EVALUATION OF THE ANTIARRHYTHMIC EFFECTS OF VERAPAMIL, PROPRANOLOL AND LIDOCAINE IN ADRENALINE INDUCED CARDIAC ARRHYTHMIAS IN DOGS

JAFRED M. A. KITAA, (B. V. M)
University of Nairobi

A thesis submitted in partial fulfillment for the degree of Master of Science in the University of Nairobi.

Department of Public Health, Pharmacology and Toxicology, Faculty of Veterinary Medicine, College of Agriculture and Veterinary Sciences, University of Nairobi.

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other University.

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J. M. A. KITAA

This thesis has been submitted for examination with our approval as University supervisors.

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DR. S. E. MITEMA, B. V. M., M. S., Ph. D.

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DR. S. W. MBUGUA, B.V.M., M. S., Ph. D.

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This work is dedicated to my loving wife Edith

and sons

Jerry, the twins Elvis and Arnold, and Gregory.

ABSTRACT

Cardiac arrhythmias can occur in any condition such as coronary heart disease or hypoxia in which impulse formation is enhanced, or in which impulse conduction is impaired, or in which a combination of these factors are present. Enhancement of automaticity of latent pacemaker cells by increased sympathetic discharge is a common cause of arrhythmias such as supraventricular and ventricular arrhythmias. Management of these arrhythmias calls for a rational use of drugs with proper diagnosis as use of the wrong drug will aggravate the existing arrhythmia and even lead to death. The purpose of this study was to evaluate two older antiarrhythmic drugs (propranolol and lidocaine) and a new antiarrhythmic drug (verapamil) in the treatment of adrenaline induced arrhythmias and observe their effects on electrocardiographic (ECG) parameters, blood pressure, some biochemical and haematologic parameters.

Twenty adult mongrel dogs of either sex were used in the first study. The dogs were divided randomly into 4 groups of 5 dogs each (n=5). The dogs were anaesthetized with halothane and then pretreated with the drugs (verapamil 0.1 mg/kg bwt., propranolol 0.06 mg/kg bwt., and lidocaine 4 mg/kg bwt.) while the controls received sterile physiological saline. All drug administrations were done intravenously using the jugular vein. Adrenaline (4 μg/kg bwt.) was administered 10 minutes after drug pretreatments. Blood was collected from the jugular vein for haematology. ECG recordings were made before drug pretreatments, after drug pretreatments,

after adrenaline administration, and 30 minutes after the first recording.

In another different study, twenty adult mongrel dogs were used in the experiment. The dogs were also randomly divided into 4 groups of 5 dogs each (n=5). The dogs were anaesthetized with halothane and then received similar drug pretreatments as in the first study except propranolol was given at a dose of 0.5 mg/kg bwt. while the controls received sterile physiological saline. Adrenaline (4 μg/kg bwt.) was administered 5 minutes after drug pretreatments. Blood was collected from the jugular vein at designated time intervals for evaluation of serum levels of calcium, α-hydroxybutyrate dehydrogenase and lactic dehydrogenase enzymes. Blood pressure was monitored via a catheter placed in the femoral artery. ECG recordings were made before drug pretreatments, after drug pretreatments, after adrenaline administration, and 30 minutes after the first ECG recording.

In the first study drug pretreatments were able to prevent death occurring while 3 dogs died in the control group. In all the dogs that died, ventricular fibrillation which was preceded by ventricular tachycardia was observed. The predominant arrhythmias that occurred were ventricular premature beats, ventricular tachycardia, and second degree heart block. The mean P wave amplitude (0.277±0.11 mV) of the lidocaine pretreated dogs was higher ($p<0.05$) than those of propranolol (0.221±0.1 mV), verapamil (0.195±0.08 mV) and control (0.148±.09 mV). Propranolol pretreated dogs

showed an increased S-T duration while for lidocaine pretreated dogs there was a decrease on adrenaline administration ($p<0.05$). Lidocaine pretreatment was able to prevent the increase in the total leucocyte count that occurred in the controls ($p<0.05$). Mean total neutrophil percentage for the lidocaine and verapamil pretreated groups were significantly less than ($p<0.05$) those of the control group.

In the second study propranolol pretreatment protected dogs from death after adrenaline administration. In control group 3 dogs died, in verapamil pretreated group 2 dogs died whereas 1 dog died in the lidocaine pretreated group. In all the animals that died ventricular fibrillations which were preceded by ventricular tachycardia occurred. The predominant arrhythmias that occurred were similar to those observed in the first study except in one propranolol pretreated dog in which sinus arrest occurred. There was significant difference ($p<0.05$) in the P wave amplitude of the verapamil pretreated group (0.19±0.06 mV) compared to the propranolol pretreated (0.14±0.05 mV) and lidocaine pretreated group (0.14±0.06 mV). There was a significant difference ($p<0.05$) in mean QRS complex between control (0.041±0.001 sec.) and lidocaine pretreated dogs (0.044±0.005 sec.).There was an apparent increase for T wave amplitude in all the groups, however, verapamil treated groups significantly increased ($p<0.05$) from 0.163±0.09 mV to 0.317±0.04 mV. Adrenaline administration caused significant increase in arterial blood pressure in all the

experimental groups ($p<0.05$). The arrhythmias, especially second degree heart block and the ventricular arrhythmias when they occurred in runs were associated with decreases in the elevated arterial pressures.

Increased trend in the serum levels of the enzyme lactic dehydrogenase and α-hydroxybutyrate dehydrogenase occurred within the first 8 hours in all the groups. However, lidocaine pretreated dogs had higher increase ($p<0.05$) compared to verapamil pretreated dogs. There was no difference in the levels of calcium between the drug pretreated groups and the control dogs.

The results obtained in this study suggests that propranolol (0.5 mg/kg bwt.) and lidocaine are superior to verapamil in the control of adrenaline induced ventricular arrhythmias in the dog at the dosages used. Pretreatments with the drugs at the dosages used in the study did not prevent increase in arterial blood pressure induced by adrenaline. Drug pretreatments did not have any clinical significant effects on the ECG parameters. Drug pretreatments did not have much effect on the levels of serum calcium and the enzymes α-hydroxybutyrate dehydrogenase and lactic dehydrogenase.

CHAPTER 1

INTRODUCTION

An arrhythmia is an abnormality in the rate, regularity, or site of origin of the cardiac impulse or a disruption in impulse conduction such that normal sequence of atrial and ventricular activation is changed (Tilley and Miller, 1986). Cardiac arrhythmias may cause symptoms varying from weakness and fatigue to sudden seizures or collapse, or both. The arrhythmias that do this include supraventricular tachycardia, atrioventricular heart block, ventricular tachycardia, ventricular fibrillation and occasionally sinoatrial arrest. These arrhythmias occur in humans as well as in animals and are of importance in veterinary medicine notably in the cat and the dog.

The rhythm disturbances affect a dog's normal haemodynamics by changing the heart rate, the regularity of the heart beats, and the time relationship of atrial and ventricular contractions. Loss of atrial assistance and regularity of the ventricles in atrial fibrillation occurs. The rhythm disturbances additionally cause a loss of synchrony in ventricular contractions, and a change in cardiac contractility independent of ventricular filling. The haemodynamic effects of rhythm disturbances are more prominent if the myocardial function at the same time is impaired by heart disease. Ventricular premature complexes can have a major effect on cardiac output as they cause the ventricles to contract too soon before the chamber has had time to adequately fill with

blood. Hence blood pressure and cardiac output drops when the premature beat occurs. An occasional ventricular premature complex can reduce coronary blood flow by 12 per cent, whereas ventricular tachycardia can reduce coronary blood flow by as much as 60 per cent (Tilley and Miller, 1986). The tissue hypoxia from poor coronary circulation can cause the animal to develop additional and more serious arrhythmias (Tilley and Miller, 1986).

Various groups of drugs are available for the management of cardiac arrhythmias amongst which the calcium channel blocking drugs are currently assuming considerable importance. These drugs reduce calcium entry into both smooth and cardiac muscle (Godfriands, 1981; Karlsberg, 1982) and have been used to control supraventricular tachycardia, reduce ventricular rate in atrial flutter and atrial fibrillation, alleviate coronary vasospasm and reduce blood pressure. Though Kane *et al.* (1981), using a rat model found that verapamil did not have significant antiarrhythmic activity, Kaumann and Aramendia (1968) had reported that dogs pretreated with intravenous verapamil before coronary occlusion did not develop ventricular fibrillation. Calcium blocking agents are also effective in reversing electrocardiographic changes associated with myocardial infarction, reducing the size of infarction and improving the perfusion of the area in experimental dogs with coronary artery occlusion (Flaim and Zelis, 1981; Vanhoutte, 1981; Bush *et al.* , 1982). The calcium channel blocker verapamil was approved in United States of America in 1981

for use in the treatment of angina pectoris and supraventricular arrhythmias whereas propranolol and lidocaine are well established in the treatment of cardiac arrhythmias. Lidocaine is widely used for the management of ventricular arrhythmias as it decreases ventricular excitability and conductivity by its effects on the "fast sodium channels". Propranolol exerts its effects by counteracting the effects of catecholamines at ß-adrenergic receptor sites.

The objectives of this study were:

- To evaluate and compare the antiarrhythmic effects of a new antiarrhythmic drug (verapamil) and the older antiarrhythmic drugs (propranolol and lidocaiine) in the treatment of adrenaline induced cardiac arrhythmias.
- To evaluate the effects of the drugs on haematology, blood pressure, extracellular calcium levels and two serum enzymes: lactic dehydrogenase (LDH) and alpha hydroxybutyrate dehydrogenase (α-HBDH).

CHAPTER 2

LITERATURE REVIEW

2.1. INTRODUCTION:

2.1.1. Calcuim channel blockers.

Calcium channel blockers are a relatively new group of drugs that have found wide usage in human medicine and may have a wide potential in veterinary medicine. These drugs reduce calcium entry into both smooth and cardiac muscle (Godfriands, 1981; Karlsberg, 1982).

The calcium channel blockers are valuable new drugs in the treatment of many cardiovascular diseases (Reves *et al.* , 1982). The calcium channel blockers have been used to control supraventricular tachycardia, reduce ventricular rate in atrial flutter and atrial fibrillation, alleviate coronary vasospasm and reduce arterial blood pressure (Karlsberg, 1982; Rouleu *et al.* , 1982; Goad, 1982). The calcium channel blockers which have been used in human medicine include verapamil, nifedipine and diltiazem among which verapamil, a synthetic papaverine derivative, has been found to have more cardiac effects. The drugs are also powerful inhibitors of vascular and extravascular smooth-muscle contractility both *in vitro* and *in vivo* (Coruzzi *et al.* , 1989) All the above three calcium channel blockers are approved for anginal therapy in humans, but only verapamil is approved for antiarrhythmic use

(Spedding, 1985; Novotny and Adams, 1986). Nifedipine is a potent, long acting vasodilator that has proved highly efficacious in relieving anginal symptoms caused by coronary vasospasm while *in vivo* it exerts no myocardial depressant effects and has no antiarrhythmic properties (Henry, 1980).

The calcium channel blocking drugs in addition to the antiarrhythmic effects have other wide effects. Rouleau *et al.*, (1982) and Ribeiro *et al.* (1982) have reported protection of hypertrophic cardiomyopathy and decreased platelet aggregation with verapamil in hamsters and rats respectively. Goad (1982) suggested that calcium channel blockers may be useful in cats with hypertrophic cardiomyopathy and hence recommended further studies for potential therapeutic applications of the drugs in veterinary medicine. Landon *et al.* (1985) reported that the calcium channel blockers nifedipine and verapamil provided protection against necrosis caused by some hepatotoxic agents in rats. In the ischaemic heart the impairment of the calcium homeostasis plays an important role in cell damage (Carafoli, 1987) both in an early and in a later phase. Amsterdam *et al.* , (1989) have reported that pretreatment with verapamil of the intact rat heart activates an ATP-dependent calcium extrusion process that may decrease cellular calcium levels in a reperfusion situation. In dogs, where partial coronary occlusion was induced, the calcium blocking agents have been effective in reversing electrocardiographic changes associated with myocardial infarction, reducing the size of the infarction, and improving the perfusion of the area (Flaim and Zelis, 1981; Vanhoutte,

1981, Bush *et al.* , 1982). This has recently been further supported by Endo *et al.* (1990) who found that gallopamil, a methoxy derivative of verapamil, administered early after coronary artery occlusion in the dog had beneficial effects on the ischaemic myocardium. The calcium channel blockers have been reported to potentiate monensin toxicosis in mice (Mitema *et al.*, 1988).

Recent work suggests that the calcium channel blocking drugs may be useful in human epilepsy (DeSarro *et al.* , 1988) however, Derlet and Albertson (1989) using a rat model have reported that these drugs may potentiate seizures and death in cocaine poisoning.

2.1.2. Verapamil.

Among the three calcium channel blockers- verapamil, nifedipine and diltiazem which have been used in human medicine, only verapamil, originally introduced as a coronary vasodilator (Haas and Hartfelder, 1962), has more cardiac effects and is approved for use in the management of arrhythmias (Spedding, 1985). Re-entrant supraventricular arrhythmias, such as paroxysmal supraventricular tachycardia, are particularly amendable to treatment with intravenous verapamil (Antman, *et al.* , 1980). Verapamil may slow the ventricular rate in the presence of atrial fibrillation or flutter (Singh *et al.*, 1978).

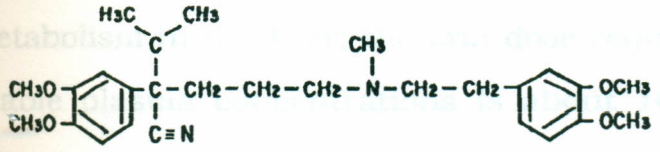
Though Kaumann and Aramendia (1968), had found that dogs pretreated with intravenous verapamil before partial coronary occlusion failed to develop ventricular fibrillation and survived for many months, Kane *et al.* (1981), reported that in dysrhythmia produced experimentally in anaesthetized rats due to coronary artery ligation verapamil had no significant antidysrhythmic activity and it increased the early post-ligation mortality. In addition, verapamil has not been shown to be efficacious in treating ventricular arrhythmias except when the arrhythmias were secondary to myocardial ischaemia (Henry, 1980).

In veterinary medicine the clinical applications for calcium channel blockers mainly involve use of verapamil for treatment of supraventricular tachyarrhythmias (Keene and Hamlin, 1986; Allert and Adams, 1987). In human medicine verapamil is used for short-term conversion of paroxysmal atrial tachycardia to sinus rhythm. Other indications are atrial fibrillation and flutter which it does by effectively reducing AV conduction hence lowering ventricular rate response. Few clinical trials have examined the antiarrhythmic efficacy of calcium channel blockade in animals with cardiac disease. Schamroth *et al.* (1972), reported a decrease in the frequency of ventricular premature beats in 11 of 23 patients treated with verapamil and disappearence of ventricular ectopic activity in the remaining 12 patients. Kittleson *et al.* (1986), observed successful termination of supraventricular tachycardia by verapamil in 8 out of 9 dogs when administered intravenously in 1 to 3 doses at the rate of 0.05 mg/kg. The

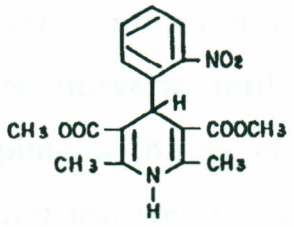
nonresponding dog developed a transient hypotensive crisis after a total verapamil dose of 0.15 mg/kg.

2.1.3. Physical and chemical properties.

Verapamil belongs to the calcium channel blocker group of drugs which have a diversity of structure, for example, verapamil and nifedipine are structurally unrelated (Fig. 1). Verapamil hydrochloride $C_{27}H_{38}N_2O_4$,HCl (491.1 mol. wt.) is a white or almost white, odourless or almost odourless, crystalline powder with a melting point of 141° to 144° C.



VERAPAMIL



NIFEDIPINE

Fig. 1 Structural formulae of some calcium channel blockers.

Verapamil hydrochloride is very soluble in chloroform (1 : 1.5), soluble in water (1 : 20) and alcohol (1 : 25). It is practically insoluble in ether.

Verapamil hydrochloride injection is a sterile

solution in water for injections at a pH of 4.5 to 6.0.

2.1.4. Preparation and routes of administration.

Verapamil hydrochloride (Isoptin®, Cordilox®) is available in tablets containing 40, 80, and 120 mg for oral administration and in 2 ml. ampoules containing 2.5 mg per ml. for intravenous administration.

2.1.5. Pharmacokinetics.

Verapamil is almost completely absorbed from the gastro-intestinal tract, but is subject to very considerable first-pass metabolism in the liver; the oral dose required to achieve comparable plasma concentrations is about 10 times higher than the intravenous dose (Schomerus *et al.* , 1976). In humans verapamil is reported to have a plasma half-life of about 7 hours following oral administration and that of its N-demethyl metabolite norverapamil, which appears in high concentrations in plasma (Kates *et al.* , 1981) is reported to be about 9 hours. After intravenous administration of verapamil the plasma half-life is shorter and its active metabolite has not been detected (Kates *et al.* , 1981). Both high pressure liquid chromatographic and gas chromatographic techniques are used to measure levels of verapamil and its major metabolites (Harapat and Kates, 1979 & 1980; Vasiliades *et al.* , 1982)

Verapamil acts within minutes of intravenous administration but its effects may last only for less than half an hour whereas it may require 2 hours to act after oral

administration with a peak effect after 5 hours. It is extensively bound to plasma proteins (Schomerus *et al.*, 1976). Keefe *et al.* (1981) in a study of verapamil protein binding in patients and healthy subjects found that approximately 90% of verapamil is bound to plasma proteins in a concentration-independent manner over the clinically observed concentration range. The drug is excreted by the kidneys in the form of its metabolite but some is also excreted in the bile into faeces.

2.1.6. Pharmacodynamics.

Verapamil has direct effects on the electrical and mechanical properties of the heart muscle and vascular smooth muscle. It substantially slows the spontaneous firing of pacemaker cells in the sinus node *in vitro* (Wit and Cranefield, 1974; Zipes and Fischer, 1974). However, in intact animals and in man heart rate slows only minimally because the effect of verapamil is partially nullified by increased sympathetic activity due to the arterial vasodilation. Verapamil increases the P-R interval (Flaim and Zelis, 1981) in sinus rhythm and thus slows ventricular rate in atrial fibrillation.

Verapamil, electrophysiologically, has a potent and specific action on the atrioventricular node. It increases the effective and functional refractory periods, hence causing AV conduction delay or block, an action that may be quite useful in supraventricular tachycardias such as atrial fibrillation or flutter. The ventricular rate can be slowed in patients with

atrial fibrillation due to slowed conduction through the AV node; however, few of these patients are converted to a normal sinus rhythm (Smith *et al.*, 1981). In the heart, studies have demonstrated that verapamil has a unique cellular action in selectively inhibiting transmembrane influx of calcium through the slow cation channels of the cardiac sarcolemma and this action is crucial to the antiarrhythmic effects of the drug. Arrhythmias caused by disturbances in either impulse formation (automaticity) or impulse conduction (reentry) are theoretically amendable to verapamil if their origin is associated with the emergence of slow response depolarization. In general, by blocking the AV node, these agents are effective in converting reentrant paroxysmol supraventricular tachycardia to a sinus rhythm (Henry, 1980; Flaim and Zelis, 1981; Karlsberg, 1982)

The most marked effect of verapamil is on the A-V node (Wit and Cranefield, 1974). In man, as well as other species, verapamil decreases the conduction velocity through the A-V node and significantly increases its functional refractory period an action that may be quite useful in supraventricular tachycardias such as atrial fibrillation or atrial flutter. The effect on A-V nodal conduction is presumably a direct result of calcium channel blockade (Husaini *et al.* , 1973; Roy *et al.* , 1974). The potent actions of verapamil on the A-V node are responsible for its effects on the ventricular response to atrial flutter or fibrillation and its ability to terminate paroxysmal supraventricular tachycardia.

Physiologically, the alteration in the intracellular concentration of calcium ions regulates the degree of shortening and force of contraction of cardiac muscle cells in response to varying haemodynamic loads. In the mammalian heart muscle excitation-contraction coupling is thought to be accomplished by the binding of calcium ions to troponin C, which by the way of a complex series of protein interactions, moves tropomyosin to a position that permits the actin-myosin interaction thus resulting in systolic contraction. Decreasing concentration of calcium ions allows this sequence to reverse resulting in diastolic relaxation. The sinoatrial and atrioventricullar nodal tissues are depolarized primarily by a calcium dependent slow channel which is also true of arterial smooth muscle. Thus, the basic actions of calcium antagonists are thus to delay or abolish in tissues in which calcium-dependent slow channel activity is important, and to weaken or abolish contractile activity, resulting in negative inotropy in the heart and vasodilation of arteries. In the heart, studies have demonstrated that verapamil has a unique cellular action in selectively inhibiting transmembrane influx of calcium through the slow cation channels of the cardiac sarcolemma an action that is crucial to the antiarrhythmic effects of the drug. *In vitro* voltage clamp experiments have shown that verapamil prolongs the recovery of slow calcium channels after depolarization (Ellrodt *et al.* , 1980; Henry, 1980). As slow calcium-dependent events are normal characteristics of automaticity in SA and AV nodal tissues, verapamil depresses their discharge rate (negative chronotropic effect) and

impulse conduction velocity (negative dromotropic effect) respectively. Verapamil does this by altering the kinetics of the calcium channels of both nodes (Antman *et al.* , 1980; Nayler and Grinwald, 1981; Zelis and Flaim, 1981). The depression of conduction velocity through AV node is demonstrated electrocardiographically by prolongation of the P-R interval (Novotny and Adams, 1986). Use of verapamil may be indicated in certain types of atrial arrhythmias and in aborting supraventricular tachycardias that depend on continuous reentry of impulses utilizing the AV node as part of the reentrant pathway (Singh *et al.* , 1980).

Verapamil does not influence intra-atrial or intraventricular impulse conduction since the normal myocardium relies upon the rapid sodium channels for depolarization and impulse conduction (Henry, 1980; McAllister, 1981; Karlsberg, 1982) while in the ventricular contractile cells the calcium ion movement contributes mostly to the plateau of the action potential (phase 2).

2.1.7. Therapeutic uses.

Verapamil is the drug of choice for abolishing acute episodes of paroxysmal supraventricular tachycardia due to A-V nodal reentry or due to anomalous A-V connections (Novotny and Adams, 1986). The drug is used for immediate reduction of ventricular response to atrial fibrillation or atrial flutter. Verapamil has been found to have significant effects on ventricular arrhythmias. Verapamil is useful in the

management of angina pectoris.

Verapamil has a potential use in preventing abortion and reducing uterine hypermotility. Studies done by Coruzzi *et al*, (1989) has indicated that calcium channel blockers are potent inhibitors of mare uterine motility *in vitro*.

2.1.8. Toxicity.

Adverse circulatory side effects of calcium channel blockade include bradycardia, various degrees of heart block, contractile depression of the heart, with reduced cardiac output and hypotension (Novotny and Adams, 1986). This combination of effects can result in decompensation of preclinical or compensated heart failure, precipitation of pulmonary oedema, and worsening of the primary ailment.

Side effects observed in humans treated with verapamil include constipation, headache, dizziness, vertigo, hypotension, fatigue, and AV block (Opie, 1980; Smith *et al.* , 1981; Karlsberg, 1982). Brodsky *et al.* , (1981), reported hepatotoxicity in one patient given verapamil.

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2.2. PROPRANOLOL.

2.2.1. Introduction.

The first drug to be shown to produce a selective blockade of ß-adrenergic receptors was dichloroisoproterenol (Powell and Slater, 1958). Studies with this drug made a substantial contribution to the understanding of the effects mediated by ß-receptors but it was not clinically used due to the fact that it had a prominent ß-receptor stimulant action, hence it is a partial agonist. Propranolol was the first ß-adrenergic antagonist to come into wide clinical use, and it remains the most important of these compounds. Propranolol is a highly potent, non-selective ß-adrenergic blocking agent with no intrinsic sympathomimetic activity.

Propranolol abolishes ventricular arrhythmias induced by digitalis due to effects both directly on the heart and, probably, on the central nervous system (Gillis, 1969). Koppes *et al.* (1980), in a study involving 32 patients two months after an uncomplicated acute myocardial infarction found that propranolol is effective in suppression of premature ventricular complexes, reducing both frequency and complexity. It is used in dogs and cats to slow the ventricular rate response to atrial fibrillation and flutter, owing to its depressant effects on the AV nodal conduction (Novotony and Adams, 1986). It is also used for suppressing supraventricular arrhythmias involving reentry pathways through the AV node. Other sympathetically mediated arrhythmias responsive to propranolol include atrial ectopic beats, paroxysmal

supraventricular tachycardia, and ventricular ectopy or tachycardia. Propranolol is very effective in supraventricular tachycardias and is an excellent prophylaxis for paroxysmal supraventricular tachycardias, including those associated with the Wolff-Parkinson-White syndrome (Shand, 1975). It is effective in abolishing inducible ventricular tachycardia or ventricular fibrillation in the dog after subacute myocardial infarction (Gang, *et al.* ,1984).

Propranolol has proved clinically useful in the treatment of supraventricular arrhythmias, including premature depolarizations and paroxysmal supraventricular or junctional tachycardia and ventricular arrhythmias (Gibson and Sowton, 1969; Singh and Jewitt, 1974; Shand, 1975). The clinical usefulness of propranolol is limited by its lack of β_1-adrenoceptor selectivity and the relatively short duration of pharmacologic effect (Muir and Sams, 1984).

2.2.2. Physical and chemical properties.

Propranolol is structurally related to its precursors: it is many times more potent than pronethalol. Propranolol and related β-antagonists have an isopropyl-substituted secondary amine on the carbon side chain a moiety which appears to be important for effective interaction with the β receptor (Fig. 2). The hydrochloride contains not less than 99% and not more than 101% $C_{16}H_{21}NO_2$,HCl (295.8 mol. wt) calculated with reference to the dried substance. It is a white odourless powder with a bitter taste which melts at about 163°C. It is

stable at pH 3 but decomposes rapidly at alkaline pH. The drug is soluble in water and alcohol (1 : 20), slightly soluble in chloroform and practically insoluble in ether.

