Arrhythmias in the isolated rat heart.

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Award date:
1985

Awarding institution:
University of Bath

ARRHYTHMIAS IN THE ISOLATED RAT HEART

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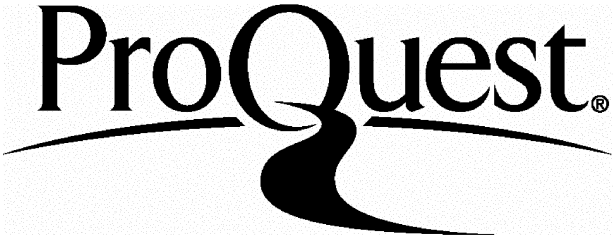
for the degree of Doctor of Philosophy

of the University of Bath

1985



Acknowledgements

Gratitude is expressed for my research supervisor, Dr. Brian Woodward for his invaluable teaching and guidance and for many hours of discussion and assistance.

I am grateful to the University of Khartoum for funding my studentship from 1982-1985.

My thanks are due to Sami El Bakbak for developing a computer programme for calculating rubidium efflux rate constant.

Finally, a special thanks to Mrs Judy Harbutt for typing of this thesis.

Triple Dedication

This thesis is dedicated to:

a) my mother Taiba

b) my father Yousif

c) my wife Zihoor

for their encouragement, support and supplication

SUMMARY

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The present study has investigated the effects of potassium, calcium and magnesium on ligation induced arrhythmias in the isolated rat heart. An attempt was made to elucidate the mechanism of action of potassium, however, more work is required to fully explain the action of this cation. Low calcium perfusion was found to reduce arrhythmias, however, calcium antagonists and magnesium, the physiological calcium antagonist, were only effective when they reduced the heart rate.

Next, the possible roles of prostaglandins, lysophospholipids and free radicals in ligation induced arrhythmias in the isolated rat heart were studied. Exogenous ZK36374 and manipulation of endogenous prostaglandins did not seem to alter arrhythmias in this model. This lack of effect *in vitro* was attributed to the absence of blood and reflex neural influences. Similarly antioxidants, free radical scavengers and manoeuvres that would be expected to alter endogenous lysophospholipids did not alter arrhythmias in this model.

Finally, $^{86}Rb^{+}$ efflux was used to investigate the effects of the following compounds and conditions on potassium loss:- magnesium, pH, hypothermia, ligation, reperfusion, fibrillation induced by altering ionic milieu, calcium paradox, adenosine, mepacrine and chlorpromazine.

CONTENTS

1.0 INTRODUCTION

1.1 Ischaemia induced arrhythmias: Clinical relevance

Sudden death in man is defined as death occurring within 1 hour of last being seen alive and represents approximately one-third of the total expression of ischaemic heart disease (Armstrong *et al.*, 1972). Sudden cardiac death in man arising from obstruction of the right coronary and left anterior descending coronary arteries is documented (Oliver, 1982). Obstruction of a coronary artery might occur as a result of coronary artery spasm, platelet thrombosis or platelet emboli (Oliver, 1982).

The rat heart develops serious arrhythmias upon occlusion of the main left coronary artery. These early arrhythmias occur within a few minutes of occlusion, peak at 10-12 minutes and then decline after 30 minutes. These life threatening arrhythmias are believed to resemble pre-hospital sudden cardiac death. A late phase of arrhythmias, which develops 4-24 hours after coronary ligation in the rat, is thought to be similar to the arrhythmias seen in the coronary care unit.

1.2 Myocardial ischaemia and metabolic changes

Ischaemia represents an imbalance between the myocardial demand for, and the vascular supply of, coronary blood (Hearse, 1980). It is different from anoxia and hypoxia in that in the case of anoxia and hypoxia, the oxygen delivery to the myocardium is either reduced or stopped while coronary flow is normal. In contrast to hypoxia and anoxia, ischaemia results in (1) retention of metabolites, (2) accumulation of intra- or extracellular ions; and (3) regional acidosis. The accumulation of these metabolites makes ischaemic hearts far more vulnerable to ventricular fibrillation

than hearts made hypoxic without reduction of flow (Bagdonas *et al.*, 1961).

Soon after the onset of ischaemia disturbances of metabolism occur. Extracellular potassium rises (Harris *et al.*, 1954), lysophospholipids (Sobel *et al.*, 1978) and cyclic AMP (Podzuweit *et al.*, 1978) increase, pH is decreased and lactic acid accumulates (Hirche *et al.*, 1980), arachidonic acid metabolites are released (Mest *et al.*, 1981; Coker *et al.*, 1981), magnesium is lost (Johnson *et al.*, 1979), free radicals are produced (Rao *et al.*, 1983) and catecholamines are released (Riemersma, 1982). Accumulation of these noxious metabolites are believed to predispose to malignant ventricular arrhythmias. For any of these metabolites to be implicated in the genesis of arrhythmias, they should satisfy certain criteria (Corr *et al.*, 1982 a).

1. A change in the concentration of the metabolite should precede the physiological derangements giving rise to arrhythmias.
2. Exposure of the sarcolemma to exogenous material at concentrations comparable to those encountered in the vicinity of the cell membrane should exert arrhythmogenic actions.
3. Conditions known to modify arrhythmias should also affect the action of the putative arrhythmic metabolite.
4. The enzymic capacity to produce any arrhythmogenic metabolite should be sufficient to produce that metabolite in arrhythmogenic concentrations.
5. Experimental modification of the concentration of the endogenous constituent with parallel exacerbation or

amelioration of arrhythmogenesis should occur despite induction of comparably severe ischaemia.

The aim of this thesis is to determine whether some of the previously mentioned metabolites mediate the electrophysiological derangements and rhythm disturbances induced by ischaemia in isolated rat heart (in this thesis ischaemia means reduction of flow in the area perfused by the left coronary artery). The above mentioned rules are applied whenever possible during discussion of the arrhythmogenic role of some of these metabolites.

Prophylaxis and treatment of ventricular arrhythmias induced by ischaemia employs relatively non-specific anti-arrhythmic agents such as procainamide which depresses membrane function or propranolol which blocks sympathoadrenal influences. Theoretically, more effective approaches may be possible with pharmacological interventions designed to antagonize the specific, local metabolic factors responsible for the initiation of these arrhythmias (Corr and Sobel, 1980).

An important characteristic of ischaemia is its heterogeneity. Varying conditions of work load and tissue perfusion create regional differences in the ischaemic myocardium. The dispersion of refractoriness to conduction between the various zones may predispose to re-entrant arrhythmias. Moreover, unidirectional conduction abnormalities between the ischaemic and the non-ischaemic zones may produce premature beats as reviewed by Hearse and Dennis (1982). Furthermore, mechanical disturbance in regional ischaemia may produce depolarization and extrasystoles (Lab, 1982).

Electrophysiological alterations which are generated by noxious metabolites and known to augment arrhythmogensis include;-

(1) A decreased (less negative) resting potential.

(2) Changes in phase 0 of the action potential.

(3) A shortening of action potential duration.

(4) An increase in the rate of spontaneous depolarization.

(for review see Opie *et al.*, 1979)

1.3 Potassium status

Potassium is the main intracellular cation. The human body contains 53 - 55 mM/kg body weight, 98% of which is confined to the cell (Edelman, 1959). Human extracellular potassium concentration is normally between 3.5 - 5.5 mM/L (Schwartz, 1978).

Extracellular potassium affects the cardiac action potential and these alterations may appear independently of intracellular potassium (Schwartz, 1978).

The high intracellular potassium concentration is maintained by an active sodium-potassium pump. This involves a $Na^+ - K^+$ dependent ATPase which requires a combination of ATP and magnesium as a substrate. It is considered by most workers that sodium is carried actively by the pump and potassium diffuses passively to replace sodium (Schwartz, 1978).

Potassium carries three different delayed outward currents during repolarization which show a phase of inward going rectification and are responsible in part for prolongation of action potential duration in Purkinje fibres and contribute

to diastolic depolarization in pacemaker fibres (Gettes, 1981).

The arrhythmogenic effect of potassium was suggested by Harris *et al.*, (1954) who reported an increase in coronary venous potassium following coronary artery ligation in the dog. A triphasic increase in extracellular myocardial potassium occurs in the isolated arterially perfused rabbit interventricular septum during ischaemia (Weiss *et al.*, 1982). A substantial rise in extracellular potassium in acute coronary artery ligated pig hearts has also been reported by Hirche *et al.*,(1980).

Although the rise in extracellular potassium after the onset of ischaemia is well established the reasons underlying such a rise are controversial. It is not brought about by acidosis or release of catecholamines (Weiss *et al.*, 1982).Since the heart is essentially an aerobic organ (Opie, 1978), it follows that restriction of oxygen supply may affect all metabolic processes in the heart. $Na^+ - K^+$ pump activity is one of these processes seriously affected during ischaemia and its inhibition might explain potassium leakage (Kunze, 1977). Balasubramanian *et al.* (1972) reported a decreased activity of $Na^+ - K^+$ ATPase during hypoxia. In contrast Rau *et al.* (1977) demonstrated maintenance of membrane $Na^+ - K^+$ pump activity through 60 minutes of anoxia. Despite this, potassium leaked out, and it was suggested that an increase in the background outward current was the mechanism for potassium loss. Suppression of $Na^+ - K^+$ pump activity with 10^{-5} M acetystrophanthidin during ischaemia does enhance potassium efflux (Weiss *et al.*, 1982). The fall in ATP content during ischaemia

is not rapid enough to be temporarily compatible with the onset of arrhythmias unless ATP is compartmentalized (Weiss *et al.*, 1982).

The "on off" characteristic of anoxia-induced potassium efflux suggests the possibility of a direct effect of oxygen on rate limiting efflux sites in the cell membrane. Coburn *et al.* (1976) have proposed the existence of an oxygen sensing system in smooth muscle and this may also be present in the myocardium.

There are many possible mechanisms whereby a rise in extracellular potassium could participate in arrhythmogenesis Increasing extracellular potassium causes the resting transmembrane potential to fall (less negative). Under such conditions cells may be depolarized to such an extent that the rapid sodium current is depressed or inactivated;this, plus the activation of slow calcium dependent action potential could lead to slow conductions and re-entry (Shigenobu *et al.*, 1972). Because of their slow rate of propagation calcium dependent action potentials have been implicated in the genesis of propagated responses and re-entry type arrhythmias. Increases in potassium outward currents shorten action potential duration (Vleugels *et al.*, 1980).

The elevated concentration of potassium within the ischaemic region and the resulting potassium gradient at the ischaemic-normal boundary produce two effects that apparently summate to generate ectopic impulses:-

a) some boundary cells certainly are exposed to potassium

concentrations within the range that increase excitability to electrical stimulation, and

b) the injury potassium potential produces currents which circulate within this narrow range that contain the hyper-excitable cells as reviewed by Harris (1966).

Injection of potassium locally into a coronary artery produces ectopic activity and fibrillation (Harris *et al.*, 1954). Further evidence in support of the concept that release of potassium in a local region of myocardium induces ectopic activity and ventricular fibrillation was provided by the findings of Hano and Harris (1963). They found that injection into a coronary artery of octylamine, a potassium releasing agent, is followed by ventricular ectopic activity while generalized global hyperkalaemia produced by the slow intravenous infusion of potassium chloride did not produce ectopic ventricular arrhythmias (Winkler *et al.*, 1938). Reduction of plasma potassium in the rat by furosemide from 3.58 to 2.8 mmol/litre significantly increased ventricular fibrillation incidence following coronary artery ligation (Abrahamsson and Almgren, 1981).

Decreased potassium loss from hearts has been associated with the antiarrhythmic actions of procaineamide, glucose/insulin and lidocaine (Opie *et al.*, 1979).

It has been reported that increases in extracellular potassium concentration abolish arrhythmias induced by strophanthidin in Purkinje fibres (Lin and Vassalle, 1980), while potassium reduces the incidence and delays the onset of ventricular fibrillation produced by coronary ligation and reperfusion in the

isolated rat heart (Lubbe *et al.*, 1978).

Experiments described in the present thesis were designed to examine the protective role of potassium in coronary artery ligation arrhythmias in the isolated rat heart. The effects of rubidium which is physiologically handled in the same way as potassium and which counteracts glycoside induced arrhythmias *in vivo* (Osman *et al.*, 1976) and abolishes arrhythmias induced by strophanthidin in Purkinjefibres (Lin and Vassalle, 1980) has also been studied.

1.4 Calcium status

Human plasma calcium concentration is closely regulated within the normal values of 2.1 to 2.7 mmole/litre. One half of this plasma calcium is in nonionized complexes. Calcium is essential for the excitability of nerve and muscle membranes, for the release of neurohumoral transmitters, for blood coagulation and for bone formation. Thus, it is important that the concentration of calcium in the plasma is kept within the above mentioned limits. Plasma calcium levels are regulated by parathyroid hormone and vitamin D which act synergestically to raise plasma calcium whereas calcitonin acts under certain conditions to lower it.

Some calcium, which is important in myocardial contractile control, is bound to the sarcolemma. Upon excitation this calcium enters the myocytes via the slow calcium channels and sodium/calcium exchange (Langer, 1984); and is believed to trigger release of large amounts of calcium from stores within the sarcoplasmic reticulum resulting in a rise in intracellular free

calcium to levels adequate for contraction of the myofilaments (Fabiato, 1981). During diastole some of this free calcium is sequestered in the sarcoplasmic reticulum, while the remainder is removed via sodium/calcium exchange and by a calcium ATPase to the extracellular space.

Many models of arrhythmias involve increasing intracellular free calcium. Administration of large doses of calcium induce fibrillation and this has been used as a method for evaluating potential antiarrhythmic drugs. The toxin aconitine produces arrhythmias by preventing closure of voltage-dependent fast sodium channels. Cardiac glycosides cause abnormal sodium accumulation by inhibiting sodium - potassium ATPase, This increased intracellular sodium depresses sodium dependent calcium extrusion and leads to calcium accumulation (Cranefield, 1977), while reducing serum ionized calcium by the addition of citrate has an antiarrhythmic action in dogs (Clusin *et al.*, 1982a). Doubling the extracellular calcium concentration and halving extracellular sodium concentration causes fibrillation *in vitro* (Woodward, 1981), while drugs enhancing calcium influx such as adrenaline cause fibrillation (Clusin *et al.*, 1982).

Despite this indirect evidence for the involvement of calcium in arrhythmogensis no increase in calcium uptake during the first 60 minutes of anoxia in the arterially perfused inter-ventricular septum of the rabbit has been demonstrated (Poole-Wilson *et al.*, 1982). In contrast earlier experiments performed in Langendorff perfused hearts of the rabbit have shown a substantial increase in calcium uptake within 15 minutes of hypoxia (Nayler *et al.*, 1976). Variations in temperature and

heart rate might account for this discrepancy (Poole-Wilson group paced the septum at 74 b/m temperature 32°C, while the Nayler group perfused the hearts at 37°C).

Interpretation of these results is made more difficult by the fact that a deleterious accumulation of calcium in ultra-structures, such as the mitochondria, could occur without any change in sarcolemmal calcium permeability (Nakanishi *et al.*, 1982) suggesting calcium redistribution rather than gain as the most detrimental factor during anoxia and ischaemia. It is also possible that the techniques available to date are not sensitive enough to measure minute changes in intracellular calcium during regional and global ischaemia.

Calcium is known primarily to mediate the voltage and time dependent slow current (Reuter, 1967). This current is activated during the plateau phase in the Purkinje fibres and during phase 0 in the SA and AV nodes.

During ischaemia the rise of extracellular potassium causes membrane depolarization. Under such conditions cells may be depolarized to such an extent that the rapid sodium current is inactivated leaving the way open for the activation and propagation of the slow calcium current by released catecholamines (Shigenobu *et al.*, 1972). These slow responses are characterized by a slow upstroke velocity, delayed recovery of excitability and slow propagation which can lead to re-entry (Cranefield and Hoffman, 1972). Action potentials showing slow response activity have in fact been recorded from partially depolarized cells adjacent to the infarcted tissue (Myerburg *et al.*, 1977)

Nonetheless, it is still questionable whether these are calcium action potentials or depressed fast action potentials. Hypoxia, ischaemia, acidosis and low ATP levels encountered during ischaemia tend to suppress the slow inward current (Sperelakis, 1984).

There are three proposed mechanisms whereby calcium causes arrhythmias:-

1. Reduced oxygen availability during ischaemia leads to failure of calcium homeostasis and raised cytosolic calcium. This in turn leads to activation of calcium dependent lipases and ATPases leading to mitochondrial calcium overload. Mitochondrial ATP production is inversely proportional to calcium content. This may result in rhythm disturbance and loss of function and structure (Nayler, 1981).
2. Calcium mediates oscillatory afterpotentials, aftercontractions, and abnormal automaticity in calcium overloaded cells, these can lead to fibrillation when calcium overload becomes severe (Clusin *et al.*, 1982).
3. Rises in cytosolic calcium increases gap junctional resistance leading to cell decoupling, conduction block and arrhythmias (De Mello, 1982).

The protective action of the calcium slow channel blocking drugs against arrhythmias is also controversial. Pretreatment of dogs with verapamil dramatically suppressed ischaemic ventricular fibrillation (Kaumann and Aramendia, 1968) while diltiazem has been reported to prolong ventricular fibrillation latency in the ischaemic dog heart (Clusin *et al.*, 1982a). Nifedipine reduced the number of arrhythmias following coronary

artery occlusion in the dog (Coker and Parratt, 1983a). In conscious rats verapamil reduced arrhythmias dose dependently (Curtis *et al.*, 1984). In contrast to these findings, verapamil has been reported to increase the incidence of mortality due to arrhythmias in the anaesthetised rats (Kane *et al.*, 1979; Clark *et al.*,, 1980). In conscious dogs diltiazem failed to protect against arrhythmias induced by coronary ligation and programmed electrical stimulation (Patterson *et al.*, 1983) and in electrically stimulated anaesthetised dogs (Patterson *et al.*, 1983) diltiazem was also shown to be inactive.

There are several criticisms regarding the above mentioned experiments:-

1. The variety of models used make it difficult to determine whether a drug is effective or not.
2. Most workers have used a single or few doses of drugs, whereas it is well known that some calcium antagonists possess a bell-shaped dose response curve (Hearse *et al.*, 1980; Nayler and Sturrock, 1984).

Protection provided by calcium antagonists in some models does not entirely implicate calcium as a mediator of arrhythmias. Some calcium antagonists possess membrane stabilizing and α receptor blocking action which could be protective (Müller *et al.*, 1982; Bayer *et al.*, 1975). Calcium antagonists also increase collateral blood flow to the ischaemic region (Henry *et al.*, 1978) and preserve ATP stores by reducing myocardial oxygen demand (Smith *et al.*, 1975). Some of the antiarrhythmic effects described above may be the result of an antiischaemic

action rather than a direct antiarrhythmic action.

Experiments in the present thesis have been designed to investigate the role of calcium, calcium antagonists and pacing on ligation induced arrhythmias in the isolated rat heart which is devoid of peripheral haemodynamic complications.

1.5 Calcium paradox

Zimmerman and Hülsman (1966) reported that perfusion of rat hearts with calcium-free media for very short periods of time, creates a situation such that upon readmission of calcium there is massive tissue disruption, enzyme release and the development of contracture. This phenomenon has been called the "calcium paradox" and its damaging effects have been attributed to sudden transmembrane fluxes of calcium. The clinical importance of the calcium paradox lies in the use of calcium-free cardioplegic solutions to arrest the heart and to protect it from undesirable ischaemic injury during open-heart surgery (Kirsch *et al.*, 1972). Intracellular calcium overload and deficiency have been implicated in the genesis of myocardial failure (Dhalla, 1976). It has been suggested that perfusion of isolated rat hearts with calcium-free media is an appropriate model for studying the effects of intracellular calcium deficiency, whereas reperfusion with normal media of calcium deprived hearts is suitable for investigating the effects of intracellular calcium overload (Yates and Dhalla, 1975). The occurrence of the calcium paradox is not restricted to the rat heart. It has been demonstrated with hearts of several mammalian species (Hearse *et al.*, 1978). This makes it likely that human hearts will be susceptible to calcium withdrawal and subsequent admission in a similar way.

There are several indices used for evaluating the magnitude of the calcium paradox and the effectiveness of proposed protective procedures. In this thesis rubidium efflux is suggested as an alternative index for evaluation of the extent of damage during the calcium paradox.

1.6 Magnesium status

The rat ventricle contains 17.3 ± 0.2 mM/kg of magnesium distributed in the mitochondria, myofibrils and complexed to AMP, ADP and ATP (Page and Polimeni, 1973). Magnesium ATP is a substrate for different ATPase enzymes. Attachment of actin and myosin filaments of cardiac muscle through cross bridges to generate the contractile process requires energy which is provided by hydrolysis of the magnesium ATP complex. Magnesium reduces the movement of extracellular calcium into the rat myocardial cell by a competitive process localized at the sarcolemmal membrane (Shine and Douglas, 1974). Changes in extracellular magnesium concentration appear to have little effect on the action potential of cardiac cells. (Shine *et al.*, 1979), unless calcium is reduced or potassium is increased. Magnesium 10 - 20 mM reduced potassium efflux and sodium influx (Shine *et al.*, 1979). Human normal plasma magnesium falls within the range of 0.83 - 0.89 mM/litre (Nordin, 1976).

Magnesium loss from human myocardium has been demonstrated after ischaemic myocardial injury and it has been suggested that a relationship between this loss and sudden death may exist (Johnson *et al.*, 1979). A higher incidence of sudden death due to ischaemic heart disease has also been noted in cities with soft drinking water which is low in magnesium and

calcium (Anderson *et al.*, 1975). In support of this interpretation some workers have claimed that serum magnesium is decreased in acute ischaemic heart disease and that myocardial tissue is deficient in magnesium in victims of fatal myocardial infarction (Johnson *et al.*, 1979; Iseri *et al.*, 1975; Dychner and Wester , 1980). Hypomagnesaemia can potentiate the contractile activity of a variety of neurohumoral substances and induce vasospasm. Hypermagnesaemia has the reverse action, i.e. it induces hyporeactivity, relaxation and vasodilation (Altura *et al.*, 1981). A number of disease states (e.g. alcoholism, atherosclerosis, eclampsia, diabetes mellitis, essential and renal hypertension) which result in elevation of blood pressure are often associated with decreased levels of magnesium (Johnson *et al.*, 1979; Szelenyi , 1973). Patients with deficiency in both potassium and magnesium were shown to be refractory to potassium repletion unless extracellular magnesium was corrected (Dyckner and Wester , 1980).

Despite the evidence that links magnesium deficiency and cardiac arrhythmias there are several criticisms of this hypothesis;

1. Moderate deficiency of extracellular magnesium failed to induce vasoconstriction in bovine coronary artery or sensitize it to agonist responses (Kalsner, 1983). It is therefore unlikely that ischaemia is due to hypomagnesaemia induced coronary spasm.
2. Magnesium levels occasionally reported in human heart disease (Iseri *et al.*, 1975) are marginally subnormal and cannot be linked to marked changes in vascular dynamics.

3. Hankin *et al.* (1970) have reported that the major dietary source of magnesium is food, and only a small amount, at most 12%, comes from water. They noted that residence in hard water areas did not ensure an intake of only hard water since approximately half of the studied subjects drank softened or treated water at home. Therefore variations in the magnesium content of drinking water are probably not sufficient to explain regional differences in cardiovascular mortality.
4. The reported marginal decrease of plasma magnesium in infarct patients could be a consequence of the infarct rather than its cause (Seelig and Heggtveit, 1974).
5. Treatment of some arrhythmias in certain clinical cases by infusion of magnesium (Johnson *et al.*, 1979; Iseri *et al.*, 1975) could be due to reduction of peripheral resistance and hence myocardial work rather than to a direct effect on the myocardium.
6. Arrhythmias associated with magnesium deficiency reported by Iseri *et al.* (1975) were also associated with possible digitalis toxicity. Therefore the role of hypomagnesaemia in this study is questionable.

In this thesis experiments have been carried out to determine whether magnesium perfused at different concentrations possess antiarrhythmic actions in the isolated coronary ligated rat model. The effects of magnesium on $^{86}Rb^{+}$ efflux have also been studied.

1.7 Prostaglandins

Prostaglandins are oxygenated derivatives of polyunsaturated fatty acids such as dihomo-γ-linolenic acid, arachidonic acid and eicosapentaenoic acid. These three fatty acids give rise to the prostaglandins of 1,2 and 3 series respectively. The most common of these prostaglandin precursors is arachidonic acid. Arachidonic acid is almost entirely bound to phospholipids. Thus formation of prostaglandins must be preceded by activation of phospholipases which release bound fatty acids. Free arachidonic acid immediately enters one of several pathways and undergoes (1) enzymatic conversion by cycloxygenase to endoperoxide intermediates; (2) enzymatic conversion by lipoxygenases to hydroperoxy acid; (3) enzymatic reacylation into phospholipids; (4) non enzymatic binding to plasma albumin. Cyclic endoperoxides (PGG_2 and PGH_2) formed by cycloxygenase decompose spontaneously to a mixture of PGE_2 and PGD_2 (Hamberg and Fredholm, 1976). The endoperoxides are converted by thromboxane synthetase to thromboxane A_2 which contracts isolated blood vessels and aggregates platelet (Christ and Van Dorp, 1972) or by prostacyclin synthetase to prostacyclin which relaxes most blood vessels and inhibits platelet aggregation. In the endothelial or smooth muscle layers of vessel wall, arachidonic acid is preferentially converted to prostacyclin. In contrast, in blood platelet arachidonic acid is preferentially converted to thromboxane A_2. Both prostacyclin and thromboxane A_2 are unstable under physiological conditions and decompose into 6-keto-$PGF_{1\alpha}$ and thromboxane B_2 respectively. Lipoxygenase converts arachidonic acid to hydroperoxy acid intermediates which form hydroxyacids or leukotrienes. Leukotrienes exhibit a number of biological effects such as contraction of bronchial smooth muscles,stimulation of

vascular permeability, and attraction and activation of leukocytes (for review see Hammerström, S., 1983).

Prostaglandin production is stimulated by ischaemia (Needleman, 1978). Release of different prostaglandins during the development of arrhythmias has been shown by different investigators (for review see Coker, 1982) and prostacyclin is the major prostaglandin released from the isolated perfused rat heart (De Deckere *et al.*, 1977). The effects of different exogenous prostaglandins have produced conflicting reports on arrhythmias produced in several models (for review see Coker, 1982). The variety of models used make it difficult to determine whether a certain prostaglandin is effective or not.

A balance in favour of thromboxane release is associated with the occurrence of a greater number of arrhythmias in coronary artery ligated greyhounds whereas fewer arrhythmias occur when the balance is in favour of prostacyclin release (Coker *et al.*, 1981). Dazoxiben (UK-37, 248) and UK38,485, thromboxane A_2 synthetase inhibitors, reduced the number of extrasystoles that occurred during the first 30 minutes of coronary occlusion and increased survival following coronary artery reperfusion in the dog (Coker *et al.*, 1982; Coker, 1984). Prostacyclin and ZK 36374, a stable prostacyclin analogue, administered intracoronary reduced the number of extrasystoles occurring during the first 30 minutes of occlusion and the incidence of ventricular fibrillation during reperfusion in anaesthetised greyhounds whereas intravenous administration of both drugs exacerbated arrhythmias (Coker and Parratt, 1983). This detrimental effect was attributed to reflex increase

in sympathetic drive initiated by systemic hypotension induced by these drugs.

Prostacyclin prevented ischaemia-induced increases in lactate and cyclic AMP in the myocardium (Rösen *et al.*, 1981) and reduced the increase in extracellular potassium in ischaemic pig hearts (Hirche, 1982).

In rat hearts LTC_4, LTD_4 and LTE_4 caused a reduction in coronary flow and spontaneous heart rate but LTB_4 had no effect on these parameters (Piper, 1983). The effects of these leukotrienes on arrhythmias are not known.

All the experiments described above have used *in vivo* models. In the present thesis experiments have been carried out using an *in vitro* model where there are no platelets. In addition, coronary flow has been controlled thus avoiding problems of interpretation when vasodilator drugs are used although redistribution of coronary flow could still occur.

1.8 Phospholipids

Cell membranes have an identical subunit molecular structure consisting of a bilayer of mixed polar lipids (phospholipids with hydrocarbon chains (hydrophobic) oriented toward the centre of the membrane forming a continuous hydrophobic phase). The hydrophilic moieties of the lipids oriented toward the cytoplasm in one direction and toward the cell exterior in the other direction, with each surface of the membrane covered by a layer of protein (Robertson, 1959). These phospholipids are essential for the activity

of membrane bound enzymes such as Na^+/K^+ ATPase, adenylate cyclase and calcium ATP ase (Weglicki, 1980). Hydrolysis of these phospholipids by phospholipase reduces the activity of these enzymes indicating particular sensitivity of membrane transport to the activity of phospholipases. The organisation of membrane phospholipids appears to determine the relative membrane permeability to ions, maintenance of membrane resistance, resting membrane potential and generation of the cardiac action potential (Corr and Sobel, 1982). The polar headgroups of membrane phospholipids are important determinants of the electrophysiological properties of membranes (De Mello, 1982). Treatment of excitable tissue with phospholipase C, which hydrolyses the ester bond between glycerol and the phosphate group and removes the polar group of the phospholipid, abolishes membrane potentials in nerve and skeletal muscle. Metabolism of phospholipids is shown in Figure 1.1.

Phospholipase A_1 and A_2 can be classified into membrane-bound and soluble forms. Enzymes of the former group are strongly bound to the plasma, Golgi or mitochondrial membranes. They generally require the presence of calcium ions for optimal catalytic activity and usually have neutral pH optimum. Soluble phospholipase A_1 and A_2 have been isolated from lysosomes. These enzymes usually have acidic pH optimum and no requirement for calcium.

Altered phospholipid metabolism is implicated as a contributory factor in membrane dysfunction, electrophysiological derangements, malignant arrhythmias and cellular injury in ischaemic myocardium. Accumulation of lysophospholipids in ischaemic hearts of several mammalian species has been reported by many investigators

(Sobel *et al.*, 1978; Chien *et al.*, 1981; Corr *et al.*,1982; Steenbergen and Jennings, 1984).

Accumulation of lysophospholipids as a result of the action of endogenous phospholipases will occur if the rate of diacyl phospholipid hydrolysis exceeds the combined rates of (1) hydrolysis of lysophospholipids by lysophospholipases and (2) reacylation of lysophosphatides and removal of lysophospholipids from membranes by binding to albumin, which has a high affinity for these amphiphilic compounds (Fiehn and Hasselbach, 1970). Activity of phospholipase A_2 is enhanced by β adrenergic stimulation as reviewed by Corr and Sobel (1982), bradykinin and ischaemia (Needleman, 1978).

Most of the processes which would diminish lysophospholipid accumulation are impaired during ischaemia. Microsomal lysophospholipase activity in normal myocardium is inhibited by a reduction of pH to 6.5 (Gross and Sobel, 1982). Acylation of lysophospholipids to phospholipids is inhibited by both acyl carnitine and glycerophosphoryl choline, both of which are known to accumulate during ischaemia (Gross and Sobel, 1982a). Lee and Sobel (1981) using radiolabelled phospholipid substrate failed to detect an increase in the activity of rabbit microsomal and mitochondrial phospholipase A_2 in the ischaemic hearts and in hearts stimulated with modulators of phospholipase A_2 including bradykinin, noradrenaline and adrenaline. They proposed that reacylation and breakdown of lysophospholipid was impaired during ischaemia and that this accounted for the accumulation of lysophosphoglycerides.

Lysophosphatidylcholine in comparable concentrations to

those found in ischaemic hearts produced several derangements including decreased resting membrane potential, overshoot of phase 0, maximal velocity of upstroke of phase 0 and action potential duration in canine Purkinje fibres (Corr *et al.*, 1979; 1981) and slow responses (Corr *et al.*,1982). Susceptibility of isolated myocytes to attack by phospholipase C is related to both the ATP content of the cell and its glycolytic activity (Higgins *et al.*, 1982). Lysophosphatidylcholine potentiates calcium accumulation in rat cardiac myocytes (Sedlis *et al.*, 1983). It inhibits myocardial Na^+/K^+ ATPase (Karli *et al.*, 1979) and therefore may augment the intracellular sodium concentration with the consequent acceleration of Na^+/Ca^{++} exchange. Furthermore lysophosphatidyl choline increases the synthesis of cyclic AMP (Ahumada *et al.*, 1979) which may augment calcium influx via phosphorylation-dependent calcium channels (Watanabe and Besch, 1974). It can form ion channels within lipid bilayers (Lee and Chang, 1977) and these channels may potentially increase both influx and efflux of calcium across the sarcolemma.

Lysophospholipids are cytolytic (Weltzien, 1979). They induce their effect by three different mechanisms (Katz, 1982). At low concentrations lysophospholipids are incorporated into biological membranes. They alter the physical state of the membrane bilayer, reducing its susceptibility to osmotic or mechanical damage and inhibiting functional properties such as excitability.

At higher concentrations mixed micelles are formed in which the added lysophosphatidylcholine forms aggregates with lipids

pulled out of the membrane into the aqueous solution around the membrane. At still higher concentrations a further loss of membrane phospholipids and physical disruption of the membrane and loss of its ability to function as a barrier occur, allowing uncontrolled calcium entry which leads to cellular death (Sedlis *et al.*, 1983).

The suggested arrhythmogenic role of lysophosphatidylcholine is met by several criticisms:-

1. In the experiments of electrophysiological effects of lysophosphatidylcholine, exogenous lysophosphatidylcholine was applied whereas it is known that the effects of exogenous lysophosphatidylcholine on tissue is different from the effects of endogenous lysophosphatidylcholine (Katz, 1982).
2. While 10 minutes of ischaemia is accompanied by an approximately 50% increase in total myocardial lysophosphatides, a 3.5 fold increase in lysophospholipids levels is observed in the hibernating ground squirrel and is probably related to enhanced membrane fluidity that allows cardiac function to be preserved at temperatures as low as 1°C (Aloia *et al.*, 1979). This relatively high lysophosphatide content is also compatible with function at normal body temperature because hibernating squirrels awaken every 8 to 12 days to experience brief periods of activity at a body temperature of 37°C.

In this thesis, experiments have been carried out to investigate the role of lysophospholipids in ligation induced arrhythmias in the isolated rat heart. This has been approached by perfusing hearts with lysophosphatidylcholine and by adding

activators and inhibitors of phospholipase to the perfusate of coronary ligated hearts.

1.9 Free radicals

Molecular oxygen has two unpaired electrons with parallel electron spin. Most organic compounds that might react with oxygen contain paired electrons. When reacting with oxygen these compounds are supposed to insert their paired electrons simultaneously into a molecule of oxygen, but this reaction is normally hindered by the spin restriction of the oxygen electrons. The result of this restriction is that ordinary molecular oxygen is relatively non-reactive. Oxygen prefers to accept electrons one at a time. This results in the formation of reduced oxygen intermediates. These reduced oxygen intermediates are the free radicals. Generation of different free radical species is shown in Figure 1.2.

These radicals if produced in living tissue are hazardous, however the normal defence system of tissues which consists of enzymes such as superoxide dismutase, catalase and glutathione peroxidase, and reducing agents such as ascorbic acid, α-tocopherol and thiol derivatives prevent the detrimental effects of these radicals.

Biological systems very rarely become anoxic (Hess and Manson, 1984) and although the rat heart has very little collateral flow (Schaper, 1984), some oxygen will reach the ischaemic tissue following coronary artery ligation.

Detailed *in vitro* radiobiological studies have demonstrated

that free radical reactions occur even at very low oxygen tensions (83% of maximum rate at PO_2 ∿ 6 mm Hg and 50% at PO_2 ∿ 1 mm Hg (Hall, 1973). Rao *et al.* (1983) found a significant increase of free radicals in ischaemic tissue following left anterior descending coronary artery ligation in the dog and a four fold increase of free radicals in coronary venous blood within 5 minutes using electron spin resonance spectrometry. Thus the low oxygen tension in ischaemic tissue is adequate to support oxygen free radical formation . During ischaemia the process of oxygen utilization by cytochrome oxidase is impaired resulting in incomplete reduction of oxygen and formation of activated oxygen species (Meerson *et al.*, 1982).

The enzyme xanthine oxidase is synthesised as xanthine dehydrogenase. The dehydrogenase form cannot transfer electrons to molecular oxygen to form hydrogen peroxide or superoxide. During ischaemia xanthine dehydrogenase is converted into xanthine oxidase by the action of a protease enzyme. The latter enzyme is activated by calcium which is thought to increase within the cell during ischaemia (Roy and McCord, 1983). The ischaemically induced depletion of cellular ATP results in an elevated concentration of AMP, adenosine, inosine and hypoxanthine. The build up of hypoxanthine, which has been demonstrated in ischaemic tissue by De Wall *et al.* (1971) serves as an oxidizable purine substrate for xanthine oxidase. In the heart the oxidase content was shown to double after 8 minutes of ischaemia (McCord, 1985). Oxidation of purine substrate by xanthine oxidase generates superoxide anion and hydrogen peroxide.

Activation of phospholipase during ischaemia leads to activation of the cycloxygenase pathway. Oxygen free radicals are generated

during the conversion of prostaglandin G_2 to prostaglandin H_2 (Kuhel *et al.*, 1980).

Catecholamines autoxidised to adrenochrome can give rise to catecholamine semiquinones, which can donate electrons to oxygen and thus generate a superoxide anion radical (Bors *et al.*, 1978).

The normal defence system of tissues seems to be impaired during ischaemia. Glutathione peroxidase activity and ascorbic acid levels fall significantly during ischaemia in the dog (Rao *et al.*, 1983). Glutathione has been shown to leak from the ischaemic rat heart (Guarnieri *et al.*, 1979) while a 16 - 18% reduction of catalase and superoxide dismutase in the rat heart has been demonstrated by Meerson *et al.* (1982).

Hydroxyl radicals attack unsaturated fatty acid side chains of membrane lipids by abstracting hydrogen and leaving a carbon radical. This radical subsequently rearranges to produce a conjugated diene and the formation of organic oxygen radicals. These organic oxygen radicals can abstract hydrogen from additional fatty acid side chains resulting in a chain reaction and the production of lipid peroxides. In the presence of metal salts these lipid peroxides decompose to give alkoxy and peroxy radicals. Increases in lipid peroxides will result in an increase in membrane fluidity, increased permeability and loss of membrane integrity (Halliwell *et al.*, 1982).

Accumulation of lipid hydroperoxides was demonstrated in

the rat by Meerson *et al.* (1982), while the antioxidant, butylated hydroxy toluene, attenuated impairment of left ventricular contractility in experimental infarction. α-Tocopherol and histidine reduced the peroxidation of unsaturated lipids in rat heart (Gauduel and Duvelleroy, 1984). Superoxide dismutase, catalase and indomethacin attenuated the depression of calcium uptake and calcium stimulated magnesium dependent ATPase activity in the canine sarcoplasmic reticulum induced by activated leukocytes (Rowe *et al.*, 1983) while superoxide dismutase reversed the effect of free radicals generated by a xanthine-xanthine oxidase system and acidosis in dog hearts (Hess *et al.*, 1984). This suggests that acidosis may contribute to free radical formation.

Experiments have been carried out to determine whether perfusion of the coronary artery ligated rat heart with ascorbic acid, glutathione, catalase and mannitol has a beneficial effect on the development of arrhythmias.

1.10 Summary of aims

Aims of this thesis are as follows:-

1. To investigate the effects of potassium on ligation induced arrhythmias in the isolated rat heart and on the ventricular fibrillation thresholds.
2. To study the effects of calcium, calcium antagonists and pacing on ligation induced arrhythmias in the isolated rat heart.
3. To investigate the effects of magnesium on ligation induced arrhythmias in the isolated rat heart and on ventricular fibrillation thresholds.

4. To study the role of prostaglandins on ligation induced arrhythmias in the isolated rat heart.
5. To investigate the role of phospholipids on ligation induced arrhythmias in the isolated rat heart.
6. To study the role of free radicals on ligation induced arrhythmias in the isolated rat heart.
7. To test the effect of magnesium on rubidium efflux.
8. To investigate the effect of fibrillation induced by changing the ionic milieu on rubidium efflux.
9. To study the effect of calcium paradox on rubidium efflux.

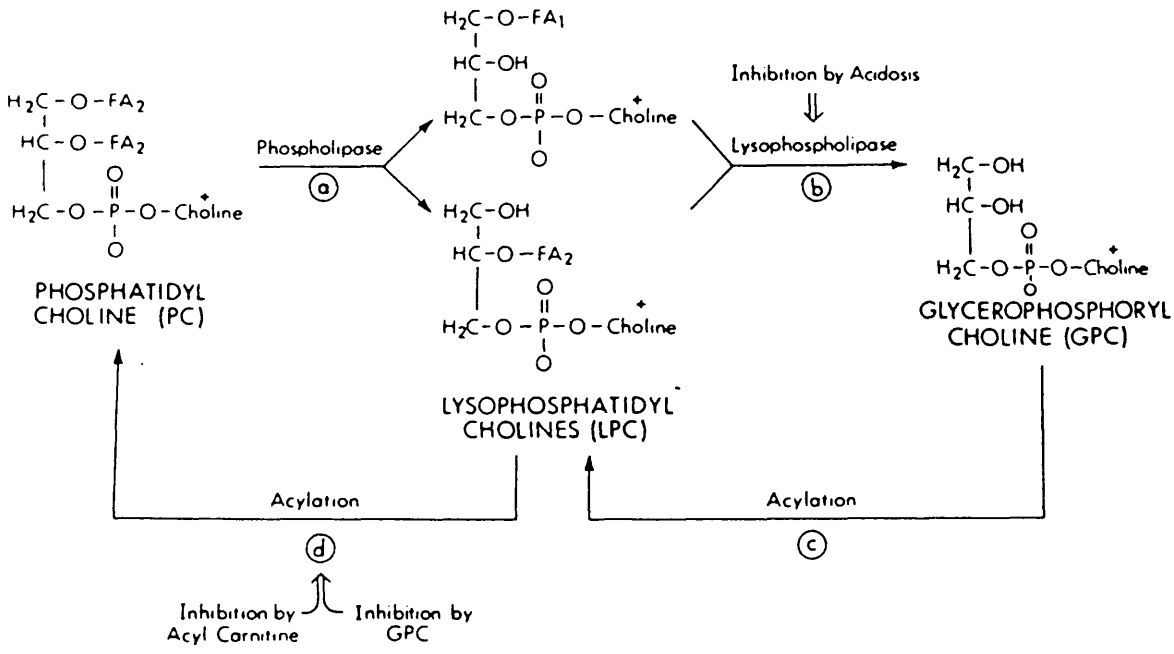


Figure 1.1. Pathways of synthesis of the major phospholipid moieties. FA = fatty acid. Redrawn from Corr *et al.* (1982a).

