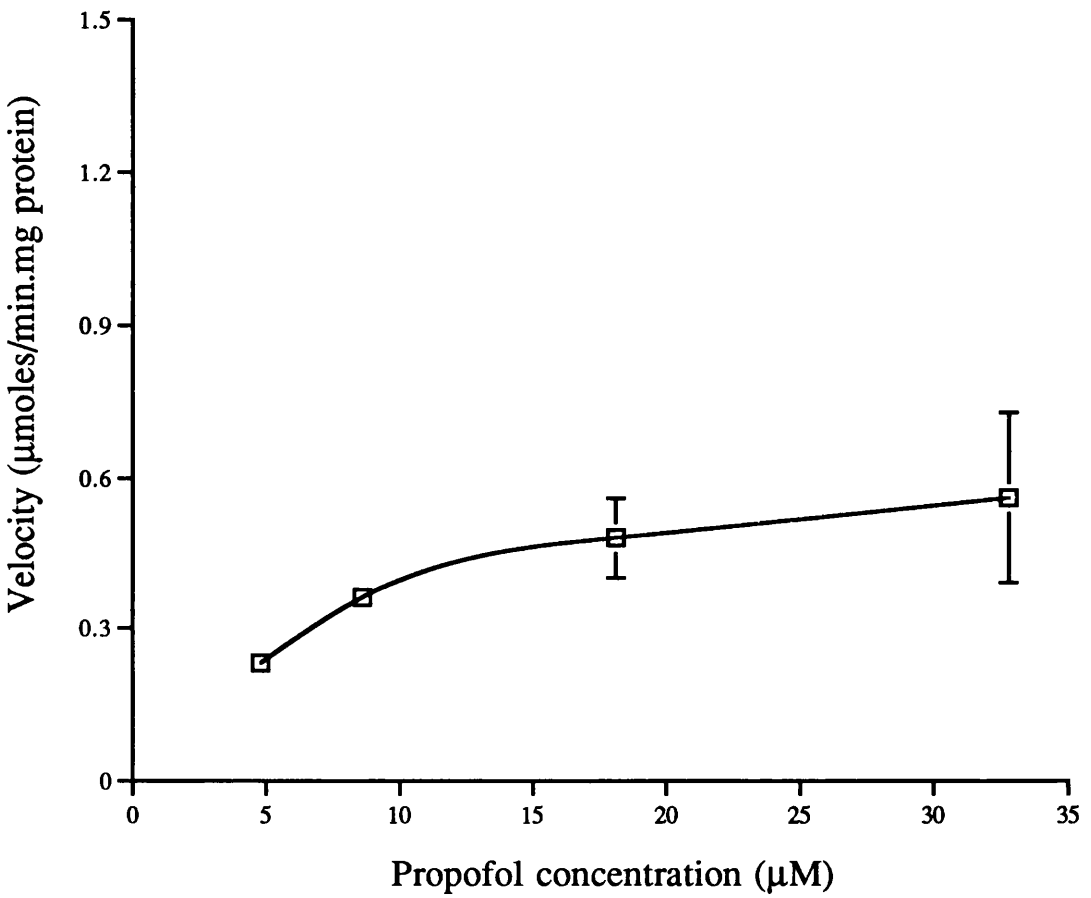

Fig 3.1.17 -The velocity of propofol degradation plotted against propofol concentrations for rat lung microsomes incubated for 15 min. Each point is the mean (+/-sem) of microsome preparations from five rats and each preparation was assayed in duplicate.

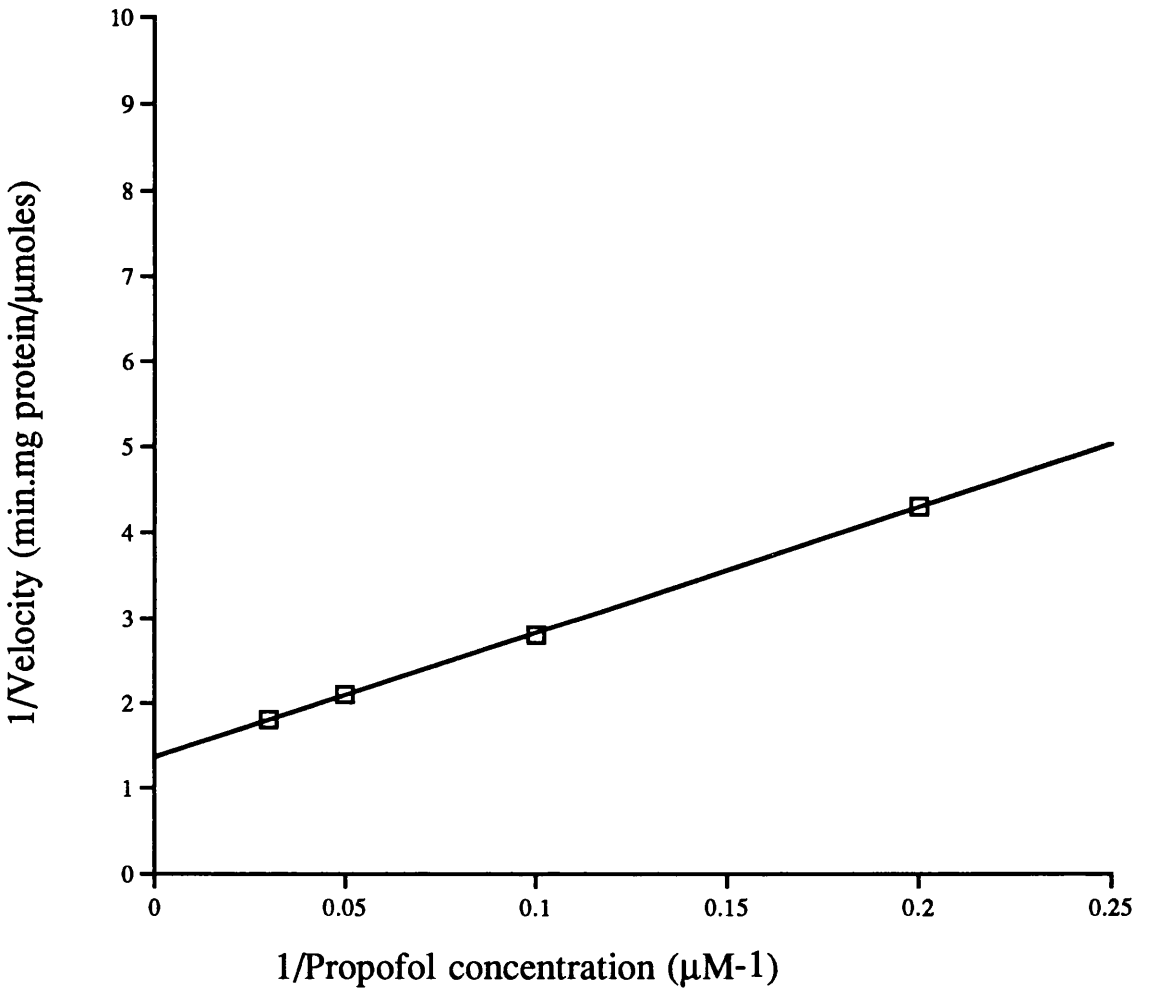

Fig 3.1.18 -Lineweaver Burk plot for propofol incubated for 15 min with rat lung microsomes. Each point is the mean of microsome preparations from five rats and each preparation was assayed in duplicate.

3.2 Pharmacokinetics and pharmacodynamics of propofol in sheep

There were no statistically significant differences between the two groups in age, and body weight. All animals were female sheep, 7 months old with a mean weight of 26.9 ±1.4 (range 22-29.5) and 25.9 ± 1.5 (range 21-29.5) for group 1 (G1) and group 2 (G2) respectively (table 3.2.1). The sheep were undergoing superficial surgery for implantation of subcutaneous tissue cages.

The mean percentage recovery of propofol blood standards (range 0.5-10 μg/ml) was 79.88 ± 0.87%. Four sets of standards (range 0.5-10 μg/ml) analysed during the same day showed a within a day coefficient of variation of 8.54 % and when the standards were analysed on different days the between assays coefficient of variation was 12.31 %.

Induction of anaesthesia was smooth in all animals in both G1 (propofol alone) and G2 (propofol and ketamine).

Endotracheal intubation was easily achieved in four animals in G1, but failed at the first attempt in one animal (05), because anaesthesia was insufficiently deep. Endotracheal intubation in this animal was only possible after ten min of propofol infusion. In G2, intubation was achieved at first attempt in all animals, but in one animal (07) it was difficult.

Two sheep in G1 (02 and 04) had a propofol infusion longer than one hour (75 and 69 min respectively). The mean duration of propofol infusion for this group was 64.8 ± 3.1 min (range 60-75 min) and the mean surgical period was 52.5±4.3 (range 42-63) with a mean time to the first skin incision of 10.9 min after induction of anaesthesia. In all animals in G2 the infusion period was 60 min. The mean duration of surgery for G2 was 49±0.6 min (range 48-51) and the mean time to skin incision was 11.0 min after induction of anaesthesia (tables 3.2.2a & 3.2.2b).

The initial infusion rate of propofol for G1 was 0.5 mg/kg/min and this was decreased twice to 0.4 and 0.3 mg/kg/min respectively (tables 3.2.3a, b, c). In G2, which received propofol and ketamine the infusion rate was decreased once. The first infusion rates were 0.3 mg/kg/min of propofol and 0.2 mg/kg/min of ketamine. These were later decreased to 0.2 mg/kg/min and 0.1 mg/kg/min for propofol and ketamine respectively (tables 3.2.4a, b). The decision to alter the infusion rate was based on the cardiovascular status of each animal during the infusion and clinical

	G1					G2				
sheep n°	01	02	03	04	05	06	07	08	09	10
WT (kg)	29	22	28	26	29.5	29.5	28	26	21	25
mean WT	26.9					25.9				
±sem	1.4					1.4				

Table 3.2.1 - Body weight (WT) for animals in G1 (01-05) which received propofol alone and animals in G2 (06-10) which received propofol and ketamine, for the induction and maintenance of anaesthesia.

sheep number	infusion time (min)	surgical time (min)
01	60	42.5
02	75	63
03	60	45
04	69	62
05	60	50
mean	64.8	52.5
±sem	3.1	4.3

Table 3.2.2a

sheep number	infusion time (min)	surgical time (min)
06	60	48
07	60	50
08	60	49
09	60	51
10	60	48
mean	60	49.2
±sem	0	0.6

Table 3.2.2b

Tables 3.2.2a,b - Infusion time and surgery duration for animals in G1 (3.2.2a) which received only propofol and for those in G2 (3.2.2b) which received propofol and ketamine for induction and maintenance of anaesthesia.