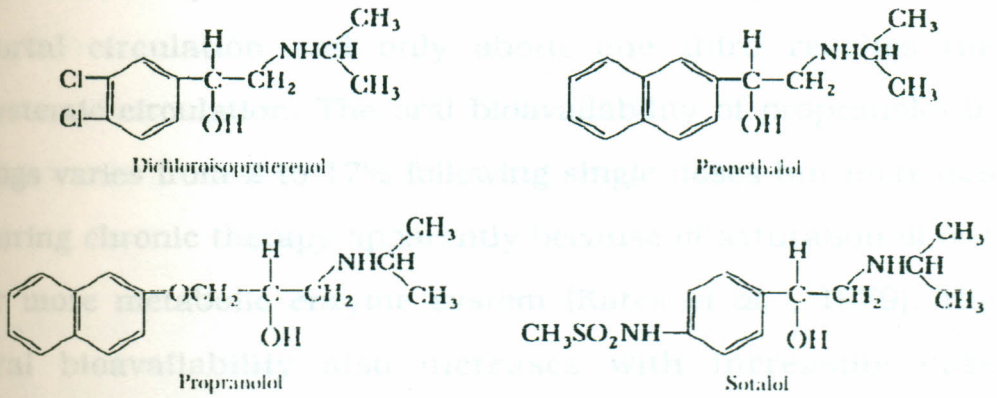


Fig. 2. Structural formulae of some ß-adrenergic blockers.

Propranolol injection is a sterile solution of propranolol hydrochloride in water containing citric acid. The content of propranolol hydrochloride is not less than 90% and not more than 110% of the stated amount and the solution has a pH of 3.0-3.5.

2.2.3. Preparation and routes of administration.

Propranolol hydrochloride (Inderal®, Anginol®, Berkolol®) is available in tablets containing 10, 40, 80 and 160 mg for oral administration, and in 1 ml. ampoules containing 1.0 mg for intravenous administration. It is also available in sustained release capsules containing 160 mg (Inderal® LA).

2.2.4. Pharmacokinetics.

Propranolol is almost completely absorbed following oral administration though much of the administered drug is metabolized by the liver during its first passage through the portal circulation and only about one third reaches the systemic circulation. The oral bioavailability of propranolol in dogs varies from 2 to 17% following single doses but increases during chronic therapy apparently because of saturation of one or more metabolic enzyme system (Kates *et al.* , 1979). The oral bioavailability also increases with increasing dose apparently due to enzyme saturation. The "first pass" elimination accounts for the low bioavailability of propranolol (Shand, 1975). The degree of hepatic extraction of propranolol becomes less as the dose is increased, suggesting that a saturation mechanism is involved (Evans *et al.*, 1973b).

Propranolol is bound to plasma proteins to the extent of 90-95% (Evans *et al.*, 1973a). The proteins are primarily α-1 acid glycoprotein and albumins. It is completely metabolized by oxidation and glucuronidation in the liver and possibly other tissues (Muir and Sams, 1984) before excretion in urine. One of the products of hepatic metabolism is 4-hyroxypropanolol which appears to exhibit ß-adrenergic blocking activity comparable to the parent compound but has a short half life. This metabolite is found in human plasma after oral , but not intravenous, administration of propranolol (Patterson *et al.*, 1970). In normal dogs 4-hyroxypropanolol has been detected in low concentrations considered

insignificant in plasma samples following both oral and intravenous administration (Muir and Sams, 1984) but may be noteworthy in disease states such as renal disorders in which the elimination of this metabolite is impaired. Other metabolites that have been identified in urine include naphthoxylactic acid, isopropylamine, and propranolol glycol. A considerable fraction of the metabolites of propranolol are apparently glucuronide conjugates (Shand, 1975).

The oxidative and conjugated metabolites are eliminated by the kidneys and in the bile. As propranolol has high lipophilicity it is extensively reabsorbed in the renal tubules hence renal elimination of the unchanged drug is unimportant.

2.2.5. Pharmacodynamics.

Though large doses of propranolol appear to exert a quinidine-like effect on the myocardium, which might contribute to the antiarrhythmic effect of the drug; the ß-adrenergic blocking activity of propranolol appears to be its major mechanism of action (Shand, 1975). Currently it is believed that ß-antagonists in therapeutic concentrations act primarily and perhaps exclusively by ß-adrenergic receptor blockade (Adams, 1986). Their local anaesthetic properties, evident in high concentrations, seems to be unimportant in acute control of cardiac arrhythmias (Adams, 1986). Gang, *et al.* (1984), explored possible mechanisms that result in timolol and propranolol reduction of the incidence of cardiac death after myocardial infarction compared the two ß-

adrenergic blockers in a dog model for inducible sustained ventricular tachycardia or fibrillation 4 to 6 days after experimental closed-chest myocardial infarction. They concluded that both drugs tended to elevate the ventricular fibrillation threshold and to prolong the ventricular effective refractory period without significantly altering the QT_c interval. This suggested a possible direct membrane depressant effect for both drugs. Propranolol produces clinical effects by occupying both β_1 and β_2 adrenergic receptors, thereby antagonizing the actions of catecholamines released either by an increased frequency of sympathetic nerve impulses from the central nervous system or by the medulla. The effects of propranolol are linked to the prevailing sympathetic tone and hence propranolol has most profound therapeutic effects in the presence of elevated sympathetic tone (Johnson and Regardh, 1976).

Propranolol reduces sinus rate and slows conduction in the atria and in the A-V node. The deceleration of AV conduction towards normal is an effect that can be useful particularly in controlling ventricular rate responses to supraventricular tachyarrhythmias such as atrial fibrillation and flutter (Adams, 1986). It also has significant effects on automaticity in cardiac Purkinje fibres. The increased firing rate of Purkinje fibres due to catecholamines is reversed by propranolol and slows the spontaneous firing.

The electrical threshold of the atria and ventricles of normal dogs is not much affected by propranolol, and the electrical threshold for ventricular fibrillation is not

consistently increased (Wallace *et al.* , 1966b). However, propranolol increases the threshold for fibrillation after experimental infarction (Gang *et al.* , 1984).

2.2.6. Therapeutic uses.

Propranolol is indicated for the management of both supraventricular and ventricular arrhythmias. Propranolol is preferred in the treatment of arrhythmias associated with phaeochromocytoma, which are due to catecholamine excess (Shand, 1975). It seems to be especially valuable in the management of arrhythmias associated with digitalis intoxication. Propranolol abolishes ventricular arrhythmias induced by digitalis by effects both directly on the heart and, probably, on the central nervous system (Gillis, 1969).

Propranolol is useful in the management of hypertension. It is also efficacious in the prophylaxis of angina pectoris as well as in the treatment of obstructive cardiomyopathy (Shand, 1975).

2.2.7. Toxicity.

The major dangers of therapy with propranolol are related to the ß-adrenergic blockade *per se*. Serious cardiac depression may develop suddenly or slowly, usually in patients whose hearts are severely compromised by disease or by other drugs such as anaesthetics. In heart failure reflex sympathetic

stimulation tends to maintain cardiac output by increasing both rate and force of myocardial contraction thus even a small degree of beta blockade may be sufficient to precipitate dramatic heart failure and life-threatening hypotension (Shand, 1975).

Propranolol can cause A-V dissociation and cardiac arrest in patients with pre existing partial heart block due to digitalis or other factors.

Another adverse reaction that has been noted is hypoglycemia especially in insulin-dependent diabetic patients (Shand, 1975; Novotny and Adams, 1986). Central nervous system side effects observed are fatigue, depression, insomnia, vivid dreams and hallucinations (Shand, 1975; Kincaid-Smith, 1984).

2.3. LIDOCAINE.

2.3.1. Introduction.

Lidocaine was introduced in 1948 as a local anesthetic and has since been widely used as such. It has also achieved prominence as an antiarrhythmic agent and is now in common use, particularly in emergency treatment, for ventricular arrhythmia that are encountered after cardiac infarction. It is a narrow-spectrum agent restricted clinically to intravenous administration in the control of life threatening ventricular arrhythmias. The antiarrhythmic action is established rapidly and safely by intravenous administration.

Lidocaine has proved to be partially useful in reverting ventricular tachyarrhythmias that develop during anaesthesia, surgery, ischaemia, gastric dilatation-volvulus complex, and other forms of trauma (Novotny and Adams, 1986).

2.3.2: Physical and Chemical properties.

Lidocaine, which is also known as Lignocaine, $C_{14}H_{22}N_2O$, (234.3 mol. wt.), is a white or slightly yellow crystalline powder with a melting point of 66° C to 69° C. It is practically insoluble in water but very soluble in ethanol, ether, and chloroform. The hydrochloride is also a white crystalline odourless powder with a melting point of 76° C to 79° C and it is very soluble in water (1 : 0.7), alcohol (1 : 1.5),

chloroform (1 : 40) and is insoluble in ether.

Lidocaine HCI Injection is a sterile aqueous solution. The content of lidocaine hydrochloride is not less than 95% and not more than 105% of the stated amounts. It is a clear colourless liquid with a pH of 5.0-7.0.

2.3.3. Preparations and routes of administration.

Preparations of lidocaine hydrochloride include injection which is available for intravenous and intramuscular administration in solutions containing 10 or 20 mg/ml in ampoules, vials, or prefilled syringes with or without epinephrine (1 : 50,000 to 1 : 200,000). There are also creams, ointments, jellies, topical solutions, and topical aerosols.

2.3.4. Pharmacokinetics.

Although lidocaine is well absorbed after oral administration, it is subject to extensive first-pass hepatic metabolism, and only about one third of the drug reaches systemic circulation. Thus after oral administration the concentrations of the drug in plasma are low and unpredictable. The drug is almost completely absorbed after intramuscular administration. About 70% of the drug is plasma bound to proteins, mostly α-1 acid glycoprotein.

Finster *et. al.* , (1972) reported that after lidocaine administration in pregnant guinea pigs it rapidly crosses the

placenta and high concentrations are found in the fetal liver, heart, and brain. Higher concentrations were found in the fetal liver than in the maternal one.

Lidocaine is metabolized primarily in the liver. The unchanged form is excreted in urine of the dog in concentrations of 10-20% while in humans essentially no lidocaine is excreted unchanged in the urine. Two metabolites have been identified in the dog from hepatic N-deethylation of lidocaine (Wilcke *et al.* , 1983a). The metabolites are monoethylglycinexylidide and glycine xylidide (Fig. 3). Burney *et al.* (1974) reported that the metabolite monoethylglycinexylidide has antiarrhythmic activity and recommended that its presence should be considered in clinical evaluation of lidocaine therapy for arrhythmias. Concentrations of the two metabolites, determined following the administration of lidocaine suggest that monoethylglycinexylidide is eliminated rapidly while glycinexylidide is more slowly eliminated (Wilcke *et al.* , 1983a).

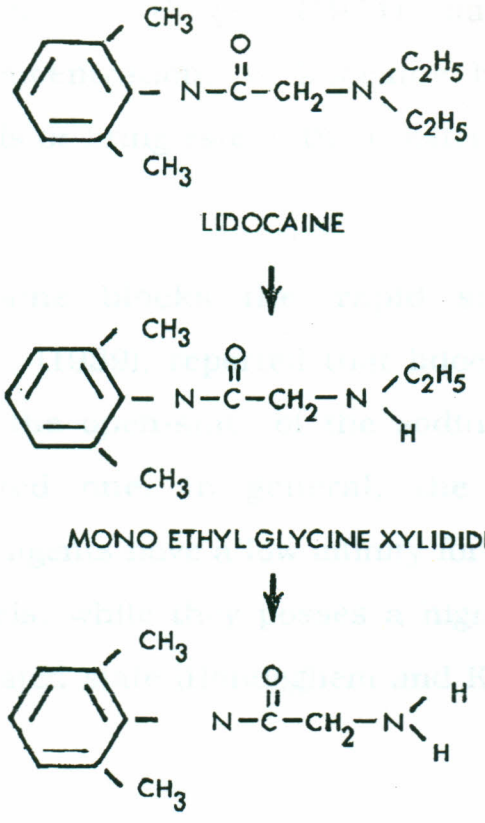


Fig. 3. Lidocaine metabolites (Burney *et al.* ,1974)

2.3.5. Pharmacodynamics.

Lidocaine exerts most of its electrophysiological effects on the heart by a direct action. It has little effect on either refractoriness or responsiveness in the atria a fact that makes it much less effective than quinidine or procainamide in slowing atrial fibrillation or atrial flutter or in converting these arrhythmias to sinus rhythm. It can abolish ventricular reentry, either causing a two-way block or by improving conduction. Gerstenblith *et al.* , (1972), reported that lidocaine increases the threshold for ventricular fibrillation.

Mandel and Bigger, (1971), have reported that therapeutic concentrations of lidocaine have no effect on action potentials or firing rate of the isolated sinus node of the rabbit.

Lidocaine blocks the 'rapid sodium channels'. Inomata *et al.* , (1989), reported that lidocaine preferentially interacts with the open-state of the sodium channel rather than the rested one. In general, the clinically useful antiarrhythmic agents have a low affinity for the rested state of sodium channels, while they posses a higher affinity for the open or inactivated state (Hondeghem and Katzung, 1984).

2.3.6. Therapeutic uses.

Lidocaine has a narrow antiarrhythmic spectrum. It is used almost exclusively to treat ventricular arrhythmias, primarily in intensive care units. Lidocaine is effective against ventricular arrhythmias caused by acute myocardial infarction, open heart surgery, digitalis, gastric dilatation-volvulus complex (Novotny and Adams, 1986). The use of lidocaine as a local anaesthetic in human, veterinary and dental medicine is considered to be outside the scope of the present review.

2.3.7. Toxicity.

The most important signs of lidocaine toxicosis involve excitation of the central nervous system, leading to

seizures. Boyes *et al.* , (1970), reported that lidocaine caused vomiting regularly at about 2.5 hours after administration in the dog. Other side effects are skeletal muscle fasciculations, disorientation, and occasional exacerbation of the ventricular arrhythmias (Novotny and Adams, 1986). Wilcke *et al.* , (1983b) have reported the signs as anxiety, vocalization, mild sedation, vomiting and defaecation with the earliest consistent sign being the appearance of tonic muscular extension. In humans these effects include focal and grand mal seizures, psychoses, drowsiness, decreased hearing, paraesthesias, disorientation, muscle twitching and rarely, respiratory arrest (Wilcke *et al.* , 1983b)

In experimental animals, overdosage of lidocaine produces death from ventricular fibrillation or cardiac arrest.

2.4. Classification of antiarrhythmic drugs.

The classification system suggested by Vaughan Williams and other workers (1984) has become the standard nomenclature model for antiarrhythmic drugs. This system is based on the observation that most of the clinically useful antiarrhythmic drugs have one predominant effect on the cardiac action potential. The antiarrhythmic drugs are hence classified into four classes (Table 1).

Class I

The dominant electrophysiologic action of Class I agents is to slow the maximal rate of Phase O depolarization of the cardiac action potential, an action due to the direct "membrane stabilizing" action owing to the ability of these agents to selectively block the fast sodium channels of the cell membrane. Harrison *et al.* , (1981), has placed the Class I agents into 3 subdivisions IA, IB, and IC due to the existence of some important dissimilarities relative to their precise effects on Phase O depolarization in the normal and abnormal cells, action potential duration and duration of refractoriness.

Class IA: The distinguishing feature of this class of drugs (Table 1) is a consistent reduction of the rate of Phase O depolarization in normal and injured cardiac cells. The drugs also uniformly prolong the cardiac action potential duration.

Class IB: These drugs (Table 1) reduce Phase O depolarization and conduction velocity in injured cardiac tissues but not in normal cells. They also have a minimal shortening effect on

normal action potential duration and refractory period.

Class IC: The drugs markedly depress the maximal rate of Phase O depolarization and conduction velocity in normal as well as abnormal cardiac cells but they exert little effect on refractoriness and action potential duration.

Class II.

These are those drugs that inhibit sympathoadrenal excitation of the heart. The clinically useful drugs in this class currently comprise only the beta-receptor antagonists.

Class III.

These are drugs that cause a rather specific prolongation of the action potential duration, thereby extending the refractory period. This effect is without significant change either in Phase 4 resting potential or Phase O depolarization.

Class IV.

These are drugs that block the calcium channels in cells.

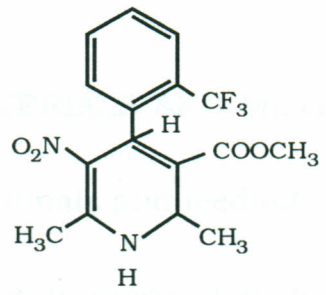
Table 1. Some of the antiarrhythmic drugs and their classification.

Class	Drugs
IA	Quinidine, procainamide, disopyramide.
IB	Lidocaine, phenytoin, tocainide, aprindine, mexiletine, ethmozin.
IC	Encainide, flecainide, lorcainide.
II	Propranolol, metoprolol, timolol, pindolol, alprenolol, nadolol, sotalol, atenolol, bunolol, bupranolol, bunitrolol, bufetolol, bufuralol, metipranolol, nifenalol, oxprenolol.
III	Bretylium, amiodarone.
IV	Verapamil, methoxyverapamil, nifedipine, diltiazem, nicardipine, perhexiline, lidoflazine, gallopamil, nitrendipine, nimodipine, flunarizine, bepridil, prenylamine, fendiline, terodiline, cinnarizine.

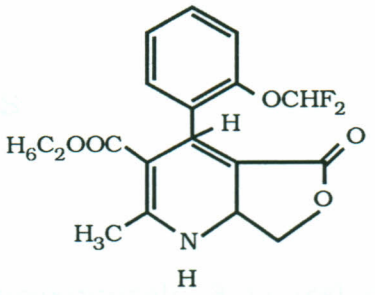
2.5. Calcium agonists.

These are dihydropyridines derivatives that increase the probability of calcium channels opening instead of blocking them. They increase calcium influx through voltage-

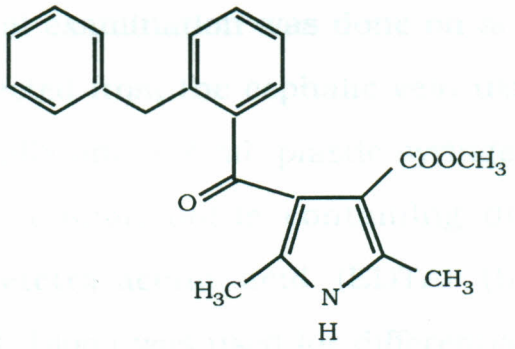
operated channels by interacting with specific dihydropyridine binding sites on the channels (Schram *et al.* , 1983). Examples of these compounds are BAY K8644 and CGP 28392 (Fig. 4). McKechnie *et al.* (1989), have reported that FPD 64176, a benzoyl pyrrole, as being a calcium channel activator with properties which are compatible with activation of voltage-operated channels.



BAY K 8644



CGP 28392



FPD 64176

Fig. 4. Structural formulae of some calcium agonists.

CHAPTER 3

EFFECTS OF ANTIARRHYTHMIC DRUGS (VERAPAMIL, PROPRANOLOL, AND LIDOCAINE) ON ELECROCARDIOGRAM AND HAEMATOLOGY IN INDUCED ARRHYTHMIAS IN DOGS.

3.1. MATERIALS AND METHODS

3.1.1. Animals and feeding.

Adult mongrel dogs (approximately 3 years) of either sex obtained from the Nairobi area were used in the study. A complete physical examination was done on acquisition. Blood (2 ml.) was collected from the cephalic vein using a 1 in. x 19 g disposable needle and a 2 ml. plastic syringe. The blood was transfered into a bijou bottle containing disodium salt of ethylenediaminetetra-acetic acid (EDTA) (BDH Chemicals Ltd., Poole, U K). Blood was used for differential cell count and blood parasite screening. The dogs were dipwashed against external parasite using formamidine (Welcare®, Wellcome Kenya Ltd. Nairobi) and dewormed against internal parasites using pyrantel pamoate (Canex®, Pfizer Inc. New York, USA.) at a dose of 14.3 mg/kg bwt. before initiation of the study. The dogs were then acclimatized for a period of at least one week before the start of the experiment.

The dogs were housed individually in the dog kennels which were cleaned every morning during the course of the

experiment. The dogs were provided with commercial dog food (Besbix®, Dog Meal, Proctar and Allan, Nairobi, Kenya) once every day at 12.00 noon and offered clean drinking water *ad libitum*. The dogs were put to sleep 3 days after the last blood sample was collected.

3.1.2. Induction of anaesthesia.

The dogs were examined and only clinically healthy dogs were used in the experiment. Anaesthesia was induced intravenously using thiopentone sodium (Intraval®, May and Baker Ltd.,Dagenhan, U. K.) at a dose of 10 mg/kg bwt. The cephalic vein was used. The animals were intubated and maintained under anaesthesia using a semi- closed circuit mixture halothane (Fluothane®, Imperial Chemical Industries, Chesire, U. K.) and oxygen (East African Oxygen Ltd., Nairobi, Kenya.).

3.1.3. Drug pre-treatments and induction of arrhythmias.

The dogs were randomly divided into 4 groups of 5 dogs each (n=5). The jugular vein area was then shaved and disinfected with spirit. All drugs mentioned below were administered intravenously using the left jugular vein. Group 1 (control) received physiological saline; group 2 received verapamil at a dose of 0.1 mg/kg bwt. (Isoptin®, Knoll AG. D-6700, Ludwigshafen, Germany.); group 3 received propranolol at a dose of 0.06 mg/kg bwt. (Inderal Injection®, Imperial

Chemical Industries PLC, Macclesfield, Chesire, U. K.) and group 4 lidocaine at a dose of 4 mg/kg bwt. (Lignomed Injection®, Regal Pharmaceuticals Ltd, Nairobi, Kenya.). All the dogs were then injected with adrenaline at a dose of 4 µg/kg bwt. 10 minutes after pre-treatment with the respective drug and prior sensitization of the heart to adrenaline by halothane.

3.1.4.1. ECG Parameter Measurements.

Each dog was placed on right lateral recumbency and held with the humeri and femora at right angles to its body and parallel to each other as described by Ettinger and Suter, (1970). The hair at the elbow and stifle joints was clipped, Kenz ECG cream applied and the ECG electrodes attached using crocodile clips. An ECG recording was done on lead II using heat sensitive paper at a recording speed of 50 mm/sec. and sensitivity of 10 mm/mV on a Kenz-ECG-103 (Suzuken Co., Ltd., Japan).

ECG recording using lead II was done on every dog before drug pretreatment; after drug treatment; then after adrenaline administration and finally 30 minutes later as the animal was recovering from anaesthesia.

The ECG recording was used to obtain the following parameters:- heart rhythm, arrhythmias/minute, heart rate, P wave height (mV), P wave width (sec), P-R interval (sec), QRS complex width (sec), R wave height (mV), S-T intervals (sec)

and depression or elevation (mV), T wave height (mV), and Q-T interval (sec).

3.1.4.2. Calculation of ECG Parameters.

At the sensitivity used of 10 mm/mV every small square height is equal to 0.1mV the height of wave was obtained by counting the number of small squares from the baseline to the peak and multiplying by 0.1 mV and since at a paper speed of 50 mm/sec. each small square on the horizontal axis is equivalent to 0.02 sec. the width of the waves and the intervals were obtained by counting the number of the small squares and multiplying by 0.02 sec.

3.1.5. Blood collection.

Blood was collected before, during, and after the ECG measurements from the jugular vein at the following time intervals: 0 hr; 1/4 hr; 1/2 hr; 1 hr; 2 hr; 4 hr; 8 hr; 12 hr; 24 hr; 48 hr; and 72 hr using a 10 ml. syringe fitted to a 19 g needle. Two millilitres of blood was put into bijou bottles containing EDTA for haematology. Blood was kept in the refrigerator at 4°C pending the various haematologic analyses.

3.1.6. Haematologic parameters.

Blood (2 ml.) for haematologic parameters - red blood

cells (RBC), white blood cells (WBC), haemoglobin (Hb), total protein (TP), packed cell volume (PCV), mean cell volume (MCV) and differential cell count - collected in the bijou bottles containing EDTA was mixed by gentle shaking. WBC, RBC, PCV, Hb, and MCV were determined using routine clinical pathology procedure (Schalm *et al.* , 1975) employing a Coulter Counter model ZM (Coulter Electronic, Inc. , Hialeah, Florida). Absolute differential count was performed on a smear stained using the May-Grunwald giemsa method. A standard 100 cells were counted and expressed as a percentage.

The microhaematocrit method described by Dacie and Lewis (1968) was used. Commercially available unheparinized (plain) capillary tubes 75 mm in length and an internal diameter of 1.3-1.5 mm were used. The tubes were filled with the uncoagulated blood until about three quarters of each tube was full of blood. The dry end of the tubes were then sealed using plasticin. The tubes were span at 9,300 x *g* for 5 minutes in a microcentrifuge (Haemofuge®, Heraeus Christ, GmBH, W. Germany.). Packed cell volume (microhaematocrit) percent was determined from the scale of Hawksley microhaematocrit reader (Hawhsley and sons Ltd. London). The buffy coat layer was not included in the reading. The plasma part was used in the determination of the total proteins. The total protein was determined using a refractometer (Atago®, SPR-T2, Japan).

3.1.7. Clinical observation.

The dogs were clinically examined for 72 hours during which temperature, pulse, respiration and appetite were recorded. Any unusual clinical signs were observed.

3.1.8. Data analysis.

Statistical analysis of data was done by analysis of variance (ANOVA) (Daniel, 1983) using an IBM computer with a panacea statistical programme. Turkey's highest significant difference (HSD) test (Daniel, 1983) was used to determine if there was a significant difference in the group means at 5% level of significance. The test statistic used in this case was:

$$HSD^* = q_{\alpha,\kappa,N-\kappa}\sqrt{\frac{MSE}{n^*_j}}$$

where

α = chosen level of significance

k = number of group means

N= total number of observations

n= number of observations in a treatment

MSE = mean square error from the ANOVA table

q = obtained by entering a HSD statistic table

n^*_j = the smallest of the two sample sizes associated with the two sample means that are to be compared

3.2. RESULTS.

3.2.1. Clinical observation.

All the dogs had good appetite throughout the 3 days of experimental period except one which had displayed muscle tremors on recovery from anaesthesia. Temperature remained between 37.9° C and 39.0° C. Three dogs in the control group died when adrenaline was administered whereas in pretreated dogs no deaths were observed.

3.2.2. Arrhythmias

The predominant arrhythmia that occured was ventricular premature complex (Fig. 5) which preceded ventricular fibrillation in the dogs that died in the control group. The other type of arrhythmia that occured was 2nd degree heart block (Fig. 6). In the control group the first arrhythmia occured after 24±5.2 seconds (20-33 sec.); for lidocaine 37.6±25.19 seconds (13-72 sec.); propranolol 25±7.39 seconds (18-34 sec.) and verapamil 17.25±4.5 seconds (15-24 sec.). There was no statistical difference in the time of onset for the first arrhythmia ($p>0.05$).