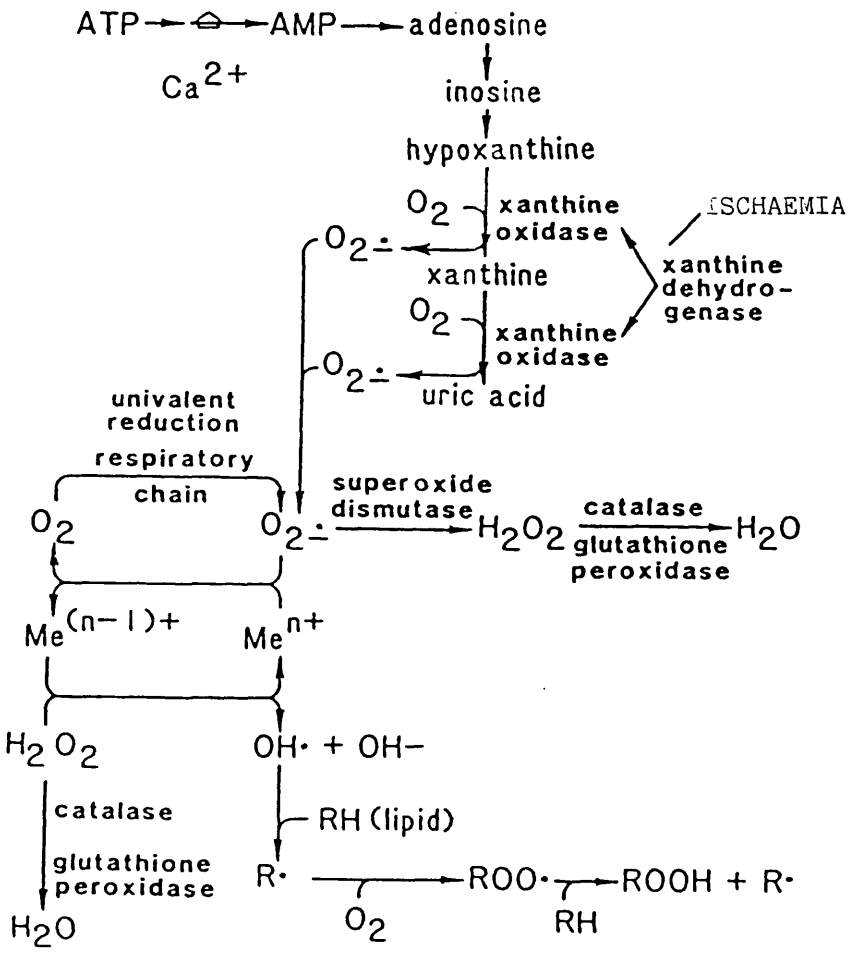


Figure 1.2. Putative relationship between degradation of ATP and the formation of superoxide and hydroxyl radicals during myocardial ischaemia $O_2^{\cdot-}$ superoxide radical OH·, hydroxyl radical ⏀ activation. Redrawn from van der Vusse and Reneman (1985).

2.0 MATERIALS AND METHODS

2.1 Rat hearts perfused by the Langendorff technique

Male Wistar rats (University of Bath strain) weighing 250 - 350 grams were used throughout the study.

Rats were stunned by a blow on the head. Hearts were exposed, excised and arrested by ice-cold perfusion media. The aorta was attached to a stainless steel cannula and perfused at a constant rate (10 mls/minute) by means of a Watson-Marlow flow inducer (HR 100).

A fine pin was passed through the apex of the heart and connected to an isometric transducer (UF 1) via a thread, this was also used to trigger a Devices rate meter (4521). Perfusion pressure was measured using a Bell and Howell transducer. Lead II was used for electrocardiography and this was displayed along with the contractility on a Gould digital storage oscilloscope OS4000 and recorded on Devices MX 216 recorder. All other recordings were made on a Devices M 19 recorder. Hearts were made to beat against a resting tension of two grams. A diagram of the equipment is shown in Figure 2.1.

2..2 Perfusion media

Initially hearts were perfused with Krebs-Henseleit buffer having the following composition (mmol/litre): NaCl 118, $NaHCO_3$ 25, KCl 4.7, KH_2PO_4 1.2, $MgSO_4$ 1.2, $CaCl_2$ 1.23 and glucose 11. It had a pH of 7.4 when gassed with 95% oxygen and 5% carbon dioxide. During preparation of this solution precautions were taken to prevent the precipitation of calcium. Perfusion fluid was pumped through a filter (Whatman No. 1) to prevent small particles from entering the coronary circulation. Temperature was

maintained at 37°C by means of a Grant SU5 circulating pump. Both temperature and pH were frequently checked.

2.3 Induction of ischaemia and arrhythmias

After setting the heart up, a loose ligature was placed around the main left coronary artery using a curved stainless steel surgical needle size 6. Five minutes before ligation calcium was increased to 2.5 mmol/litre and potassium decreased to 2.5 mmol/litre (by decreasing KCl). Coronary artery ligation was produced by tightening the ligature and the electrocardiogram was recorded for 30 minutes following ligation. The protocol of the experiments is shown in Figure 2.2. Whenever reperfusion of heart was required, the thread was passed through a fine polythene tube to form a snare This was then held in place with a small bulldog clip. To reperfuse the ischaemic tissue the clip was released.

The E.C.G. parameters recorded were the number of premature ventricular contractions (PVC's), the incidence, onset and duration of ventricular tachycardia (V.T.) and incidence, onset and duration of ventricular fibrillation (V.F.). Premature contractions recorded during runs of tachycardia were included in the total PVC's count.

2.4 Pacing Langendorff perfused hearts

Hearts were paced with bipolar stimulating electrodes placed on the metal aortic cannula and on the atrioventricular groove. The right atrium was removed and hearts were stimulated at rates between 200 and 450 beats min^{-1} with square wave pulse of 2 mseconds duration and 10V amplitude, delivered by Grass S48 stimulator via

Grass S[U5A isolation unit.

2.5 Evaluation of ventricular fibrillation thresholds

Hearts were set up and paced at 300 beats.min^{-1} with bipolar stimulating electrodes using one channel of a Grass S88 two channel stimulator as described in Section 2.1. The second channel of the stimulator was synchronized with the first channel and with a second stimulator (Grass S48). The second stimulator was used to deliver a train of pulses (2 mseconds pulses every 5 mseconds for 70 mseconds delivered 10 mseconds after the peak of the R wave) from the S48 with a controlled delay after the pacing stimulus with the use of the arrhythmic pulse facility of the S88. Trains were delivered via a Grass S1U5A stimulus isolation unit to bipolar stimulating electrodes positioned across the ventricular wall. The positioning of the electrodes and the use of the isolation units prevented interference of stimulus with the ECG recording. The second channel of the S88 stimulator also triggered a single sweep of a Narco TM storage oscilloscope. Equipment used is shown in Figure 2..3.

Experimental procedure

After stabilising the heart for 15 minutes an arrhythmic stimulus was commenced with a stimulus delivered 10 mseconds after the peak of the R wave. Stimulation was initiated at 1 mA and increased at 20 second intervals in 2 mA increments until ventricular fibrillation was obtained. If fibrillation occurred the heart was allowed to stabilize for 1 minute before repetition of the same stimulus. Fibrillation was considered to occur when a chaotic electrical activity appeared with associated collapse

of contractility which persisted for 800 mseconds. Ventricular fibrillation threshold was taken as the current which produced fibrillation on two successive occasions.

2.6 Determination of $^{86}Rb^{+}$ efflux rate constant using liquid scintillation counting

Construction of quench curve:-

A quench curve was constructed using the external standard channel ratio. 4 mililitres of scintillation fluid (Opti phase 'Safe') and 0.02 µCi of $^{86}Rb^{+}$ were added to two mini-vials. To tubes 2,3,4,5,6,7,8, 9 and 10 were added 20, 40, 60, 80, 100, 120, 140, 160 and 180 µl of CCl_4 respectively. Then the volume was completed to 5 mililitres with distilled water to simulate the emulsion system found in the experimental conditions and the tubes were capped. The activity in 0.02 µCi of $^{86}Rb^{+}$ was counted in terms of dpm and entered in the machine. The samples were then loaded into the LKB 1215 liquid scintillation counter which had been programmed to count the samples and to construct automatically a quench curve. This curve was stored in the counter's memory so that dpms for all subsequent samples were calculated automatically. A typical quench curve is shown in Figure 2.4.

2.7 $^{86}Rb^{+}$ loading procedure

The rat heart was setup and perfused with Krebs-Henseleit solution containing calcium 1.2 mM, potassium 2.5 mM unless otherwise indicated for a control period of 5 minutes. $^{86}Rb^{+}$ (0.2µCi/ml) was then added for 10 minutes. $^{86}Rb^{+}$ was then washed out using the above mentioned Krebs' solution. The effluent collected for the first 14 minutes was discarded, aliquots were then collected every minute for 15 minutes. The perfusate was

then switched to the one being studied. One millilitre of perfusate was added to four millilitres of scintillation fluid (Opti phase 'Safe') and counted in an LKB 1215 scintillation counter using an external standard channel ratio method for 10,000 counts or 10 minutes. At the end of the experiment the heart was blotted with tissue paper, chopped into small pieces and left to dissolve in 5 mls of 1N KOH. After 48 hours the dissolved heart was neutralized with 5 mls of 1N HCl to prevent chemiluminescence. The decay during the 48 hours was allowed for. Then one millilitre of the neutralized material was added to 4 mls of the scintillation cocktail and counted.

2.8 Treatment of $^{86}Rb^{+}$ efflux results

The efflux rate constant of $^{86}Rb^{+}$ was calculated by dividing the counts in the perfusate by the number of counts contained in the hearts during the collection period (Dürbin and Jenkinson, 1961).

$$\text{Efflux rate constant } min^{-1} = \frac{\text{Counts in perfusate}}{\text{Tissue counts x collection time (min)}}$$

The mean of the last 5 samples of the control period were compared with the mean of the last 5 samples of the treatment period. However, in the early experiments only the percentage increase in $^{86}Rb^{+}$ counts were calculated.

2.9 Effect of ventricular fibrillation on $^{86}Rb^{+}$ efflux

Hearts were set up as in Section 2.1 and perfused with Krebs-Henseleit solution containing potassium 2.5 mM, calcium 1.2 mM. In order to determine if ventricular fibrillation affects rubidium efflux, hearts were made to fibrillate in the absence of ligation by the use of arrhythmogenic solutions. The following

arrhythmogenic solutions were used:-

a) Ca^{++} 5 mM K^{+} 1.2 mM Mg^{++} 1.2 mM

b) Ca^{++} 5 mM Na^{+} 59 mM K^{+} 5.9 mM

c) Ca^{++} 5 mM Mg^{++} 0 mM K^{+} 2.5 mM

Changes were made by altering KCl concentration. In the case of 59 mM NaCl, sucrose was added to maintain normal osmolarity.

2.10 Effect of calcium paradox on $^{86}Rb^{+}$ efflux

Hearts were set up as in Section 2.1 and perfused with Krebs-Henseleit solution (K^{+} 2.5, Ca^{++} 1.2 mM). The hearts were then perfused with calcium free solution for 2, 4, 6, 8 and 10 minutes followed by calcium repletion. Samples of perfusate were collected every minute for evaluation of $^{86}Rb^{+}$ and protein efflux. Percentage rise in resting tension and recovery of developed tension were also calculated.

2.11 Estimation of protein

Protein was determined by the Lowry method (Lowry *et al.*, 1951).

The following three stock solutions were prepared:

(1) 5% $CuSO_4.5H_2O$

(2) 10% NaK tartarate

(3) 2% Na_2CO_3 in 0.1 N NaOH.

Solution A was prepared by adding 1 ml of the 5% $CuSO_4.5H_2O$ and 1 ml of 10% NaK tartarate to 8 mls water.

Solution B was prepared by adding 1 ml of solution A to 50 mls of 2% Na_2CO_3 in 0.1 N NaOH. Folin Ciocalteau reagent (phenol) was diluted 1 in 1 with water just before use.

A standard curve was constructed using 10 - 200 µg bovine serum albumin (Figure 2.5).

Procedure

0.5 ml samples were added to solution B and left for 10 min at room temperature. Then 0.5 ml of Folin Ciocalteau reagent was added, mixed and read at 610 nm after 30 minutes using a spectrophotometer.

2.12 Determination of dry weight/wet weight ratio

In experiments where protein estimation was performed, hearts were blotted with tissue paper and wet weight was determined. Hearts were then left overnight in a hot oven (100°C). The next day hearts were cooled to room temperature and weighed to determine the dry weight/wet weight ratio.

2.13 Statistical analysis

Results are expressed as the mean ± standard error of mean. Paired and unpaired t-test were used as appropriate.

Assessment of the data relating to premature ventricular contractions during occlusion was by Wilcoxon's rank sum test for unpaired data. Statistical differences between incidences of ventricular tachycardia and ventricular fibrillation were assessed by Chi-Squared analysis. Regression lines were fitted by least squares analysis. $P < 0.05$ was considered to be significantly different from the appropriate control.

2.14 Drugs used

Drug	Manufacturer
Adenosine	Sigma
Adrenaline	BDH
Arachidonic acid	BDH
Ascorbic acid	BDH
Aspirin	Sigma
Atropine	Sigma
BW 755C	Wellcome
Carbachol	BDH
Catalase	Sigma
Dazoxiben	Pfizer
Diltiazem	Pfizer
Eicosatetraynoic acid	Roche
Glutathione	Sigma
Indomethacin	MSD
Lysophosphatidylcholine	Sigma
Mannitol	BDH
Mepacrine	Sigma
Nafaztrom	Bayer
N-butyl imidazole	Koch Light Laboratories
Ouabain	BDH
$^{86}RbCl$	Amersham
Verapamil	Abbot
ZK 36374	Schering

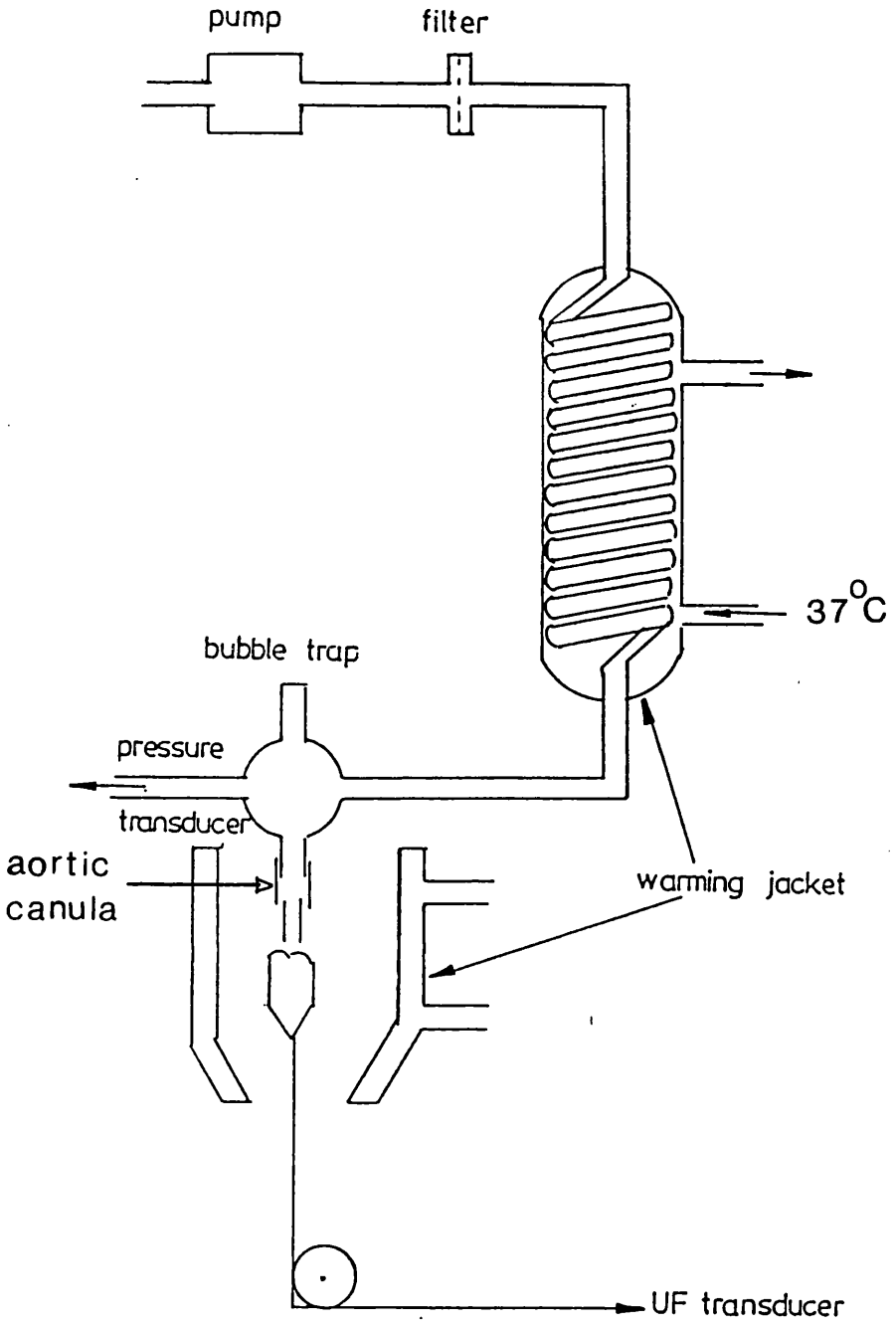


Figure 2.1 Diagrammatic representation of perfusion apparatus.

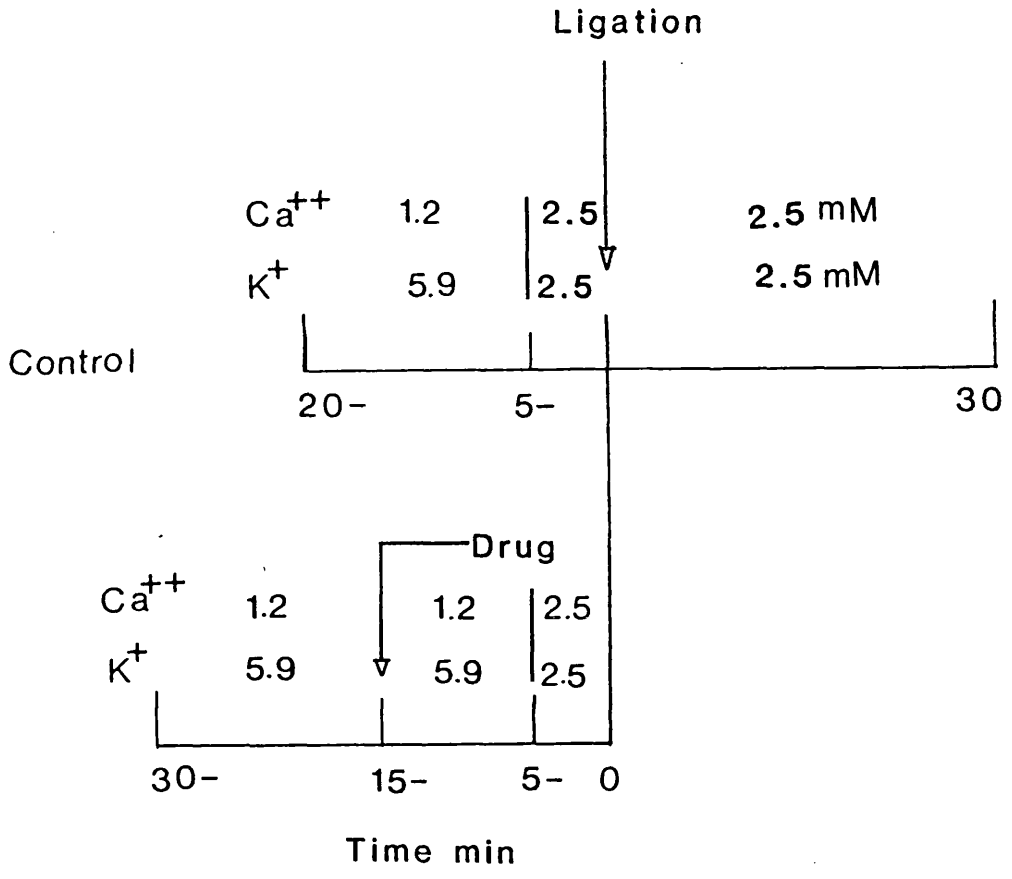


Figure 2.2. Diagrammatic representation of protocol of experiments.

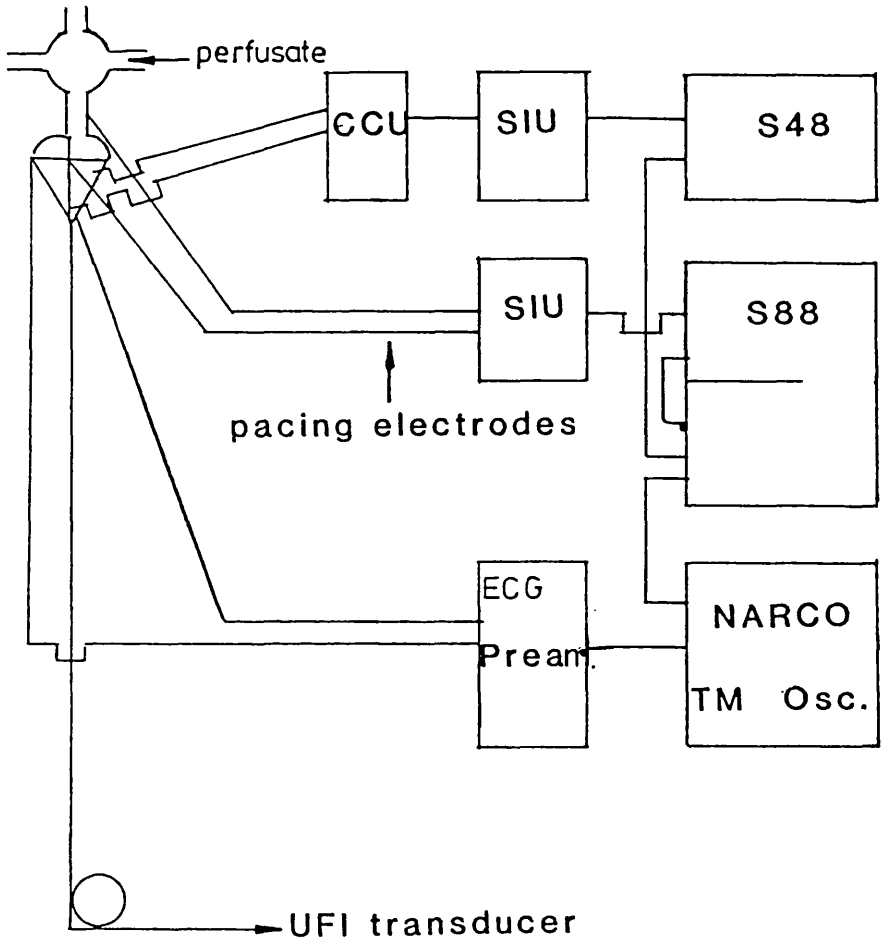


Figure 2.3 Diagrammatic representation of instrumentation for the assessment of ventricular fibrillation threshold by a train of stimuli.

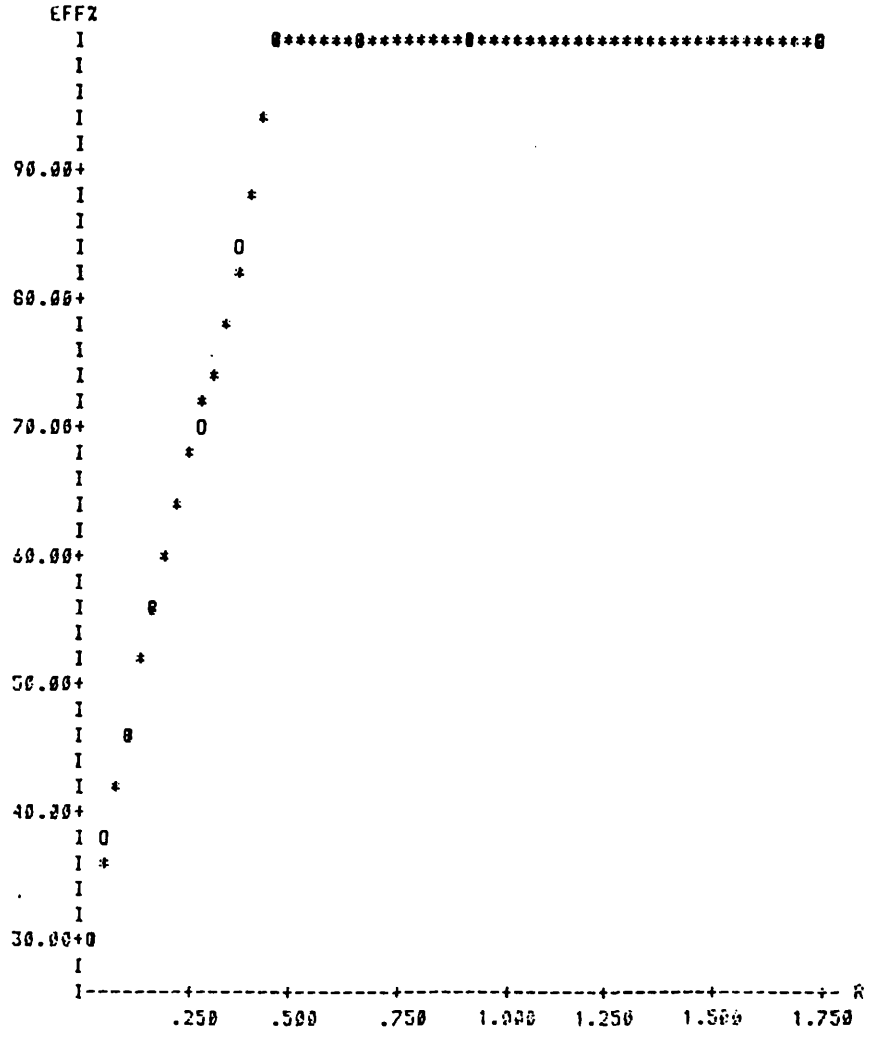


Figure 2.4. Quench curve for $^{86}Rb^{+}$ determined by the LKB 1215 liquid scintillation counter.

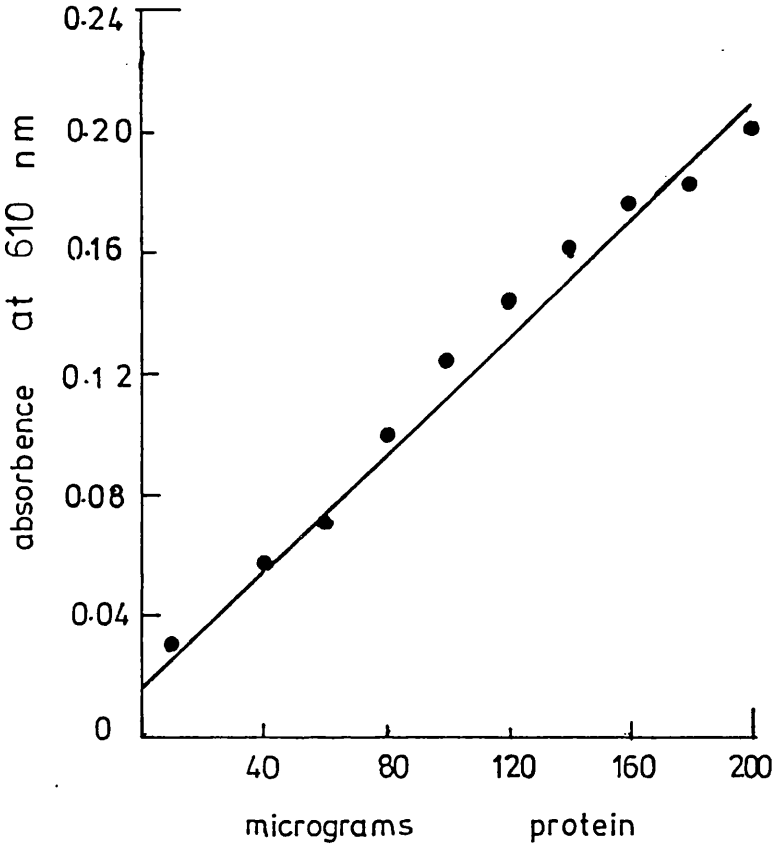


Figure 2·5 Typical calibration curve for protein.

CHAPTER 3

In this chapter the effects of perfusate potassium, calcium and magnesium on ligation induced arrhythmias in the isolated rat heart are described. The effects of these ions on the haemodynamics of the perfused heart are also considered. Inhibition of Na^+-K^+ ATPase with ouabain and the use of drugs that shorten action potential duration (carbachol and adrenaline) are used to investigate the mechanism of action of potassium on ligation induced arrhythmias. The effects of potassium and magnesium on ventricular fibrillation thresholds are also described.

3.1 Coronary ligation induced arrhythmias

Hearts were initially stabilised in modified Krebs-Henseleit solution containing 1.2 mM calcium and 5.9 mM potassium. Five minutes prior to ligation the ionic milieu was adjusted according to the required experimental conditions.

The criteria for a successful ligation were an increase in perfusion pressure (P.P.), a decrease in developed tension (D.T.), a rise in resting tension (R.T.) and discolouration of the ischaemic area on the left ventricle. A typical example is shown in Figure 3.1.

Ventricular tachycardia was diagnosed if more than four or more consecutive morphologically similar ventricular extrasystoles occurred. Fibrillation was diagnosed if chaotic fractionated irregularities persisted for more than two seconds (Figure 3.2).

3.2 Effects of potassium on ligation induced arrhythmias

Potassium experiments were performed in the presence of 2.5 mM calcium. Potassium 1.2 - 10 mM induced a concentration dependent reduction in the incidence ($P < 0.05$, $r = 0.957$) and duration ($P < 0.02$, $r = 0.877$) of ventricular fibrillation (Figure 3.3). Potassium also delayed the onset of fibrillation ($r = 0.955$, $P < 0.01$) in those hearts that developed this type of arrhythmia (Figure 3.4).

Potassium within the concentration range of 1.2 to 5.9 mM had no substantial effect on the number of premature contractions or on the incidence and duration of ventricular tachycardia, but at a concentration of 10 mM it significantly reduced all of these arrhythmias (Figure 3.3).

The major haemodynamic effect of potassium was to produce a concentration dependent vasodilation ($P < 0.01$, $r = -0.945$) (Figure 3.5). at the concentrations used, potassium had no significant effect on developed tension or heart rate.

3.3 Effects of postligation administration of potassium on ligation induced arrhythmias

In the clinical situation drugs are usually applied after infarction has taken place. This experiment was carried out to test if postligation administration of potassium was beneficial. Postligation administration of 10 mM potassium significantly ($P < 0.05$) reduced the incidence and duration of ventricular fibrillation (Figure 3.6).

3.4 Effects of replacing potassium with rubidium on ligation induced arrhythmias

4.7 mM rubidium in the presence of 1.2 mM potassium significantly reduced the incidence and duration of ventricular fibrillation. There was no significant difference between the antiarrhythmic effects elicited by this combination of rubidium and potassium and the effect of 5.9 mM potassium (Figure 3.6).

3.5 Effects of ouabain on ligation induced arrhythmias

It is possible that potassium exerts its antiarrhythmic effects by stimulating the sodium potassium pump. This experiment was carried out to test whether inhibition of this pump can aggravate arrhythmias.

Ouabain (10^{-5} and 5×10^{-5}M) had no effect on ligation induced arrhythmias ($n = 9$). At these concentrations ouabain did not significantly alter heart rate, perfusion pressure or developed tension. Increasing the ouabain concentration to 10^{-4} M caused the resting tension to rise, but the developed tension was not increased.

3.6 Effects of carbachol on ligation induced arrhythmias

Accumulation of extracellular potassium in the ischaemic region would be expected to shorten action potential duration in that area. This would elicit an inhomogeneity in action potential duration between the ischaemic and non-ischaemic regions which may result in electrophysiological derrangements. It is possible that potassium exerts its antiarrhythmic effects by producing shortening of action potential duration in the non-ischaemic region thus reducing the heterogeneity between the ischaemic and non-ischaemic

region. Carbachol, which shortens the action potential duration by increasing potassium efflux, was used to see if by shortening the action potential duration a similar protective effect could be obtained to that seen with potassium.

Carbachol (10^{-6} M) did not alter any measured parameter of coronary ligation induced arrhythmias, however it did significantly reduce heart rate from 230 ± 18 to 105 ± 17 beats/minute (n = 5) ($P < 0.05$) and increased perfusion pressure from 53 ± 4 to 93 ± 9 mm Hg ($P < 0.05$). To counteract any possible protective effect which might be elicited by low heart rate, carbachol treated hearts were paced at the initial heart rate 10 minutes before ligation (stimulus parameters were 6V, 2 mseconds duration).

3.7 Effects of adrenaline on coronary ligation induced arrhythmias

This experiment was carried out to test whether inducing homogenous shortening of action potential duration with adrenaline could produce an antiarrhythmic effect similar to potassium. Adrenaline (10^{-7} M) did not signficiantly alter any measured arrhythmia parameter, although there was a tendency for the incidence and duration of ventricular fibrillation to increase. Perfusion pressure was significantly decreased from 56 ± 4 to 43 ± 1 mm Hg ($P < 0.05$) (n = 5) and the heart rate was significnatly ($P < 0.05$) increased from 213 ± 18 to 280 ± 11 beats/minute following the addition of adrenaline.

3.8 Effects of calcium on coronary ligation induced arrhythmias

These experiments were done to investigate the role of calcium on coronary ligation induced arrhythmias. Different concentrations

of calcium were perfused to test whether low extracellular calcium can protect against these arrhythmias. In these experiments a potassium concentration of 2.5 mM was used. 0.61, 1.23 and 2.5 mM calcium did not significantly alter the incidence or duration of ventricular fibrillation (Figure 3.7), however, decreasing extracellular calcium did delay the onset of ventricular fibrillation ($r = -0.999$, $P < 0.05$) (Figure 3.8). 0.6 mM calcium significantly ($P < 0.05$) reduced the number of premature ventiruclar contractions (Figure 3.7) and delayed the time required to reach maximum instability after coronary ligation from 12 to 20 minutes (Figure 3.9).

3.9 Effects of verapamil on coronary ligation induced arrhythmias

Verapamil was used to test whether blocking the slow calcium channels was protective against ligation induced arrhythmias.

Verapamil (10^{-8}, 5×10^{-8} and 10^{-7} M) perfused for 15 minutes prior to ligation and during ligation failed to exert any protective effect on arrhythmias although with 5×10^{-8} M ($n = 15$) there was a tendency for the incidence of ventricular fibrillation to decrease. 5×10^{-8} verapamil significantly ($P < 0.01$) reduced developed tension from 9.4 ± 1 to 6.5 ± 0.8 grams while 10^{-7} M verapamil ($n = 11$) significantly reduced ($P < 0.01$) both developed tension from 8.4 ± 0.7 to 4.6 ± 0.5 grams and perfusion pressure from 87 ± 10 to 63 ± 7 mm Hg. At these concentrations of verapamil there was no significant effect on heart rate.

3.10 Effects of diltiazem on coronary ligation induced arrhythmias

10^{-7} M diltiazem had no significant effect on the incidence and duration of ventricular fibrillation, however increasing the

concentration to 10^{-6} M significantly ($P < 0.05$) reduced the incidence and duration of ventricular fibrillation (Figure 3.10). Diltiazem induced a concentration dependent reduction of developed tension, perfusion pressure and heart rate. The effects of verapamil and diltiazem on perfusion pressure, developed tension and heart rate are shown in Figure 3.11. Unlike low calcium,neither drug affected the onset of ventricular fibrillation.

Pacing of hearts at 300 beats/minute reversed the protective effect of 10^{-6} M diltiazem (Figure 3.10). Diltiazem solvent, ethanol, was used as a control.

3.11 Effects of electrical pacing on coronary artery ligation arrhythmias

Pacing of hearts at 200, 250, 300, 350 and 450 beats/minute did not have any significant effect on the incidence of ventricular fibrillation, however there was a significant ($P < 0.05$) negative correlation between heart rate and onset of ventricular fibrillation ($r = -0.924$) (Figure 3.12).

3.12 Effects of magnesium on ligation induced arrhythmias

Perfusion of hearts with 0.6, 1.2. 2.4 and 3.6 mM magnesium did not affect the incidence or duration of ventricular fibrillation, but 4.8 mM magnesium significantly ($P < 0.05$) reduced both the incidence and duration of ventricular fibrillation. 4.8 mM magnesium produced 31% reduction in heart rate from 245 ± 11 to 169±76 bpm which could contribute to its antiarrhythmic action. This is supported by the fact that pacing hearts at 300 beats/minute reversed the protective effect of 4.8 mM magnesium (Figure 3.13).

Magnesium also produced a concentration dependent reduction in developed tension and perfusion pressure.

3.13 Effects of magnesium and potassium on ventricular fibrillation thresholds

Electrically induced fibrillation causes an increase in the resting tension and irregularities in the electrocardiogram. A typical example of electrically induced ventricular fibrillation is shown in Figure 3.14.

Perfusion with magnesium (0.6 - 4.8 mM) did not induce any significant effect on ventricular fibrillation thresholds. On the other hand, potassium 5.9 mM significantly ($P < 0.01$) increased the ventricular fibrillation threshold when compared with 2.5 mM potassium (Figure 3.15).

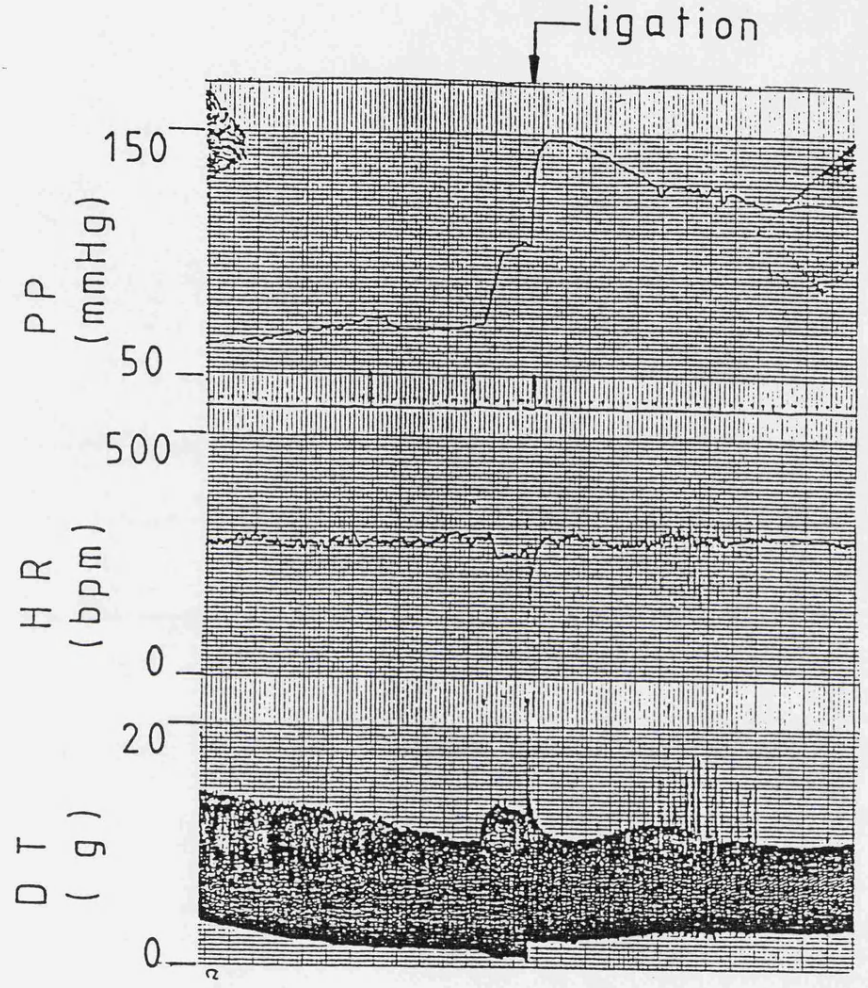


Figure 3.1. An example of an experiment in which the criteria for a successful ligation were satisfied. Note the increase in perfusion pressure (P.P.), rise in resting tension and the decrease in developed tension (D.T.) occurring on ligation.

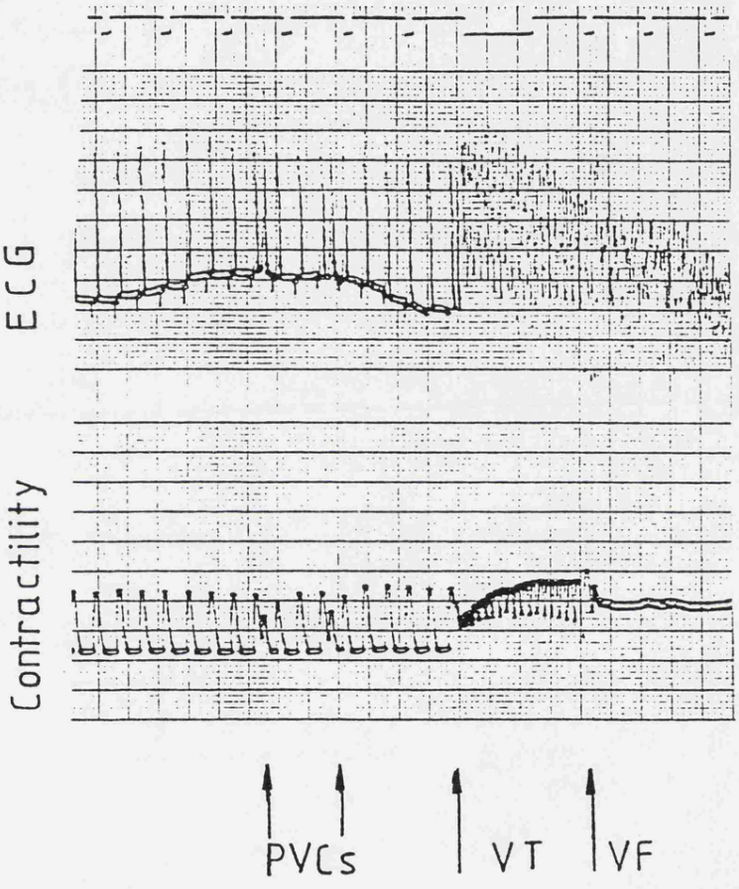


Figure 3.2. An example of an experiment in which premature ventricular contractions (P.V.C.s), tachycardia (V.T.) and fibrillation (V.F.) occurred following coronary artery ligation.

