MAGNESIUM SULPHATE REVERSAL OF ESTABLISHED BUPIVACAINE ELECTROPHYSIOLOGICAL CARDIOTOXICITY

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DECLARATION

I, Anthony Raddon Reed, hereby declare that the work on which this thesis is based is my original work (except where acknowledgements indicate otherwise), and that neither the whole work nor any part of it has been, is being, or is to be submitted for another degree in this or any other university.

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Signed by candidate

Date: 24th June 1998

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MAGNESIUM SULPHATE REVERSAL OF ESTABLISHED BUPIVACAINE ELECTROPHYSIOLOGICAL CARDIOTOXICITY

1. INTRODUCTION

Bupivacaine is an amide-type local anaesthetic, and is popular because of its anaesthetic properties - high potency, dense sensory and motor blockade, and long duration of action. However bupivacaine is probably more cardiotoxic than any other local anaesthetic in use today. The United States Food and Drug Administration withdrew approval of 0.75% bupivacaine for use in obstetric anaesthesia in 1984, owing to reports of cardiac arrest with this concentration. In lower concentrations bupivacaine remains useful for regional and local anaesthesia.

Bupivacaine is similar to lignocaine in its central nervous system toxicity; however its tendency for cardiotoxicity would seem greater.

Bupivacaine toxicity is a major hazard in anaesthesia. When bupivacaine toxicity occurs, it is frequently manifest as disturbances of cardiac

conduction leading to ventricular fibrillation and is particularly intractable to all forms of treatment. The mortality, from bupivacaine toxicity, is considerable. In a 1985 report to the F.D.A. the manufacturers of bupivacaine reported 12 cases of cardiac arrest- 10 of them fatal[3]. Despite this, bupivacaine is the most widely used regional anaesthetic drug because of its advantages of quality of block and duration of action. There are, however, no satisfactory mechanisms for treating the cardiac disturbances that occur in cases of toxicity. Three recent cases experienced by the Groote Schuur Group, have suggested that magnesium sulphate may, uniquely, reverse bupivacaine toxicity. The first, a paediatric case having a caudal anaesthetic block developed ventricular arrhythmia's which reversed immediately following a 20mg/kg intravenous dose of magnesium sulphate. The second was a case at the Somerset Hospital following an epidural block where, again, intractable arrhythmias occurred which responded to infusions of magnesium sulphate. A 3rd case occurred in a child following a caudal block using bupivacaine. A bizarre ventricular arrhythmia was treated initially with magnesium sulphate (10mg/kg) with a good result.

A literature search reveals that one previous study[11] has analysed the interaction between bupivacaine and magnesium. In this study, magnesium was administered at the same time as bupivacaine in a canine model. Magnesium sulphate was shown to protect against the prolonged QT interval and the prolongation of the QRS complex. This study did not investigate the potential role of magnesium in treating established bupivacaine arrhythmia's. It therefore appears important to investigate this potential role for magnesium, as it may offer the first really effective form of therapy for what is currently a potentially lethal complication.

2. CARDIAC ELECTROGENESIS AND ION CHANNELS

ION CHANNELS IN THE HEART

ELECTROPHYSIOLOGICAL CONSIDERATIONS

2.1 ION CHANNELS IN THE HEART

A repetitive, precisely choreographed sequence of currents through families of Na^+, Ca^{++}, K^+ and Cl^- channels is responsible for generating the normal sinus rhythm of pacing potentials, and it is the transmission of these signals through specialized conduction pathways, and cardiac action potentials which trigger the process of "excitation-contraction" coupling. The latter series of events depends on a separate set of ion channels and results in the release of intracellular Ca^{++} which produces the physical interaction between actin and myosin resulting in contraction. An understanding of the different types of channels, how they regulate flow of ions across the membrane and how the sequence is arranged, is necessary to be able to understand how bupivacaine's cardiac effects are mediated. Only then may we begin to speculate as to how any specific treatments may work. An advance in our ability to treat toxicity may occur by chance. A breakthrough in our ability to treat bupivacaine toxicity may help to explain the mechanisms of bupivacaine's toxic effects.

The fundamental units of cardiac excitability are ion channels, complex glycoproteins embedded in the plasma membrane of cardiac myocytes. There are many different types of cardiac ion channels and ion pumps which together orchestrate a complex process that results in the cardiac cell's action potential.

2.2 ELECTROPHYSIOLOGICAL CONSIDERATIONS

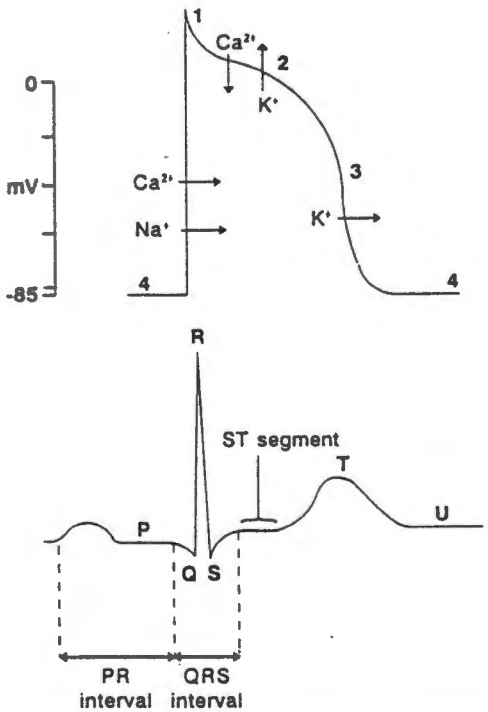


Fig. 1. The upper panel illustrates a ventricular myocardial cell action potential. The lower panel shows a schematic QRS-T complex. The figure shows that the upstroke of the action potential (phase 0) occurs as sodium and then calcium move across the cell membrane from the extracellular to the intracellular compartment. This portion of the action potential is responsible for the QRS complex of the ECG which is recorded as the ventricular cells are sequentially depolarised. The plateau of the action potential (phase 2) results from the balance between the inward movement of calcium ions and the outward movement of potassium ions. This portion of the action potential is responsible for the ST segment of the ECG. The phase of rapid repolarisation (phase 3) occurs as potassium ions move across the membrane from the intracellular to the extracellular compartment. This portion of the action potential is responsible for the T wave of the ECG which reflects the sequential rapid repolarisations of the ventricular cells [from Harper & Gettes (1988), with permission].

Phase 0 (V_{max}) of the action potential is a sodium dominated current in atrial and ventricular myocardium and also in His-Purkinje fibres whereas the SA and AV nodes phase 0 is dominated by calcium currents. The cumulative upstrokes of the atrial action potentials are responsible for the p waves on the body surface ECG. The cumulative upstrokes of the ventricular action potential are responsible for the QRS complex. The maximal rate of rise (V_{max}) is a major determinant of the conduction velocity and duration of the QRS complex on the ECG. Slowing of V_{max} by sodium channel blockade is therefore reflected as an increase in the QRS duration on the body surface ECG.

The rapid upstroke is followed by phase 1 and this is followed by a prolonged period of slow repolarization (phase 2) which results from a balance between inward and outward currents. The inward currents are predominantly carried by calcium ions and the outwards currents by potassium ions. This plateau is reflected on the body surface ECG by the ST segment. The phase of rapid repolarization (phase 3) results from the enhanced outward movement of intracellular potassium ions across the cell membrane. The rapid repolarization phase restores the membrane to its resting potential and corresponds to the relative refractory period - this time is reflected on the body surface ECG as the T wave.

3. BUPIVACAINE

3.1 CHEMICAL PROPERTIES

Bupivacaine is a member of the amide group of local anaesthetic agents and is a structural homologue of mepivacaine. It consists of a lipophilic aromatic group linked to a hydrophilic tertiary amine group by linked by an intermediate amide chain which is an important determinant of the duration of action [117]. Bupivacaine has a pKa of 8.1 at 25°C and this value is close enough to blood pH that significant changes in the ratio of ionized to un-ionized drug species have been shown to occur during acid-base imbalances [106].

Bupivacaine is 96% protein bound and although highly protein bound at lower concentrations, the ratio of free to bound drug increases rapidly as concentration increases [1]. The 2 proteins involved in binding are alpha-1-acid glycoprotein (AAG) and albumin [70] Binding to AAG is a high affinity-but saturable process. This is important as these two proteins are not as abundant in infancy as in childhood and adults. A decrease in protein binding of bupivacaine of up to 50% [106] has been reported in the foetus and neonate, and may last until 6 months of age.

3.2 MECHANISMS OF ACTION

It is a tertiary base and is administered as bupivacaine hydrochloride [B-HCL] which is water soluble. After injection the amine base is liberated by the relative alkalinity of the tissue. The tissue will therefore contain both the ionised [BH^+] and the unionised [B] whose relative proportions will depend on the pH of the solution and the pKa. The non-ionized base diffuses through the nerve sheath, perineuronal tissues and neuronal membrane to reach the nerve axoplasm where it partially dissociates again.

$$B + H^+ <\!\!> BH$$

In the ionized form BH^+ it enters the sodium channel from the axoplasm and either occludes the channel or results in channel closure.

Sodium channels exist in activated-open, inactivated-closed, and rested-closed states during various phases of the action potential. In the resting nerve membrane, sodium channels are distributed evenly between the rested-closed and inactivated-closed states. By selectively binding to sodium channels in inactivated-closed states, local anaesthetic molecules, especially bupivacaine stabilize these channels in this configuration and prevent their change to rested-closed and activated-open states in response to nerve fibre impulses. Sodium channels in the inactivated-closed state are not permeable to sodium and thus conduction of nerve impulses in the form of propagated action potentials cannot occur. It has been speculated that local anaesthetics, including bupivacaine, bind to specific sites located on the inner portion of sodium channels, as well as obstructing sodium channels near their external openings to maintain these channels in the inactivated-closed states.

Frequency dependent blockade is frequently seen with local anaesthetics including bupivacaine. Sodium channels tend to recover from local anaesthetic-induced conduction blockade between action potentials and to develop additional conduction blockade each time sodium channels open during an action potential(frequency dependent blockade). Therefore local anaesthetic molecules can gain access to receptors only when the sodium channels are in the activated-open (lignocaine) or inactivated-closed (lignocaine + bupivacaine) states. For this reason selective blocking of nerve fibres may be related to the characteristic frequencies of activity of a nerve, as well as to its anatomical properties such as diameter. So a nerve that is being repetitively stimulated is more sensitive to blockade than one in the resting state.

4. CARDIAC EFFECTS OF BUPIVACAINE

4.1 EVIDENCE IN HUMANS
4.2 EVIDENCE IN ANIMALS

4.1. EVIDENCE IN HUMANS

Initially bupivacaine was thought to be a safe local anaesthetic agent and in 1966 during development trials it was even given intravenously in doses of up to 0.75 mg/kg to volunteers [17].

In 1975, Scott [66] performed a study which would probably now be considered unethical. Five medically qualified subjects to received infusions of bupivacaine, etidocaine and lignocaine to determine the features of toxicity. They received bupivacaine and etidocaine at 10mg/min by infusion until a total dose of 125mg had been given or either the subject or an observer thought that symptoms were too severe. In 2 subjects the bupivacaine infusions were terminated at 80 and 105mg after severe objective signs of toxicity. The etidocaine group all received the full 125 mg dose. The major difference concerned the frequency and severity of lightheadedness and muscle twitching. Four of the five bupivacaine subjects had symptoms and 2 of them severe enough muscle twitching to require termination of the infusion. None of the etidocaine infusion had any objective, observer-noted signs of toxicity. Numbness of the tongue and circum-oral tissues was observed in all but 1 of the infusions and probably reflected the vascularity of these tissues, giving rise to sufficiently high concentrations of the drugs at terminal nerve endings to

cause numbness in these areas. The peak plasma concentrations were similar in the bupivacaine and etidocaine groups despite the mean dose of bupivacaine being slightly lower at 112mg compared to 125mg for etidocaine. The disappearance of etidocaine from the plasma was considerably faster than for bupivacaine. Of note in their discussion is that all the signs and symptoms of toxicity were referable to the CNS. They note cardiovascular alterations which included an increase in heart rate and blood pressure by almost 20 %, and yet failed to see any significance in these changes as they observed no ECG changes. Current thinking on the cardiac effects of toxic doses of bupivacaine includes the possibility of some autonomic nervous system activation and the observed tachycardia and hypertension may well have been evidence of this phenomenon.

Moore et al [63] published a series of 11080 anaesthetics with only 15 systemic toxic reactions. Unrecognised intravascular injections were responsible for 13 of the cases and rapid absorption after intercostal block with typical doses in the other two.

Edde [7] reported a case of cardiac arrest(ventricular fibrillation) associated with needle withdrawal after an interscalene brachial plexus block which they attributed to a subarachnoid injection. Following early intubation, adrenaline and defibrillation he made a full recovery. The patient had multiple problems including chronic renal failure, hypertension, cardiac failure and hypocalcaemia. Albright included this case in his editorial[17], describing 6 patients, in whom he thought that unrecognised intravascular injection had most probably occurred.

Cottrell [16] in one of the early descriptions of the haemodynamic effects of bupivacaine pointed out that the commercially prepared preparation of

bupivacaine and 1:200 000 adrenaline may be associated with profound cardiovascular changes. Both patients being were invasively monitored during thoracic surgery, and both developed cardiac compromise following intercostal nerve blocks with bupivacaine at the end of an extended period of cardiovascular stability[16]. The first case involved the performance of an intercostal block using 112 mg bupivacaine(with 1:200 000 adrenaline)which resulted in a transient increase in arterial pressure followed by sudden profound hypotension which failed to respond to IV calcium chloride, ephedrine and sodium bicarbonate. Finally noradrenaline infusion was required for 12 hours to maintain an adequate perfusion pressure.

The second case also involved an intercostal block using 75 mg bupivacaine in 1:200 000 adrenaline. An arterial bleed was noted at the injection site of one of the intercostal blocks. Immediately following bupivacaine injection this patient developed frequent multifocal premature ventricular contractions and following an initial surge in blood pressure it progressively fell over 20 minutes despite a 50 mg bolus of lignocaine, which appeared to halt the premature ventricular contractions. Blood loss had been minimal and the blood pressure failed to respond to 3000ml of Ringer's lactate and only following a unit of whole blood did the pressure increase. Of note was that the post-operative ECG was unchanged from baseline.

Of note is that the one patient (described by Cottrell) failed to respond to calcium chloride, ephedrine and sodium bicarbonate and only responded to a nor-adrenaline infusion. The second patient's ventricular extrasystoles responded to intravenous lignocaine although the blood pressure was only restored with a transfusion of blood. Cottrell [16] concluded that caution should taken with the doses used via the intercostal route, particularly in the face of cardiac failure and general anaesthesia.

Albright [17] during 1979, in an early editorial on bupivacaine toxicity comments on 6 anecdotal cases of sudden cardiovascular collapse associated with rapid intravenous injection of bupivacaine and etidocaine. All the cases occurred in operating rooms under the direct supervision of anaesthesiologists following negative aspiration tests. Sudden cardiovascular collapse(ventricular fibrillation or ventricular tachycardia, asystole, or complete heart block with P waves only) occurred almost immediately after rapid injection of the local anaesthetic agent, so that antecedent hypoxia was probably not an aetiological factor. It would seem that only one of these 6 cases resulted in death and that the other cases responded to prolonged resuscitation. He concluded that local anaesthetics, including bupivacaine may result in almost simultaneous seizures and cardiovascular collapse.

In October of 1983 Albright made a personal presentation to the Food and Drug Administration's Anesthetic and Life Support Advisory Committee [93]. He reported a series of 49 cases of cardiac events, either cardiac arrest or ventricular tachycardia requiring cardioversion. These were all cases within a 10 year period in the United States. The majority of these cases were in woman receiving 0.75% epidural bupivacaine for analgesia in labour.

Table 1

	Number of patients	Dose of bupivacaine(mg)	Deaths
Non obstetric	14	50-360	5
Obstetric			
0.5% bupivacaine	8	75-135	6
0.75% bupivacaine	27	50-180	10
Total	49		21

The report to the FDA resulted in the withdrawal of 0.75% bupivacaine from obstetric use in the United States. Albright's editorial and the attention given to his representation to the FDA probably resulted more importantly in an increased awareness of the dangers of local anaesthetic toxicity. The resultant introduction of test doses(with or without adrenaline), fractionated dosing and increased patient monitoring have probably all been responsible for increased safety associated with the use of local anesthetic agents.

In a further report in the 1984 Winter/Spring news letter of the Society of Obstetric Anesthesia and Perinatology [93], Albright reported 52 deaths following bupivacaine, 31 of which were maternal and 5 which were infant deaths. Unfortunately this information remains unpublished.

The British literature initially supported the use of bupivacaine for I.V.R.A until some early case reports described CNS toxicity soon after injection

despite apparently functional tourniquets [78,116]. When the Committee on Safety of Medicines (UK) records were examined in 1981 [78] it was noted that between 1963 and 1976 a total of 14 cases of toxicity with bupivacaine had been reported. Three cases had reported seizures during epidural anaesthesia with bupivacaine and one of these had been fatal. Quite possibly this patient may have experienced accompanying cardiac toxicity. In 1978 the first case of seizure with I.V.R.A. and bupivacaine was reported. In 1979 five cases were reported, four of them during I.V.R.A. [78]. This sparked some interest in comparing the efficacy and toxicity of various local anaesthetic agents for I.V.R.A. [77,85,92,116]. At the same time the Department of Health and Social Security (DHSS) in the United Kingdom had been informed of five deaths directly attributable to I.V.R.A.[115]. The patients were all healthy and being treated for minor conditions in accident and emergency departments. All cases involved bupivacaine and separate tourniquets, all procedures were performed without a designated anaesthetist. This DHSS report prompted an editorial in the British Medical Journal [115] which came out strongly in favour of better training about equipment use and the presence of an anaesthetist to supervise the anaesthesia. Whilst the same editorial provided good evidence that prilocaine was as effective as bupivacaine and the toxicity profile better the author failed to recommend that bupivacaine was to be discouraged from use in I.V.R.A.[115].

Davis [9] describes another successful resuscitation following early institution of supportive therapy during awake surgery for epileptic focus excision.

Mallampati [10] describes a successful resuscitation with early seizure termination, intubation and ventilation following presumed intravascular injection during brachial plexus block.

Hasselstrom et al [40] studied 8 human volunteers. He observed the effect of producing plasma bupivacaine levels approximating those achieved during epidural anaesthesia. Hasselstrom and workers [40] found that plasma levels around 1.8ug/ml produced a 10-15% increase in heart rate accompanied by a steady and progressive increase in blood pressure(10-15 mmHg) and a decrease in cardiac output of up to 20 %. They could find no evidence of a change in CVP, oxygen consumption, plasma cortisol, blood lactate, FFA's or blood glucose although there was a small rise in plasma catecholamine levels. This they felt indicated that there was probably no major activation of the sympathetic nervous system and it was concluded that some of the changes were possibly due to some inhibition of cardiac sympathetic nerve activity as had been shown with lignocaine previously [40] . In addition they felt that the poor correlation of total plasma bupivacaine concentration with the cardiovascular effects was probably suggestive of differences in protein binding, intracellular transport and sensitivity.

Mazoit et al [70] looked at the pharmacokinetics of bupivacaine in 13 infants undergoing caudal anaesthesia for hernia repair and measured plasma levels and free fraction to determine the kinetics. It was shown that following 2.5mg/kg doses the plasma levels peaked at 10-60 minutes following injection and that the peak plasma levels varied enormously from 0.55-1.93 ug/ml. The area under the curve (AUC) also showed wide variation with a range of 201-1107 ug/min/ml. The free fraction showed a negative correlation with age and this was evident until 6 months of age. They concluded that the increased free fraction reported previously in

neonates was evident and was present until 6 months of age and that therefore practitioners should be careful with the doses administered in young infants.

A case was reported [43] of ventricular fibrillation induced by accidental flushing of the pericardium with bupivacaine. A single DC shock was necessary to cardiovert the patient and he made a good recovery.

Ved et al [46] described 2 cases of cardiac toxicity following caudal anaesthesia in infants. Both cases received standard doses of bupivacaine and soon after being turned into the prone position were observed to have cardiovascular collapse which was accompanied by ventricular tachycardia in both cases, one of which remitted spontaneously and one received 1mg/kg lignocaine. Neither case had evidence of analgesia or paralysis, suggesting intravenous injection, and both had no sequelae.