All the dogs in the control showed arrhythmias occuring. In the dogs pretreated with verapamil one dog had no arrhythmias occuring, another one had only one isolated 2nd degree heart block occuring while the remaining 3 dogs had premature ventricular complexes (Fig. 5) occuring for a short duration of time. In the propranolol pretreated group, one dog did not develop any arrhythmias on adrenaline

administration while the rest developed arrhythmias. There was one dog that developed intermittent 2nd degree heart block, another one had 2nd degree heart block that proceeded on to ventricular premature complexes while 2 of the dogs in this group only developed ventricular complexes. On the other hand all the dogs pretreated with lidocaine developed ventricular premature complexes which were paroxysmal in one dog. On the basis of occurrence and severity of the arrhythmias verapamil followed by propranolol were better than lidocaine in prevention of arrhythmias.

4.2.3. ECG Parameters.

4.2.3.1. Heart rate.

Verapamil and lidocaine treated dogs showed a significant increase in heart rate compared to the control dogs following administration of adrenaline ($p<0.05$). There was a significant difference between lidocaine and propranolol treated groups ($p<0.05$). The heart rate in the propranolol treated dogs and control dogs decreased following adrenaline administration (Fig. 7).

3.2.3.2. P wave.

Lidocaine pretreated dogs' mean of 0.277±0.11 mV was different ($p<0.05$) from that of the control dogs' of 0.148±0.09 mV and verapamil dogs' of 0.195±0.08 mV, however, the values were all within the normal range. There was no apparent differences in the duration of the P wave ($p>0.05$).

3.2.3.3. P-R interval.

Propranolol was associated with an apparent increase in the P-R interval on adrenaline administration even greater than that in the control group. On the other hand verapamil and lidocaine were able to decrease the P-R interval due to adrenaline (Fig. 8). These were however, found not to be statistically significant ($p>0.05$).

3.2.3.4. QRS complex and R wave.

The QRS complex duration was not apparently affected by the drugs under investigation but all the drugs caused a slight increase in the R wave millivoltage on adrenaline administration (Fig. 9). The increase being more apparent with propranolol pre-treated dogs. However, statistical significance was found to be between lidocaine and control; lidocaine and verapamil treated groups ($p<0.05$).

3.2.3.5. S-T duration.

Propranolol caused a reduction in the S-T duration but on adrenaline administration the S-T duration increased back to original. In the group receiving verapamil there was no change in the S-T duration on administration of adrenaline. Lidocaine was apparently more effective in reduction of the S-T duration on administration of adrenaline (Fig. 10) with a statistical significant difference being found between lidocaine and propranolol treated groups ($p<0.05$).

3.2.3.6. S-T segment depression and elevation.

Verapamil caused an apparent increase in the S-T depression whereas propranolol and lidocaine caused a decrease. On adrenaline administration there was an apparent reduction in the depression in the case of verapamil but not with lidocaine, that is, lidocaine was apparently able to prevent the reduction in S-T depression due to adrenaline. Lidocaine also caused an apparent reduction in the S-T elevation.

3.2.3.7. T wave.

All the drugs were able to prevent increase of the T wave millivoltage (Fig. 11) on adrenaline administration with propranolol being more effective followed by verapamil and lidocaine apparently being the least effective (Fig. 12), however, they were found not be significant ($p>0.05$). The increase in the control dogs' mean of 0.159±0.05 mV after saline to 0.433±0.09 mV after administration of adrenaline was statistically significant ($p<0.05$).

3.2.3.8. Q-T interval (sec).

Adrenaline caused a slight decrease in the Q-T interval an action that seemed to have been counteracted by all the drugs (Fig.13). Significant difference was found between verapamil and lidocaine in relation to the control. A significant difference was also found between lidocaine and propranolol treated groups ($p<0.05$).

3.3. Haematologic parameters

Changes were noticed in the haematologic parameters in the first eight hours. On administration of adrenaline in the control group there was an initial decrease of PCV from 38% to 36% in the first half hour before increasing to a maximum of 45% two hours later (Table 2). No major changes occured in the dogs pretreated with the respective drugs though there was a significant difference between the verapamil and lidocaine treated groups ($p<0.05$).

The control group had an apparent steady increase in RBC from 6.42 $x10^6$ at 15 minutes to 7.67 x 10^6 at 2 hours. This effect seem to have been counteracted by all the drugs with significant differences between control and verapamil, verapamil and propranolol, and verapamil and lidocaine ($p<0.05$) (Table 3).

There was an apparent steady increase in the total leucocyte count in the control group from 8,800 at 15 minutes to 16,500 at 8 hours. A similar pattern of increase occured to a lesser extent with the drug treated animals but only slightly in case of lidocaine treated group which was statistically significant ($p<0.05$) (Table 4). The leucocyte count for the lidocaine treated group was significantly less than that of verapamil ($p<0.05$).

The control group had a drop in the percentage total neutrophils from 75% at 0 hour to 63.5% at 1 hour before increasing to a maximum of 81% at 8 hours (Table 5). A

similar increase occurred for propranolol without the initial drop with a maximum at 4 hours while only slight increases occurred in case of verapamil and lidocaine. Analysis of variance showed that the various treatments had a very significant effect on total neutrophils ($p<0.05$) which was found to be due to the variation between the control and propranolol. The neutrophil percentage was higher ($p<0.05$) in the verapamil group than in the propranolol group.

There was a slight increase in the mean lymphocyte percentage of the control group from 0 hour to 1 hour after which there was a decrease from 34% to 17% at 8 hours. There was also a decrease but to a lesser extent in case of verapamil and propranolol treated groups. There was no apparent decrease in the lidocaine treated group, however, there was an increase from 4 hours to a peak at 24 hours.

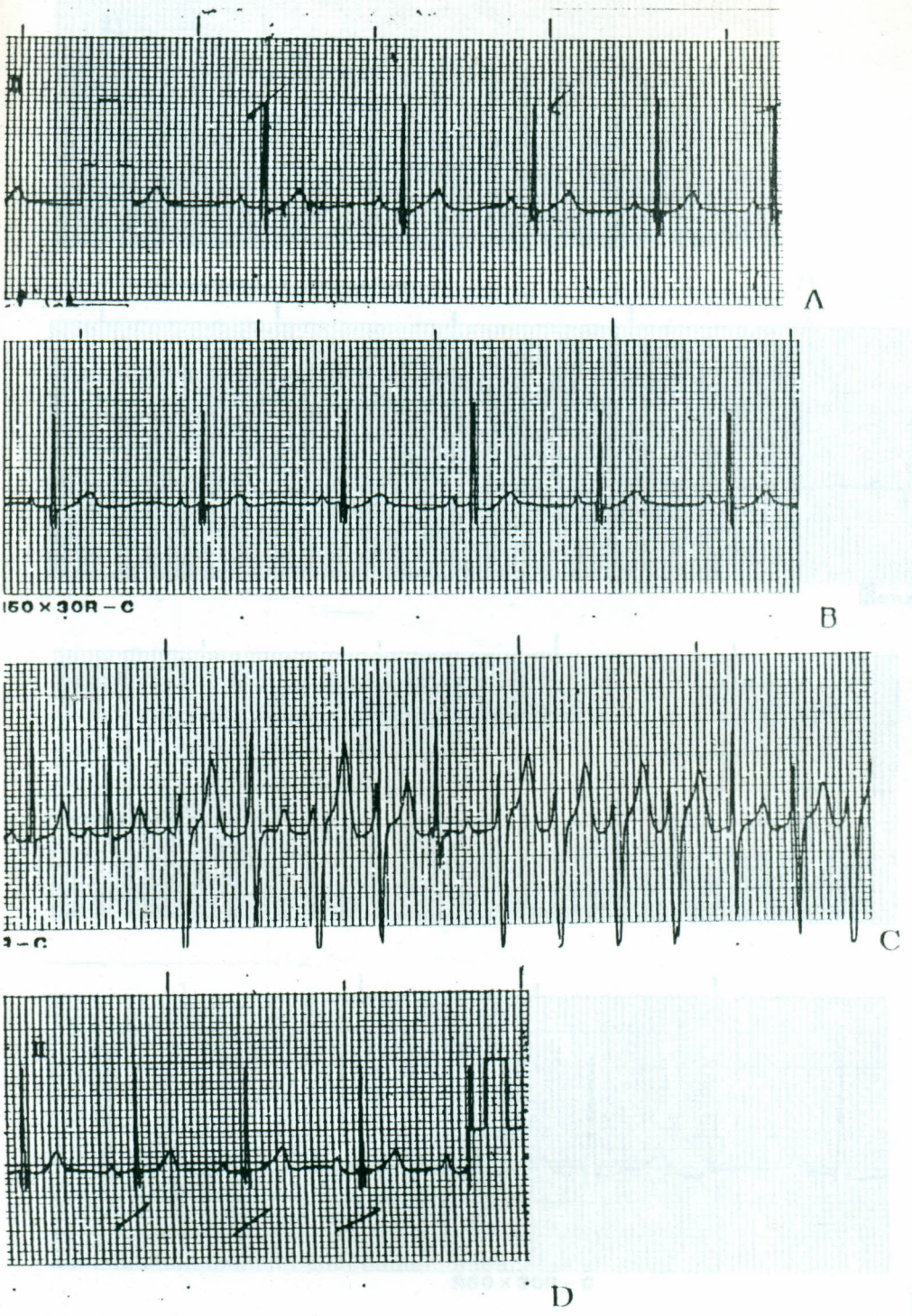


Fig. 5 ECG scan (A) showing the electrocardiographic complexes before any drug administration, (B) after administration of physiological saline, (C) ventricular tachycardia, (D) normal sinus rhythm on recovery from anaesthesia (Dog no. A1C). Recording lead II, Paper speed 50 mm/sec., and sensitivity 1 mm= 0.1 mV

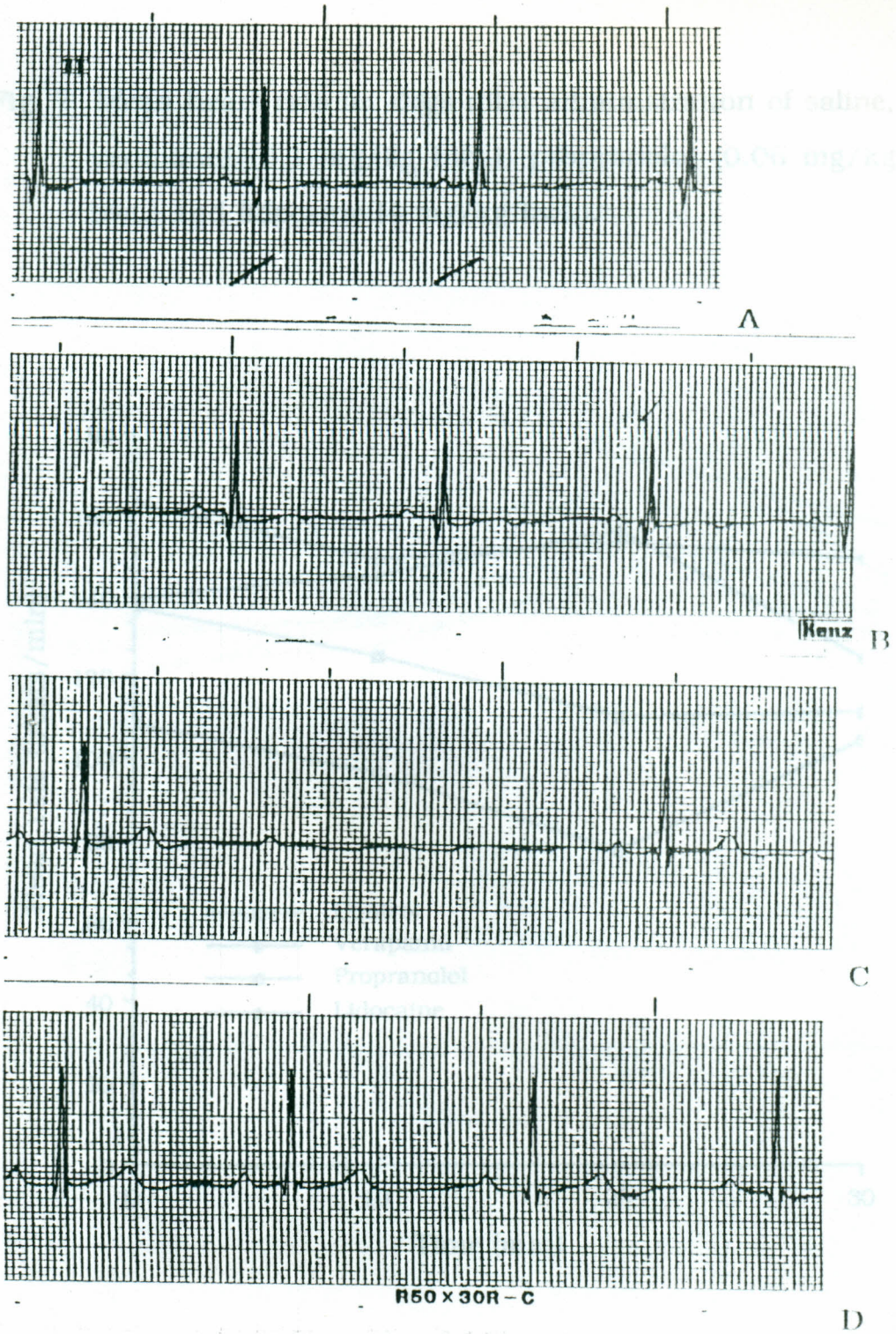


Fig. 6 ECG scan (A) normal sinus rhythm before any drug treatment, (B) after physiological saline, (C) 2nd degree heart block after adrenaline administration, (D) normal sinus rhythm 30 minutes later. Recording lead II, Paper speed 50 mm/sec., and sensitivity 1 mm= 0.1 mV

Fig. 7 Mean heart rate for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.06 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

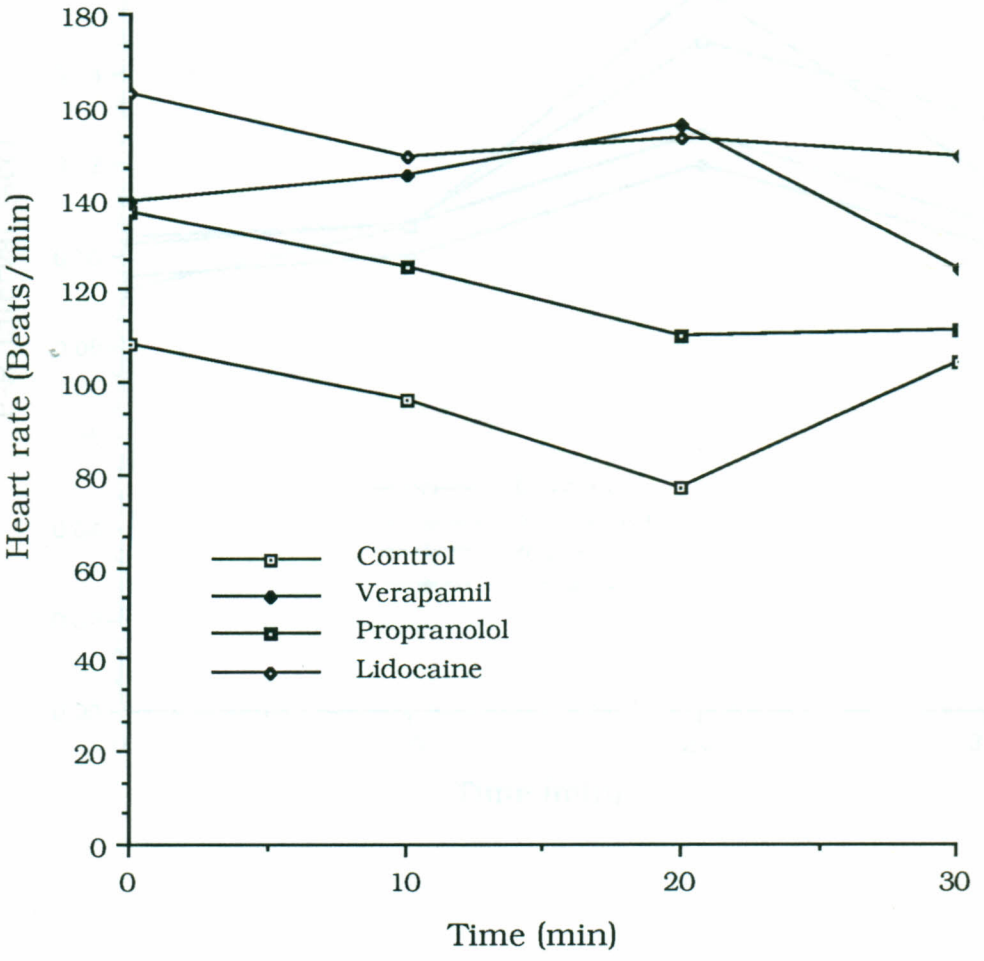


Fig. 8 Mean P-R interval for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.06 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

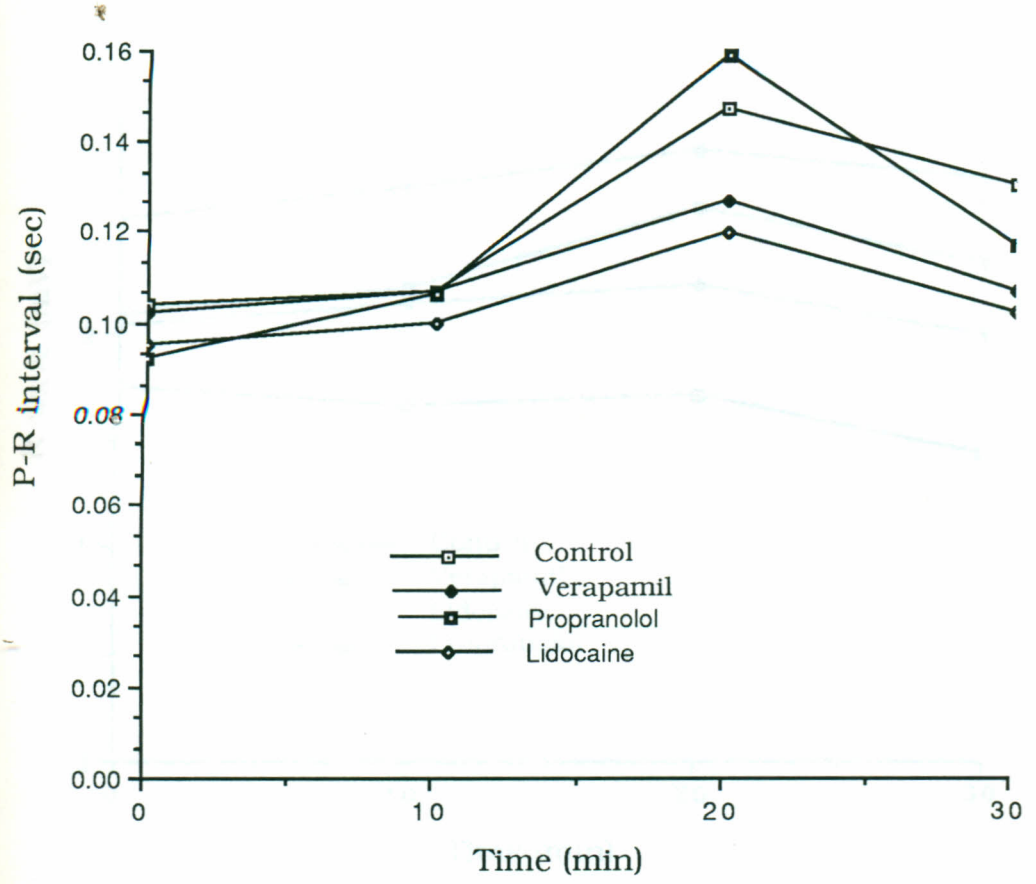


Fig. 9 Mean R wave millivotage for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.06 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

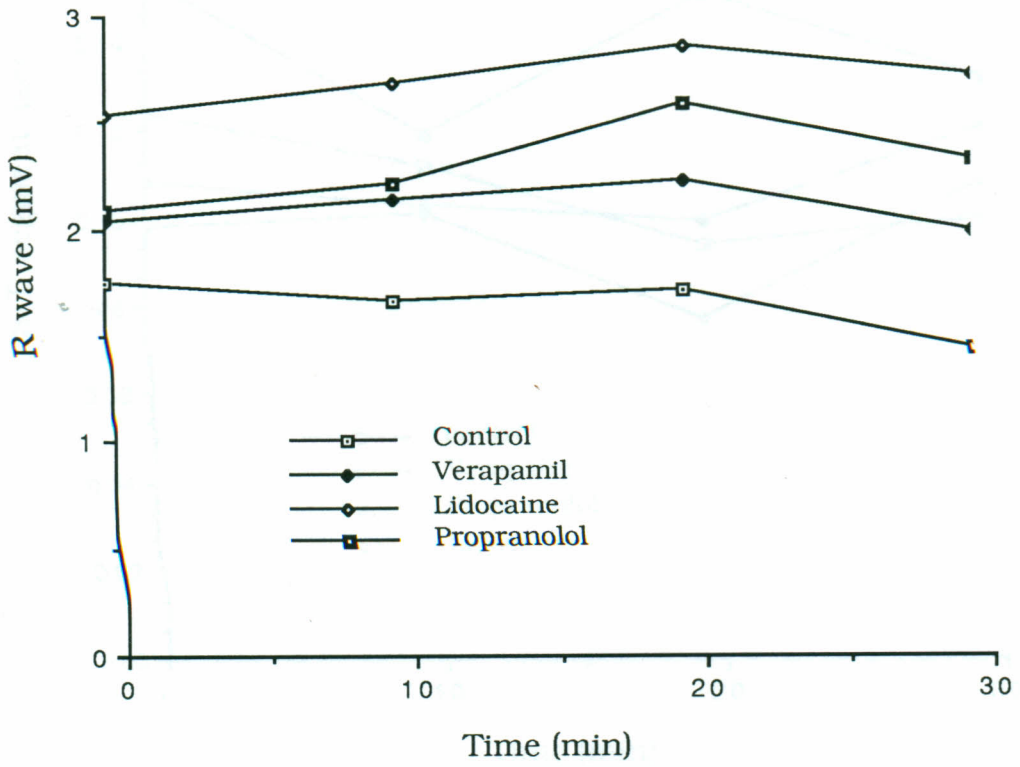
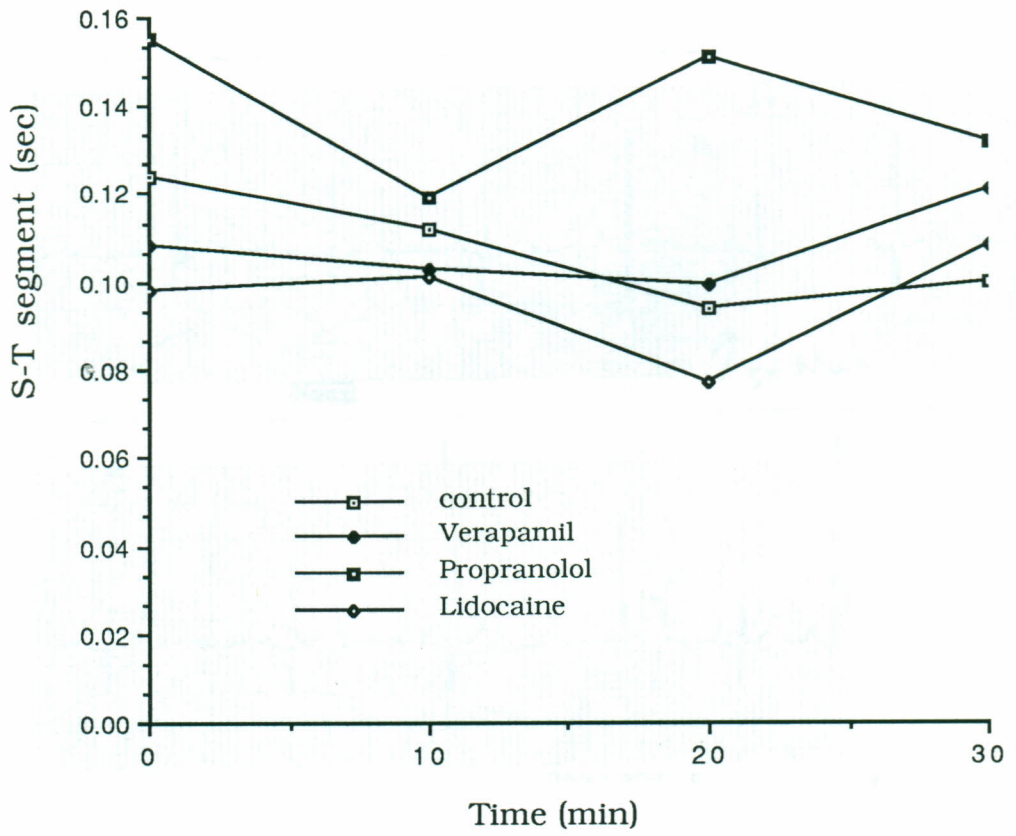


Fig.10 Mean S-T segment duration for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.06 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).