Initial rate - 0.5 mg/kg/min

sheep n°	period	total dose
01	0-30	435
02	0-35	385
03	0-10	140
04	0-20	260
05	0-32	472

Table 3.2.3a

First alteration - 0.4 mg/kg/min

sheep n°	period	total dose
01	30-45	174
02	35-45	88
03	10-20	112
04	20-30	104
05	32-60	330

Table 3.2.3b

Second alteration - 0.3 mg/kg/min

sheep n°	period	total dose
01	45-60	130
02	45-75	198
03	20-60	336
04	30-69	304
05	-	-

Table 3.2.3c

Tables 3.2.3a, b, c - Period (min) for each infusion rate applied (a: the initial infusion rate; b: the first alteration; c: the second alteration) and the total dose of propofol (mg) received during each period for animals in G1 which were anaesthetised with propofol. Time zero (0) is the time the infusion started, which was immediately after induction of anaesthesia.

Initial rate: - propofol - 0.3 mg/kg/min
- ketamine - 0.2 mg/kg/min

sheep n°	period	total dose	
		p	k
06	0-18	159	106
07	0-31	260	174
08	0-31	242	161
09	0-30	189	126
10	0-30	225	150
	41-60	142	95

Table 3.2.4a

First alteration: - propofol - 0.2 mg/kg/min
- ketamine - 0.1 mg/kg/min

sheep n°	period	total dose	
		p	k
06	18-60	248	124
07	31-60	162	81
08	31-60	150	75
09	30-60	126	63
10	30-41	45	22

Table 3.2.4b

Tables 3.2.4a, b - Period (min) for each infusion rate applied (a: initial infusion rate; b: first alteration) and the total dose (mg) of propofol (p) and ketamine (k) received during each period, for animals in G2 which were anaesthetised with propofol and ketamine. Time zero (0) is the time the infusion started, which was immediately after induction of anaesthesia.

assessment of the depth of anaesthesia. Consequently, the time of alteration varied between animals.

All animals in G1 and G2 were in a light plane of surgical anaesthesia during the infusion period. Intermittent skin twitching was present in two animals in G1 (35 and 40 min after induction of anaesthesia), when the second surgical area was being clipped and this lasted 2 and 3 min respectively. One animal in G2 reacted to skin touch but not to skin incision, 35 min after induction. No purposeful movements were seen and the degree of analgesia appeared to be satisfactory.

The mean total dose (induction + infusion) of propofol received by animals in G1 was 801 ± 42 mg of which 694 ± 23 mg was given during the infusion period. G2 animals received a mean dose of propofol of 469.6 ± 23.2 mg of which 389.9 ± 19.4 mg was given during the infusion and a mean dose of ketamine of 267.3±15.3 mg, of which 235.5±13.4 was given during infusion (table 3.2.5a, b).

Recovery from anaesthesia was rapid and without excitement in all animals in both groups. The mean time to endotracheal extubation was 2.8 ± 0.4 and 5.3 ± 0.9 min, to sternal recumbency, 6.3 ± 1.2 and 11.2 ± 1.7 min and to standing unaided, 10.9 ± 1.6 and 15.1 ± 2.2 min after infusion was switched off, for G1 and G2 respectively (tables 3.2.6a, b). There was a significant difference between the time to endotracheal extubation and sternal recumbency between the two groups ($P<0.05$).

Pulse rate, arterial blood pressure and respiratory rate were well maintained throughout the infusion period. In G1, the mean pulse rate fell from 105 ± 15 beats/min at 5 min to 95 ± 9 beats/min at 45 min. The mean systolic arterial blood pressure fell from 114 ± 10 mm Hg at 5 min to 98 ± 3 mm Hg at 30 min and the mean respiratory rate varied between 16 ± 1 and 19 ± 7 breaths/min during the infusion period. For animals in G2, the mean pulse rate varied between 102 ± 12 and 107 ± 12 beats/min throughout anaesthesia. The mean systolic arterial blood pressure fell from 127 ± 17 mm Hg at 5 min to 96 ± 7 mm Hg at 30 min. The mean respiratory rates for this group were higher than for G1 at all time points throughout anaesthesia and ranged from 26 ± 3 to 45 ± 9 breaths/min (figs 3.2.1, 3.2.2 and 3.2.3 respectively). One animal in G1 (02) and two animals in G2 (08; 09) were apnoeic post induction for 7, 2 and 3 min respectively. These animals were ventilated manually with 100% oxygen until spontaneous breathing resumed.

For animals in G1, the mean blood propofol concentration at the time of skin incision was 4.9 ± 1.2 μg/ml (range 2.98-7.10). The maximum individual concentration of propofol in blood was achieved between 5 and 30 min after the beginning of the infusion and was between 4.8 and 10.3 μg/ml. At the time the

sheep n°	ind (mg)	inf (mg)	total (mg)
01	116	739.5	855.5
02	88	671	759
03	112	588	700
04	104	668.2	772.2
05	118	802.4	920.4
mean	107.6	693.8	801.4
±sem	5.5	36.2	38.7

Table 3.2.5

	ind (mg)		inf (mg)		total (mg)	
sheep n°	p	k	p	k	p	k
06	88.5	59	407.1	230.1	495.6	289.1
07	84	38	422.8	254.8	506.8	282.8
08	78	26	392	236	470	262
09	63	21	315	189	378	210
10	75	25	412.5	267.5	487.5	292.5
mean	77.7	31.8	389.9	235.5	467.6	267.3
±sem	4.4	6.9	19.4	13.4	31.8	15.3

Table 3.2.5b

Table 3.2.5a, b - Induction dose (ind), infusion dose (inf) and total dose (total) of propofol (p) and ketamine (k), received by animals in G1 (3.2.5a) anaesthetised with propofol alone and by animals in G2 (3.2.5b) anaesthetised with propofol and ketamine.

sheep number	time to endotracheal extubation	time to sternal recumbency	time to standing unaided
01	4	10.5	12.5
02	2	5	11
03	3	6	15
04	3	7	11
05	2	3	5
mean	2.8	6.3	10.9
±sem	0.4	1.2	1.6

Table 3.2.6a

sheep number	time to endotracheal extubation	time to sternal recumbency	time to standing unaided
06	2.5	9.5	13
07	4	7	11
08	7	12.5	22.5
09	7	17	18
10	6	10	11
mean	5.3	11.2	15.1
±sem	0.9	1.7	2.2

Table 3.2.6b

Table 3.2.6a, b - Anaesthetic recovery times (min) - time to endotracheal extubation, time to sternal recumbency and time to standing unaided, measured from the time the infusion was switched off, for animals in G1 (3.2.6a), anaesthetised with propofol alone and animals in G2 (3.2.6b) anaesthetised with propofol and ketamine.