McCloskey and colleagues [67] reported three cases of bupivacaine toxicity in children following caudal infusion postoperatively. Case one involved a 3.98 kg full-term neonate who received a 2.5mg/kg bolus and 2 further boluses of 1.87mg/kg at 1.5 and 3 hours after initial bolus. Then 2 hours post surgery an infusion of 2.5mg/kg/hour was commenced and 10 hours later a bradycardia and hypotension were noticed and the serum bupivacaine concentration at the time was 5.6ug/ml. Cardiac massage and ventilation followed by 10ug/kg adrenaline intravenously resulted in ventricular tachycardia and this only settled following 3 boluses of lignocaine and 5mg/kg phenytoin. The VT recurred 2 hours later and failed to respond to bretylium(4mg/kg) but responded to diazepam(0.25mg/kg) and phenytoin(7mg/kg) given to control accompanying seizures.

Case 2 was 8 years old and received similar doses of bupivacaine intraoperatively and an analgesic infusion of 1.67mg/kg/hour of 0.25% bupivacaine and 1:200000 adrenaline. 25 hours after infusion commenced 2 generalized convulsions occurred associated with serum bupivacaine level of 6.6ug/l.

Case 3 was a 4 year old weighing 12 kg who received a 2.5mg/kg bolus and 3 further boluses of 1.67mg/kg followed by an infusion at 1.67mg/kg/hour. 26 hours later the catheter was resited for technical reason and 8 hours thereafter seizures commenced associated with serum bupivacaine concentrations of 10.2ug/ml.

Roitman et al [44] reported a case of successful cardiac resuscitation following an intercostal nerve block using 100mg bupivacaine in a patient receiving digoxin and metoprolol (a beta-blocker). The patient required a surprisingly short period (15 min) of CPR and this led to some speculation regarding the potential cardioprotective or cardiopriming action of these cardiac drugs. de Jong in a letter to Anesthesia and Analgesia [30] felt that the reason the outcome was good was that patient was conscious and therefore the postulated central action of bupivacaine causing cardiotoxicity was allowed to occur at a lower plasma level than if the patient were anaesthetised. Hence the appearance of cardiotoxicity before seizures. He felt that the literature supports the postulate that CNS toxicity is more likely to occur in anaesthetised patients compared to conscious patients as under anaesthesia the central cardiac stimulatory effects are suppressed and hence higher plasma levels are required to produce cardiac toxicity ; therefore CNS toxicity presents first.

Whereas the reporting authors had postulated that the cardiac glycoside and beta blocker combination was an aggravating combination which acted by lowering the threshold for cardiac toxicity, (and hence the occurrence

of cardiovascular collapse at lower plasma levels allowed a rapid resuscitation); de Jong [30], felt that these drugs may be useful in the treatment or prevention of bupivacaine cardiotoxicity.

Brown et al [38] looked prospectively at 25697 regional anaesthetics in a large teaching institution over 7 years specifically analysing the incidence of seizures and associated cardiovascular changes. He found that the incidence varied depending on the type of block (caudal>brachial plexus>epidural route), and that even amongst the different approaches to the brachial plexus there was a differing incidence. He found that the practitioners' experience of blocks during that period was not associated significantly with seizure occurrence.

Table 2 Overall Seizure Rates During Regional Anaesthesia[38]

Anaesthetic	Total procedures	Seizures	Seizure/1000
Caudal	1295	9	6.9
Brachial Plexus Blocks	7532	15	2.0
Axillary	6620	8	1.2
Interscalene	659	5	7.6
Supraclavicular	253	2	7.9
Epidural	16870	2	0.1

Bupivacaine was the local anaesthetic most frequently associated with seizures, and in spite of 16 patients developing seizures after bupivacaine blocks, none experienced acute cardiovascular collapse [38]. This data may be of use in predicting which patients are at risk of cardiac toxicity. One

could postulate that the procedures with a high incidence of CNS toxicity are likely to have a lower incidence of cardiovascular toxicity, but in similar ratios to CNS toxicity.

Peutrell [68] looked at the bupivacaine levels associated with continuous extradural infusions in 8 children aged 3-12 months . They received a mean initial dose of 1.2mg/kg and an infusion at a mean rate of 0.38mg/kg/hour. The bupivacaine concentrations are represented in Table 3.

Table 3 Time Course of Peripheral Venous Total Plasma Concentration

	Bupivacaine concentration(ug/ml)					
Patient No.	4 h	8 h	16 h	24 h	32 h	..40 h
1	0.21	0.36	0.78	1.26	0.81	
2	0.91					
3	0.96	0.96	0.55	0.60	0.79	
4	1.0	0.36	0.51	0.54	0.66	
5	1.0	0.8	0.67			
6	0.89	1.15	0.92	0.83		
7	0.55	0.70	0.79	0.6	0.87	0.36
8	0.91	1.01	1.11	1.3	2.02	

Patient 8 showed clear evidence of accumulation with time. The authors concur with the view that babies less than 6 months of age have increased potential for bupivacaine toxicity due to increased free fraction [70].

Seizures have been reported [68] to occur at plasma concentrations of bupivacaine as low as 2ug/ml. This work of Peutrell [68] showed that even at infusion levels within the limits of current guidelines that infants can show accumulation to levels above that shown to be associated with seizures. Peutrell [68] also comments on work by Wolf analysing plasma concentrations of bupivacaine in 20 children between 2 and 48 months of age, which showed that 2 children, weighing 10 and 8.3 kg had concentrations of 2.5 and 3.7ug/kg after 24 hours of an epidural bupivacaine infusion. No toxic side effects were reported. Larsson and colleagues looked at 7 children under 7 months and measured plasma bupivacaine concentrations at 6 and 12 hours following an initial dose of 1.5-1.9mg/kg bupivacaine with adrenaline. They then infused bupivacaine at 0.5-0.83 mg/kg/hour without adrenaline and showed an accumulation at 6-12 hours with a mean concentration of 1.59ug/ml(range 1.2-2.1) at 6 hours and 2.06ug/ml(range 1.53-2.98) at 12 hours. Two children had CNS side effects.

Sullivan and Abbott [47] reported 2 cases of suspected cardiac toxicity following accepted doses of intra-articular bupivacaine, one having had a fracture into the joint which may have contributed to the rapid absorption. Both cases exhibited ventricular tachycardia and the outcome was favourable.

4.1.(1) SUMMARY OF HUMAN EXPERIENCE:

Cardiac toxicity was an unrecognised problem during the early use of bupivacaine and it was not until Albright published his early observations that clinicians became aware of the potentially lethal complications of this drug. At the same time the British literature had been reporting cases of toxicity[78,,92,115]. This prompted a surge in clinical case reports and in

animal experimental work in attempts to explain the complication. More importantly, it resulted in an increased awareness amongst ordinary clinicians, and the incidence of case reports has decreased significantly over the 2nd decade following Albright's editorial.

Mulroy's review of epidural anaesthesia in 1997 [95] summarised the incidence of systemic complications with epidural anaesthesia over the past 4 decades. The incidence of systemic complications in the 3 series between 1955 and 1964 was between 1.1 and 12.0%. Since Albright's editorial in 1979 the incidence published has been between 1.0 and 0.01%. Two large studies involving a total of 34309 epidural anaesthesia patients, had incidences of systemic reactions (unspecified) of 0.1 and 0.01%.

A prospective questionnaire looked at the serious complications related to regional anaesthesia during 1996 in France [114]. They had a low response rate of 736 from 4927 questionnaires. They asked anaesthetists to document problems over a 5 month period during which data was collected from 103730 regional anaesthestics (40640 spinal, 30413 epidural, 21278 peripheral blocks and 11229 I.V.R.A block). There were 98 reported complications but one had inadequate details [114]. When the remaining 97 were analysed there were 32 cardiac arrests, 7 of the fatal. Twenty-six of the deaths were during spinal anaesthesia and accounted for 6 of the deaths, the small doses used in spinal blocks are highly unlikely to account for a systemic cardiac effect of local anesthetic agents. Seizures attributable to high plasma levels of local anaesthetics occurred in 23 patients but no cardiac arrest occurred in this group[114].

The authors of these reports express the view that the increased awareness and the use of specific techniques such as aspiration and adrenaline-containing test doses account for the improved safety.

In conclusion toxicity with bupivacaine remains a serious hazard and the factors which influence it are:

* Total dose of drug
* Site of injection
* Protein binding [105, 106]
* Early seizure termination
* Oxygenation
* Cardiac failure, has been shown [104] to result in higher concentrations of lignocaine during an infusion preceded by a loading dose. These results suggest that the clearance of lignocaine, which is twice that of bupivacaine[105], is limited by the hepatic blood flow. This may be compounded by the fact that many of these patients have an acidosis which means that the lipophilic portion of the molecule decreases. The low cardiac index means that the perfusion of many organs will be decreased. These factors can initially give high concentrations of local anaesthetic agents in the circulation and relatively well perfused organs such as the heart and brain may receive excessively large amounts of the drug resulting in toxic effects [104].

*Acid base status

As pointed out above it is not only the total concentration of the drug that must be taken into consideration but also the drug concentration in terms of the prevailing acid-base status and how that may affect the state of dissociation and the ionised fractions [104].

The incidence of toxic manifestations would seem to have decreased over the last three decades. This is possibly as a result of:

* **The use of test doses**
* **Attention to equipment**
* **Awareness**

  Anaesthetic literature and textbooks have highlighted the problems relating to bupivacaine cardiotoxicity, and there is no doubt that current anaesthetists appreciate the toxicity of bupivacaine far more than their earlier counterparts.

* **Dedicated anaesthesia staff**

  The change in practice as recommended in the UK [115] led to a dedicated anaesthetist being present during I.V.R.A. In addition anaesthetic departments had to accept more responsibility for training casualty officers, on an ongoing basis about the equipment, drugs and resuscitation of patients during I.V.R.A.

There are no well proven treatments for established toxicity apart from early seizure termination and oxygenation. This is owing to the poor understanding of the mechanism of the cardiac effects. Furthermore researches are restricted to animal studies in their attempts to elucidate the mechanisms of toxicity and any potential treatments. Data derived from various species of laboratory animals may not be applicable to humans, as there would seem to be significant differences in the electrophysiology of mouse / rat hearts and those of higher mammals [personal communication, J Vandenberg, Dept of Biochemistry, Cambridge University, UK]. Vandenberg and others believe that the more prominent early repolarization in mouse action potentials(AP's) gives rise to a shorter AP duration. The transient calcium current occurs during the AP in humans as

opposed to following the AP in small rodents, so that the relationship of channel opening to the depolarization is not comparable. Similarly the "switching off" of the sodium current is more due to "closing of an activation gate rather than opening of an inactivation gate". These concerns need to be considered when interpreting many of the studies on bupivacaine including the present study which was conducted in rats..

4.2. EVIDENCE IN ANIMALS

The severe cardiac effects associated with bupivacaine largely preclude human experimentation. Animals have become the accepted source of intact and isolated tissue models for the toxic effects of bupivacaine. In this section the toxicity profile, as determined in animal models, is reviewed. The following section summarises work done in animals to establish the mechanisms of bupivacaine's toxic effects. Finally previously explored treatment options are discussed.

4.2.(1) TOXICITY PROFILE

Local anaesthetic drugs vary not only in the doses required to produce toxicity but also in the response of different systems to toxic serum levels, as well as in the final mechanism of death. Nancarrow et al [79] studied the toxic dose and the mechanism of death in sheep. They subjected chronically invasively monitored animals to increasing doses of one of the local anaesthetic drugs on successive days until death occurred. The mean fatal dose of lignocaine was 30.8mg(-+5.8mg), for bupivacaine was 3.7(-+1.1mg) and that for ropivacaine was 7.3mg (-+1.0mg). Thus the ratio of toxic doses was approximately 9:1:2. In four out of four lignocaine treated animals, respiratory depression with bradycardia and hypotension without arrhythmias was the cause of death. Three out of four bupivacaine treated animals died after the sudden onset of ventricular tachycardia / fibrillation without hypoxia or acidosis. The fourth bupivacaine animal died in a similar manner to the lignocaine animals. Three out of the five animals given ropivacaine died in a similar manner to animals in the lignocaine

group. However unlike the lignocaine treated group these animals also had periods of ventricular arrhythmia's. The remaining two ropivacaine sheep died of sudden onset ventricular arrhythmia's / fibrillation.

Tanz et al [1] showed in isolated guinea pig hearts that 3ug/ml bupivacaine produced rhythm disturbances in 50% of preparations. The commonest arrhythmia's were 2:1 heart block, bigemini, pulsus alternans and trigemini[1]. These authors [1] also showed a significant decline (to 58% by 90 minutes) in coronary blood flow in isolated vertebrate hearts exposed to 3ug/ml of bupivacaine. This correlated with a concomitant decrease in contractility and M_{VO2} consumption. Isolated pig coronary arteries were then exposed to bupivacaine and no coronary constriction could be demonstrated. It was therefore concluded that the decrease in coronary flow was a consequence of the decrease in metabolic needs[1].

Bupivacaine has been associated with other arrhythmias including widening of the QRS, increased QRS amplitude and an increased number of ectopic beats with a predominance of nodal and ventricular arrhythmias [1].

Studies on guinea pig hearts seemed to indicate that the relative cardiotoxicity between lignocaine and bupivacaine [24] was the same as their anaesthetic potency ratio. Nath et al[24] used an intact pig model with selective cannulation of the left anterior descending coronary artery to compare the action of bupivacaine and lignocaine on cardiac contractile force and electrophysiology without interference from the CNS. This required the intracoronary administration of the drug and excluded any effect on the SA and AV nodes. Using sequential increments of either drug they were able to demonstrate the ECG changes of the drugs without CNS interference and the bupivacaine animals demonstrated the well described changes of QRS widening, QT interval increasing and a change in T-wave

polarity and electrical axis. Predictably PR interval was unchanged as the drug was delivered to the ventricle only and therefore nodal and high His-bundle changes were not expected. Both lignocaine and bupivacaine produced dose dependent cardiodepression peaking 5 sec after the injection. Following the high doses a 30 % average reduction in left ventricular contraction (LV dP/dT) and an increase in left ventricular end diastolic pressure (LVEDP) of 25% was recorded. The cardiodepressant ratio was comparable with the local anaesthetic ratio of 1:4. These results were in accordance with other studies showing that the primary causes of death from bupivacaine cardiotoxicity are conduction disturbances and ventricular fibrillation. The 4mg/kg dose of bupivacaine resulted in ventricular fibrillation induced cardiac arrest in all 7 animals receiving this dose. The equivalent lignocaine dose of 16mg/kg resulted in no deaths but this group of survivors were then exposed to 32 and 64 mg/kg lignocaine and 3 of the 5 animals receiving 64 mg/kg died in a similar manner to those receiving bupivacaine.

Kotelko et al [13] injected sheep (tracheotomised, with oxygen supplementation and thiopentone seizure termination) with equivalent (low dose) obstetric epidural doses of bupivacaine and (double dose) high dose, and found that all sheep had nodal or ventricular arrhythmia's. The most common ECG abnormality was widening of the QRS complex which occurred in all animals regardless of the dose. Other abnormalities included supraventricular tachycardia, atrioventricular conduction blocks, ventricular tachycardia, multiform premature ventricular contractions and ST-T wave changes. ST-T wave depression of 2mm from baseline control was considered significant.

Rosen et al [5] studied sheep receiving bupivacaine under conditions of mild hypoxia and moderate hypercarbia. They found that in sheep, receiving a low dose (2.1.mg/kg) of bupivacaine, that 3 out of 6 animals had significant haemodynamic changes and that all sheep had evidence of serious ECG changes or arrhythmias. One of the animals died. Animals given high dose (4.2 mg/kg) also had evidence of significant ECG changes and despite vigorous resuscitative attempts became hypotensive and died. The most common ECG abnormality was wide QRS-bradycardia, occurring in most animals regardless of the dose. Two-thirds of the animals given high dose bupivacaine had electromechanical dissociation (EMD) and refractory asystole.

de Jong [8] studied cats and found that adequately ventilated and oxygenated cats were able to survive twice the convulsant dose of bupivacaine, and if hypotension was corrected with ephedrine they could survive 3 times the convulsant dose. They found that regular nodal and ventricular arrhythmias appeared with bupivacaine and etidocaine but not with lignocaine. They also found that the QRS complex began to widen and increase in amplitude before the dose of bupivacaine given reached 1 mg/kg. All bupivacaine treated animals had arrhythmias by the time that seizures started.

Nancarrow et al [79] studied sheep receiving a toxic dose. They compared the mechanism of death employing different local anaesthetics and found that although bupivacaine is approximately four times more potent that lignocaine as a local anaesthetic, it is approximately nine times more lethal. Therefore the margin of safety would be smaller after the inadvertent intravenous injection of bupivacaine than an equipotent dose of lignocaine. This they felt was not based on a greater uptake, of bupivacaine

into the brain and myocardium, as they could demonstrate no significant difference in brain and blood concentrations following lethal doses. Bupivacaine [79] may be arrhythmogenic in the absence of marked hypoxia, respiratory or metabolic acidosis, hyperkalaemia or hypotension. However there is work showing that acidosis, hyperkalaemia and other local conditions may decrease the threshold for bupivacaine toxicity, and Nancarrow's work showing that acidosis failed to increase tissue concentrations in myocardium and brain suggests that the effect of acidosis may not be mediated through greater brain or myocardial uptake. This work [79] showed that the percentage of each agent isolated from the brain and myocardium after fatal doses was similar for all three agents studied. They conclude that their results support the proposition that the observed differences in cardiotoxicity between agents are due to qualitative differences with respect to their effects on cardiac electrophysiology, and not because of disproportionate uptakes into the myocardium

Concern about the poor outcome from cardiopulmonary resuscitation following cardiac arrest with bupivacaine has led to work on potentially safer long acting local anaesthetic agents. The related amino-amide local anaesthetic drug ropivacaine is thought to possess a safer CNS-cardiac toxicity ratio and is prepared as a pure S-enantiomer. Feldman et al [32] established the convulsant dose of bupivacaine and ropivacaine in beagle dogs and then in a blinded fashion gave twice that dose and managed any cardiac changes aggressively with closed cardiac massage, adrenaline, bretylium, atropine and direct current cardioversion. The intravenous administration of 4.3mg/kg of bupivacaine and 4.9mg/kg of ropivacaine (the convulsive doses) resulted in overt seizure activity in all animals. All animals survived without cardiovascular support. Within 30 seconds of administration of the convulsive dose and in conjunction with overt seizure

activity, a 54% increase in heart rate occurred in both groups. After abolition of seizures, heart rates in both groups returned to pre-drug values and remained there. The convulsive dose of either drug caused no significant changes in the PR or QT interval on the ECG. The QRS duration was increased approximately 30 % in both groups between 1 and 3 minutes after local anaesthetic administration. Significant increases in systolic, diastolic and mean arterial pressure occurred in both groups for the first minute following drug injection and concurrent with seizure activity.

Following twice the convulsant dose the results were different. Twice the convulsant dose of ropivacaine resulted in seizures which were treated with intravenous thiamylal after which the dogs were intubated and ventilated with oxygen enriched air. 83% required no further resuscitation, one dog received adrenaline and bretylium and a second had a brief period of ventricular ectopy which settled spontaneously. All ropivacaine treated dogs survived. In the bupivacaine group all dogs had successful control of seizures with thiamylal and respiratory support was instituted. 67% of dogs required no further treatment; however 2 dogs died despite resuscitative measures including multiple doses of adrenaline, manual chest compressions, atropine, bretylium and DC cardioversion. They concluded that in their study designed to mimic the clinical occurrence of bupivacaine cardiotoxicity that the results were in accordance with those of Chadwick [51] where 20% of cats were unresuscitatable. In this study 33% of the dogs receiving bupivacaine died despite resuscitation attempts.

Chadwick's study [51] involved 20 cats which were anaesthetised, paralysed with pancuronium and ventilated to avoid the possible effect of acidosis, hypoxia and hypercarbia. They used EEG seizure activity as a CNS endpoint and a mean arterial pressure (MAP) of 10mmHg as cardiovascular endpoint.

They then infused bupivacaine at a rate of 4mg/kg/min and lignocaine at 16mg/kg/min and recorded the times to each endpoint.