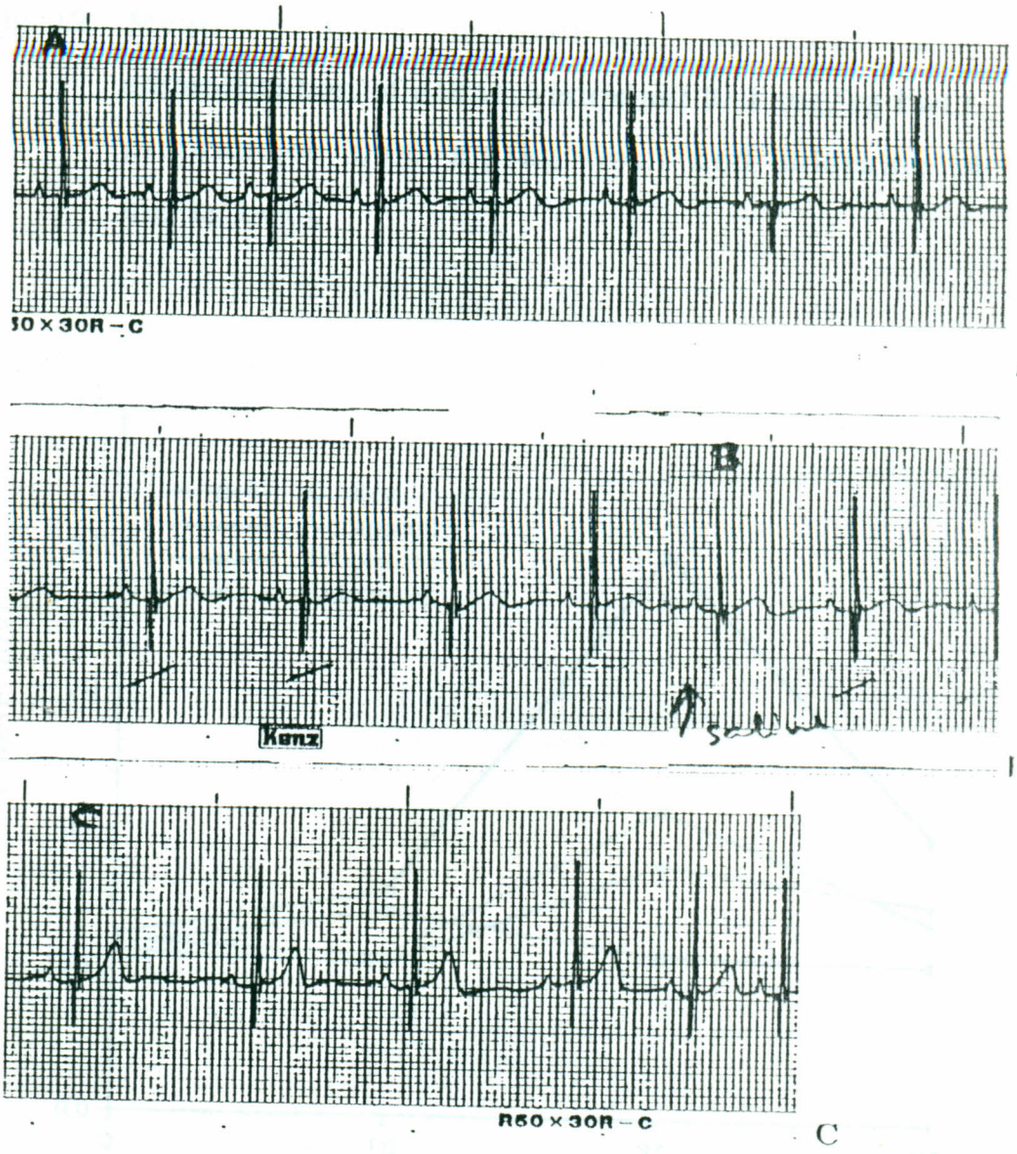


Fig. 11. ECG scan showing (A) T wave before any drug administration, (B) after administration of physiological saline and (C) increased T wave after adrenaline administration (Dog no. A1B). Recording lead II, Paper speed 50 mm/sec., and sensitivity 1 mm= 0.1 mV.

Fig. 12 Mean T wave millivoltage for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.06 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

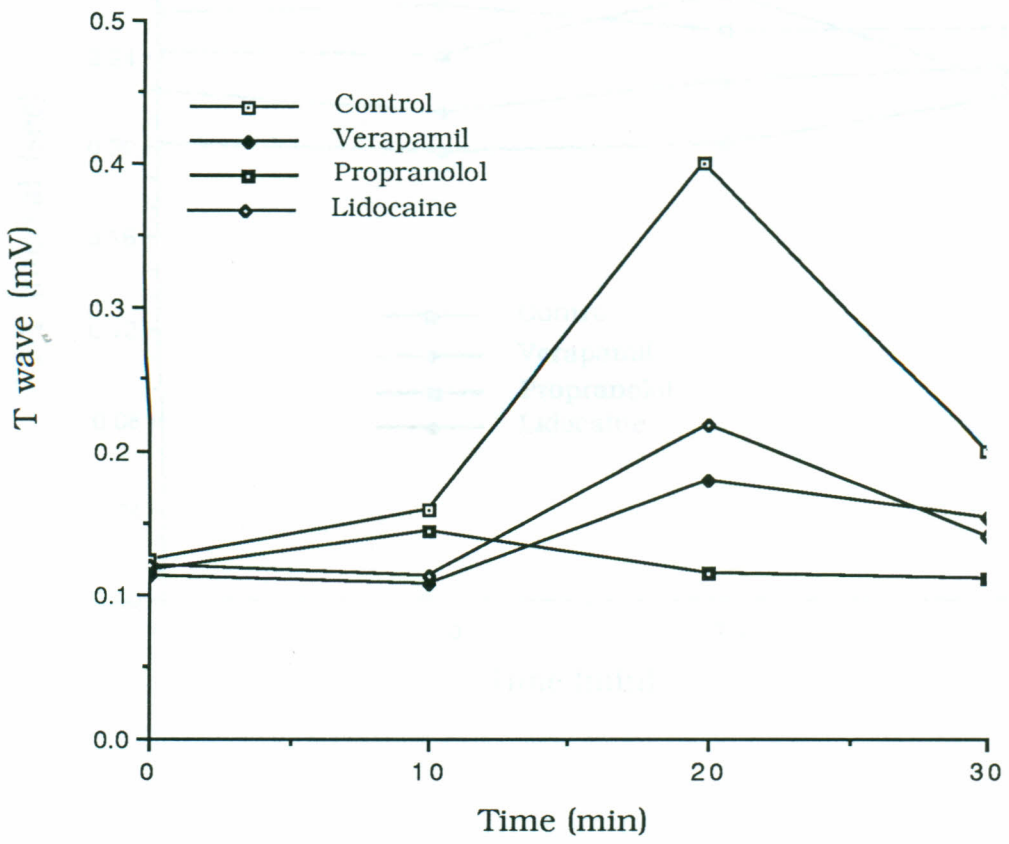


Fig. 13 Mean Q-T interval for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.06 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

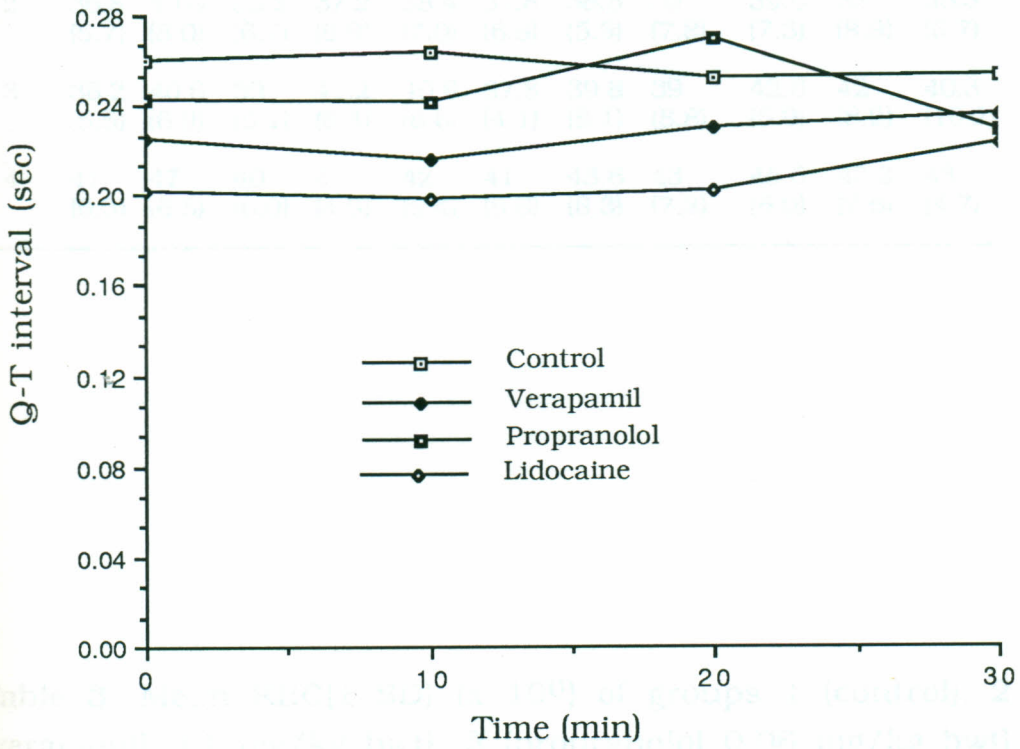


Table 2. Mean PCV (± SD) of groups 1 (control), 2 (verapamil 0.1 mg/kg bwt), 3 (propranolol 0.06 mg/kg bwt) and 4 (lidocaine 4 mg/kg bwt).

	Time of study (hours)										
Group	0	1/4	1/2	1	2	4	8	12	24	48	72
1	38 (9.9)	37 (7.1)	36 (8.5)	42 (9.2)	45 (5.7)	38 (9.9)	43 (7.1)	40 (11.3)	38 (2.8)	39 (5.7)	39 (8.5)
2	36.8 (5.7)	40.6 (8.0)	35.2 (6.7)	37.2 (6.9)	38.4 (7.0)	37.8 (6.8)	38.8 (5.9)	39 (7.8)	39.6 (7.3)	38 (8.9)	35.3 (6.7)
3	36.2 (5.5)	40.6 (6.9)	39 (6.1)	41.2 (6.4)	40.6 (6.6)	37.8 (4.1)	39.8 (8.1)	39 (8.8)	42.6 (5.9)	42 (8.2)	40.3 (10.2)
4	41 (6.3)	47 (6.5)	40 (6.0)	41 (4.9)	42 (9.4)	41 (9.8)	43.6 (8.3)	43 (7.2)	45.5 (6.0)	42.2 (7.6)	43 (4.7)

Table 3. Mean RBC(± SD) (x 10^6) of groups 1 (control), 2 (verapamil 0.1 mg/kg bwt), 3 (propranolol 0.06 mg/kg bwt) and 4 (lidocaine 4 mg/kg bwt).

	Time of study (hours)										
Group	0	1/4	1/2	1	2	4	8	12	24	48	72
1	6.43 (1.00)	6.42 (0.74)	6.98 (1.70)	7.20 (1.21)	7.67 (0.69)	6.88 (0.34)	7.56 (1.03)	7.28 (0.69)	7.10 (0.21)	7.20 (0.79)	7.17 (1.39)
2	5.81 (1.14)	6.65 (1.4)	5.58 (0.94)	6.00 (1.09)	6.40 (1.18)	6.25 (1.00)	6.35 (1.18)	6.19 (1.54)	6.21 (1.32)	6.06 (1.43)	5.97 (1.3)
3	6.20 (0.78)	7.09 (1.27)	6.57 (1.19)	7.14 (1.25)	7.07 (1.09)	6.34 (0.76)	7.06 (1.27)	6.89 (1.15)	7.37 (0.78)	7.46 (1.67)	7.02 (1.15)
4	6.43 (1.26)	7.73 (1.05)	6.30 (1.03)	6.75 (1.34)	7.16 (1.59)	7.11 (1.74)	7.21 (1.94)	6.83 (1.42)	7.65 (1.34)	6.96 (1.52)	6.91 (0.73)

Table 4. Mean WBC (± SD) (x 10^3)of groups 1 (control), 2 (verapamil 0.1 mg/kg bwt), 3 (propranolol 0.06 mg/kg bwt) and 4 (lidocaine 4 mg/kg bwt).

	Time of study (hours)										
Group	0	1/4	1/2	1	2	4	8	12	24	48	72
1	9.05 (4.17)	8.8 (3.39)	10.85 (7.28)	10.80 (5.94)	12.10 (6.36)	13.15 (6.43)	16.50 (8.34)	15.30 (6.22)	13.15 (5.57	11.70 (2.83)	12.05 (5.05)
2	6.98 (2.42)	8.44 (2.65)	7.20 (2.74)	8.82 (4.29)	10.38 (2.90)	9.36 (3.73)	12.56 (0.34)	13.35 (4.64)	10.38 (2.24)	10.12 (6.13	11.88 (3.75)
3	7.20 (3.40)	8.22 (4.08)	8.25 (3.10)	9.56 (4.43)	9.92 (3.68)	10.24 (3.12)	12.73 (5.54)	9.78 (3.97)	10.42 (3.54)	10.04 (2.72)	10.03 (2.42)
4	6.54 (0.87)	8.36 (1.85)	6.40 (0.86)	7.22 (1.57)	8.24 (1.07)	8.16 (2.16)	8.96 (2.92)	9.03 (2.12)	9.55 (2.98)	7.84 (1.43)	8.23 (0.70)

Table 5. Mean TN (± SD) (%) of groups 1 (control), 2 (verapamil 0.1 mg/kg bwt), 3 (propranolol 0.06 mg/kg bwt) and 4 (lidocaine 4 mg/kg bwt).

	Time of study (hours)										
Group	0	1/4	1/2	1	2	4	8	12	24	48	72
1	75 (4.2)	67 (5.7)	66.5 (2.1)	63.5 (2.1)	66.5 (2.1)	76.5 (2.1)	81 (0)	75 (4.2)	73 (0)	71.5 (10.6)	69.5 (5.0)
2	67.2 (23.1)	60.4 (13.2)	67 (17.1)	70 (14.2)	70.2 (14.0)	68.8 (12.0)	73.8 (6.9)	73 (10.2)	69 (11.7)	66 (15.9)	67.3 (9.1)
3	51.8 (6.8)	55.6 (9.2)	54.5 (8.3)	59 (10.6)	56.6 (12.0)	70.2 (5.8)	65.5 (3.9)	62.8 (6.4)	63 (14.9)	58.2 (14.0)	58 (7.7)
4	66 (15.0)	62.6 (10.5)	63.4 (15.2)	64.8 (9.1)	65 (13.8)	69.2 (6.6)	68.8 (9.86)	64.8 (12.9)	63.8 (11.1)	58 (14.9)	60.3 (9.7)

3.4. DISCUSSION

Three dogs died among the control group whereas no deaths were observed among the drug pretreated groups. The deaths were due to ventricular fibrillation which was preceded by premature ventricular beats. These results show that verapamil pretreatment prevented ventricular fibrillation from developing and are in agreement with those of Kaumann and Aramendia (1968) who found that dogs pretreated with intravenous verapamil before coronary artery ligation did not develop ventricular fibrillation. Dogs with dilatative cardiomyopathy frequently have ventricular arrhythmias and the most likely cause of sudden death in these dogs is postulated to be ventricular fibrillation (Rush and Keene, 1989).

In a study of human patients with ventricular tachycardia, Harrison and Alderman (1971) found that lidocaine completely abolished ventricular premature beats when the plasma concentrations of the drug was 6-7 μg/ml. Similar results were obtained in dogs by Wilcke *et al.* (1983b). In the present study lidocaine pretreated dogs still had premature ventricular beats occuring on adrenaline administration even though lidocaine had been reported to increase the arrhythmogenic dose of adrenaline both in humans and dogs anaesthestized with halothane (Johnston *et al.* , 1976 and Chapin *et al.* , 1980). This observed difference could have been due to a decrease in plasma lidocaine concentration by the time of adrenaline administration though

in the present study the plasma concentrations of the drug were not measured.

Propranolol is reported to be effective in controlling ventricular arrhythmias both in humans and dogs (Woosley *et al.* , 1979; Muir, 1986). Gang *et al* (1984) reported that propranolol abolished ventricular fibrillation in dogs with myocardial infarction. In this study propranolol prevented ventricular fibrillation from developing in dogs with induced arrhythmias. The finding that death was prevented from occuring after pretreatment is in agreement with that of Koppes *et al* (1980) who observed suppression of premature beats after acute myocardial infarction and hence prevention of sudden death in human patients. This drug prevented development of arrhythmias in one dog whereas in the others 2nd degree heart block and ventricular premature beats were observed which were, however, not as predominant as those in the control dogs. A reduction in premature ventricular beats had also been reported by Winkle *et al.* (1978).

In this study lidocaine did not cause any alteration in the P-R, QRS, and QT intervals which was in agreement with the findings of Smith *et al.* (1972) though following adrenaline administration the P-R interval increased but not significantly. Although Kaumann and Aramendia (1968) found that verapamil prolonged the P-R interval in dogs with coronary artery ligation and similar results were obtained by Heng *et al.* (1975) in humans, the present study did not show any significant increase in the P-R interval due to verapamil pretreatment. Drug pretreatments did not cause any

significant ($p>0.05$) alterations in the QRS and QT intervals on adrenaline administration suggesting that adrenaline did not cause any alterations on the rate of depolarization or repolarization of the ventricles. Lack of effect on depolarization or repolarization of action potentials by verapamil in patients with sinus rhythm has been reported by Heng *et al.* (1975). Adrenaline acts on adrenergic receptors in the heart to exert both positive inotropic and chronotropic effects hence resulting in higher demands for oxygen, lack of which causes hypoxia. Hypoxia and ischaemic conditions in the myocardium cause an increase in the T wave amplitude (Bolton, 1975). In this study administration of adrenaline significantly ($p<0.05$) increased the T wave amplitude from 0.159 ± 0.05 mV to 0.433 ± 0.09 mV in the control dogs suggesting that the oxygen demand for the myocardium had increased. Furthermore in this study since the polarity of the T wave changed in some of the animals, the cause for the increased T wave amplitude was most likely due to hypoxia. The T wave amplitude increase occurred just prior to the start of the arrhythmias which tends to suggest that in halothane anaesthetized dogs adrenaline induced arrhythmias may be precipitated by increased oxygen demands leading to myocardial hypoxia. The hypoxia causes the ventricular myocardium to become irritable and stimulate ventricular arrhythmias which could lead to ventricular fibrillations a more serious consequence of ventricular myocardial irritability (Bolton, 1975). Verapamil was apparently superior in prevention of adrenaline induced arrhythmias in these halothane anaesthetized dogs. Pretreatment with this drug

prevented arrhythmias from occurring in one dog and in another dog only one isolated 2nd degree heart block occurred. Propranolol and lidocaine did not have much effect probably due to low concentrations of the drugs in the myocardium at the time of adrenaline administration.

The number of the various circulating blood cells vary with normal physiological conditions. The considerable variations that normally exist among a given population are attributed to sex, age, nutrition, ambient temperature and sexual cycle. Therefore the normal values listed are usually considered as guide lines rather than rigid criteria. In this study the haematologic parameters remained within the normal species range with only total neutrophils for the control 8 hour sample being slightly above the normal. Increase in neutrophils accomplished by mature cells suggest that the effect is mainly one of redistribution of cells already available within the capillary beds (Schalm *et al.*, 1975). Most of the changes were noted in the first 8 hour period from the initiation of the study. The increase in these parameters were due to stress hence release of blood reserves from the spleen, bone marrow and the shunting of blood from the non essential organs such as the intestines which occurs when adrenaline is released or administered into the blood stream.

CHAPTER 4

EFFECTS OF ANTIARRHYTHMIC DRUGS (VERAPAMIL, LIDOCAINE, AND PROPRANOLOL) ON SOME CARDIOVASCULAR AND BIOCHEMICAL PARAMETERS

4.1. MATERIALS AND METHODS.

4.1.1. Animals and feeding.

The source of animals and their feeding was as described in section 3.1.1 of experiment 1.

4.1.2. Induction of anaesthesia.

Anaesthesia was induced and maintained as described in section 3.1.2 of experiment 1.

4.1.3. Drug treatments and induction of arrhythmias.

These were done as in section 3.1.3 of experiment 1 except propranolol was administered at a dose of 0.5 mg/kg bwt. Adrenaline was administered 5 minutes after treatment with the respective drug.

4.1.4. ECG measurements and calculation.

Measurement of ECG parameters and calculation was done as described in sections 3.1.4.1 and 3.1.4.2 of experiment 1.

4.1.5. Blood pressure.

A 16-gauge catheter was placed into the femoral artery as described by Rawling *et al.* , (1970), and used for recording arterial blood pressure. The catheter was flushed with heparinized physiological saline. Blood pressure was measured, using a noncompliant fluid-filled system and a type 4-327-L221 transducer (Consolidated electrodynamics, England) was connected to a pen recorder (Lectromed Ltd. Jersey, Channel Island, U. K.). The pressure transducer was placed at the level of the heart in the laterally recumbent dogs. At the end of the experiment the catheter was removed and the femoral artery double ligated with silk. The area was flushed with 5,000 IU crystalline penicillin. The subcutaneous tissue was sutured with 2/0 catgut and the skin closed with 2/0 nylon in a simple interrupted pattern.

4.1.6. Blood collection.

Blood (8 ml) was collected during ECG and blood pressure measurements and thereafter from the jugular vein at the following time intervals- 0 hr, 1/4 hr, 1/2 hr, 1 hr, 2 hr, 4 hr, 8 hr, 12 hr, 24 hr, 48 hr and 72 hr using a 10 ml syringe fitted with a 19 g needle. The blood was put into clean sterile universal bottles for biochemical analysis. Blood was kept in the refrigerator at 4°C pending the various analyses.

4.1.7 Biochemical parameters.

Blood collected for biochemical determination was centrifuged at 2,300 x *g*. for 10 minutes. Serum was then

harvested and stored in the refrigerator at 4°C pending assay for lactic dehydrogenase (LDH), α-hydroxybutyrate dehydogenase (HBDH) and calcium.

4.1.7.1. Lactic dehydrogenase

The lactic dehydrogenase enzyme (LDH) was determined using the kinetic method according to IFCC recommendations using Eurodiag kits (Eurodiag, Ermont Cedex, Paris, France). The LDH activity was determined by the reaction:

$$\text{Pyruvate} + \text{NADH} + H^+ \xrightleftharpoons{\text{LDH}} \text{L Lactate} + NAD^+$$

The reaction mixture was prepared by mixing 3 ml of the buffer and the freeze-dried NADH. The reaction mixture (1 ml) was put into a test tube and the sample (20 µl) was added, mixed gently and incubated at 30° C for one minute. The change in optical density (OD) per minute was measured every 30 seconds for 3 minutes at 340 nm using a model ASA 24 spectrophotometer (Coultronics, SA, Margency 95580 Andilly). The activity of LDH present in the sample was calculated by the following formula:

$$\Delta \text{ O.D./min} \times 8\,095$$

Where the Δ O.D/min. was greater than 0.100 serum was diluted 1/10 with sodium chloride solution (9 g/l) and the results multiplied by 10.

4.1.7.2. alpha-Hydoxybutyrate dehydrogenase

The α-hydroxybutyrate dehydrogenase enzyme (HBDH) was determined according to the optimised kinetic method recommended by DGKC using Eurodiag kits (Eurodiag, Ermont Cedex, Paris, France). The HBDH activity in the specimen is determined by the reaction:

$$\alpha\text{-ketobutyrate} + NADH + H^+ \xrightleftharpoons{\alpha\text{- HBD}} \alpha\text{-hydroxybutyrate} + NAD^+$$

The reaction mixture was prepared by mixing 3 ml of distilled water and the freeze-dried NADH. The reaction mixture (1 ml) was put into a test tube and the sample (30 µl) was added, mixed gently and incubated at 25° C for one minute. The change in optical density per minute was measured every 1 minute for 3 minutes at 340 nm using a model 25 spectrophotometer (Beckman®, Instruments Inc. , USA). The activity of α-HBDH present in the sample was calculated by the following formula:

$$\Delta \text{ O.D./min} \times 5\,450$$

Where the Δ O.D/min. was greater than 0.100 serum was diluted 1/10 with sodium chloride solution (9 g/l) and the results multiplied by 10.

4.1.7.3. Calcium.

This was determined colorimetrically using the o-cresolphthalein complexone, without deproteinization method. A commercially available diagnostic kit was used

(Boehringer Mannheim GmBH).

The reagents and sample were pipetted into test tubes as tabulated below:

	Reagent blank	Standard	Sample
Standard	-	0.05 ml	-
Serum	-	-	0.05 ml
Buffer	1.00 ml	1.00 ml	1.00 ml
Chromogen	1.00 ml	1.00 ml	1.00 ml

The tubes were then mixed gently and absorbance of sample (A_{sample}) and standard (A_{std}) was read against the blank after 5 minutes at 575 nm using a model ASA 24 spectrophotometer (Coultronics, SA, Margency 95580 Andilly). A control was also run in exactly the same way as the sample.

The concentration of calcium (mg/100 ml) in the serum was obtained from the formula:

$$c = 8 \times \frac{A_{sample}}{A_{std}}$$

Where the concentration of calcium exceeded 15 mg/100 ml the sample (0.1 ml) was diluted with redistilled water (0.1 ml), the assay repeated and the result multiplied by 2.

4.1.8. Clinical observation.

The dogs were clinically examined for 72 hours during which temperature, pulse, respiration and appetite were recorded. Any unusual clinical signs were observed.

4.1.9. Data analysis.

Statistical analysis of data was done by analysis of variance (ANOVA) (Daniel, 1983) using an IBM computer with a panacea statistical programme. Turkey's highest significant difference (HSD) test (Daniel, 1983) was used to determine if there was a significant difference in the group means at 5% level of significance. The test statistic used in this case was:

$$HSD^* = q_{\alpha,\kappa,N-\kappa}\sqrt{\frac{MSE}{n^*_j}}$$

where

α = chosen level of significance

k = number of group means

N= total number of observations

n= number of observations in a treatment

MSE = mean square error from the ANOVA table

q = obtained by entering a HSD statistic table

n^*_j = the smallest of the two sample sizes associated with the two sample means that are to be compared

4.2. RESULTS.

4.2.1. Clinical observation.

All the dogs had good appetite throughout the experimental period. Temperatures ranged between 37.9° C and 39° C. Heart rate, pulse and respiration remained within the normal species range. Three dogs (60 %) in the control group died when adrenaline was administered whereas only two (40 %) in the verapamil and one (20 %) in the lidocaine pretreated dogs died. No death occurred in the propranolol pretreated dogs.

4.2.2. Arrhythmias.

The predominant arrhythmia that was observed was ventricular premature beats which developed into ventricular tachycardia (Fig. 14) proceeding into ventricular fibrillation in the dogs (Fig. 15) that died during the experiment. Second degree heart block (Fig. 16) occurred in one dog in the verapamil pretreated dogs (dog no. B2E) and in one dog in the lidocaine pretreated dogs (dog no. B4D). There was slowing of the heart rate in all the propranolol pretreated dogs with one dog developing ventricular premature beats. The surviving dogs in the control group developed ventricular tachycardia which occurred in episodes. In the control group the first arrhythmia occured after 19.4±4.39 seconds (12-23 sec.); for lidocaine 26.33±13.05 seconds (16-41 sec.); verapamil 22.6±14.12 seconds (21-38 sec.). Only one dog developed ventricular arrhythmias after 187 seconds in the propranolol

pretreated dogs. These were however found not to be statistically significant ($p>0.05$).

4.2.3. Blood pressure.

Increases in blood pressure were noted in all experimental dogs except one verapamil pretreated dog (dog no. B2A). This particular dog did not also develop any arrhythmia during the experiment. The arrhythmias, especially the 2nd degree heart block, that occurred on adrenaline administration were associated with decreases in the blood pressure.