Figure 3.3. Effects of potassium on coronary artery ligation arrhythmias in the rat heart *in vitro*

(a) Duration of ventricular fibrillation (sec)

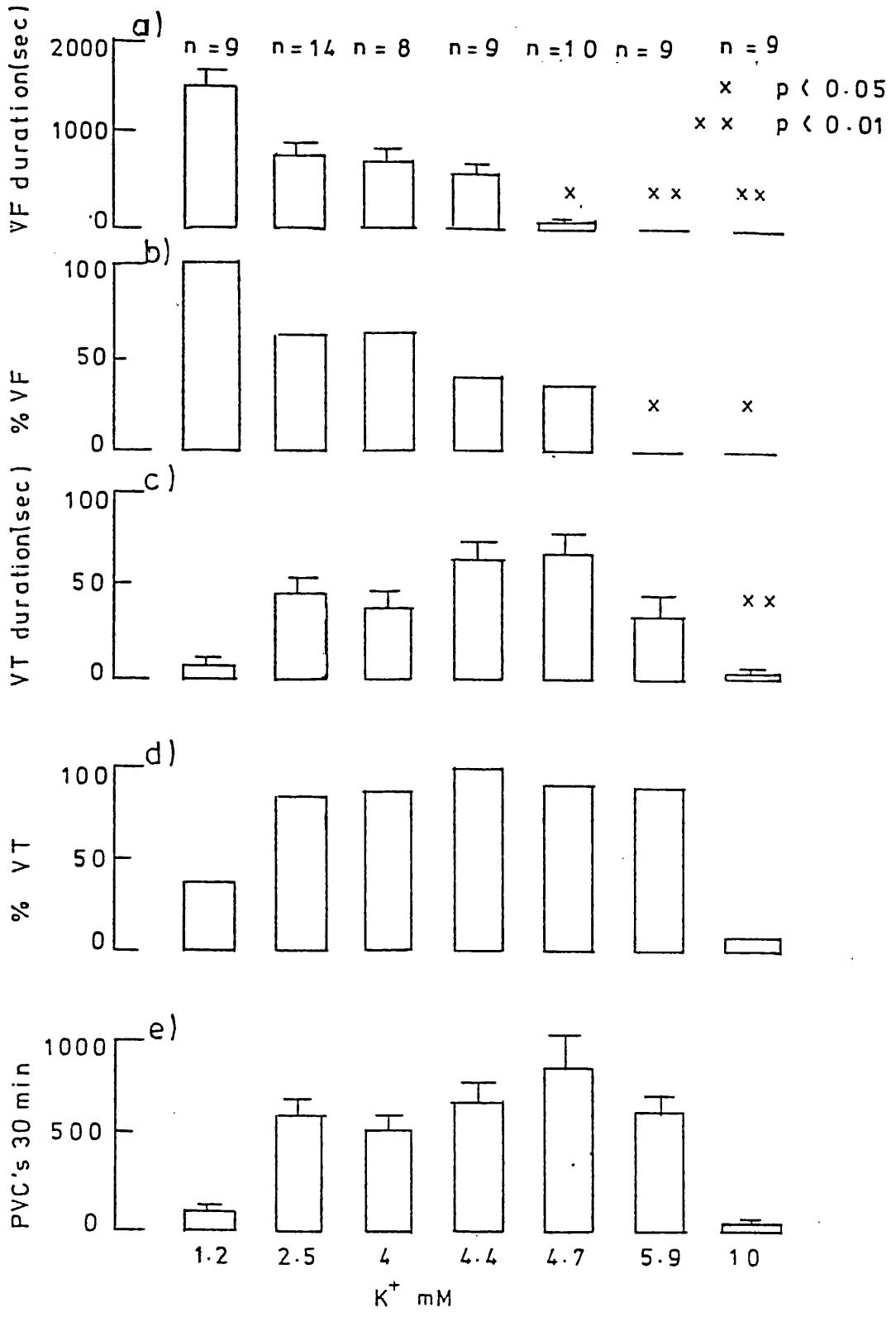
(b) Percentage incidence of ventricular fibrillation,

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia,

(e) Premature ventricular contractions/30 min

p compared with 2.5 mM potassium.


a)
n = 9
n = 14
n = 8
n = 9
n = 10
n = 9
n = 9
x p (0.05
x x p (0.01
VF duration(sec)
2000
1000
0
b)
% VF
100
50
0
c)
VT duration(sec)
100
50
0
d)
% VT
100
50
0
e)
PVC's 30 min
1000
500
0
1.2
2.5
4
4.4
4.7
5.9
10
K+ mM

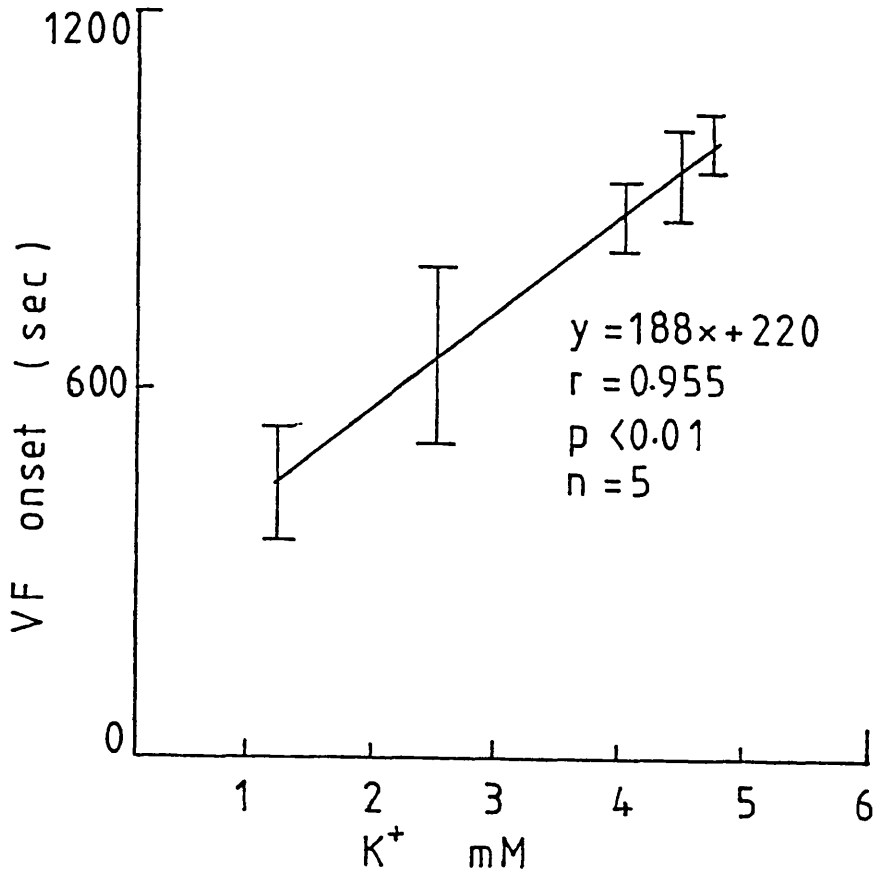


Figure 3.4. Relation between perfusate potassium concentration and onset of ventricular fibrillation (VF).

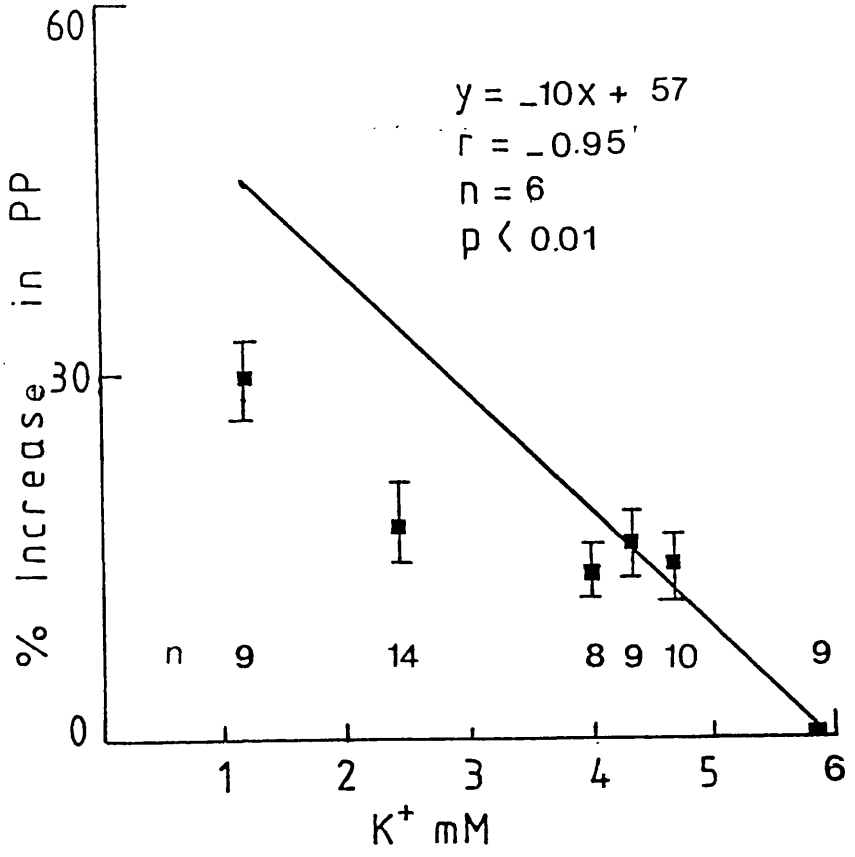


Figure 3.5.

Relationship between perfusate potassium concentration and percentage increase in perfusion pressure (PP) before coronary ligation from control potassium level of 5.9 mM. Perfusion pressure at 5.9 mM potassium was 61 ± 1, n = 68.

Figure 3.6. Effects of 10 mM potassium given immediately after coronary artery ligation and 4.7 mM rubidium + 1.2 mM potassium given before ligation on coronary artery ligation arrhythmias in the rat *in vitro*.

(a) Duration of ventricular fibrillation (sec)

(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions/ 30 min,

Control potassium was 2.5 mM.

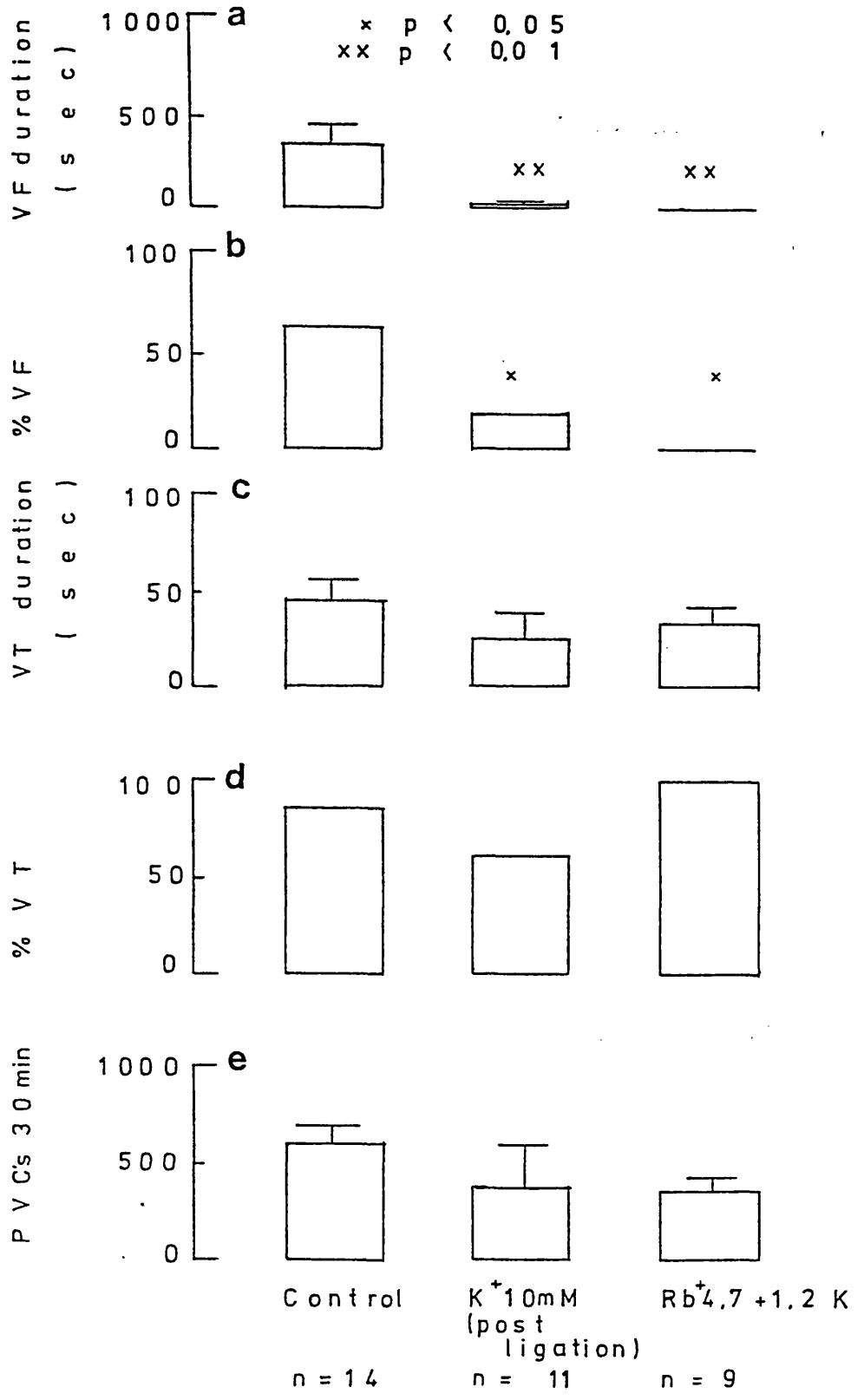

VF duration (sec)
1000
500
0
a
x p < 0,05
xx p < 0,01
xx
xx
% VF
100
50
0
b
x
x
VT duration (sec)
100
50
0
c
% VT
100
50
0
d
PVCs 30 min
1000
500
0
e
Control
K+ 10mM (post ligation)
Rb+ 4,7 +1,2 K
n = 14
n = 11
n = 9

Figure 3.7 Effects of calcium concentration on coronary artery ligation induced arrhythmias

(a) Duration of ventricular fibrillation (sec)

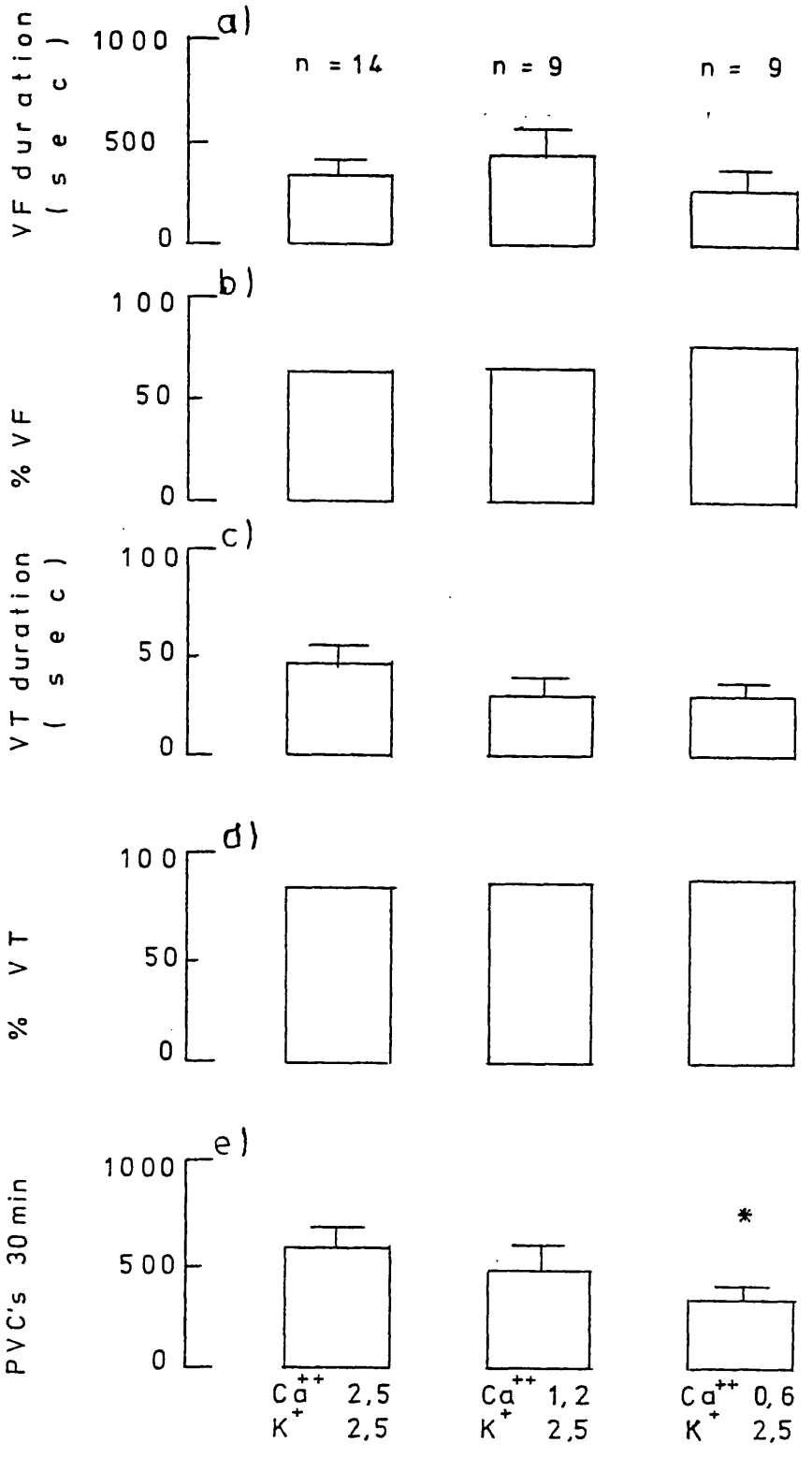
(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contraction/3o min.

*$P < 0.05$ compared with 2.5 mM calcium and 2.5 mM potassium.


a)
VF duration (sec)
1000
500
0
n = 14
n = 9
n = 9
b)
% VF
100
50
0
c)
VT duration (sec)
100
50
0
d)
% VT
100
50
0
e)
PVC's 30 min
1000
500
0
*
Ca++ 2,5
K+ 2,5
Ca++ 1,2
K+ 2,5
Ca++ 0,6
K+ 2,5

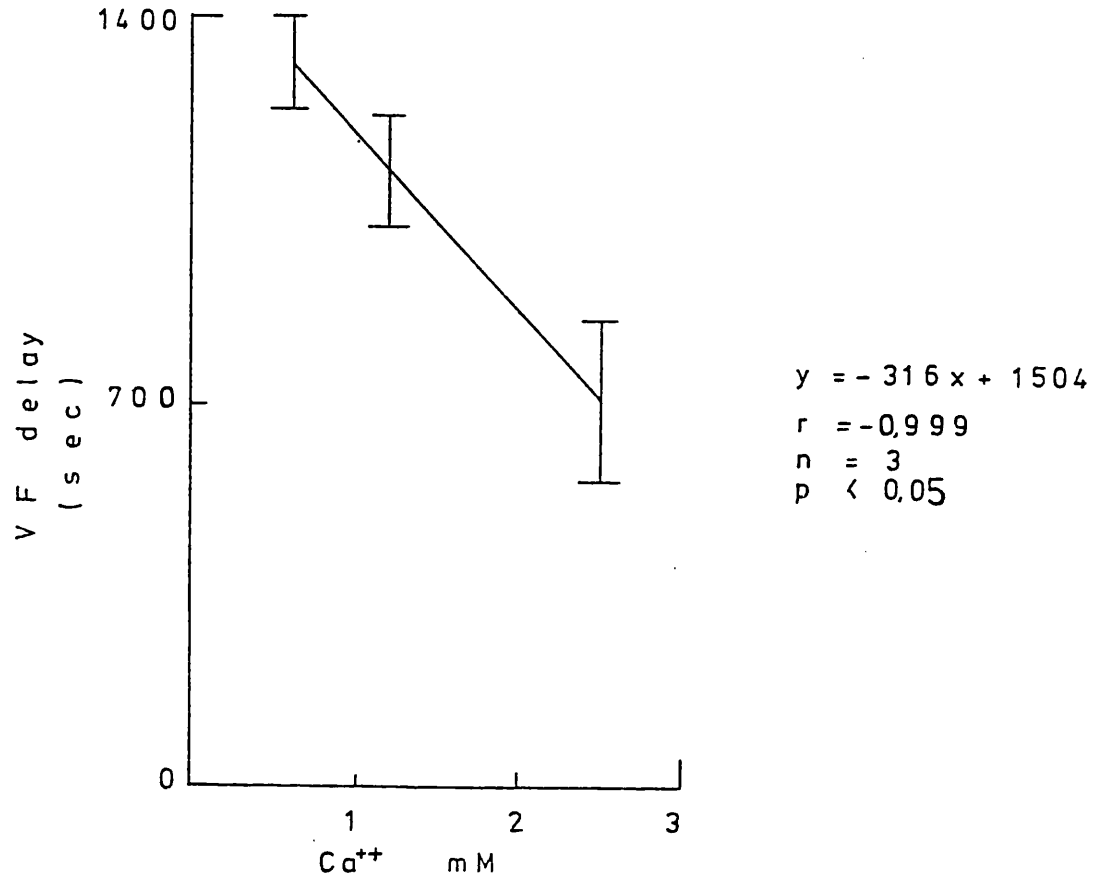


Figure 3.8 Effects of calcium concentration on the onset of ventricular fibrillation following coronary artery ligation.

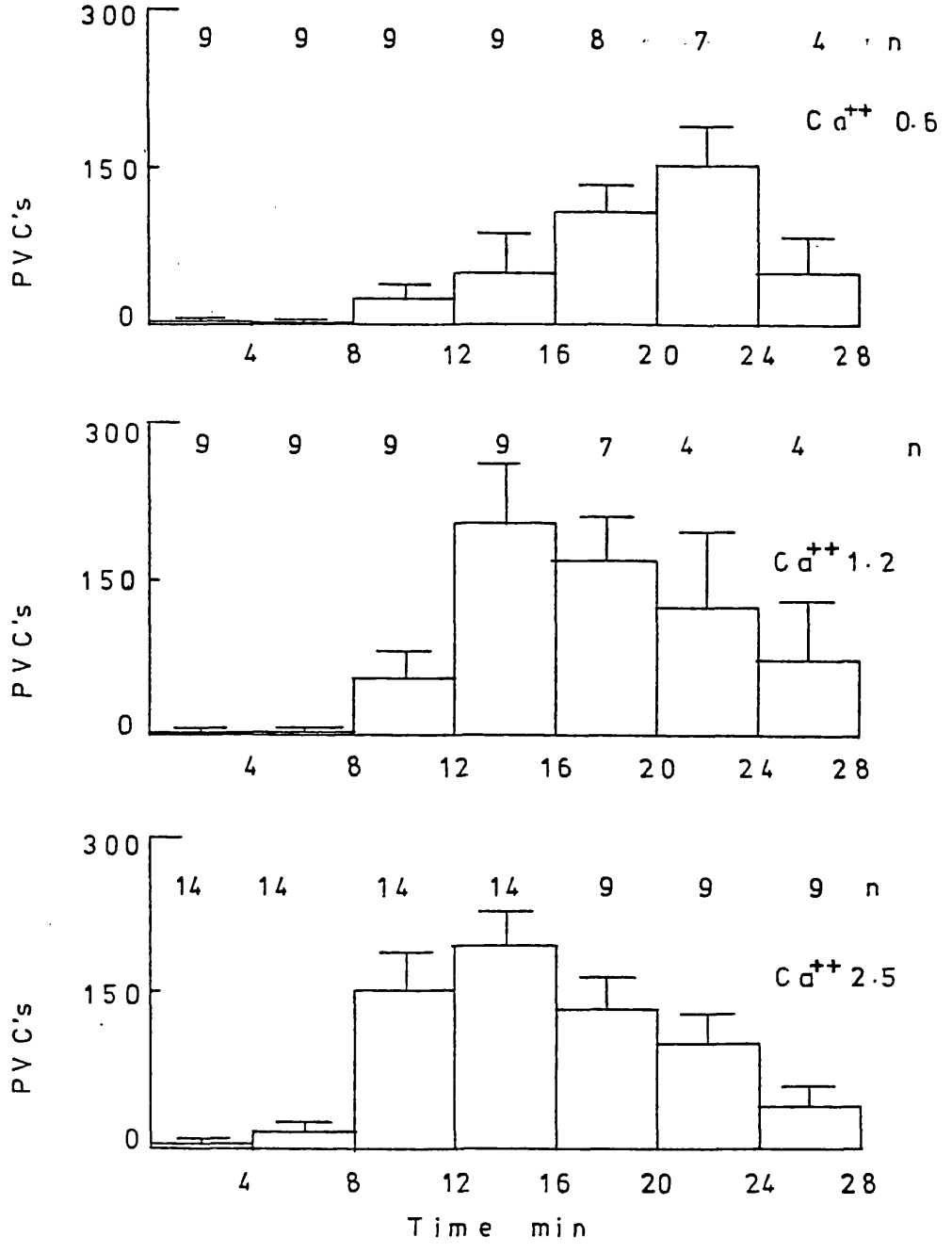


Figure 3.9. Time course of the occurrence of premature ventricular contractions during coronary artery ligation in the presence of 0.6 mM, 1.2 mM and 2.5 mM calcium. Note the delay in the peak elicited by reducing extracellular calcium to 0.6 mM.

Figure 3.10. Effects of diltiazem concentration of the perfusate during coronary artery ligation on:

(a) Duration of ventricular fibrillation in seconds

(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia in seconds

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions/30 minutes

Note the reversal of the protective effect, elicited by 10^{-6} M diltiazem by pacing the hearts to 300 beats/minute. Control was 0.0001 v/v ethanol.

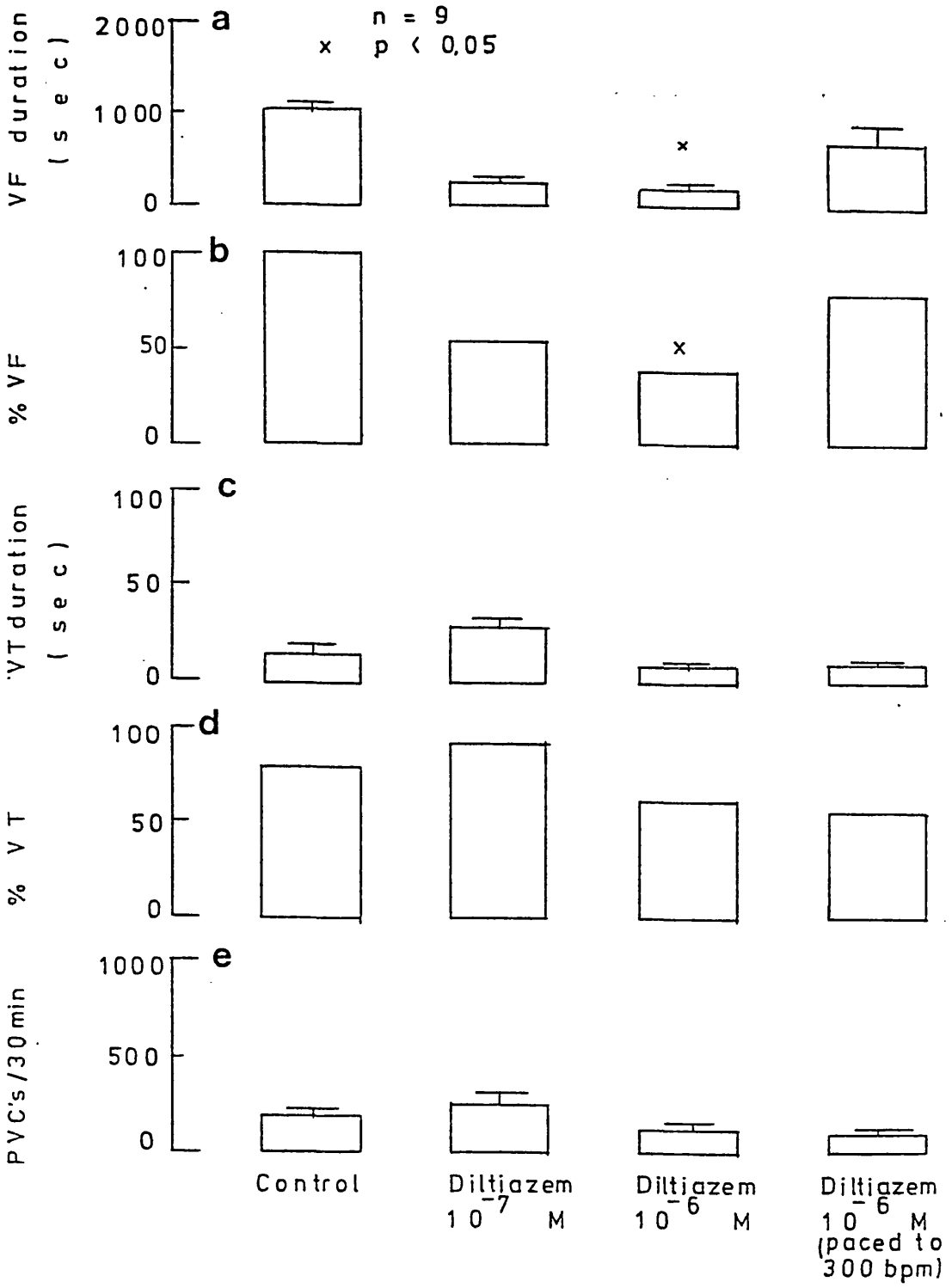
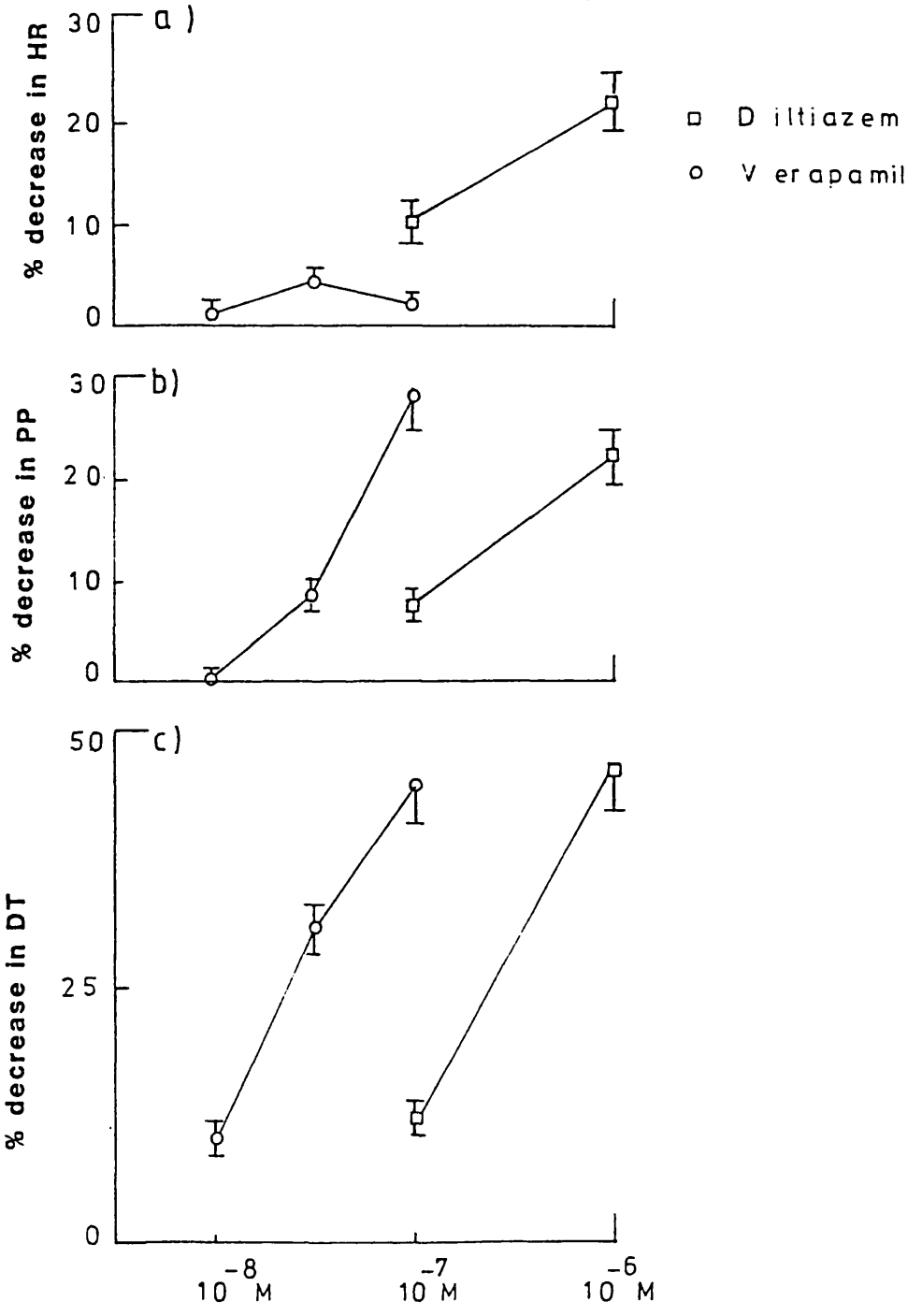

n = 9
x p < 0,05
a
VF duration (sec)
2000
1000
0
b
% VF
100
50
0
c
VT duration (sec)
100
50
0
d
% VT
100
50
0
e
PVC's/30 min
1000
500
0
Control
Diltiazem 10⁻⁷ M
Diltiazem 10⁻⁶ M
Diltiazem 10⁻⁶ M (paced to 300 bpm)

Figure 3.11. Comparison of the effects of verapamil and diltiazem on (a) Developed tension DT (b) Perfusion pressure (PP), (c) Heart rate HR. The control values of each treatment were as follows:

	DT(g)	PP (mm Hg)	HR(b/m)	n
Verapamil 10^{-8}M	9.9±0.7	46 ± 3	257 ± 7	5
Verapamil $5x10^{-8}$M	9.4 ± 1	64 ± 7	255 ± 9	15
Verapamil 10^{-7}M	8.4±0.7	87 ±10	259 ± 9	11
Diltiazem 10^{-7}M	10.1±0.6	61 ± 5	237 ± 5	9
Diltiazem 10^{-6}M	9.7±0.6	64 ± 8	252 ±14	9


a)
b)
c)
% decrease in HR
% decrease in PP
% decrease in DT
30
20
10
0
50
25
Diltiazem
Verapamil
10^{-8} M
10^{-7} M
10^{-6} M

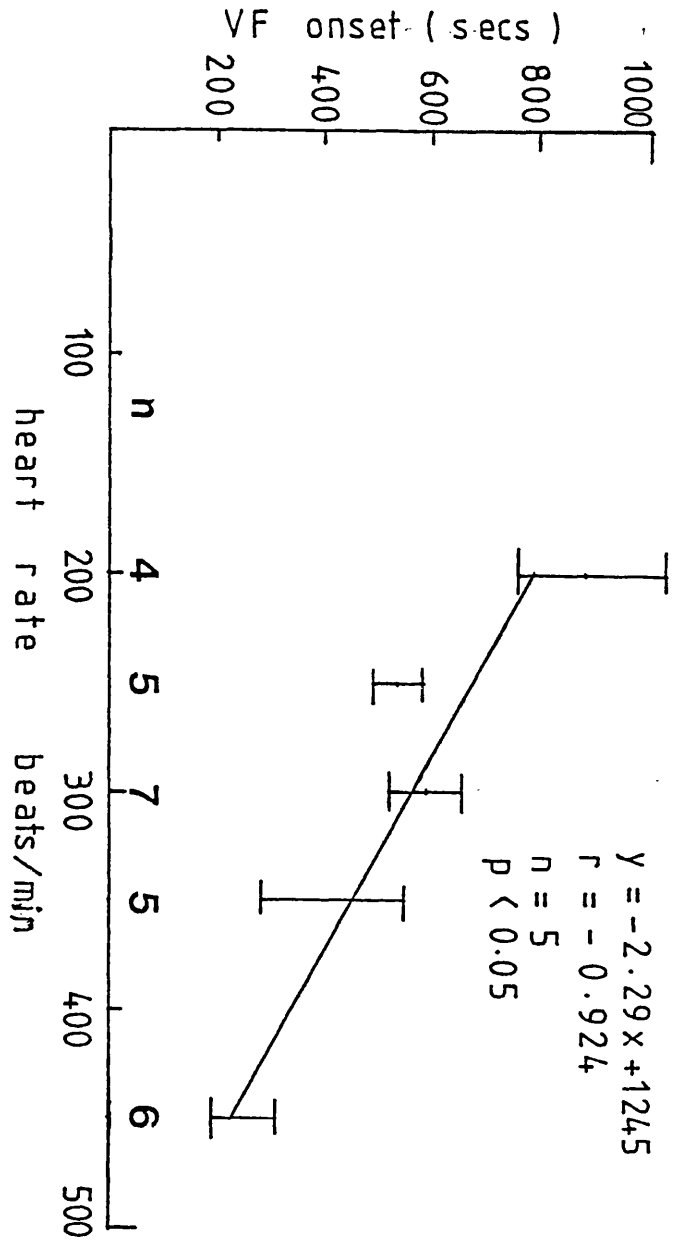


Figure 3.12. Relationship between pacing rate and onset of ventricular fibrillation.

Figure 3.13. Effects of magnesium concentration on:

(a) Duration of ventricular fibrillation in seconds

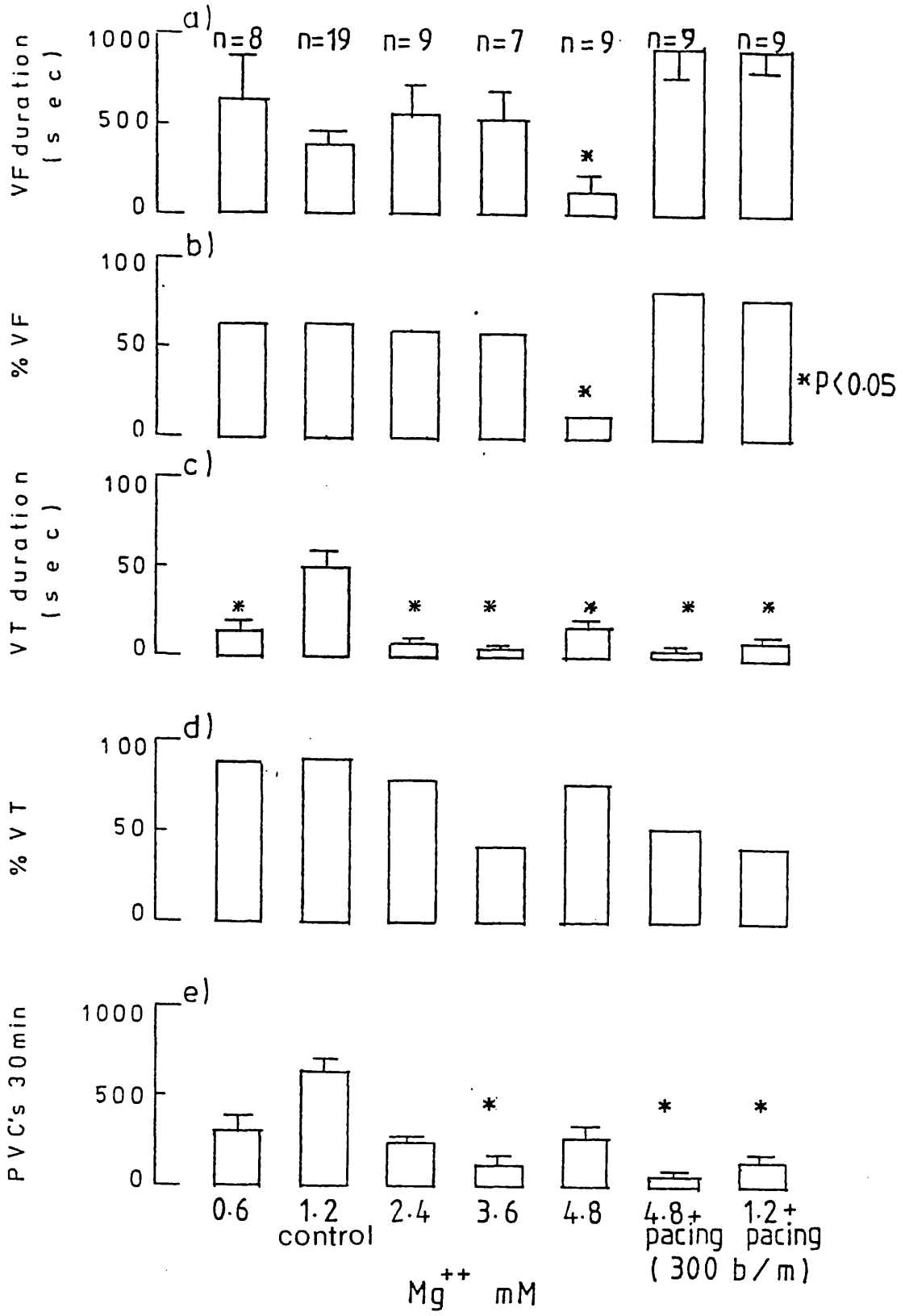
(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia in seconds

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions/30 minutes

Note the reversal of the protective effect, elicited by 4.8 mM magnesium, by pacing hearts at 300 beats/ minute. Results compared with 1.2 mM magnesium.


a)
VF duration (sec)
1000
500
0
n=8
n=19
n=9
n=7
n=9
n=9
n=9
*
b)
% VF
100
50
0
*
*P<0.05
c)
VT duration (sec)
100
50
0
*
d)
% VT
100
50
0
e)
PVC's 30min
1000
500
0
*
0.6
1.2 control
2.4
3.6
4.8
4.8+ pacing (300 b/m)
1.2+ pacing
Mg++ mM

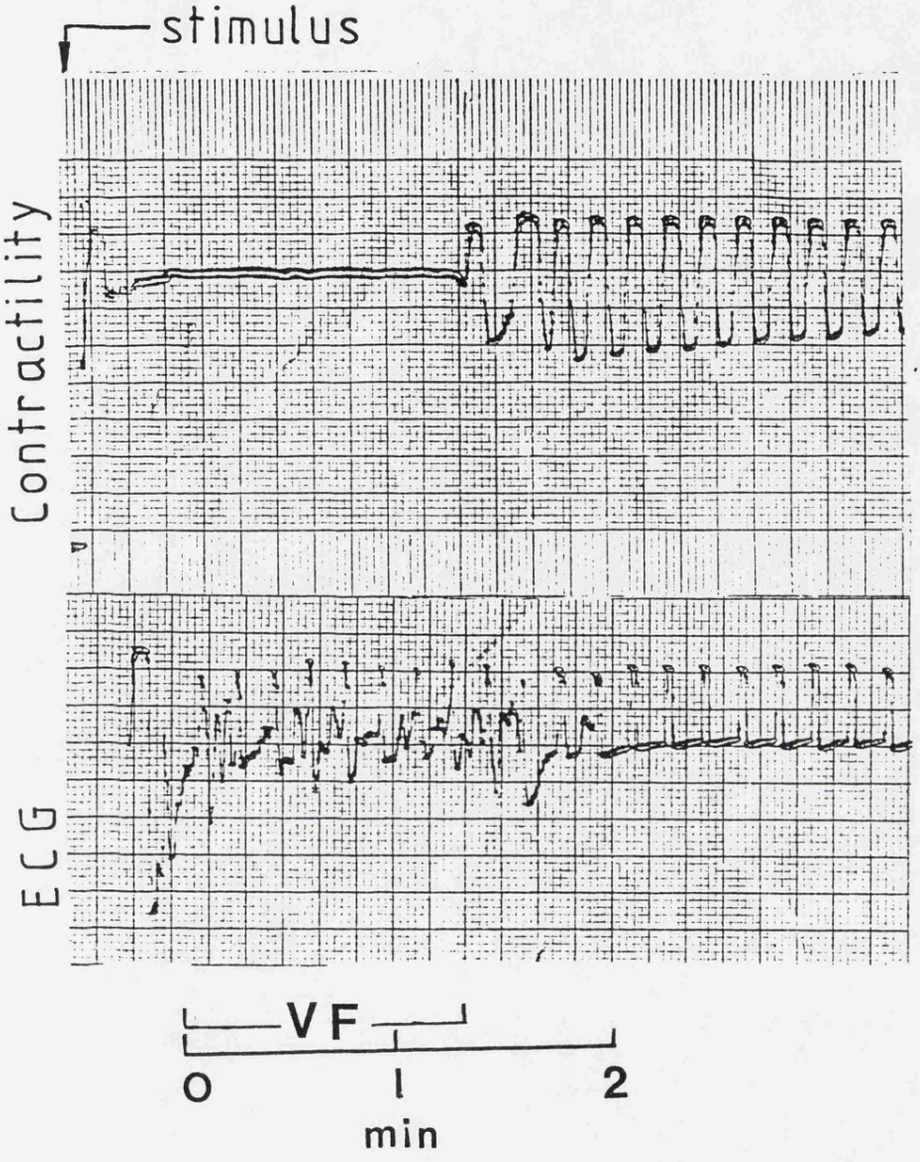


Figure 3.14. An example of the effect of ventricular fibrillation induced by the train of stimuli method on:

(a) Developed tension

(b) Electrocardiogram

(Noise due to the pacing impulse is seen in the E.C.G.)