They found that the onset of electrical seizure activity was virtually the same in both groups. Despite the earlier onset of electrocardiographic changes in the bupivacaine group, mean arterial pressure was greater and remained so for longer than in the lignocaine group. They established that the CV/CNS toxicity ratio was 4.0 with lignocaine and 4.8 with bupivacaine. Despite very high lignocaine plasma levels all animals were resuscitatable, while 2 of the bupivacaine group were not resuscitatable although this was not statistically significant.

The use of pancuronium in this study may have influenced the results in the light of more recent evidence implicating the central activation of the sympathetic nervous system in bupivacaine cardiotoxicity. The value attached to the CV/CNS toxicity ratios needs to be interpreted in the light of the assumptions made at the design stage when they equated an infusion rate of 4mg/kg and 16mg/kg per minute to be equal for bupivacaine and lignocaine respectively. Presumably this based on the commonly quoted potency equivalents of 0.5% bupivacaine being equipotent with 2% lignocaine.

The echocardiographic evaluation of bupivacaine cardiotoxicity in dogs was studied by Coyle [36] . The temporal relationship with the ECG changes was studied in 9 pentobarbital anaesthetised dogs. They found that the overall sequence of electrocardiographic changes was that initial systolic dysfunction was followed by right ventricular dilatation. This right ventricular dilatation was so profound that it was associated with massive septal shift into the left ventricle. These significant changes of systolic function occurred before the ECG changes of serious arrhythmias.

de La Coussaye et al [25] undertook an elegant study to test the hypothesis that bupivacaine facilitates the occurrence of ventricular reentrant dysrhythmias. They used isolated rabbit hearts in a standard Langendorff perfusion system. An endocardial cryotechnique was employed to destroy most of the myocardium except the epicardium, which was then studied, using high resolution ventricular epicardial mapping to determine whether reentrant phenomenon were taking place. There were 5 intact rabbit hearts maintained as controls. These intact hearts produced no spontaneous dysrhythmias during exposure to 0.2, 0.5, and 1.0 ug/ml bupivacaine. However during exposure to 5ug/ml bupivacaine, ventricular tachycardia occurred in three of five hearts. By programmed electrical stimulation using up to three premature beats and maximum pacing rate, ventricular fibrillation was induced in all hearts during the control (no bupivacaine). During administration of 0.2ug/ml bupivacaine, ventricular fibrillation was induced in 3 of 5 hearts using the same protocol. In the remaining 2 hearts only sustained monomorphic ventricular tachycardia and non sustained monomorphic ventricular tachycardia could be induced. During administration of 0.5 ug/ml, the spectrum of dysrhythmias induced by programmed electrical stimulation was completely different. In 1 of 5 hearts ventricular fibrillation was induced, whilst the other 4 hearts could only be induced into sustained monomorphic ventricular tachycardias. After the endocardium was frozen, all measurements were performed during ventricular pacing because no spontaneous atrioventricular conduction was present. No spontaneous ventricular dysrhythmias were observed. One preparation exhibited sustained monomorphic ventricular tachycardia after rapid pacing during control perfusion. However in 3 of 6 preparations, sustained monomorphic ventricular tachycardia was induced at 0.2ug/ml bupivacaine. Epicardial mapping demonstrated that all these ventricular tachycardias were based on reentry. At bupivacaine

concentrations greater than 0.2ug/ml no ventricular arrhythmia was induced. Analysis of the complete sequence of activation during initiation of ventricular tachycardias showed a marked slowing of conduction, arcs of conduction block and reentry around these arcs of conduction block. This was considered the first evidence that ventricular reentrant tachycardia could be induced by bupivacaine. No dysrhythmias could be induced at concentrations greater than 0.2 ug/ml bupivacaine in the frozen heart. This may be explained by the inability to pace the heart rapidly at greater concentrations of bupivacaine. This bupivacaine inexcitability had also been previously described by Moller et al [6] and Lacombe et al [75] in rabbit hearts.

Mets et al [21] observed the cumulative effects of bupivacaine and lignocaine in rats. They studied 71 rats and allowed them to breathe spontaneously following intraperitoneal pentobarbitone. They then received infusions of lignocaine (2%), bupivacaine (0.5%) or both (1% and 0.25% respectively). A control group received saline intravenously. Once the infusions were begun the haemodynamic and respiratory variables were monitored until arrest of either occurred. The times to respiratory and cardiac arrest were similar in the lignocaine, bupivacaine and mixture groups. Interestingly they noted that in all animals respiratory arrest occurred before cardiovascular collapse. They used the cumulative doses of lignocaine and bupivacaine administered separately to determine a lignocaine to bupivacaine ratio; this was found to be 3.36. They could therefore determine lignocaine-equivalent doses and use these to interpret the mixture. They concluded that the mixture of lignocaine/bupivacaine was no more toxic than the individual compounds. however their was no difference in the lignocaine-equivalent doses of the 3 groups and it can therefore be concluded that bupivacaine and lignocaine are additively

toxic. Interestingly they only observed one seizure and attributed this low incidence to the use of pentobarbital.

In a study in isolated dog hearts observing the effect of differing concentrations of lignocaine and bupivacaine on electrophysiology, Wheeler et al [73] studied the drugs' effects on the intact conduction system. They found that both lignocaine and bupivacaine reduced the spontaneous rate of isolated dog ventricles, lignocaine exhibited a cessation of right atrial electrical and mechanical activity more so than bupivacaine, and noradrenaline (0.2microgram/ml) was successful in reversing these changes. Using micro electrodes to impale the sinus node region they were able to track electrical activity in that area and the results are recorded in the following table: [From 73, page 205]

Table 4 Transitional Cell Action Potential Characteristics

Transitional Cell Action Potential Characteristics

Solution	N_h	N_i	Rate (min^{-1})	MDP (mV)	Takeoff Potential (mV)	Overshoot (mV)	MRD (V/sec)	APD_{50} (msec)	Phase 4 Depolarization	
									Early (V/sec)	Late (V/sec)
Control	7	22	86 ± 15	−62 ± 7	−54 ± 5	4.4 ± 4.2	11.5 ± 11.0	129 ± 13	.045 ± .026	.0056 ± .0049
Lidocaine (25–35 μg/ml)	3	7	67 ± 2*	−61 ± 6	−53 ± 6	2.0 ± 5.2	4.1 ± 2.9	143 ± 17*	.026 ± .008	.0058 ± .0014
Bupivacaine (1.75–2.5 μg/ml)	3	5	86 ± 14	−60 ± 3	−54 ± 2	1.2 ± 8.0	2.0 ± 1.2*	143 ± 7*	.036 ± .013	.0084 ± .0078
Significance			$P < .05$	NS	NS	NS	$P = .05$	$P < .05$	NS	NS

Figures stated as mean ± standard deviation. N_h = number of hearts; N_i = number of impalements (one AP per impalement); MDP = maximum diastolic potential; MRD = maximum rate of depolarization; APD_{50} = action potential duration at 50% repolarization; NS = not significant.

Significance indicates differences between groups in the columns; *denotes a significant difference from control.

Significant changes were observed in maximum rate of depolarization during bupivacaine exposure (control =11.5 ± 11.0 V/sec, bupivacaine =2.0 ± 1.2 V/sec).

When loss of atrial mechanical activity occurred it was often in the presence of sino-atrial activity but in the absence of conduction (and no action potentials) through surrounding "transitional tissues". They termed

this "sinus exit block". This sinus exit block was reversible by the administration of noradrenaline and may reflect noradrenaline's augmentation of the slow inward current, a well known action of beta-adrenergic agonists [73].

When they analysed the action potential in the interventricular septum a small change in the peak amplitude of the action potential spike was found, with no change in the plateau height or resting potential, but bupivacaine increased the action potential duration, although not in a dose dependent manner.

Wheeler and co-workers' results [73] correlated with previous work showing a concentration dependent decrease in indices of contractility.

Detailed study of atrioventricular conduction in the presence of bupivacaine showed that atrial activity conveyed to the AV node was conducted to the septum but did not produce excitation of the atrium. However "bizarre" localized blocks and conduction patterns were demonstrated within the AV node and these were described as being conditions which predispose to re-entrant arrhythmias. They were even able to observe premature septal beats which arose from the AV node, with re-entry being the likely mechanism of the extra beats.

Interesting observations [73] were made on the effects of bupivacaine on action potentials (AP) in the different areas of automaticity, conduction and contraction, and it was concluded that this may shed some light on the area of the heart specifically influenced by bupivacaine. They showed that bupivacaine was 20 times more potent than lignocaine in depressing AP properties in atrial cells. In septal cells the AP changes were more variable, although the depression of V_{max} was more pronounced with bupivacaine. Septal cells also showed an action potential lengthening with

bupivacaine. Increased duration of ventricular muscle action potential occurred with bupivacaine and this also occurs with drugs such as quinidine, which like bupivacaine also predisposes to Torsade de Pointes. Despite these increases in AP duration, some isolated Purkinje Fibres exhibited decreased action potential duration as has been shown with lignocaine. This differing effect on the action potential of Purkinje fibres and ventricular muscle may be associated with Torsade de Pointes and ventricular fibrillation.

Arlock [111] studied the action of lignocaine, bupivacaine, and ropivacaine on guinea pig papillary muscle sodium channels. He demonstrated a depression of the plateau of the action potential in paced myocardium by all the drugs. In higher doses the action potential overshoot was decreased and the action potential shortened in paced preparations. Different stimulation protocols were used to assess use-dependent block, evaluate recovery from use-dependent block, and to evaluate the relative contribution of activation-dependent and inactivation-dependent block. They demonstrated recovery intervals, following 19 conditioning impulses, of 0.186s for lignocaine, 1.4s for ropivacaine and 2.1s for bupivacaine. Analysing these data they estimated that the percent of drug-blocked channels at the end of the 19th beat was 88% for the bupivacaine compared to 48% and 75% for the lignocaine and ropivacaine respectively. This confirms that bupivacaine acts on the sodium channels in a use-dependent manner in depressing V_{max} of the action potential.

The second part of the study analysed the effect of different stimulation rates on V_{max}. In the presence of bupivacaine the steady state level of V_{max} decreased progressively as the stimulation rate increased from 0.05 to 3.3 Hz. This confirms the rate dependent nature of block of the sodium channels by bupivacaine [111]. In addition it was confirmed that block

increased with increasing duration of depolarisation. This is in accordance with other work showing that the sodium channels remain in the inactivated state and hence more susceptible to bupivacaine when the action potential is prolonged. During the final part of the experiment preparations were paced with fast trains, of stimuli, with an exceptionally short inter-stimulus interval and a very short action potential plateau, which demonstrated very little difference between bupivacaine and ropivacaine. In addition the onset was slower than with lignocaine. This is compatible with the work of Hondeghem, Katzung and others [76,111,3] suggesting that lignocaine has higher affinity for activated channels; while bupivacaine and ropivacaine seem to bind preferentially to inactivated channels. Despite the lack of difference in action potential measurements they demonstrated a significant decrease in contractility in the bupivacaine preparations. The mechanisms are unclear [111] but a reduction in calcium influx due to calcium channel blockade, reduction of calcium influx due to shortening of the action potential blockade, inhibition of calcium efflux from the sarcoplasmic reticulum, or a reduction of intracellular calcium due to reduced intracellular sodium activity and inhibition of the sodium-calcium exchange mechanism are possible explanations.

Pitkanen [110] studied the effects of 3 different local anaesthetics on inotropy and chronotropy in isolated beating and perfused rabbit hearts. Whilst the model could be flawed in that stability was defined as the achievement of 3 consecutive readings within 20% of each other, it is still worthwhile considering as some of the work does agree with the findings of other investigations. Nine concentrations of local anaesthetics were studied 6, 20 and 40 micrograms/ml of lignocaine, and 1, 6 and 13 micrograms/ml each of bupivacaine and ropivacaine. During drug

exposure a significant slowing in spontaneous heart rate was demonstrated, even at low concentrations in the case of bupivacaine. This is contrary to previous studies in intact animals, where there is often an early increase in blood pressure and heart rate [32,73] and also contrary to our experience with infusions of bupivacaine. A human study looking for evidence of autonomic activation [40] similarly showed an early tachycardia and I postulate that this is evidence of central nervous system involvement in intact animals. Thus there may be considerable evidence supporting central nervous system involvement in intact humans and animals.

Pitkanens group [110] produced a 62 % depression of left ventricular systolic pressure with the 6ug/ml bupivacaine which was significantly different to that produced by an equal concentration of ropivacaine. However this isolated heart model excludes possible CNS mediated cardiovascular effects of bupivacaine. The following are the results obtained in the 13ug/ml groups:

Ropivacaine 13ug/ml n=6	Bupivacaine 13ug/ml n=6
2 cases of AV block	2 "almost complete" A-V block
2 cases nodal/idioventricular rhythm with AV dissociation	4 severe ventricular tachycardia with 2 or more foci
1 ventricular ectopy interrupted by beats seemingly preceded by p-wave	
1 animal had no ECG changes	

Their conclusion, in agreement with the literature, was that a majority of the ventricular arrhythmias seen with bupivacaine are re-entrant in nature.

Nath and co-workers [93] performed randomised cross-over experiments in pigs anaesthetised with pentobarbitone. They administered lignocaine or bupivacaine into isolated anterior descending coronary arteries in a trial designed to eliminate any CNS mediated cardiovascular influences. There was a dose-dependent depression of the left ventricle in the same ratio as the anaesthetic potency of the two drugs. However comparable prolongation of the QRS interval was obtained at a ratio of 1:16. It was therefore concluded that bupivacaine was 4 times as potent as lignocaine in depressing myocardial contractility, and 16 times as potent when considering electrophysiological toxicity [93].Therefore bupivacaine may have a 4 times higher electrophysiological effect than myocardial depressant effect.

The "***modulated receptor hypothesis***" has been used to describe the interaction of bupivacaine with sodium channels. According to this hypothesis blockade of sodium channels results from interaction of drugs with a specific receptor site associated with the sodium channel. Drug affinity for this site is dependent upon the state of the channel, i.e. the rate constants describing the affinity and dissociation of the drug from the receptors are different for each channel state and for different drugs [3].

Bupivacaine is similar to lignocaine in that it has a high affinity for inactivated channels, but unlike lignocaine it has relatively low affinity for open channels [3]. Furthermore bupivacaine may also differ in the manner of its interaction with the rested channels. The channel block

cannot be reduced markedly by hyperpolarising the membrane, which suggests that bupivacaine may slowly or incompletely dissociate from the rested channels. The higher potency of bupivacaine over lignocaine at physiological heart rates results from both a higher affinity for sodium channels as well as a qualitative difference in its kinetics of interaction with cardiac sodium channels. The estimated dissociation constant for bupivacaine and inactivated sodium channels is 0.9uM(0.3ug/ml) whilst a conservative estimate for lignocaine is 10uM(2.3ug/ml) [3] .

Lignocaine blocks both open and inactivated sodium channels very rapidly and recovery at diastolic potentials is very rapid. Thus lignocaine may be considered to block sodium channels in a "fast-in-fast-out" manner. The kinetics of block onset and recovery for bupivacaine are different. Recovery from block at diastolic potentials is slow whilst development of block is slow at low concentrations but fairly rapid at higher concentrations. Thus bupivacaine blocks sodium channels in a "slow-in-slow-out" manner (at low concentrations) and in a "fast-in-slow-out" manner (at concentrations >0.2ug/ml). Because recovery from block is always slow the block accumulates even at very low heart rates.

These differences make bupivacaine much more potent in depressing V_{max} in ventricular muscle. Depression of V_{max} will result in slowed conduction of cardiac action potentials. In an intact heart this can result in prolonged P-R intervals and a widened QRS complex. Slowed conduction also can result in unidirectional block and re-entry, which in turn can result in the production of unifocal or multifocal ventricular ectopics and ventricular tachycardias [3]. The proven ability [3] of bupivacaine in concentrations above 1ug/ml to shorten the action potential duration may explain changes in the S-T interval.

Symanski and Gettes [22], in a review of the effect of drugs on the electrophysiology of the heart, comment on the effects of decreasing the velocity of V_{max}. The rate of maximal rise of the action potential upstroke (phase 0) is a major determinant of conduction velocity and duration of the QRS complex on the body surface ECG. When the sodium current is completely inhibited, the calcium current may still be activated and be of sufficient degree to initiate an action potential, but V_{max} and conduction will be much slowed [22] In general the class I anti-arrhythmic drugs cause sodium channel blockade and thereby reduce the V_{max} of the action potential upstroke, slow impulse conduction, and prolong the QRS complex on the body surface ECG. It is difficult to correlate what seems to be happening to AP's in individual cardiac cells (nodal, conducting and contractile myocardial) and the body surface ECG. The effect of an isolated sodium channel blockade on an individual myocyte would be to decrease V_{max}, the degree of depolarization and shorten the action potential duration. However many drugs, such as class I anti-arrhythmic agents can both decrease V_{max} and then shorten, lengthen or leave the AP plateau duration unchanged. These problems are compounded by the evidence that different areas of the myocardium may be affected in a different manner by the same drug. Thus attempts to use body surface ECG to interpret the effects of drugs affecting the electrophysiology at individual cell level is not only difficult, but possibly highly inaccurate.

Clarkson et al [3] studied isolated guinea pig hearts looking at the onset and offset of bupivacaine's cardiac effects and their relationship to stimulation rate and diastolic membrane potential. They recognised that the V_{max} they were measuring in phase 0 of the action potential was not necessarily an exact measure of sodium ion flow but that it had been validated for this

purpose [3]. In the presence of bupivacaine 1 ug/ml, V_{max} after a long period of rest was no different from control. Upon application of a train of beats V_{max} declined with each successive action potential until in steady-state conditions a new V_{max} value was reached in about 10 beats. The steady state depression was strongly dependent on the number of times that the channels were used per unit of time. When bupivacaine was compared with lignocaine whilst looking at the rate dependency it was found [3] that lignocaine was far more sensitive to changes in heart rate through the physiological range. This difference indicates that their potency ratios may not be identical at different heart rates. A further technique was used to determine when sodium channels were being blocked by each drug, and association and dissociation constants were derived for the individual drugs; in particular, this could be related accurately to different stages of the action potential. Bupivacaine was found to have a high affinity for the channel in an "inactivated -closed" state whereas lignocaine had high affinities for the "open" and "closed - inactivated" states. It was concluded that bupivacaine like lignocaine has depressant effects on the cardiac sodium channels that are both "time" and "voltage" dependent. The blockade of sodium channels increases as stimulation rate is increased or the membrane potential becomes more depolarized.

For lignocaine, recovery of block was very rapid [3] and occurred during diastole so that accumulation only occurs at faster than physiological heart rates. The recovery characteristics of bupivacaine are different in that the dissociation constant is low at diastolic potentials and the drug therefore accumulates even at slow heart rates. These differences make bupivacaine much more potent at depressing V_{max} in ventricular muscle at physiologic heart rates.

The cardiac depressant effects of bupivacaine can be attenuated by reducing the heart rate, prevention of diastolic depolarization and

shortening of the action potential duration. Therefore large changes in heart rate such as can be brought about by abolishing a tachycardia could reduce the depressant action of bupivacaine on cardiac conduction and thus be beneficial in reducing its cardiac toxicity [3]. In addition even a small amount of diastolic depolarization would be expected to greatly increase the depressant effect of bupivacaine on conduction. Thus conditions known to depolarize the cardiac cell membrane(eg. hyperkalaemia, severe hypoxia) are expected to increase bupivacaine's depressive effects. Manipulations that shorten the duration of the plateau may be beneficial in treating bupivacaine toxicity, while conditions that lengthen the action potential duration may be expected to worsen toxicity. Unfortunately we know of no drug that markedly shortens the action potential and is not itself toxic[3]

Interestingly no correlation could be demonstrated between heart rate and predisposition to dysrhythmias. No study has specifically investigated this effect in the intact animal. The investigators also expressed concern that about the use of hexamethonium, was a general ganglion blocker. They could not exclude it from exerting a protective effect, possibly by from blocking some pathway in the parasympathetic nervous system [31] .