The period taken for the increase in blood pressure to occur after adrenaline administration were:- control 11±2.12 seconds (range 9 to 14); verapamil 9.6±6.43 seconds (range 6 to 15); propranolol 12.8±6.53 seconds (range 8 to 24); and lidocaine 13.2±6.14 seconds (range 9 to 24)m ($p>0.05$). Maximum increase in blood pressure occurred after 44.2±22.33 seconds (range 24 to 71) in the control dogs; 29.2±20.41 (range 0 to 52) verapamil; 110.8±38.74 seconds (range 72 to 155) propranolol; and 49.8±33.27 (range 17 to 102) lidocaine pretreated groups. The maximum increase took much longer to be attained in the propranolol treated dogs as compared to the other groups ($p<0.05$).

Reduction in systolic, diastolic, and mean arterial blood pressure were observed in all the groups. Increase occurred in all the groups after administration of adrenaline, with the

least increase in the verapamil pretreated group (Fig. 17). There was however, no statistical difference among the groups ($p>0.05$).

4.2.4. ECG Parameters

4.2.4.1. Heart rate and R-R interval

The various groups had no significant differences on heart rate even though the propranolol pretreated dogs showed an apparent decrease. There was also a marked increase in the R-R interval after administration of adrenaline (Fig. 18). A slight increase was noted in the R-R interval for lidocaine pretreated group (Fig. 19).

4.2.4.2. P wave

There was no significant difference in the duration of P wave though the lidocaine pretreated group showed an increase in contrast to the decrease that occurred in the control and verapamil pretreated groups. No apparent difference in P wave amplitude was noted.

4.2.4.3. P-R interval

There was an apparent increase in the propranolol and lidocaine pretreated groups (Fig. 18) while only a slight increase occurred in the verapamil group (Fig. 20). These were however, not statistically significant ($p>0.05$).

4.2.4.4. QRS complex and R wave

Slight changes occurred in the QRS complex duration and R wave amplitude. Statistical analysis showed that the means for the QRS complex duration for lidocaine (0.044±0.005 sec) was higher ($p<0.05$)than that for the control dogs (0.041±0.001 sec). These were however, within the normal species range.

4.2.4.5. S-T segment

Slight reduction in duration were found. Minor elevation and depression of the segment were observed in the various groups.

4.2.4.6. T wave

Lidocaine administration caused a slight reduction in the T wave amplitude compared to the control group. Increases in amplitude ($p>0.05$) occurred just before ventricular arrhythmias (Fig. 15) which were higher than in the control for the verapamil and lidocaine pretreated groups and less in the propranolol pretreated group(Fig. 21).

4.2.4.7. Q-T interval

Propranolol caused a slight increase in the Q-T interval. Propranolol and lidocaine pretreated groups had increased Q-T interval after adrenaline administration.

4.2.5. Biochemical parameters

Increase in the serum levels of lactic dehydrogenase occurred with the maximum increase at 4 hours for lidocaine and propranolol, and 8 hours for the control and verapamil. The increase was least in verapamil followed by propranolol (Table 6). There was a significant difference ($p<0.05$) in lactic dehydrogenase levels between the verapamil and lidocaine pretreated groups of dogs.

The serum levels of α-hydroxybutyrate dehydrogenase remained within the normal range established by readings from 28 mongrel dogs of 23.6 - 118.1 IU/L. Slight increase in the serum levels of α-hydroxybutyrate dehydrogenase with the peaks at 2 hours for verapamil, 8 hours for lidocaine and control. Similar increase was observed in the control and lidocaine groups, slight increase in verapamil group and none in the propranolol group (Table 7) ($p>0.05$).

Though there was a statistical difference between the mean serum calcium levels ($p<0.05$), the changes that occurred were similar (Table 8) and the values were all within the canine species normal range.

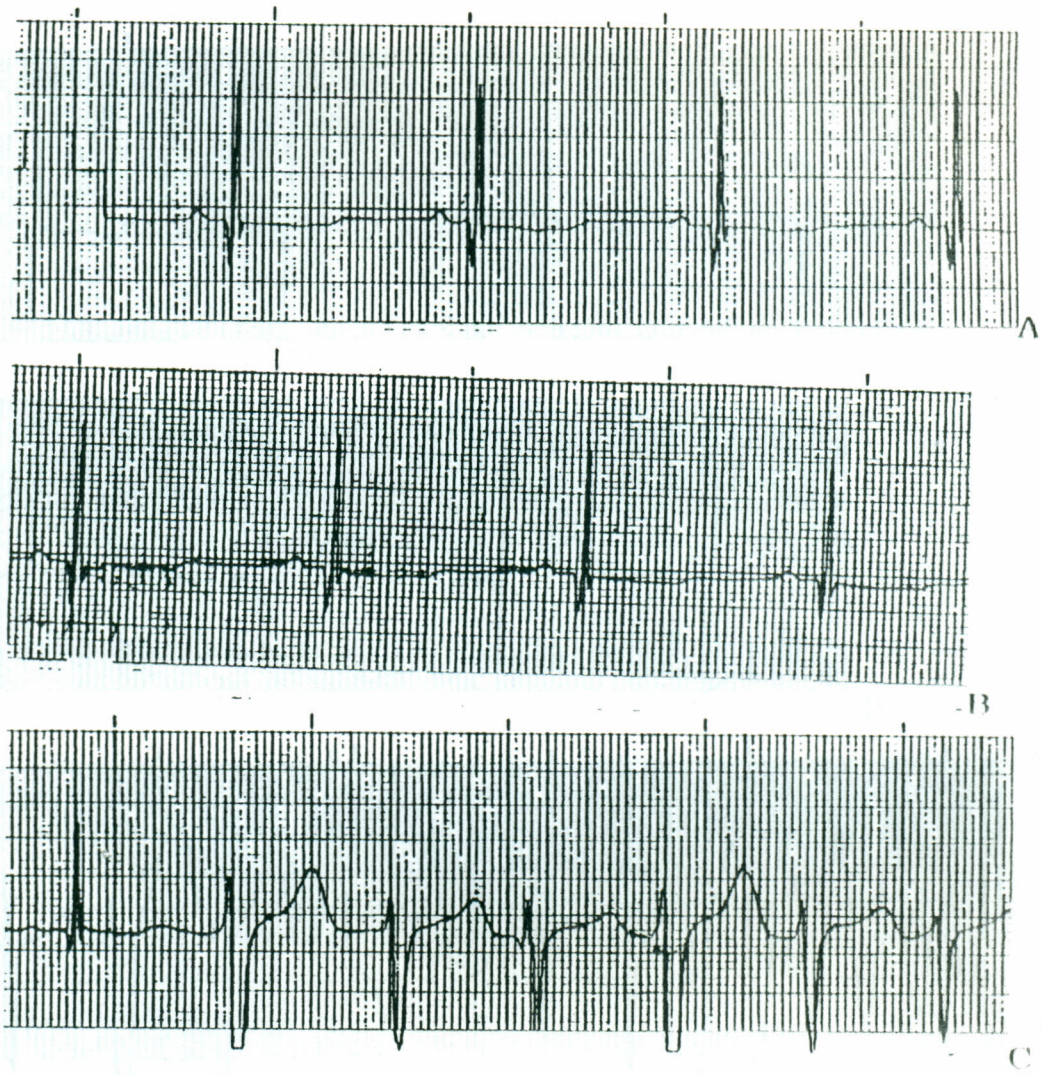


Fig. 14 ECG scan (A) showing the electrographic complexes before any drug administration, (B) after physiological saline, and (C) ventricular tachycardia after adrenaline administration (Dog no. B1B). Recording lead II. Paper speed 50 mm/sec., and sensitivity 1 mm= 0.05 mV.

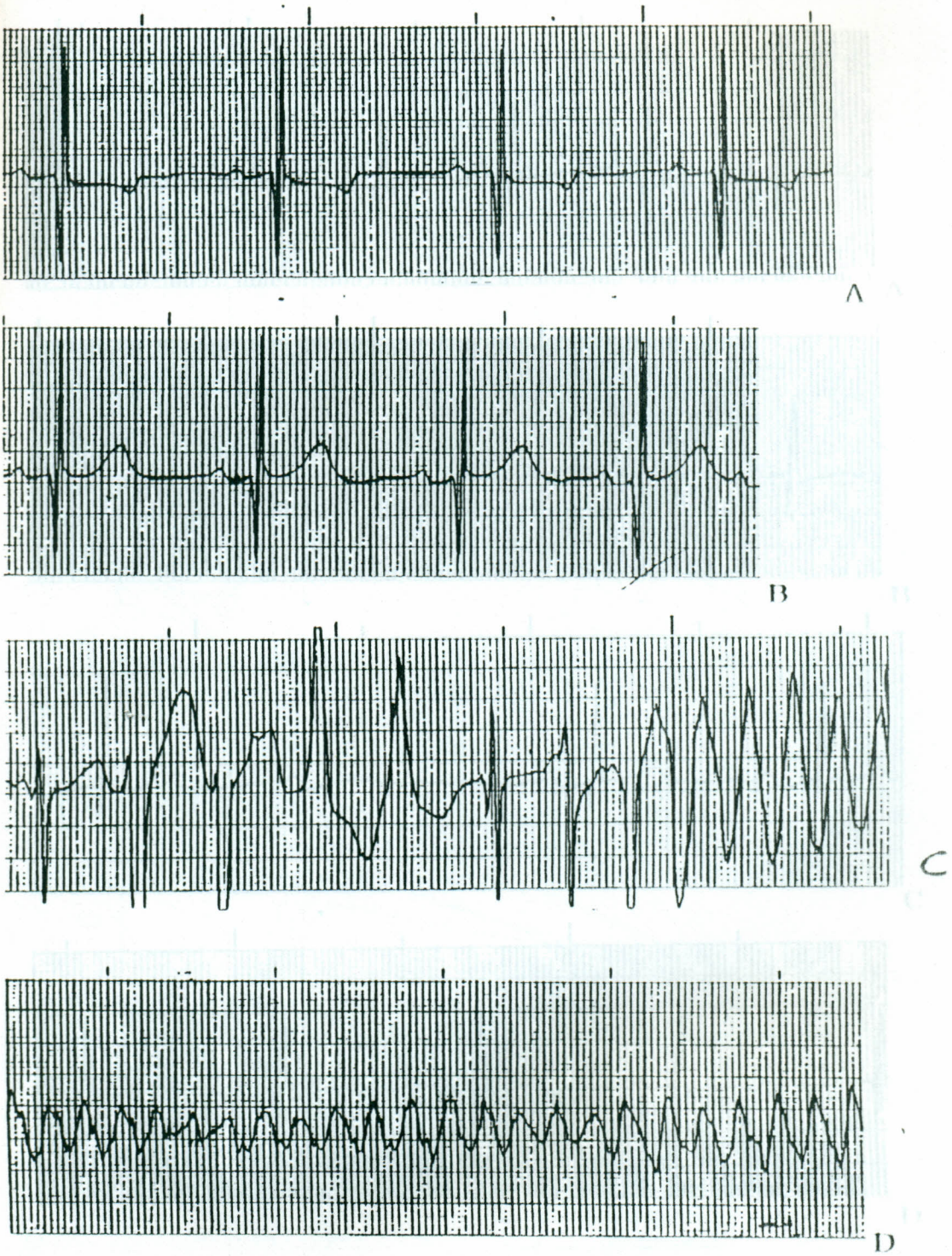


Fig. 15 ECG scan showing the electrocardiographic complexes (A) after physiological saline, (B) increased T wave millivoltage which then (C) ventricular tachycardia and ventricular flutter which proceeded into (D) ventricular fibrillation before death (Dog no. 1C). Recording lead II, Paper speed 50 mm/sec., and sensitivity 1 mm= 0.1 mV.

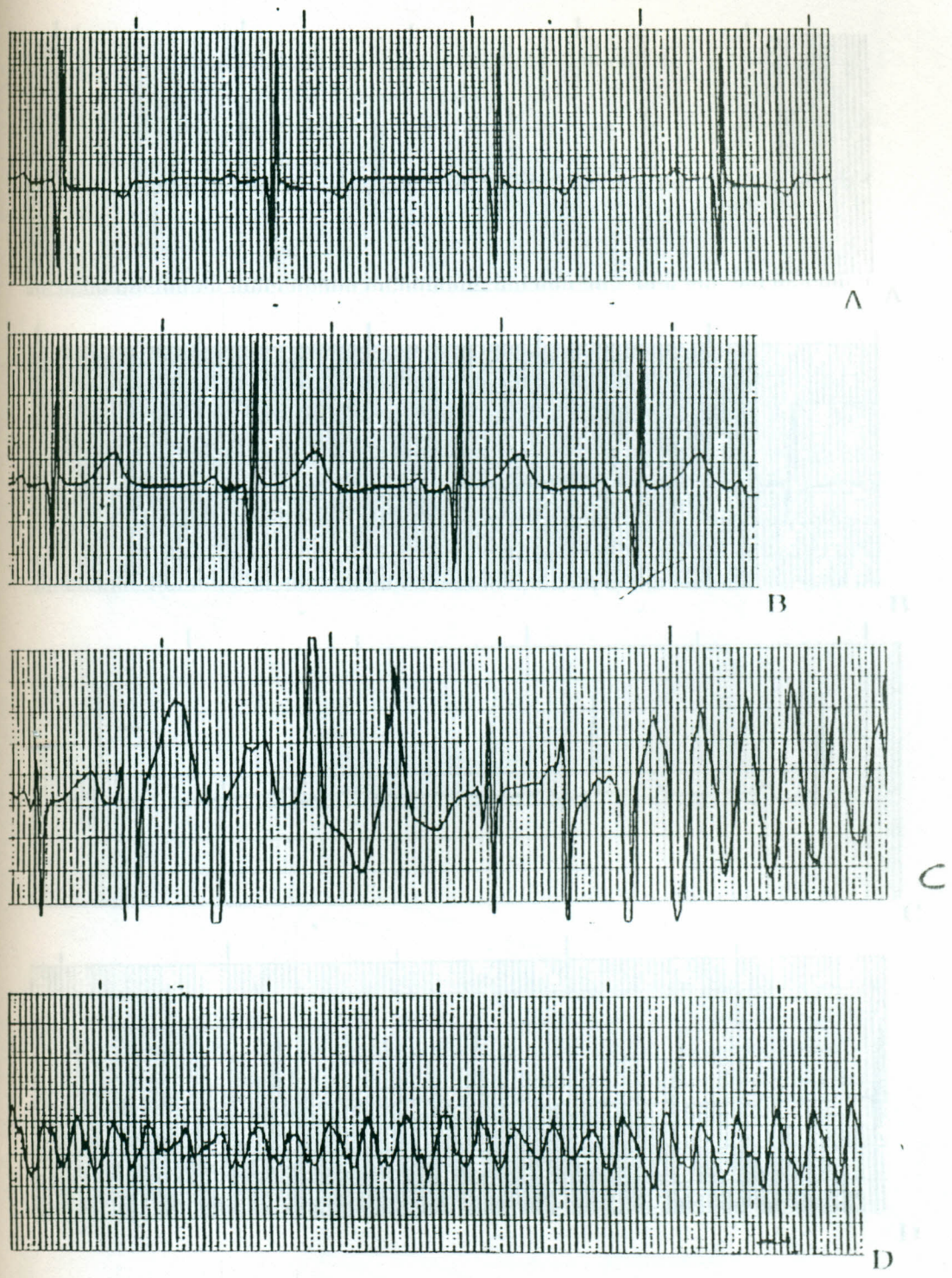


Fig. 15 ECG scan showing the electrocardiographic complexes (A) after physiological saline, (B) increased T wave millivoltage which then (C) ventricular tachycardia and ventricular flutter which proceeded into (D) ventricular fibrillation before death (Dog no. 1C). Recording lead II, Paper speed 50 mm/sec., and sensitivity 1 mm= 0.1 mV.

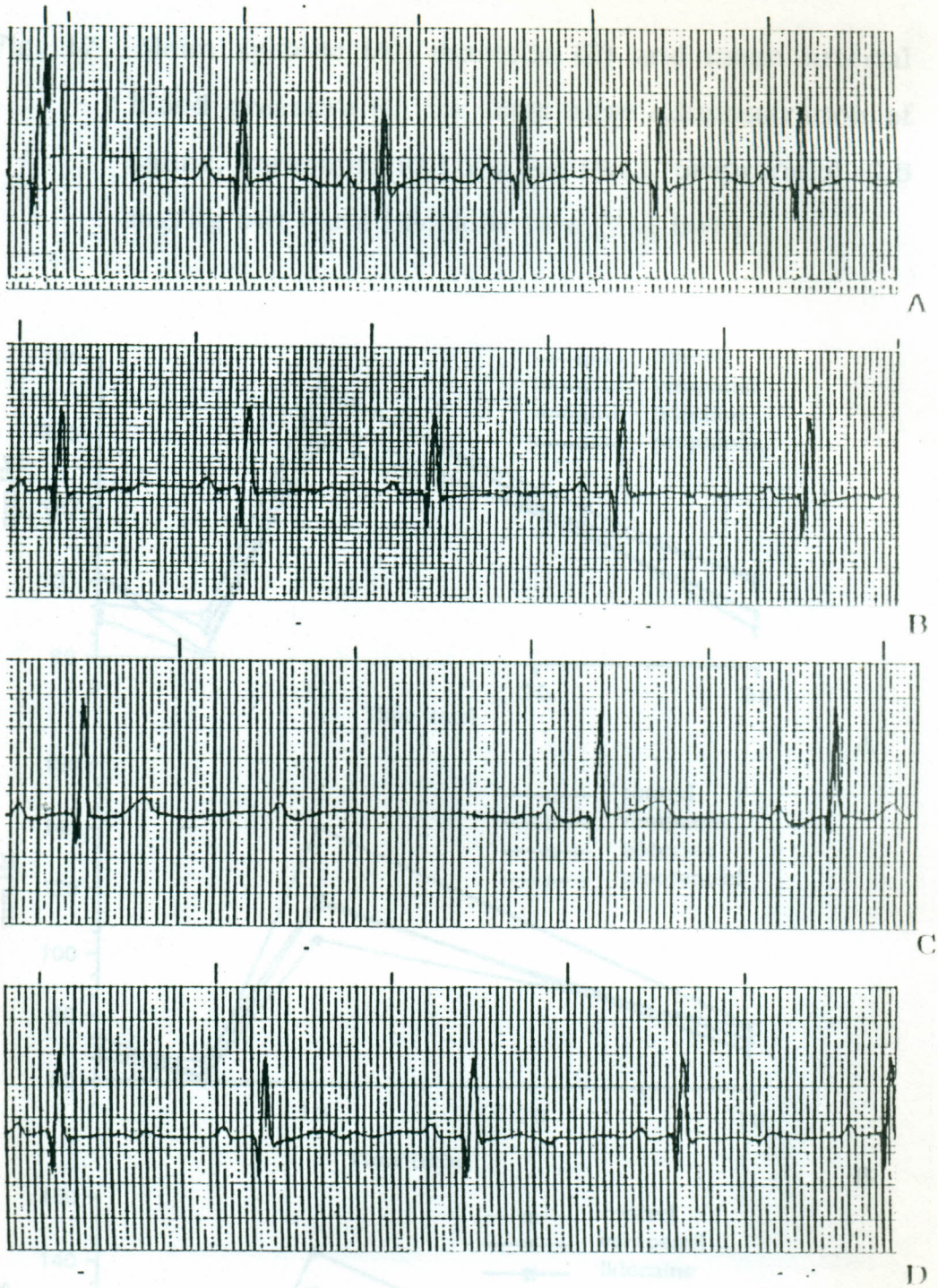
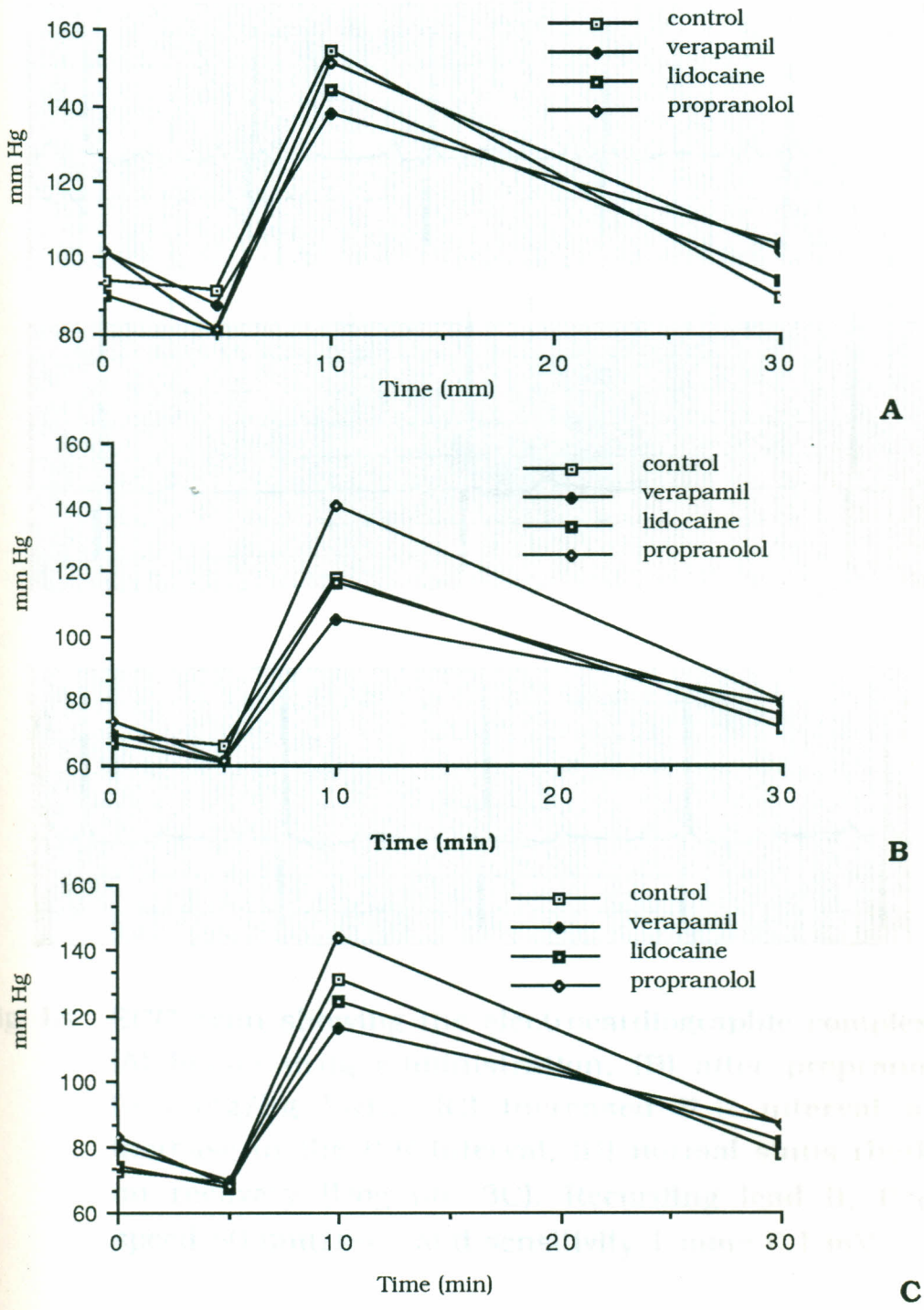


Fig. 16 ECG scan showing the electrocardiographic complexes (A) before drug administration, (B) after verapamil (0.1 mg/kg bwt.), (C) 2nd degree heart block, and (D) normal sinus rhythm on recovery (Dog no. B2E). Recording lead II, Paper speed 50 mm/sec., and sensitivity 1 mm= 0.1 mV.

Fig. 17 Mean systolic (A), diastolic (B) and mean arterial blood pressure (C) for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.5 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).



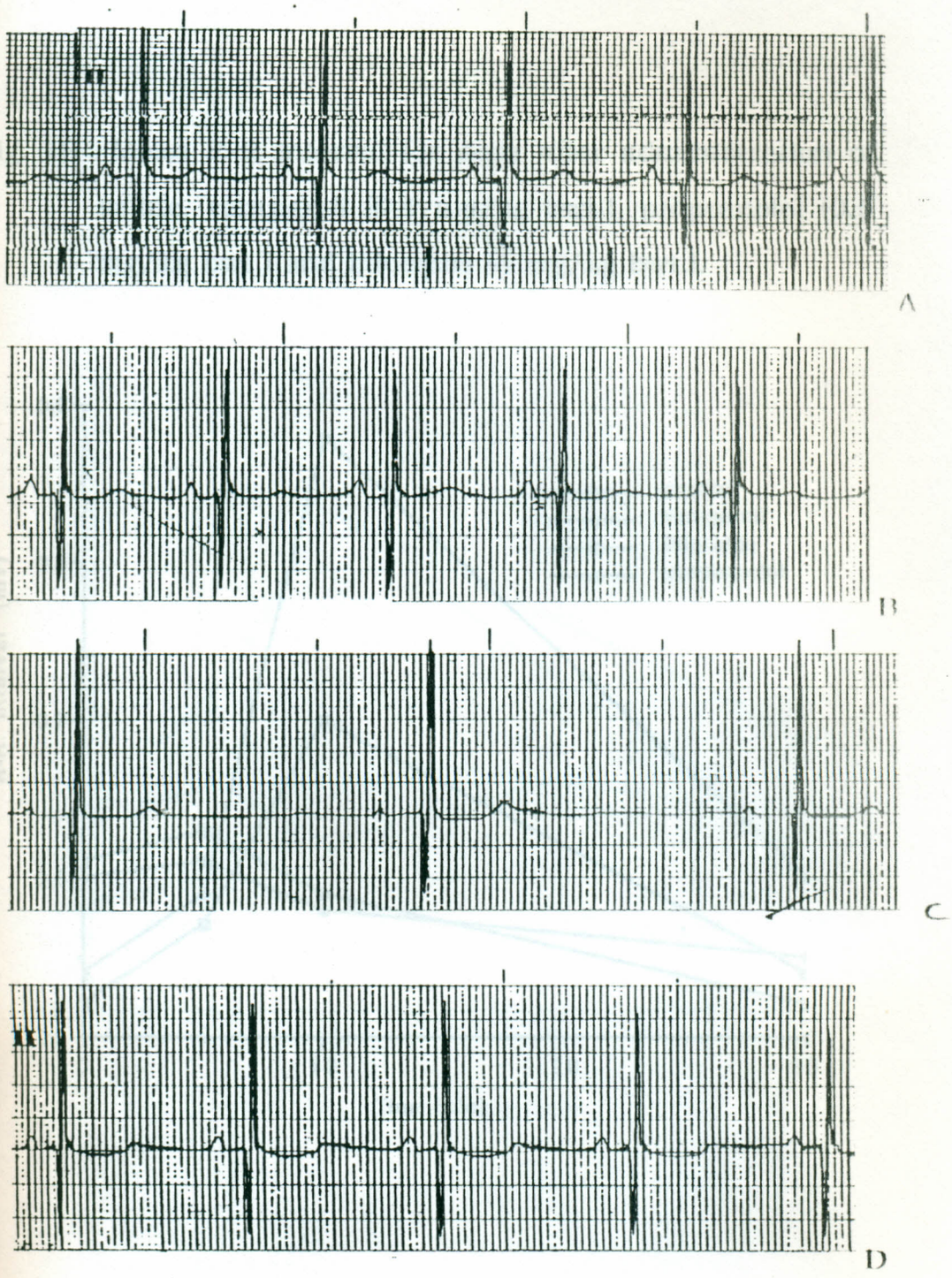


Fig. 18 ECG scan showing the electrocardiographic complexes (A) before drug administration, (B) after propranolol (0.5 mg/kg bwt.), (C) increased R-R interval and increase in the P-R interval, (D) normal sinus rhythm on recovery (Dog no. 3C). Recording lead II, Paper speed 50 mm/sec., and sensitivity 1 mm= 0.1 mV.