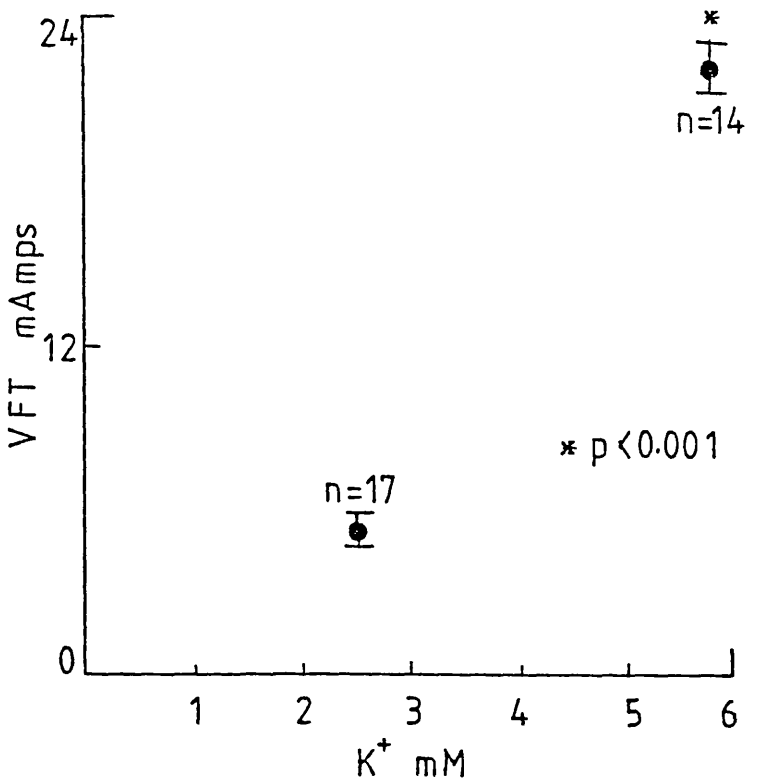


Figure 3.15. Effects of potassium on ventricular fibrillation threshold (mAmps)

3 DISCUSSION

3.a Acute coronary ligation as a model of arrhythmias

Although all coronary occlusion models have drawbacks such as the inability to precisely predict the time of onset of arrhythmias or even whether arrhythmias will or will not develop, and variability due to the site of coronary occlusion, they still have been very helpful in predicting antiarrhythmic efficacy. Coronary occlusion probably bears a closer resemblance to the clinical situation than many other arrhythmia models.

Animals like the dog, the pig and the rat develop an early phase of arrhythmias following coronary occlusion which last for about 30 minutes and which can further be divided into phase 1a and 1b (Meesmann, 1982; Podzuweit, 1982; Fagbemi, 1985). These early arrhythmias are thought to resemble a sudden cardiac attack in man.

3.b The rat heart as an experimental model of arrhythmias

The rat is cheaper compared to other experimental animals and its small size will save drugs, especially costly newly synthesised compounds.

The rat heart like healthy man has low collateral flow (Schaper , 1984) and upon ligation an almost homogenous ischaemia is induced. The low level of collateral flow means that in the absence of inotropic or chronotropic effect any actions relating to arrhythmias are more likely to be the result of direct effects on the electrical activity of the heart and not the result of non specific anti-ischaemic actions.

Drugs routinely used for the treatment of cardiac arrhythmias

in man such as lignocaine and propranolol are effective in the rat (Kane *et al.*, 1981).

Despite these advantages which favour the use of the rat as an experimental model, the rat does have some disadvantages.

1. Unlike man, the rat ECG lacks a definite ST segment. Infant rats do show an ST segment, which begins to disappear at about 20 days of age. The lack of the ST segment is due to the fact that rat ventricular muscle does not exhibit a prominent plateau phase during repolarization and its total duration is considerably shorter than that of most other mammalian species. The rat also has a briefer conduction time and shorter refractory period compared to other species. This accounts for the shorter PR interval and the ability of the rat heart to conduct impulses from atrium to ventricle at the faster heart rate characteristic of this species (Spear, 1981).
2. The rat exhibits a marked seasonal variation in the occurrence of ventricular fibrillation following coronary ligation *in vivo* (Abrahamsson and Almgren, 1981). The mechanism behind this variability is at present not known but may involve seasonal variation in hormonal, nervous and metabolic activity.
3. Electrophysiological differences exist between small and large hearts. Small hearts are prone to spontaneous defibrillation, particularly if a reent rant or circus mechanism is invoked as the cause of arrhythmias. Thus ventricular fibrillation in the rat may be easily reversed by drugs which produce only slight changes in action-potential duration or refractory period (Bargey and Beil, 1983).

3.c The isolated rat heart as a model of arrhythmia

The time course of premature contractions, elicited upon ligation of the rat heart *in vitro*, is similar to that seen *in vivo* (Clark *et al.*, 1980 and this thesis). They peak about 10 minutes post-ligation in both cases, though they decline earlier *in vivo*. This might be due to the higher *in vivo* heart rate which might accelerate ischaemic tissue death rendering it electrically quiescent.

The isolated rat heart has the advantage of being devoid of neural hormonal and haemodynamic influences and hence interpretation of results is less complicated than the *in vivo* model. Moreover the variation in arrhythmias encountered *in vivo* can be largely avoided.

However, isolated hearts perfused by the Langendorff technique have several drawbacks.

1. It can be questioned that the coronary artery ligated isolated heart is not truly ischaemic, this is because high coronary flow rates are usually used. This is a consequence of absence of haemoglobin in the perfusion fluid because haemoglobin has a higher carrying capacity for oxygen than water. But lack of coronary flow probably means that ischaemia is still occurring.
2. Hearts perfused by the Langendorff mode perform no volume work as there is no left atrial filling. This could affect the severity of arrhythmias.

In conclusion, the isolated rat heart is a useful model for screening antiarrhythmic drugs and possesses many advantages which seem to outweigh its drawbacks.

3.d Effects of potassium on ligation inducd arrhythmias in the isolated rat heart

It is clear from Figures 3.3 and 3.4 that potassium delayed and reduced ventricular fibrillation in a concentration related manner. Potassium within the concentration range 1.2 - 5.9 mM did not affect the incidence or duration of ventricular tachycardia, however, increasing potassium concentration to 10 mM reduced both incidence and duration of ventricular tachycardia. In the case of 1.2 mM potassium the onset of ventricular fibrillation was so rapid that it allowed little chance for ventricular tachycardia and premature ventricular contractions to develop.

These results are in agreement with the finding of Abrahamsson and Alm gren (1981) who obtained an increase in the incidence of ventricular fibrillation by reducing plasma potassium in the anaesthetized rat using furosemide.

A similar protective effect of potassium was noticed in reperfusion arrhythmias in the isolated rat heart (Lubbe *et al.*, 1978) and in strophanthidin - induced arrhythmias in canine Purkinje fibres (Lin and Vassalle, 1980).

These results also concur with the clinical observations that relate low plasma potassium with the occurrence of arrhythmias (Gettes, 1981) and tend to confirm that coronary artery ligated isolated rat heart does behave in a similar way to other species with regard to arrhythmogenesis .

The main haemodynamic effect of potassium is the induction

of concentration dependent fall in perfusion pressure (Figure 3.5) which is a measure of coronary dilatation. This vasodilation effect could benefit the ischaemic region by increasing the low collateral flow to the forementioned area. However, vasodilatation could direct flow to the non ischaemic region at the expense of the ischaemic region and hence cancel that beneficial effect, because total flow cannot be increased in these experiments and ischaemic vessels are already maximally vasodilated.

The beneficial effects of diltiazem over verapamil has been attributed to flow increase in the marginal ischaemic zone (Thuillez *et al.*, 1983). The possibility exists that potassium could induce such an effect and hence preserve the viability of that zone, however, more extensive study is required to prove this hypothesis. Potassium has recently been shown to depress calcium dependent depolarizations in guinea-pig heart (Hiraoka *et al.*, 1985). This could also contribute to its antiarrhythmic action.

The finding that postligation administration of potassium reduced arrhythmias (Figure 3.6) although not as effective as preligation administration, along with the fact that the rat heart possesses very low collateral circulation suggest that the site of action of potassium is possibly in the non-ischaemic zone rather than in the ischaemic zone i.e. reducing K^+ heterogeneity across ischaemia-normal border.

The antiarrhythmic effect of potassium has been ascribed to stimulation of Na^+/K^+ ATPase by this ion (Skou, 1957). Such stimulation would result in hyperpolarization by restoring the

electrolyte gradient and increasing the net outward current. However, in the present study ouabain which inhibits Na^+/K^+ pump failed to increase the incidence of fibrillation. This could be due to the fact that rat hearts are resistant to ouabain (Erdman *et al.*, 1980) and this is evident by the fact that no increase in heart rate or developed tension was produced by the concentrations used in the present study.

An argument not in favour of the suggestion of Skou is that during ischaemia potassium is already high in the ischaemic zone and should have stimulated the sodium pump.

Potassium leakage in response to ischaemia has been reported in the dog (Harris *et al.*, 1954), pig heart (Hirche *et al.*, 1980) and the rabbit interventricular septum (Weiss and Shine, 1982). This rise in extracellular potassium was found to be accompanied by shortening of the action potential duration and an increase in conduction time in the ischaemic myocardium (Weiss and Shine, , 1982a). The shortening of action potential duration and the increase in conduction time in the ischaemic zone by leaked potassium might produce electrical inhomogeneity between ischaemic and normoxic zones in the heart. When differences in action potential duration exist in adjacent areas, current flow from the site of the longest action potential duration to that of the shorter duration, might in the latter area induce an extrasystole when the current is strong enough to depolarize these cells to threshold (Janse *et al.*, 1980). High perfusate potassium might produce electrical homogeneity in the heart by shortening the action potential in the normoxic zones. However, drugs which shorten the action potential

duration such as carbachol and adrenaline failed to protect against ligation induced arrhythmias in the present study. This could imply:

1. Shortening the action potential duration is not the sole electrophysiological mechanism whereby potassium induces its protective effect.
2. Or potassium might act by an unidentified mechanism.
3. Or carbachol and adrenaline possess arrhythmogenic influences which outweighed any possible protective effects.

In fact carbachol has been shown to increase potassium permeability in guinea-pig intestine (Bolton and Clark , 1981) whereas adrenaline augments the slow inward current (Shimoni *et al.*, 1984).

A possible mechanism for the protective effect of potassium is that high perfusate potassium may reduce potassium loss from cells in the borderline ischaemic zone where perfusion is reduced but not absent as suggested by Lubbe *et al.* (1978).

Potassium reduced calcium overload in canine Purkinje fibres (Lin and Vassalle, 1980). Recently, delayed after depolarizations have been observed in guinea-pig ventricular muscle with low potassium (Hiraoka *et al.*, 1985). These delayed after depolarizations are assumed to be an important factor for the genesis of arrhythmias. The antiarrhythmic effect of high perfusate potassium could possibly be ascribed to abolition of these delayed after depolarizations.

3.e Effects of rubidium on ligation induced arrhythmias

Rubidium induced a similar protective effect to that of potassium

against ligation induced arrhythmic along with a similar effect on perfusion pressure (Figure 3.6).

A protective effect of rubidium against strophanthidin-induced arrhythmias in canine Purkinje fibres was reported by Lin and Vassalle (1980). Probably the mechanisms responsible for potassium antiarrhythmic effect also apply to rubidium.

Two beneficial practical conclusions could be drawn from these potassium experiments:

1. During *in vivo* experiments of arrhythmias plasma potassium should be monitored because surgical trauma and haemorrhage enhance plasma potassium and hence could contribute to the variability seen in acute *in vivo* experiments.
2. Human extracellular potassium concentration is 3.5 - 5 mM (Schwartz, 1978). These levels fluctuate due to acidosis, glucose and insulin(Schwartz, 1978) and catecholamine administration (Linton *et al.*, 1982). Hence insuring proper potassium intake and maintaining high plasma potassium levels might serve as a prophylactic against sudden cardiac death.

In conclusion, potassium possesses a striking antiarrhythmic effect in the isolated coronary ligated rat heart. The mechanism underlying this effect still requires further investigation.

3.f. Effects of calcium on ligation induced arrhythmias

Reducing calcium in the perfusate from 2.5 to 0.6 mM had no

effect on the incidence or duration of ventricular fibrillation, however, it did delay the onset of ventricular fibrillation, reduced the number of premature beats and delayed the time required to reach maximum instability from 12 to 20 minutes (Figures 3.7, 3.8 and 3.9).

The reduction in the number of premature contractions produced by 0.6 mM calcium could be due to the fact that the time required to reach maximum instability was delayed and the counting of the premature contractions was performed only for 30 minutes.

These results are in harmony with the findings of Clusin *et al.* (1982a), who increased ventricular fibrillation latency in the dog by reducing serum ionized calcium by chelation with citrate.

Calcium ions have been implicated as one mediator of the adverse effects of myocardial ischaemia and arrhythmias (Clusin *et al.*, 1982) and calcium ions mediate slow responses (Reuter, 1967). These slow responses, because of their much slower rate of propagation, are thought to play an important role in abnormal automaticity (Isenberg , 1977). However, the possibility of slow current generation in the ischaemic area is questionable as hypoxia,acidosis and low ATP levels encountered during ischaemia tend to suppress the slow inward current (Sperelakis,1984) and increase of calcium uptake during the early minutes of ischaemia is controversial (Poole-Wilson *et al.*, 1982), although the hearts in the present study did demonstrate a rise in resting tension which is an indication of increase of intracellular free calcium (Figure 3.2). Therefore the possibility exists that calcium does increase but this increase

cannot be substantiated with the present technology.

The protective effect of low calcium could be explained by the low extracellular calcium reducing calcium influx which in turn would reduce the ionic current giving rise to abnormal automaticity and slow responses.

The main haemodynamic influence of calcium is the induction of a concentration dependent increase in developed tension up to 2.5 mM whereafter the developed tension levels off. Reduction in contractility by low calcium may reduce ischaemic tissue demand for oxygen and decrease requirements for high energy phosphates thus preventing tissue damage. Decreased calcium influx might also spare high energy phosphates required for calcium sequestration. The influx of calcium its f might injure cellular organelles by activation of phospholipases or by calcium loading of the mitochondria (Shine and Douglas, 1983). Protection of the mitochondria from calcium loading might facilitate recovery of high energy phosphate production. A low calcium perfusate may also reduce gap junctional resistance leading to improvement of conduction and hence protection against arrhythmias (De Mello, 1982).

3.g Effects of verapamil on ligation induced arrhythmias

Verapamil over the concentration range used did not reduce the incidence or the duration of ventricular fibrillation, although there was a tendency for the middle concentration (5×10^{-8} M) to protect against ligation induced arrhythmias.

This bell-shaped dose response curve of verapamil has been

reported by Hearse *et al.* (1980) in enzyme and protein leakage studies and by Nayler and Sturrock (1984) in catecholamine release experiments.

Studies with verapamil have provided conflicting data as to its efficacy in preventing ligation induced arrhythmias. It has been reported to reduce arrhythmias dose dependently *in vivo* in the conscious rats (Curtis *et al.*, 1984). On the other hand, it has been shown to increase the incidence of mortality in anaesthetized rats (Kane *et al.*, 1979; Clark *et al.*, 1980).

Verapamil is well-established as treatment for supra-ventricular arrhythmias. The doses used by Kane and Clark (bolus injection of 0.1 mg/kg) appear to correspond with those used clinically for supraventricular arrhythmias and there is no reason to assume that such a dose will continuously suppress ischaemic induced ventricular arrythmias. To prove that administration of low doses of verapamil was the reason underlying verapamil's failure to suppress ventricular fibrillation in other studies, Curtis *et al.* (1984) used higher doses (2 - 20 mg/kg). They obtained a dose-dependent reduction in arrhythmias with an ED_{50} of 6 mg/kg However, these doses produced plasma concentrations in the μmolar range and rats with large occluded zones died before the onset of arrhythmias.

3.h Effects of diltiazem on ligation induced arrhythmias

Diltiazem 10^{-7} M did not reduce ventricular fibrillation incidence or duration. Increasing the concentration to 10^{-6} M reduced both incidence and duration of ventricular fibrillation (Figure 3.10). However it induced a 25% reduction in heart rate. 10^{-6} M diltiazem

is almost equivalent to 10^{-7} M verapamil in reducing developed tension and perfusion pressure. On the other hand verapamil at the concentrations used had a negligible effect on heart rate (Figure 3.11). The antiarrhythmic effect of diltiazem may be attributed to its effect on heart rate. This is supported by the fact that pacing hearts at 300 beats/min reversed this protective effect. However, it is also possible that electrical stimulation releases neurotransmitter substances that may aggravate arrhythmias. But control hearts would also have this release.

This disparity in action of calcium channel blockers is expected because they are of heterogenous chemical structure. It has been demonstrated that diltiazem increased regional myocardial blood flow and contractility in the marginal ischaemic area in the dog and preserved the viability of this zone while verapamil did not (Thuillez *et al.*, 1983). Therefore it is also possible that these effects may contribute to the antiarrhythmic effect of diltiazem seen at high concentrations.

The failure of verapamil and diltiazem to produce a similar effect to low calcium implies that if calcium accumulation is involved in the genesis of ligation induced arrhythmias, then the calcium slow channel is not the major route whereby calcium gets into the cell. Calcium may gain access through the sodium/calcium exchange process which is probably not affected by calcium antagonists.

3.i Effect of electrical pacing on ligation induced arrhythmias

Pacing hearts at different rates did not affect the incidence

of ventricular fibrillation, however, a negative correlation between heart rate and onset of ventricular fibrillation was obtained (Figure 3.13). It was reported by Clusin *et al.* (1982) that the average rate of calcium influx increases with heart frequency. Therefore electrical pacing increases calcium influx in a beat to beat manner raising cytosolic calcium leading in turn to activation of calcium dependent lipases, ATPases and mitochondrial calcium overload. This may result in rhythm disturbances (Nayler, 1981).

Electrical pacing induces vasodilatation. This vasodilatation cannot be attributed to a direct effect on calcium fluxes, however, it is possible that electrical stimulation releases vasodilator substances such as adrenaline or prostacyclin. Alternatively the negative inotropic effect induced by electrical stimulation may physically reduce vascular compression hence induce vasodilatation.

These results with electrical pacing lend further support for the involvement of calcium in the genesis of arrhythmias, however, the possibility again exists that electrical stimulation releases other neurohumoral substances which could aggravate arrhythmias.

3.j Effects of magnesium on ligation induced arrhythmias

Magnesium over the range 0.6 - 3.6 mM failed to protect against ligation induced arrhythmias in the isolated rat heart, however, 4.8 mM magnesium significantly reduced both incidence and duration of ventricular fibrillation(Figure 3.14).

Magnesium within the concentration range used in the present

study produced concentration dependent reduction in heart rate, developed tension and perfusion pressure. These results indicate that increase in extracellular magnesium must be extreme in order to reduce significantly the incidence of ventricular fibrillation. The protective effect of high extracellular magnesium could be attributed to the marked decrease in heart rate. In support of this, pacing hearts at 300 beats/min reversed the protection induced by 4.8 mM magnesium. The bradycardia and the negative inotropic effect elicited by 4.8 mM magnesium could protect the heart by reducing calcium influx and preserving high energy phosphate levels (Nayler, 1981). The fact that halving extracellular magnesium concentration did not significantly increase the incidence of ventricular fibrillation indicates that moderate changes in extracellular magnesium concentration does not alter cardiac rhythm. Human plasma magnesium levels fall within the range of 0.82 - 0.89 mM and do not vary enormously. Distinctly abnormal plasma values (i.e. 0.3 - 0.4 mM) are rarely encountered, except in condition such as renal failure and acute alcoholism (Kalsner, 1983). Therefore, marginally subnormal magnesium levels reported in humans do not seem to play an essential role in rhythm disturbance. Moreover, changes in extracellular magnesium concentration appear to have little effect on the action potential of cardiac cells (Shine *et al.*, 1979).

Therefore the present results do not confirm an association of cardiovascular death with magnesium deficiency and are in accord with Kalsner (1983) findings.

3.k Ventricular fibrillation thresholds as an index of electrical instability

Fibrillation is often preceded by a rapid ventricular tachyarrhythmia or a premature ventricular contraction falling within the vulnerable phase of the preceding beat (R-on-T phenomenon). The advantage of the ventricular fibrillation threshold technique is that it allows one to study the influence of single factors on ventricular vulnerability in a controlled system free of many of the variables associated with fibrillation in the intact animal. Passing current during the vulnerable period of the E.C.G. serves to increase artificially the amount of inhomogeneity between adjacent ventricular fibres.

The clinical relevance of this technique is questionable. However, effective antiarrhythmic drugs in man are effective using this technique (Lubbe *et al.*, 1975).

3.l Effects of potassium and magnesium on ventricular fibrillation threshold

5.9 mM potassium significantly elevated the ventricular fibrillation threshold compared to 2.5 mM potassium (Figure 3.16). While magnesium over the range 0.6 - 4.8 mM failed to induce any significant effect on the ventricular fibrillation threshold. This protective effect of high perfusate potassium is possibly due to stimulation of Na^+/K^+ pump or reduction of calcium overload and improvement of cell-to-cell communication (De Mello, 1982) and confirms the findings demonstrated with potassium in coronary ligated hearts.

The failure of magnesium to increase the ventricular fibrillation

threshold is also in agreement with the previous results of ligation induced arrhythmias. The protective effect of 4.8 mM magnesium in the latter experiments was possibly due to the marked reduction in heart rate. In the ventricular fibrillation thresholds experiments hearts were paced to 300 bpm and this electrical pacing might have abolished the possible protective effect of 4.8 mM magnesium.

CHAPTER 4

In this chapter the role of prostaglandins, lysophospholipids and free radicals on ligation induced arrhythmias in the isolated rat heart are examined. Inhibitors of enzymes encountered in arachidonic acid and phospholipid metabolism, general antioxidants and free radicals scavengers were used as tools in this investigation.

4.1 Effects of prostaglandins on coronary artery ligation induced arrhythmias in the isolated rat heart

In vitro studies have shown that a balance in favour of thromboxane release is associated with the occurrence of a greater number of arrhythmias whereas fewer arrhythmias occur when the balance is in favour of prostacyclin release (Coker et al., 1981). In the present study, experiments were designed to see if such a balance is of any importance in *in vitro* model devoid of blood and neural influences.

The metabolic pathways of arachidonic acid are shown in Figure 4.1 along with the enzyme inhibitors used in this study .

4.1a Effects of ZK36374 on ligation induced arrhythmias in the isolated rat heart

ZK36374, a stable prostacyclin analogue (Coker and Parratt, 1983) was perfused to see if it possessed any antiarrhythmic action in the isolated rat heart. ZK 36374 (2×10^{-8} M, 10^{-6} M) did not significantly alter the incidence and duration of ventricular fibrillation. ZK 36374 (2×10^{-8} M) reduced the developed tension from 8.1 ± 0.5 to 7.1 ± 0.4 grams and perfusion pressure from 88 ± 10 to 60 ± 5 mm Hg ($P < 0.05$) ($n = 8$) while ZK 36374 (10^{-6} M) reduced the developed tension from 11.5 ± 1.2 to 8.5 ± 1 grams

($P < 0.05$) and the perfusion pressure from 92 ± 7 to 58 ± 3 mm Hg ($P < 0.05$) ($n = 8$). Neither concentration affected heart rate. Hearts perfused with ZK 36374 solvent were used as controls.

4.1b Effects of a combination of ZK 36374 (2×10^{-8} M) and indomethacin (10^{-7} M) on ligation induced arrhythmias

It is possible that any protective effect of ZK 36374 in the previous experiment was masked by thromboxane A_2 produced in the heart. Indomethacin was used to prevent possible thromboxane A_2 synthesis by inhibiting the cyclooxygenase enzymes.

The indomethacin and ZK 36374 combination did not significantly affect the incidence and duration of ventricular fibrillation, however, it did reduce both developed tension and perfusion pressure ($P < 0.05$) ($n = 10$).

4.1c Effects of arachidonic acid on coronary ligation induced arrhythmias

Arachidonic acid, which is the precursor of prostaglandins, prostacyclin and thromboxane (Needleman, 1978), was used to test if arachidonic acid or endogenously produced arachidonic acid metabolites possessed any antiarrhythmic action.

Arachidonic acid (10^{-6} M) did not alter ligation induced arrhythmias or haemodynamic properties of the 9 hearts used in this experiment.

4.1d Effects of aspirin and indomethacin on ligation induced arrhythmias in the isolated rat heart

Aspirin and indomethacin are cyclo oxygenase inhibitors (Higgs

and Vane, 1983). They are used in the present study to see if inhibition of cyclooxygenase protected against ligation induced arrhythmias.

Aspirin (10^{-5} M) and indomethacin (10^{-6} M and 10^{-5} M) failed to alter any of the measured parameters of arrhythmias and haemodynamic properties of the hearts, although there was a tendency for the duration of ventricular fibrillation to increase (n 6-8).

4.1 e Effects of N-butyl imidazole (10^{-6} M) and dazoxiben (10^{-5}M) on ligation induced arrhythmias

N-butyl imidazole (Bowman and Rand, 1980) and dazoxiben (Coker *et al.*, 1982) are thromboxane A_2 synthetase inhibitors. Indomethacin and aspirin non selectively inhibit both thromboxane and prostacyclin synthesis.

N-butyl imidazole (10^{-6} M) did not reduce the incidence and duration of ventricular fibrillation, however, it initially reduced the perfusion pressure from 71 ± 4 to 61 ± 4 ($P < 0.05$) and then increased it to 90 ± 7 mm Hg (n = 10). N-butyl imidazole at this concentration did not affect developed tension or heart rate.

Dazoxiben failed to alter any of the measured parameters of arrhythmias or haemodynamic properties of the hearts.

4.1f Effects of BW 755C and 5, 8, 11, 14 eicosatetraynoic acid (ETYA) on ligation induced arrhythmias

The possibility that the increased ventricular fibrillation duration induced by aspirin and indomethacin was due to leukotrienes synthesis prompted more work to investigate this pathway. BW 755C

and ETYA are dual inhibitors of cyclo-oxygenase and lipoxygenase enzymes. (Honn and Dunn, 1982).

BW 755C (4, 10 and 20 mg/L) induced a bell-shaped effect on the incidence of ventricular fibrillation (Figure 4. 2). BW 755C imparted a yellowish colour on the hearts, the intensity of which was dependent on the concentration of the drug (BW 755C is colourless in aqueous solution). BW 755C reduced ($P < 0.05$) developed tension from 10.3 ± 0.7 to 8.4 ± 0.7 grams while 20 mg/L BW 755C reduced ($P < 0.01$) developed tension from 11.8 ± 0.7 to 8.4 ± 0.5 grams ($n = 8-10$). BW 755C at the concentrations used did not affect the heart rate or the perfusion pressure.

ETYA (5 and 10 mg/L) did not protect against ligation induced arrhythmias or affect the haemodynamic properties of the hearts.

4.1 Effects of nafazatrom on ligation induced arrhythmias

Nafazatrom is a lipoxygenase inhibitor and prostacyclin synthesis inducer (Honn and Dunn, 1982). Nafazatrom (3.9×10^{-5} M) was sonicated in 100 µl ethyl alcohol and dispersed in one litre of Krebs solution. It significantly ($P < 0.05$) reduced both the incidence and duration of ventricular fibrillation (Figure 4.3). but it did not affect the haemodynamic properties of the hearts.

4.2g Effects of lysophosphatidylcholine on ligation induced arrhythmias

Increases of lysophospholipids during ischaemia are documented and implicated in cardiac rhythm disturbances (Corr *et al.*, 1979, 1981, 1982). The following experiments were performed to see if perfusion of isolated hearts with lysophosphatidylcholine and the use of phospholipase stimulators and inhibitors could affect the

incidence of ventricular fibrillation. Chloroform was used as a solvent for lysophosphatidylcholine.

The concentration of lysophosphatidylcholine determined by Corr *et al.* (1981) in the ischaemic canine myocardium (0.2 mM), which they used for their study of electrophysiological changes and calcium up-take, caused a sudden collapse of contractility in the isolated perfused rat heart. 10^{-5} M lysophosphatidylcholine induced a gradual decrease in developed tensions and a gradual rise in resting tension which culminated in ventricular fibrillation. On the other hand 10^{-6} M lysophosphatidylcholine had no effect on parameters of arrhythmias and tension. This suggests an all or none effect of lysophosphatidylcholine on tension and arrhythmias.

2×10^{-6} M lysophosphatidylcholine (initially dissolved in 20 µl chloroform and dispersed in one litre of Krebs solution) elicited a significant ($P < 0.01$) increase in the incidence and duration of ventricular fibrillation in the presence or absence of coronary artery ligation (Figures 4.4 and 4.5). In these hearts developed tension gradually decreased, followed by uncoupling which culminated in ventricular fibrillation. In these experiments the colour of the hearts became whitish. Control hearts in these experiments were perfused with 20 µl/L chloroform. Also in these experiments, control hearts were perfused by 4.4 mM potassium because an increase in ventricular fibrillation incidence was expected.

4.2a Effects of lysophosphatidylcholine in presence of 10 mM potassium on ligation induced arrhythmias

It is possible that lysophosphatidylcholine produces its arrhythmic effect by increasing calcium influx (Sedlis *et al.*, 1983).

Potassium was increased to 10 mM to reduce calcium influx (Lin and Vassalle, 1980). 10 mM potassium protected against the detrimental effect of lysophosphatidylcholine (Figure 4.6).

4.2b Effects of mepacrine on ligation induced arrhythmias

In the previous experiments exogenously applied lysophosphatidylcholine induced fibrillation. It is possible that endogenously produced lysophosphatidylcholine has a different effect. Mepacrine, chloropromazine and trifluperazine were used to inhibit phospholipase A_2 while bradykinin was used to stimulate it (Porozaleck *et al.*,1982; Needleman *et al.*, 1975).

Mepacrine 10^{-7} M and 10^{-6} M reduced the incidence and duration of ventricular fibrillation (Figure 4.7). 10^{-6} M mepacrine also reduced ($P < 0.05$) developed tension. Neither concentration had any significant effect on perfusion pressure or heart rate.

4.2c Effects of chlorpromazine and trifluperazine on ligation induced arrhythmias

Neither chlorpromazine (10^{-7} M and 10^{-6} M) nor trifluperazine (10^{-7} M) had any effect on ligation induced arrhythmias or the haemodynamic properties of the hearts ($n = 9$).

4.2d Effects of bradykinin on ligation induced arrhythmias

Bradykinin (7.5 ng/ml and 15 ng/ml) perfused in the presence of 5.9 mM potassium (because an increase in the incidence of ventricular fibrillation was expected), did not affect ligation induced arrhythmias. Bradykinin 15 ng/ml reduced perfusion pressure from 77 ± 6 to 53 ± 5 mm Hg ($P < 0.05$). No significant effect was

induced on developed tension or on heart rate (N = 9-10).

4.3 Effects of antioxidants and free radical scavengers on ligation induced arrhythmias

The antioxidants ascorbic acid and glutathione; mannitol which is a hydroxyl radical scavenger, and catalase which catalyses the reduction of hydrogen peroxide to water were used to investigate the possible role of free radicals in ligation induced arrhythmias. The concentrations of ascorbic acid, mannitol and glutathione used were found to be effective in the more serious reperfusion arrhythmias in the rat heart (Woodward and Zakaria, 1985).

4.3a Effects of ascorbic acid on ligation induced arrhythmias

Ascorbic acid 10^{-3} M did not significantly alter arrhythmias or the haemodynamics of the hearts.

4.3b Effects of glutathione on ligation induced arrhythmias

Glutathione 10^{-3} M failed to exert any protective effect against ligation induced arrhythmias. A significant ($P < 0.05$) reduction in developed tension from 9.8 $\pm$ 0.3 to 8.8 $\pm$ 0.3 grams and perfusion pressure from 53 $\pm$ 4 to 45 $\pm$ 3 was noticed with no significant effect on heart rate (n = 9).

4.3c Effects of mannitol on ligation induced arrhythmias

Mannitol 20 mM/L did not alter arrhythmias or haemodynamic properties of the hearts.

4.3d Effects of catalase on ligation induced arrhythmias

Catalase 3000 μ/ml did not protect against ligation induced arrhythmias or affect the haemodynamic properties of the hearts.

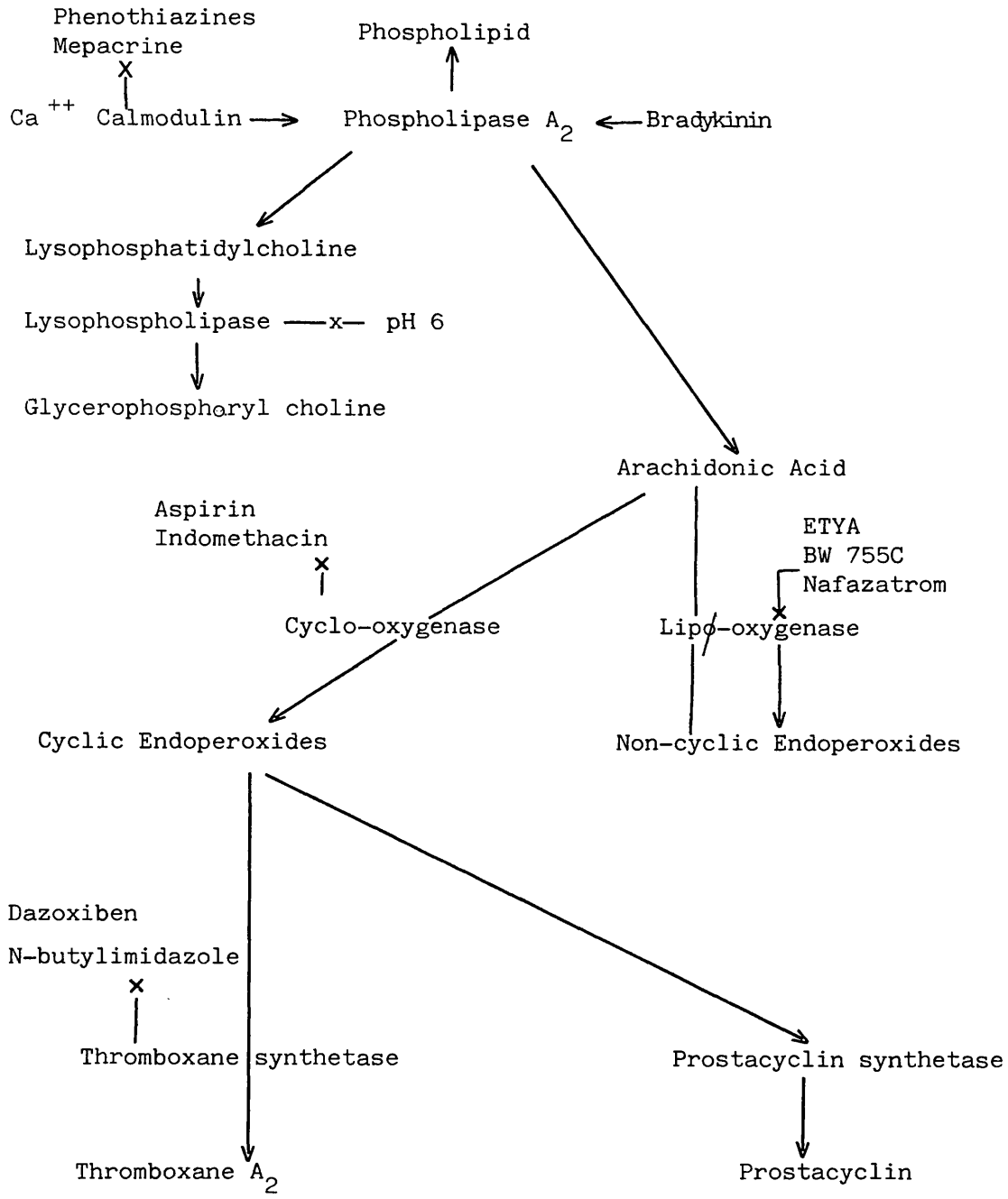


Figure 4.1. Diagram illustrating arachidonic acid metabolic pathways. Inhibitors and stimulants of enzymes of these pathways used in this study are shown.

x - sites of inhibition

Figure 4.2. Effects of perfusate concentration of BW 755C on:

(a) Duration of ventricular fibrillation (sec)

(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions/30 min.

Control calcium was 2.5 mM and potassium 2.5 mM.

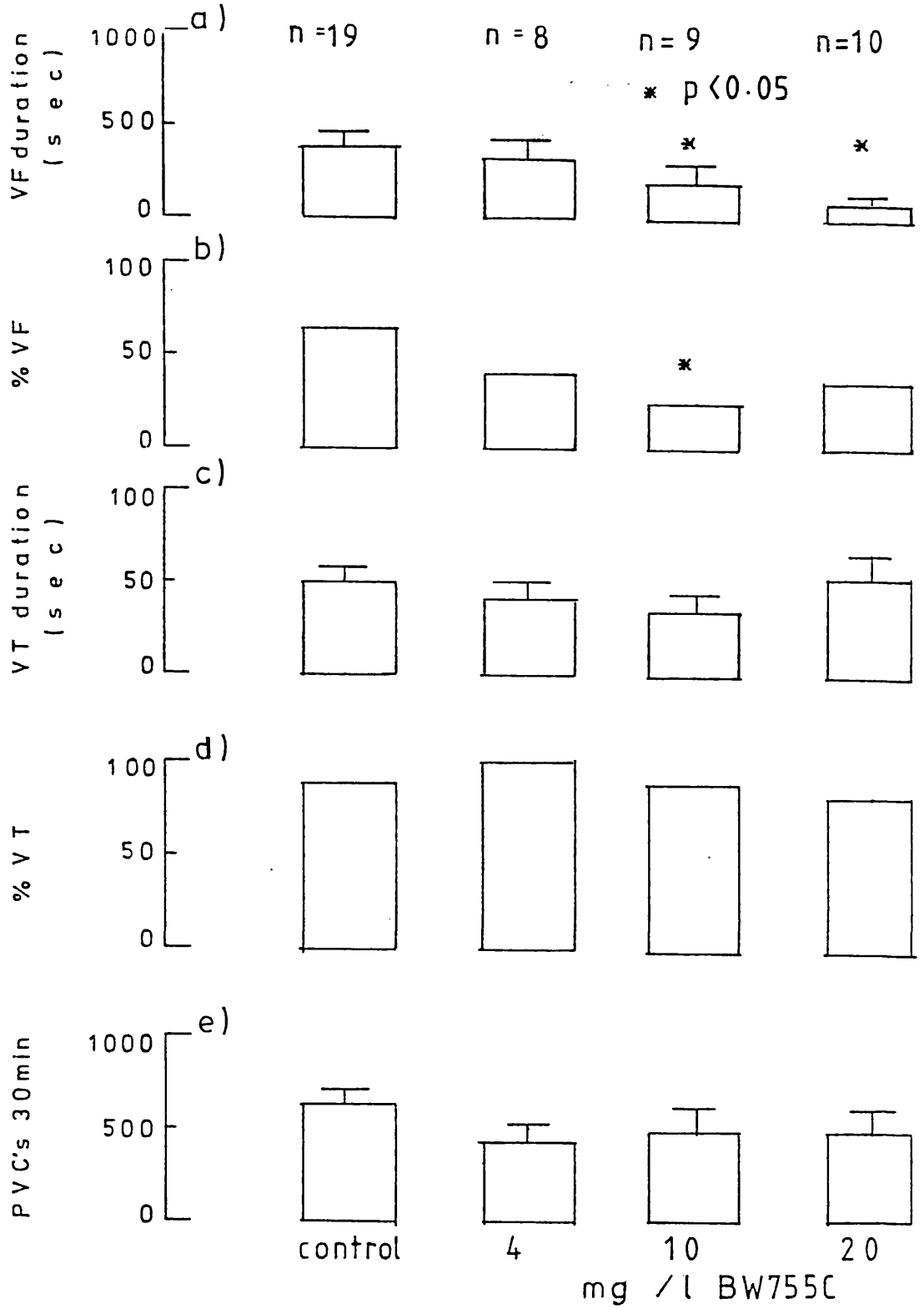

a)
VF duration (sec)
1000
500
0
n =19
n = 8
n= 9
n=10
* p <0.05
b)
% VF
100
50
0
c)
VT duration (sec)
100
50
0
d)
% VT
100
50
0
e)
PVC's 30min
1000
500
0
control
4
10
20
mg / l BW755C

Figure 4.3. Effects of nafazatrom on:

(a) Duration of ventricular fibrillation (sec)

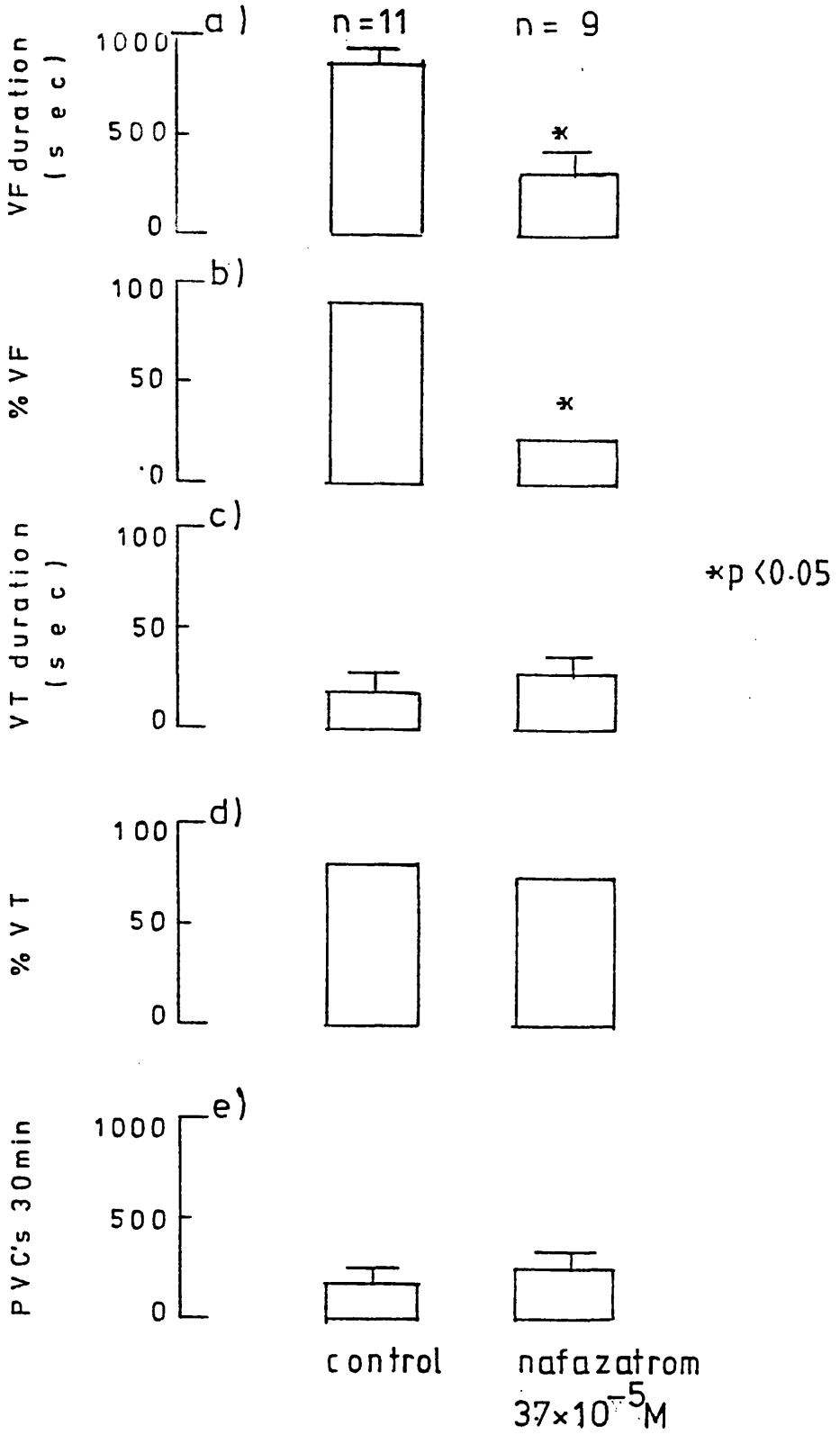
(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions /30 min

Control calcium was 2.5 mM and potassium 2.5 mM.


a)
n=11
n= 9
VF duration (sec)
1000
500
0
*
b)
% VF
100
50
0
*
c)
VT duration (sec)
100
50
0
d)
% VT
100
50
0
e)
PVC's 30min
1000
500
0
control
nafazatrom
37×10⁻⁵M

*p <0.05

Figure 4.4. Effects of lysophosphatidylcholine perfused in ligated hearts on:

(a) Duration of ventricular fibrillation (sec)

(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions/30 min.