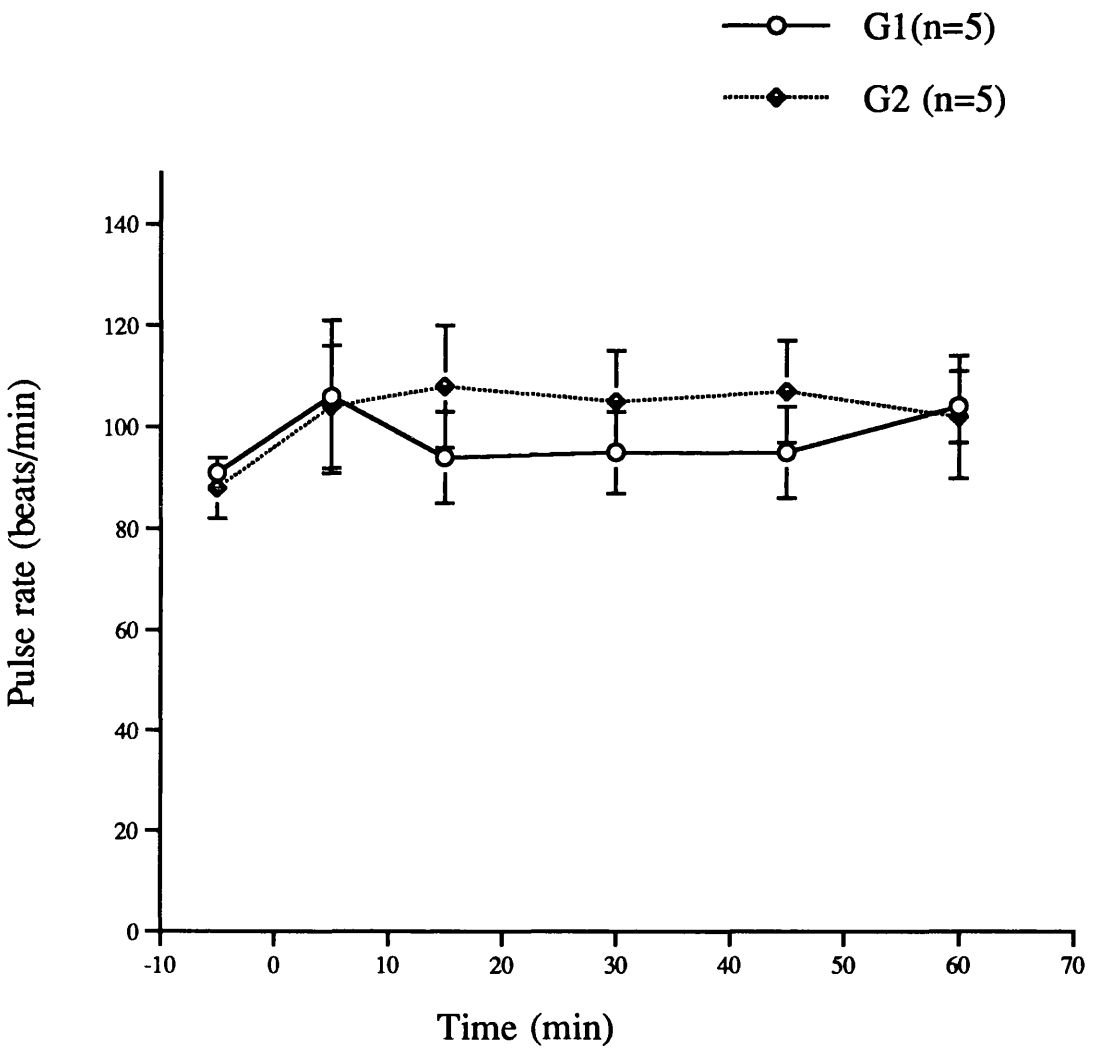

Fig 3.2.1- Mean pulse rate (+/-sem), before and during anaesthesia for animals which received propofol only (G1) and animals which received propofol and ketamine (G2), for induction and maintenance of anaesthesia. The infusion started at time 0, immediately after induction of anaesthesia.

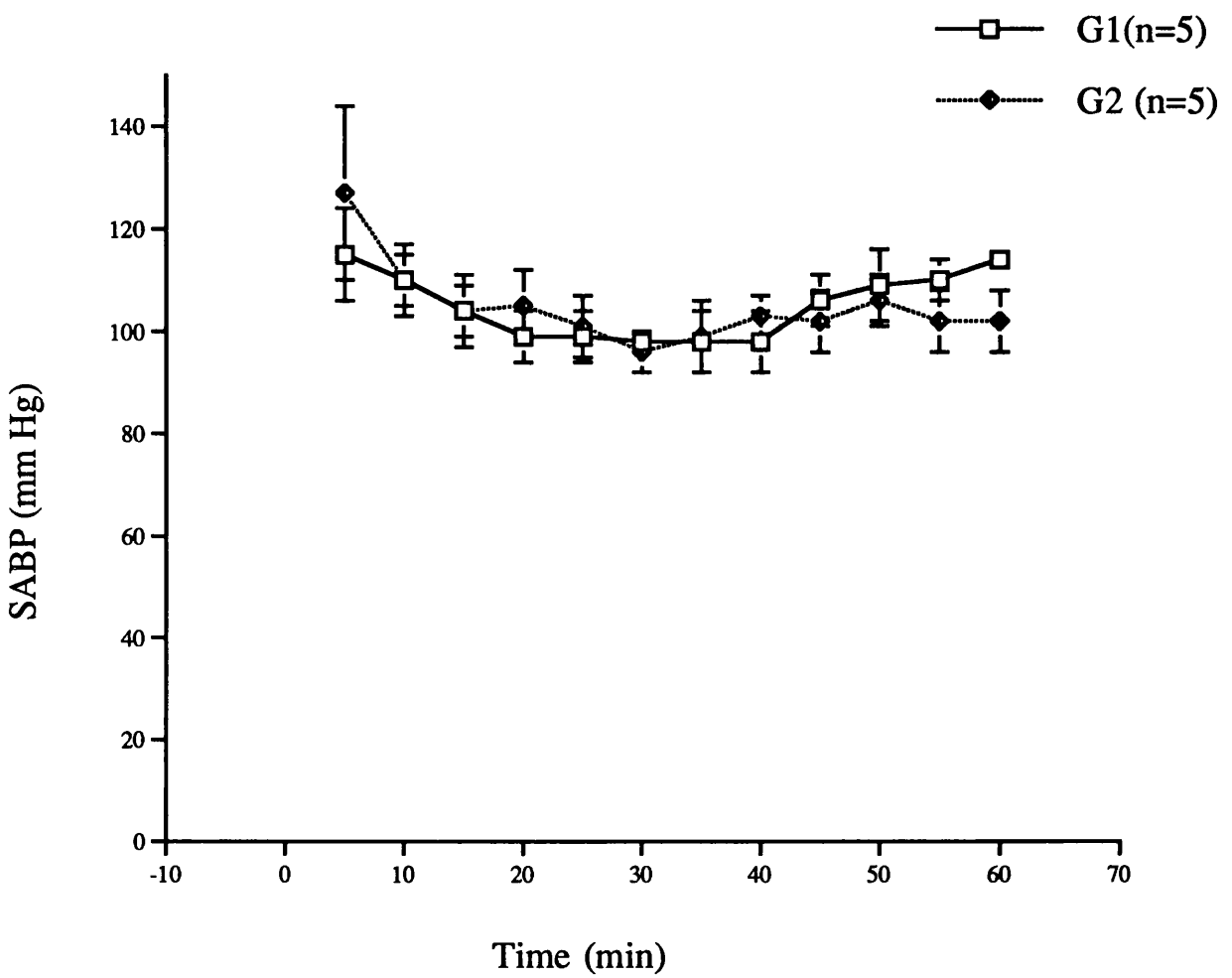

Fig 3.2.2-Mean systolic arterial blood pressure (SABP) (+/-sem), during anaesthesia for animals in group 1(G1), which received only propofol and animals in group 2(G2), which received propofol and ketamine, for induction and maintenance of anaesthesia. The infusion started at time 0, immediately after induction of anaesthesia.

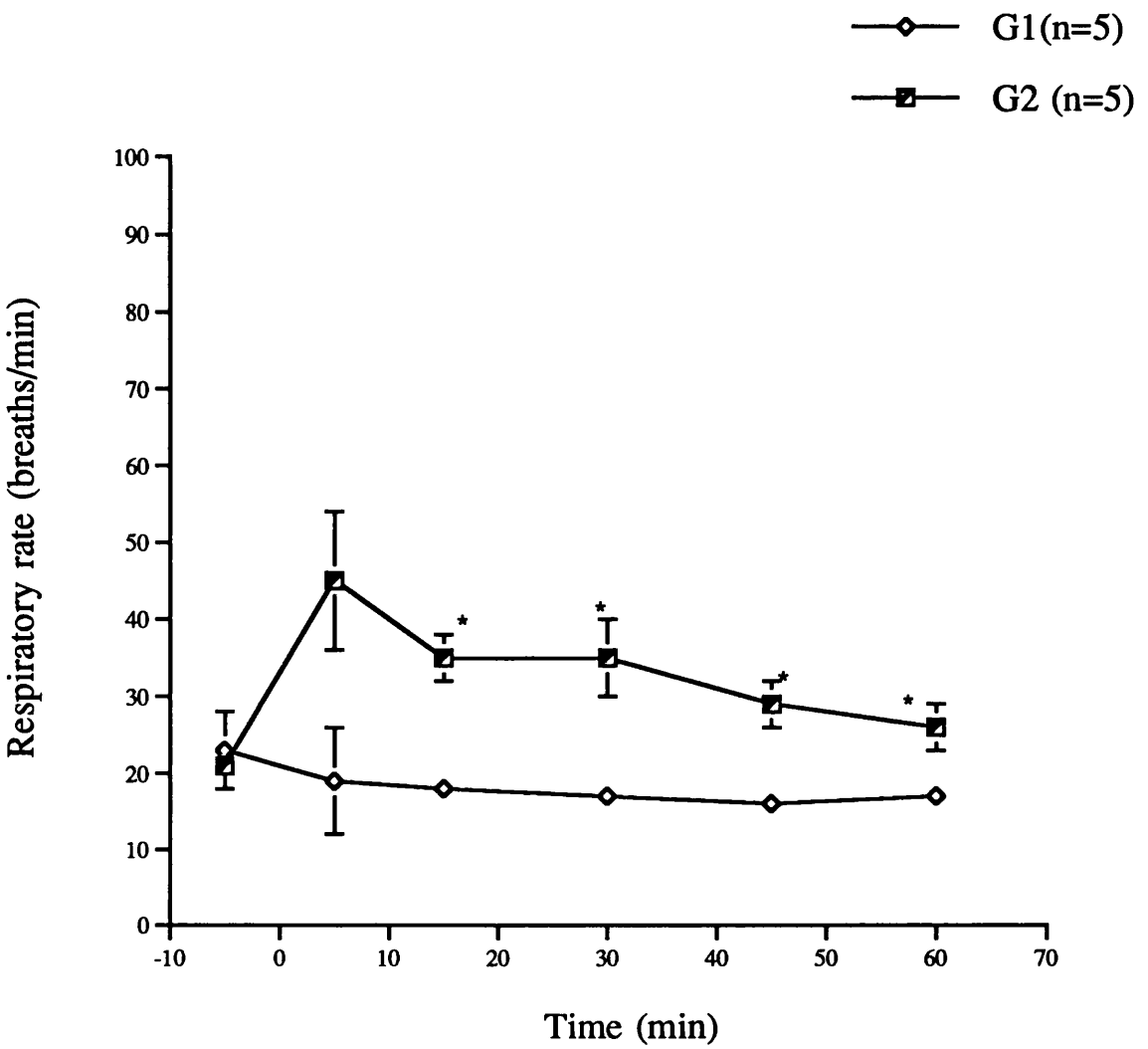

Fig 3.2.3 - Mean respiratory rate (+/-sem), before and during anaesthesia for animals in G1 anaesthetised with propofol and animals in G2 anaesthetised with propofol and ketamine. The infusion started at time 0, immediately after induction of anaesthesia. * significantly different from G1, $P<0.05$.

infusion was switched off, the mean blood propofol concentration was 4.9 ± 0.6 μg/ml (range 4.1-7.1) (table 3.2.7a). Propofol in blood was detected in all animals until 4h after the infusion had been switched off, but in some animals it was detected for a further 5h (table 3.2.8; figs 3.2.4 & 3.2.5).