Fig. 19 Mean R-R interval for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.5 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

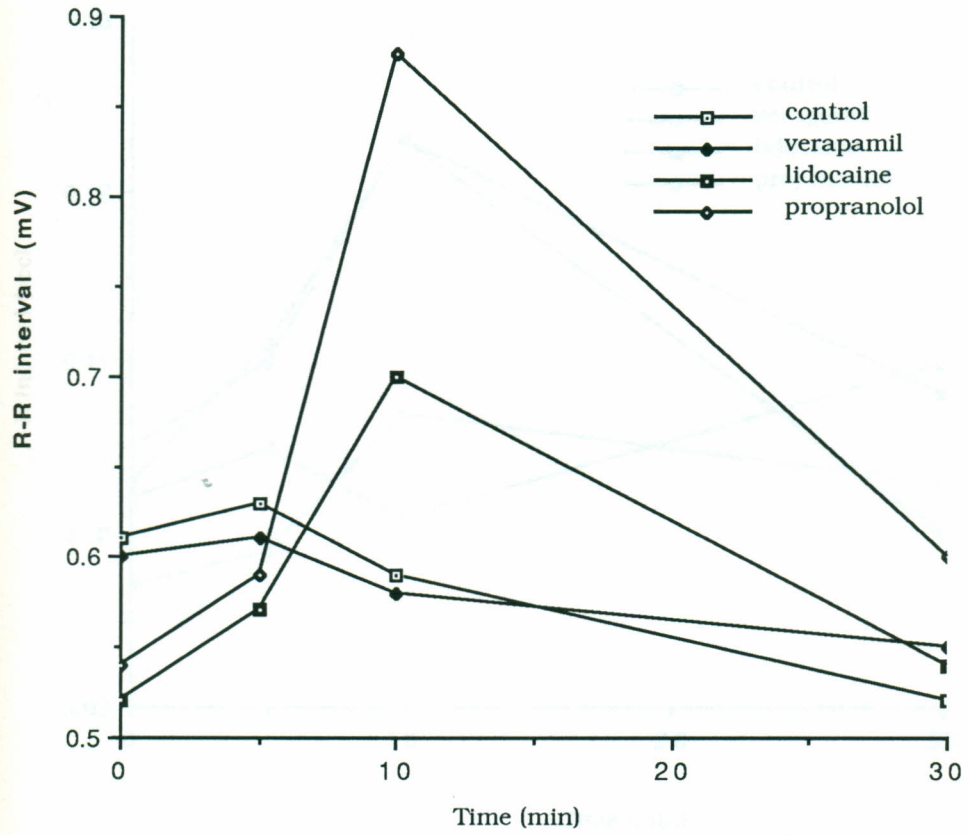


Fig. 20 Mean P-R interval for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.5 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

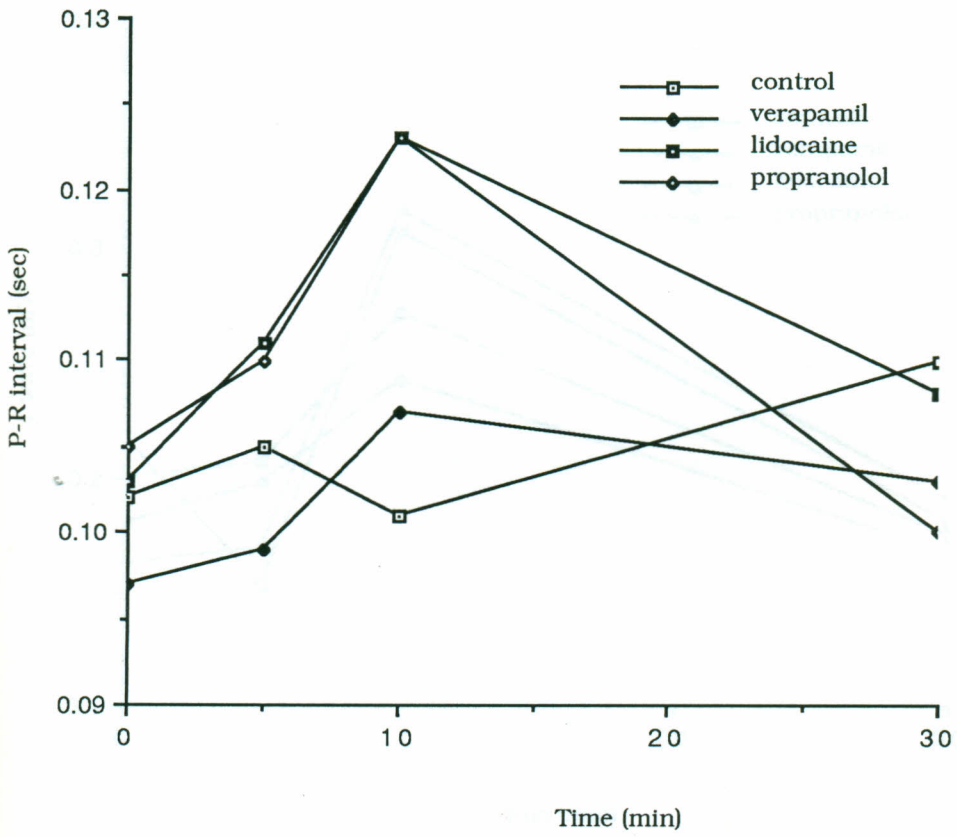


Fig. 21 Mean T wave millivoltage for dogs after administration of saline, verapamil (0.1 mg/kg bwt.), propranolol (0.5 mg/kg bwt.) and lidocaine (4 mg/kg bwt.).

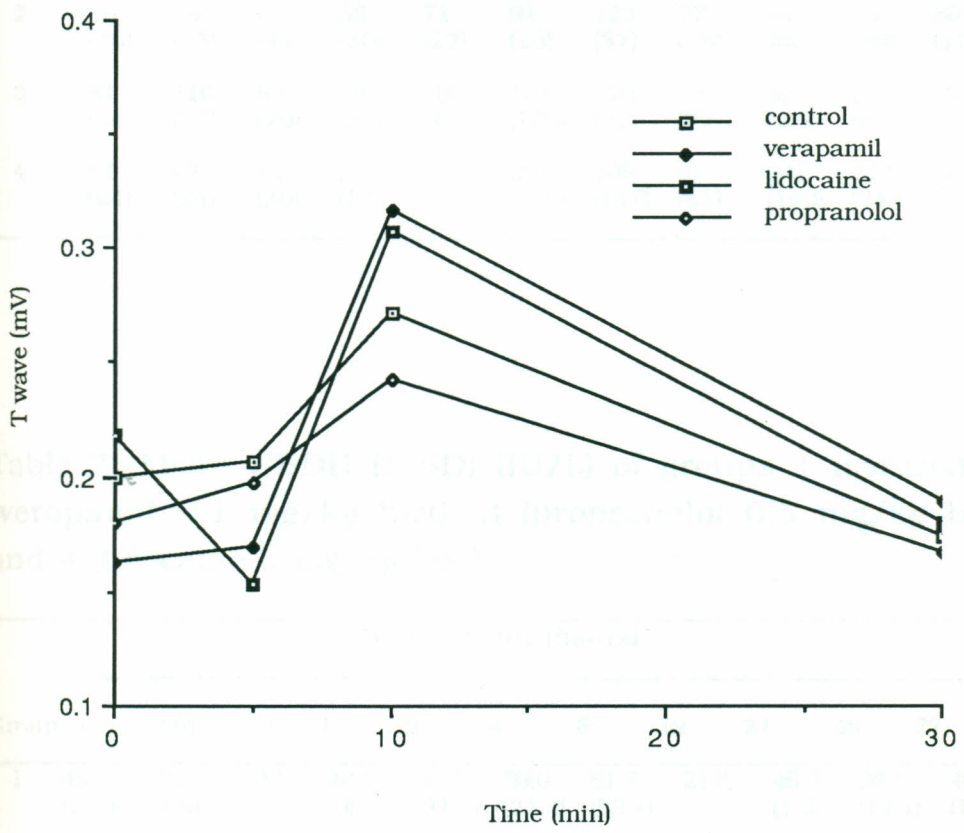


Table 6. Mean LDH (± SD) (IU/L) of groups 1 (control), 2 (verapamil 0.1 mg/kg bwt), 3 (propranolol 0.5 mg/kg bwt) and 4 (lidocaine 4 mg/kg bwt).

	Time of study (hours)										
Group	0	1/4	1/2	1	2	4	8	12	24	48	72
1	103 (106)	183 (34)	88	169 (69)	169 (45)	196 (134)	307 (342)	67	123 (87)	131 (141)	163 (13)
2	36 (19)	89 (43)	55 (41)	58 (30)	71 (23)	91 (19)	123 (37)	77 (28)	94 947)	95 (29)	86 (17)
3	81 (48)	110 (57)	83 (29)	76 (29)	105 (44)	164 (105)	119 (31)	73 (27)	82 (22)	115 (81)	128 (83)
4	81 (24)	77 (24)	71 (30)	57 (13)	120 (85)	232 (200)	208 (147)	91 (41)	247 (163)	210 (247)	60 (19)

Table 7. Mean HBDH (± SD) (IU/L) of groups 1 (control), 2 (verapamil 0.1 mg/kg bwt), 3 (propranolol 0.5 mg/kg bwt) and 4 (lidocaine 4 mg/kg bwt).

	Time of study (hours)										
Group	0	1/4	1/2	1	2	4	8	12	24	48	72
1	45.8 (11.4)	31.8 (3.9)	29.1	49.1 (0)	55.4 (21.8)	59.0 (24.4)	81.7 (59.1)	21.8	46.3 (1.3)	39.1 (14.1)	40.0 (10.3)
2	32.0 (5.4)	37.0 (9.2)	41.8 (21)	32.1 (5.2)	60.6 (8.6)	43.6 (23.6)	55.7 (25.1)	36.3 (11.4)	66.0 (26.5)	31.5 (9.2)	45.3 (4.6)
3	66.9 (38.1)	67.6 (35.5)	45.4 (33.8)	53.1 (29.2)	48.3 (7.8)	49.1 (15.8)	41.1 (12)	38.8 (17)	40.3 (7.2)	45.8 (34.1)	48.3 (13.8)
4	44 (13.1)	45 (8.2)	58.6 (42.5)	36.3 (7.4)	43.6 (18)	72.5 (44.8)	78.8 (87.3)	41.3 (16.5)	66.8 (28.5)	67.7 (59.5)	35 (10.1)

Table 8. Mean Calcium (± SD) (mg/100 ml) of groups 1 (control), 2 (verapamil 0.1 mg/kg bwt), 3 (propranolol 0.5 mg/kg bwt) and 4 (lidocaine 4 mg/kg bwt).

	Time of study (hours)										
Group	0	1/4	1/2	1	2	4	8	12	24	48	72
1	10.3 (0.7)	9.86 (1.5)	10.9	9.6 (1.6)	9.2 (1.2)	9.3 (0.3)	9.9 (0.2)	10.6	10.3 (1.0)	9.8 (0.4)	9.6 (0.9)
2	10.3 (1.7)	10.2 (1.6)	10.0 (0.9)	10.5 (1.4)	10.3 (1.2)	9.9 (0.6)	10.5 (0.2)	10.7 (1.0)	11 (1.5)	10.7 (0.2)	10.7 (0.9)
3	11.0 (0.9)	11.2 (0.8)	11.4 (1.5)	11 (1.5)	11.5 (1.4)	10.3 (0.9)	9.8 (1.3)	10.3 (0.7)	10 (1.2)	9.8 (0.7)	10.6 (0.6)
4	9.9 (1.1)	9.8 (1.3)	10.0 (1.1)	10.4 (0.3)	10.2 (1.0)	9.9 (0.9)	9.5 (1.3)	10.0 (0.8)	10.3 (0.6)	10.4 (1.2)	10.6 (2.0)

4.3. DISCUSSION

Death was observed in all the groups except the propranolol pretreated group. In all the dogs that died ventricular fibrillation which was preceded by ventricular tachycardia were observed hence the occurrence of ventricular tachycardia may be an important indication of impending fatal ventricular fibrillation. The finding that propranolol pretreatment prevented ventricular arrhythmia from occuring is in agreement with that of Gang *et al* (1984) who observed termination of ventricular fibrillation in dogs with myocardial infarction. Suppression of premature beats by propranolol after acute myocardial infarction has also been reported in humans (Koppes *et al.*, 1980). In the present study ventricular premature beats occurred in only one dog out of the 5 propranolol pretreated dogs as compared to the control group where all the dogs developed arrhythmias. Propranolol pretreatment was thus able to protect the dogs against adrenaline induced ventricular arrhythmias.

Ventricular premature beats occurred in three dogs with one (Dog no. B4F) dying after the arrhythmia progressed to ventricular fibrillation. These results are in contrast with those reported by other researchers, but supports the fact that lidocaine is a short acting antiarrhythmic drug that requires a constant infusion in order to maintain therapeutic plasma levels. Harrison and Alderman (1971) have reported lidocaine plasma concentrations of 6-7 µg/ml to completely abolish ventricular premature beats in humans.

In this study 2 dogs in the verapamil pretreated group died after developing ventricular tachycardia that proceeded to ventricular fibrillation. The difference between the findings of this study and that of Kaumann and Aramendia (1968) could be due to the dosages used and the mode of inducing the arrhythmias. They used 0.79 mg/kg of verapamil and coronary artery ligation to induce arrhythmias while in the present study 0.1 mg/kg of verapamil and adrenaline was used to induce arrhythmias. The present finding moreover, seems to be in agreement with published report that intravenous verapamil is not very effective in the treatment of ventricular arrhythmias, although it has been shown to reduce the frequency of premature ventricular beats, especially in acute myocardial infarction (Singh *et al.*, 1978).

Halothane has depressant effect on the myocardium. This may have contributed to the apparent increases in the R-R intervals in all groups of animals in this study. Adrenaline administration decreased the R-R interval in the control group a reflection of the increase in heart rate, while in the propranolol and lidocaine pretreated dogs the increased interval could have been due to further depression of the myocardium. Adrenaline exerts positive chronotropic effect by its action on β_1-adrenoceptors in the heart while lidocaine depresses myocardial excitability a difference in mode of action which could account for the increase in the R-R interval in these group of dogs. This increase is also in agreement with the fact that this drug depresses spontaneously depolarizing Purkinje fibres even in very low

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concentrations (Singh and Hauswirth, 1974). Propranolol blocks the beta adrenergic receptors in the myocardium leaving the heart open to the parasympathetic tone which could account for the observed decrease in the heart rate. In this group of dogs adrenaline administration caused a further decrease in the heart rate (increase in the R-R interval) due to stimulation of α-receptors in the myocardium.

Atrioventricular conduction was not affected by the drugs used in this study as there was no significant change in the P-R interval. The T wave amplitude increased just prior to the occurrence of arrhythmias. This could have been due to hypoxia.

The heart is innervated by both the sympathetic and parasympathetic nervous systems which continuously discharge. The normal blood pressure is maintained by baroreceptors present in the carotid sinus and the aortic arch in conjunction with the vasomotor centre in the medulla oblongata. The baroreceptors and the vasomotor centre coordinates such that when there is an increase in blood pressure increased vagal discharge results in negative chronotropic and inotropic effects lowering the pressure. In the blood vessels, there is inhibition of the sympathetic tone to the vessels, hence cholinergic innervation causes vasodilation, decrease in peripheral resistance and resultant decrease in blood pressure. The opposite is true when blood pressure decreases.

Adrenaline causes increases in blood pressure by its action both centrally in the heart and peripherally on the blood vessels. In the heart the effect is due to stimulation of ß and α receptors resulting in increased rate and force of contraction. The increased rate is due to rapid entrance of Na^+ into the cells causing the threshold potential to be reached quickly and depolarization to spread rapidly, increased availability of Ca^{++} is responsible for the increased force of contraction. In the present study, adrenaline caused an increase in blood pressure irrespective of whether arrhythmias occurred or not which suggests that the mechanism by which the increase in blood pressure was occurring is different to that by which the arrhythmias were being caused in these halothane anaesthetized dogs. Propranolol is a $ß_1$ and $ß_2$ receptor blocker (Adams, 1988). Propranolol blocks $ß_1$-receptors in the heart and $ß_2$-receptors in the blood vessels causing depression of the heart rate and peripheral resistance thus resulting in decreased output. This is most pronounced in a heart which was under the dominance of the sympathetic tone. In the present study, propranolol administration resulted in some reduction in arterial blood pressure. Adrenaline administration however, increased arterial blood pressure. This could have been due to stimulation of the α receptors resulting in a decrease in heart rate and increase in contractility due to increased Ca^{++} movement into the cells. Moreover, peripherally α stimulation by adrenaline is stronger than $ß_2$ stimulation resulting in predominant arteriolar contraction (Kittleson and Knowlen,

1986).

Acute haemodynamic changes following administration of calcium antagonists are primarily due to arteriolar vasodilation. Heng *et al.* 1975, reported a mild but transient fall in blood pressure occurring in some patients after verapamil administration. In the present study, this hypotensive effects of verapamil were masked by adrenaline induced increase in blood pressure. Catecholamines may attenuate the sensitivity of the cardiovascular system to the hypotensive effects of calcium channel blockers (Achike and Dai, 1990). Whereas verapamil acts to decrease blood pressure by affecting the Ca^{++} channels in the smooth muscles of the blood vessels hence causing vasodilation (Singh and Roche, 1977) adrenaline increases blood pressure by its effects on α receptors which causes vasoconstriction.

In cardiac myopathy the serum enzymes α-HBDH and LDH increases and hence could act as an indicator to the existing problem during clinical laboratory evaluation. Boyes *et al.*, (1970) have reported in their work that repeated treatments with lidocaine over a period of 90 days was not associated with any changes in clinical laboratory tests. In the present study, drug pretreatments did not have any clinically significant changes in the biochemical tests done.

CHAPTER 5

GENERAL DISCUSSION AND CONCLUSIONS

5.1. GENERAL DISCUSSION.

Catecholamines are thought to increase intra-cellular calcium binding and hence the observed positive inotropy (Kittleson and Knowlen, 1986). They act by stimulating the β_1 and α receptors in the heart. Stimulation of α adrenoceptors by adrenaline results in increased cardiac output, excitability and rate. Sustained stimulation of α and β adrenoceptors can lead to increased myocardial metabolism and oxygen consumption. Increased myocardial oxygen demand could result to hypoxia in the myocardium. Myocardial ischaemia and hypoxia have been postulated to be inciting causes of most cardiac arrhythmias associated with gastric dilatation volvulus in the dog (Muir and Lipowitz, 1978). In this study myocardial hypoxia occurred as depicted by the increase in the T wave amplitude which occurred prior to the ventricular arrhythmias. In the intact heart, sympathetic stimulation or catecholamine infusion leads to an increase in pacemaker activity at all levels. In the ventricular and Purkinje fibres they cause a moderate decrease in the refractory period (Gibson and Sowton, 1969). This together with the fact that halothane sensitizes the myocardium to adrenaline resulted in the observed ventricular arrhythmias. Ventricular premature beats are often life-threatening because they may progress to

ventricular fibrillation and death (Wilcke *et al.*, 1983a) In this study, all dogs that developed ventricular fibrillation died during the experiment.

Propranolol reduces cardiac activity by diminishing or preventing ß-adrenergic stimulation. It reduces the rate and force of contraction of the heart and decreases the rate of conduction of impulses through the conducting system. In the present study, propranolol when given at 0.5 mg/kg bwt. was able to prevent adrenaline induced arrhythmias. At this higher dose however, some undesirable effects like sinus bradycardia were noted. Bradycardia and atrioventricular block have been reported to occur after adrenaline administration 1 hour after propranolol (Kram *et al.*, 1974). Control of ventricular arrhythmias by propranolol has been reported (Woosley *et al.*, 1979; Koppes *et al.*, 1980; and Gang *et al.*, 1984). Propranolol protected the animals from the adrenaline induced arrhythmias as it is a ß receptor blocker. Propranolol blocks the ß receptors which cause vasodilation therefore the increase in blood pressure that occurred in this study was due to the powerful vasoconstriction due to adrenaline action on α receptors. This increase in blood pressure also explains the bradycadia that was observed in the propranolol pretreated dogs particularly those receiving 0.5 mg/kg dosage. This bradycadia could have occurred as a result of reflex vagal activity (Adams, 1988).

Verapamil is a calcium channel blocker (Godfriands, 1981 and Karlsberg, 1982). In the heart, verapamil selectively inhibits transmembrane influx of calcium through the slow

cation channels of the cardiac sarcolemma, an action that is crucial to the antiarrhythmic action of the drug (Adams, 1988). The slow calcium-dependent events are normal characteristics of automaticity in SA and AV nodal tissues hence verapamil alters the kinetics of the calcium channels of both nodes (Antman *et al.*, 1980; Nayler and Grinwald, 1981; and Zelis and Flaim, 1981) resulting in depression of their discharge rate and impulse conduction respectively. Adrenaline administration was associated with the occurrence of arrhythmias in the verapamil pretreated dogs suggesting that these arrhythmias may not have been associated with calcium movements in the slow calcium channels in the nodal tissues. Verapamil prolongs the effective refractory period of the AV node and suppresses its conduction velocity which can be restored by exposure to catecholamines (Antman et al., 1980). Adrenaline restores SA nodal automaticity in verapamil treated fibres (Wit and Cranefield, 1974). These are due to the fact that ß-adrenergic agonists increase the magnitude of slow inward current by elevation of cyclic AMP. Verapamil does not affect intra-atrial and intraventricular impulse conduction (Husaini *et al.*, 1973; Roy *et al.*, 1974) since the normal myocardium relies upon the rapid sodium channels for depolarization and impulse conduction (Henry, 1980; McAllister, 1981; and Karlsberg, 1982).

In automatic cells (like those of the bundle of His and its Purkinje ramifications in the ventricles) catecholamines increase the rate of diastolic depolarization hence increase the rate of impulse formation by these cells showing

pacemaker activity (Gibson and Sowton, 1969). This could explain why the arrhythmias occurred in the verapamil pretreated dogs as verapamil prolongs atrioventricular nodal refractoriness by a direct action on the slow channels in the node proximal to the bundle of His (Braunwald, 1982). This is of clinical importance as it represents the mechanism through which nodal-reentrant supraventricular tachycardia is abolished and ventricular response in atrial flutter and fibrillation controlled (Heng *et al.*, 1975 and Braunwald, 1982). Moreover, drugs with positive inotropic properties such as the ß-adrenergic agonists act not only by augmenting Ca^{++} influx through the slow channels of the surface membrane at the time of depolarization but also by enhancing the release of Ca^{++} from intracellular stores which are not affected by calcium channel blockade and by increasing the sensitivity of the contractile system to Ca^{++}. No death occurred from ventricular fibrillation in the first experiment whereas 2 dogs died after developing this arrhythmia in the second experiment. This disparity could have been due to the duration allowed for the drug to exert its maximal antiarrhythmic action and hence protect the dogs from ventricular fibrillation and the subsequent death. This could be in agreement with the fact that after intravenous administration peak effects for verapamil are seen in 10-15 minutes (Stone *et al.*, 1980). Verapamil does not appear to be an effective drug for the treatment of ventricular arrhythmias (Singh et al., 1978 and Opie, 1980). This is further corroborated by the finding in the present study that verapamil was not effective in prevention of ventricular

arrhythmias induced by adrenaline in these halothane anaesthetized dogs.

Lidocaine is a short acting antiarrhythmic drug. Veterinarians have been using lidocaine hydrochloride for short-term (24 hr or less) emergency control of ventricular arrhythmias. The usual treatment method is to administer a 4 mg/kg intravenous bolus of lidocaine and immediately begin constant intravenous infusion at 50 μg/kg/min.(Muir and Lipowitz, 1978). In the first experiment all the 5 dogs developed ventricular arrhythmias whereas only 3 dogs with one dying developed the arrhythmia in the second experiment. Considering that the dose of lidocaine used in both experiments was the same the difference in the observation could only be explained by the time allowance before adrenaline was administered. For lidocaine to control ventricular arrhythmias a sustained infusion is required as its antiarrhythmic action wears off very fast. On the basis of the pharmacokinetic properties of lidocaine, its constant infusion assures a constant plasma concentration (Bigger, 1975). The one dog that died from ventricular fibrillation in the second experiment could have been from individual idiosyncrasy.

5.2. CONCLUSIONS

From the results obtained in this study the following general conclusions may be derived:

1. Propranolol remains the drug of choice in the

management of adrenaline induced ventricular arrhythmias in the dogs.

2. Propranolol is effective in protecting against adrenaline induced cardiac arrhythmias in halothane anaesthetized dogs when given at a dose of 0.5 mg/kg bwt. but not when given at lower doses (0.06 mg/kg bwt).

3. Verapamil intravenously is not a useful drug in the control of adrenaline induced arrhythmias in the dogs.

4. Drug pretreatments did not affect the levels of the serum enzyme LDH and α-HBDH in the dogs with adrenaline induced arrhythmias.

5. Drug pretreatments did not affect the serum calcium levels in dogs with adrenaline induced arrhythmias.

6. Drug pretreatments did not have any clinical significant effects on the electrocardiographic parameters.

7. Pretreatments with propranolol, lidocaine, and verapamil did not protect the dogs against adrenaline induced increase in arterial blood pressure.

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Appendix 1: ECG parameters for the control group receiving physiological saline.