Control calcium was 2.5 mM and potassium 4.4. mM.

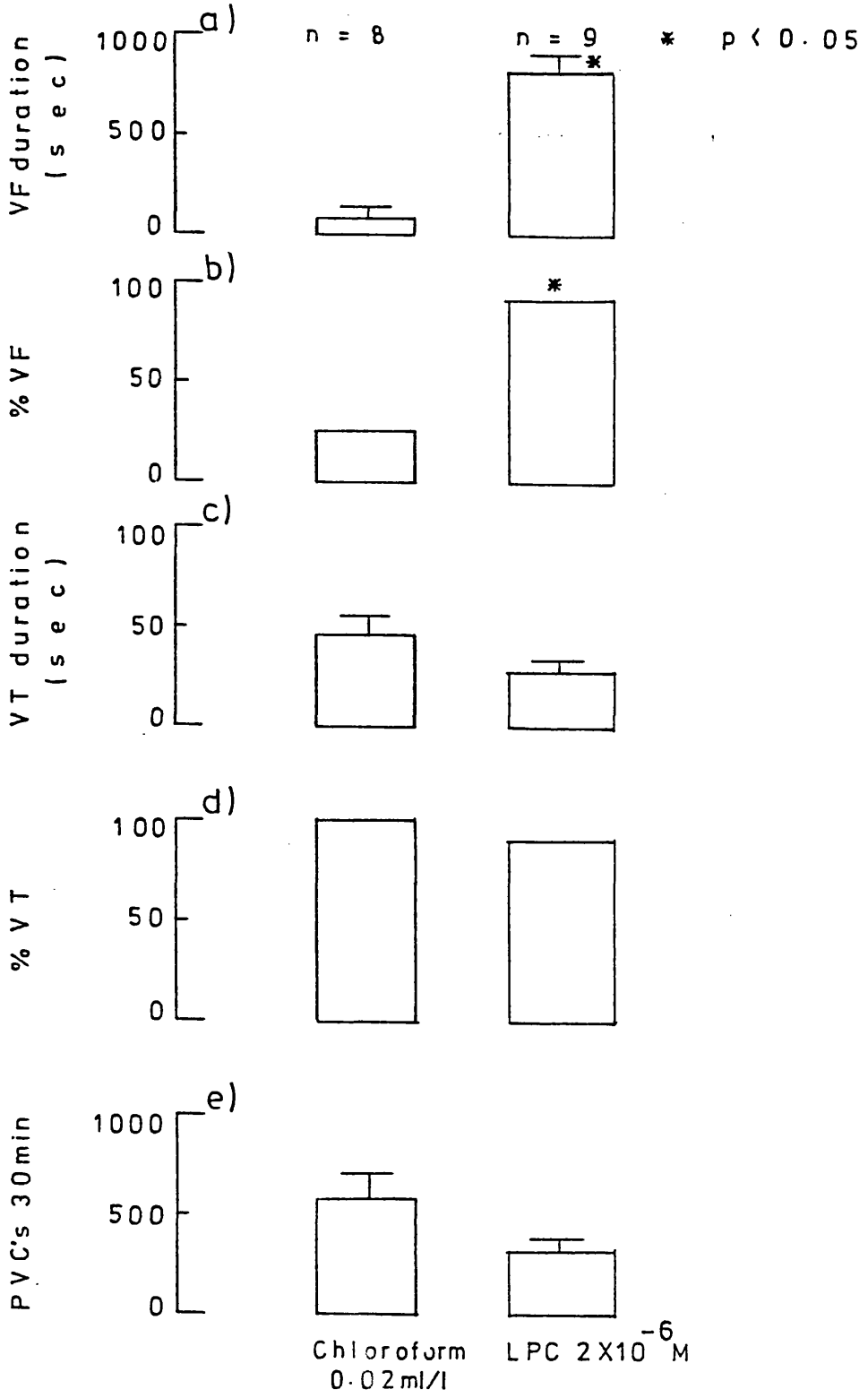

a)
n = 8
n = 9
* p < 0.05
VF duration (sec)
1000
500
0
b)
% VF
100
50
0
c)
VT duration (sec)
100
50
0
d)
% VT
100
50
0
e)
PVC's 30min
1000
500
0
Chloroform 0.02ml/l
LPC 2X10⁻⁶M

Figure 4.5. Effects of lysophosphatidylcholine perfused in non ligated hearts on:

(a) Duration of ventricular fibrillation (sec)

(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions/30 min.

Control calcium was 2.5 mM and potassium 4.4. mM.

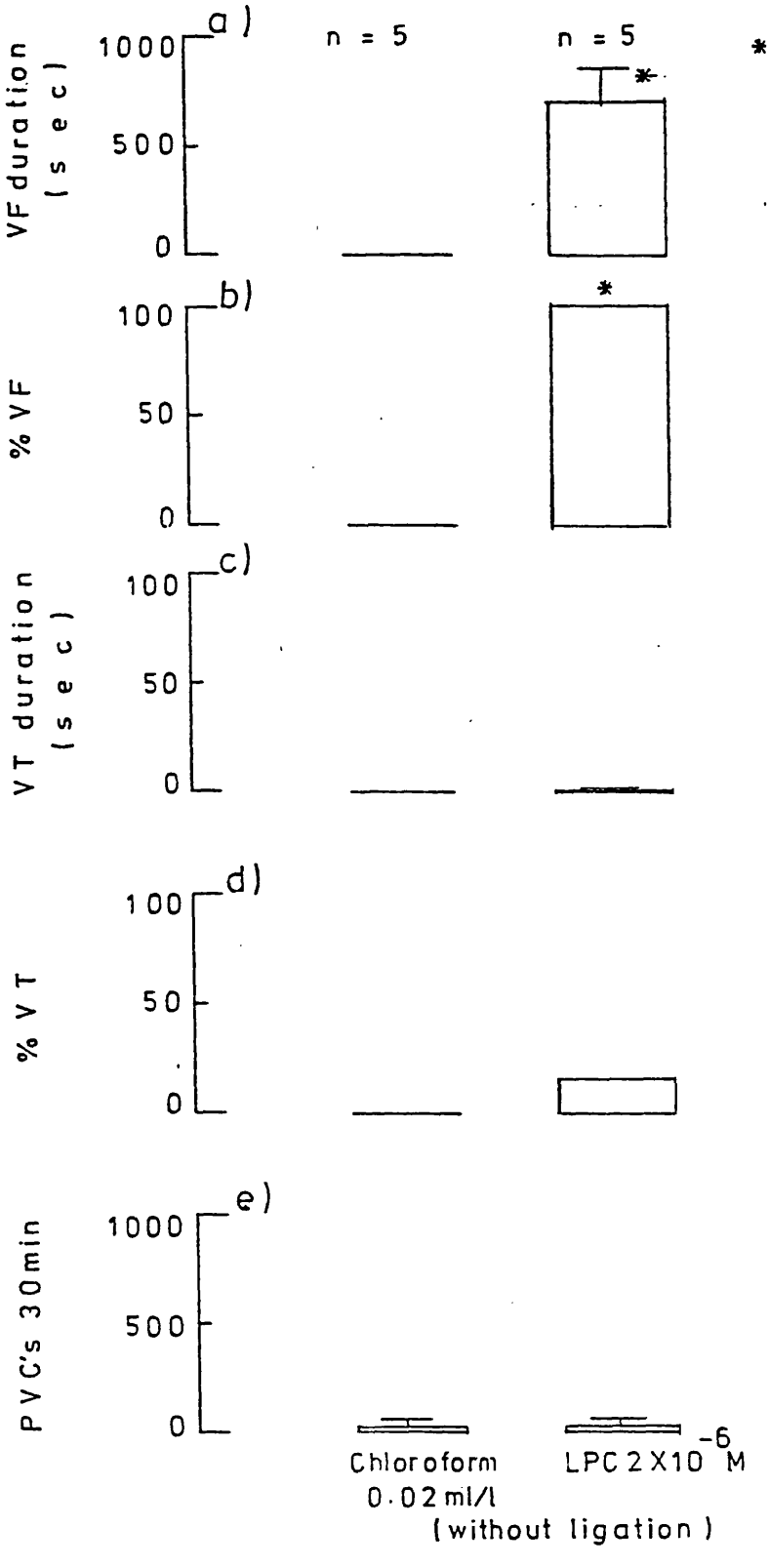

a)
n = 5
n = 5
* p < 0.01
VF duration (sec)
1000
500
0
b)
% VF
100
50
0
c)
VT duration (sec)
100
50
0
d)
% VT
100
50
0
e)
PVC's 30min
1000
500
0
-6
Chloroform 0.02 ml/l
LPC 2X10 M
(without ligation)

Figure 4.6. Effects of 10 mM potassium on arrhythmia parameters induced by lysophosphatidylcholine. These parameters are:

(a) Duration of ventricular fibrillation

(b) Incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Incidence of ventricular tachycardia

(e) Premature ventricular contractions/30 min.

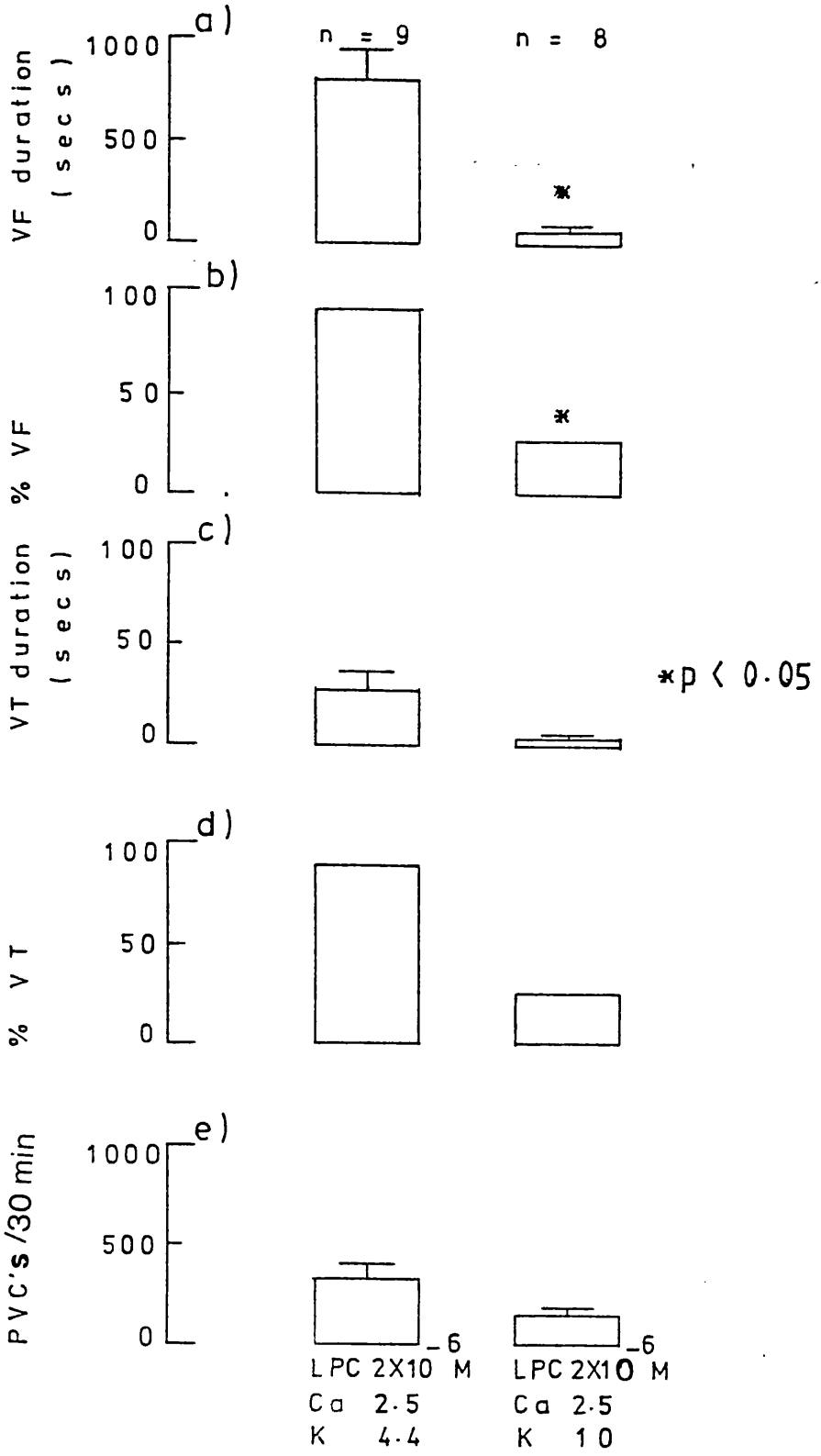

a)
VF duration (secs)
1000
500
0
n = 9
n = 8
*
b)
% VF
100
50
0
*
c)
VT duration (secs)
100
50
0
*p < 0.05
d)
% VT
100
50
0
e)
PVC's/30 min
1000
500
0
6
6
LPC 2X10 M
Ca 2·5
K 4·4
LPC 2X10 M
Ca 2·5
K 10

Figure 4.7. Effects of mepacrine on:

(a) Duration of ventricular fibrillation (sec)

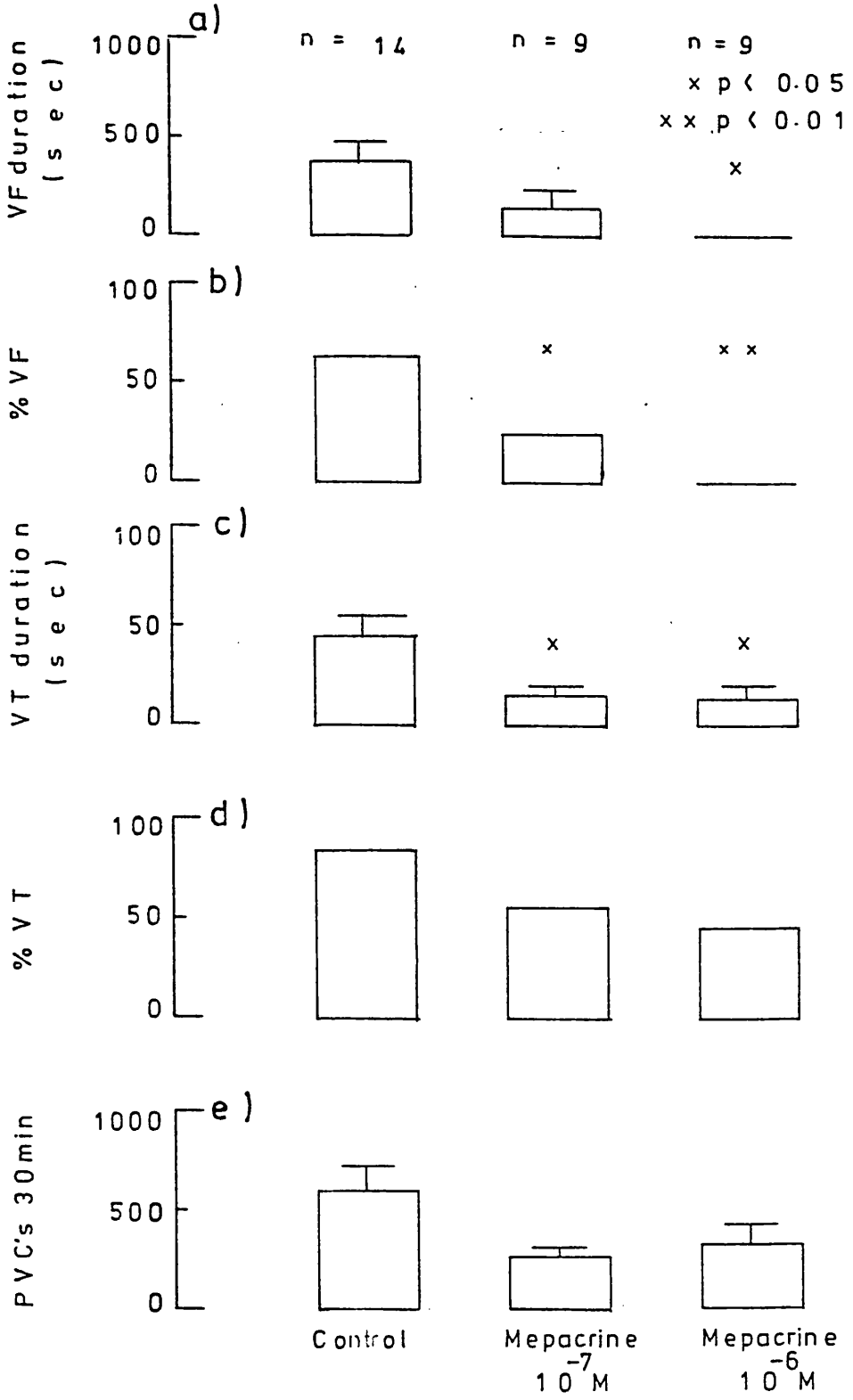
(b) Percentage incidence of ventricular fibrillation

(c) Duration of ventricular tachycardia (sec)

(d) Percentage incidence of ventricular tachycardia

(e) Premature ventricular contractions/30 min

Control calcium was 2.5 mM and potassium 2.5. mM.


a)
b)
c)
d)
e)
VF duration (sec)
% VF
VT duration (sec)
% VT
PVC's 30min
1000
500
0
100
50
0
n = 14
n = 9
n = 9
x p < 0.05
xx p < 0.01
Control
Mepacrine 10^-7 M
Mepacrine 10^-6 M

4.0 DISCUSSION

4.1.1 Effects of modulations of prostaglandins on coronary artery ligation induced arrhythmias in the isolated rat heart

Release of prostaglandins during ischaemia has been reported by many authors (for review see Coker, 1981), while many classes of antiarrhythmic drugs can inhibit prostaglandin synthesis (Das, 1981).

In addition some inhibitors of prostaglandin synthesis have been shown to possess antiarrhythmic properties (Das, 1981; Moschos and Haider, 1978; Johnston *et al.*, 1983). Thromboxane has been implicated in the development of ventricular fibrillation (Coker *et al.*, 1981; Coker *et al.*, 1982) while prostacyclin has been reported to be antiarrhythmic (Coker and Parratt, 1983; Johnston *et al.*, 1983).

Almost all of the work related to prostaglandins and arrhythmia has been done *in vivo* while little is known about the effect of these local hormones in *in vitro* models of arrhythmias devoid of blood, neural and hormonal influences.

4.1.ii Effects of ZK 36374 on ligation induced arrhythmias in the isolated rat heart

In contrast to the *in vivo* findings of Coker and Parratt (1983), ZK 36374 failed to reduce the incidence of ventricular fibrillation in the isolated rat heart. The protective *in vivo* effect of ZK 36374 and prostacyclin could be explained because of their potent inhibitory effect on platelet aggregation (Needleman and Kaley, 1978). As platelets are absent in the isolated rat heart this could explain why ZK 36374 did not affect arrhythmias in the *in vitro* model. *In vivo* thromboxane induced platelet aggregation may obstruct collateral circulation to the ischaemic zone and hence aggravate

the ischaemic injury. Also ZK 36374 induced reduction in peripheral resistance *in vivo* might reduce heart load.

The failure of the combination of ZK 36374 and indomethacin to prevent arrhythmias *in vitro* indicates that ZK 36374 does not possess an antiarrhythmic effect which is masked by the production of thromboxane as indomethacin would inhibit thromboxane production, however, there could be no thromboxane production *in vitro* due to the absence of platelets. Similarly, arachidonic acid, the precursor of prostaglandins, did not protect against ligation induced arrhythmias. However, if it had any protective effect (possibly attributed to prostacyclin synthesis) this could be abolished by thromboxane or leukotrienes synthesis if these metabolites are detrimental.

4.1.iii Effects of aspirin and indomethacin on ligation induced arrhythmias

Aspirin and indomethacin have the disadvantage of blocking the synthesis of both prostacyclin and thromboxane. 10^{-5} M aspirin - which is used in the present study - has been shown to induce a 20% inhibition of human synovial prostaglandins synthesis *in vitro* whereas indomethacin is 257 times more potent (Crook *et al.*, 1976). 300 ng/ml/min indomethacin was found to completely inhibit prostacyclin synthesis in the rabbit heart (Needleman, 1976). This is approximately between 10^{-6} and 10^{-5} M. However no data is available about the effect of these drugs in the rat *in vitro*.

The present results are in contrast to the *in vivo* findings of Moschos *et al.* (1982) and Johnston *et al.* (1983). They are also against the postulation that salicylate ion possesses an antiarrhythmic effect independent of cyclo-oxygenase inhibition (Johnston *et al.*, 1983).

The increase in the duration of ventricular fibrillation in the case of aspirin and indomethacin could be accounted for as inhibition of cyclo-oxygenase leaves more arachidonic acid available for conversion to leukotrienes (Higgs and Vane, 1983) which could have detrimental effects as suggested by the protective effect of BW 755C and nafazatrom.

4.1.iv Effects of N-butyl imidazole and dazoxiben on ligation induced arrhythmias

In order to keep the prostacyclin/thromboxane balance in favour of prostacyclin, thromboxane synthetase selective inhibitors have been used in the present study. 2 mg/kg i.v. dazoxiben reduced coronary sinus thromboxane B_2 concentration by 30% in the dog (Coker *et al.*, 1982). If the extracellular fluid volume is 0.31 litre/kg - 0.61 litre/kg (Bowman and Rand, 1980) this dose of 2 mg/kg approximately equals a concentration of 2×10^{-5} M - 10^{-5} M which is similar to that used in this thesis (10^{-5} M).

Although there is no available data about the effects of these drugs on the isolated rat heart, it could be concluded, that thromboxane synthesis inhibition has no protective role on ligation induced arrhythmias in the rat heart *in vitro*. This is in contrast to the *in vivo* findings of Coker *et al.*, (1982) in the dog.

The failure of ZK 36374, arachidonic acid, aspirin, indomethacin, N-butylimidazole and dazoxiben to alter ligation induced arrhythmia in the isolated rat heart strongly suggests that prostaglandins and thromboxane are not implicated in the genesis of arrhythmias in this model. Effects reported in the *in vivo* models could be

attributed to the presence of platelets or to extracardiac neural and hormonal influences.

4.1.v Effects of ETYA, BW 755C and nafazatrom on ligation induced arrhythmias

The possibility that the increase in ventricular fibrillation duration induced by aspirin and indomethacin is due to leukotriene synthesis prompted more work to investigate this pathway. However, no information is available about the presence of leukotrienes in the isolated rat heart. Despite this, drugs which affect leukotriene , synthesis in other tissues were examined. 5, 8, 11, 14 - eicosatetraynoic acid is a dual inhibitor of both lipoxygenase and cycloxygenase. It inhibits tumor cell lipoxygenase with an ED_{50} of 0.9 μM (Honn and Dunn, 1982). BW 755C inhibits the synthesis of prostaglandins, hydroxy acids and leukotrienes possibly by an antioxidant or reducing mechanism (Higgs and Vane, 1983). Nafazatrom is a prostacyclin synthesis inducer and lipoxygenase inhibitor. It inhibited tumor cell lipoxygenase at ED_{50} = 3 μ M (Honn and Dunn, 1982).

BW 755C reduced the incidence of ventricular fibrillation in a concentration dependent manner with a maximum effect at 10 μg/ml whereafter the protective effect was reduced (Figure 4.2).

BW 755C also induced a concentration dependent change in the colour of the hearts associated with reduction in developed tension. The reason for this change is not clear but it could be postulated that BW 755C possesses a toxic effect; judged by the change of colour, which antagonises its antiarrhythmic effect.

The ability of BW 755C and nafazatrom to reduce the incidence of ventricular fibrillation (Figure 4.3) may implicate lipoxygenase products in the gensis of arrhythmias in the isolated rat heart. However, the failure of ETYA to reduce arrhythmias complicates the interpretation of results.

It could be postulated that 1) lipoxygenase pathway is arrhythmogenic but ETYA has a very toxic effect that counteracts its beneficial effect or 2) lipoxygenase pathway has no role in the gensis of arrhythmias and BW 755C and nafazatrom have an unidentified anti-arrhythmic mechanism

Further investigation is required to evaluate the role of leukotrienes in the isolated rat heart and to examine the direct electrophysiological effects of some of these compounds. Coker and Parratt (1984) ascribed the protective effect of nafazatrom in anaesthetized dogs to its effect on prostacyclin release. However, this mechanism does not explain the actions of this compound in the isolated rat hearts. For ZK 36374 and manoeuvres that would be expected to increase prostacyclin release failed to affect arrhythmias in the isolated rat heart.

4.2.i Effects of lysophospholipids on ligation induced arrhythmias

The ability of lysophosphatidylcholine to precipitate fibrillation in the normoxic heart (Figures 4.4, 4.5) is in agreement with the findings of Man and Choy (1982). It is also in harmony with the findings of Corr *et al.* (1979) that normal and ischaemic myocardium deteriorates in response to exogenous lysophosphoglycerides.

This effect could be attributed to the cytolytic effect of lysophosphatidylcholine (Weltzien, 1978) and potentiation of calcium accumulation (Sedlis *et al.*, 1979). Other detrimental effects of lysophosphatidylcholine which may possibly precipitate arrhythmias are inhibition of myocardial Na^+-K^+ ATPase (Karli *et al.*, 1979) increased synthesis of cyclic AMP (Ahumada *et al.*, 1979) and the formation of ion channels within lipid bilayers (Lee and Chang, 1977) that may potentially increase both influx and efflux of calcium ions across the sarcolemma.

The concentration of lysophosphatidylcholine, that caused colapse of contractility and fibrillation in the present study, is almost 1/100th the concentration estimated by Corr *et al.* (1981) in the ischaemic zone and which they used for electrophysiological and calcium overload studies. This suggests that the concentration of lysophosphatidylcholine found in the rabbit by Corr *et al.* (1981), is either overestimated or that endogenous lysophosphatidylcholine behaves differently from exogenous lysophosphatidylcholine. In fact the effects of low concentrations of added lysophosphatides on calcium transport by phospholipase C treated membranes were shown to be opposite to those of higher concentrations in the native membrane (Katz , 1982).

This together with results on drugs affecting prostaglandins prompted a study of phospholipases A_2 inhibitors which should decrease lysophosphatidylcholine levels.

4.2.ii Effects of mepacrine, chlorpromazine and trifluperazine on ligation induced arrhythmias

Mepacrine and the phenothiazines are calmodulin inhibitors

(Prozialeck and Weiss, 1982). Calmodulin has been shown to activate phospholipase A_2 (Prozialeck and Weiss, 1982). These compounds are also able to form complexes with phospholipids and thereby prevent enzymatic attack by phospholipases (Blackwell and Flower, 1983).

IC_{50} values for bovine brain calmodulin were 28, 40 and 42 μmolar for trifluperazine, chlorpromazine and mepacrine respectively (Prozialeck and Weiss, 1982). However, no data is available for the rat heart. However, if similar sensitivity is assumed for the rat heart, it can be seen that the concentrations used in the present study were by far less and would account for some of the negative findings.

The ability of mepacrine to suppress ligation induced arrhythmias (Figure 4.7) and the failure of chlorpromazine and trifluperazine can either mean:

1. Lysophospholipids are involved in the genesis of arrhythmias, but the concentrations of chlorpromazine and trifluperazine used were too low to inhibit phospholipase A_2. This possibility could be ruled out by evaluating the effect of these drugs on rat heart phospholipase.
2. Lysophospholipids are not involved in the genesis of arrhythmias and mepacrine acts by some other mechanism possibly local anaesthetic. This is supported by the structural similarity of this drug with quinidine, this possibility could be confirmed by evaluating the electrophysiological effects of mepacrine on the heart.

4.2.iii Effects of bradykinin on ligation induced arrhythmias

7.5 ng/ml bradykinin was shown to stimulate maximum production of prostaglandins in the rabbit heart (Needleman *et al.*, 1975). The concentrations used in the present study (7.5 ng/ml and 15 ng/ml) are therefore likely to stimulate phospholipase unless the rat heart is less sensitive to bradykinin.

The failure of bradykinin to elicit an increase in the incidence of ventricular fibrillation is in agreement with Lee and Sobel (1981). This group failed to detect an increase in the activity of ischaemic rabbit heart microsomal and mitochondrial phospholipase A_2 in the presence of bradykinin.

4.3.i Effects of antioxidants and free radical scavengers on ligation inducd arrhythmias

Free radicals cause accumulation of lipid hydroperoxides (Meerson *et al.*, 1982), inhibition of Na^+/K^+ ATPase (Acosta *et al.*, 1984) and depression of calcium uptake by the sarcoplasmic reticulum (Rowe *et al.*, 1983).

There is considerable data implicating free radicals in ischaemically induced myocardial damage, however, their possible role in arrhythmia production is not clear.

The free radical scavengers and antioxidants at the concentrations used in the present study were shown to prevent the more serious reperfusion arrhythmias *in vitro* (Woodward and Zakaria, 1985). However, the failure of these compounds to exert a protective effect in ligation induced arrhythmias may imply a different aetiology for the reperfusion arrhythmias. The process

of reperfusion involves a sudden influx of oxygen into the ischaemic region. This coupled with impairment of the oxidation-phosphorylation system and accumulation, during ischaemic period of reducing equivalents, will create conditions favouring free radical generation (Meerson *et al.*, 1982).

CHAPTER 5

Extracellular potassium increases following ischaemia and this may be an important factor leading to the development of arrhythmias. In this chapter, the effects of magnesium, pH, hypothermia, contractility and antiarrhythmic drugs on $^{86}Rb^{+}$ efflux from the isolated rat hearts have been studied. $^{86}Rb^{+}$ has been used as a marker for potassium as it behaves in a similar manner to potassium and is more convenient to use. The effects of electrical pacing and non ischaemically induced arrhythmias on $^{86}Rb^{+}$ efflux have also been examined in order to see if arrhythmias can themselves affect $^{86}Rb^{+}$ efflux.

5.1 Effects of magnesium on $^{86}Rb^{+}$ efflux rate constant (e.r.c.).

These experiments were carried out to investigate the effects of magnesium on rubidium efflux rate constant.

Increasing extracellular magnesium concentration from 1.2 mM to 4.8 mM reduced ($p < 0.01$) $^{86}Rb^{+}$ e.r.c. from 0.035 ± 0.003 to 0.024 ±0.001 min^{-1}. Reperfusion with normal perfusate (Mg^{++}, 1.2 mM) re-established the initial e.r.c. values (Figure 5.1).

Perfusion of three hearts with magnesium (0.6 - 4.8 mM) elicited a concentration dependent effect on e.r.c. (Figure 5.2) ($P < 0.01$, $r = -0.969$), heart rate (Figure 5.3) ($P < 0.01$, $r = -0.988$), developed tension (Figure 5.4) ($P < 0.001$, $r = -0.994$) and perfusion pressure (Figure 5.5) ($P < 0.05$, $r = -0.972$). The change in e.r.c. brought about by magnesium was linearly correlated with change in heart rate (Figure 5.6) ($P < 0.01$, $r = -0.986$), developed tension (Figure 5.7) ($P < 0.01$, $r = -0.983$) and perfusion pressure (Figure 5.8) ($P < 0.01$, $r = -0.966$).

The effects of magnesium on e.r.c. could be due to its action on heart rate, developed tension or perfusion pressure rather than a direct effect on e.r.c. In order to test this a further series of experiments were carried out.

5.2 Effects of magnesium (4.8 mM) on e.r.c. in hearts paced at 300 b/min

In order to assess whether the correlation between e.r.c. and heart rate is causative, hearts were paced at 300 b/min 5 minutes before magnesium (4.8 mM) was perfused. In these paced hearts the e.r.c. was still reduced ($P < 0.05$) by magnesium (4.8 mM) when compared with 1.2 mM magnesium (Figure 5.9a). However, the reduction in e.r.c. was less pronounced than that produced in the absence of pacing (18% in presence and 30% in absence). This implies that reduction in heart rate induced by magnesium may account for some of the reduction in e.r.c. In these electrically paced hearts the reduction in e.r.c. induced by magnesium was still accompanied by a reduction ($p < 0.05$) in developed tension (Figure 5.9b).

5.3 Effects of electrical pacing at different rates on e.r.c.

For further assessment of the effects of heart rate on e.r.c., hearts were paced at different rates (250, 300, 350, 400 and 450 bpm). Pacing at these rates did not significantly affect e.r.c., however, electrical pacing was accompanied by a decrease in developed tension ($p < 0.01$, $r = -0.996$) (Figure 5.10).

5.3 Effects of calcium and verapamil on e.r.c.

In order to examine the effect of a reduction in developed tension on e.r.c., developed tension was altered by perfusing hearts with different concentrations of calcium (0.6, 1.2, 2.4, 3.6 and 4.8 mM) and one concentration of the calcium antagonist, verapamil (10^{-7} M).

Perfusion of hearts with 0.6, 1.2, 2.4 mM calcium produced a concentration dependent increase in contractility, above this concentration the contractility tended to level off. The two concentrations which increased developed tension (1.2 and 2.4 mM) produced a significant increase ($P < 0.05$) in e.r.c. (Figure 5.11).

Verapamil (10^{-7}M) significantly reduced developed tension and e.r.c. ($p < 0.01$) (Figure 5.12).

For further investigation of the effect of developed tension on e.r.c., developed tension was reduced by increasing hydrogen ion concentration.

5.4 Effects of pH on e.r.c.

Reduction of perfusate pH to 6.7 and 5.2 was achieved by omitting 18 millimole or 25 milimole of sodium bicarbonate respectively. Sodium ion concentration was adjusted by adding sodium chloride. Reduction of pH to 6.7 induced a slight initial increase in developed tension followed by a decrease ($p < 0.001$); perfusion pressure was initially decreased ($P < 0.01$). This was then followed by an increase which was significant ($p < 0.01$). Heart rate and e.r.c. were both reduced ($p < 0.01$) (Figure 5.13).

Reduction of pH to 5.2 reduced developed tension ($p < 0.001$) and e.r.c. ($p < 0.01$) while perfusion pressure was increased ($p < 0.05$). The weak contractility in this experiment failed to trigger the rate meter (Figure 5.13).

5.5 Effects of hypothermia on e.r.c.

Perfusate temperature was reduced from 37°C to 23°C in order to reduce the heart rate.

reduced ($P < 0.001$) e.r.c. despite the increase ($P < 0.01$) in developed tension (Figure 5.14).

5.6 Effects of adenosine on e.r.c.

To investigate the relation between reduction in e.r.c. and reduction in perfusion pressure produced by magnesium the vasodilator adenosine was used. Adenosine (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} M) induced a concentration dependent effect on perfusion pressure with no significant effect on e.r.c. (Figure 5.15).

5.7 Effects of ligation and reperfusion on % increase in $^{86}Rb^{+}$ counts

Extracellular increases of potassium following coronary artery ligation might be an important factor leading to the development of ventricular arrhythmias. It was of interest to see if potassium loss preceded the development of arrhythmias.

In these experiments % change in $^{86}Rb^{+}$ counts was used instead of e.r.c. because they were some of the earlier experiments when no measurement of rate was performed.

No increase in $^{86}Rb^{+}$ counts was noticed upon ligation of coronary artery and that could be due to the low collateral circulation in the rat heart (Figure 5.16). Because no increase in $^{86}Rb^{+}$ counts was detected during ligation hearts were reperfused to see if $^{86}Rb^{+}$ released during ischaemia could be washed out. In these experiments hearts were perfused with 10 mM potassium to prevent fibrillation upon reperfusion. Increasing potassium to 10 mM itself increased ($p < 0.01$) e.r.c. (Figure 5.17). Perfusate was collected for 35 seconds following reperfusion to calculate the percentage increase in counts. An example

of the effect of ligation and reperfusion is shown in Figure 5.16.

Ligation for 5, 10, 13 and 15 minutes produced a time dependent increase in counts during reperfusion ($P < 0.05$, $r = 0.095$) (Figure 5.18).

Increases of extracellular potassium during reperfusion in these experiments could be due to inhibition of Na^+/K^+ pump activity during ischaemia (Kunze, 1977). To assess the possible contribution of inhibition of Na^+/K^+ pump activity to the leakage of potassium during ischaemia, hearts were perfused with ouabain.

5.8 Effects of ouabain on e.r.c.

Ouabain (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4} M) did not exert any significant effect on e.r.c., heart rate or developed tension.

5.9 Effects of ventricular fibrillation on e.r.c.

Because it was not possible to measure a change in e.r.c. during arrhythmia development in the coronary ligated rat heart, arrhythmias were induced by altering the ionic milieu to see if arrhythmias themselves affect e.r.c. Fibrillation in these models was found to be consistent and predictable. Three models were attempted and these were:-

(a)	Ca^{++} 5 mM	Na^+ 59 mM	K^+ 5.9 mM
(b)	Ca^{++} 5 mM	Na^+ 118 mM	K^+ 1.2 mM
(c)	Ca^{++} 5 mM	Mg^{++} 0	K^+ 2.5 mM

(a) Ca^{++} 5 mM, Na^+ 59 mM, K^+ 5.9 mM

In the experiments using model (a) sucrose was added to compensate for reduction of osmolarity produced by halving sodium

chloride concentration. After introducing the arrhythmogenic solution a, samples were taken every 15 seconds. Fibrillation in this model occurs 180 ±15 seconds (n = 3) after changing the composition of the perfusate. When fibrillation took place rubidium e.r.c. had already started to increase and a greater increase in e.r.c. took place after the onset of ventricular fibrillation (Figure 5.19a) but this increase was not maintained.

(b) Ca^{++} 5 mM, K^{+} 1.2 mM.

In this model, fibrillation occurs 187 ± 7 seconds after changing the composition of the perfusate. After altering the ionic milieu a transient increase in e.r.c. took place. On the onset of ventricular fibrillation a greater increase in e.r.c. occurred (Figure 5.19b) which was not maintained.

(c) Ca^{++} 5 mM, Mg^{++} 0, K^{+} 2.5 mM.

In this model, fibrillation occurs 210 ± 54 seconds after altering the ionic composition of the perfusate. A gradual increase in e.r.c. took place before the onset of ventricular fibrillation. (Figure 5.19c).

In the previous three models of arrhythmias increase in e.r.c. accompanied the onset of fibrillation. Further experiments were carried out to try to dissociate the occurrrence of ventricular fibrillation from the change in e.r.c.

5.10 Effects of mexiletine (5-10 mg/litre) and verapamil 10^{-6}M on fibrillation induced by 5 mM calcium, 59 mM sodium chloride and 5.9 mM potassium

Drugs were applied 15 minutes before altering the ionic milieu

5 and 10 mg/litre mexiletine alone reduced ($P < 0.01$) e.r.c. but after altering the ionic milieu a gradual increase in e.r.c. still occurred. Despite this both concentrations of mexiletine prevented the development of ventricular fibrillation within 7 minutes.

Verapamil 10^{-6} M reduced ($P < 0.01$) e.r.c. and prevented the incidence of ventricular fibrillation. Upon altering the ionic milieu a gradual increase in e.r.c. still took place (Figure 5.20).

5.11 Effects of mexiletine on fibrillation induced by 5 mM calcium, 1.2 mM potassium

10 mg mexiletine prevented ventricular fibrillation and reduced ($P < 0.01$) e.r.c. Upon altering the ionic composition of the perfusate e.r.c. increased only for 2 minutes before levelling off. (Figure 5.21).

5.12 Effects of mexiletine on fibrillation induced by 5 mM calcium, 0 mM magnesium and 2.5 mM potassium

10 mg/L mexiletine reduced ($P < 0.01$) e.r.c.. $^{86}Rb^{+}$ e.r.c. increased after altering the ionic milieu but was reduced compared to control . Ventricular fibrillation was also prevented (Figure 5.22).

5.13 Effects of mexiletine and verapamil on the haemodynamics of the isolated hearts

5 mg/L mexiletine reduced non significantly the heart rate and developed tension while 10 mg/L mexiletine significantly ($P < 0.05$) reduced both developed tension and heart rate. Neither concentration had a significant effect on perfusion pressure (Figure 5.23).

10^{-6} M verapamil reduced the developed tensions from 8.4 ± 0.5 to 3.9 ± 0.4 grams and the e.r.c. from 0.029 ± 0.001 to 0.021 ± 0.001 min^{-1}. The low contractility did not trigger the rate meter.

5.14 Evaluation of $^{86}Rb^{+}$ efflux during calcium paradox

$^{86}Rb^{+}$ detected following reperfusion in the isolated rat heart could be due to wash out of accumulated $^{86}Rb^{+}$ during coronary occlusion. Some of this increase in $^{86}Rb^{+}$ efflux could also be due to the direct effect of reperfusion as reperfusion injury may be caused by calcium overload (Shine and Douglas, 1983), as this can be induced by the calcium paradox. The effects of this on $^{86}Rb^{+}$ efflux were examined.

5.15 Effects of duration of calcium depletion on % increase in $^{86}Rb^{+}$ counts

This is one of the early experiments where % increase in counts was used instead of e.r.c.

Perfusate was depleted of calcium for 2, 4, 6, 8 and 10 minutes. Experiments were arranged in such a way that calcium repletion took place 40 minutes after the start of $^{86}Rb^{+}$ washout so that efflux could be compared with the control values at this time. One minute after calcium depletion hearts were uncoupled and perfusion pressure fell significantly ($P < 0.01$) from 70 ± 5 to 54 ± 2 mm Hg ($n = 16$).

At the time of calcium repletion there was a 52% increase in perfusion pressure. This rise in perfusion pressure was not dependent on the period of calcium depletion.

Re-admission of calcium following depletion for 2 and 4 minutes resulted in 80 ± 6 and 86 ± 4 recovery of developed tension respectively and loss of $^{86}Rb^{+}$.

On the other hand, calcium repletion after depletion for 6, 8 and 10 minutes resulted in loss of mechanical and electrical activity, protein leakage, increases in $^{86}Rb^{+}$ efflux and a rise in resting tension (Figures 5.24, 5.25, 5.26).

Tissue water content was not affected by time of calcium depletion.

5.16 Effects of hypothermia and low calcium perfusion (both appplied during the period of calcium depletion) on calcium paradox.

Hypothermia and perfusion with 0.25 mM calcium both during calcium depletion period (for 6 minutes) resulted in complete contractile recovery and preservation of $^{86}Rb^{+}$ and protein. (Figure 5.27). Ca^{++} 0.25 mM was used because any concentration higher than 0.05mM Ca^{++} during the calcium deprivation was shown to protect hearts against the damage caused by calcium readmission by Alto and Dhalla (1979).

5.17 Effects of mepacrine and chlorpromazine on e.r.c.

The phospholipase A_2 inhibitor meparcine (10^{-7} and 10^{-6} M) had an antiarrhythmic action (4.2c) while chlorpromazine another phospholipase inhibitor failed to exert any antiarrhythmic effect at the dosage used (10^{-7} and 10^{-6} M).

Experiments were designed to see whether these antiarrhythmic

concentrations of mepacrine had any effect on e.r.c. Perfusion of hearts with mepacrine (10^{-7}, 10^{-6}, 10^{-5} and 10^{-4}) reduced the e.r.c. and developed tension in a concentration dependent manner (Figure 5.28).