For animals in G2, the mean blood propofol concentration at the time of skin incision was 2.2 ± 0.3 μg/ml (range 1.2-2.9). The maximum individual concentration of propofol in blood was achieved between 5 and 30 min after the start of the infusion and was in the range 2.2 - 4.7 μg/ml; at the time the infusion was switched off, the blood propofol concentration was 2.3 ± 0.2 μg/ml (range 1.8-2.8) (table 3.2.7b). Propofol was measured in all sheep for 2h after the infusion was stopped, but in some animals it was detected for a further 4h (table 3.2.9; figs 3.2.4 & 3.2.5).

For animals in G1, the mean area under the concentration-time curve (AUC_{obs}) for propofol was 6.6 ± 1.0 μg.h/ml and the mean area under the first moment curve ($AUMC_{obs}$) was 4.7 ± 0.9 $\mu g.h^2/ml$. The mean residence time (MRT_{obs}) was 0.71 ± 0.04 h, mean whole body clearance (CLb) 82 ± 11 ml/kg.min and the volume of distribution at steady state (Vdss), 3.4 ± 0.5 L/kg. (table 3.2.10a).

The mean AUC_{obs} for animals in G2, was 2.5 ± 0.3 μg.h/ml and the mean $AUMC_{obs}$ was 1.8 ± 0.2 $\mu g.h^2/ml$. The mean MRT_{obs} was, 0.71 ± 0.05 h, mean CLb of 129 ± 16 ml/kg.min and mean Vdss, 5.46 ± 0.72 L/kg (table 3.2.10b).

time	sheep number						
(min)	01	02	03	04	05	mean	±sem
2	6	4.4	6.2	2.6	1.3	4.1	1.0
5	4.4	4.6	6.5	3.8	2.2	4.3	0.7
10	-	-	7.1	4.7	3	4.9	1.2
15	8.4	5.5	6.6	5.2	3.2	5.8	0.8
30	9.8	6.9	4.6	5.4	4.8	6.3	1.0
45	10.3	6.6	4.1	3.9	4.6	5.9	1.2
60	7.1	5.1	4.1	4.1	4.4	4.9	0.6
69	-	-	-	4.2	-		
75	-	5.5	-	-	-		

Table 3.2.7a

time	sheep number						
(min)	06	07	08	09	10	mean	±sem
2	2.5	1.8	-	1.9	1.3	1.9	0.2
5	2.5	2.4	4.7	2.2	2.0	2.8	0.5
10	1.2	3.3	3.0	2.5	2.1	2.2	0.3
15	1.0	1.7	2.5	3.0	1.2	1.9	0.4
30	1.3	2.3	2.8	3.6	2.2	2.4	0.4
45	1.3	1.7	2.2	2.8	1.6	1.9	0.3
60	1.7	2.4	2.2	2.6	2.8	2.3	0.2

Table 3.2.7b

Table 3.2.7a, b - Blood propofol concentration (µg/ml) during the infusion period, for animals in G1 (3.2.7a) which received propofol only and animals in G2 (3.2.7b) which received propofol and ketamine for induction and maintenance of anaesthesia.

time	sheep number						
(min)	01	02	03	04	05	mean	±sem
2	3.2	1.7	2.2	1.8	1.8	2.1	0.3
5	2.3	0.9	1.2	1.1	1.0	1.3	0.3
10	2	0.8	0.7	0.7	0.8	1.0	0.2
15	1.3	0.4	0.6	0.7	0.4	0.7	0.2
30	0.6	0.2	0.2	0.2	0.2	0.3	0.1
45	0.3	0.1	0.1	0.2	0.1	0.2	0.0
60	0.2	0.07	0.07	0.1	0.08	0.1	0.0
90	0.2	0.07	0.05	0.06	0.06	0.08	0.02
120	0.1	0.05	0.03	0.05	0.01	0.05	0.02
180	0.06	0.02	0.02	0.02	0.02	0.03	0.01
240	0.03	0.01	0.01	0.01	0.02	0.02	0.00
360	0.02	ND	0.01	0.01	0.01	0.01	0.00
540	0.01	ND	ND	ND	ND	ND	ND
1320	ND	ND	ND	ND	ND	ND	ND
1440	ND	ND	ND	ND	ND	ND	ND

Table 3.2.8 - Blood propofol concentration (μg/ml) after the infusion was switched off (at time 0), for animals in G1 (n=5) which were anaesthetised with propofol.

ND - not detected

time	sheep number						
(min)	06	07	08	09	10	mean	±sem
2	0.5	0.6	1.1	1.5	0.9	0.9	0.2
5	0.2	0.3	0.5	0.6	0.5	0.4	0.1
10	0.2	0.2	0.3	0.3	0.3	0.3	0.0
15	0.08	0.4	0.5	0.2	0.3	0.3	0.1
30	0.08	0.1	0.2	0.1	0.2	0.1	0.0
45	0.06	0.06	0.1	0.04	0.1	0.07	0.01
60	0.02	0.05	0.1	0.03	0.08	0.06	0.02
90	0.02	0.06	0.09	0.02	0.04	0.05	0.01
120	0.01	0.03	0.04	0.01	0.04	0.03	0.01
180	ND	0.02	0.03	0.01	0.02	0.02	0.01
240	ND	0.02	0.02	ND	0.02	0.01	0.00
360	ND	0.01	ND	ND	0.01	ND	ND
540	ND	ND	ND	ND	ND	ND	ND
1320	ND	ND	ND	ND	ND	ND	ND
1440	ND	ND	ND	ND	ND	ND	ND

Table 3.2.9 - Blood propofol concentration (μg/ml) after the infusion was switched off (at time 0), for animals in G2 which were anaesthetised with propofol and ketamine.

ND - not detected

sheep n°	AUC_{obs}	$AUMC_{obs}$	MRT_{obs}	CLb	Vdss
01	9.9	8.0	0.81	49.7	2.4
02	7.7	5.6	0.73	74.2	3.2
03	5.7	3.3	0.59	73.2	2.6
04	5.1	3.4	0.73	96.7	3.9
05	4.5	3.3	0.71	116.7	5.1
mean	6.6	4.7	0.71	82	3.4
±sem	1.0	0.9	0.04	11	0.5

Table 3.2.10a

sheep n°	AUC_{obs}	$AUMC_{obs}$	MRT_{obs}	CLb	Vdss
06	1.5	0.9	0.59	180.7	6.4
07	2.4	1.8	0.78	127.8	6.0
08	3.1	2.1	0.69	98.2	4.1
09	3.2	2.0	0.62	94	3.5
10	2.3	1.9	0.85	143.8	7.3
mean	2.5	1.8	0.71	129	5.5
±sem	0.3	0.2	0.05	16	0.7

Table 3.2.10b

Tables 3.2.10a, b - Area under the concentration-time curve [(AUC_{obs}); µg.h/ml)], area under the first moment curve [($AUMC_{obs}$); µg.h²/ml], mean residence time [(MRT_{obs}); h], whole body clearance [(Clb) ml/kg.min] and the volume of distribution at steady state [(Vdss); L/kg] of propofol in both G1 (3.2.10a), anaesthetised with propofol only and G2 (3.2.10b), anaesthetised with propofol and ketamine.

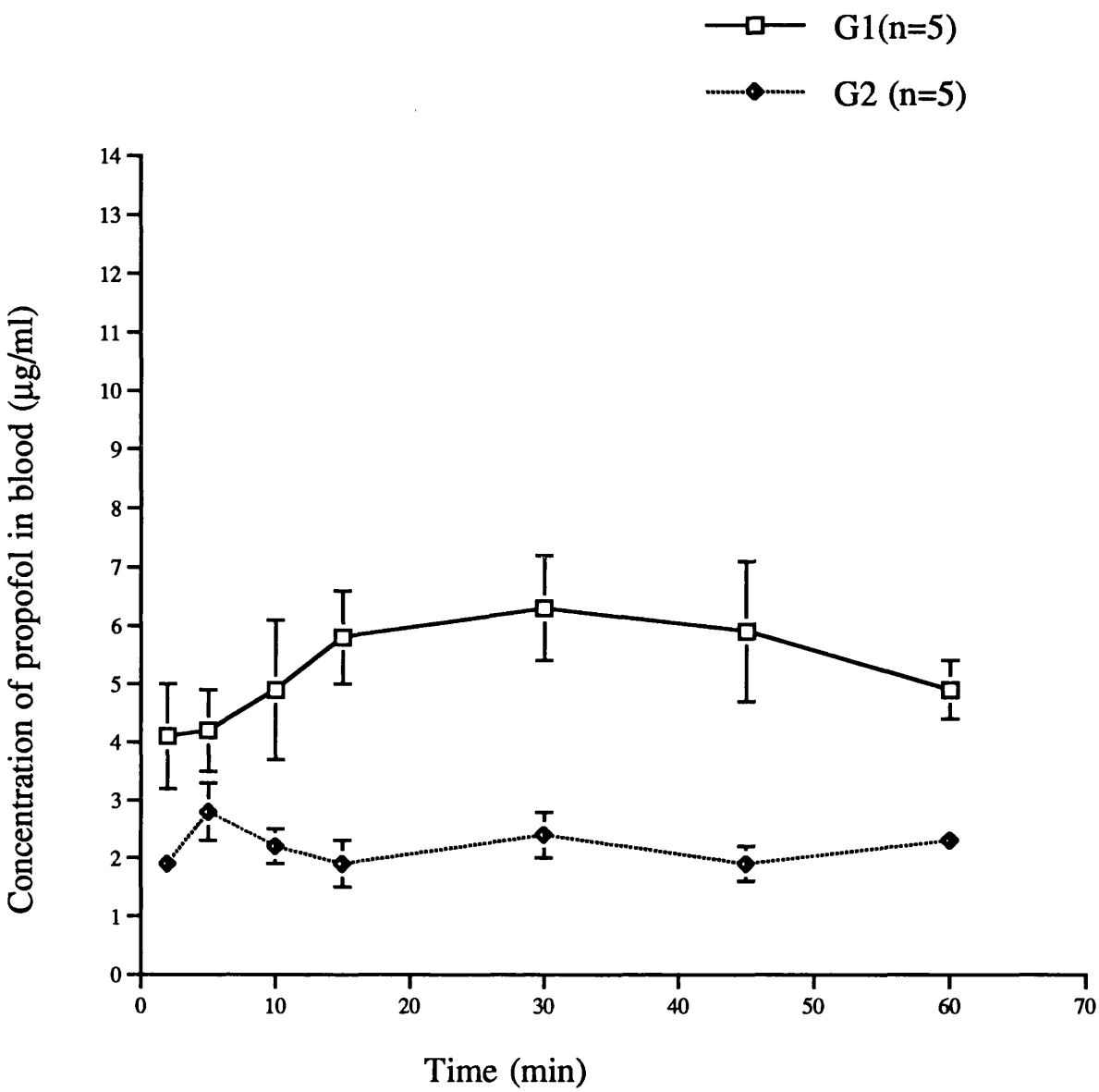

Fig 3.2.4 -Blood propofol concentration (+/-sem), during the infusion period for animals in G1, anaesthetised with propofol only and animals in G2 anaesthetised with propofol and ketamine. The infusion started at time 0, immediately after induction of anaesthesia.