Dog no.	Heart rhythm	Arrhythmias /min	Heart rate	P wave (sec)	P wave (mV)	P-R interval (sec)	QRS (sec)	R (mV)	S-T (sec)	S-T Depr. (mV)	S-T Elev. (mV)	T (mV)	Q-T Interval (sec)
A1A													
Before treatment	NSR	_	80	0.04	0.1	0.1	0.04	1.475	0.165	0.05	0	0.05 (-)	0.255
Drug	NSR	_	93	0.047	0.1	0.1	0.04	1.4	0.16	0.1	0	0.083 (-)	0.253
Adrenaline	2nd DHB	20	60	0.057	0.183	0.153	0.04	1.7	0.107	0.017	0	0.333 (+)	0.243
30 min later	NSR	_	107	0.06	0.183	0.113	0.04	1.333	0.1	0.017	0	0.1 (-)	0.233
A1B													
Before treatment	SAr.	_	94	0.053	0.183	0.12	0.04	1.55	0.06	0	0	0.183 (+)	0.287
Drug	SAr.	_	80	0.047	0.183	0.12	0.04	1.55	0.053	0	0	0.167 (+)	0.3
Adrenaline	VPB	Died											
A1C													
Before treatment	NSR	_	167	0.039	0.4	0.077	0.04	2.886	0.113	0.134	0	0.1 (-)	0.193
Drug	NSR	_	120	0.04	0.2	0.101	0.04	2.8	0.113	0.12	0	0.214 (-)	0.227
Adrenaline	VPB	Died											
A1D													
Before treatment	NSR	_	120	0.043	0.05	0.097	0.036	1.25	0.19	0.017	0	0.05 (+)	0.287
Drug	NSR	_	100	0.033	0.02	0.087	0.037	1.1	0.127	0	0	0.15 (-)	0.26
Adrenaline	VPB	Died											
AiE													
Before treatment	NSR	_	120	0.043	0.05	0.097	0.036	1.25	0.19	0.017	0	0.05 (+)	0.287
Drug	NSR	_	100	0.033	0.02	0.087	0.037	1.1	0.127	0	0	0.15 (-)	0.26
Adrenaline	VPB	Died											

Appendix 2: ECG parameters for the dogs receiving 0.1 mg/kg verapamil

Dog no.	Heart rhythm	Arrhythmias/min.	Heart rate	P wave (sec.)	P wave (mV)	P-R interval (sec.)	QRS (sec.)	R wave (mV.)	S-T (sec.)	S-T Dep. (mV)	S-T elev. (mV)	T wave (mV)	Q-T interval (sec.)
A2A													
Before treatment	NSR	_	200	0.047	0.2	0.08	0.03	1.3	0.083	0	0	0.067(+)	0.183
Drug	NSR	_	214	0.047	0.167	0.08	0.033	1.883	0.08	0.017	0	0.033(+)	0.173
Adrenaline	NSR	_	180	0.04	0.183	0.07	0.034	1.8	0.057	0	0	0.24 (+)	0.193
30 min. later	NSR	_	120	0.04	0.133	0.088	0.035	1.583					
ADB													
Before treatment	NSR	_	140	0.047	0.2	0.113	0.04	2.214	0.132	0.08	0	0.08	0.213
Drug	NSR	_	140	0.047	0.14	0.137	0.04	2.2	0.14	0.12	0	0.1	0.21
Adrenaline	VPB	80	160	0.047*	0.047	0.12	0.04*	2.4*	0.11*	0.06*	0*	0.134*	0.228*
30 min later	NSR		120	0.05	0.2	0.133	0.04	2.28	0.167	0.14	0	0.2 (-)	0.26
A2C													
Before treatment	NSR	_	120	0.041	0.1	0.097	0.04	1.923	0.087	0	0	0.083(-)	0.217
Drug	NSR	_	120	0.04	0.1	0.097	0.04	1.9	0.087	0	0	0.1 (-)	0.2
Adrenaline	2nd DHB	6	100	0.04	0.107	0.147	0.04	2.0	0.13	0	0	0.123(+)	0.297
30 min. later	NSR	_	107	0.04	0.1	0.103	0.04	1.9	_	0	_	_	_
A2D													
Before treatment	SAr.	_	87	0.043	0.233	0.13	0.04	3.017	0.19	0.083	0	0.1 (+)	0.297
Drug	SAr.	_	113	0.043	0.183	0.133	0.043	2.95	0.143	0.133	0	0.133(+)	0.293
Adrenaline	VT & 2nd DHB	193	193	0.067	0.183	0.207	0.04	2.86	0.12	0.067	0	0.5 (+)	0.23
30 min later	NSR	_	133	0.05	0.417	0.113	0.04	2.5	0.113	0.017	0	0.183 (-)	0.257
A2E													
Before treatment	NSR	_	147	0.057	0.25	0.09	0.04	1.7	0.05	0	0	0.233 (+)	0.217
Drug	NSR	_	140	0.057	0.233	0.09	0.04	1.7	0.063	0	0.05	0.167 (+)	0.22
Adrenaline	VPB	93	147	0.067	0.283	0.1	0.04	1.9	0.057	0	0.067	0.2 (+)	0.183
30 min. later	NSR	_	140	0.06	0.333	0.1	0.04	1.633	0.087	0	0	0.183 (+)	0.216

* Readings from immediately after arrhythmogenic region.

Appendix 3: ECG parameters for dogs receiving 0.06 mg/kg propranolol

Dog no.	Heart rhythm	Arrhythmias/min.	Heart rate	P wave (sec.)	P wave (mV)	P-R interval (sec.)	QRS (sec.)	R wave (mV.)	S-T (sec.)	S-T Dep. (mV)	S-T elev. (mV)	T wave (mV)	Q-T interval (sec.)
A3A													
Before treatment	NSR	_	160	0.035	0.4	0.041	0.04	2.157	0.137	0.067	0	0.067(+)	0.228
Drug	NSR	_	180	0.044	0.35	0.077	0.0414	2.57	0.123	0.003	0	0.03 (+)	0.2
Adrenaline	NSR	_	140	0.046	0.3	0.067	0.04	2.85	0.1	0	0	0.107	0.215
30 min. later	NSR	_	140	0.05	0.22	0.087	0.04	2.84	0.093	0.013	0	0.05	0.203
A3B													
Before treatment	NSR	_	100	0.043	0.15	0.107	0.04	1.373	0.1	0.067	0	0.2 (-)	0.26
Drug	NSR	_	100	0.067	0.107	0.113	0.04	1.333	0.113	0.05	0	0.167 (-)	0.267
Adrenaline	2nd DHB	6	54	0.047	0.117	0.16	0.04	1.767	0.12	0.1	0	0.2	0.287
30 min. later	NSR	_	107	0.045	0.09	0.158	0.033	1.75	0.127	0.033	0	0.2 (-)	0.26
A3C													
Before treatment	NSR	_	113	0.04	0.2	0.126	0.04	3.3	0.147	0.2	0	0.134 (-)	0.247
Drug	SAr.	_	100	0.043	0.186	0.14	0.04	3.246	0.113	0.06	0	0.234 (-)	0.267
Adrenaline	2nd DHB & VPB	100	200	0.04	0.166	0.3	0.04	3.734	0.167	0	0	0.1	0.287
30 min. later	SAr.	_	73	0.037	0.114	0.137	0.04	3.08	0.203	0.134	0	0.04 (-)	0.243
A3D													
Before treatment	NSR	_	193	0.043	0.4	0.08	0.04	1.65	0.23	0.067	0	0.1 (+)	0.217
Drug	NSR	_	127	0.04	0.333	0.1	0.04	2.033	0.12	0.033	0	0.183 (-)	0.24
Adrenaline	VPB	53	60	0.053	0.167	0.147	0.04	2.283	0.143	0.033	0	0.167(+)	0.283
30 min. later	NSR	_	120	0.04	0.3	0.1	0.04	2.033	0.147	0.067	0	0.15 (+)	0.243
A3E													
Before treatment	NSR	_	120	0.05	0.233	0.107	0.04	1.917	0.16	0	0	0.083(+)	0.257
Drug	NSR	_	120	0.05	0.2	0.1	0.04	1.867	0.127	0	0	0.1 (-)	0.23
Adrenaline	VPB	20	67	0.05	0.183	0.173	0.04	1.85	0.073	0	0	0.117(+)	0.237
30 min. later	SAr.	_	113	0.043	0.2	0.103	0.04	1.917	0.09	0	0	0.117(+)	0.19

Appendix 4: ECG parameters for dogs receiving 4 mg/kg lidocaine

	Heart rhythm	Arrhythmias /min	Heart rate	P wave (sec)	P wave (mV)	P-R interval (sec)	QRS (sec)	R wave (mV)	S-T (sec)	S-T Depr. (mV)	S-T Elev. (mV)	T (mV)	Q-T Interval (sec)
A4A													
Before treatment	NSR	_	140	0.05	0.25	0.11	0.04	1.133	0.127	0.073	0	0.167 (-)	0.19
Drug	NSR	_	127	0.043	0.2	0.11	0.04	1.317	0.127	0	0	0.1 (-)	0.18
Adrenaline	VPB	60	80	0.047	0.233	0.157	0.04	1.55	0.083	0	0.017	0.133 (+)	0.213
30 min later	SAr.	_	127	0.05	0.217	0.117	0.04	1.25	0.127	0	0	0.14 (-)	0.233
A4B													
Before treatment	NSR	_	180	0.055	0.383	0.093	0.04	2.783	0.103	0	0.023	0.05 (-)	0.2
Drug	NSR	_	173	0.52	0.5	0.09	0.04	2.966	0.08	0	0.046	0.08 (+)	0.18
Adrenaline	VPB	80	173	0.053	0.266	0.092	0.04	3.1	0.073	0	0.026	0.04 (-)	0.157
30 min later	NSR	_	207	0.037	0.2	0.08	0.04	3.334	0.067	0	0.034	0.234 (+)	0.18
A4C													
Before treatment	NSR	_	214	0.05	0.2	0.1	0.04	2.234	0.063	0	0.2	0.134 (-)	0.16
Drug	NSR	_	200	0.05	0.2	0.1	0.04	2.234	0.073	0	0	0.1 (-)	0.173
Adrenaline	VPB	100	160	0.05	0.2	0.11	0.04	2.766	0.06	0	0	0.6 (-)	0.207
30 min. later	NSR	_	190	0.06	0.2	0.105	0.04	2.5					
A4D													
Before treatment	NSR	_	120	0.028	0.483	0.09	0.03	2.467	0.077	0	0	0.19 (+)	0.217
Drug	NSR	_	100	0.043	0.2	0.1	0.04	2.8	0.077	0	0	0.183(+)	0.217
Adrenaline	VT	233	247	0.04*	0.383*	0.117*	0.04*	2.85*	0.067*	0.033*	0*	0.15 (+)*	0.207*
30 min. later	SAr.	_	100	0.037	0.333	0.11	0.04	2.65	0.087	0.073	0	0.107(+)	0.24
A4E													
Before treatment	SAr.	_	160	0.043	0.466	0.08	0.04	4.034	0.12	0.046	0	0.06 (-)	0.243
Drug	SAr.	_	147	0.04	0.226	0.1	0.04	4.1	0.15	0.066	0	0.1 (-)	0.24
Adrenaline	VPB	53	107	0.05	0.166	0.123	0.04	3.966	0.103	0.114	0	0.166(+)	0.22
30 min. later	SAr.	_	120	0.04	0.234	0.1	0.04	3.866	0.15	0	0	0.08 (-)	0.24

* Readings from immediately after arrhythmogenic region.

Appendix 5: ECG parameters for dogs receiving physiological saline

Dog no.	Heart rhythm	R-R interval	Arrhythmias in min.	Heart rate	P wave (sec.)	P wave (mV)	P-R interval (sec.)	QRS (sec.)	R wave (mV.)	S-T (sec.)	S-T Dep. (mV)	S-T elev. (mV)	T wave (mV)	Q-T interval (sec.)
B1A														
Before treatment	SAr	0.59	-	100	0.043	0.117	0.08	0.04	2.15	0.083	0	0.017	0.283	0.283
Drug	SAr	0.58	-	107	0.047	0.117	0.08	0.04	2..117	0.077	0	0	0.25	0.3
Adrenaline	VPB	0.6	VF -Death	113	0.035	0.125	0.08	0.04	2.0	0.14	0	0.05	0.175	0.275
B1B														
Before treatment	SAr	0.61	-	100	0.05	0.2	0.097	0.04	3.667	0.187	0.1	0	0.2 (-)	0.3
Drug	SAr	0.63	-	100	0.05	0.267	0.1	0.04	3.667	0.183	0.1	0	0.2 (-)	0.3
Adrenaline	VPB	0.5	140	120	0.04	0.2	0.11	0.04	4.0	0.12	0	0	0.3 (-)	0.25
30 min. later	NSR	0.46	-	133	0.05	0.133	0.1	0.04	3.5	0.067	0.067	0	0.147 (-)	0.28
B1C														
Before treatment	SAr	0.61	-	100	0.047	0.133	0.12	0.04	1.983	0.157	0.1	0	0.2	0.26
Drug	SAr	0.62	-	100	0.047	0.1	0.127	0.04	2.067	0.147	0.083	0	0.233	0.26
Adrenaline	VPB	0.48	VF-Death	113	0.043	0.117	0.107	0.04	2.217	0.08	0.033	0	0.4	0.267
B1D							-							
Before treatment	SAr	0.62	-	100	0.04	0.2	0.113	0.043	3.3	0.193	0.033	0	0.2 (-)	0.313
Drug	SAr	0.67	-	93	0.043	0.2	0.123	0.043	3.367	0.2	0.033	0	0.2 (-)	0.323
Adrenaline	VPB	0.59	93	153	0.047	0.2	0.113	0.04	3.8	0.13	0	0	0.2 (+)	0.23
30 min. later	NSR	0.57	-	120	0.47	0.2	0.12	0.043	3.2	0.19	0.1	0	0.2 (-)	0.333
B1E														
Before treatment	SAr	0.61	-	100	0.04	0.133	0.1	0.04	1.5	0.217	0.05	0	0.117 (-)	0.32
Drug	SAr	0.65	-	100	0.04	0.117	0.097	0.04	1.483	0.22	0.05	0	0.15 (-)	0.33
Adrenaline	VPB	0.77	VF-Death	80	0.043	0.133	0.093	0.04	1.5	0.22	.083	0	0.283 (-)	0.347

Appendix 6: ECG parameters for dogs receiving Verapamil 0.1 mg/kg

Dog no.	Heart rhythm	R-R interval	Arrhythmias in min.	Heart rate	P wave (sec.)	P wave (mV)	P-R interval (sec.)	QRS (sec.)	R wave (mV.)	S-T (sec.)	S-T Dep. (mV)	S-T elev. (mV)	T wave (mV)	Q-T interval (sec.)
B2A														
Before treatment	SAr	0.55	-	113	0.04	0.2	0.08	0.04	1.817	0.107	0	0	0.317	0.287
Drug	SAr	0.57	-	107	0.05	0.233	0.08	0.04	1.917	0.116	0.017	0.017	0.317	0.297
Adrenaline	SAr	0.58	-	100	0.053	0.267	0.09	0.04	1.95	0.127	0	0	0.3	0.3
30 min. later	NSR	0.54	-	120	0.053	0.283	0.093	0.04	1.433	0.123	0	0	0.25	0.3
B2B														
Before treatment	SAr	0.70	-	93	0.04	0.167	0.1	0.04	3.2	0.177	0.133	0	0.133 (-)	0.267
Drug	SAr	0.63	-	100	0.04	0.2	0.09	0.04	3.067	0.21	0.133	0	0.2 (-)	0.283
Adrenaline	VPB	0.61		107	0.043	0.1	0.103	0.04	3.2	0.203	0	0	0.267(+)	0.32
B2C														
Before treatment	SAr	0.77	-	87	0.05	0.167	0.113	0.047	2.567	0.133	0.05	0	0.167	0.333
Drug	SAr	0.9	-	73	0.053	0.2	0.12	0.047	2.4	0.21	0.083	0	0.067	0.337
Adrenaline	VPB	0.68		100	0.047	0.15	0.11	0.047	2.383	0.12	0.05	0	0.367	0.293
B2D														
Before treatment	NSR	0.56	-	120	0.05	0.117	0.103	0.053	1.2	0.133	0.05	0	0.1	0.26
Drug	NSR	0.5	-	120	0.047	0.133	0.107	0.05	1.267	0.147	0.05	0	0.1	0.26
Adrenaline	VPB & 2nd DHB	0.61	10	87	0.047	0.15	0.15	0.053	1.583	0.083	0	0	0.35	0.247
30 min. later	NSR	0.58	-	100	0.04	0.15	0.107	0.047	1.2	0.14	0.017	0	0.117	0.28
B2E														
Before treatment	NSR	0.42	-	140	0.047	0.3	0.09	0.04	3.2	0.16	0.033	0	0.1 (+)	0.25
Drug	NSR	0.44	-	140	0.05	0.2	0.1	0.04	3.133	0.137	0	0	0.167(+)	0.233
Adrenaline	VPB	0.37	78	173	0.04	0.2	0.083	0.043	3.2	0.08	0.033	0	0.3 (-)	0.2
30 min. later	NSR	0.54	-	120	0.053	0.2	0.11	0.04	3.0	0.153	0.067	0	0.2 (-)	0.247

Appendix 7: ECG parameters for dogs receiving 0.5 mg/kg propranolol

Dog no.	Heart rhythm	R-R interval	Arrhythmias in min.	Heart rate	P wave (sec.)	P wave (mV)	P-R interval (sec.)	QRS (sec.)	R wave (mV.)	S-T (sec.)	S-T Dep. (mV)	S-T elev. (mV)	T wave (mV)	Q-T interval (sec.)
B3A														
Before treatment	SAr	0.49	-	127	0.033	0.05	0.107	0.04	2.783	0.093	0.033	0	0.117	0.237
Drug	SAr	0.61	-	100	0.04	0.167	0.1	0.04	2.933	0.127	0.033	0	0.117	0.24
Adrenaline	VPB	0.49	14	120	0.05	0.133	0.103	0.04	3.217	0.123	0.067	0	0.117	0.22
30 min. later	SAr	0.61	-	100	0.033	0.083	0.113	0.04	2.833	0.15	0	0.033	0.05	0.253
B3B														
Before treatment	NSR	0.53	-	100	0.04	0.2	0.1	0.04	2.25	0.115	0	0	0.1	0.235
Drug	NSR	0.5	-	127	0.04	0.2	0.1	0.04	2.033	0.117	0	0	0.083	0.237
Adrenaline	Bradycadia	0.92	-	67	0.04	0.133	0.14	0.04	2.717	0.143	0.067	0	0.133	0.28
30 min. later	NSR	0.56	-	110	0.04	0.2	0.1	0.04	2.15	0.165	0.125	0	0.1	0.265
B3C														
Before treatment	NSR	0.7	-	80	0.053	0.133	0.123	0.04	1.7	0.12	0.033	0	0.467	0.32
Drug	NSR	0.78	-	80	0.05	0.15	0.137	0.04	1.65	0.12	0.033	0	0.5	0.327
Adrenaline	Bradycardia	1.42	-	53	0.043	0.1	0.147	0.047	2.033	0.137	0.067	0	0.417	0.32
30 min. later	NSR	0.67	-	100	0.053	0.133	0.12	0.043	1.9	0.233	0.083	0	0.35	0.423
B3D														
Before treatment	NSR	0.5	-	120	0.043	0.2	0.1	0.04	2.133	0.143	0.05	0	0.133	0.257
Drug	NSR	0.51	-	120	0.053	0.2	0.11	0.04	2.117	0.157	0.083	0	0.133	0.267
Adrenaline	Bradycardia	0.77	-	80	0.06	0.133	0.127	0.04	2.3	0.04	0	0.05	0.383	0.3
30 min. later	NSR	0.52	-	120	0.05	0.167	0.064	0.04	2.483	0.197	0.05	0.033	0.1	0.3
B3E														
Before treatment	NSR	0.47	-	140	0.04	0.1	0.093	0.04	1.833	0.12	0.033	0	0.083	0.227
Drug	NSR	0.56	-	133	0.04	0.1	0.103	0.047	1.8	0.163	0.033	0	0.15	0.293
Adrenaline	Bradycadia	0.78	-	87	0.037	0.05	0.1	0.047	2.083	0.177	0.1	0	0.167	0.293
30 min. later	NSR	0.66	-	100	0.04	0.1	0.103	0.043	1.8	0.187	0.083	0	0.233	0.323

Appendix 8 ECG parameters for dogs receiving 4 mg/kg lidocaine

Dog no.	Heart rhythm	R-R interval	Arrhythmias in min.	Heart rate	P wave (sec.)	P wave (mV)	P-R interval (sec.)	QRS (sec.)	R wave (mV.)	S-T (sec.)	S-T Dep. (mV)	S-T elev. (mV)	T wave (mV)	Q-T interval (sec.)
B4A														
Before treatment	NSR	0.55	-	107	0.04	0.133	0.097	0.04	1.05	0.133	0.033	0	0.283	0.34
Drug	NSR	0.62	-	100	0.043	0.117	0.103	0.04	1.033	0.157	0.033	0	0.217	0.327
Adrenaline	Bradycardia	0.92	-	67	0.04	0.1	0.113	0.04	1.35	0.163	0	0	0.15	0.47
30 min. later	NSR	0.49	-	127	0.053	0.183	0.1	0.04	0.817	0.12	0.05	0	0.167	0.347
B4B														
Before treatment	NSR	0.36	-	173	0.04	0.083	0.1	0.04	1.017	0.077	0	0	0.04	0.173
Drug	NSR	0.48	-	120	0.047	0.117	0.107	0.043	1.083	0.123	0	0	0.03	0.22
Adrenaline	NSR	0.46	-	133	0.047	0.1	0.11	0.047	1.183	0.087	0	0.017	0.1	0.227
30 min. later	NSR	0.48	-	120	0.05	0.1	0.11	0.05	0.717	0.09	0	0	0.037	0.203
B4C														
Before treatment	SAr	0.57	-	107	0.047	0.2	0.117	0.04	4.167	0.133	0	0	0.333	0.28
Drug	SAr	0.58	-	100	0.053	0.2	0.12	0.04	4.6	0.16	0.067	0	0.2	0.267
Adrenaline	VPB & 2nd HDB	1.1	5	73	0.077	0.2	0.17	0.057	4.6	0.1	0.133	0	0.733	0.253
30 min. later	SAr	0.59	-	100	0.05	0.2	0.12	0.04	4.133	0.137	0.033	0	0.333	0.297
B4D														
Before treatment	NSR	0.62	-	100	0.04	0.05	0.103	0.04	1.2	0.21	0.05	0	0.1	0.32
Drug	NSR	0.61	-	100	0.04	0.05	0.113	0.043	1.25	0.193	0.067	0	0.083	0.317
Adrenaline	VPB	0.57	46	113	0.043	0.083	0.13	0.043	1.417	0.08	0.017	0.033	0.333	0.32
30 min. later	NSR	0.61	-	100	0.037	0.067	0.1	0.043	1.2	0.21	0.067	0.15	0.183	0.323
B4E														
Before treatment	NSR	0.52	-	120	0.05	0.2	0.1	0.05	2.2	0.137	0.117	0	0.333 (-)	0.247
Drug	NSR	0.58	-	100	0.05	0.2	0.11	0.05	2.4	0.14	0.183	0	0.233 (-)	0.243
Adrenaline	VPB	0.43	Death	133	0.047	0.183	0.09	0.047	2.25	0.073	0	0.017	0.217(+)	0.23

Appendix 9: α-HBDH, LDH and calcium serum levels in dogs receiving physiological saline.

Dog no.	0 hr	1/4 hr	1/2 hr	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr	48 hr	72 hr
B1A											
HBDH (IU/L)	45										
LDH (IU/L)	19										
Calcium (g/100 ml)	10.95										
B1B											
HBDH (IU/L)	63.58	29.07		49.05	70.85	76.3	123.53	21.8	45.42	49.05	47.23
LDH (IU/L)	281	207		218	200	291	548	67	184	230	172
Calcium (g/100 ml)	9.36	8.78		8.45	8.38	9.47	9.79	10.59	10.99	9.47	8.93
B1C											
HBDH(IU/L)	32.7										
LDH(IU/L)	27										
Calcium (g/100 ml)	9.85										
B1D											
HBDH (IU/L)	47.23	34.52	29.07	49.05	39.97	41.78	39.92		47.23	29.07	32.7
LDH (IU/L)	110	159	88	120	137	101	65		61	31	154
Calcium (g/100 ml)	11.05	10.94	10.86	10.72	10.08	9.05	10.03		9.53	10.08	10.18
B1E											
HBDH (IU/L)	39.97										
LDH (IU/L)	79										
Calcium (g/100 ml)	10.46										

Appendix 10: α-HBDH, LDH and calcium serum levels in dogs receiving 0.1 mg/kg verapamil.

Dog no.	0 hr	1/4 hr	1/2 hr	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr	48 hr	72 hr
B2A											
HBDH (IU/L)	27.25	38.15	39.97	38.15	50.87	30.88	65.4	39.97	54.5	32.7	43.6
LDH (IU/L)	43	135	35	92	81	101	264	104	94	73	67
Calcium (g/100 ml)	12.82	12.06	11.06	12.05	11.61	10.47	10.29	9.56	9.39	10.80	10.25
B2B											
HBDH (IU/L)	34.52										
LDH (IU/L)	6										
Calcium (g/100 ml)	10.58										
B2C											
HBDH (IU/L)	38.15										
LDH (IU/L)	37										
Calcium (g/100 ml)	8.02										
B2D											
HBDH (IU/L)	34.52	45.42	63.58	29.07	67.22	159.87	74.48	45.42	96.28	39.97	61.77
LDH (IU/L)	35	81	29	36	87	69	112	79		127	246
Calcium (g/100 ml)	9.71	9.70	9.44	10.00	10.15	9.27	10.62	11.28	11.22	10.91	10.07
B2E											
HBDH (IU/L)	25.43	27.25	21.8	29.07	63.58	29.07	27.25	23.62	47.23	21.8	74.48
LDH (IU/L)	59	50	202	46	45	102	93	49	141	84	265
Calcium (g/100 ml)	10.21	8.91	9.45	9.39	9.25	9.82	10.63	11.39	12.27	10.48	11.65

Appendix 11: α-HBDH, LDH and calcium serum levels in dogs receiving 0.5 mg/kg propranolol.