No significant effect was induced by chlorpromazine (10^{-7}, 10^{-6} and 10^{-5} M) but a concentration of 10^{-4} M, which caused a marked reduction of contractility induced a significant ($P < 0.001$) reduction in e.r.c. (Figure 5.29).

Neither drug had any significant effect on heart rate.

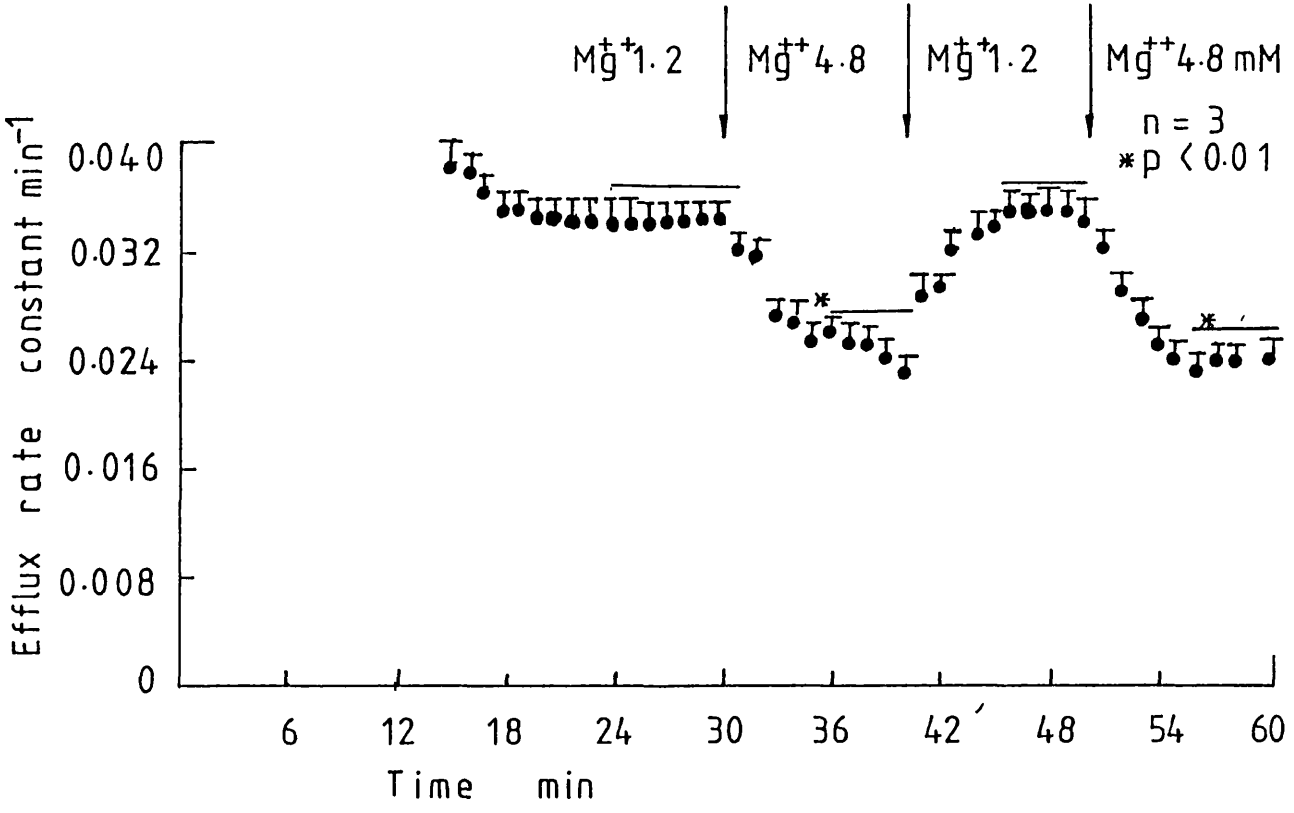


Figure 5.1. Effect of 4.8 milimolar magnesium sulphate on $^{86}Rb^{+}$ efflux rate constant (min^{-1}). The mean of the last five control samples is compared with the mean of the last five treated samples as indicated by the bars. Calcium concentration was 1.2 mM and potassium 2.5 mM.

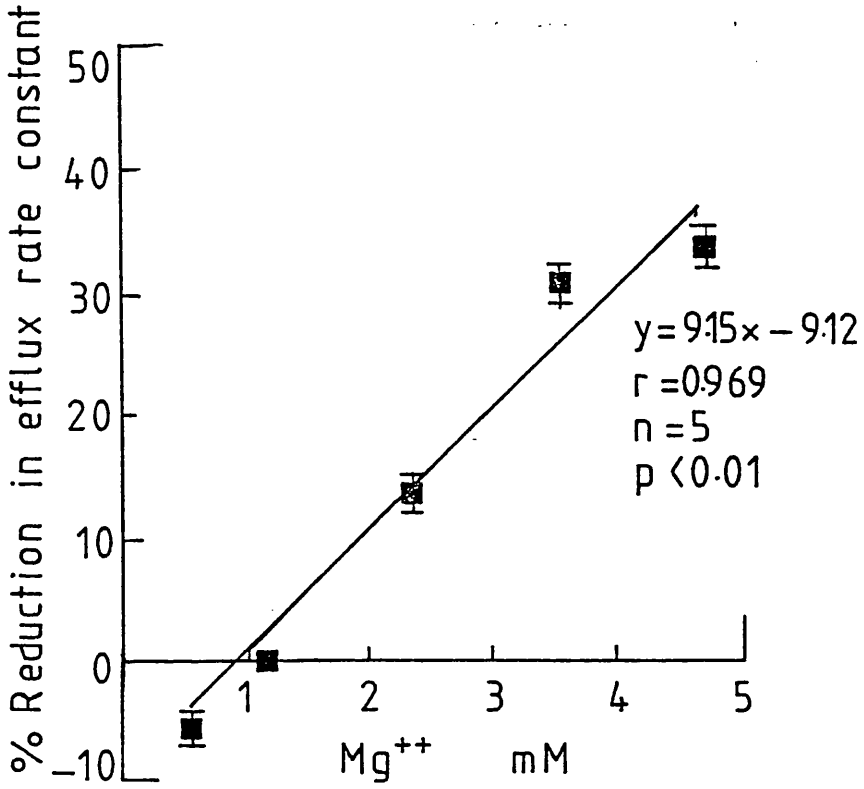


Figure 5.2. Relationship between magnesium concentration and $^{86}Rb^{+}$ efflux rate constant. 3 hearts were used in each group. Control e.r.c. at 1.2 mM magnesium was 0.026 ± 0.001 min^{-1}.

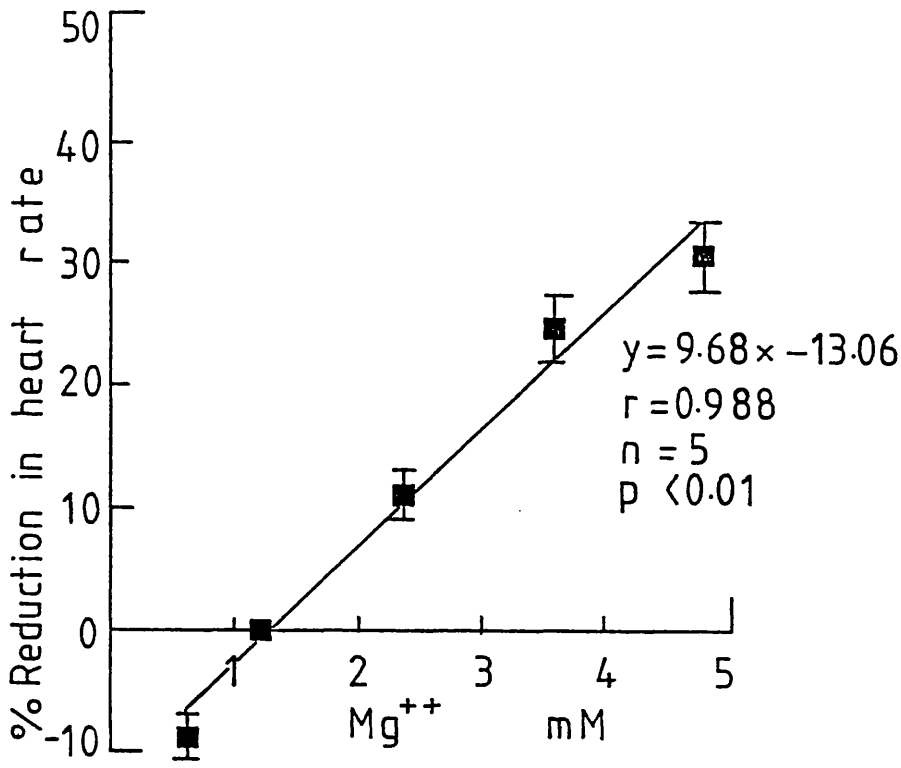


Figure 5.3. Relationship between magnesium concentration and heart rate. Every point is the mean of 3 hearts. Control heart rate at 1.2 mM magnesium was 213 ± 9 b/min

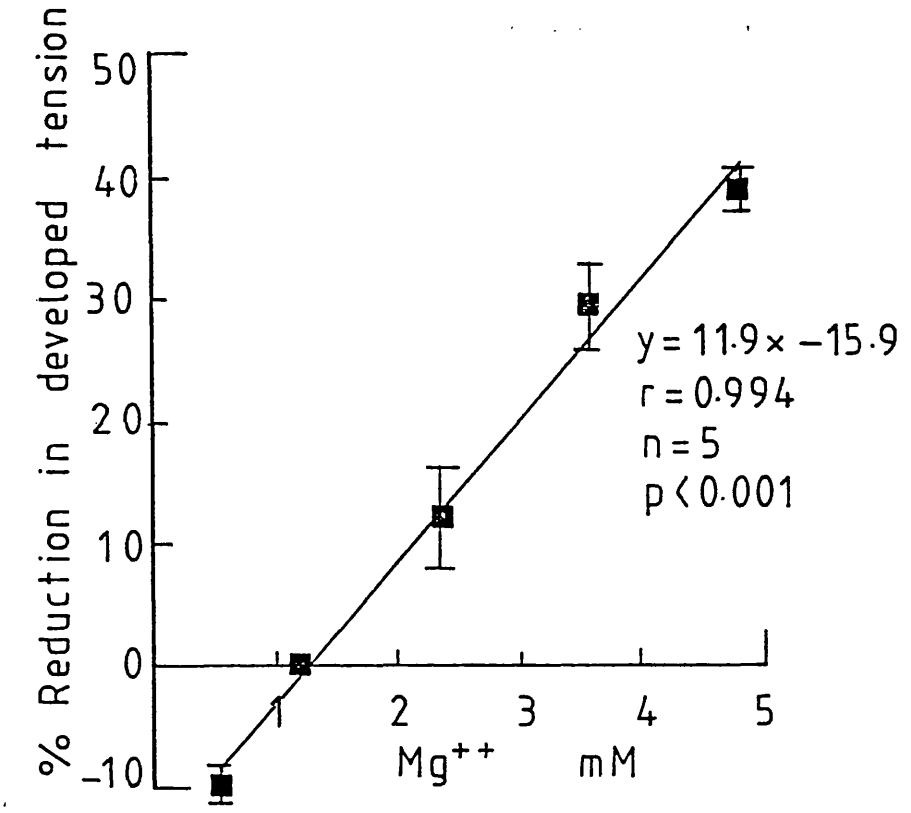


Figure 5.4. Relationship between magnesium concentration and % reduction in developed tension. Each point is the mean of 3 hearts. Control developed tension at 1.2 mM magnesium was 8.8 ± 1 grams.

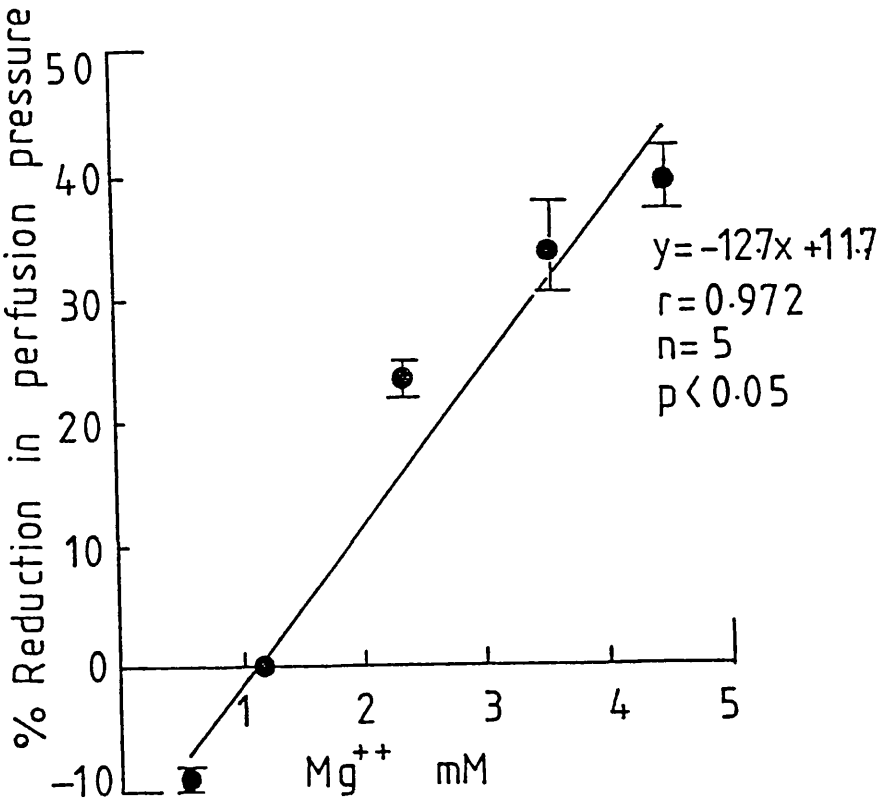


Figure 5.5.. Relationship between magnesium concentration and percentage reduction in perfusion pressure. Each point is the mean of 3 hearts. Control perfusion pressure at 1.2 mM magnesium was 104 ± 6 mm Hg.

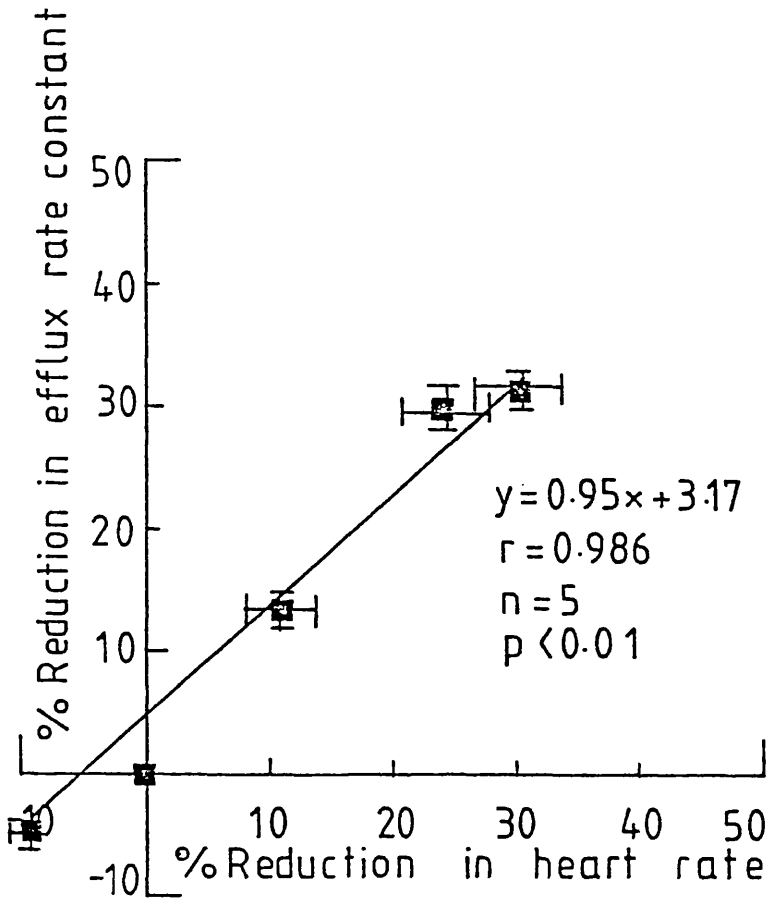


Figure 5.6. Relationship between percentage reduction in heart rate and percentage reduction in $^{86}Rb^{+}$ efflux rate constant when both variables were manipulated by magnesium. Every point is the mean of 3 hearts. Control heart rate at 1.2 mM magnesium was 213 ± 9 b/min and control, e.r.c. was 0.026 ± 0.001 min^{-1}.

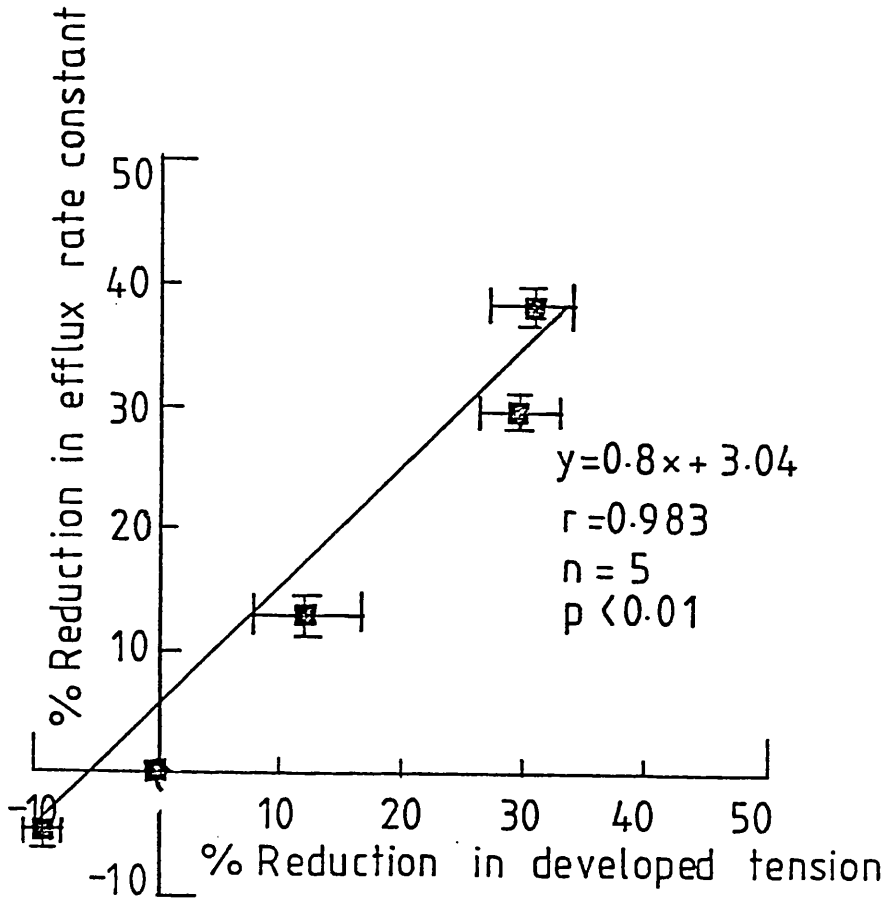


Figure 5.7. Relationship between percentage reduction in developed tension and percentage reduction in efflux rate constant when both variables were manipulated by magnesium concentration. Each point is a mean of 3 hearts. Control developed tension at 1.2 mM magnesium was 8.8 ± 1 grams and e.r.c. 0.026 ± 0.001 min^{-1}.

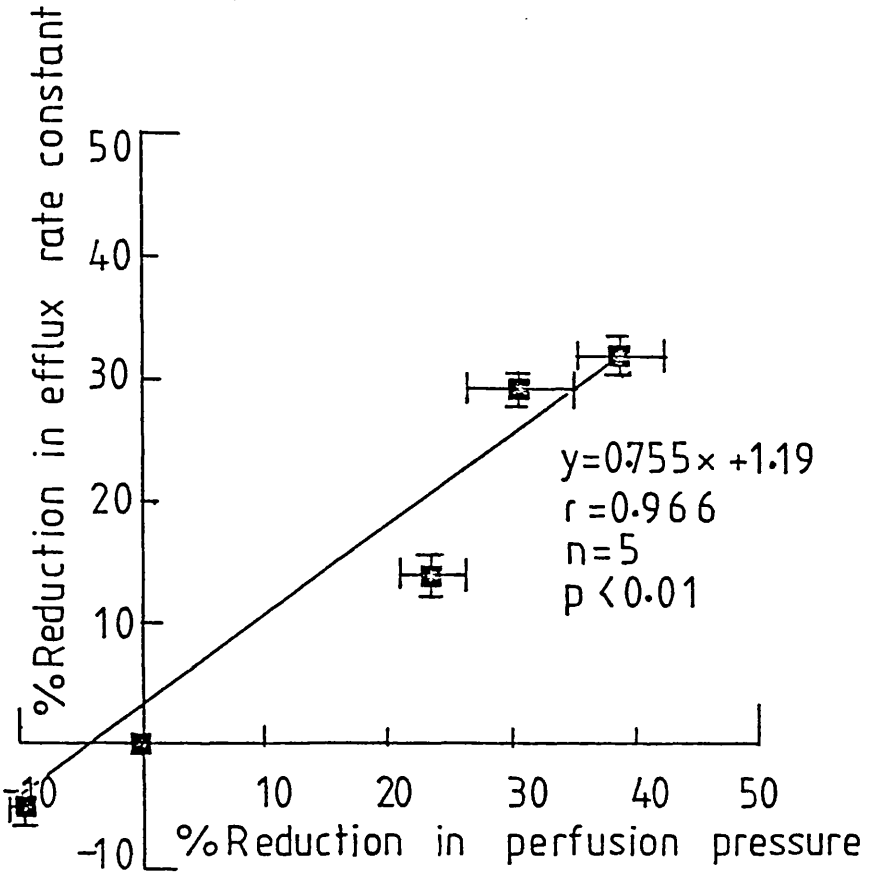


Figure 5.8. Relationship between percentage reduction in perfusion pressure and percentage reduction in $^{86}Rb^{+}$ efflux rate constant when both variables were manipulated by magnesium concentration. Each point was the mean of 3 hearts. Control perfusion pressure at 1.2 mM magnesium was 104 ± 6 mm Hg and e.r.c. 0.026 ± 0.001 min^{-1}.

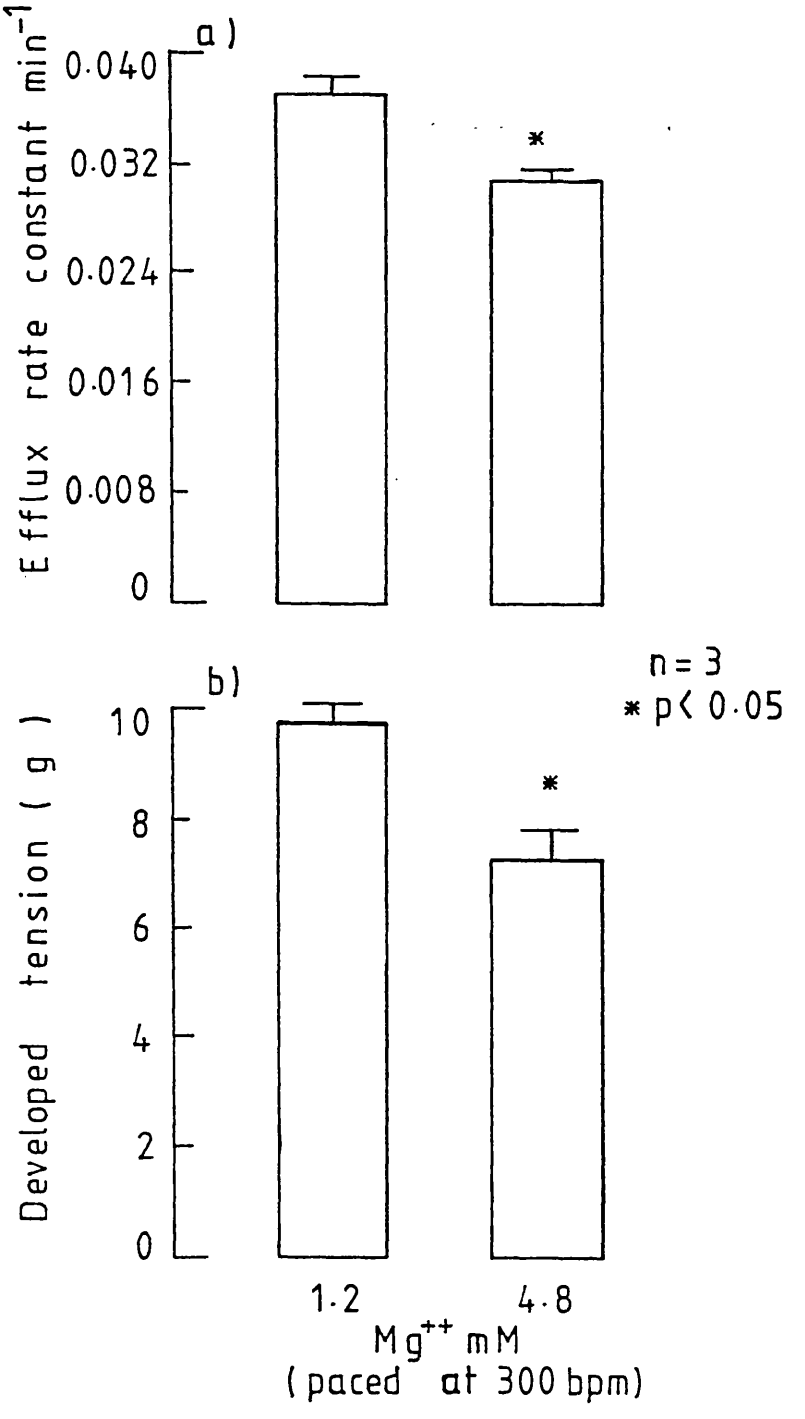


Figure 5.9. Effect of 4.8 mM magnesium on

(a) $^{86}Rb^{+}$ efflux rate constant (min^{-1}) of hearts paced at 300 b/min

(b) Developed tension (g).

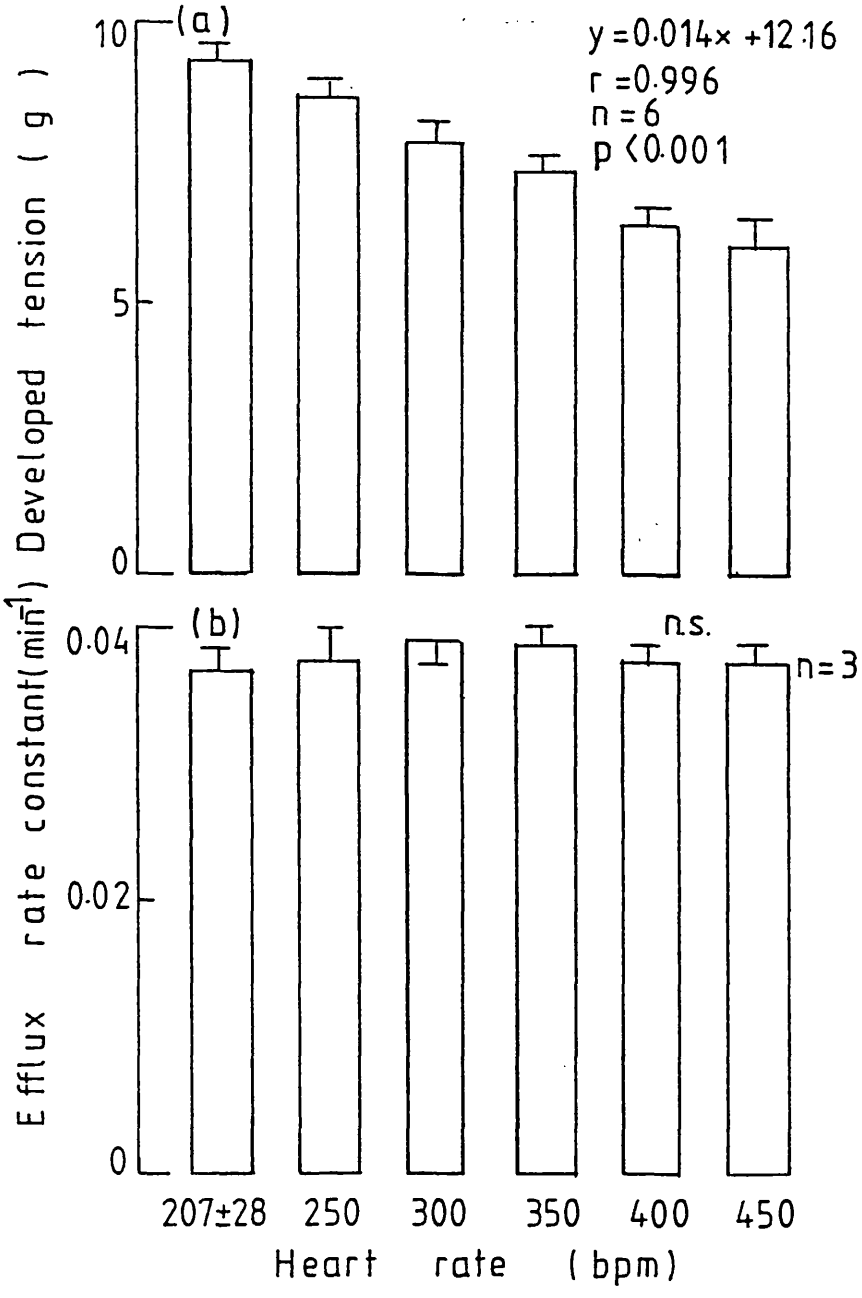


Figure 5.10. Effects of pacing hearts at different rates on

(a) Developed tension (g)

(b) $^{86}Rb^{+}$ efflux rate constant (min^{-1})

Potassium concentration here is 5.9 mM.

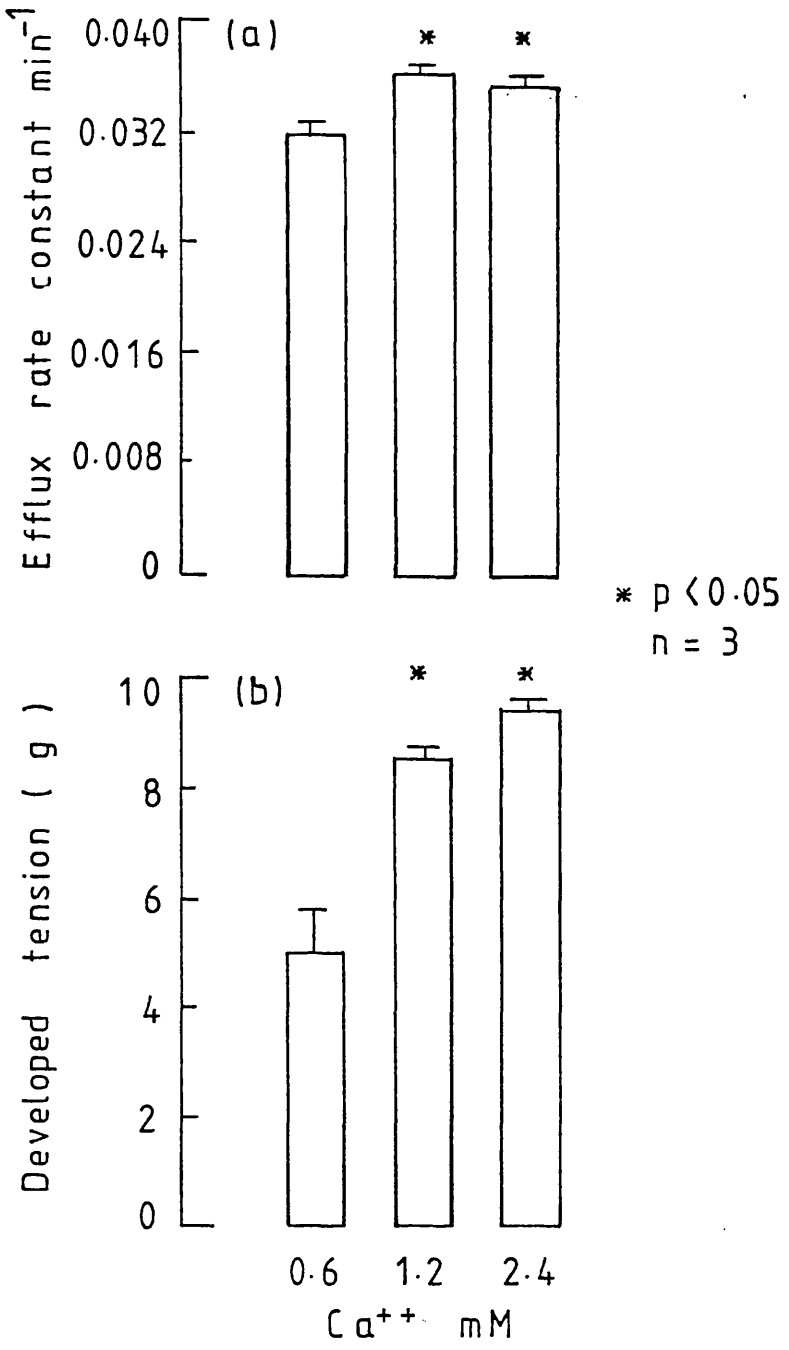


Figure 5.11. Effects of calcium concentration on

(a) $^{86}Rb^{+}$ efflux rate constant (min^{-1})

(b) Developed tension (g)

Potassium concentration here is 5.9 mM. 0.6 mM calcium is considered control.

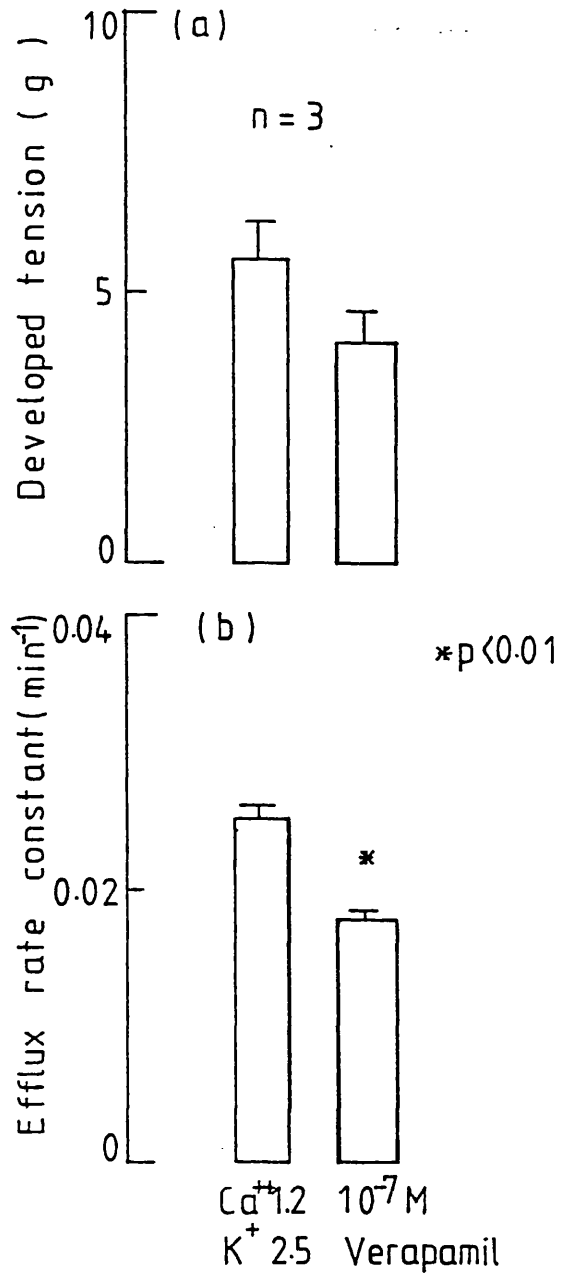


Figure 5.12. Effects of verapamil 10^{-7} on

(a) Developed tension (g)

(b) $^{86}Rb^{+}$ efflux rate constant (min^{-1})

Figure 5.13. Effects of pH 6.7 and pH 5.2 on

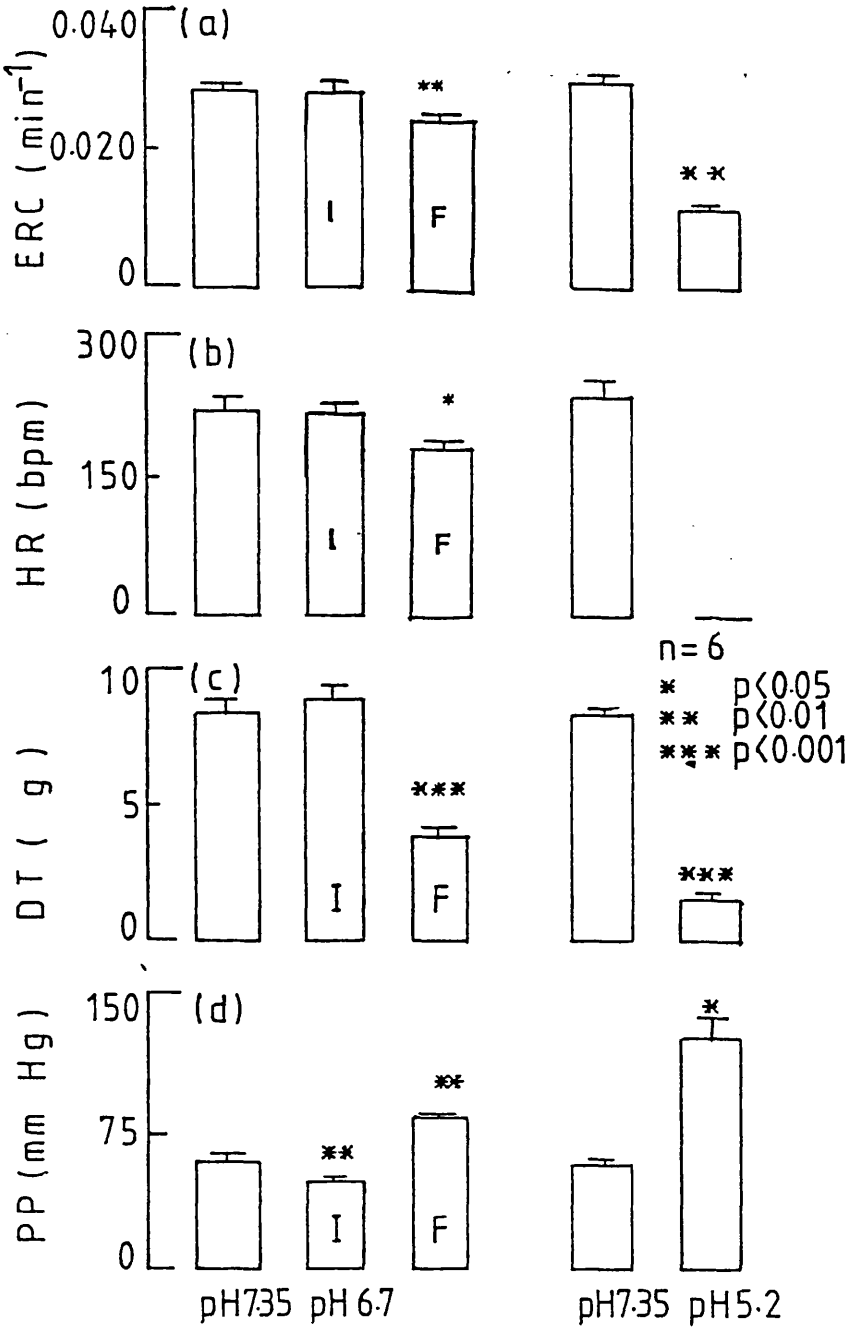
(a) $^{86}Rb^{+}$ efflux rate constant (ERC) (min^{-1})

(b) Heart rate (HR) (Beats/min)

(c) Developed tension (DT) (g)

(d) Perfusion pressure (PP) (mm Hg)

Developed tension and perfusion pressure showed biphasic change when pH was decreased to 6.7. (I) represents initial change and (F) represents final steady state change. In the case of pH 5.2 the developed tension did not trigger the rate meter.


(a)
ERC (min⁻¹)
0.040
0.020
0
I
F
**
**
(b)
HR (bpm)
300
150
0
I
F
*
(c)
DT (g)
10
5
0
I
F
***
***
(d)
PP (mm Hg)
150
75
0
I
F
**
*
n=6
* p<0.05
** p<0.01
*** p<0.001
pH7.35
pH 6.7
pH7.35
pH 5.2

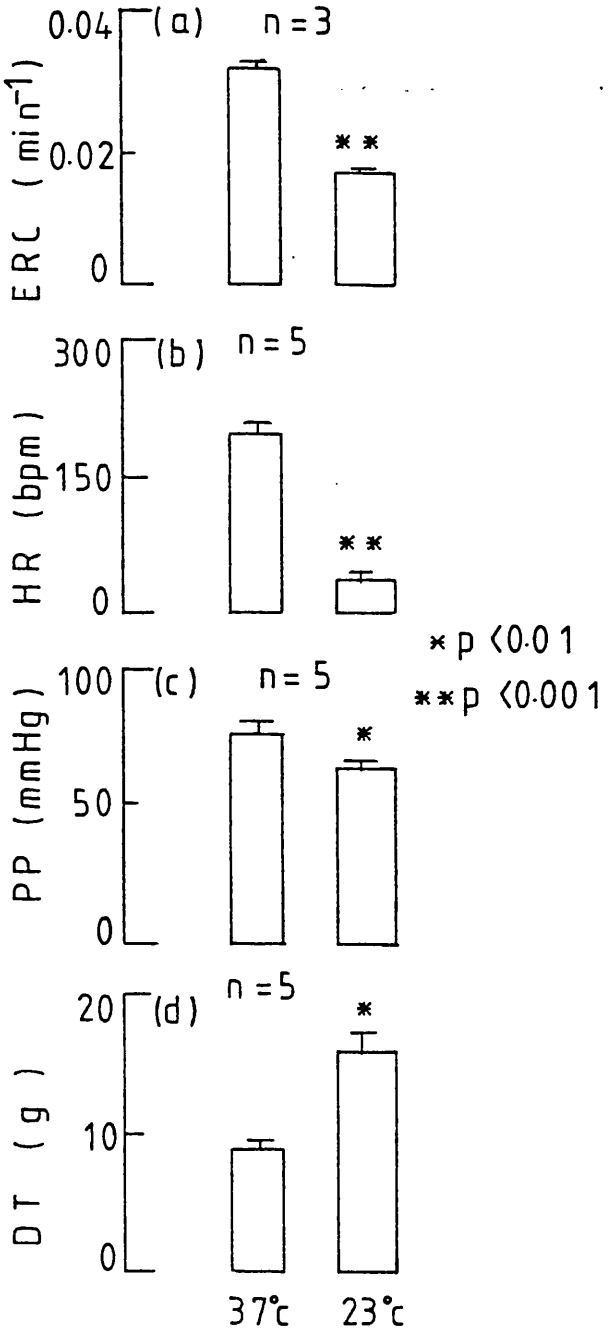


Figure 5.14. Effects of hypothermia (23°C) on:

(a) $^{86}Rb^{+}$ efflux rate constant (ERC) (min^{-1})

(b) Heart rate (HR) (bpm)

(c) Perfusion pressure (PP) (mm Hg)

(d) Developed tension (DT) (g).