Lynch [4] studied various mechanisms which might account for the cardiac effects of bupivacaine, compared and contrasted with another lipid soluble drug (etidocaine) and a water soluble drug (lignocaine) and found that the effects of 1.15ug/ml and 2.88ug/ml bupivacaine was very similar to the equivalent concentrations of etidocaine [4]. A dose- and frequency-dependent depression of contractility was observed which correlated with previous work by Courtney [15] and Clarkson and Hondeghem [3]. Lignocaine also caused contractile depression which was more profound at

the low frequency range studied; 9.37ug/ml lignocaine caused a pattern of depression that was similar to that of a tenfold lower concentration of the more lipid soluble drugs [4]. With regard to action potential duration lignocaine caused a significant decrease at all frequencies up to 2 Hz. Bupivacaine and etidocaine were shown to have a similar effect but the reduction was not uniformly significant at all frequencies[4]. It was concluded that there was a reverse frequency dependent depression of myocardial contractility. These effects contrasted sharply with the local anaesthetic effect on the rate of depolarization of the fast action potential, which is used as a measure of sodium ion influx through sodium channels [4].

4.2.(1.1) SUMMARY OF TOXICITY PROFILE

1. BUPIVACAINE vs LIGNOCAINE

Doses

The cardiac effects of bupivacaine begin to be seen at plasma levels of 2-4ug/ml [1] and increase progressively with increasing concentration. The equivalent changes are only seen at lignocaine concentrations of 20-30ug/ml. These levels of bupivacaine can be reached during caudal [68,70] and epidural anaesthesia and analgesia. Several studies have suggested that the negative electrophysiological and inotropic effects are not equal for bupivacaine and lignocaine [51,73]. They suggest that bupivacaine has electro- physiological effects which outweighs the negative inotropic effects. One study [24] demonstrated that the ratio of depression of contractility between the bupivacaine and lignocaine was 4:1, which is the same as their anaesthetic potency ratio. However the electrophysiologic toxicity ratio was 16:1, indicating the profound effects of bupivacaine on electrophysiological conduction [24].

Mechanisms

Several studies [21,32,48,79] analysing the mechanisms of death have indicated that respiratory failure occurs before severe cardiac manifestations. Nancarrow's study [79] seemed to indicate that lignocaine resulted primarily in respiratory failure whereas mortality due to ropivacaine and bupivacaine in sheep has a combination of respiratory and cardiac causes.

2. THE CARDIOVASCULAR EFFECTS OF BUPIVACAINE

Inotropy

Both in-vitro [1, 4, 72, 87, 93] and in-vivo [20, 24, 40, 54, 87, 93] studies have shown that bupivacaine has profound negative effects on contractility that would seem to be independent of any electrophysiological (of specialised conduction tissue) effect as it remains demonstrable in isolated of myocardium and myocytes.

Chronotropy

Isolated hearts [73, 110,41] demonstrate a simple dose-response curve when bupivacaine concentration is plotted against decreasing heart rate. However intact animals [14, 42, 53, 32, 35, 54] and human studies [40] seem to indicate a bi-phasic response to increasing concentrations of bupivacaine. This is quite possibly the postulated central stimulatory action of bupivacaine is absent in the denervated isolated hearts and that only its direct action on the cardiac pacemakers and conducting tissue is present in this situation. Some support for this postulate is the attenuating effect, on bupivacaine induced arrhythmia's, seen by Bernards [53] when animals were primed with benzodiazepines, which are known to suppress the output of the sympathetic nervous system.

Electrophysiology

Depression of cardiac conduction appears to be one of the main mechanisms of the cardiac effects of bupivacaine. This is largely dependent on blockade of the cardiac sodium channel and is dependent both on use and the state of the sodium channel. Blockade of the sodium channels develops during the upstroke and plateau of the action potential and dissipates during the diastolic interval

between beats. Hence the effect of bupivacaine is both state- (of the channel) and time-dependent [111].

It seems that the severe ventricular arrhythmias following large doses are the result of reentrant phenomena [11, 25, 26, 73, 88, 112]. However many different dysrhythmias have been described including increased PR intervals [3,24], broad QRS complexes [3,5,8,20,24,26,35,42,47,51] , bradycardia [5,26,30,35,42,49,51,67,73,102], bigemini[1,8], trigemini[1], heart block [1,5,12,17,20,30,35,42,45,51], pulsus alternans [1,12], nodal beats [2,8], ventricular ectopic beats [1,6,8,12,16,42], idioventricular rhythm [8,11,35,91], bundle branch blocks [8], ventricular tachycardia [9,10,11,12,17,20,21,25,32,46,47, 56,91], prolonged QT interval[11,24], Torsade de Pointes[73], ventricular fibrillation [1,7,17,24,25,32,43,56,90] , electro- mechanical dissociation [5] and asystole [5,17,42,51]

Echocardiography

An echocardiographic study [36] of the effects of bupivacaine in dogs at concentrations less than those required to produce ECG changes of toxicity, demonstrated systolic dysfunction followed by acute right ventricular dilatation with shift of the septum into the left ventricle.

There is no equivalent study in humans or large primates and therefore difficult to interpret.

4.2.(2) POSTULATED MECHANISMS OF TOXICITY

4.2.(2.1) ROLE OF SODIUM CHANNELS

The local anaesthetic effect of bupivacaine is mediated by its action on sodium channels and a large component of the cardiac toxicity is similarly mediated by sodium channel blockade. The major consequence is a slowing of V_{max} of phase 0 of the cardiac action potential. The cardiac effects are predominantly in the specialised conducting system of the His-Purkinje system and then in atrial and ventricular muscle as these areas rely on sodium channels for early and rapid depolarization. Calcium channels are able to play a role in a slower depolarization, in the His-Purkinje system and the atrial and ventricular myocardium if sodium channels are completely blocked. This is in contrast to the sino-atrial and atrio-ventricular nodes which rely on calcium channels for their phase 0 depolarization. One of the possible effects of V_{max} depression is a slowing of depolarization which can be reflected as QRS complex prolongation on body surface ECG recordings.

Hyponatraemia has the potential to decrease the transmembrane sodium gradient and therefore possibly potentiate the effect of fast sodium channel blockade. Work by Bertrix and co-workers [61] studied the effect of altering the sodium concentration - they demonstrated that dog hearts in a hyponatraemic environment were able to be induced to show the features of bupivacaine toxicity at much lower plasma levels. In addition the production of hypernatraemia was shown to reverse some of the cardiotoxic changes induced by bupivacaine. Profound hyponatraemia (serum sodium 100-110) on its own was shown to produce abnormalities similar to those produced by moderate hyponatraemia and bupivacaine.

4.2.(2.2) ROLE OF CALCIUM CHANNELS

Calcium has been implicated in the cardiac effects seen with bupivacaine. Calcium is important in the action potential conduction in the atrio-ventricular pathway (the SA and AV nodes in particular). Tanz et al [1] exposed isolated vertebrate hearts to additional calcium and could show no reversal of bupivacaine's cardiac toxicity. Calcium influx initiates and controls the force of contraction in the process of excitation-contraction coupling, therefore the determination of whether bupivacaine affects the slow inward current is essential to our understanding of the mechanism of the cardiac toxicity of local anaesthetics

Various calcium channel antagonists [27, 87] have been studied in association with bupivacaine cardiotoxicity, probably as a result of French work by Eledjam and de la Coussaye [123] suggesting that slow calcium dependent channels may be responsible for a component of bupivacaine's cardiotoxicity.

Coyle and Sperelakis [64] looked at the ongoing controversy as to whether bupivacaine has any action on the calcium slow inward current channels [64]. . They studied 12 isolated guinea pig hearts perfused in Tyrodes solution. They elevated the K^+ concentration to 26mM which raised the resting membrane potential to -46mV and inactivated the fast Na^+ channels. Slow-rising action potentials(AP) were induced with isoprotenerol added to the perfusate. Low concentrations of lignocaine or bupivacaine did not affect the V_{max} of the slow action potentials (AP's). Pharmacological concentrations of bupivacaine depressed V_{max} by 50%

whereas lignocaine in equivalent concentrations had no effect. Bupivacaine also caused a significant depolarization of the resting potential to -39mV. Washout of lignocaine was rapid and isoprotenerol excitability was restored in 3 minutes whereas the bupivacaine washout was significantly longer. The bupivacaine concentration necessary to produce a V_{max} depression of 50% for the slow action potentials (AP's) was similar to that required to depress the fast(Na^+) channel V_{max} by a similar amount. This data suggests that bupivacaine does inhibit the slow inward calcium current and that the concentration required to produce a 50% decrease in V_{max} of the slow action potentials in this study is similar to those shown to produce the same degree of suppression of V_{max} in fast action potentials as demonstrated by Clarkson and Hondeghem [3]

The phase 0 of the cardiac action potential in both the SA node and the AV node is largely calcium dependent rather than sodium channel dependent as in the His fibres, ventricular conducting tissue and cardiac myocytes. De La Coussaye et al [41] studied isolated frog atria to look at the effects of increasing concentrations of bupivacaine, and showed a contributory effect of calcium channel inhibition. They used the double sucrose gap technique. This involves placing a short segment of isolated fibres in a bath where a small segment in the centre is isolated by 2 streams of iso-osmotic sucrose solution, thereby creating an artificial node of Ranvier. The central pool between the 2 sucrose streams can be perfused with physiological fluid or with test solution. The inside potential of the test area is patch-clamped and stimulating voltage can be applied and current flow across the membrane can be measured. They used $3x10^{-7}$ M tetrotoxin which inhibits the fast inward sodium current [41]. Bupivacaine was added to the central test solution at varying concentrations and its effect measured. They showed that bupivacaine inhibited the slow-inward

current in a concentration dependent fashion. This moderate calcium inhibitory effect might explain sinus bradycardia and the slowing of AV node conduction observed in anaesthetised dogs following bupivacaine infusion. Bupivacaine depressed not only the amplitude but also the kinetics of the slow-inward current. The time to reach peak current is increased and this allows for increased time for calcium to enter the cells. This is counterbalanced to some extent by a decrease in the slow inward current amplitude. De La Coussaye feels that this does not explain a significant role for calcium and that other factors such as inhibition of energy metabolism may be implicated in bupivacaine's ability to decrease contractility.

Despite concerns about the accuracy of using frogs [41] to represent effects of bupivacaine in humans the authors felt that their results possibly explain the sinus bradycardia and slowing of AV node conduction induced by bupivacaine.

Hyman et al [27] looked at the effect of Nimodipine in 84 rats and pretreated 2 groups with 200 and 500 ug/kg of nimodipine respectively and had 2 groups of placebo controlled dogs. They found that the 200ug/kg nimodipine pretreatment protects against fatal cardiotoxicity of LD_{50} and LD_{90} bupivacaine although 500ug/kg did not.

Other work [87] has suggested that the calcium antagonists may potentiate bupivacaine's effect on the heart. Recent work by Herzig [87] looked at the interaction between various calcium antagonists and bupivacaine, and tried to clarify which of the calcium channel antagonists would increase the negative inotropy of bupivacaine. In addition they compared the action of one calcium channel antagonist (nitrendipine) against various local

anaesthetic agents to see if any of the local anaesthetics were particularly sensitive to the effects of calcium antagonists.

The first part of the experiment used isolated guinea pig atria suspended in Tyrodes solution. The influence of the calcium antagonists on inotropy were recorded using concentration response-curves. They evaluated the increase in potency by determining the half-inhibitory concentration (EC_{50}) of bupivacaine for each preparation. They showed an increase in negative inotropic potency for bupivacaine in the presence of the dihydropyrodine derivatives (felodipine, nifedipine, nitrendipine), whereas the amphiphilic calcium antagonists led to a smaller change. The degree of potentiation of the negative inotropic effect, as caused by nitrendipine, differed between the various local anaesthetics, bupivacaine and ropivacaine being affected to a greater extent.

The second part of the experiment consisted of isolated guinea pig hearts suspended in Tyrodes solution with 10% gelatin to help prevent myocardial oedema (by maintaining some oncotic pressure). Strain and pressure gauges were placed in the cardiac chambers and the surrounding blood vessels to record changes in pressures and contractility with time. The same technique using dose-response curves was used with differing concentrations of the local anaesthetics, with and without the presence of a low concentration of nifedipine. The effects of bupivacaine on cardiac inotropy, and hence cardiac function, were enhanced in the presence of nifedipine. The effects of bupivacaine on cardiac rhythm remained unchanged.

Arlock's work [111] demonstrates bupivacaine's attenuation and block of automaticity at potentials where the calcium-conductance is high. This may indicate that bupivacaine is affecting the transmembrane calcium current. The decreased inotropic effect of bupivacaine, independent of its sodium

channel effect, may be due to depression of the transmembrane calcium current which is responsible for triggering the contractile apparatus.

4.2.(2.3) ROLE OF POTASSIUM CHANNELS

Agents that alter the duration of the plateau phase of the cardiac action potential may affect the cardiotoxicity of bupivacaine, because bupivacaine induced block of cardiac sodium channels develops mostly during the action potential plateau when sodium channels are inactivated. Cardiac action potential duration is principally controlled by potassium currents flowing through several different voltage-dependent potassium channels. If the action potential were prolonged due to potassium channel block, this might contribute an additional mechanism for local anaesthetic cardiotoxicity because of the increased number of open or inactivated sodium channels.

Avery [118] found in well ventilated and oxygenated dogs that mild hyperkalaemia produced cardiotoxicity at a significantly lower dose than in the normokalaemic state.

Courtney [98] studied frog hearts to see if bupivacaine had a blocking effect on potassium channels and found that it resulted in a significant blockade of the I_k (delayed rectifier potassium current) and I_{ki} (inward rectifier potassium current) potassium channels at concentrations which produce the well described sodium channel block. Potassium channel block may therefore account for part of bupivacaine's reported cardiotoxicity.
Following work by Clarkson and Hondegmens [3] which showed that bupivacaine preferentially bound to the inactivated sodium channels [62] other researchers realized that this inactivated state predominates during the depolarized plateau phase of the action potential. Thus any condition

increasing the time that sodium channels spend in the inactivated state (by prolonging the action potential duration) might be expected to enhance the blockage of sodium channels. The duration of the cardiac action potential is controlled by currents flowing through a variety of voltage dependent potassium channels. These include I_{to} (transient outward potassium channel), I_k (delayed rectifier potassium current) and I_{ki} (inward rectifier potassium current). Agents which inhibit these currents would be expected to increase the duration of the action potential. The studies by Courtney [98] and others [62] had already reported that bupivacaine was a potent blocker of the delayed rectifier potassium current. Castle [62] therefore studied the various potassium channels in isolated rat ventricular myocytes using a patch-clamp technique. He was able to show that bupivacaine is a potent inhibitor of I_{to} in rat ventricular myocytes. Reports prior to Castle's study showed conflicting results concerning bupivacaine's effect on I_{ki} and this study [62] seemed to confirm that bupivacaine has no effect, even at millimolar concentrations, on I_{ki} . The studies looking at bupivacaine's effect on potassium channels are still rather conflicting. Bupivacaine has been shown to increase the action potential duration in frog and canine atrial wall muscle and also in canine ventricular septal wall. However the action potential duration has been shown to be decreased by bupivacaine [62] in canine and rabbit Purkinje fibres. It is possible that there may be regional differences in bupivacaine's effect on action potential duration. This may result in areas differing in excitability which in turn could precipitate re-entrant arrhythmias. So whilst there is no doubt that bupivacaine's inhibition of fast sodium channels accounts for the majority of its cardiotoxicity, one can not exclude that blockage of potassium channels - by prolonging the time sodium channels spend in the inactivated state - may potentiate the effects on sodium channels.

Boban et al [45] set out to test the hypothesis that ATP- sensitive potassium channel openers could attenuate the bupivacaine induced atrioventricular block. They studied isolated and perfused guinea pig hearts and infused bupivacaine at two concentrations to produce first degree AV block (4ug or 1.15mg/ml) and second degree heart block (15-20uM or 4.31-5.71ug/ml) respectively. During a stable AV block they administered the potassium channel openers (pinacidil and bimikalim). In the presence of bupivacaine, pinacidil and bimikalim both attenuated the atrioventricular (AV) block induced by bupivacaine by approximately 20 %. The beneficial effect of bimikalim was reversed by glibenclamide which is a specific blocker of ATP-dependent potassium channels. This seems to support the mechanism of the other 2 drugs as being through the K channel. In addition the 2nd degree AV block induced by higher doses of bupivacaine was converted to 1st degree block by the either pinacidil or bimikalim. This work strongly suggests that the AV blocking effect of bupivacaine can be partly treated with K channel openers which have the effect of accelerating cardiac repolarization.

Recent modeling of potassium channels in proarrhythmia by Starmer [88] found that the cardiac vulnerable period during which stimulation results in unidirectional block was prolonged by actions that slowed conduction (especially secondary to sodium channel block) of an excitation wave front. Thus class 1 agents inherently amplify the probability of initiating reentry secondary to unsuppressed premature excitation. In a general sense, sodium and potassium currents are complimentary: one depolarizes and the other repolarizes. With sodium channel blockade the cellular antiarrhythmic property (prolongation of recovery of excitability) leads to a multicellular proarrhythmic property (prolongation of the vulnerable period) that destabilises bidirectional wave front formation. Similarly with

potassium channel blockade, the cellular antiarrhythmic property derived from prolongation of the action potential duration [prolonged refractoriness] might translate to a multicellular proarrhythmic property by prolonging the wavelength (AP duration . velocity) and destabilising some other aspect of propagation [88]. Recognising that there may be several different modes of initiation of Torsade de Pointes they used computer modeling in the setting of reduced potassium currents. They ignored the mechanistic basis for reentrant initiation and hypothesized that for some situations the "initiating" and "maintenance" processes are distinct and can be explored separately. Recent work by their group had shown that potassium channel blockade by quinidine reduced the vulnerable period during which reentrant arrhythmias could be generated. The reentrant wave front has been observed to be a spiral wave rotating around a small core region or it can drift in response to local inhomogeneities in the medium. Theoretical studies have shown that the properties of the medium can influence the tip of the spiral waveform and that shifts in the spiral tip are reflected in the ECG. If the spiral tip rotates around a single point then a reentrant cycle will follow the same path and be reflected as a monomorphic ECG pattern. Conversely, if the tip rotates around a non-stationary region then the reentrant pathways differ and are reflected as a polymorphic ECG pattern.

In this work they looked at polymorphic ECG's in a uniform medium when the action potential was prolonged. Action potential duration (APD) prolongation was achieved either by reducing the magnitude of the generalised potassium conductance, mimicking either channel block or hypokalaemia, although similar results could have been obtained by any intervention which prolongs the action potential duration. The conclusions from this modeling suggest that non-sustained tachycardias and ventricular fibrillation seen with long QT syndrome(leading to Torsade de

Pointes) and hypokalaemia, or in the presence of potassium channel block are compatible with a reentrant process in the setting of diminished repolarizing currents [88].

In summary potassium channels are involved and may play a role in prolonging individual action potentials. This is supported by the action of specific potassium channel openers which reverse the effect that bupivacaine has on prolonging the action potential plateau. Prolongation of the action potential allows the sodium channels to remain in the closed-inactivated state for longer allowing preferential binding of bupivacaine. Action potential lengthening also is thought to predispose to reentrant arrhythmias and magnesium may shorten the action potential duration and therefore help in reversing bupivacaine's effect.

4.2.(2.4) ROLE OF AUTONOMIC NERVOUS SYSTEM

Early in the use of amide local anesthetics it was realised that lignocaine stimulated the heart through a central mechanism with a resultant increase in cardiac output and mean arterial pressure [103].

The autonomic nervous system has recently been implicated in the cardiotoxicity of bupivacaine and research has become focused in this area. Bernards and Artru [31] looked in anaesthetised rabbits and found that intracerebroventricular administration of small doses of bupivacaine induced hypertension and cardiac arrhythmias. Moreover, the intracerebroventricular administration of midazolam, known to potentiate the activity of gamma-amino-butyric-acid(GABA)-ergic neurones that

inhibit the activity of outflow neurones of the autonomic nervous system, suppressed bupivacaine induced hypertension and arrhythmias.

In one of the earliest papers implicating the sympathetic nervous system in bupivacaine cardiotoxicity Bernards [53] looked at the effect of midazolam and diazepam premedication on the CNS and CVS toxicity of bupivacaine. Two previous studies had provided inconclusive evidence of the role of benzodiazepines and they therefore looked at 3 groups (n=10) of awake swine, one group receiving saline, one diazepam 0.15mg/kg and another midazolam 0.06mg/kg as premedication. All animals were invasively monitored and aggressively resuscitated once cardiovascular collapse occurred. They found that the control group developed dysrhythmias earlier than the diazepam/midazolam groups. Blood pressure and heart rate increased during the first 2 minutes of bupivacaine in the control group but not in the benzodiazepine groups. The dose of bupivacaine to produce CVS collapse and the plasma levels did not differ amongst the groups. Animals receiving the benzodiazepines were less likely to experience seizures and the diazepam animals were less successfully resuscitated. They postulated that the increase in heart rate and blood pressure seen in the control group was on the basis of sympathetic activation just as it had been shown to stimulate the amygdala to cause seizures.