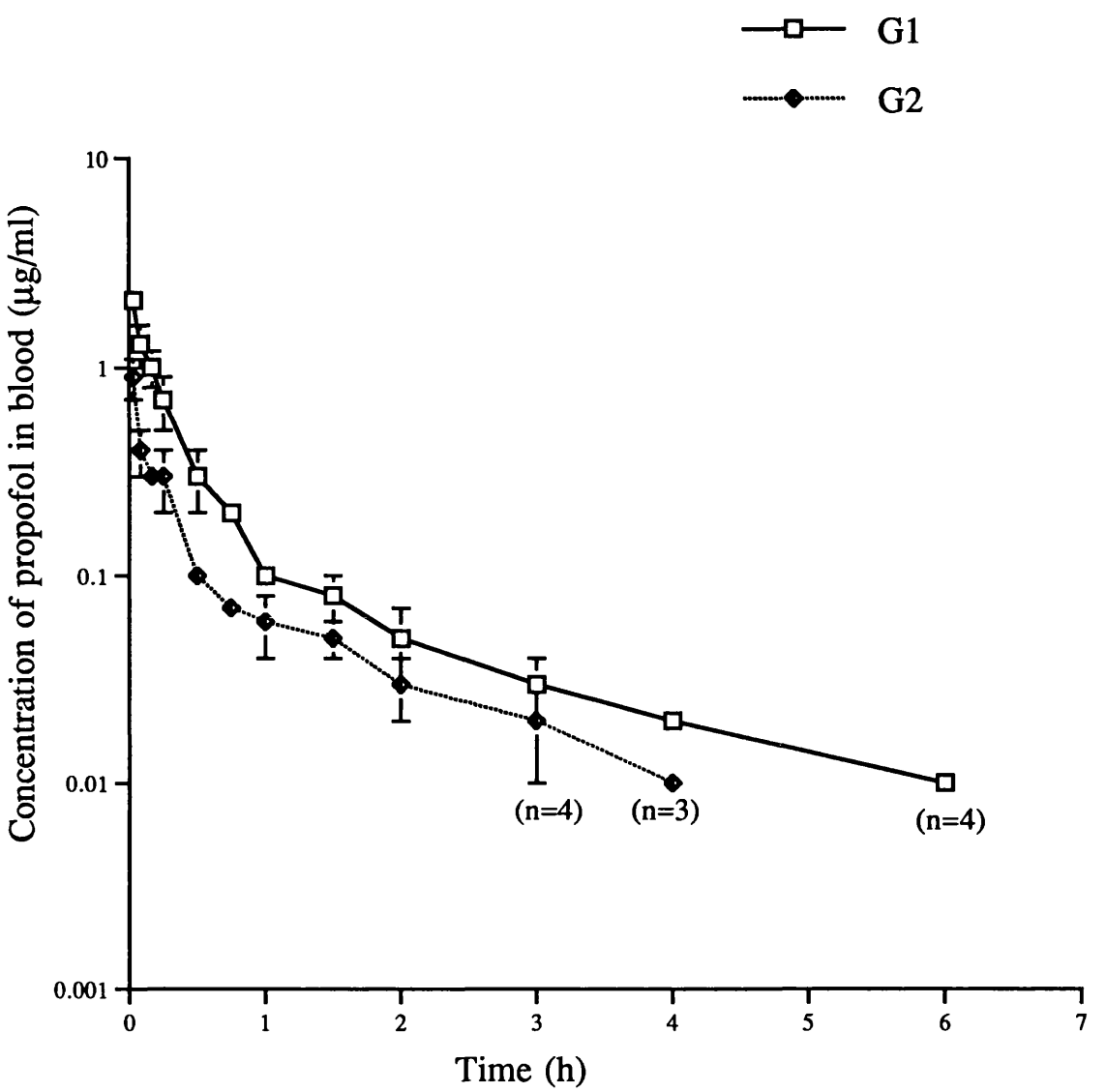

Fig 3.2.5 - Semilogarithmic plot of blood propofol concentration (+/-sem), *versus* time after the infusion was switched off (at time 0), for animals in G1, anaesthetised with propofol only and animals in G2 anaesthetised with propofol and ketamine. n=5 except where indicated in parentheses.

4. DISCUSSION

4.1 Degradation and interactions of propofol with other drugs *in vitro*

Several problems were encountered in the studies of propofol degradation by microsome preparations. Firstly, to date there are only two reports in the literature on drug metabolism *in vitro* involving propofol, neither of which reported the measurement of propofol (Janicki et al., 1992; Baker et al., 1993). Moreover, both of these groups used only one incubation time point, 15 and 45 min respectively, in studies of the effect of propofol on drug metabolism. Consequently, it was necessary to carry out a pilot study to optimise the incubation conditions. Although tissue from only one animal was used for each incubation time point, the data demonstrated clearly that the oxidative degradation of propofol by rat hepatic microsomes was rapid. This highlighted a second potential problem. Since the amount of propofol degraded even after 5 min of incubation was in excess of 5%, standard calculations of enzyme kinetic constants were not possible as the conditions of the reaction did not comply with the assumptions on which the Michaelis Menten equation are based (Segel, 1975). In order to obtain linear kinetics and calculate the maximal velocity, Vmax, and the Michaelis constant, Km, the reaction must proceed to a negligible extent during the course of the assay (<5% of substrate used). If a substantial fraction of the substrate is used during the assay, the Km and Vmax values determined from the reciprocal plot of velocity against substrate concentration will be in error. However, in 1971, Lee & Wilson showed that the arithmetic mean substrate concentration, ½([So] +[St]) where So is the initial substrate concentration and St is the substrate concentration at the incubation time, t, is an excellent approximation of the substrate concentration and with this value the modified Lineweaver Burk plot can yield reliable estimates of the kinetic constants Vmax and Km even when the reaction has proceeded to a considerble extent (circa 4% error with 50% substrate utilization).

The kinetic constants in these studies were obtained by using the modified Lineweaver Burk equation , therefore, the percentage error in the results obtained is likely to be small. An incubation time of 15 min was chosen in an attempt to permit drug interaction studies to yield reliable data. For further studies with propofol in hepatic microsome preparations, the most appropriate incubation time would be set to 5 min or less, although in practice this is difficult for technical reasons.

It was also observed in this study that when the incubation time was greater than 30 min, the concentrations of propofol remaining were very variable and inconsistent, probably due to instability of the mixture, but further studies are necessary to

support this suggestion, since the incubation was only carried out once. However, these preliminary data cast some doubt on the consistency of the results reported by Janicki *et al.* (1992) who used a 45 min incubation time when studying the effects of propofol on the degradation of alfentanil and sufentanil by liver microsomes from pigs and humans.

The rate of degradation of propofol was proportional to the concentration which is in accordance with that predicted by the Michaelis Menten equation. The decrease on the rate of propofol degradation with time was presumably due to the decrease in the saturation of the enzyme, however, it could have been due to product inhibition. This highlighted a third problem encountered with studies on propofol degradation *in vitro.* The degradation of propofol was measured on the basis of substrate disappearance and not product formation. Ideally the accumulation of one or more products of propofol degradation would be desirable. This would have allowed better enzyme kinetic analyses, and a study of the ratio of substrate disappearance and product formation. Furthermore, it would have been desirable to study the possibility of product inhibition of cyto P450. However, it was not possible to obtain reference standards of the metabolites due to the difficulty in synthesising these compounds (I. Cockshott, personal communication). It has been shown that the major metabolites of propofol in humans and animals are propofol glucuronide and the glucuronic and sulphate conjugates of its hydroxylated metabolite (Simons *et al*, 1988, 1991a,b), although the proportion of metabolites produced varies with species. However, no information on what metabolites were produced, their time course of production or the effect of drug interactions on specific metabolites was acquired from the studies reported here.

The concentrations of propofol used in these studies were intended to reflect a range of blood propofol concentrations including those which would be encountered during anaesthesia and those which would allow determination of Km and Vmax. The concentrations of substrate chosen to generate the reciprocal plot, should be around the Km value, otherwise accurate determinations are not possible. The mean Km values recorded for rat hepatic tissues ranged from 43.6μM - 48.9μM and the range of concentrations used during incubation spanned this value.