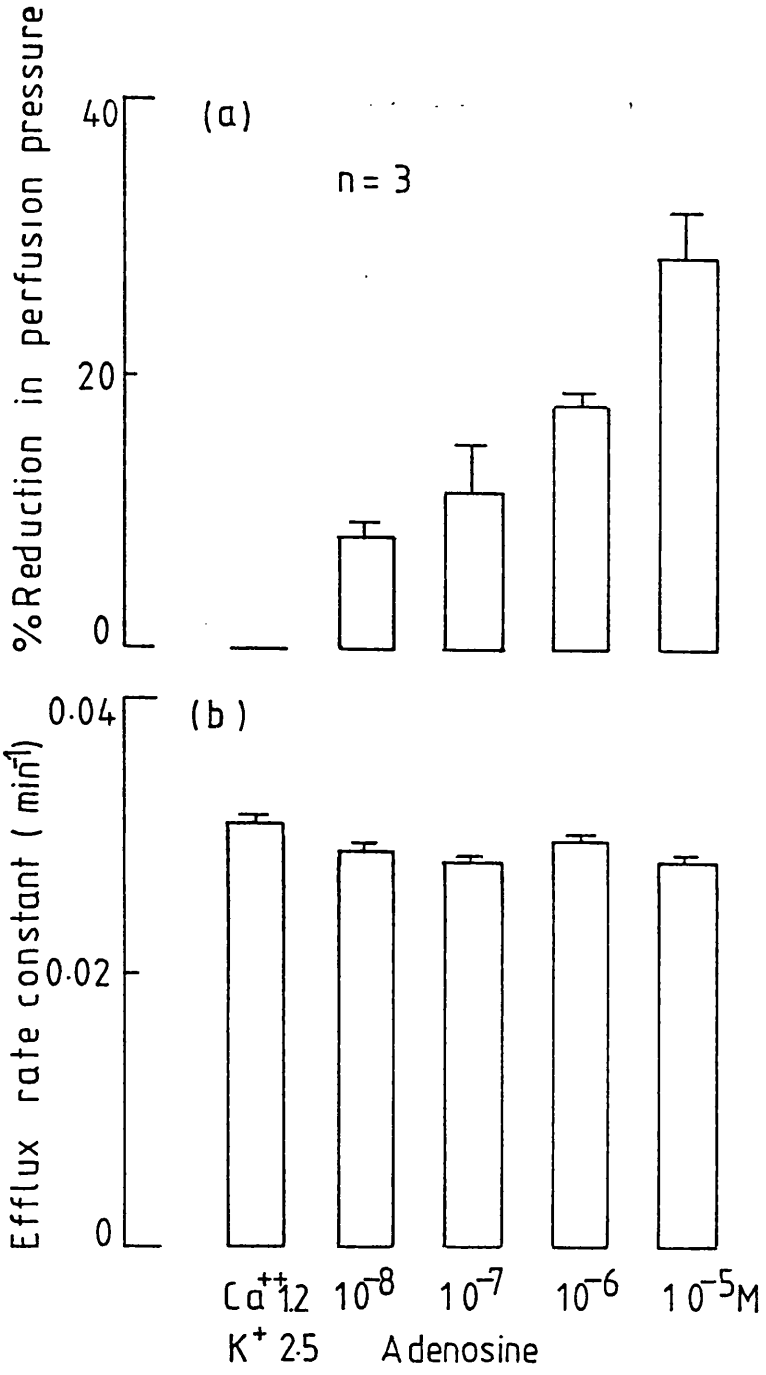


Figure 5.15. Effects of adenosine concentration on:

(a) % reduction in perfusion pressure (control perfusion pressure was 86 ± 9 mm Hg, n=3)

(b) $^{86}Rb^{+}$ efflux rate constant (min^{-1})

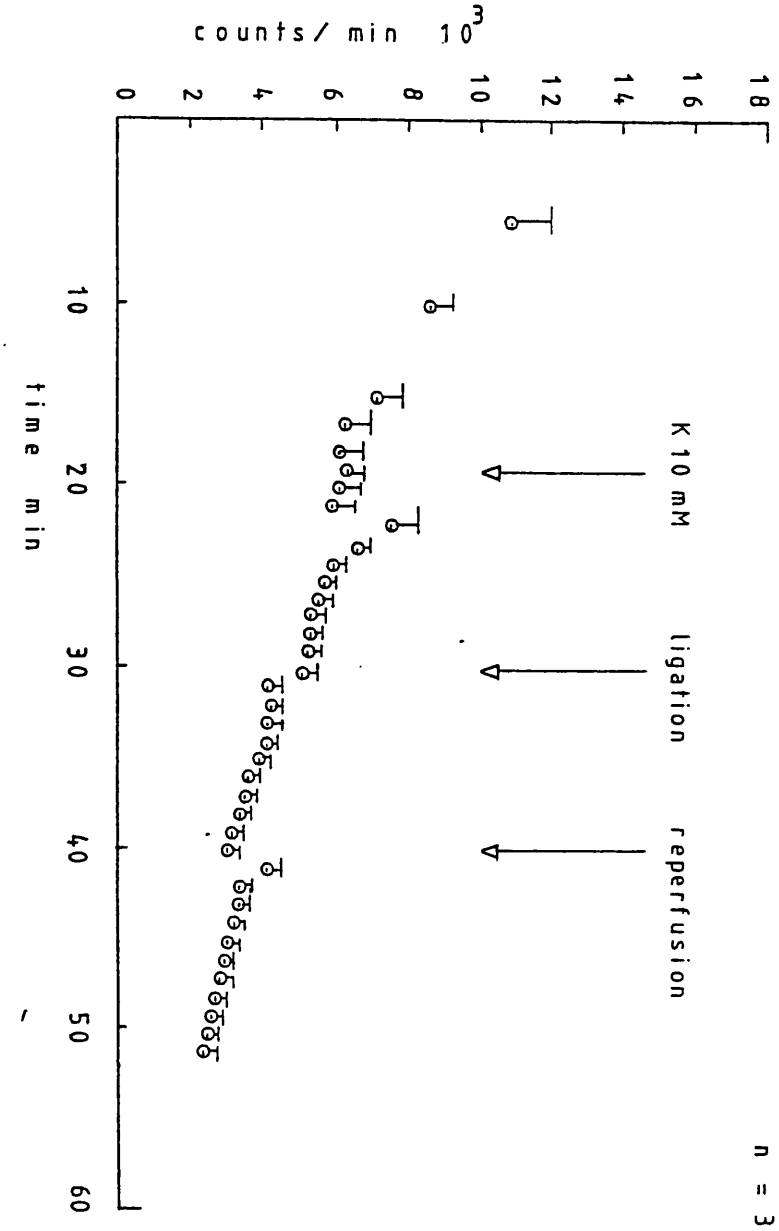


Figure 5.16. Effects of ligation for 10 minutes and reperfusion on $^{86}Rb^{+}$ counts/minute.

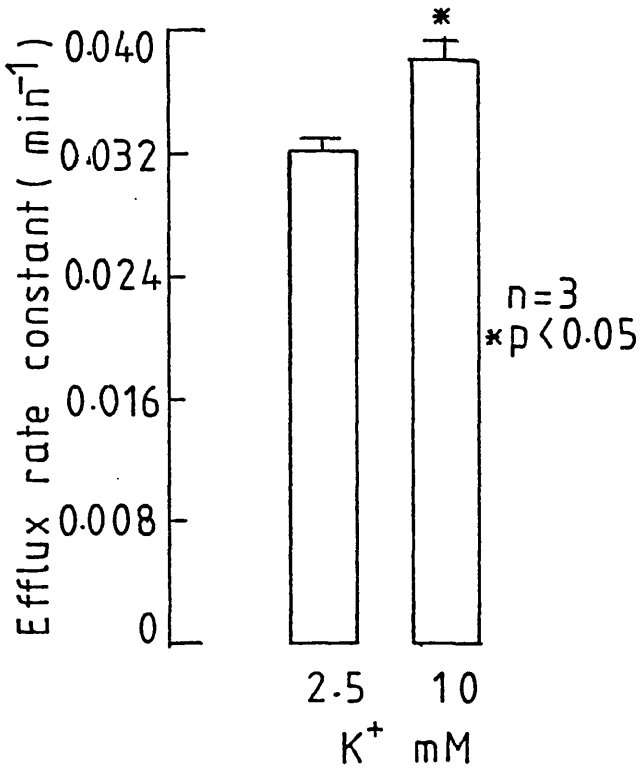


Figure 5.17. Effect of 10 mM potassium on $^{86}Rb^{+}$ efflux rate constant (min^{-1})

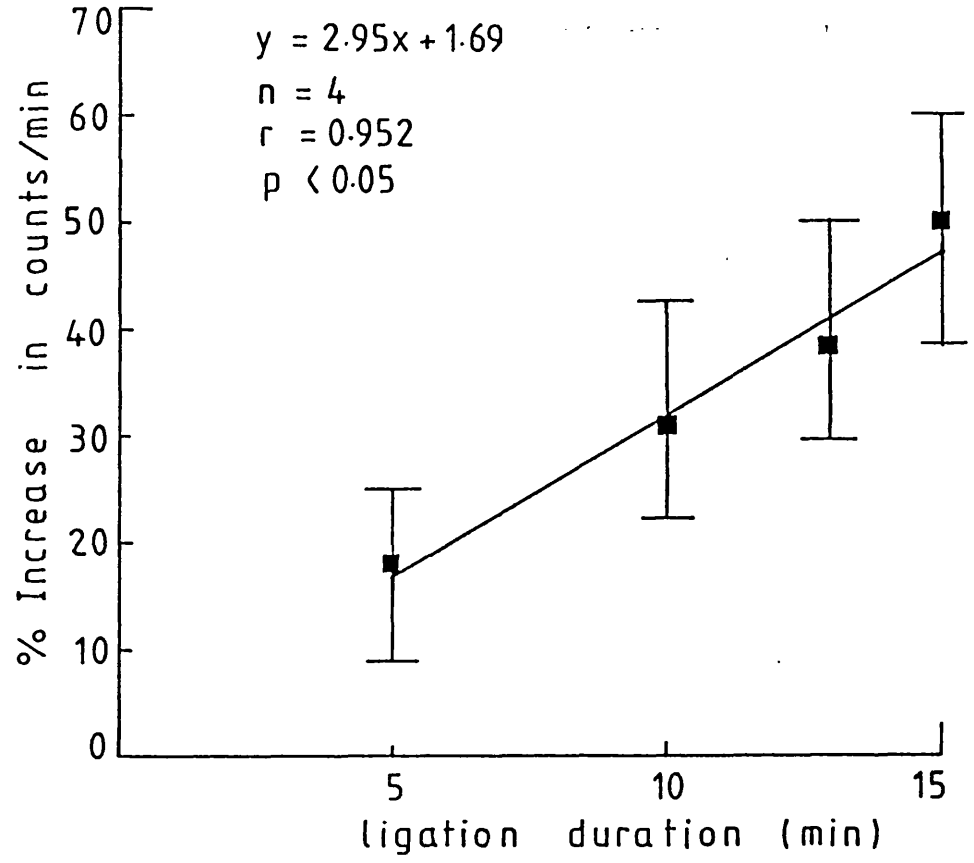


Figure 5.18. Relationship between ligation duration (minutes) and % increase in $^{86}Rb^{+}$ counts per minute. Potassium concentration was 10 mM. Each point is the mean of 3 hearts.

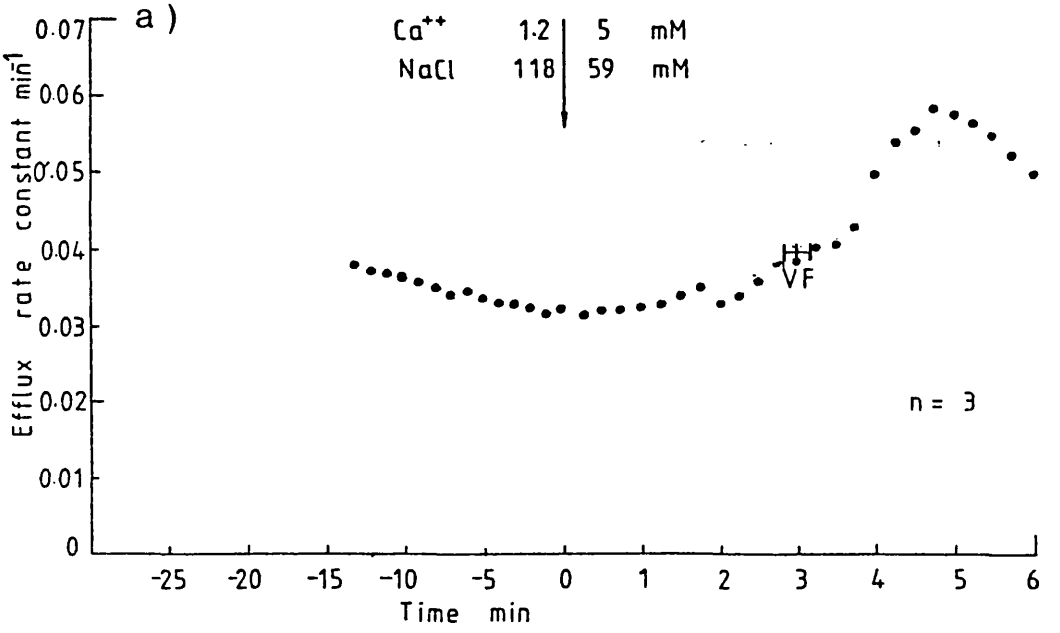
Figure 5.19. Time course of the effects of:

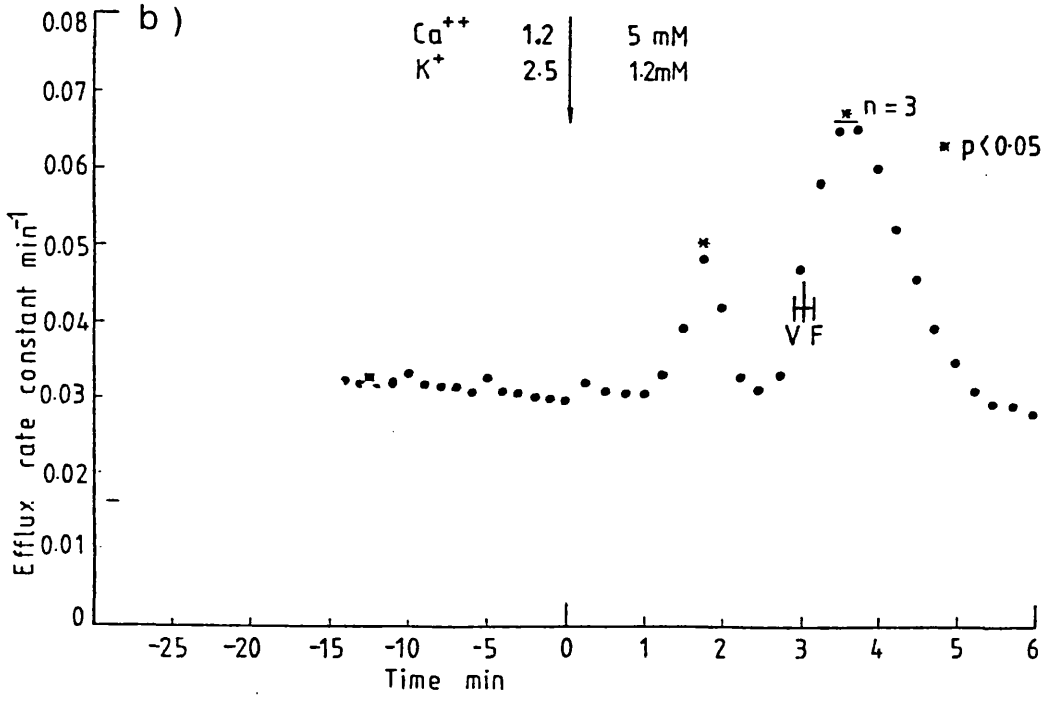
(a) Ca^{++} 5 mM, Na^{+} 59 mM

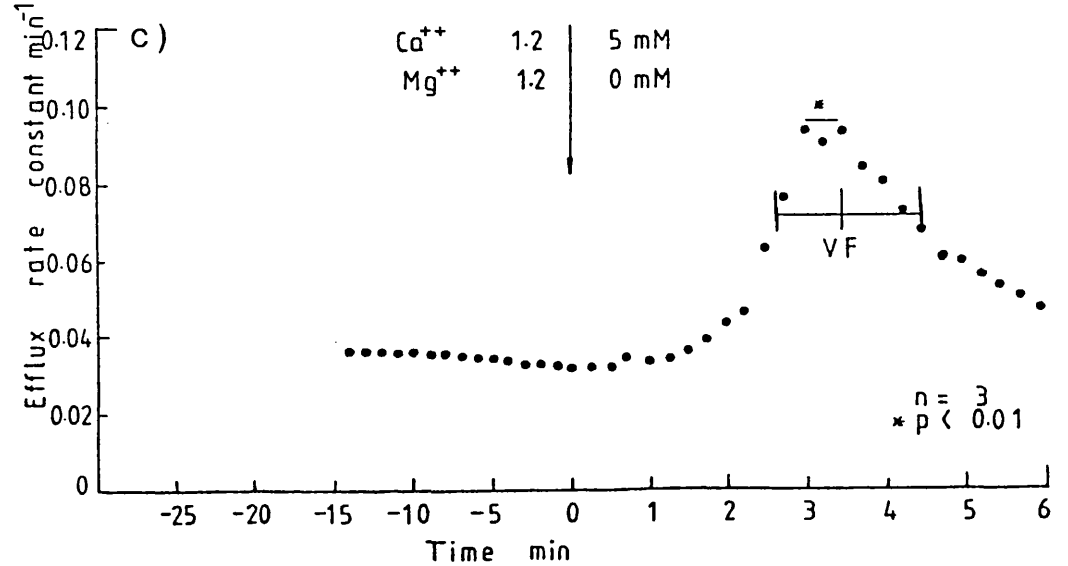
(b) Ca^{++} 5 mM, K^{+} 1.2 mM

(c) Ca^{++} 5 mM, Mg^{++} 0 mM

on $^{86}Rb^{+}$ efflux rate constant (min^{-1}).


a)
Ca++ 1·2 5 mM
NaCl 118 59 mM
VF
n = 3
Efflux rate constant min-1
Time min


b)
Ca++ 1·2 5 mM
K+ 2·5 1·2mM
n = 3
* p<0·05
VF
Efflux rate constant min-1
Time min


c)
Ca++ 1·2 5 mM
Mg++ 1·2 0 mM
VF
n = 3
* p < 0·01
Efflux rate constant min-1
Time min

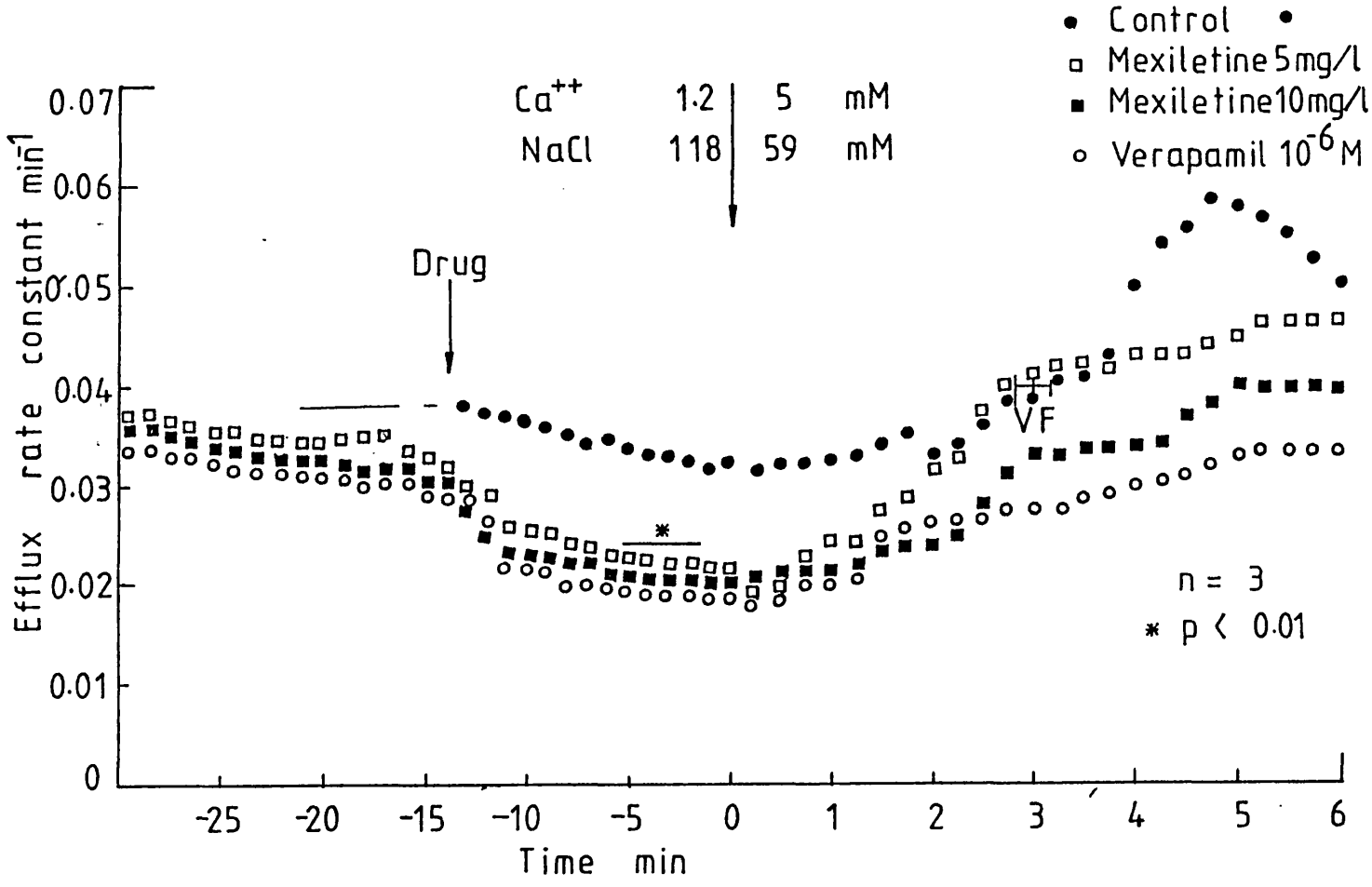


Figure 5.20. Time course of the effect of 5 mM calcium and 59 mM sodium chloride on $^{86}Rb^{+}$ efflux rate constant and the effect of mexiletine (5 and 10 mg/L) and verapamil 10^{-6}M on changes of efflux rate constant induced by this ionic milieu. Standard error of mean is omitted for the purpose of clarity. Points indicated by bars are compared.

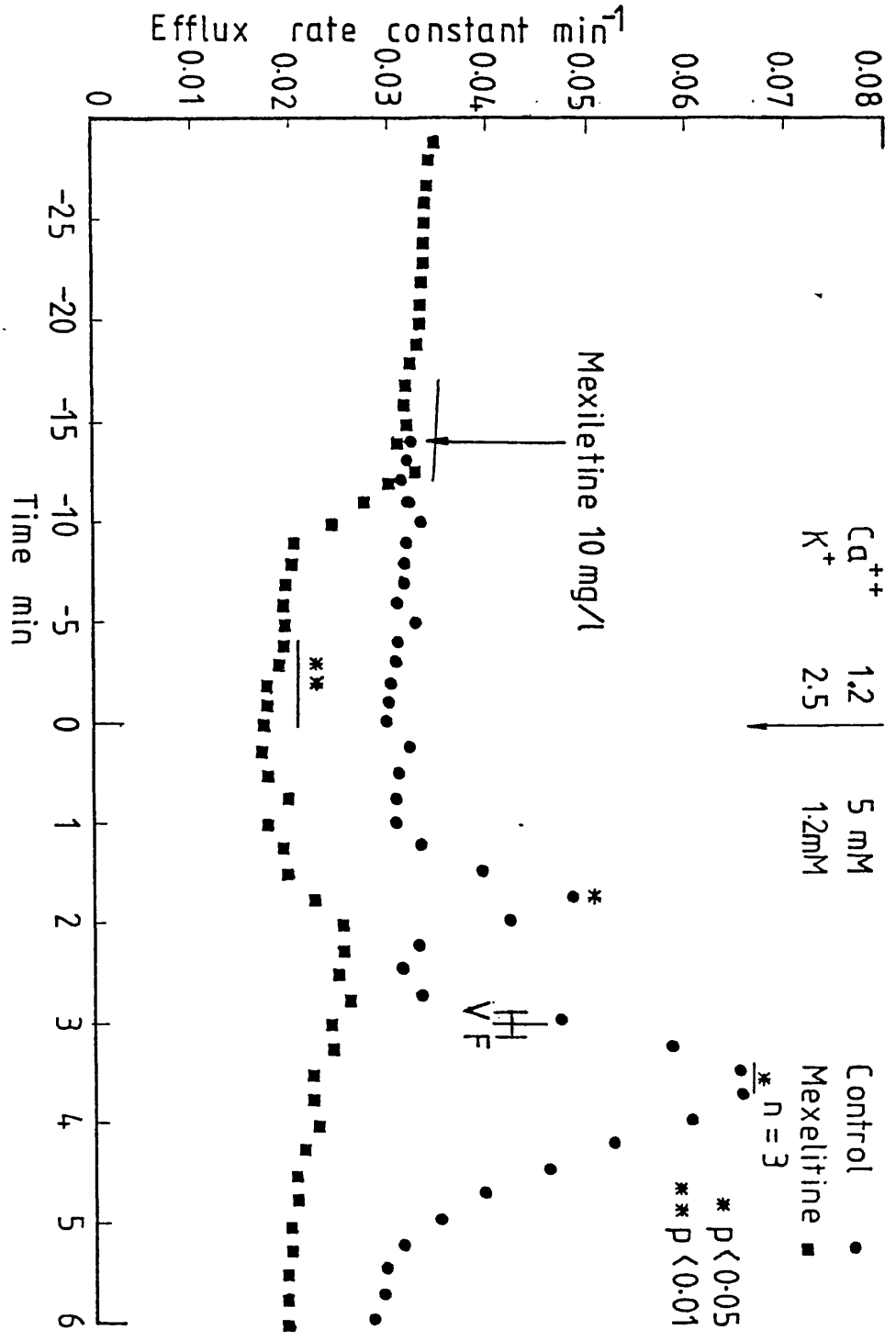


Figure 5.21. Time course of the effect of 5 mM calcium and 1.2 mM potassium on $^{86}Rb^{+}$ efflux rate constant and the effect of 10 mg/L mexiletine on changes in $^{86}Rb^{+}$ efflux rate constant induced by this ionic milieu. Standard error of means is omitted for the purpose of clarity. Points indicated by bars are compared.

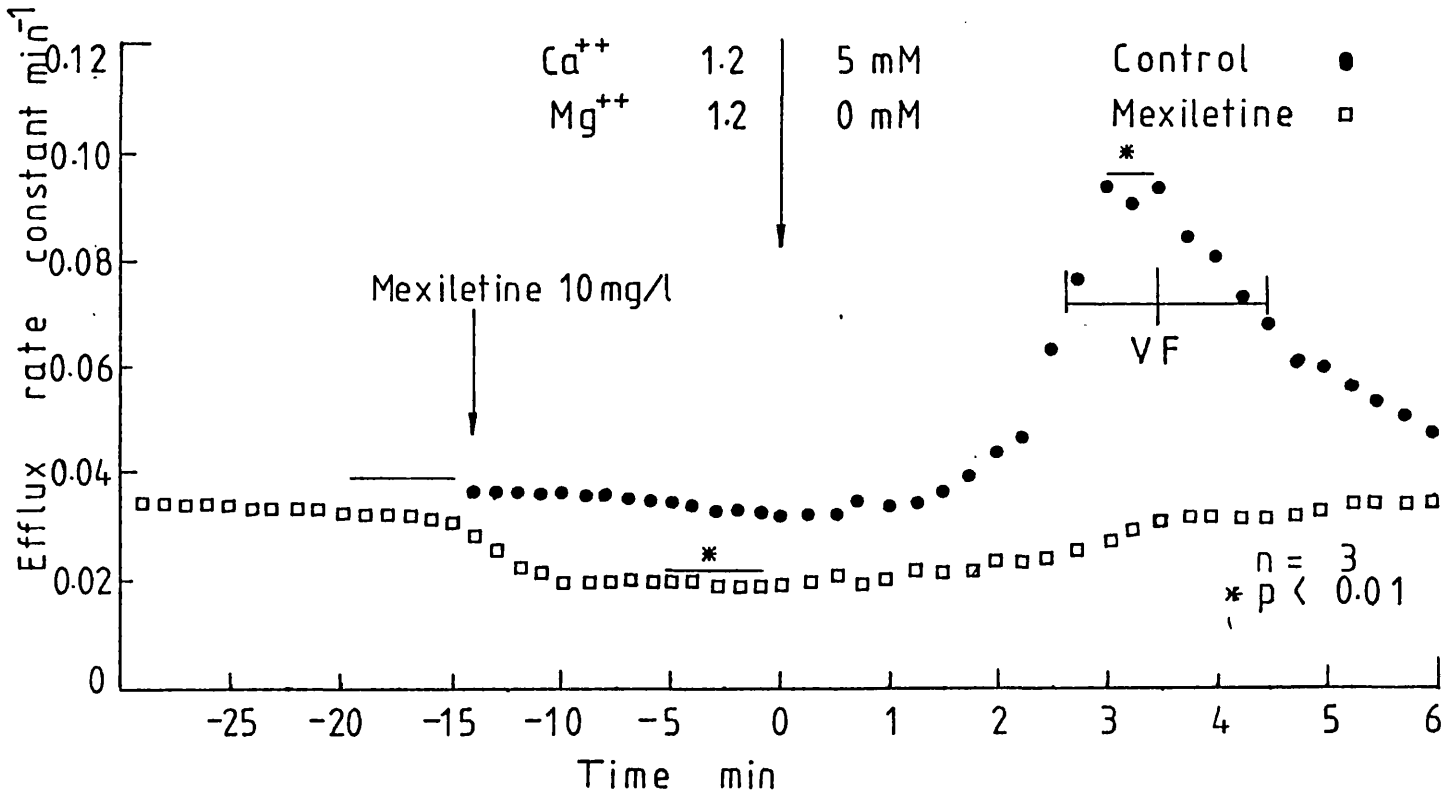


Figure 5.22. Time course of the effect of 5 mM calcium and 0 mM magnesium on $^{86}Rb^{+}$ efflux rate constant and the effect of 10 mg/L mexiletine on the changes in $^{86}Rb^{+}$ efflux rate constant induced by this ionic milieu. Standard error of mean is omitted for clarity. Points indicated by bars are compared.

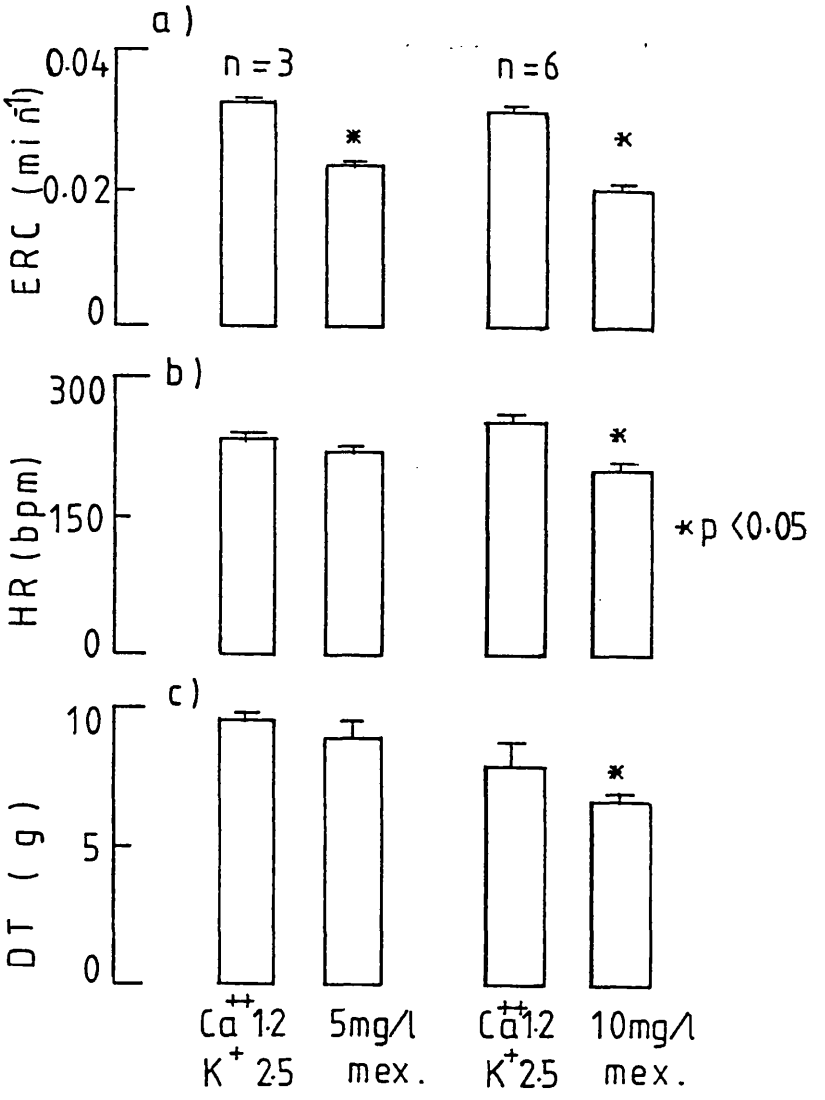


Figure 5.23. Effects of mexiletine (5 and 10 mg/litre) perfused for 15 minutes before altering ionic milieu on:

(a) $^{86}Rb^{+}$ efflux rate constant (min^{-1}) (ERC)

(b) Heart rate (b/m) (HR)

(c) Developed tension (g) (DT)

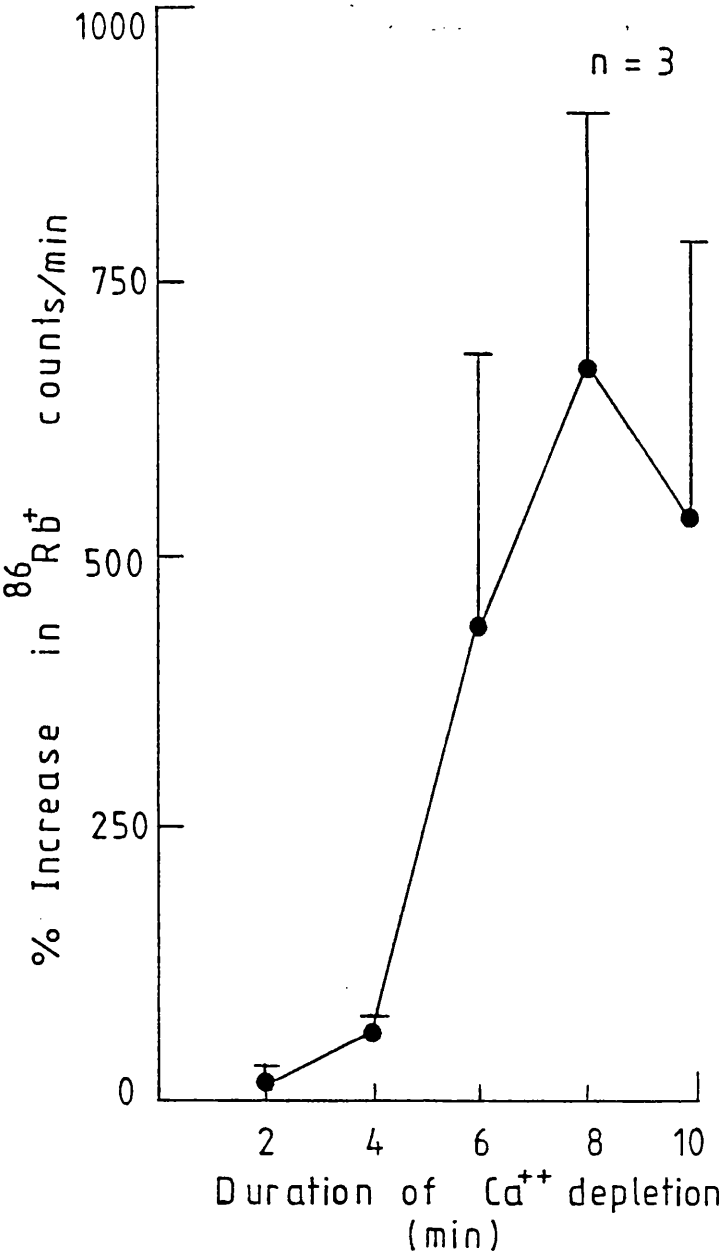


Figure 5.24. Effects of calcium free period on percentage increase in $^{86}Rb^{+}$ counts/min during calcium repletion. Control calcium was 1.2 mM.

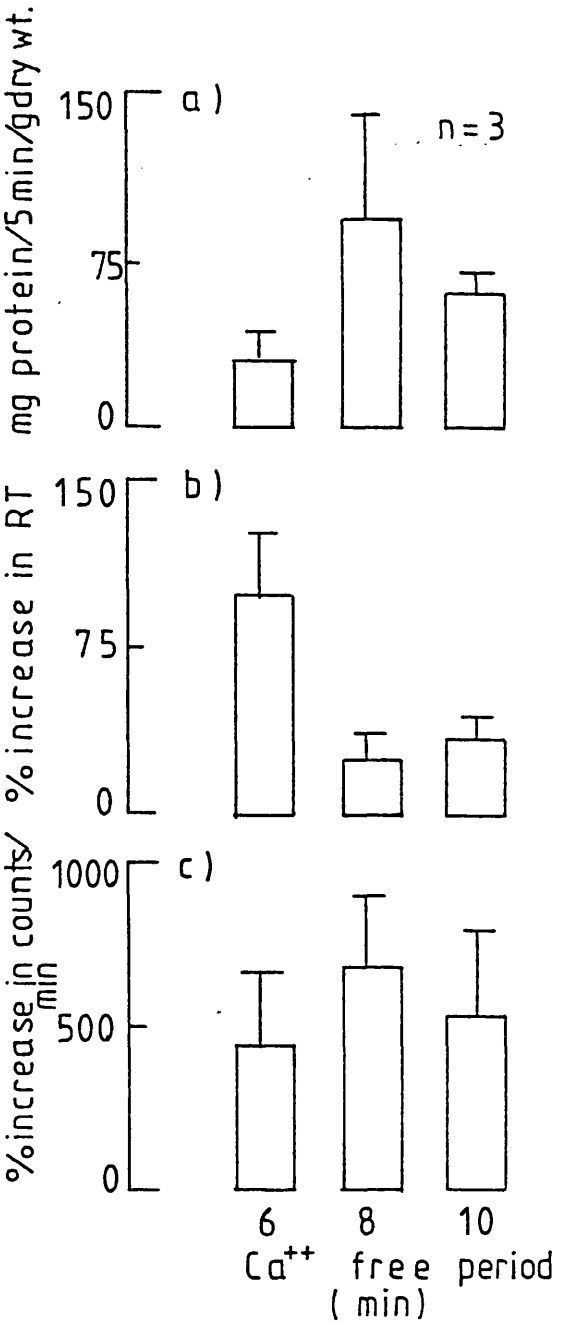


Figure 5.25. Effects of calcium repletion after 6, 8, and 10 minutes of calcium free periods on

(a) Protein leakage (mg/5 min/gram dry weight)

(b) Percentage increase in resting tension

(c) Percentage increase in $^{86}Rb^{+}$ counts/min

Note the dissociation of the index of contracture (% rise in resting tension) from the other two indices. Control calcium was 1.2. mM.

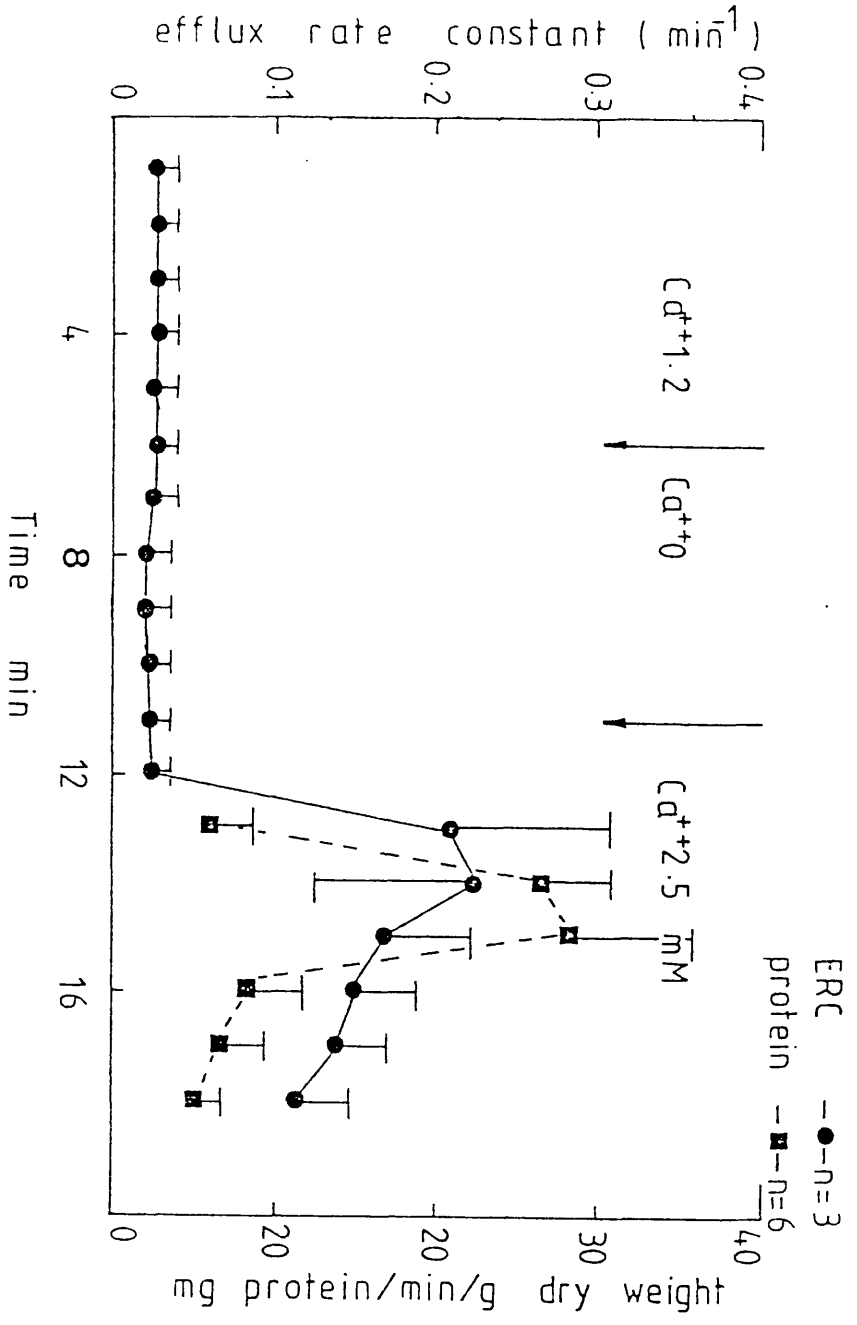


Figure 5.26. Time course of $^{86}Rb^{+}$ efflux rate constant during control, calcium free and calcium repletion period and time course of protein leakage(mg protein/g dry weight/min) during calcium repletion.