De La Coussaye et al [35] looked at the putative cardioprotective effects of hexamethonium in the presence of cardiotoxic plasma levels of bupivacaine. They attempted to determine the mechanism by which the autonomic nervous system may plays a role in bupivacaine cardiotoxicity and their model consisted of closed chest, pentobarbital anaesthetised dogs in 3 groups- the control group getting bupivacaine alone, the second

pretreated with 0.2mg/kg atropine and the 3rd pretreated with intravenous hexamethonium. Animals in the hexamethonium group were also paced via the right atrium to obtain a heart rate similar to the control group. The bupivacaine infusion in the control group significantly decreased heart rate, lengthened PR, atria-His, His-ventricle and QTc intervals; and QRS widening. Atropine pretreatment did not modify cardiac disturbances induced by bupivacaine . Hexamethonium pretreatment attenuated QRS widening and QTc lengthening but worstened the bradycardia, atria-His and PR intervals. They concluded that any drug which maintains better cardiac conduction after bupivacaine as demonstrated by decreased lengthening of the HV or QRS interval, should also prevent the occurrence of ventricular arrhythmias. They argue that the deleterious activation of the autonomic nervous system is actually activation of the sympathetic nervous system. They concluded by speculating that it may be the increased heart rate which is really the cause of the increased toxicity as a result of a use dependent block.

Bernards et al [31] looked at the intracerebroventricular installation of control CSF, Muscimol (GABA agonist), picrotoxin (GABA chloride channel blocker) and intravenous hexamethonium. These were followed by intravenous bupivacaine infusions. They had previously shown that intracerebroventricular administration of midazolam, a drug that enhances GABA activity, terminated both the cardiac dysrhythmias and hypertension. The increased sympathetic nervous system outflow could result from bupivacaine mediated blockade of the GABA-ergic neurons that are known to tonically inhibit brainstem sympathetic outflow. Midazolam was effective in terminating the dysrhythmias and hypertension possibly because it restored the lost GABA -ergic inhibition. However the animal models do not yet explain whether the sympathetic nervous system plays a

part during accidental intravenous infusion. The plasma levels of bupivacaine which produced cardiac dysrhythmias in the muscimol and hexamethonium groups was significantly greater than the control group. The plasma concentration of bupivacaine that produced cardiac dysrhythmias in the picrotoxin group was no different from control. In all groups, cardiac dysrhythmias initially consisted of premature ventricular contractions often progressing to multifocal ventricular ectopics, bigemini, trigemini or runs of ventricular tachycardia. No animal developed ventricular fibrillation. In all animals cardiovascular collapse resulted from electromechanical dissociation. Their finding that muscimol pretreatment resulted in an increased threshold for bupivacaine induced cardiac arrhythmias was consistent with their hypothesis that GABA ergic receptors are involved. However they found that picrotoxin pretreatment did not decrease the arrhythmia threshold as expected. They speculate that it was their conservative dose of picrotoxin that resulted in this lack of results. They had arrived at the conservative dose due to an earlier trial during which they were able to demonstrate that picrotoxin itself was able to induce arrhythmias in sufficient dose. Despite the results showing an increased threshold for dysrhythmias in hexamethonium and muscimol groups, it did not alter the threshold for cardiovascular collapse. It is therefore important to question whether the increased dysrhythmia threshold is clinically significant. Death from intravenous bupivacaine occurs from either ventricular dysrhythmias or electromechanical dissociation (EMD) depending on species and individual variability. However it is unclear why either should predominate in some species or individuals. It would be nice to speculate that in those species and individuals who develop ventricular dysrhythmias, that increasing the dysrhythmia threshold may also raise the threshold for cardiovascular collapse [31]. Interestingly they found no correlation between heart rate

and predisposition to dysrhythmias. No study seems to have investigated this effect in the intact animal. They also expressed concern that hexamethonium was a general ganglion blocker and that therefore they could not exclude it from a protective effect from blocking some pathway in the parasympathetic nervous system.

Following evidence that the CNS may be involved in the cardiotoxicity of bupivacaine, Richter et al [59] looked, in rats, at the effects of arrhythmogenic doses of bupivacaine on the activity of brain medullary cells. They were able to demonstrate that intravenous administration of bupivacaine results in consistent patterns of cellular inhibition in the nucleus tractus solitarius cells. This is in agreement with reports describing the direct placement of bupivacaine in the nucleus tractus solitarius. Cellular inhibition was often preceded by a period of excitation in cortical cells. These changes were felt to be consistent with excitatory input to the nucleus tractus solitarius [54].

Gerard [54] attempted to help sort out the conflicting literature concerning the cardiovascular effects of diazepam and bupivacaine by looking at the interaction of the two drugs at therapeutic concentrations rather than the toxic plasma levels at which they had both been studied previously. Using chronically instrumented mongrel dogs they had 4 groups- group 1 receiving saline 1ml/min for 30 minutes; group two receiving diazepam 0.2mg/kg bolus; group 3 bupivacaine 0.4mg/kg bolus and a bupivacaine infusion at 15 microgram/kg/min. Group 4 received the diazepam and bupivacaine regimes.

The results [54] of this were:

Table 5.

	Diazepam	Bupivacaine	Combination
SVR	Down at 5min	Unchanged	Down at 5-10 min
HR	Up at 5 min	Up at 30 min	Up at 5-10 min
CO	Up at 5 min	Unchanged	Up at 5-10 min
AP	Unchanged	Unchanged	Unchanged
LV dP/dt	Unchanged	Up	Unchanged

SVR = Systemic vascular resistance HR = Heart rate

CO = Cardiac output LVdP/dt= Myocardial contractile force

The primary effect of the diazepam was to induce early vasodilatation, SVR decreased at 5 min, with an accompanying increase in HR and CO. Aortic pressure and LV dP/dt (as a measure of myocardial contractile force) remained unchanged. The plain bupivacaine group showed a delayed (30 min) increase in heart rate and LV dP/dt, without any change in cardiac output or aortic pressure. The combined effect of the 2 drugs was a decreased SVR and increased heart rate and cardiac output which occurred at 5 minutes. The heart rate was maintained throughout the experiment and was not accompanied by any increase in myocardial contractility, unlike the bupivacaine alone.

These results failed to show the dysrhythmic effects seen at toxic doses. Bupivacaine alone exerts a sympathomimetic effect which is blunted by diazepam. Bupivacaine and diazepam may be a potentially dangerous combination as the diazepam could blunt the early warning signs of bupivacaine toxicity This may be particularly important during epidural block when the sympathomimetic effect may be important in counteracting vasodilatation and care should be exercised when using the combination in those with impaired cardiac function.

Sympathetic tone blockade was investigated by de La Coussaye [52] in dogs. They had a control group, a second group received 0.2mg/kg atropine and a third group received 0.2mg/kg propanolol. They all received 4mg/kg bupivacaine over 10 seconds and various cardiovascular parameters were recorded. In all groups the main effect was an impairment of infranodal conduction, especially that of the fast inward sodium current. The atropine group was no different from control and they concluded that the parasympathetic system therefore played no part. The propanolol group showed increased susceptibility and this manifest mainly as an increase Atria-His conduction time and a greater decrease in mean arterial pressure [52].

Clonidine has been investigated fairly extensively as an agent for attenuating and treating bupivacaine cardiotoxicity. Clonidine is known to prolong the duration of sensory and motor block produced by bupivacaine and the combination has been used by constant infusion via the epidural route for post operative analgesia. de Kock et al [28] looked at 2 groups of rats, one placebo and one pre-treated with clonidine 5ug/kg. The rats were anaesthetised with intraperitoneal pentobarbital and tracheotomised and ventilated. 15 Minutes following the clonodine/placebo they received a bupivacaine infusion at 2mg/kg/min. They showed that the dose necessary to produce an isoelectric EEG was 58.6 +/- 14.9 mg/kg in the clonidine group compared to 22 +/-6.4 mg/kg in the placebo group($P<0.001$)

In their second experiment they found ventricular dysrhythmias first occurring after 10.9 +/- 4.5 min in the clonidine group and 3.2 +/-1.0 min in the placebo group($P<0.01$)

The mechanism of this antidysrhythmogenic effect is not elucidated. A central site of action is the most probable explanation. The alpha-2 agonists

potently inhibit the neuronal firing rate from the locus coeruleus, leading to a decrease in sympathetic outflow. This action may decrease the release of noradrenaline at the cardiac neuroeffector junctions. If the early signs after bupivacaine overdose are caused by increased autonomic nervous system outflow, as hypothesised by Bernards and Artu [31], clonidine pretreatment may be adequate prevention.

In addition the clonidine induced bradycardia may be another reason why it increases the threshold for cardiotoxicity. Bupivacaine blocks the cardiac sodium channels in a " fast in, slow out" fashion subject to use dependence. A slower heart rate before bupivacaine overdose allows more time for the sodium channel to remain in the closed, configuration, during which time the affinity of the bupivacaine for the channel is reduced and bupivacaine may move away from its binding site.

De La Coussaye et al [26] felt that there are two problems in bupivacaine cardiotoxicity; firstly the electrophysiologic changes and then the haemodynamic disturbance.

They felt that the main electrophysiologic disturbance induced by bupivacaine was a potent inhibition of the fast inward current resulting in a slowing of ventricular conduction velocities, which are well known to cause reentry phenomenon and hence ventricular arrhythmias. Therefore they felt it illogical to try and treat cardiotoxicity with another antiarrhythmic drug. They had previously observed that clonidine slightly shortens the variables of ventricular conduction velocities represented by the His-ventricular (HV) interval and QRS duration in anaesthetised dogs. They therefore investigated the hypothesis that clonidine would reverse the slowing of ventricular conduction velocities induced by large doses of bupivacaine. The haemodynamic disturbances resulted from a bupivacaine induced depression of myocardial contractility and therefore contractility

need to be improved. Hence they felt the need to add an inotrope to the clonidine and selected dobutamine, postulating that adrenaline would be more arrhythmogenic. In anaesthetised dogs they looked at five groups all of whom received atropine 0.2mg/kg to inhibit vagal responses on the sinus and atrioventricular nodes.

The groups were:

Group 1 saline control

Group 2 bupivacaine only

Group 3 bupivacaine followed by clonidine(0.01gm/kg)

Group 4 bupivacaine and dobutamine infusion(5ug/kg/min)

Group 5 bupivacaine/clonidine and dobutamine

They found that the bupivacaine group showed significant bradycardia, prolonged the PR(>50%), AH(>30%) and HV(>100%) intervals; and also significantly increased the QRS(>100%) duration. The haemodynamic effects included a 50 % decrease in LV dP/dt max and increased LVEDP(>50%).

Comparison with the clonidine/bupivacaine group showed that the dose of clonidine (following bupivacaine) briefly shortens the PR interval, decreases QRS duration (3-5min) and shortens the HV interval (3-5min). Clonidine also significantly enhances bupivacaine induced bradycardia The dobutamine following bupivacaine group showed a dobutamine increased LVdP/dt max but no change in electrophysiologic parameters or LVEDP between groups 4 and 2. The combined treatment significantly improved the PR and HV intervals, decreases the QRS duration previously impaired by the bupivacaine and increases LV dP/dt. Moreover the combination was more effective in bringing about these changes than clonidine alone.

A further study by the group led by de La Coussaye [34] also looked at the possible receptor mechanisms for the cardio-protective action of clonidine in the face of toxic bupivacaine plasma concentrations. They used various interventions in intact dogs to block various aspects of the autonomic nervous system. They were able to postulate that it was through activation of the cardiac vagus nerves, through ganglionic nicotinic receptors that clonidine had its positive effect on bupivacaine toxicity. It would seem that it was not a direct action via acetylcholine at the nerve terminals but possibly through an associated neuropeptide such as neuropeptide Y [34].

Pitkanen [110] when looking at isolated perfused rabbit hearts found that even at low concentrations of bupivacaine there was a sharp decrease in spontaneous heart rate. This evidence in denervated organs could be confirmation of the CNS effect on toxicity as other work and our own experience in the laboratory is that an initial stimulation of the CVS occurs with an increase in mean arterial pressure accompanied by an increase in heart rate.

4.2.(2.5) ROLE OF LIPID SOLUBILITY AND FREQUENCY DEPENDENT BLOCK

Courtney [15] looked at isolated frog sciatic nerves and measured the height of the action potential in response to single stimuli and to repetitive stimuli. He found that all the nerves treated with the highly lipid soluble agents (bupivacaine, tetracaine, etidocaine) took longer to develop conduction block and also to recover from 40 Hz stimuli than the moderate lipid solubility agents (procaine, lignocaine, prilocaine, mepivicaine). The highly lipid soluble agents required relatively large numbers of impulses to reach maximum effect of use in conduction block. They felt that as normally sodium channels recover from the inactive state with a time constant of 5-10msec, which accounts for the normal refractory period following an impulse and that their data, obtained during potassium channel blockade, suggested that the frequency dependent conduction block is due to an alteration in the process of sodium channel inactivation by local anaesthetic drugs. This was despite previous studies [15] showing that for the extremely hydrophilic drugs opening of the sodium channel is necessary to permit drug to and unbind from the receptor.

4.2.(2.6) ROLE OF ENANTIOMO-SELECTIVITY

Bupivacaine is a racemic mixture of 2 enantiomers. It has been long known that in some mammals the S(-) enantiomer is of equal or higher potency for nerve blocking than the R(+) enantiomer [20] but with less local and general toxicity. Thus it is not surprising that the enantiomers have differing effects on the heart. Some studies have shown that the S(-) enantiomer is less cardiotoxic than the R(+) enantiomer in animal models.

However with our current knowledge unable to describe the exact mechanism of bupivacaine cardiotoxicity, and particularly that there may be multiple sites of action on both sides of the blood brain barrier; it needs a concerted research effort to determine the exact role of the 2 enantiomers. Another significant difference between the 2 enantiomers may be the role of differential protein binding and hence differences in hepatic clearance of drug [20].

Vanhoutte et al [80] looked at the different actions of the 2 enantiomers of bupivacaine on cardiac electrophysiology in isolated pieces of guinea-pig hearts. They found that the 2 enatiomers had differing effects and properties in vitro. R(+) bupivacaine caused a stronger reduction of V_{max}. A mean apparent dissociation constant of 16 microM was found for the reduction of V_{max} at 1 Hz by R(+) bupivacaine versus 39 microM for S(-) bupivacaine. Conversely the anaesthetic potency of S(-) bupivacaine seems to be larger or equal to that of R(+) bupivacaine.

At 1 Hz an important amount of use-dependent block was present resulting from a block of sodium channels during the action potential and incomplete blocking during the diastolic period. R(+) bupivacaine unblocked significantly slower than did S(-) bupivacaine at all tested membrane potentials. They concluded that the fact that R(+) bupivacaine has a greater effect on V_{max} and action potential duration is possibly the explanation for the higher in vivo toxicity. Reduction of V_{max} slows conduction. Too much slowing of conduction and shortening of the action potential duration predisposes to reentrant phenomena. This may indicate a specific receptor site being involved at the sodium channel rather that a non-specific effect on disturbing the membrane.

4.2.(2.7) ROLE OF TISSUE UPTAKE

Concern has been raised about the possible ion trapping of bupivacaine within neural and myocardial tissue under hypoxic and acidotic conditions such as occur during convulsions and cardiac arrest. This argument has been used in attempts to explain the apparent resistance to resuscitative efforts following bupivacaine induced cardiac arrest. Nancarrow's work [79] found that the percentage of each agent isolated from the brain and myocardium after fatal doses was similar for all three agents studied. Acidosis failed to increase tissue concentrations in myocardium and brain suggests that the effect of acidosis may not be mediated through greater brain or myocardial uptake. They conclude that their results support the proposition that the observed differences in cardiotoxicity between agents are due to qualitative differences with respect to their effects on cardiac electrophysiology, and not because of disproportionate uptakes into the myocardium.

The study by Morishima et al [14], whilst comparing the toxicity of lignocaine and bupivacaine in nonpregnant and pregnant sheep, also correlated blood and tissue levels in the brain and myocardium in an attempt to explain the mechanism of toxicity. Their results showed that during a bupivacaine infusion that convulsions were associated with hypertension and tachycardia. The dosage given was correlated with the plasma level by means of a ratio. Significantly higher dosage and blood concentration ratios were noted in the lignocaine group. The tissue/blood concentration ratios of bupivacaine were significantly higher than those for lignocaine in the lungs and the adrenals. The tissue/blood ratios were also greater in the hearts and livers of bupivacaine group but did not reach statistical significance.

At present there is little evidence to support "ion trapping of bupivacaine" in cardiac tissue as a contributory factor to its severe cardiac actions.

4.2.(2.8) ROLE OF DRUG INTERACTIONS

Several studies looking at the possible role of various ion channels and also the studies attemting treatment are indirectly studies examining drug interactions. Timour's study [56] looked specifically at the effect of various drug interactions, with bupivacaine, on the heart. They found that a prolongation of right ventricular conduction time was enhanced by cibenzoline, disopyramide and propanolol. These changes were not due to significant blood pressure changes altering clearance of bupivacaine. The increase in conduction disorders with cibenzoline and disopyramide were not accompanied by significant changes in the effective refractory period and therefore reentrant dysrhythmias were a likely consequence. They could show that increasing right ventricular conduction time by more than 100% resulted in ventricular tachycardia and fibrillation.

Two other drugs that they studied, clomipramine and verapamil had only moderate or no effects when combined with bupivacaine. They felt that the potentiation by class 1c drugs such as cibenzoline could have been predicted in view of its ability to block fast sodium channels. Although the class 1a drugs such as disopyramide have less of an effect on fast sodium channels it is not surprising that they had a conduction prolonging effect.

4.2.(3) POTENTIATING/ATTENUATING FACTORS

4.2.(3.1) ROLE OF MAGNESIUM

The only work looking at the effect of hypermagnesaemia on the cardiotoxicity of bupivacaine is that of Solomon et al [11]. They studied 10 mongrel dogs and induced hypermagnesaemia in one group with a loading dose (140mg/kg) followed by an infusion (80mg/kg/hr) to maintain plasma Mg++ at 2.42 ± 0.18 mmol/l (baseline Mg++ 0.67 ± 0.03mmol/l) [1mmol/l is equal to 24mg/l]. The animals were intubated and ventilated. An infusion of bupivacaine was commenced and at the onset of seizure activity and cardiovascular collapse (as determined by pre-defined criteria) the total dose and plasma concentration of bupivacaine was noted. All animals maintained a sinus rhythm until at least 6mg/kg bupivacaine had been delivered. The bupivacaine infusion resulted in PR and QRS interval prolongation in both groups. PR interval prolongation was not statistically different between groups at 6mg/kg. Widening of the QRS complex in the control group occurred after the infusion of 3mg/kg bupivacaine but in the magnesium pretreated group the QRS prolongation only occurred after 6mg/kg had been infused. By then the Mg^{++} group also had statistically evident QRS prolongation but the control group had lengthened significantly more than the Mg^{++} group. This study showed that Mg++ did not increase the convulsant threshold but was effective in preventing the cardiac dysrhythmias associated with bupivacaine toxicity in dogs. However it was unable to help explain the mechanism of bupivacaine cardiac toxicity and seems to be the only trial looking at the role of magnesium in the prevention of the cardiac effects of bupivacaine. The model is however not the one most applicable to the clinical scenario of bupivacaine toxicity.

It would be of greater clinical relevance to test whether magnesium would be useful in treating the established cardiac effects of bupivacaine.

4.2.(3.2) ROLE OF HYPOXIA/ACIDOSIS

Albright's editorial [17] followed 6 anecdotal cases -to his knowledge- of sudden cardiovascular collapse associated with rapid intravenous injection of bupivacaine. All the cases occurred in operating rooms under the direct supervision of anaesthesiologists following negative aspiration tests. Sudden cardiovascular collapse (ventricular fibrillation or ventricular tachycardia, asystole, or complete heart block with P waves only) occurred almost immediately after rapid injection of the local anaesthetic agent, so that he felt that antecedent hypoxia probably was not an aetiologic factor.