In humans, there appears to be a wide variation in blood concentrations of propofol associated with the induction of sleep and the abolition of responses to surgical stimuli, with the concurrent use of other drugs making comparisons difficult (Spelina *et al.*, 1986; Schafer *et al.*, 1988; Dixon *et al.*, 1990). In human volunteers, the blood concentration required to induce sleep varied from as low as 1.64 μg/ml to as high as 6.38 μg/ml (Schuttler *et al.*, 1985), which correspond to 9μM and 37μM

respectively, while the blood concentrations required to abolish responses to surgical stimulation in humans appear to be in excess of 5μg/ml (29μM) (Turtle *et al.*, 1987). In dogs undergoing superficial body surface surgery, blood propofol levels in excess of 5μg/ml were also required to maintain satisfactory surgical conditions (Nolan and Reid, 1993), while in female rats, liver propofol concentrations as high as 132μM are achieved following a single anaesthetic dose (Simons et al., 1991a). Thus, it was considered appropriate to choose the concentration range from 28μM to 140μM in rat microsome studies.

It has been shown *in vivo* that species variations in the persistence of propofol in blood following i.v. administration exist. In goats, Reid *et al.* (1993) did not detect propofol in blood 2h after bolus administration, while in rats, dogs and humans, propofol can still be detected 8 h or later after the end of administration (Cockshott *et al.*, 1990; Simons *et al.*, 1991a; Nolan & Reid, 1993). These differences are probably the result of species variation in the capacity to metabolise propofol. In this study, no significant difference was observed between the rate of propofol degradation by microsomal hepatic cyto P450 obtained from rat and sheep. The Km values for rats and sheep were similar (42.0 ± 7.1μM in sheep; 48.9 ± 2.2μM in rat). and confirm the similarities that exist between these species for cyto P450 oxidative metabolism *in vitro* (see review by Smith, 1991).

Of interest was the observation that Sprague Dawley rats degraded propofol more rapidly than Wistar rats. This observation was based on a small number of animals, however, it indicated the possibility that breed differences may exist in the ability of animals to metabolise propofol. Recent work by Zoran *et al.* (1993) reported significant differences in the whole body clearance of propofol between greyhounds and mixed breed dogs *in vivo*; the clearance rate for greyhounds was half that obtained in mixed breed dogs (54.0 ± 10.0 ml/kg.min cf 114.8 ± 50.0 ml/kg.min), although, the value reported for greyhounds is comparable to that obtained for mixed breed dogs by Nolan *et al.* (1993). Strain differences in the metabolism of hexobarbitone have been observed in mouse, manifested as increased sleeping time after a single dose (Alvan, 1992). Thus, despite the lack of significant differences between sheep and rat in the rate of degradation of propofol and the affinity of cytochrome P450 for propofol recorded in this study, the possibility exists of other interspecies and interbreed variation in propofol degradation.

At concentrations equal or greater than 10 mM, 1-aminobenzotriazole (ABT) has been shown to inhibit (by alkylation of the prostetic heme) by approximately 70% the activity of several isoenzymes of cyto P450, with no detectable effect on other

components of the endoplasmic reticular mixed function oxidase systems such as NADPH cytochrome c reductase (FMO) and cytochrome b_5 (Mugford *et al.*, 1992).

The inhibition of cyto P450 with ABT, 12 mM, caused a dramatic reduction in the rate of propofol degradation which suggests that the oxidative degradation of propofol is extensively cyto P450-dependent within hepatic microsomes. The degree of inhibition observed was dependent on the concentration of propofol used, less inhibition of propofol degradation at higher initial concentrations of propofol, which suggests that the inhibition is competitive.

The identities of isoenzymes that catalyse ABT metabolism have not yet been extensively studied, however, ABT appears to affect isoenzymes responsible for ethoxycoumarin and ethoxyresourfin O-deethylation (P450 1A & 2B) and lauric acid hydroxylation (P450 4A1) (Mugford *et al.*, 1992). These workers indicated that there are P450 isoenzymes which are not affected by ABT, however, their identities are still unknown. There is a need for further enzymatic studies to clarify whether the residual rate of degradation of propofol observed in the presence of ABT is a result of degradation from P450 subfamilies which do not recognise ABT as a substrate, or whether there is a contribution from other enzymatic systems such as the flavin-monooxigenase system (FMO). However, it is clear that an enzymatic dependence exists between the isoenzymes inhibited by ABT and the degradation of propofol.

Inhibition of cyto P450 activity of rat hepatic microsomes by propofol was reported by Baker *et al.* (1993). They found propofol (25μM - 1000μM) to be a dose dependent inhibitor of benzphetamine demethylation and aniline hydroxylation in rat liver microsomes at concentrations of 25μM and higher, but to be ineffective at inhibiting enflurane metabolism. Benzphetamine is metabolised by the isoenzyme P450 2B1, and aniline by P450 2B1 and to a small extent by P450 2C6. while enflurane is metabolised by P450 2E1. The objective of their work was to identify the effectiveness and selectivity of propofol in inhibiting drug metabolism and they demonstrated that propofol could function as an effective and selective inhibitor of drug metabolism. Because the greatest degrees of propofol inhibition occurred in microsomes from phenobarbitone - treated animals, they demonstrated that propofol was most effective at inhibiting the isoenzymes P450 2A1 and P450 2B1, phenobarbitone - inducible forms of cytochrome P450. It is worth commenting that the incubation of propofol with hepatic microsomes for 15 min would probably result in a considerable decrease in the 'actual' propofol concentration, and therefore it is possible that these workers have underestimated the capacity of propofol to inhibit these isoenzymes, since they did not measure propofol concentrations.

Propofol dose - dependently inhibited the N-demethylation of aminopyrine by rat hepatic microsomes. Concentrations as low as 5.6μM did not significantly interfere with aminopyrine N-demethylation, but the inhibition (mean, 34.9%) was effective at doses of 56 μM, a concentration achievable during clinical anaesthesia. The broad, yet selective capacity of propofol to inhibit the activity of cyto P450 isoenzymes has many implications for the concomitant use of drugs during propofol anaesthesia *in vivo*. It is also possible that the interaction of propofol with different enzyme sites may vary and that inhibition at one isoenzyme might be competitive, while at other sites, noncompetitive inhibition might occur. These possibilities need to be studied further with selective ligands, inhibitors and inducers for the particular isoenzymes involved. Thus, it appears likely that propofol has the potential to alter drug metabolism, depending on the drug administered and its metabolic pathway.

Aminopyrine significantly inhibited the degradation of propofol, and significantly increased the Km value for propofol without affecting Vmax, indicating that the inhibition was competitive in nature. Further studies are necessary to determine whether aminopyrine combines to the active site of the enzyme which degrades propofol (true competitive inhibition), thus indicating that aminopyrine and propofol share the same isoenzymes for degradation, or whether it binds to a site other than the active one (feedback inhibition). The data obtained from the studies with ABT and aminopyrine thus suggest that the P450 2B and 2C isoenzymes are involved in the oxidative degradation of propofol since aminopyrine is preferentially metabolised by the isoenzyme P450 2C11.

Ketamine and alfentanil are drugs which are potentially suitable for use in conjunction with propofol. Both ketamine and alfentanil undergo extensive hepatic metabolism, both are metabolised by cytochrome P450 and demethylation is the major pathway for both drugs. Ketamine also undergoes hydroxylation, while dealkylation and aromatic hydroxylation occur during the metabolism of alfentanil (Waterman & Livingston, 1978b; Meuldermans *et al.*, 1987). Since both ketamine and alfentanil undergo demethylation, and propofol degradation is inhibited by aminopyrine, an effective substrate for demethylation processes, it was considered possible that either of these drugs might inhibit propofol metabolism. These drugs were used at one concentration only, one that would reflect clinically effective plasma concetrations. Alfentanil plasma concentrations between 148 and 285 ng/ml have been reported to be clinically satisfactory in combined infusions with propofol (Schuttler *et al.*, 1988), consequently, a concentration of 200 ng/ml was chosen for study, while the ketamine concentration used was 5 μg/ml, since Waterman & Livingston (1978b) indicated that sheep awake from ketamine anaesthesia at plasma concentration of approximately 1μg/ml. The consequences of inhibition of propofol

degradation *in vivo* would be to raise propofol blood levels and prolong anaesthesia. Indeed, Gepts *et al.* (1988) reported that when propofol and alfentanil were used together to maintain anaesthesia, the activity of each drug was potentiated. Neither ketamine nor alfentanil, had a significant effect on the microsomal hepatic oxidative degradation of propofol. Ketamine increased the Km value for propofol (mean values, 46.5μM to 53.2μM), but this difference was not significant, while alfentanil did not change either the Vmax or the Km of propofol. Janicki *et al.* (1992), reported that propofol inhibited dose-dependently (3μM - 100μM) the oxidative metabolic degradation of alfentanil and sufentanil by hepatic microsome preparations from pig and humans. The major metabolite of propofol is formed by hydroxylation of its phenol ring and alfentanil also undergoes some hydroxylation, therefore, it is possible that the interactions between propofol and alfentanil involve cyto P450 isoenzymes which perform hydroxylation reactions. However, since hydroxylation is not the major pathway for alfentanil degradation (Meuldermans *et al.*, 1987), it is considered unlikely that significant interference of alfentanil with propofol degradation will occur *in vivo* unless very high doses of alfentanil are used. Indeed work *in vivo* has suggested that alfentanil does not affect significantly the pharmacokinetics of propofol (Browe *et al.*, 1992; Bostek *et al.*, 1992).

Ketamine did not alter the degradation of propofol by liver microsomes, which suggests that the isoenzymes responsible for propofol degradation do not have a high affinity for ketamine, and that no interaction between the two drugs *in vivo* is likely to occur. However, it is known that ketamine induces liver enzymes *in vivo*, increasing its own metabolism (Livingston & Waterman, 1978), therefore, the potential for alteration of propofol metabolism by ketamine in whole animals cannot be totally excluded. It would be of interest to test this potential *in vitro* using hepatocellular cultures, since such tissue cultures have the potential to display liver enzyme up- and down-regulation, which cannot occur with microsome preparations.