Dog no.	0 hr	1/4 hr	1/2 hr	1 hr	2 hr	4 hr	8 hr	12 hr	24 hr	48 hr	72 hr
B3A											
HBDH (IU/L)	34.52	32.7	19.98	19.98	45.42	38.15	41.78	56.32	47.23	39.97	56.32
LDH (IU/L)	29	82	37	39	66	86	65	37	63	26	39
Calcium (g/100 ml)	10.71	12.01	12.88	11.74	13.83	10.27	8.90	10.35	9.03	10.12	9.74
B3B											
HBDH (IU/L)	87.2	89.02	41.78	49.05	47.23	39.97	21.8	14.53	49.05	34.52	30.88
LDH (IU/L)	94	197	85	85	84	95	122	76	112	93	64
Calcium (g/100 ml)	12.12	10.75	9.57	8.77	10.65	9.49	8.77	9.72	9.12	8.94	10.77
B3C											
HBDH (IU/L)	25.43	25.43	38.15	32.7	61.77	58.13	54.5	94.47	34.52	29.07	56.32
LDH (IU/L)	95	91	79	63	165	240	239	313	87	141	235
Calcium (g/100 ml)	10.19	10.46	10.92	10.81	10.57	9.29	10.19	10.54	10.30	10.82	10.59
B3D											
HBDH (IU/L)	118.08	99.92	23.62	70.85	41.78	36.33	45.42	29.07	34.52	19.98	36.33
LDH (IU/L)	39	48	117	127	71	90	126	73	58	75	113
Calcium (g/100 ml)	11.74	12.18	13.04	12.96	11.22	11.44	11.81	11.29	11.82	9.53	11.33
B3E											
HBDH (IU/L)	69.03	90.83	103.55	92.65	45.42	72.67	41.78	43.6	36.33	105.37	61.77
LDH (IU/L)	146	134	108	74	138	311	143	68	91	241	188
Calcium (g/100 ml)	10.36	10.77	10.38	10.64	10.96	10.86	9.21	9.52	9.50	9.68	10.64

Appendix 13: Hematological parameters from the dog no. A1A treated with physiological saline.

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	49.7	47.2	55.4	55.9	56.7	52.8	55.4	53.3	50.1	51.7	55.1
PCV. (M) (%)	45	42	42	48	49	45	46	48	40	43	45
TP. (g/100 ml)	8.2	7.2	7.4	8.2	8.4	8.0	7.8	8.0	7.4	7.2	8.4
Hb. (g/100 ml)	16.2	16.7	18.9	18.3	19.0	17.9	18.6	18.1	17.5	19.3	18.7
RBC. ($\times 10^6$)	7.14	6.94	8.18	8.05	8.15	7.12	8.28	7.76	7.24	7.76	8.15
WBC. count	12,000	11,200	16,000	15,00	16,600	17,700	22,400	19,700	17,100	13,700	15,600
TN. (%)	72	71	68	65	65	78	81	72	73	79	66
Immat. N. (%)	0	1	0	1	0	2	4	3	8	0	1
Lymph. (%)	27	26	28	31	31	19	15	23	19	21	32
Monocyt. (%)	0	1	0	0	0	1	0	0	0	0	0
Eosinoph. (%)	1	1	4	3	4	0	0	2	0	0	1
Basoph. (%)	0	0	0	0	0	0	0	0	0	0	0
MCV. (cu microns)	72	71	70	72	72	71	70	71	72	70	70

Appendix 14: Hematological parameters from the dog no. A1C treated with physiological saline.

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	35.5	36.3	36.6	39.8	42.8	40.1	42.8	40.0	43.0	41.8	39.3
PCV. (M) (%)	31	32	30	35	41	31	38	32	36	35	33
TP. (g/100 ml)	6.2	6.0	6.8	6.4	7.0	7.0	6.2	6.4	6.0	6.4	6.2
Hb. (g/100 ml)	12.3	13.5	12.9	13.8	15.2	14.2	11.7	14.2	14.0	13.9	13.2
RBC. ($\times 10^6$)	5.72	5.9	5.78	6.34	7.18	6.64	6.83	6.79	6.95	6.64	6.19
WBC. count	6,100	6,400	5,700	6,600	7,600	8,600	10,600	10,900	9,200	9,700	8,500
TN. (%)	78	63	65	62	68	75	81	78	73	64	73
Immat. N. (%)	1	0	1	0	0	2	0	0	0	1	2
Lymph. (%)	21	36	34	37	32	22	19	22	27	33	22
Monocyt. (%)	0	0	0	0	0	0	0	0	0	0	0
Eosinoph. (%)	0	1	0	1	0	1	0	0	0	2	3
Basoph. (%)	0	0	0	0	0	0	0	0	0	0	0
MCV. (cu microns)	64	63	65	65	62	63	65	61	64	65	65

Appendix 15: Hematological parameters from the dog no A2A treated with verapamil.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	52.4	57.7	48.6	51.3	57.2	54	54.9	58	60	58	52.4
PCV. (M)	(%)	46	50	45	43	47	45	46	49	49	52	40
TP.	(g/100 ml)	5.4	5.2	5.0	5.4	5.4	5.8	6.0	6.2	5.6	5.8	5.2
Hb.	(g/100 ml)	18.8	19.9	17.8	16.8	19.6	18.5	18.8	20.3	19.7	17.1	19.6
RBC.	($x 10^6$)	7.39	8.20	6.87	6.80	7.86	7.54	7.96	8.17	8.01	8.11	7.57
WBC.	count	9,300	9,500	8,500	8,600	13,700	13,600	13,400	9,600	8,500	7,000	8,900
TN.	(%)	83	72	83	83	73	84	81	79	82	69	76
Immat. N.	(%)	2	2	2	2	2	1	1	0	1	0	0
Lymph.	(%)	10	18	12	13	19	13	14	18	16	28	19
Monocyt.	(%)	0	0	0	0	0	0	0	0	0	0	0
Eosinoph.	(%)	5	7	3	2	5	2	4	3	1	3	5
Basoph.	(%)	0	0	0	0	0	0	0	0	0	0	0
MCV.	(cu microns)	74	73	73	78	76	75	72	74	78	75	72

Appendix 16: Hematological parameters from the dog no. A2B treated with verapamil.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	39.9	40.8	36.8	57.1	40.1	40.1	39.4	37.2	37.7	38.1	36.6
PCV. (M)	(%)	37	37	35	35	34	32	35	35	35	33	30
TP.	(g/100 ml)	6.0	6.0	5.5	6.0	6.4	5.2	6.0	5.8	6.0	7.0	7.0
Hb.	(g/100 ml)	14.6	14.3	12.3	12.9	13.0	12.4	13.9	12.6	12.8	12.8	12.8
RBC.	($x 10^6$)	5.79	6.12	5.40	5.87	5.96	5.58	5.85	5.52	5.41	5.59	5.48
WBC.	count	3,800	4,700	4,100	4,800	8,400	3,700	8,700	10,900	11,700	4,500	8,900
TN.	(%)	27	41	49	48	48	56	64	60	54	41	56
Immat. N.	(%)	0	0	0	1	0	0	4	1	0	0	0
Lymph.	(%)	70	51	47	41	48	41	30	34	41	55	41
Monocyt.	(%)	0	1	0	0	0	0	0	0	0	0	0
Eosinoph.	(%)	3	7	4	9	4	3	2	5	5	4	3
Basoph.	(%)	0	0	0	0	0	0	0	0	0	0	0
MCV.	(cu microns)	70	68	69	69	69	72	69	69	71	70	68

Appendix 17: Hematological parameters from the dog no. A2C treated with verapamil.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	34.3	36	33.6	32.6	36.7	38.0	37.1	37.0	34.2	31.4	33.8
PCV. (M)	(%)	32	30	29	28	32	30	31	31	30	29	29
TP.	(g/100 ml)	8.4	8.2	8.2	8.4	9.0	9.8	9.6	9.0	9.6	8.4	9.2
Hb.	(g/100 ml)	10.7	11.0	9.8	9.4	11.0	12.5	12.3	11.5	11.4	10.9	10.6
RBC.	(x 10^6)	4.35	4.63	4.32	4.17	4.72	4.96	4.74	4.56	4.52	4.14	4.51
WBC.	count	9,200	10,400	8,300	8,200	8,100	9,400	17,000	20,00	13,200	11,600	13,000
TN.	(%)	70	53	54	65	68	63	80	83	79	85	73
Immat. N.	(%)	0	1	0	2	0	0	2	2	0	0	0
Lymph.	(%)	21	33	38	24	27	32	16	14	17	11	19
Monocyt.	(%)	0	0	0	0	0	0	0	0	0	0	0
Eosinoph.	(%)	9	13	8	9	5	5	2	1	4	4	8
Basoph.	(%)	0	0	0	0	0	0	0	0	0	0	0
MCV.	(cu microns)	79	79	79	79	79	78	79	82	77	76	75

Appendix 18: Hematological parameters from the dog no. A2D treated with verapamil.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	48.2	52.4	42.1	48.4	49.2	51.3	48.8	50.7	52.7	49.5	53.0
PCV. (M)	(%)	37	47	38	45	45	44	40	41	42	41	42
TP.	(g/100 ml)	7.2	6.8	7.2	7.2	7.2	7.2	7.2	7.2	7.2	7.2	6.6
Hb.	(g/100 ml)	15.1	18.7	15.5	17.2	17.8	16.7	18.4	18.8	12.9	10.8	17.1
RBC.	(x 10^6)	6.31	7.69	5.99	6.76	7.02	6.56	6.47	6.51	6.40	6.18	6.33
WBC.	count	7,300	10,900	10,900	16,000	13,400	11,600	14,100	12,900	10,700	20,100	16,700
TN.	(%)	82	71	87	81	86	62	72	70	68	65	64
Immat. N.	(%)	0	0	0	0	0	0	0	0	1	0	0
Lymph.	(%)	16	27	13	19	14	30	24	27	26	30	27
Monocyt.	(%)	0	0	0	0	0	0	0	0	0	0	0
Eosinoph.	(%)	2	2	0	0	0	8	4	3	5	5	9
Basoph.	(%)	0	0	0	0	0	0	0	0	0	0	0
MCV.	(cu microns)	78	71	72	74	73	80	78	81	85	82	86

Appendix 19: Hematological parameters from the dog no A2E treated with verapamil.

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	36.0	45.0	36.8	44.6	43.7	45.9	48.5	-	47.0	48.8	-
PCV. (M) (%)	32	39	29	35	34	38	42	-	42	35	-
TP. (g/100 ml)	7.2	7.0	7.0	7.6	7.6	7.6	7.8	-	7.4	7.6	-
Hb. (g/100 ml)	10.7	16.4	13.7	15.8	15.7	14.2	17.3	-	16.9	14.9	-
RBC. ($x 10^6$)	5.21	6.61	5.33	6.39	6.43	6.60	6.73	-	6.69	6.30	-
WBC. count	5,300	6,700	5,200	6,500	8,300	8,500	9,600	-	7,800	7,400	-
TN. (%)	74	65	62	73	76	79	72	-	62	70	-
Immat. N. (%)	0	0	0	0	0	0	0	-	0	0	-
Lymph. (%)	16	28	24	20	14	11	21	-	29	11	-
Monocyt. (%)	0	0	0	0	1	0	0	-	0	0	-
Eosinoph. (%)	10	7	14	7	9	10	7	-	9	11	-
Basoph. (%)	0	0	0	0	0	0	0	-	0	0	-
MCV. (cu microns)	70	71	70	72	70	72	75	-	72	72	-

Appendix 20: Hematological parameters from the dog no. A3A treated with propranolol (0.06 mg/kg bwt.).

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	44.3	49.1	50.8	53.4	47.4	432.7			53.5	52.4	
PCV. (M) (%)	35	43	42	48	38	38			47	44	
TP. (g/100 ml)	7.2	7.0	8.0	7.2	7.4	7.0			7.4	8.0	
Hb. (g/100 ml)	16.5	17.2	18.3	18.4	16.9	15.2			20.9	19.2	
RBC. ($x 10^6$)	6.59	7.17	7.63	7.69	7.01	6.38			8.00	7.84	
WBC. count	8,200	8,300	10,200	12,600	12,600	11,100			12,200	12,800	
TN. (%)	57	60	58	69	72	72			84	77	
Immat. N. (%)	3	0	2	2	0	2			0	0	
Lymph. (%)	37	34	33	22	27	22			16	23	
Monocyt. (%)	0	0	0	0	0	0			0	0	
Eosinoph. (%)	3	6	7	7	1	4			0	0	
Basoph. (%)	0	0	0	0	0	0			0	0	
MCV. (cu microns)	69	71	69	72	70	71			70	69	

Appendix 21 : Hematological parameters from the dog no. A3B treated with propranolol (0.06 mg/kg bwt.).

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	37.5	40.9		44.9	46.1	45.8	44.3	38.5	46.1	40.9	39
PCV. (M) (%)	34	31		41	37	36	35	30	39	34	30
TP. (g/100 ml)	7.6	7.6		8	8	7.6	7.8	7.4	7.8	7.6	7.0
Hb. (g/100 ml)	12.5	12.5		15.2	15.1	13.4	15.1	15.2	15.5	14.4	13.1
RBC. ($\times 10^6$)	5.98	5.98		7.17	7.29	6.43	7.38	6.65	7.22	6.55	6.39
WBC. count	3500	3,500		5,000	6,000	7,300	8,200	7,300	5,200	6,600	7,200
TN. (%)	58	66		65	56	78	69	68	63	69	66
Immat. N. (%)	0	0		0	0	0	0	0	1	0	2
Lymph. (%)	38	32		29	36	20	29	29	30	26	29
Monocyt. (%)	0	0		0	0	0	0	0	0	0	0
Eosinoph. (%)	4	2		6	8	2	2	3	6	5	4
Basoph. (%)	0	0		0	0	0	0	0	0	0	0
MCV. (cu microns)	64	70		65	66	74	63	59	67	65	

Appendix 22: Hematological parameters from the dog no. A3C treated with propranolol (0.06 mg/kg bwt.).

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	51.8	56.7	49.4	59.7	59.2	48.5	53	53.4	54	53.9	56.1
PCV. (M) (%)	45	45	43	45	47	43	45	45	45	48	49
TP. (g/100 ml)	7.0	6.4	6.6	7.4	7.2	6.2	7.0	7.2	7.0	7.0	7.2
Hb. (g/100 ml)	16.5	20.4	17.2	19.7	19.3	15.5	17.6	17.4	18.1	17.0	18.3
RBC. ($\times 10^6$)	6.88	8.13	6.98	7.93	7.89	6.22	7.14	7.24	7.46	7.39	7.77
WBC. count	12,500	14,500	11,100	15,400	14,200	13,800	19,500	15,600	14,200	12,800	12,700
TN. (%)	55	56	1	52	50	69	66	62	54	8	50
Immat. N. (%)	0	0	0	0	0	0	0	0	0	0	0
Lymph. (%)	37	40	38	38	35	29	23	29	36	39	43
Monocyt. (%)	0	0	0	0	0	0	0	0	0	0	0
Eosinoph. (%)	8	4	11	10	15	10	0	10	10	13	7
Basoph. (%)	0	0	0	0	0	0	0	0	0	0	0
MCV. (cu microns)	78	73	73	78	78	78	77	76	75	75	75

Appendix 23: Hematological parameters from the dog no. A3D treated with propranolol (0.06 mg/kg bwt.).

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	46.3	60.2	47	52.6	56.7	52.6	57	55.8	60.8	70.6	53.4
PCV. (M) (%)	37	48	41	41	48	40	48	48	48	51	49
TP. (g/100 ml)	8.0	7.0	7.4	8.0	7.8	7.8	7.8	7.8	8.0	7.2	7.6
Hb. (g/100 ml)	14.4	19.6	15.3	17.5	16.8	17.4	19.0	17.9	18.2	20.0	18.4
RBC. ($x 10^6$)	6.61	8.52	6.81	7.93	7.91	7.39	8.37	8.20	8.18	10.0	8.17
WBC. count	6,100	8,700	7,500	9,000	10,500	12,300	15,000	7.2	8,600	8,600	11,200
TN. (%)	46	55	64	65	64	70	60	67	69	52	63
Immat. N. (%)	0	0	0	0	0	0	0	0	0	0	0
Lymph. (%)	36	30	24	21	24	19	25	26	19	34	27
Monocyt. (%)	0	0	0	0	0	0	0	0	0	0	0
Eosinoph. (%)	18	15	12	14	12	18	15	7	12	14	10
Basoph. (%)	0	0	0	0	0	0	0	0	0	0	0
MCV. (cu microns)	72	74	72	69	75	74	71	71	77	73	72

Appendix 24 : Hematological parameters from the dog no. A3E treated with propranolol (0.06 mg/kg bwt.).

	0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC) (%)	36.2	38.5	33.0	33.4	35.4	35.3	35.9	36.2	40.0	37.5	39.3
PCV. (M) (%)	30	36	30	31	33	32	31	33	34	33	33
TP. (g/100 ml)	6.0	6.0	6.0	6.6	6.6	6.2	6.2	6.2	6.2	6.2	6.2
Hb. (g/100 ml)	12.3	14.6	12.3	12.8	11.6	12.0	10.1	12.0	-	11	9.5
RBC. ($x 10^6$)	4.93	5.63	4.86	4.97	5.25	5.21	5.33	5.46	5.98	5.52	5.73
WBC. count	5,700	6,100	4,200	5,800	6,300	6,700	8,200	9,000	11,900	9,400	9,000
TN. (%)	43	41	45	44	41	62	67	54	66	45	53
Immat. N. (%)	0	0	0	0	0	0	0	0	0	0	0
Lymph. (%)	35	43	41	29	39	23	19	24	23	38	35
Monocyt. (%)	0	0	0	1	0	0	0	0	0	0	0
Eosinoph. (%)	12	16	14	26	20	15	14	10	11	17	12
Basoph. (%)	0	0	0	0	0	0	0	0	0	0	0
MCV. (cu microns)	75	70	69	69	69	69	69	69	69	70	70

Appendix 25: Hematological parameters from the dog no. A4A treated with lidocaine.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	51.4	58.6	44.9	52.2	67.0	56.8	58.8	58.8	59.4	58.4	58.2
PCV. (M)	(%)	47	48	41	43	46	47	50	50	47	47	47
TP.	(g/100 ml)	7.8	7.2	7.2	7.2	7.4	7.4	7.0	7.0	7.0	7.8	7.4
Hb.	(g/100 ml)	18.2	20.3	16.7	18.4	19.2	19.8	14.8	19.6	19.4	21.2	19.0
RBC.	($\times 10^6$)	8.02	9.70	7.08	8.00	8.52	8.68	9.88	8.48	8.69	8.71	7.54
WBC.	count	6,500	11,100	6,700	7,500	8,100	10,200	13,600	11,800	13,300	10,100	9,100
TN.	(%)	72	62	66	65	81	76	80	81	78	76	68
Immat. N.	(%)	0	0	0	0	0	0	3	0	0	0	1
Lymph.	(%)	16	25	25	21	13	16	14	16	19	15	24
Monocyt.	(%)	0	0	0	0	0	0	0	0	0	0	0
Eosinoph.	(%)	2	13	9	4	5	8	3	3	3	9	7
Basoph.	(%)	0	0	0	1	0	0	0	0	0	0	0
MCV.	(cu microns)	67	70	66	68	68	68	69	72	71	70	67

Appendix 26: Hematological parameters from the dog no. A4B treated with lidocaine.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	42	45.6	38.5	46	39	37.2	39.3	40.8	43.7	37.0	43.5
PCV. (M)	(%)	32	37	31	34	30	29	32	35	36	30	38
TP.	(g/100 ml)	8.0	7.8	8.2	8.4	8.0	8.4	7.4	7.6	8.6	8.0	8.0
Hb.	(g/100 ml)	12.7	14.5	11.6	12.9	10.4	11.5	12.1	13.5	13.2	11.9	13.5
RBC.	($\times 10^6$)	5.53	6.00	5.05	5.47	5.02	4.86	5.10	5.56	5.81	5.20	5.90
WBC.	count	5,500	5,953	5,400	6,100	6,700	5,200	6,700	7,800	6,000	8,300	8,100
TN.	(%)	48	50	50	57	54	66	62	51	51	65	47
Immat. N.	(%)	0	0	0	0	1	1	1	2	0	1	4
Lymph.	(%)	50	45	45	39	42	30	35	43	56	31	48
Monocyt.	(%)	0	0	0	0	0	0	0	0	0	1	0
Eosinoph.	(%)	2	5	5	4	3	3	2	4	3	2	1
Basoph.	(%)	0	0	0	0	0	0	0	0	0	0	0
MCV.	(cu microns)	77	78	77	85	79	78	78	75	77	74	74

Appendix 27: Hematological parameters from the dog no. A4C treated with lidocaine.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	37	42	35	38.1	43.9	41.7	41.7	42.4	-	43	-
PCV. (M)	(%)	40	48	40	39	36	35	38	39	-	40	-
TP.	(g/100 ml)	8.4	8.2	8.4	8.2	8.0	8.0	7.6	7.4	-	7.9	-
Hb.	(g/100 ml)	14.4	16.7	14.0	16.8	15.3	14.0	13.2	14.7	-	13.8	-
RBC.	(x 10^6)	4.84	6.49	5.47	5.26	5.93	5.66	5.60	5.72	-	5.52	-
WBC.	count	7,600	8,700	6,900	7,900	8,400	6,700	6,300	7,000	-	7,100	-
TN.	(%)	52	57	47	55	53	60	56	59	-	43	-
Immat. N.	(%)	0	0	0	0	0	0	0	0	-	0	-
Lymph.	(%)	35	27	35	32	34	31	32	28	-	44	-
Monocyt.	(%)	0	0	1	0	0	0	0	0	-	0	-
Eosinoph.	(%)	12	16	17	13	13	9	12	13	-	13	-
Basoph.	(%)	0	0	0	0	0	0	0	0	-	0	-
MCV.	(cu microns)	75	76	74	73	76	75	76	76	-	79	-

Appendix 28: Hematological parameters from the dog A4D treated with lidocaine.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	50.7	58.7	53.6	50.3	59.5	64.1	57.4	-	59.7	54.9	50.6
PCV. (M)	(%)	47	55	49	45	54	54	47	-	50	49	47
TP.	(g/100 ml)	7.2	7.0	7.0	7.6	8.2	8.2	8.0	-	7.8	7.4	7.6
Hb.	(g/100 ml)	16.3	20.0	16.9	16.0	19.9	17.4	19.8	-	19.5	17.3	17.8
RBC.	(x 10^6)	7.05	8.02	7.48	6.95	8.45	8.48	8.16	-	8.61	7.66	7.31
WBC.	count	5,900	7,700	5,600	5,300	8,300	8,700	9,500	-	9,500	7,300	8,300
TN.	(%)	80	66	70	70	72	75	76	-	62	54	67
Immat. N.	(%)	1	0	0	0	0	0	0	-	0	0	0
Lymph.	(%)	19	33	30	30	26	24	24	-	37	34	26
Monocyt.	(%)	0	0	0	0	0	0	0	-	0	1	0
Eosinoph.	(%)	0	1	0	0	2	1	0	-	1	2	7
Basoph.	(%)	0	0	0	0	0	0	0	0	0	0	0
MCV.	(cu microns)	75	74	75	75	73	79	73	-	72	75	72

Appendix 29: Hematological parameters from the dog no. A4E treated with lidocaine.

		0 Hr.	1/4 Hr.	1/2 Hr.	1 Hr.	2 Hr.	4 Hr.	8 Hr.	12 Hr.	24 Hr.	48 Hr.	72 Hr.
PCV. (CC)	(%)	47.6	59.1	45.9	57.0	53.8	55.9	57.1	-	56.5	54.2	-
PCV. (M)	(%)	39	48	39	46	46	40	51	-	45	45	-
TP.	(g/100 ml)	7.2	7.2	7.0	7.4	7.2	7.2	7.4	-	7.0	7.2	-
Hb.	(g/100 ml)	17.4	22.3	12.8	19.4	14.5	11.1	22.8	-	20.0	16.9	-
RBC.	($\times 10^6$)	6.73	8.44	6.43	8.07	7.59	7.86	7.32	-	7.47	7.69	-
WBC.	count	7,200	8,300	7,400	9,300	9,700	10,000	8,700	-	9,400	6,400	-
TN.	(%)	78	78	84	77	-	69	70	-	64	42	-
Immat. N.	(%)	0	0	0	1	-	1	0	-	1	0	-
Lymph.	(%)	17	18	11	19	-	28	26	-	28	49	-
Monocyt.	(%)	0	1	0	0	-	0	1	-	0	0	-
Eosinoph.	(%)	5	3	5	3	-	2	3	-	7	9	-
Basoph.	(%)	0	0	0	0	-	0	0	-	0	0	-
MCV.	(cu microns)	73	73	74	74	74	74	81	-	79	74	-

Appendix 30: Systolic, diastolic and mean blood pressure (mmHg) for the control dogs receiving physiological saline and adrenaline .

Dog no.	Before Treatment			Physiological saline			Adrenaline			30 minutes later		
	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP
B1A			52			52			135			
B1B	87	63	71	82	57	65.33	150	118	128.67	110	94	99.33
B1C	98	76	83.33	97	74	81.67	145	100	115			
B1D	89	62	71	84	59	67.33	172	138	149.33	69	49	55.67
B1E	103	76	85	102	73	82.67	150	116	127.33			

Appendix 31: Systolic, diastolic and mean blood pressure (mmHg) for the control dogs receiving verapamil (0.1 mg/kg bwt.) and adrenaline .

Dog no.	Before Treatment			Physiological saline			Adrenaline			30 minutes later		
	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP
B2A	107	69	81.67	99	75	83	99	77	84.33	99	77	84.33
B2B	106	66	79.33	90	62	71.33	147	102	117			
B2C	83	58	66.33	66	44	51.33	121	100	107			
B2D	106	78	87.33	89	59	69	190	135	153.33	106	80	88.67
B2E	106	76	86	94	63	73.33	133	112	119	105	79	87.67

Appendix 32: Systolic, diastolic and mean blood pressure (mmHg) for the control dogs receiving Propranolol (0.5 mg/kg bwt.) and adrenaline .

Dog no.	Before Treatment			Physiological saline			Adrenaline			30 minutes later		
	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP
B3A	113	97	102.33	105	87	93	167	164	165	110	95	100
B3B	134	88	103.33	66	55	58.67	159	151	153.67	110	92	98
B3C	98	67	77.33	84	57	66	141	123	129	104	70	81.33
B3D	79	58	65	75	56	62.33	149	136	140.33	103	84	90.33
B3E	84	59	67.33	76	51	59.33	140	127	131.33	80	56	64

Appendix 33: Systolic, diastolic and mean blood pressure (mmHg) for the control dogs receiving lidocaine (4 mg/kg bwt.) and adrenaline .

Dog no.	Before Treatment			Physiological saline			Adrenaline			30 minutes later		
	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP	Systolic	Diastolic	MBP
B4A	74	57	62.67	68	53	58	137	123	127.67	93	80	84.33
B4B	90	64	72.67	84	59	67.33	143	110	121	93	66	75
B4C	110	85	93.33	95	77	83	173	139	150.33	103	87	92.33
B4D	80	52	61.33	76	54	61.33	117	84	95	88	68	74.67
B4E	97	74	81.67	81	61	67.67	139	125	129.67			