Work by both Mets' [21] and Nancarrow [79], in rats and sheep respectively demonstrated that respiratory depression occurs before the serious cardiac affects with bupivacaine toxicity.

Mallampati reported [10] a case of successful resuscitation which they attributed to the early aggressive institution of cardio-respiratory support. At the time they concluded that there was mounting clinical evidence that prevention of hypoxia was associated with a good outcome.

Kotelko et al [13] studied tracheostomised ewes which had rapid treatment of seizures and never became hypoxic or hyperkalaemic. All of the animals receiving standard epidural doses of bupivacaine developed serious arrhythmias. However their mortality rate was very low and Kotelko postulated that, considering other evidence, hypoxia and acidosis potentiate

the cardiotoxicity of bupivacaine and that under conditions of hypoxia and acidosis the incidence of fatal arrhythmias may have been higher.

De Jong [8] also studied cats and found that adequately ventilated and oxygenated cats were able to survive twice the convulsant dose. If hypotension was also corrected with ephedrine they could survive 3 times the convulsant dose. They found that regular nodal and ventricular arrhythmias appeared with bupivacaine and etidocaine but not with lignocaine. They also found that the QRS complex began to widen and increase in amplitude within 1 mg/kg of infusion. All bupivacaine treated animals had arrhythmias by the time that seizures started. They showed that, within limits, in well ventilated and oxygenated cats - that the support of ventilation and the correction of hypotension with ephedrine- were vital elements in resuscitating animals given cardiotoxic doses of bupivacaine.

Chadwicks' study [51] involved 20 cats which were anaesthetised, paralysed with pancuronium and ventilated to avoid the possible effect of acidosis, hypoxia and hypercarbia. They used EEG seizure activity as a CNS endpoint and a MAP of 10mmHg as cardiovascular endpoint and used infusion rates of 4mg/kg/min for bupivacaine and 16mg/kg/min for lignocaine and recorded the times to each endpoint.

They found that the, onset time of electrical seizure activity was virtually identical in both groups. Despite earlier onset of electrocardiographic changes in the bupivacaine group, mean arterial pressure was greater and remained so for longer than in the lignocaine group. They established that the CVS/CNS toxicity ratio was 4.0 with lignocaine and 4.8 with bupivacaine. Despite very high lignocaine plasma levels all animals were resuscitatable; two of the bupivacaine group were not resuscitatable although this was not statistically significant.

The use of pancuronium in this study may have influenced the results in the light of more recent evidence implicating the central activation of the sympathetic nervous system in bupivacaine cardiotoxicity. The value attached to the CVS/CNS toxicity ratios needs to be interpreted in the light of the assumptions made at the design stage when they equated an infusion rate of 4mg/kg and 16mg/kg per minute to be equal for bupivacaine and lignocaine respectively. Presumably this was based on the commonly quoted clinical potency equivalents of 0.5% bupivacaine and 2% lignocaine.

Heavner [42] looked at the effect of hypoxia in intact pigs and demonstrated that ventilated pigs with fractional inspired oxygen content ($Fi-O_2$) of 0.1 and 0.15 showed a decreased threshold for the CNS and cardiovascular toxicity of bupivacaine. In addition Heavner observed an initial increase in heart rate in the $Fi-O_2$ 0.3 group and noted an increased baseline in the other 2 groups. This is interesting as hypoxia has been shown to be a potent stimulant of the sympathetic nervous system and bupivacaine has been postulated to have similar actions. Therefore the increase in the 0.3 group could be hidden in the other groups who may already have had sympathetic stimulation via a hypoxic stimulus.

Others [93] studied sheep rendered acidotic and hypoxaemic and gave a similar dose to those used by Kotelko [13]. They found the incidence of electrocardiographic changes similar in both studies but whereas only one bupivacaine animal died in the first study, 100% of bupivacaine treated animals died after the higher dose in the this study.

4.2.(3.3) ROLE OF PREGNANCY

There was concern about the high number of obstetric cases of bupivacaine cardiotoxicity occurring in the literature[48] . Some studies had suggested that pregnant animals may be more susceptible to bupivacaine toxicity than to other local anaesthetic agents.

Morishima et al [14] compared the toxicity of lignocaine and bupivacaine in nonpregnant and pregnant sheep. They demonstrated that during bupivacaine infusion convulsions were associated with hypertension and tachycardia. The dosage given was correlated with the plasma levels by means of a ratio. Significantly higher dosage and blood concentration ratios were noted in the lignocaine group.

Similar doses of bupivacaine [14] were needed to produce convulsions in pregnant and nonpregnant animals. However a lower dose of bupivacaine was necessary to produce cardiotoxicity in the pregnant animals. Although a small study it seemed to indicate that pregnancy was a predisposing factor to bupivacaine cardiotoxicity.

Santos et al [48] looked at the comparative systemic toxicity of ropivacaine and bupivacaine in pregnant and nonpregnant ewes. They found that the onset of bupivacaine's toxic manifestations occurred in the following sequence: convulsions, hypotension, apnoea, and circulatory collapse. There were no significant differences between nonpregnant and pregnant animals in the doses or serum concentrations required to elicit toxic manifestations. In nonpregnant animals, similar doses and serum concentrations of ropivacaine and bupivacaine were associated with the onset of convulsions and circulatory collapse. In pregnant ewes, greater doses of ropivacaine as compared bupivacaine were required to produce

convulsions and circulatory collapse. The proportion of animals manifesting a malignant arrhythmia as the terminal event was similar amongst all groups.

Moller and Covino [84] exposed rabbits to 4 days of progesterone before examining the effects of bupivacaine and ropivacaine on the then isolated hearts and found the progesterone pre-treated animals were more sensitive to the effects of bupivacaine. This was not present in the hearts exposed to ropivacaine. They speculated as to whether the effects seen were on the basis of the ropivacaine being a single enantiomer or whether it was the different side chains of the 2 drugs that accounted for the difference. A further unanswered question is whether progesterone influences drugs binding to the sodium channel in excitable membranes?

In summary the effect of pregnancy on bupivacaine toxicity is still undecided but the hormonal changes, and progesterone in particular may potentiate the cardiotoxic effects.

4.3 SUMMARY OF PROPOSED CARDIAC ACTION OF BUPIVACAINE

The cardiac effects of toxic doses of bupivacaine appear to be threefold:

* Pronounced negative chronotropism and inotropism
* Cardiac conduction disturbances -Sodium Channel
  -Potassium Channel
  -Calcium Channel
* Central stimulation of sympathetic nervous system

Depression of cardiac conduction via sodium channel blockade appears to be one of the primary mechanisms of bupivacaine's cardiac toxicity. However there would appear to be evidence that other ion channels may be involved and that the onset and offset may be not only activity-dependent but also different in character. Blockade of sodium channels develops during the upstroke and plateau of the action potential and dissipates during the diastolic interval between beats. Thus both changes in heart rate and in transmembrane diastolic potential, as well as any manuoever that alters the duration of the action potential plateau, may alter drug action [3].

The contribution of potassium channels seems to account for the increased duration of the action potential and accounts for the effect of specific potassium channel blockers in attenuating the effects of bupivacaine. In addition, the calcium channels seem implicated in some of the activity during the lengthened action potential duration and also once profound sodium channel blockade has seriously undermined the sodium channels' role in myocardial cellular depolarization.

5. RECOMMENDED TREATMENT

5.1 LIGNOCAINE

Lignocaine is a class I anti-arrhythmic drug and is useful for the treatment of ventricular arrhythmias. Lignocaine has been suggested as a treatment for the cardiac toxicity of bupivacaine, apart from a postulate based on the modulated receptor hypothesis there would appear to be little evidence for this indication.

Although lignocaine has been used to treat ventricular arrhythmias induced by bupivacaine [46,67], this is contradictory to many other laboratory studies, including work from our laboratory [21], showing additive effects.

De Jong and Davis [9] suggested that lignocaine could be used to treat the ventricular arrhythmias of bupivacaine toxicity. They performed a study in 34 cats given double the convulsant dose of bupivacaine followed by diazepam to stop convulsions. Six of these cats had persistent arrhythmias (only one ventricular tachycardia) which were successfully converted by a bolus dose of lignocaine.

Another group [124] reportedly compared the ability of bretylium and lignocaine to stop pacing induced ventricular arrhythmias following cardiotoxic doses of bupivacaine to "open chest" dogs. In contrast to de Jong and Davis' study, they found that lignocaine enhanced the cardiotoxic effects of bupivacaine by reducing the threshold for ventricular tachycardia. Bretyllium raised the threshold and reversed established bupivacaine induced cardiovascular dysfunction.

The cardiac and respiratory toxicity of lignocaine and bupivacaine have been shown to be additive in rats by researchers at our institution [21].

The modulated receptor hypothesis depends on the dissociation constants for bupivacaine and lignocaine being relatively long and short, respectively. Suggestions that the lignocaine would competitively antagonise the bupivacaine do not allow for lignocaine's dissociation constant being so short that bupivacaine would rapidly return to the receptor and bind for a relatively protracted time.

De La Coussaye et al [26] felt that since the main electrophysiological disturbance induced by bupivacaine was a potent inhibition of the fast inward current, resulting in a slowing of ventricular conduction velocities- which are well known to cause reentry phenomenon and hence ventricular arrhythmias- it was illogical to try and treat cardiotoxicity with another antiarrhythmic drug, such as lignocaine.

A very recent paper by Fujita and co-workers [90] studied the infusion of lignocaine (1%), bupivacaine (0.25%) or lignocaine (1%) and bupivacaine (0.25%) mixture when infused into the anterior descending artery of pig hearts. The bupivacaine depressed the ventricular wall function significantly more than the lignocaine or the combination. However the mixture of lignocaine when infused with the bupivacaine raised the threshold for ventricular fibrillation as compared to the bupivacaine group. Whilst this is good evidence for raising the threshold for ventricular fibrillation it does not equate to being able to use it clinically to treat established toxicity.

5.2 ADRENALINE

The initial assumption was that adrenaline was a first line drug for the treatment of bupivacaine cardiotoxicity. However Heavner [33] in a letter questioned the wisdom of this suggestion as their unpublished study on treatment in rats showed that aggressive use of adrenaline to treat bupivacaine toxicity resulted in marked hypertension and dangerous and often terminal ventricular dysrhythmias. In addition, Feldman [32] concluded that the use of adrenaline to treat cardiovascular collapse following bupivacaine overdose was controversial and that vasopressor drugs with less direct cardiac effects may be more beneficial.

Several reports of bupivacaine toxicity resistant seemingly resistant to adrenaline had caused some concern [69] and Butterworths group speculated that it was bupivacaine's interference in beta-receptor cAMP production that could explain this resistance. Butterworth et al [69] showed that bupivacaine inhibits cAMP production in an equipotent fashion compared with ropivacaine and mepivacaine. This inhibition of catecholamine induced cAMP production is early in the production pathway [33]. In a further study they showed that bupivacaine displaced beta-adrenergic receptor ligands from the receptor at concentrations associated with cardiovascular toxicity. However there seems to be some debate about evidence for the competitive nature of these interactions. The initial assumption was that adrenaline was a first line drug for the treatment of bupivacaine cardiotoxicity. However if the effect is not competitive then adrenaline may not be the ideal agent.

Moore, Crawford and Scurlock [74] suggested that the addition of adrenaline might prevent or treat the myocardial depression caused by bupivacaine.

However this could not be confirmed in animals rendered hypoxic and acidotic and given bupivacaine both with and without adrenaline.

Kambam and co-workers [91] studied the addition of adrenaline and phenylephrine to bupivacaine. In a rather crude experiment with rats they were able to demonstrate that adrenaline and phenylephrine increased the incidence of death from toxicity from 20% to 90% in animals given 1:200 000 adrenaline or phenylephrine together with a standard dose of bupivacaine. The same dose of the vasopressors had no mortality at all when given by itself.

The recent review by Kerin and Somberg on proarrhythmia [96] produces several good reasons why adrenaline may not be the ideal agent to use concurrently with bupivacaine. The sodium channel blockers create the conditions of disequilibrium between conduction and refractoriness at ventricular level which allow for reentry to occur. Beta-adrenergic blockers have been shown to be protective against the development of these ventricular arrhythmias. In addition local release of catecholamines by various proarrhythmic agents is postulated to be the cause of their proarrhythmic properties and the use of beta-blockers attenuates this effect[96].

5.3 NORADRENALINE

A case report [16] described the use of noradrenaline to correct the haemodynamic consequences of bupivacaine. In addition Wheeler [73] demonstrated that "sinus exit block" induced by toxic levels of bupivacaine could be reversed by noradrenaline.

5.4 BETA-BLOCKERS

A case report [44] of a successful resuscitation in a patient receiving digoxin and a beta-blocker initiated speculation that the combination allowed easier resuscitation. Others believed that the combination lowered the threshold for toxicity and a study in animals [52] seemed to support this.

5.5 BENZODIAZEPINES

Numerous studies support the use of benzodiazepines to attenuate the cardiotoxic actions of bupivacaine [31,53,54,]. Intracerebroventricular instillation of benzodiazepines has been shown to potentiate the activity of GABA-ergic neurones [31] and suppress bupivacaine induced hypertension and arrhythmias.

Bernard's study [53] is cause for some concern with regard to the benzodiazepines' effect in combination with bupivacaine. Animals pretreated with diazepam showed increased thresholds for arrhythmias but not cardiovascular collapse and they were then more difficult to resuscitate.

5.6 ATROPINE

Several studies [34,35,52] have shown that atropine has no effect on bupivacaine toxicity.

5.7 BRETYLIUM

A group [124] led by Kasten compared the ability of bretylium against that of lignocaine to stop pacing induced ventricular arrhythmias following cardiotoxic doses of bupivacaine to "open chest" dogs. In contrast to de Jong and Davis' study they found that lignocaine enhanced the cardiotoxic effects of bupivacaine by reducing the threshold for ventricular

tachycardia. Bretylium raised the threshold and reversed established bupivacaine induced cardiovascular dysfunction.

5.8 CALCIUM ANTAGONISTS

Despite concern and evidence [87] about the potentiating effect of calcium antagonists on bupivacaine cardiotoxicity, a group [27] have shown that a specific dose (200ug/kg) of nimodipine offered some protection. The use of calcium antagonists as treatment cannot be supported with current evidence.

5.9 CLONIDINE AND DOBUTAMINE

Clonidine is known to potentiate the sensory and motor block of bupivacaine and is used clinically in the epidural space. Animal studies have shown raised thresholds for CNS and cardiotoxicity with clonidine pretreatment. The mechanism is not understood, but possible explanations include the slowing of heart rate by clonidine which is thought to decrease the cardiotoxic effect of bupivacaine (modulated receptor hypothesis).

The second possible mechanism is a central action at alpha-2 receptors which have been shown to decrease sympathetic outflow from the locus coeruleus [28]. Clonidine has been shown to shorten the His-Purkinje and QRS duration. de La Coussaye [26] demonstrated that clonidine reversed the bupivacaine induced increases in QRS duration and PR interval in dogs although it exaggerated the bupivacaine induced bradycardia. The combination of dobutamine was a further improvement as it showed the same positive electrophysiologic effects with a maintained heart rate and improved ventricular contractility.

5.10 HEXAMETHONIUM

Although the ganglion blocker, hexamethonium, has been shown to raise the threshold for toxicity [31,35] it has not been shown to be effective once toxicity has been established. Its use as treatment for established toxicity cannot be recommended.

5.11 PREVENTION OF HYPOXIA/ACIDOSIS

This is well established and is reviewed under this heading in the animal evidence section earlier.

5.12 PACEMAKER

Although there are no case reports describing the use of pacemakers for the management of bupivacaine cardiac toxicity, it is certainly an option to be entertained as the toxicity of a given serum concentration perfused through the intact heart seems to be dominated by conduction disturbance. Pitkanen's study [110] looked at isolated, perfused rabbit hearts and compared the ability of the hearts to be paced at atrial and ventricular levels with differing concentrations of lignocaine, bupivacaine and ropivacaine. Half of the hearts could not be atrially paced at 6 microgram/ml bupivacaine and 13 microgram/ml ropivacaine. All the ropivacaine hearts (at 13microgam/ml) could be paced in the ventricle and only one of six at 13 microgram/ml bupivacaine. Following washout of the local anaesthetics all hearts could be paced at baseline voltages. This finding may indicate that the use of an external or internal pacemaker during resuscitation from local anaesthetic overdose may be useful.

6. MAGNESIUM

6.1 MAGNESIUM METABOLISM

Magnesium is the 4th most common cation in the body. Total body stores average 1000mmol and the normal serum range is considered 0.7-1.25 mmol/l. Approximately 50 % is contained in bone and 20-30% in skeletal muscle. The heart also has a high concentration of magnesium. Only 1% of this magnesium is in the extracellular compartment. Extracellular magnesium is 55% unbound and ionised, 33% is bound to proteins and the remaining 12% is complexed to anions.

Magnesium's physiological role is complex. Magnesium has multiple sites of action in basic cellular processes and is a metabollic cofactor in hundreds of enzymatic processes, especially those involved in energy metabolism. Magnesium is required for protein and nucleic acid synthesis and for several mitochondrial processes. Effects of magnesium on calcium mediated systems and the sodium-potassium-ATPase may be critical in explaining some of the cardiac actions of magnesium. Magnesium's action on calcium within cells includes:

-Inhibition of sarcolemmal calcium flux

-Competition with calcium for binding sites on actin

-Modulation of the cAMP system.

In addition magnesium is involved in ion transport systems, especially the Na-K-ATPase system where magnesium serves as a cofactor in this important membrane enzyme system and thereby influences the transport of sodium and potassium across cell membranes.

6.2 CELLULAR BASIS FOR THE ELECTRICAL ACTIONS OF MAGNESIUM

Several different potassium channels exist within cardiac cells. Intracellular magnesium promotes inward rectification of potassium through some of the potassium channels including the acetylcholine sensitive potassium channels[113]. These effects on the potassium currents increase intracellular potassium and prolong the action potential. Intracellular magnesium also appears to affect the delayed rectifier potassium channel that is responsible for membrane repolarization after an action potential.

Magnesium has long been recognised as a physiological calcium channel blocking agent. Increased intracellular magnesium inhibits calcium entry through the dihydropyridine-sensitive sarcolemmal channels (so called "slow channels") [113]. The effects of magnesium on the calcium channel are to shorten the action potential duration. The physiological significance of these effects is uncertain but the potential exists for magnesium to control calcium currents across the membrane.

6.3 EFFECT OF MAGNESIUM ON THE HEART

Mg^{++} has a long history as an anti-arrhythmic, especially for paroxysmal atrial tachycardia, atrial fibrillation, ventricular extrasystoles, and prolonged QT interval, and is also the treatment of choice for Torsade de Pointes.

McLean [72], in a review on the therapeutic uses of magnesium, was of the opinion that " given its lack of serious lasting toxicity that consideration should be given to adding magnesium to any therapeutic arsenal for supraventricular and ventricular arrhythmias".

Several studies have shown a negative chronotropic effect on the heart [113]. Neither atropine or propanolol alters this effect suggesting that the effect of magnesium on the sinus node is independent of the autonomic nervous system. In humans magnesium infusions have predominantly shown no significant change in heart rate but isolated case reports have described magnesium induced bradycardia. There are reports[113] of an increased sinoatrial conduction time in normal persons given magnesium sulphate. A number of studies have reported AH interval prolongation, although others [113] found no clear prolongation but concluded that the effect may well be dose dependent. In extreme hypermagnesaemia QRS widening does occur. Intravenous magnesium does seem to increase the effective, functional and relative refractory periods of the AV node.

Electrophysiologic studies [72] have demonstrated that a magnesium infusion prolongs the PR interval in normal hearts. This is then followed by increased sinoatrial conduction time, an increased atrioventricular

nodal refractory period and no effect on atrial or ventricular refractoriness or conduction.

The relationship between magnesium and QT interval is of particular interest. There is evidence for mild prolongation of the QT interval in dogs fed a magnesium deficient diet, although the possibility of other electrolyte abnormalities is present. Animal studies could show, in the setting of hypocalcaemia, that low magnesium causes prolongation of the action potential duration and the QT interval. Clinical support for isolated hypomagnesaemia causing increased QT interval or for exogenous magnesium decreasing QT interval is scant. Despite Torsade de Pointes being predisposed to by prolongation of the QT interval, and magnesium being uniquely successful in its treatment, there remains no evidence that magnesium normalises the QT interval even after abolishing the arrhythmia [113].