There are many studies indicating that the lung plays an active role in the uptake and metabolism of drugs (Orton *et al.*, 1973). The *in vitro* study with rat lung microsome preparations confirms speculation from studies *in vivo*, which suggested that the high clearance of propofol might be due to an extra hepatic site of metabolism (Lange *et al.*, 1990; Veroli *et al.*, 1992). The data reported here indicate that lung tissue can degrade propofol and it is considered likely that this is one of the sites which contributes to the high body clearance of propofol *in vivo*, at least in rats. The mean Km value, 13μM, indicates that the affinity of propofol for the enzyme degrading it is high and is similar to the affinity of propofol for hepatic microsomal enzymes. However, the range of Km values was large, from 2.9μM - 30.2 μM and further work should be done to characterise the enzyme kinetics in this

tissue. Since there is interspecies differences in the degradation of xenobiotics, it cannot be assumed that propofol is degraded by the lung in all species. It is difficult to estimate the contribution of lung tissue to the degradation of propofol compared with hepatic tissue, since the concentration of protein in the lung was much lower than the liver (and the concentrations of propofol used were also lower). Matot *et al.* (1993), reported that in cats there is a substantial extraction of propofol by the lung, therefore, it is important to consider the potential for drug metabolsim in this tissue. Moreover, the lung is an organ which receives a high blood flow (equivalent to cardiac output), and consequently could help account for the high body clearance reported in species such as the goat (Reid *et al.*, 1993). Further studies are required to characterise and quantify lung metabolism of propofol *in vitro*.

4.2 Pharmacokinetics and pharmacodynamics of propofol and propofol/ketamine infusions in sheep.

In vitro techniques for studying drug metabolism have been developed for scientific, economical and ethical reasons, and the information generated from such work is useful. However, *in vitro* alternatives generally cannot replace whole animal experiments, but they provide clear indications for subsequent *in vivo* research. The results obtained from the degradation of propofol and the apparent lack of effect of ketamine on propofol metabolism *in vitro* suggested that this drug combination might be suitable to study *in vivo*. Consequently the use of propofol/ketamine infusions was studied qualitatively i.e. suitability for body surface surgery, and quantitatively, by characterising the kinetics and dynamics of propofol when coadministered with ketamine. Sheep were considered to be an appropriate model for this purpose. Firstly, data obtained from *in vitro* studies indicated that there are no observed difference in the Km and Vmax values for sheep and rats; secondly, the practicalities of TIVA and repeat blood sampling indicated that rats would be difficult to handle, and thirdly, the pharmacokinetics of ketamine in sheep indicated that it was rapidly metabolised (Waterman & Livingston, 1978b) and thus might be a useful agent to administer by infusion.

To date, there is a lack of information on the concomitant use of propofol and ketamine for anaesthesia, only two reports in humans have been found (Mayer *et al.*, 1990; Schuttler *et al.*, 1991). Both groups reported that total intravenous anaesthesia with propofol and ketamine was very satisfactory and that propofol and ketamine induced fewer disturbances in physiological parameters than total intravenous anaesthesia with propofol and fentanyl.

General anaesthesia in ruminant animals is associated with the risk of regurgitation of ruminal contents which may lead to aspiration pneumonia and death (Hall & Clarke, 1991). The rapid recovery from anaesthesia in sheep given a bolus dose of propofol before maintenance of anaesthesia with halothane (Waterman, 1988), indicated the potential for use of propofol in a total intravenous anaesthetic technique in this species, where a rapid recovery from anaesthesia is essential. Many regimens of anaesthesia have been suggested as being suitable for use in small ruminants (Taylor, 1991), however none of those discussed included TIVA. The use of a variable infusion rate was considered important and of more clinical value than using a constant-rate infusion, because it permits the anaesthetist to titrate the intravenous dose to a desired effect, according to the status of each individual animal.

The initial infusion rate of propofol alone, 0.5 mg/kg/min, was based on data and clinical experience gained by Nolan & Reid (1993) in dogs undergoing the same surgical procedure, and premedicated with the same drug combination. It was anticipated that ketamine would have a propofol-sparing effect and consequently, the initial propofol infusion rate for sheep receiving propofol and ketamine was lower, 0.3 mg/kg/min. The combination of an induction dose of propofol, 4mg/kg, followed by a variable infusion rate was satisfactory clinically for superficial surgery in sheep. Similarly, an induction dose of propofol, 3 mg/kg, and ketamine, 1 mg/kg, and a variable infusion of the two drugs proved satisfactory for induction and maintenance of anaesthesia. In both groups, the first skin incision was made 10 min after the infusion began, and the degree of analgesia appeared to be satisfactory. Reid *et al.* (1993), reported that a bolus dose of 4 mg/kg was satisfactory for induction of anaesthesia in goats, where anaesthesia was maintained with a gaseous anaesthetic, while previously, Waterman (1988) had indicated that a mean propofol induction dose of 3.8 mg/kg was satisfactory in sheep. Nolan & Reid (1993) reported that induction of anaesthesia with propofol 4 mg/kg prior to maintenance with a zero order infusion of propofol (0.4 mg/kg/min) and 67% nitrous oxide was satisfactory for superficial surgery in dogs, although these authors commented that this anaesthetic regime induced 'light' surgical anaesthesia. The inclusion of ketamine allowed a sparing of the dose of propofol necessary for induction and maintenance of anaesthesia. Although infusion rates were adjusted according to clinical need and physiological status, no other attempt was made to equate the plane of anaesthesia in both groups. Indeed it is considered unlikely that the depth of anaesthesia in both groups was the same, and it is possible that the total dose of propofol used in sheep receiving ketamine could have been reduced further. One animal in the propofol group and two in the propofol/ketamine group were apnoeic after induction of anaesthesia, but spontaneous breathing resumed shortly

after manual ventilation was performed. The higher incidence of apnoea in sheep receiving propofol/ketamine than in the propofol group might suggest that the animals were not in the same plane of anaesthesia and that a smaller dose of propofol would have been adequate. However, apnoea post induction is a normal feature during propofol anaesthesia in humans and animals (Morgan & Legge, 1989; Watkins *et al.*, 1987; Nolan *et al.*, 1993) and the group numbers were small (n=5).

The quality of anaesthesia was satisfactory in both groups of sheep and no reaction to the surgical procedures was observed. Both systolic arterial blood pressure and pulse rate, indicators of cardiovascular function, were well maintained throughout the infusion period. In both groups there was an increase of approximately 15% from the value taken before induction, for the pulse rate 5 min after the infusion began, while 30 min after the infusion began, the systolic arterial blood pressure had fallen in both groups by approximately 15% from the value taken at the start of the infusion. Mild hypotension is a feature of propofol anaesthesia in humans (Langley & Heel, 1988), although this is dose dependent.

The respiratory rate was significantly higher during the infusion period for animals in the propofol/ketamine group compared to those sheep that received propofol alone, possibly due to the inclusion of ketamine, since respiratory depression was not recorded in the other group. Respiratory depression is not a feature of clinical doses of ketamine (White *et al.*, 1982), but is often associated with propofol anaesthesia. Repiratory rate is a fairly crude marker of respiratory function and arterial blood gas analysis or end tidal carbon dioxide concentration recordings would have been more informative.

Schuttler *et al.* (1988) & Puttick *et al.* (1992) showed that marked individual variations in blood concentrations of propofol regularly occur during infusion of propofol, a finding observed similarly in dogs by Nolan & Reid (1993). In this study there were variations in blood concentrations of propofol and in the time of achievement of maximal blood concentrations, however, this was probably a reflection of differences in individual infusion rates. The use of a variable infusion rate determined by clinical and cardiovascular parameters was surprisingly successful in achieving a steady state blood propofol concentration in both groups of sheep. The levels of ketamine in plasma were not measured and it is not known if steady state levels of this drug were also achieved with the total amount infused.

The persistence of propofol in blood, in these sheep (last detectable propofol, 9h) was less than the 25h reported for dogs undergoing propofol infusion for a similar period (Nolan & Reid., 1993), suggesting that sheep metabolise propofol faster than

dogs. This has also been previously reported for the barbiturate anaesthetic, pentobarbitone (Rae, 1962).

The recovery times observed in sheep anaesthetised with propofol alone were markedly shorter than those reported by Nolan & Reid in dogs (1993), 2.8 ± 0.4 cf 7.6 ± 1.0 min for endotracheal extubation, 6.3 ± 1.2 cf 20.8 ± 6.3 min for sternal recumbency and 10.9 ± 1.6 cf 30.7 ± 8.0 min for standing unaided for sheep and dogs respectively. Sheep that received propofol and ketamine took significantly longer to be extubated and to roll into sternal recumbency than sheep that had been anaesthetised with propofol alone, but there were no differences in the time to standing. This may indicate that the plasma ketamine concentrations as the infusion was switched off were higher than necessary and highlights the need to measure plasma concentraions of all drugs administered in a multi-drug infusion technique.

Pharmacokinetic analysis of the data showed propofol to have a large volume of distribution in sheep. The volume of distribution at steady state (3.4 ± 0.5 L/kg) was greater than that reported in goats, 2.56 ± 0.32 L/kg (Reid *et al.*, 1993), but smaller than reported for dogs, 6.51 ± 0.52 L/kg (Nolan & Reid, 1993). The clearance of propofol in goats (275.0 ± 38.5 ml/min.kg) is higher than that obtained for sheep receiving propofol alone in this study (82 ± 11 ml/kg.min), however, propofol in sheep is more rapidly cleared than in dogs, 50.1 ± 3.9 ml/kg.min (Nolan & Reid, 1993), and in humans, 2.09 ± 0.65 L/min (Shafer *et al.*, 1988). No significant differences were recorded between the two goups of sheep although the mean whole body clearance of propofol and the Vdss was higher for sheep that received ketamine. These data suggest that ketamine does not alter the pharmacokinetics of propofol in sheep. However, in this study the total dose of propofol administered was lower in the group which received propofol/ketamine than in the group that received propofol alone and although other workers have reported that propofol does not exhibit dose-dependent pharmacokinetics (Gepts *et al.*, 1987), further studies are necessary to confirm this in sheep. In addition, only 5 animals were included in each group and if larger numbers were used, significant differences in whole body clearance and Vdss may have been demonstrated.

In conclusion, the studies reported in this thesis, suggest that the rapid recovery observed from propofol anaesthesia is related to the high rate of degradation of this drug by the hepatic enzyme systems, and suggest that the lung may be an important organ in degrading propofol. Cytochrome P450 enzymatic systems have an important role in propofol degradation and it is hypothesised that P450 2B and 2C subfamilies are involved in the degradation of propofol. Since the degradation of propofol in microsomes was extremely rapid, it would be interesting to study the

degradation of propofol by hepatocellular cultures where both microsomal and cytosolic enzymatic systems are present, intracellular drug distribution takes place and inhibition/induction studies can be monitored. Secondly, it is clear that propofol, at concentrations found in blood during clinical anaesthesia, has the potential to influence significantly the rate of metabolism of other drugs and *vice versa.* However, these potential interactions have to be evaluated on the basis of the individual drugs coadministered with propofol. Finally, it is important to emphasise that propofol alone or when combined with other drugs is potentially very useful for total intravenous anaesthesia, and that propofol/ketamine infusions are worthy of further development in animal species.

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APPENDIX

animal n°	protein	cyto P450
R1	16	1.0
R2	17	0.99
R3	14	1.0
R4	16	0.54
R5	17	0.65
R6	18	0.92
R7	17	0.65

Table A1 - Concentration of protein (mg/gr of liver) and Cytochrome P450 (cyto P450; nmol/mg of protein) of hepatic microsome preparations, from rats used in the study of the time course of the degradation of propofol by hepatic microsomes. The rats from R1-R7 were used for incubations corresponding to 5, 10, 15, 20, 30, 45 and 60 min respectively.

animal n°	protein	cyto P450
R1	16	1.0
R2	17	0.9
R3	16	0.7
R4	15	0.6
R5	14	0.69
R6	17	0.52

Table A2 - Concentration of protein (mg/gr of liver) and Cytochrome P450 (cyto P450; nmol/mg of protein) of hepatic microsome preparations, from rats used in the study of the inhibition of cytochrome P450 activity by of propofol.

animal n°	protein	cyto P450
R1	18	0.54
R2	16	0.88
R3	17	0.6
R4	15	0.66
R5	15	0.61
R6	14	0.58

Table A3 - Concentration of protein (mg/gr of liver) and Cytochrome P450 (cyto P450; nmol/mg of protein) of hepatic microsome preparations, from rats used in the study of the possibility of extra cytochrome P450 degradation of propofol.

animal n°	protein	cyto P450
R1	15	0.72
R2	16	0.76
R3	15	0.89
R4	15	0.51
R5	16	0.69
R6	16	0.6
R7	15	0.71
R8	14	0.49
R9	16	0.47
R10	14	0.6
R11	16	0.83
R12	15	0.67
R13	16	0.78
R14	15	0.48
R15	15	0.51
R16	15	0.61
R17	14	0.59
R18	14	0.78

Table A4 - Concentration of protein (mg/gr of liver) and Cytochrome P450 (cyto P450; nmol/mg of protein) of hepatic microsome preparations, from rats used in the study of the effect of other drugs on the cytochrome P450 degradation of propofol. Animals R1 to R6 were used in experiments with propofol and ketamine; R7 to R12 in experiments with propofol and alfentanil and from R13 to R18 in experiments with propofol and aminopyrine.

animal n°	protein	cyto P450
S1	19	0.66
S2	19	0.49
S3	18	0.54
S4	15	0.62
S5	20	0.59
S6	19	0.60

Table A5 - Concentration of protein (mg/gr of liver) and Cytochrome P450 (cyto P450; nmol/mg of protein) of hepatic microsome preparations, from rats used in the study of the degradation of propofol by sheep cytochrome P450 enzymatic system (a comparison with sheep).

animal n°	protein
R1	1
R2	1.25
R3	1.83
R4	1.5
R5	1.9

Table A6 - Concentration of protein (mg/gr of liver) and Cytochrome P450 (cyto P450; nmol/mg of protein) of hepatic microsome preparations, from rats used in the study of the degradation of propofol by lung cytochrome P450 enzymatic system

In. Conc	05 min	10 min	15 min	20 min	30 min
28	16	15	15	14	14
56	36	36	33	32	29
84	60	57	56	51	46
140	112	107	101	99	84

Table A7 - Initial propofol concentration (In. Conc, μM) and propofol concentrations (μM) used for the graphical representations of the velocity of propofol degradation plotted against propofol concentrations in the study of the time course (5, 10, 15, 20, 30 min) of propofol degradation by rat hepatic microsomes.

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	18.1	15.6	18.2	17.6	14.9	15.8	16.7	0.6
56	41.3	37.0	45.8	42.5	34.72	37	39.7	1.7
84	67.3	61.7	70.7	67.5	57.6	60.2	64.2	2.1
140	120.6	120.6	127.3	118.5	110.2	112.8	118.3	2.5
28*	24.8	24.6	27.1	26.9	27.5	25.8	26.1	0.5
56*	51.5	52.2	56.8	54.4	53.4	51	53.2	0.9
84*	77.8	79.9	79.9	79.7	81.3	77.6	79.4	0.6
140*	136.9	135.5	140.2	136	133.3	129.5	135.2	1.5

Table A8 - Initial propofol concentration (I.C., μM) and propofol concentrations (μM) used for the graphical representations of the velocity of propofol degradation plotted against propofol concentrations in the study of propofol degradation by rat hepatic microsomes in the presence or absence of 1-aminobenzontriazole (*).

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	14.1	14.7	14.7	14.9	14.7	14.7	14.6	0.1
56	28.9	32.0	32.9	34.2	32.3	32.2	32.1	0.7
84	46.4	52.0	55.5	54.5	54.1	52.3	52.4	1.3
140	86.2	97.7	103.6	105.0	101.2	99.7	98.9	2.7
28*	14.2	14.9	14.9	15	15.1	14.9	14.8	0.1
56*	29.8	32.3	33.7	34.8	33.3	32.8	32.8	0.7
84*	47.1	52.5	55.3	56.2	54.7	53.2	53.2	1.3
140*	86.9	96.5	103.3	105.5	100.1	98.0	98.4	2.7

Table A9 - Initial propofol concentration (I.C, μM) and propofol concentrations (μM) used for the graphical representations of the velocity of propofol degradation plotted against propofol concentrations in the study of the degradation of propofol by rat hepatic microsomes in the absence or in the presence of ketamine (*).

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	15.2	15.9	16.4	14.6	15	15.7	15.5	0.3
56	34.5	36	37.2	32	32	36.2	34.6	0.9
84	59.3	61.6	59.9	50.6	54.9	58.2	57.4	1.6
140	114.8	111	111	93.6	102.8	107.9	106.8	3.1
28*	15.2	15.6	16.5	14.7	15.1	15.8	15.5	0.2
56*	34.6	35.8	36.1	32.0	33.5	34.8	34.5	0.6
84*	59	62.2	59.2	50.6	53.5	57.3	57.0	1.7
140*	117.4	123.8	110.2	91.0	102.6	108.5	108.9	4.8

Table A10 - Initial propofol concentration (I.C, μM) and propofol concentrations (μM) used for the graphical representations of the velocity of propofol degradation plotted against propofol concentrations in the study of the degradation of propofol by rat hepatic microsomes in the absence or in the presence of alfentanil (*).

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	14.8	14.8	14.5	14.0	14.7	14.6	14.6	0.1
56	33.7	33.5	33	34.4	33.1	31.7	33.2	0.4
84	53.7	59.4	52.9	56.0	55	50.5	54.6	1.2
140	104.2	111	99	112	102.4	92.8	103.6	3.0
28*	17.4	17.7	17.1	18.5	16.9	16.9	17.4	0.2
56*	37.9	39.4	37.2	40.8	35.6	35.4	37.7	0.9
84*	60.6	64.1	58.4	62.0	59.5	55.8	60.1	1.2
140*	109.1	115.1	109.6	114.2	107.1	99.2	109.0	2.3

Table A11 - Initial propofol concentration (I.C, μM) and propofol concentrations (μM) used for the graphical representations of the velocity of propofol degradation plotted against propofol concentrations in the study of the degradation of propofol by rat hepatic microsomes in the absence or in the presence of aminopyrine (*).

I.C.	R1	R2	R3	R4	R5	R6	mean	±sem
28	16.4	14.3	14.6	14.2	14.1	14.0	14.6	0.4
56	41.5	33.2	33.0	31.8	29.7	28.4	32.9	1.9
84	73.7	54.7	55.4	53.3	49.5	48.0	55.8	3.8
140	124.6	106.4	109.1	101.8	99.1	98.7	106.6	4.0
28*	15.2	15.9	16.4	14.6	15	15.7	15.5	0.3
56*	34.5	36	37.2	32	32	36.2	34.6	0.9
84*	59.3	61.6	59.9	50.6	54.9	58.2	57.4	1.6
140*	114.8	111	111	93.6	102.8	107.9	106.8	3.1

Table A12 - Initial propofol concentration (I.C, μM) and propofol concentrations (μM) used for the graphical representations of the velocity of propofol degradation plotted against propofol concentrations in the study of the degradation of propofol by sheep and rat* hepatic microsome preparations.

I.C.	R1	R2	R3	R4	R5	mean	±sem
5.6	4.8	4.4	4.5	5.1	5.0	4.8	0.1
11	8.5	8.1	8.4	9.3	8.9	8.6	0.2
28	16.6	17.0	17.6	20.0	19.1	18.1	0.6
56	28.8	31.9	31.2	37.1	35.0	32.8	1.5

Table A13 - Initial propofol concentration (I.C, μM) and propofol concentrations (μM) used for the graphical representations of the velocity of propofol degradation plotted against propofol concentrations in the study of the degradation of propofol by rat lung microsome preparations.