Rowlands [99] in his book on ECG interpretation concludes that hypermagnesaemia induces ECG changes similar to hyperkalaemia and these are manifest as prolongation of the PR interval and widening of the QRS complexes. Others [72] have suggested that cellular excitability might increase with magnesium deficiency and this has led to some speculation as to whether magnesium would therefore be of much use as an anti-arrhythmic in the face of normal magnesium levels. Further work [72] looked at thresholds for ventricular premature beats and fibrillation in dog hearts. They demonstrated a significant increase in threshold required to produce either premature ventricular contractions or ventricular fibrillation after magnesium chloride had been infused. Although they failed to record serum magnesium levels there was no reason to suspect that this group of healthy dogs had a deficiency of magnesium. They felt that

they were observing a physiologic effect of magnesium which they postulated to be based on modulation of transmembrane ion fluxes.

Although magnesium is now accepted therapy for Torsade de Pointes, its role in the more commonly seen monomorphic ventricular tachycardias is not as clear. A single study [113] in humans found that 7 of eleven patients with haemodynamically stable monomorphic ventricular tachycardia converted to a supraventricular rhythm within 10 minutes after starting an infusion of 2 grams magnesium sulphate over 1 minute. The only reported side effect was that all patients experienced a warm flushing sensation.

Two electrophysiologic studies [72] seemed to suggest that the greatest effect of magnesium infusion is on the nodal conducting tissue rather than on atrial or ventricular myocardium. This property may explain why magnesium has such good properties for blocking or slowing re-entrant circuits at the level of the node. In addition magnesium may exert its effect in terminating supraventricular arrhythmias through the same mechanism.

Re-entrant circuits may play a role in the genesis of some ventricular and supraventricular arrhythmias. Automaticity changes may also contribute to both arrhythmia development and treatment. Magnesium's effect on decreasing ventricular excitability and a similar effect in the atria may explain its role in multifocal atrial tachycardia [72].

Other work [72] demonstrated a raised arrhythmia threshold in magnesium pre-treated dogs using an ischaemic canine myocardium model.

Magnesium therapy has been shown by Iseri et al [109] to be effective for ventricular arrhythmias intractable to either potassium replacement (if hypokalaemic) or the antiarrhythmics lignocaine or bretylium, even with normal serum magnesium levels.

A study by Viskin [119] compared the use of magnesium against adenosine for the treatment of supraventricular arrhythmias. Although less successful than adenosine, the electrophysiological measurements suggested that magnesium's mechanism of action was via conduction suppression in accessory pathways as well as in atrioventricular nodal tissue.

In 1990 Solomon et al [11] showed that magnesium sulphate, when given to establish therapeutic concentrations prior to bupivacaine infusion, raised the threshold for the initiation of the cardiac toxicity.

Recently Akazawa et al [50] looked at the safety of boluses of magnesium under sevoflurane anaesthesia. They looked at the effects of 3 different doses of magnesium in both sinus rhythm and atrial pacing. The boluses of magnesium(30, 60 and 90mg/kg) significantly increased serum magnesium concentrations and significantly prolonged A-V nodal conduction time during sinus rhythm, intra-atrial and A-V nodal conduction time during atrial pacing and total ventricular conduction time at doses greater than 30 mg/kg. His-Purkinje conduction time was increased at doses greater than 60 mg/kg. RR and PR intervals and QRS duration were increased at doses greater than 30 mg/kg during both sinus rhythm and atrial pacing. QTc interval remained unchanged during sinus rhythm. Their conclusion was that magnesium is safe during sevoflurane anaesthesia (up to 1.0 MAC),

even in high doses and that it may be used if indicated for cardiac arrhythmias and hypertension during sevoflurane anaesthesia.

Magnesium has been shown to reduce conduction velocity of cardiac cells via a decrease in maximum upstroke velocity by blocking the sodium channel, although relation between upstroke velocity and conduction velocity may not be as direct as previously thought. Because Purkinje figures and ventricular muscle have almost the same ionic mechanisms for development of action potential, with conduction velocities dependent on the rate of rise of depolarization carried by the fast inward sodium current, significant prolongation of His-Purkinje and ventricular conduction time during both sinus rhythm and atrial pacing may explained by the sodium current blocking action of magnesium[50].

QT interval reflects the average action potential duration of ventricular muscle and is determined mainly by the balance between outward potassium and inward calcium permeability during ventricular repolarization. Because QTc interval remained unchanged, significant prolongation of QT interval during sinus rhythm can be attributed to dose related decrease in heart rate [50].

More recent work by Nakaigawa and Akazawa [97] looked at the effects of 60, 90 and 120 mg/kg doses of magnesium sulphate on the cardiovascular system and myocardial metabolism. They used 9 mongrel dogs anaesthetised with pentobarbitone and fentanyl. They found that all 3 doses produced dose dependent decreases in systolic and mean blood pressure, heart rate, left ventricular dP/dt max. Stroke volume was increased and systemic vascular resistance decreased which they speculated may account for the unchanged cardiac output. The decreased afterload allowing the decreased contractility to be compensated for and hence preserved the pump function of the heart. The ECG changes were a significant increase in QRS duration

and corrected QTc interval but this was dose independent. ST segment, P wave and T wave configuration was unchanged at all doses. A dose dependent increase in sinus cycle length, PQ and QT intervals was seen with the magnesium. A possible shortcoming with this study is the use of the muscle relaxant, pancuronium which has nicotinic receptor inhibitory actions [34]. These inhibitory actions may interfere with the interpretation of some of the data; which they themselves attribute to possible sympathetic activation. The work was in agreement with others [97] who could show that magnesium may produce short-lived blockade of sympathetic ganglia and prevents the stimulating effects of potassium and acetylcholine on superior cervical ganglia in cats.

Many studies looking at the effect of magnesium on the myocardium appeared to indicate that magnesium had little effect in changing the action potential of Purkinje fibres. However these studies [109] had mostly been carried out in Tyrodes solution which contains 7.14mg/dl of free calcium. This amount is more than that present in normal plasma and may be enough to resist the action of magnesium. Chesnais et al [120] looked at the effect of reducing the calcium concentration- they observed that when the calcium concentration was reduced, magnesium shortened the plateau phase and the duration of the action potential. This was interpreted to indicate that magnesium had probably blocked the slow inward current carried by sodium ions. It is conceivable that magnesium had blocked the entrance of any calcium that was present. The pacemaker cells, in the SA and AV nodes, are largely dependent on calcium influx for phase 0 of the action potential and other studies [109] have shown in giant squid axons that the calcium influx can be blocked by magnesium.

7. RATIONALE FOR I.V. MAGNESIUM AS THERAPY FOR BUPIVACAINE CARDIOTOXICITY

Multiple descriptions of the use of magnesium for the treatment of arrhythmias led to the successful use of magnesium in some particularly resistant cases of bupivacaine cardiotoxicity at Red Cross Children's Hospital. A literature search revealed previous work by Solomons [11] who showed an increase in arrhythmia threshold when a therapeutic plasma level of magnesium had been attained.

The increased threshold seen by Solomons prompted a laboratory study to investigate the use of magnesium sulphate in established bupivacaine cardiotoxicity. Administration of bupivacaine to humans via the intravenous route was precluded by the known severe cardiotoxicity; thus an animal model was employed.

8. EXPERIMENTAL PROTOCOL

The objective of this study was to attempt to mimic the cardiotoxicity as seen in the clinical setting and to test the hypothesis that magnesium sulphate would reverse established bupivacaine cardiotoxicity. Therefore it was proposed that readily visible (qualitative) changes on the ECG monitor, as used in theatre, should be studied. A literature review revealed other studies looking at measured parameters of the recorded ECG, and therefore this method was considered. One previous study used the animals' baseline as the normal for the study and then defined deviations greater than 25% from the baseline as being abnormal. In this study we experienced a large variation in baseline parameters between rats, and there was concern about the ability to make accurate measurements from recorded ECG traces. Therefore a blinded, independent and senior cardiac anaesthetist was asked to make a qualitative interpretation of the rhythm strips.

8.0 METHOD

8.1 Ethical Approval:

Ethical Committee approval was obtained for the use of 10 rats in a pilot study and 20 rats in a blinded, randomised and controlled study.

8.2 Pilot Study:

10 rats were used in a pilot study to determine the average bupivacaine infusion rates necessary to establish cardiotoxicity. These animals were also used to determine the effective therapeutic dose of magnesium sulphate. Initial doses used were 40mg/kg, based on the loading doses used in humans to achieve therapeutic levels in eclampsia. These proved to produce profound hypotension that took

several minutes to reverse. A 20mg/kg dose of magnesium sulphate was found to reverse established bupivacaine toxicity as determined by ECG criteria, and to produce moderate hypotension that was short lived.

8.3 Power analysis:

Power analysis revealed that if we could successfully produce arrhythmias in 90% of rats studied, and if magnesium was successful in reversing the arrhythmias in at least 50% of the animals, then wee would need only twelve (12) animals in total to demonstrate the effect. As we were not certain that we would be able to induce toxicity in 90% of animals, we proposed to study twenty (20) rats (10 in each group) . The control group would simply have bupivacaine infusion continued and would receive a saline placebo when the treatment group received the magnesium dose.

8.4 Study Design:

All animals studied were male Long-Evans rats weighting 250-350g, from the University of Cape Town colony. Following termination of the experiment rats were euthanased with a bolus dose of bupivacaine.

Two rats were studied each day in the microsurgical laboratory.

Rats were anaesthetised with 60mg/kg of intraperitoneal pentobarbitone.

Once adequate anaesthesia was established as measured by absence of response to snout stimulation they were placed supine on a cork board and the snouts fixed to the board using a rubber band.

Endotracheal intubation was performed using a modified paediatric Macintosh laryngoscope and individually manufactured endotracheal tubes.

Rats were then ventilated with air. A tidal volume of 15 ml/kg was utilised at a rate of 60 breaths per minute using a Harvard Apparatus Rodent Respirator. The ventilator settings remained unadjusted for the duration of the experiment so that the tight control of rat weights provided similar degrees of ventilation for the two groups.

Two intravenous lines were placed in the opposite femoral veins using an operating microscope. One was primed with 0.5% isobaric bupivacaine [Macaine, Adcock-Ingram] and the other primed with either the magnesium sulphate in a concentration of 20mg/ml or saline which would be given in an equivalent volume.

An arterial line [24G Jelco] was placed in one femoral artery under direct vision using the operating microscope. This was connected to a primed and heparinised Deltran II Disposable Pressure transducer [Utah Medical Products].

A Siemens Sirecrust 961 Critical Care monitor was used to record the invasive blood pressure and a 3 lead ECG using 3 needle electrodes placed subcutaneously in the left and right axillae and over the lower margin of the left praecordium. Standard lead II was monitored

and recorded. A Siemens Siredoc 220 printer was used to record the monitor data at a paper speed of 25mm per second.

Following the stimulation of preparation the animals were allowed a 15 minute timed "stabilization period" before the experiment.

Following stabilisation the bupivacaine infusion was commenced at 4ml/hour and increased by 1 ml/hr every 4 minutes until cardiac toxicity was established by the following criteria:

-ventricular arrhythmias or fibrillation

-multifocal atrial arrhythmias

-grossly visible prolongation of the QRS complex

-any AV conduction worse than 1st degree block

The prime outcome measures were the incidence of reversal of cardiac toxicity (as defined above) seen as:

-abolition of ventricular arrhythmias or ventricular fibrillation

-abolition of multifocal atrial cardiac arrhythmia's

-reversal of a prolonged QRS complex

-improvement of any AV conduction block.

Once toxicity was established the infusion rate of bupivacaine was maintained, an arterial blood sample of 2ml was obtained and the volume replaced by 2 ml of normal saline. The ECG was recorded and then the pre-randomised treatment (magnesium sulphate 20mg/kg or an equal volume of saline) given by a rapid bolus over 1-2 seconds. ECG monitoring was maintained and any changes recorded.

Two minutes after the initial treatment, the infusion was terminated and if ECG features were still considered abnormal then a second dose of treatment or placebo given.

Rats then remained under observation for a further 10 minutes and any changes were recorded.

The ECG recordings and the time from starting the bupivacaine infusion were presented in chronological order to a blinded observer(a senior cardiac anaesthetist) with the following information:

-time of trace

-baseline trace labeled

-early toxicity labeled

-established toxicity trace labeled

-trace immediately post treatment

-trace at two minutes post treatment

-trace at three minutes post treatment

-trace at four minutes post treatment

-traces from any other times where noteworthy changes had occurred, were presented with the relevant time indicated.

The blinded, independent observer had been given a copy of the protocol and was asked to comment in detail on the rate, rhythm, PR interval and QRS complex in each trace, and then to make a decision about any changes between traces, and to label changes as one of the following:

-no response

-worse

-good response

9. RESULTS

The mean weight of the male Long-Evans rats was 293g ± 16.4g. The was no significant difference between the groups.

Arterial samples were taken from the 2nd rat studied each day and blood gases analysed. An incomplete sample(8 rather than 10) was due to technical difficulties obtaining enough blood from the rats at the end of the experiment. The quantity of blood required for analysis precluded us from taking specimens earlier in the experiment. Blood gas analysis was done to audit our ventilation (see appendix 1.) and the results indicate that all the checked animals were adequately ventilated and oxygenated. Despite concerns regarding statistical analysis of such a small sample the analysis is included to demonstrate the lack of any differences.

Four of nine rats in the magnesium group received a second dose of the treatment and five of the ten rats in the placebo group received a second dose. There is no significant difference in the volume of fluid that the two groups received.

Groups were compared using Fisher's Exact test.

Two samples of the ECG traces from one rat in each group are included as examples of the data presented for analysis by the blinded observer (in appendix 3).

9.1 RESULTS AT 2 MINUTES

RAT	CONTROL		MAGNESIUM
1	No response	2	Good response
3	No response	5	Good response
4	Good response	7	Line out
6	No response	9	Good response
8	No response**	11	Good response
10	No response	12	Good response
14	Worse**	13	Good response
15	No response	16	No response
19	No response	17	Good response
20	No response**	18	No response**

	NO RESPONSE	GOOD RESPONSE	
CONTROL	9	1	
			p=0.005
MAGNESIUM	2	7	

[Fisher's exact test]

9.2 RESULTS AT 3 MINUTES

RAT	CONTROL		MAGNESIUM
1	No response*	2	Good response*
3	No response	5	Good response
4	Good response	7	Line out
6	No response	9	Good response
8	No response	11	Good response
10	Worse	12	Good response
14	Worse	13	Good response
15	Worse	16	No response
19	No response	17	Good response
20	No response	18	Good response

	NO RESPONSE	GOOD RESPONSE	
CONTROL	9	1	
			p=0.0019
MAGNESIUM	1	8	

[Fisher's Exact test]

9.3 RESULTS AT 4 MINUTES

RAT	CONTROL		MAGNESIUM
1	No response	2	Good response
3	No response	5	Good response
4	Good response	7	Line out
6	No response	9	Good response
8	Good response	11	Good response
10	No response	12	Good response
14	No response	13	Good response
15	No response	16	No response
19	No response	17	Good response
20	Good response	18	Good response

	NO RESPONSE	GOOD RESPONSE	
CONTROL	7	3	
			p=0.015
MAGNESIUM	1	8	

[Fisher's Exact test]

9.4 TOTAL BUPIVACAINE DOSES

(Doses in mg of bupivacaine)

RAT	CONTROL		MAGNESIUM
1	9.8	2	16.1
3	7.2	5	12.8
4	4.9	7	Line out
6	19.7	9	12.8
8	7.2	11	9.8
10	23.7	12	12.8
14	19.7	13	12.8
15	16.1	16	16.1
19	16.1	17	23.7
20	4.9	18	19.7
	Mean=12.93		Mean=15.1
	SD =6.92		SD =4.27

Students t-test (assuming unequal variances)

p = 0.413 = NO SIGNIFICANT DIFFERENCE BETWEEN GROUPS

Three rats (underlined) in the control group (Rats 4, 8 and 20) received far smaller total doses of bupivacaine than the rest of the rats. Two of these rats (4 and 20) were noted at the time to have "very early toxicity". Rat number 4 was the only control rat which seemed to respond within 2 minutes to the saline placebo. By 4 minutes after initial treatment rats 8 and 20 were the only other two control rats which seemed to respond to placebo

If these three rats as a group are compared to all the remaining rats then : the three rats in the control group which responded to placebo received a mean dose of bupivacaine of 5.7mg ± 1.3mg[Mean ± SD]

The remaining 16 rats received a mean dose of bupivacaine of 15.7mg ± 4.8mg

When the doses of bupivacaine were compared using Student's t-test

$p = 0.003$ = Highly significant difference

These results indicate that the responders in the control group were the rats receiving significantly less bupivacaine than the remaining rats as a group. This probably explains their early response to termination of the bupivacaine infusion. The results are still significant despite the apparent response of these three rats in the control group. It was decided not to exclude them from the statistical analysis as they would only serve to increase the significance of the results.

10. DISCUSSION

Bupivacaine cardiotoxicity, as reviewed earlier, remains a serious complication when it occurs, and no effective treatment is known.

The results of this study show that in intact rats magnesium produces a more rapid resolution of bupivacaine induced electrophysiological changes than placebo. The improvements are in rhythm and electrical conduction, although this is often at the expense of potentiating the bradycardic effects of bupivacaine toxicity. Whilst the bradycardia remains a problem it is potentially more amenable to therapy than the changes in rhythm and conduction which magnesium sulphate reversed. The opportunity therefore exists to explore the possibility of combining magnesium with a positive chronotrophic agent such as dobutamine [26].

This study was performed in an intact animal model and this raises several questions about its significance. The use of animals is necessary in view of the severe consequences of the drug at toxic levels in humans. However there is concern about extrapolating this kind of data to humans, particularly in the light of concerns (as previously discussed by Van den Berg-personal communication) about the different roles and timing of ion channel opening in the rat myocardial action potential when compared to primates. A further compounding factor is that pentobarbitone suppresses the central nervous system including the sympathetic nervous system and has been considered to raise the threshold for bupivacaine cardiotoxicity. However it was used uniformly in this study, so that there was no bias between groups. Pentobarbitone has an action similar to thiopentone and other general anaesthetics which are often used concurrently with local anesthetic drugs. Unfortunately due to differences in preparation times for

each animal there may have been differences in the state of anaesthesia of the animals. The single dose of intraperitoneal pentobarbitone seemed to provide adequate anaesthesia for the duration, and the only control was the timed period allowing animals to recover from the surgical stimulation. The table giving the bupivacaine doses provides some idea of differences between the groups as the step-wise increases in infusion rate were all timed. An additional concern is the use of surface ECG recordings in an attempt to determine what the drug is doing to individual action potentials in different areas of the intact heart. Analysis of the surface ECG limits ones ability to make deductions and hypotheses about what is happening at a cellular level with regard to bupivacaine's toxicity and magnesium's therapeutic action.

The mechanism of bupivacaine cardiac effects remains poorly understood but current knowledge seems to support the involvement of several ion channels in the direct cardiac effects. Bupivacaine certainly decreases V_{max} of the action potential. This effect is almost certainly mediated by blockade of the sodium channels responsible for phase 0 of the action potential. If the decrease in V_{max} was an isolated effect then one would expect that the extent of depolarisation would be decreased, accompanied by a decrease in length of action potential plateau and duration.

However evidence suggests that toxic doses of bupivacaine lengthen the action potential duration largely by prolonging the plateau duration. A study looking at the dose-response relationship between the action potential duration and bupivacaine concentrations in isolated hearts demonstrated that at low concentrations the action potential plateau's were shortened, suggesting a predominance of sodium channel blockade. At concentrations above 5mM(1.43ug/ml) [73] the action potential plateau

duration increased suggesting that either potassium or calcium channel block was now dominant. This is based on knowledge that lengthening of the action potential duration is always due to a mechanism independent of the cause of the decrease of V_{max} (J Vandenberg-personal communication).

Bupivacaine has however been shown to increase the action potential duration in frog and canine atrial wall muscle and also in canine ventricular septal wall. However the action potential duration has been shown to be decreased by bupivacaine [62] in canine and rabbit Purkinje fibres. Therefore it is possible that there may be regional differences in effect of bupivacaine on action potential duration and this may result in areas differing in excitability, i.e. dispersion, which in turn could precipitate re-entrant arrhythmias [3,62].

Wheeler's group [73] showed that, in the presence of bupivacaine, atrial activity conveyed to the AV node was conducted to the septum but did not produce excitation of the atrium. They were able to demonstrate "bizarre" localized blocks and conduction patterns within the AV node which predispose to re-entrant arrhythmias. Premature septal beats, were observed to arise from the AV node, with re-entry being the likely mechanism of the extra beats. Bupivacaine was shown to be 20 times more potent than lignocaine in depressing action potential(AP) properties in atrial cells. In septal cells the AP changes were more variable although the depression of V_{max} was more pronounced with bupivacaine. Septal cells also showed an action potential lengthening with bupivacaine. Ventricular muscle action potential lengthening occurred with bupivacaine and this also occurs with drugs such as quinidine which, like bupivacaine, also predispose to Torsade de Pointes.

The effect on the body-surface ECG of decreasing V_{max} in the ventricular muscle mass is to increase the QRS complex duration. The effect of increasing the duration of the action potential plateau and therefore the action potential duration is to increase the QT interval. An increase in QRS complex duration accompanied by an increased QT interval are almost universal in studies looking at the electrophysiological changes seen in bupivacaine cardiotoxicity. Both these findings are well known to be associated with an increased risk of reentry and with resultant ventricular arrhythmia's, especially Torsade de Pointes, which has often been associated with bupivacaine cardiotoxicity.

Evidence for the initiation of Torsade de Pointes seems to point to a significant contribution of reentrant phenomenon and also possibly independently to a role for QT prolongation. Torsade de Pointes tends to develop in association with antiarrhythmic drugs that block sodium channels and prolong the QT interval [89]. However not all sodium channel blockers predispose to reentrant phenomena. An index of degree of dispersion of the sodium channel blocking effect would seem more important. Evidence would support the notion that increased dispersion of repolarization is strongly correlated with the risk of development of Torsade de Pointes. This has recently been reviewed by Nattel [121] when considering pro-arrhythmia by sodium channel blockers. Drugs thought to induce Torsade de Pointes have been found to produce spatial and temporal APD heterogeneity in the ventricle which may predispose to induction of re-entry phenomena by early after depolarization's(EAD's). Facilitators of the long QT syndrome predisposing to EAD's include bradycardia, hypokalaemia and hypomagnesaemia. Nattel also speculates that the drug induced EAD's upstroke is generated by the L-type calcium current- which

may explain the susceptibility to arrhythmias with hypomagnesaemia and may explain why magnesium is a useful therapeutic agent for EAD induced arrhythmias. Indeed there may be a specific population of deep subepicardial myocardial muscle cells with APD behaviour which resembles Purkinje Fibres [121] and which equally readily display EAD's in response to various drugs, predisposing to Torsade de Pointes. Further evidence of the role of EAD's comes from in-vivo work [121] showing that drug induced (using a potassium channel blocker model predisposing to Torsade de Pointes) EAD's and/or ventricular arrhythmia's were suppressed by magnesium, diltiazem, beta-blockers and sodium channel blockers [121].

Electrolyte abnormalities, including hypokalaemia and hypomagnesaemia may prolong repolarization and precipitate ventricular arrhythmias [89]. The argument for reentrant cycles inducing Torsade de Pointes is not complete and several issues remain unresolved, although the observations that dispersion of repolarization and delayed activation of the ventricle are present in patients with the long QT syndrome provides further rationale for the implication of reentry in Torsade de Pointes[89].

De La Coussaye's epicardial mapping of reentrant pathways [25] during bupivacaine toxicity in isolated rabbit hearts provides good evidence for reentrance as an initiating factor in bupivacaine toxicity.

The beneficial effect of magnesium in the treatment of Torsade de Pointes is not clear, but it is assumed that it is related to its ability to stabilise membrane potential, thereby reducing the excitability of the cell membrane [96]. Magnesium's effect in treatment of Torsade de Pointes

occurs despite it seeming to have no effect on the prolonged QT interval which is thought to predispose to the arrhythmia [113]. There is speculation that magnesium's calcium blocking properties may depress some forms of automaticity, such as triggered activity, which may underlie the genesis of Torsade de Pointes [89]. Indeed recent work [122] has attempted to quantify the role of EAD's, bradycardia potentiated AP lengthening and interventricular dispersion of AP lengthening in acquired Torsade de Pointes; and the role of magnesium in the reversal of ventricular dispersion. Verduyn's group [122] demonstrated that in Torsade de Pointes susceptible dogs that interventricular APD dispersion correction by magnesium sulphate was accompanied by an inability to induce Torsade de Pointes. This was in contrast to no demonstrated change in intraventricular APD in either ventricle when they were studied separately.

Magnesium has long been recognised as a physiological calcium channel blocking agent. Increased intracellular magnesium inhibits calcium entry through the dihydropyridine-sensitive sarcolemmal channels (so called "slow channels") [113]. The effects of magnesium on the calcium channel are to shorten the action potential duration. [113] The physiological significance of these effects is uncertain but the potential exists for magnesium to control calcium currents across the cell membrane.

Bupivacaine induced lengthening of the action potential plateau produces QT interval prolongation. Reentry due to lengthening of the action potential, accompanied by prolonged QT interval, is widely accepted as playing a significant role in bupivacaine electrophysiological toxicity often leading to Torsade de Pointes. Magnesium has been shown to shorten the duration of the action potential plateau [113] and this is a possible

explanation for the early correction of the electrophysiological changes seen in this study.

Bupivacaine toxicity can be devastating when it occurs and this study, whilst not providing a cure, does offer some direction in terms of new forms of treatment. In addition it may open up new ways of approaching the mechanism of bupivacaine cardiotoxicity, as magnesium may be play a role at a cardiac level, in the central nervous system, or at both sites.

Further studies are needed to elucidate the exact mechanism and site of action of magnesium sulphate in the setting of the complex cardiac electrophysiological abnormalities induced by bupivacaine.

Appendix 1. ARTERIAL BLOOD GASES

Rat	Group	pH	pO_2	pCO_2	BE
4.	Control	7.47	17.8	4.0	-1.2
10.	Control	7.47	12.92	3.45	-3.8
11.	Magnesium	7.45	15.3	5.02	2.5
12.	Magnesium	7.48	15.18	3.95	-0.9
13.	Magnesium	7.49	14.28	3.94	-0.2
14.	Control	7.51	11.55	2.83	-5.1
18.	Magnesium	7.55	13.92	2.80	-3.5
19.	Control	7.55	14.77	3.32	-0.3

	CONTROLS	MAGNESIUM
pO_2	14.26.± 2.7	14.67 ± 0.67
pCO_2	3.4 ± 0.48	3.92 ± 0.9
pH	7.5 ± 0.03	7.495 ± 0.041
Base excess	-2.6 ± 2.23	-0.52 ± 2.46

Appendix 2: Toxicity Criteria

Controls

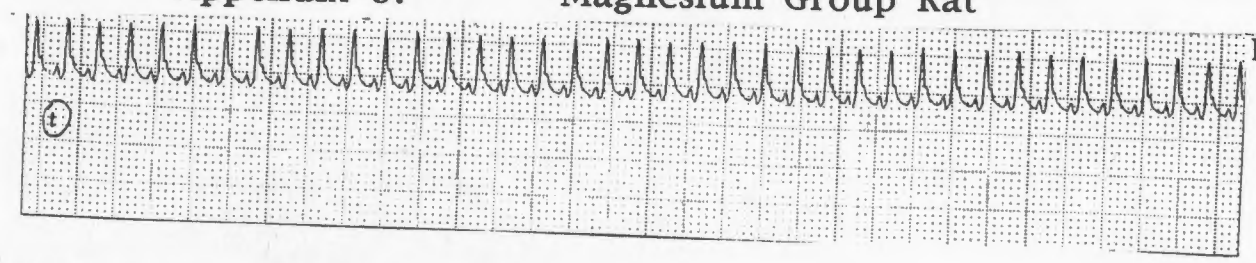
Rat	Toxicity Criteria
1	sinus bradycardia
3	Sinus bradycardia, 2nd degree AV block(Wenkebach), intraventricular conduction disturbance
4	Sinus bradycardia with dropped beats, intraventricular conduction delay, AV block(possible mobitz II)
6	Severe sinus bradycardia, 2nd degree AV block
8	Sinus bradycardia, 2nd degree AV block progressing from type I to type II
10	Sinus bradycardia, 2nd degree AV block(type I), slow ventricular rate
14	Sinus bradycardia, 2nd degree(typeII) AV block
15	Sinus bradycardia, 1st degree AV block, intraventricular conduction delay
19	Sinus bradycardia, 1st degree AV block, poor intraventricular conduction
20	Regular rhythm, possible SVT with aberrant conduction

Magnesium:

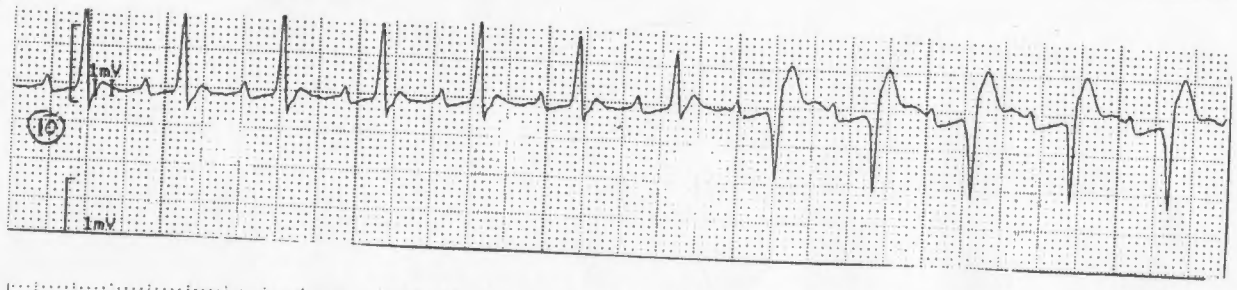
Rat	Toxicity Criteria
2	sinus bradycardia, QRS widening and intraventricular conduction delay
5	Sinus bradycardia, 1st degree AV block, wide QRS
9	Sinus bradycardia, QRS widening, dropped beats
11	Sinus bradycardia, intraventricular conduction disorder, very wide QRS

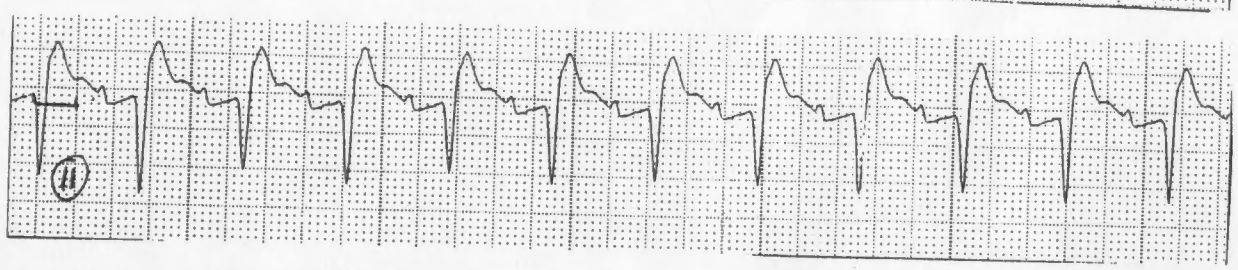
12	Sinus bradycardia, 1st degree AV block, intraventricular conduction delay
13	Sinus bradycardia, 1st degree AV block, intraventricular conduction delay
16	Sinus bradycardia, 2nd degree AV block(typeI)
17	1st degree AV block, severe intraventricular conduction block
18	Marked sinus bradycardia,1st degree AV block, poor intraventricular conduction

Appendix 3. Magnesium Group Rat

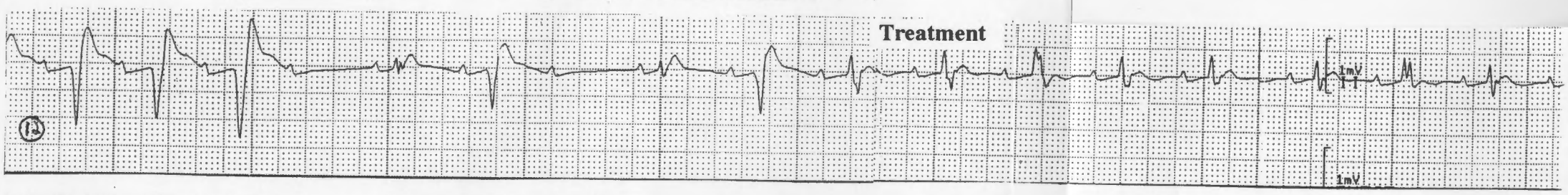


Baseline

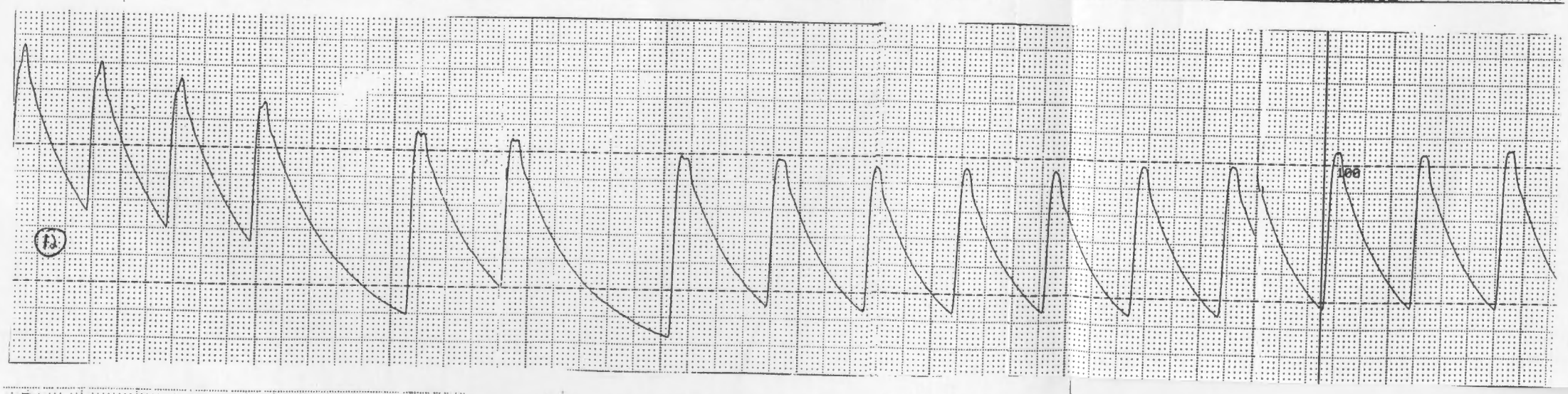


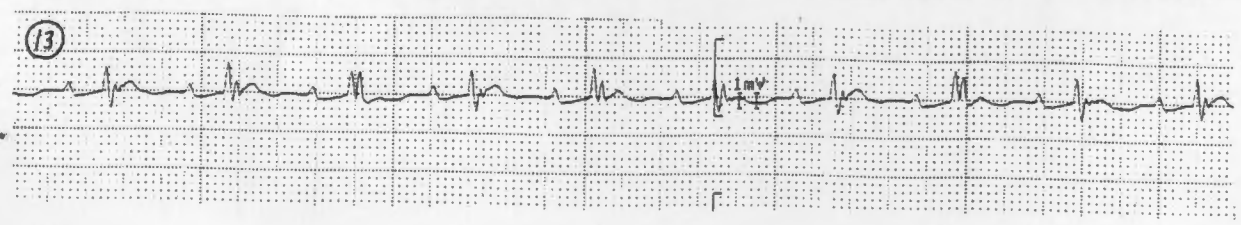


Toxicity

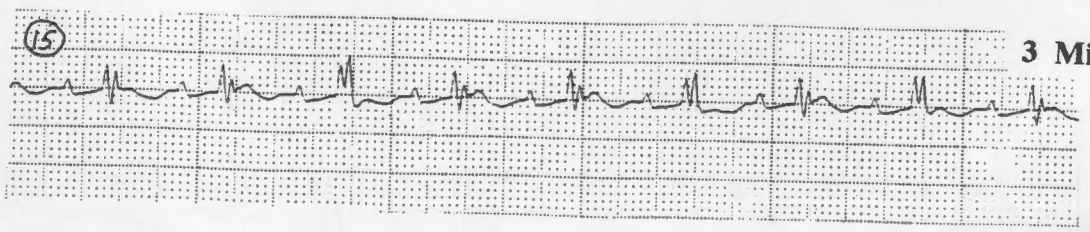


Treatment



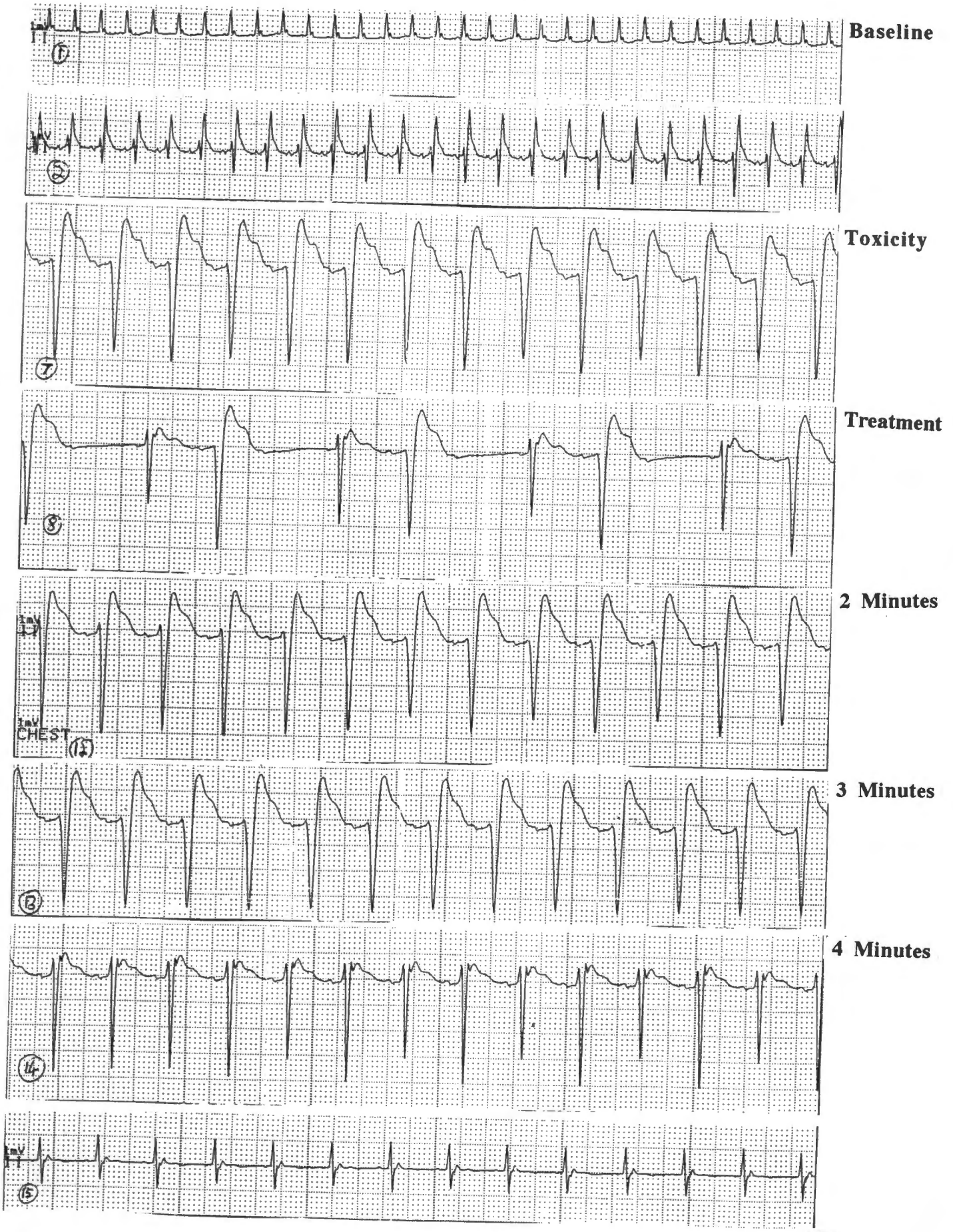


2 Minutes



3 Minutes

Appendix 3. Control Group Rat



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