Figure 5.27. Effects of hypothermia (23°C) and low calcium perfusion (0.25 mM) both during 6 minutes of calcium depletion on:

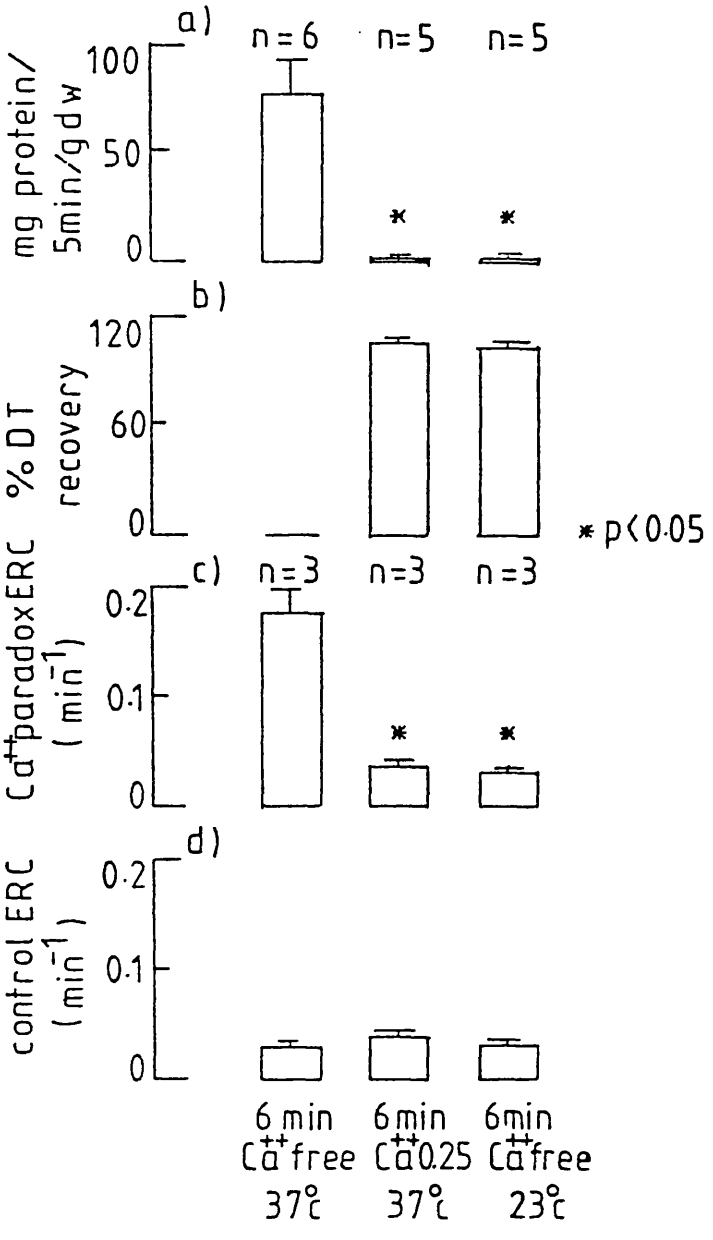
(a) Protein leakage (mg protein/5 min/g dry weight) during Ca^{++} repletion

(b) Percentage recovery in developed tension (DT) during Ca^{++} repletion

(c) $^{86}Rb^{+}$ efflux rate constant (min^{-1}) (ERC) during calcium repletion

(d) $^{86}Rb^{+}$ efflux rate constant (min^{-1}) (ERC) during control period.

Control calcium was 1.2. mM.


a)
n=6
n=5
n=5
mg protein/
5min/g d w
100
50
0
b)
% D T
recovery
120
60
0
* p<0.05
c)
n=3
n=3
n=3
Ca++paradoxERC
(min-1)
0.2
0.1
0
d)
control ERC
(min-1)
0.2
0.1
0
6 min
Ca++ free
37°C
6 min
Ca++ 0.25
37°C
6min
Ca++ free
23°C

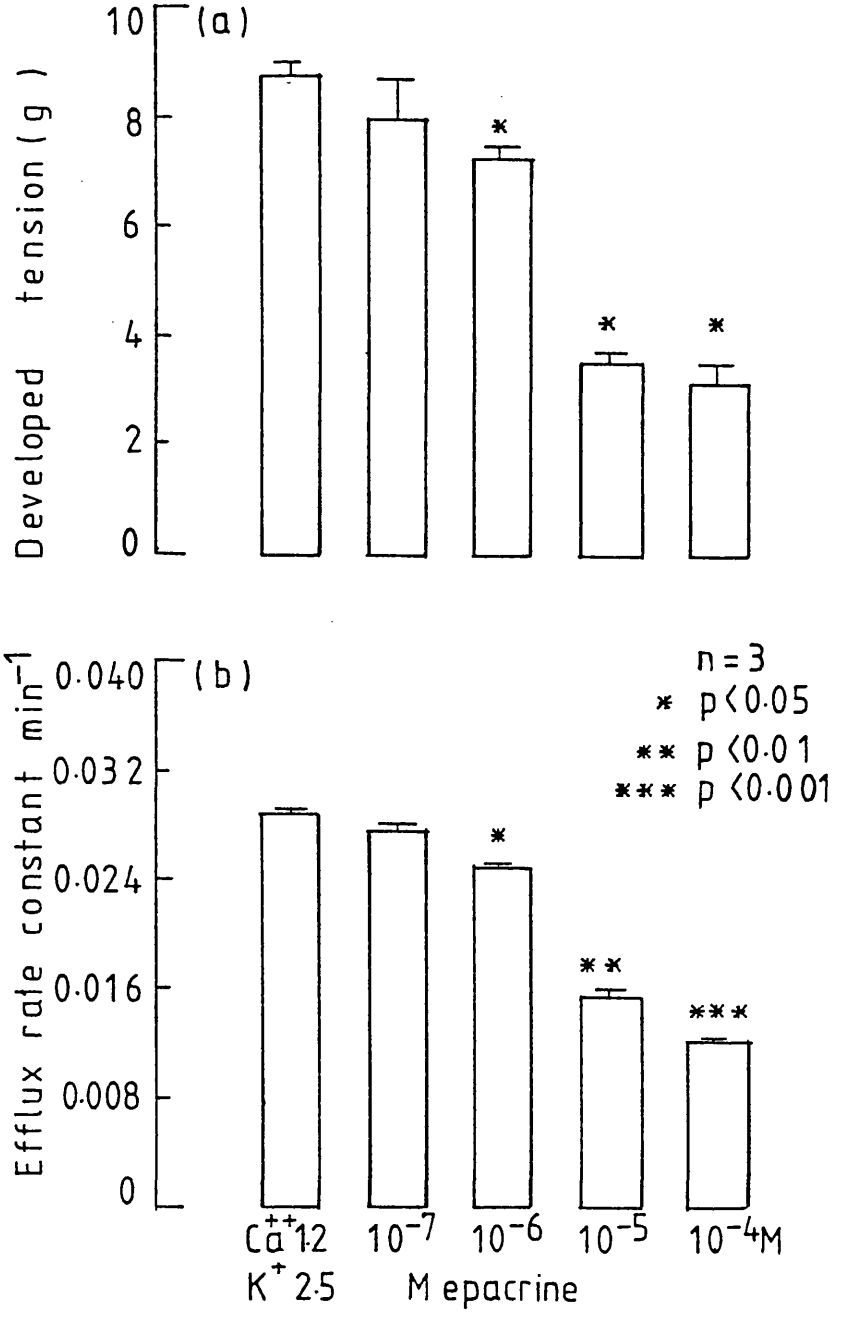


Figure 5.28. Effects of mepacrine on:

(a) Developed tension (grams)

(b) $^{86}Rb^{+}$ efflux rate constant (min^{-1})

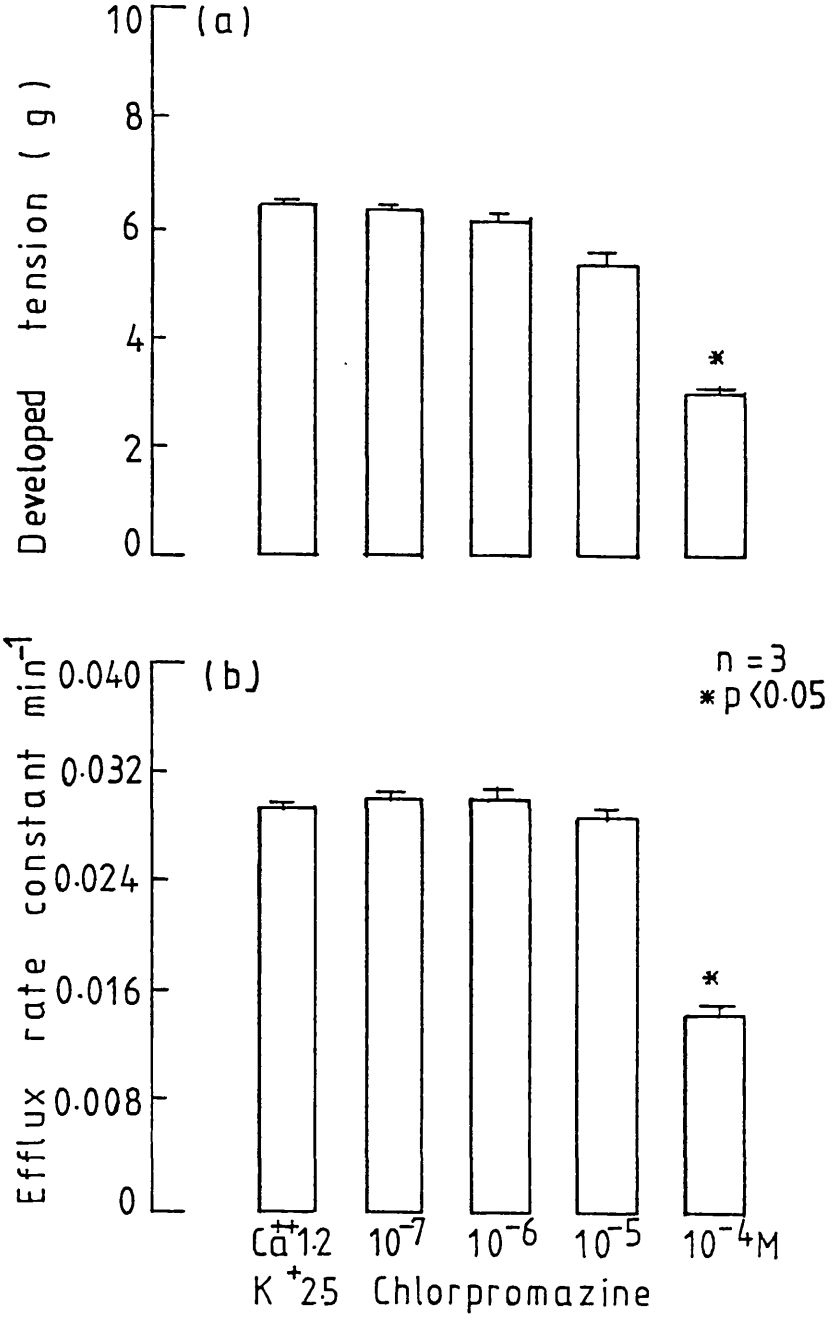


Figure 5.29. Effects of chlorpromazine on

(a) Developed tension (grams)

(b) $^{86}Rb^{+}$ efflux rate constant (min^{-1})

CHAPTER 5. DISCUSSION

5.a Use of $^{86}Rb^{+}$ as a tracer of potassium fluxes

Because $^{42}K^{+}$ has a short half-life (12.4 hours), $^{86}Rb^{+}$ whose half decay time is 18.7 days, has been used as a substitute as it can replace potassium electrophysiologically. However, $^{86}Rb^{+}$ does have a blocking effect on the potassium permeability of the cell membrane in skeletal muscle by inhibiting the Na^{+}/K^{+} pump (Adrian, 1964). $^{86}Rb^{+}$ has been used as a tracer of potassium ions for investigations in pancreatic islets cells (Henquin, 1977) and membrane transport systems such as the sodium potassium pump (Tomita and Yamamoto, 1971). $^{86}Rb^{+}$ and $^{42}K^{+}$ were shown to be lost from a single homogenous compartment and the rate coefficient of $^{86}Rb^{+}$ loss was not significantly different from that of $^{42}K^{+}$ in smooth muscle of canine trachea (Imaizumi and Watanabe, 1981).

The biological half life of $^{86}Rb^{+}$ in the present study in the isolated rat heart was found to be 21.2 $\pm$1 minutes, n = 30, which is far less than that reported in canine tracheal smooth muscle (140.8 $\pm$ 19.9 minutes, n=8) (Imaizumi and Watanabe, 1981). This difference is possibly due to the higher metabolic and mechanical activity of the rat heart.

5.b Effects of magnesium on $^{86}Rb^{+}$ efflux rate constant (e.r.c.)

The results in Figures 5.1 and 5.2 demonstrate that magnesium induces a concentration dependent reduction in e.r.c. This reduction in e.r.c. is accompanied by a decrease in developed tension, heart rate and perfusion pressure (Figures 5.3, 5.4, 5.5, 5.6, 5.7 and 5.8).

These results are in agreement with the findings of Shine and Douglas (1974), however, in the present study changes of e.r.c. are shown with lower concentrations of magnesium than those reported by Shine and Douglas.

The rapid effect of magnesium on e.r.c. suggests an effect at the sarcolemma membrane. Mangesium might inhibit a pathway for potassium exchange in the rat ventricle, however, an effect on haemodynamics is not excluded as magnesium reduced heart rate, developed tension and perfusion pressure.

5.c Reduction in heart rate as a possible mechanism for reduction of e.r.c.

The failure of magnesium to induce similar reduction in e.r.c. in hearts electrically paced at 300 bpm (Figure 5.9) indicates that the decrease in heart rate induced by magnesium contributes to some extent to its effect on $^{86}Rb^{+}$ efflux. The remaining component is possibly due to a direct magnesium effect and/or the negative inotropic effect of magnesium. However, electrical stimulation of hearts at different rates did not induce a significant increase in e.r.c. (Figure 5.10). One possible explanation for this discrepancy is that the rat heart shows a negative staircase effect with pacing and any increase in e.r.c. due to increase in heart rate could be masked by the decrease in e.r.c. induced by the negative inotropic effect of pacing, however, the possibility that activation of the Na^{+}/K^{+} pump by electrical pacing, which may compensate for the possible increase in e.r.c. (Kléber, A.G., 1983) could not be ruled out.

5.d Negative inotropic effect of magnesium as a possible mechanism of e.r.c. reduction

In order to investigate the contributary role of magnesium negative inotropism to its effect on e.r.c., developed tension was manipulated by perfusion with different concentrations of calcium and one concentration of verapamil.

The fact that increasing concentrations of calcium induced an increase in developed tension, and significant increases in e.r.c. (Figure 5.11) implies that changes in contractile force can affect the e.r.c. The finding that the concentration dependent increase in developed tension produced by calcium were not accompanied by a concentration dependent effects on e.r.c. is possibly due to the fact that the calcium concentration in these experiments was not added progressively (1.2, then 0.6 then 2.4 mM) and to the small change in e.r.c. The small change in e.r.c. induced by calcium demonstrates that the decrease in developed tension caused by magnesium could be responsible for a small component of the decrease in e.r.c.

These results are in contrast with those of Shine and Douglas (1979). In their experiment, 5 mM calcium reversed the depression of 20 mM magnesium on developed tension but did not affect $^{42}K^{+}$ exchange, however. Shine and Douglas:

1. Applied a different method of counting rate (effluent counts min^{-2}) which is probably not as accurate as the method used in the present study because it does not relate the effluent count to the tissue count.
2. May be their technique was not sensitive enough to measure the minute changes induced by calcium as they failed to detect changes in $^{42}K^{+}$ efflux with magnesium concentrations less than 5 mM.

These results suggest the presence of Ca-activated K^{+} channels in the heart, however, the evidence that such channels exist is equivocal. (Eisner and Vaughan-Jones, 1983). This is partly due to technical difficulties encountered in identifying an individual ionic current amongst the many currents that exist in the heart. In addition

an increase in intracellular calcium will produce an intracellular acidification which will shorten the action potential duration and increase the potassium outward current. Moreover, increasing intracellular calcium will cause a fall in ATP which in turn will activate the potassium current that is activated by ATP depletion. (Eisner and Vaughan-Jones, 1983; Akinori and Tohru, 1985).

These two currents could be confused with a Ca-activated K^+ current since many manoeuvres which increase intracellular calcium also decrease intracellular ATP and pH.

The negative inotropic effect of verapamil was accompanied by a reduction in e.r.c. (Figure 5.12), however, verapamil's negative inotropic action does not account for the whole of the e.r.c. decrease. A 27% reduction in developed tension produced by verapamil, elicited a 70% reduction in e.r.c. while a 72% reduction in developed tension produced by halving the calcium concentration elicited only an 11% reduction in e.r.c., which suggests an additional unidentified mechanism for verapamil's action.

5.f Effects of reduction of developed tension by reducing pH on e.r.c.

In order to simulate the negative inotropic effect induced by magnesium, developed tension was reduced by reducing pH.

The simultaneous reduction of developed tension, heart rate and e.r.c. induced by increased hydrogen ion concentration (Figure 5.13) lends further support for the correlation between these parameters. The negative inotropic effect of acidosis is accounted for by a reduction of calcium influx (Fry and Poole-Wilson, 1979), however,

an effect on calcium-troponin interaction or calcium release from the sarcoplasmic reticulum is not excluded (Tsien, R.W., 1976). Replacement of calcium bound to the sarcolemmal membrane by H^+ might explain the initial transient increase in developed tension.

Intracellular pH falls after the onset of ischaemia (Steenbergen *et al.*, 1977). This fall in pH is apparently protective against further possible ischaemic injury as the acidosis induced reductions in contractility reduces tissue demand for oxygen and hence preserves high energy phosphates (Steenbergen *et al.*, 1977). The preservation of high energy phosphates could explain the decrease in the potassium loss. (Akinori and Tohru, 1985).

5.f Effect of reducing heart rate by hypothermia on e.r.c.

In order to simulate reduction of heart rate induced by magnesium, heart rate was reduced by hypothermia. Reduction of heart rate to 42 ± 5 bpm by reducing temperature to 23°C was associated with a decrease in e.r.c. despite the increase in developed tension (Figure 5.14). In one ex periment (not shown here) a reduction of temperature to 29°C reduced the heart rate to 150 bpm and increased e.r.c. These results indicate that the effect of hypothermia on e.r.c. is a resultant of two opposing effects, these are the increase in developed tension and the decrease in heart rate. The results of hypothermia lend further support for a positive correlation between heart rate and developed tension on one hand and e.r.c. on the other hand.

5.g Effect of vasodilation induced by adenosine on e.r.c.

In order to investigate the contribution of vasodilation induced by magnesium to its effect on e.r.c., vasodilation was induced by

different concentrations of adenosine.

The failure of adenosine to affect e.r.c. despite the marked vasodilatation (Figure 5.15) indicates that vasodilatation does not play a major role in regulating e.r.c. The magnesium induced reduction in e.r.c. is probably not mediated by the vasodilator action of magnesium. The preserving effect of magnesium on intracellular potassium may account for protection against ischaemia induced cardiac necrosis in rats reported by Seelig, M.S. (1974).

5. h Effects of ligation and reperfusion on % increase in $^{86}Rb^{+}$ counts

The failure to detect an increase in $^{86}Rb^{+}$ count during coronary artery ligation (Figure 5.16) is similar to the findings of Abrahamsson *et al.*, 1983), who studied ^{3}H-noradrenaline. They failed to detect an increase of labelled noradrenaline upon coronary artery ligation in the perfused rat heart. This could be accounted for because the low collateral circulation of the rat heart did not allow wash out of the labelled material. However, upon reperfusion a marked increase in $^{86}Rb^{+}$ washout is seen (Figure 5.16). It is not clear whether this change is merely due to washout of $^{86}Rb^{+}$ during ischaemia, or to ischaemia and reperfusion damage, however, the linearity of the change in $^{86}Rb^{+}$ with time may suggest that this change is due only to washout of accumulated $^{86}Rb^{+}$ (Figure 5.18). Moreover, the fact that the effect is not persistent does not support a role for reperfusion damage initiating further $^{86}Rb^{+}$ efflux.

The present results of $^{86}Rb^{+}$ efflux during ischaemia are consistent with the findings of Harris *et al.* (1954), Weiss *et al.* (1982) and Hirche *et al.* (1980) who studied potassium efflux.

The net cellular potassium loss must result from an increase in potassium efflux or from a decrease in potassium influx brought about by reduced Na^+/K^+ pumping or from a combination of these two processes (Kléber, A.G., 1984).

The failure of ouabain (10^{-7} - 10^{-4} M) to induce an increase in $^{86}Rb^+$ washout could be attributed to the fact that rat hearts are relatively resistant to this drug. But rat heart Na^+/K^+ ATPase activity is half-maximally inhibited by ouabain in a concentration of 5.9×10^{-5}M) (Erdmann, 1980). This may suggest that the increase in $^{86}Rb^+$ efflux is not the result of a decrease in Na^+/K^+ pump activity during the ischaemic period.

5.i. Effects of potassium on $^{86}Rb^+$ e.r.c.

The increase in e.r.c. induced by high potassium, used in the previous experiment to prevent fibrillation, (Figure 5.17) is in agreement with Bartschat and Blaustein (1985). This potassium-stimulated $^{86}Rb^+$ efflux is presumably due to depolarizing cells.

5.j Effects of induction of arrhythmias by altering the ionic milieu on e.r.c.

The present experiments were performed to see if potassium loss precedes the development of arrhythmias.

Most models used for the study of antiarrhythmic drugs for the prevention of sudden cardiac death produce variable and non predictable arrhythmias. However, Karli (1958) showed that low potassium or sodium in the perfusate coupled with a high calcium concentration favoured the development of ventricular fibrillation. These manoeuvres are believed to inhibit Na^+/K^+ ATPase leading to an increase of

intracellular calcium via Na^{+}/Ca^{++} exchange. The clinical relevance of fibrillation in this model is questionable. However, antiarrhythmic drugs, effective in man were found to be effective in this model (Woodward, 1980).

The three models used to induce fibrillation (Figure 5.19) demonstrated an increase of $^{86}Rb^{+}$ e.r.c. before the onset of ventricular fibrillation although the time course is different for every model. The model of (Ca^{++} 5 mM, Mg^{++} 0) is not good due to the inconsistency of the incidence of ventricular fibrillation. The major increase in $^{86}Rb^{+}$ e.r.c. in these models which develop after the onset of ventricular fibrillation could be attributed to the contracture which normally accompanies the onset of fibrillation.

The increase in e.r.c. which preceded fibrillation in all 3 models could be ascribed to an increase of intracellular calcium. This is evident from the increase in resting tension which preceded the onset of ventricular fibrillation.

The increase of potassium permeability that preceded ventricular fibrillation is possibly the cause of ventricular fibrillation. This is in line with the findings of Harris *et al.* (1954). They induced ectopic activity and fibrillation by locally injecting potassium into a coronary artery of the dog.

The protective effect against the development of ventricular fibrillation in these models induced by mexiletine and verapamil (Figures 5.20, 5.21, 5.22) could be attributed to the ability of these drugs to reduce potassium loss. Although the arrhythmogenic solutions reversed the reduction in e.r.c. produced by these drugs, but the e.r.c. seems to stabilize at a lower elvel than that what was associated

with the development of ventricular fibrillation. However, it is not known whether these drugs delay or simply prevent the incidence of ventricular fibrillation for these drugs were studied only for 7 minutes after changing the ionic milieu.

The simultaneous reduction of heart rate, developed tension and e.r.c. induced by mexiletine (Figure 5.23 and Section 5.13) lends further support for the positive correlation of these parameters.

5.k Evaluation of $^{86}Rb^{+}$ efflux during calcium paradox

In the previous arrhythmogenic models increases in intracellular calcium would be expected and this could be the cause of arrhythmias. Reperfusion of calcium deprived hearts with normal media is a suitable model for investigating the effects of calcium overload (Yates and Dhalla, 1975). In agreement with Alto and Dhalla (1979) calcium repletion after a period of calcium deprivation induced potassium loss which was dependent on the period of calcium deprivation (Figure 5.24).

In the present study mechanical activity was not lost completely when calcium was deprived for periods less than 6 minutes, however, readmission of calcium was accompanied by reduction of contractile recovery and $^{86}Rb^{+}$ loss. Calcium depletion for a longer period produced on calcium readmission, loss of mechanical activity, $^{86}Rb^{+}$ and protein loss (Figures 5.25, 5.26). This protein leakage may mean that the loss in $^{86}Rb^{+}$ is not due to specific calcium effect on $^{86}Rb^{+}$ but to a more generalised cell damage. The loss of protein during calcium repletion is in agreement with the findings of Hearse *et al.* (1980).

The present results have shown a dissociation between some of the indices of the calcium paradox (Figure 5.25). The magnitude of contracture does not parallel $^{86}Rb^{+}$ efflux and protein loss. Such a dissociation of calcium paradox indices has been shown by Baker *et al.* (1984) between calcium delivery, enzyme leakage and cell damage.

In many experimental studies only one or two indices are used, the assumption being made is that these are sufficient to define the extent of cell damage. This assumption may be dangerous as these indices are not necessarily parallel.

Protection against the calcium paradox induced damage by hypothermia and a low calcium concentration perfusion during the calcium deprivation period (Figure 5.27) is in agreement with the findings of Alto and Dhalla (1977). They have shown that calcium concentrations higher than 0.05 mM during the calcium deprivation period protect hearts against the damage caused by calcium readmission. This protective effect of low calcium could be explained because above a minimal calcium concentration, no damage of calcium fucose bridges occurs (Frank *et al.*, 1977). The protective effect of hypothermia applied during the calcium depletion period is possibly due to the fact that the more crystalline structure of the cell membrane at low temperatures allows the membrane to bind calcium more firmly and hence protect the membrane structure (Grinwald and Nayler, 1981).

5.1 Effects of mepacrine and chlorpromazine on e.r.c.

The concentration dependent reduction on e.r.c. induced by mepacrine and the failure of chlorpromazine to induce a similar effect (Figures 5.28, 5.29) is consistent with the effects of these drugs on ligation

induced arrhythmias (4.2c). The antiarrhythmic action of mepacrine could possibly be attributed to its effect on potassium movement. The simultaneous reduction of developed tension produced by mepacrine lends further support for the correlation between e.r.c. and developed tension. Quinine, a structural analogue of mepacrine has been shown to block Ca-activated potassium channels in many tissues (Bartschat and Blaustein, 1985), however, further investigation is required to elucidate the mechanism of action of mepacrine.

6.0 FINAL DISCUSSION

6.1 Contribution of haemodynamic effects to the antiarrhythmic effects of drugs and ions on ligation induced arrhythmias in the isolated rat heart

A decrease in perfusion pressure, which is a measure of vasodilation, is thought to protect hearts against ischaemic damage by increasing flow through collateral vessels to the ischaemic zone. An increase of flow will salvage the ischaemic tissue by providing substrates and removing noxious metabolites. However, in the present study there seems no correlation between the antiarrhythmic effect of the drug and its vascular effect (Table I). While potassium reduced both parameters, ZK 36374 reduced perfusion pressure with no effect on arrhythmias. On the other hand, BW 755C, nafazatrom and mepacrine, which did not affect perfusion pressure, significantly reduced arrhythmias. This dissociation of the two parameters could be attributed to the low collateral circulation of the rat heart (Schaper, 1984). If anything, a coronary dilator might be expected to exacerbate damage by causing coronary steal.

Similarly, no correlation exists between a decrease of developed tension and ligation induced arrhythmias in the isolated rat heart. A decrease of developed tension would be expected to protect the ischaemic tissue by reducing oxygen demand, preserving high energy phosphates, and reducing calcium influx. Potassium and nafazatrom exerted their antiarrhythmic effect without affecting the developed tension while mepacrine and BW 755C reduced both parameters. On the other hand, ZK 36374 and verapamil reduced developed tension with no significant effect on arrhythmias. Potassium, BW 755C, nafazatrom and mepacrine exerted their antiarrhythmic effect without affecting the heart rate, however, magnesium and diltiazem did not induce any antiarrhythmic effect unless they reduced the heart rate.

Table 1. Summary of the effects of some of the drugs and ions used in the present study on ligation induced arrhythmias and haemodynamic properties of the hearts; ↑ and ↓ denote increase and decrease respectively whereas - denotes no effect.

Drug or ion	Perfusion pressure	Developed tension	Heart rate	Arrhythmias
Potassium	↓	-	-	↓
Magnesium	↓	↓	↓	↓
ZK 36374	↓	↓	-	-
Verapamil	↓	↓	-	-
Diltiazem	↓	↓	↓	↓
Aspirin	-	-	-	-
Indomethacin	-	-	-	-
Dazoxiben	-	-	-	-
BW 755C	-	↓	-	↓
Nafazatrom	-	-	-	↓
Mepacrine	-	↓	-	↓

In conclusion, vasodilation and reduced developed tension do not have an important contribution to antiarrhythmic mechanism but they serve as adjuvant to the mechanisms of certain drugs. However, a decrease in heart rate does seem to confer some antiarrhythmic effect. This was seen with magnesium and diltiazem, the beneficial effect of which was prevented by electrical pacing.

6.2 Potassium efflux as a factor in the development of arrhythmias

Potassium loss has been implicated in the gensis of arrhythmias (for citation of references, see Introduction section). Hence a drug that reduces potassium loss would be expected to be a potential anti-arrhythmic agent. In the present study the antiarrhythmic effects of mepacrine and mexiletine have been attributed to their potassium sparing effect (Table 2). However, potassium which is a potential antiarrhythmic ion, increased potassium efflux but potassium would itself reduce heterogeneity between the ischaemic and non-ischaemic area.

Table 2. Summary of the effects of certain drugs and ions on arrhythmias and $^{86}Rb^{+}$ efflux rate constant.

Drug or ion	$^{86}Rb^{+}$ e.r.c.	Arrhythmias
Mepacrine	↓	↓
Mexiletine	↓	↓
Chlorpromazine	-	-
Potassium	↑	↓

Table 3. Comparison of the effects of magnesium and mepacrine on $^{86}Rb^{+}$ e.r.c. and ligation induced arrhythmias.

	% reduction in $^{86}Rb^{+}$ e.r.c.	Effect on arrhythmias
Magnesium		
2.4 mM	14	-
4.8 mM	31	↓
Mepacrine		
10^{-7}M	3	↓
10^{-6}M	13	↓↓

However, this association between potassium preservation and antiarrhythmic effect of a drug is met by a reservation. A slight effect on $^{86}Rb^{+}$ e.r.c. was associated with antiarrhythmic effect in the case of mepacrine whereas the same effect on $^{86}Rb^{+}$ e.r.c. was not associated with antiarrhythmic effect in the case of magnesium (Table 3). This can only be explained as mepacrine possessed another antiarrhythmic mechanism and that potassium preservation served as an adjuvant to that mechanism.

6.3 Conclusions

The isolated rat heart is a simple preparation and useful for screening of antiarrhythmic drugs. In this preparation (1) Potassium possesses a striking antiarrhythmic effect in the isolated coronary ligated rat heart. The mechanism underlying this effect requires further investigation.

2. Calcium has detrimental effects on ligation induced arrhythmias, however, calcium antagonists failed to protect against this type of arrhythmia .

3. The results in the present thesis do not confirm an association between magnesium deficiency and arrhythmias.

4. Prostaglandins do not seem to be directly implicated in ligation induced arrhythmias in this model.

5. Perfusion of hearts with lysophosphatidylcholine has a detrimental effect on arrhrythmias whereas free radical scavengers have no effect.

6. Magnesium has a striking effect on $^{86}Rb^{+}$ efflux which seems to

be associated with heart rate and developed tension.

7. Ventricular fibrillation induced by ionic manipulation is preceded by an increase in $^{86}Rb^{+}$ efflux.

8. Readmission of calcium to hearts after deprivation is accompanied by an increase in $^{86}Rb^{+}$ efflux which seems to be due to generalized cellular damage. This is evidenced by protein leakage.

6.4 Suggestions for future work

1. Evaluation of effects of potassium on regional flow and myocardial function in the non-ischaemic zone, marginal zone and ischaemic zone.

2. Comparison of the effects of mepacrine and phenothiazines on the electrophysiology of the heart and phospholipase activity.

3. Evaluation of the effects of BW 755C and nafazatrom on the electrophysiology of the heart and lipoxygenase activity.

4. Examination of the effect of lysophosphatidylcholine on $^{86}Rb^{+}$ efflux.

5. Evaluation of the effects of varying pH and temperature on $^{86}Rb^{+}$ efflux in paced hearts.

6. Comparison of the effects of metabolic and respiratory acidosis on $^{86}Rb^{+}$ efflux.

7. Use of rise in $^{86}Rb^{+}$ efflux caused by anoxia and reoxygenation for investigation of the effects of calcium antagonists and free radical scavengers.

8. Use of $^{86}Rb^{+}$ efflux increase as a calcium paradox index for investigation of the route of calcium influx during calcium readmission and the effect of calcium antagonists.

9. Bartschat and Blaustein (1985) employed a sensitive method for evaluation of $^{86}Rb^{+}$ efflux which could measure changes in $^{86}Rb^{+}$ efflux in every second. This method could be employed to give more accurate results for investigations of the effect of arrhythmias on $^{86}Rb^{+}$ efflux.

REFERENCES

Abrahamsson, T. and ALmgren, O., 1981, Ventricular fibrillation following coronary artery ligation in the rat. In: The rat electrocardiogram in pharmacology, eds. Budden,R., Detweiler, D.K.and Zbinden, G. Pergamon Press, pp. 239-242.

Abrahamsson, T., ALmgren, O. and Carlsson,L., 1983. Ishaemia-induced noradrenaline release in the isolated rat heart: Influence of perfusion substrate and duration of ischaemia. J. Mol. Cell Cariology 15, 821-830.

Acosta, D., Combs, A..B. and Ramos, K., 1984. Attenuation by anti-oxidants of Na^+/K^+ ATPase inhibition by toxic concentrations of isoproterenol in cultured rat myocardial cells. J. Mol. Cell. Cardiol. 16, 281-284.

Adrian, R.H., 1964. The rubidium and potassium permeability of frog muscle membrane. J. Physiol. 175, 134-159.

Ahumada, G.G., Bergmann, S.R., Carlson, E., Corr, P.B. and Sobel, B.E. 1979. Augmentation of cyclic AMP content induced by lysophosphatidyl-choline in rabbit hearts. Cardiov. Res. 13, 377-382.

Akinori, N. and Tohru, S., 1985. Membrane current through adenosine triphosphate-regulated potassium channels in guinea-pig ventricular cells. J. Physiol. 363, 463-480.

Aloia, R.C., Pengelley, E.T., Bolen, J.L. and Rouser, G., 1979. Changes in phospholipid composition in hibernating ground squirrel, *Citellis lateralis* and their relationships to membrane function at reduced temperatures. Lipids Vol. 9, No. 12, 993-999.

Alto, L.E. and Dhalla, N.S., 1979. Myocardial cation contents during induction of calcium paradox. Am. J. Physiol. 237(6): H713-719.

Altura, B.M., Altura, B.T., Carella, A. and Turlapaty, P.D.M.V., 1981. Hypomagnesmia and vasoconstriction: possible relationships to etiology of sudden death in ischaemic heart disease and hypertensive disease. Artery 9(3), 212-231.

Anderson, T.W.,Leriche, W.H. and Mackay, J.S., 1975. Sudden death and ischaemic heart disease. New Eng. Med. J. 280-805.

Armstrong, A., Duncan, B., Oliver, M.F., Julian, D.G., Donald, K.W., Fulton, M., Lutz, W. and Morrison, S.L., 1972. Natural history of acute coronary heart attacks. A community study. Br. Heart J., 34, 67-80.

Bagdonas, A.A. Stuckey, J.H., Piera, J., Amer. N.S. and Hoffman, B.F., 1961. Effects of ischemia and hypoxia on the specialized conducting systems of the canine heart. Am. Heart J., 61, 206-218.

Baker, J.E., Kemmenoe, B.H., Hearse, D.J. and Bullock, G.R., 1984. Calcium delivery and time: factors affecting the progression of cellular damage during the calcium paradox in the rat heart. Cardiovas. Research 18, 361-370.

Balasubramanian, V., McNamara, D.B. and Singh, J.N. and Dhalla, N.S., 1973. Biochemical basis of heart function - X reduction in the Na^+/K^+ stimulated ATPase activity in failing rat heart due to hypoxia. Can. J. Phys. Pharmacology 51, 504-510.

Bartschat, D.K. and Blaustein, M.P., 1985. Calcium-activated potassium channels in isolated presynaptic nerve terminals from rat brain. J. Physiol. 361, 441-457.

Bayer, R., Kalusche, D., Kaufmann, R.and Mannhold, R., 1975. Inotropic and electrophysiological actions of verapamil and D600 in mammalian myocardium. Arch. Pharmacol. 290, 81-97.

Bergey, J.L. and Beil, M.E., 1983. Antiarrhythmic evaluation of naloxone against acute coronary occlusion-induced arrhythmias in pigs. European J. Pharmacol. 90, 427-431.

Blackwell, G.J. and Flower, R.J., 1983. Inhibition of phospholipase. Br. Med. Bull 39(3), 260-264.

Bolton, T.B. and Clark, J.P., 1981. Effects of histamine, high potassium and carbachol on $^{42}K^{+}$efflux from longitudinal muscle of guinea-pig intestine. J. Physiol. 320, 347-361.

Bors, W., Michel, C., Saran, M., Lengfelder, E., 1978. The involvement of oxygen radicals during the autoxidation of adrenaline. Biochem. Biophys. Acta 540(1), 162-172.

Bowman, W.C. and Rand, M.J., 1982.Textbook of Pharmacology, Cambridge.

Chien, K.R., Reeves, J.P., Buja,L.M.,Bonte, F., Parkey, R.W. and Willerson, J.T., 1981. Phospholipid alterations in canine ischemic myocardium. Temporal and topographical correlations with Tc-99m-PPi accumulation and an *in vitro* sarcolemmal calcium permeability defect. Circ. Res. 48: 711-719.

Christ, E.J. and van Dorp, D.A., 1972. Comparative aspects of prostaglandin biosynthesis in animal tissues. Advances in the bioscience Vol. 9, Ed. Raspe, O., New York, Pergamon Press, pp. 35-38.

Clark, C., Foreman, M.I., Kane, K.A., McDonald, F.M. and Parratt, J.R. 1980. Coronary artery ligation in anaesthetized rats as a method for the production of experimental dysrhythmias and for the determination of infarct size. J. Pharmac. Metho. 3, 357-368.

Clusin, W.T., Bristow, M.R., Bain, D.S., Shroeder, J.S., Jaillon, P. and Harrison, D.C., 1982a. The effects of diltiazem and reduced serum ionized calcium on ischaemic ventricular fibrillation in the dog. Circ. Res. 50: 518-526.

Clusin, W.T., Bristow, M.R., Karagueuzian, H.S., Katzung, B.G. and Schroeder, J.S., 1982. Do calcium-dependent ionic currents mediate ischaemic ventricular fibrillation? The Amer. J. Cardiol. 49, 606-612.

Coburn, R.F., Grubb, W. and Aronson, K., 1976. Effect of cyanide on oxygen sensitive tension in rabbit aorta (Abstract) Federation Proc. 35, 698-

Coker, S.J., Parratt, J.R., Ledingham, I.McA. and Zeitlin, I.J., 1981. Thromboxane and prostacyclin release from ischaemic myocardium in relation to arrhythmias. Nature 291, 323-324.

Coker, S.J. and Parratt, J.R., Ledingham, I.McA. and Zeitlin, I.J., 1982. Evidence that thromboxane contributes to ventricular fibrillation induced by reperfusion of the ischaemic myocardium. J. Molec. Cell. Cardiol. 14, 483-485.

Coker, S.J., 1982. Early ventricular arrhythmias arising from acute myocardial ischaemia: Possible involvement of prostaglandins and thromboxanes. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press, London pp. 217-237.

Coker, S.J. and Parratt, J.R., 1983. Prostacyclin-antiarrhythmic or arrhythmogenic? Comparison of the effects of intravenous and intracoronary prostacyclin and ZK36374 during coronary artery occlusion and reperfusion in anaesthetized grey hounds. J. Cardiovas. Pharmacol. 5(4), 557-567.

Coker, S.J. and Parratt, J.R., 1983[a] Nifedipine reduces arrhythmias but does not alter prostanoid release during coronary artery occlusion and reperfusion in anaesthetized grey hounds. J. Cardiovas. Pharmacol. 5, 406-417.

Coker, S.J., 1984. Further evidence that thromboxane exacerbates arrhythmias. Effects of UK38485 during coronary artery occlusion and reperfusion in anaesthetized greyhounds. J. Mol. Cell. Cardiol. 16, 633-641.

Coker, S.J. and Parratt, J.R., 1984. The effects of nafazatrom on arrhythmias and prostanoid release during coronary artery occlusion and reperfusion in anaesthetized greyhounds. Mol. Cell. Cardiol. 16, 43-52.

Corr, P.B., Cain, M.E., Witkowski, F.X., Price, D.A. and Sobel, B.E., 1979. Potential arrhythmogenic electrophysiological derangements in canine Purkinje fibres induced by lysophosphoglycerides. Circ.

Res. 44, 822-832.

Corr, P.B. and Sobel, B.E., 1980. The role of biochemical factors in ventricular dysrhythmia accompanying ischaemia. In: Advances in Cardiology 27, 346-360. Ed. Vogel, J.H.K., Karger, Basel.

Corr, P.B., Snyder, D.W., Cain, M.E., William, A., Crafford, J.R., Gross, R.W. and Sobel, B.E., 1981. Electrophysiological effects of amphiphiles on canine Purkinje fibres. Circ. Res. 49, 354-363.

Corr, P.B. and Sobel, B.E., 1982. Amphiphilic lipid metabolism and ventricular arrhythmias. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press, London, pp. 199-218.

Corr, P.B., Synder, D.W., Lee, B.I., Gross, R.W., Keim, C.R. and Sobel, B.E., 1982. Pathophysiological concentrations of lyosphosphatides and the slow response. Am. J. Physiol. 243, H187-H195.

Corr, P.B., Gross, R.W. and Sobel, B.E., 1982a. Arrhythmogenic amphiphilic lipids and the myocardial cell membrane, 1982, J. Mol. Cell. Cardiol. 14, 619-626.

Cranefield, P.F. and Hoffman, B.F., 1972. Conduction of the cardiac impulse. Characteristics of very slow conduction. J. Gen. Physiol. 59, 227.

Cranefield, P.F., 1977. Action potential,after potentials and arrhythmias Circ. Res. 41, 415-423.

Crook, D.,Collins, A.J., Bacon, P.A. and Chan, R., 1976. Prostaglandin synthetase activity from human rheumatoid synovial microscomes. Ann. Rheum. Dis. 35, 327-332.

Curtis, M.J., MacLeod, B.A. and Walker, M.J.A., 1984. Antiarrhythmic actions of verapamil against ischaemic arrhythmias in the rat. Br. J. Pharmacol. 83, 373-385.

Das, U.N., 1981. Prostaglandins and cardiac arrhythmias. A new concept I.R.C.S. Med. Sci. 9(5), 385-387.

DeDekere, E.A.M., Nugteren, D.H. and Ten Hoor, F., 1977. Prostacyclin is the major prostaglandin released from the isolated perfused rabbit and rat heart. Nature Vol. 268, 160-163.

De Mello, W.C., 1972. Membrane lipids and cardiac electrogenesis. In: Electrical phenomena in the heart. Ed. De Mello, W.C., Academic Press, New York, pp. 89-110.

De Mello, W.C., 1982. Intercellular communication in cardiac muscle. Circ. Res. Vol. 51, 1-9

De Wall, R.A., Vasko, K.A., Stanley, E.., Kezdi, P., 1971. Response of the ischaemic myocardium toallopurinol. Am. Heart J. 82, 363-370.

Dhala, N.S., 1976. Involvement of membrane system in heart failure due to calcium overload and deficiency. J. Mol. Cell. Cardiol. 8, 661-667.

Durbin, R.P. and Jenkinson, D.H., 1961. The effect of carbachol on the permeability of depolarized smooth muscle to inorganic ions. J. Physiol. 157, 74-89.

Dyckner, T. and Wester, P.O., 1980. Relation between potassium, magnesium and cardiac arrhythmias. Acta Med. Scand. Supp. 647, Electrolytes and cardiac arrhythmics pp. 67-73.

Edelman, I.S. and Leibman, J., 1959. Anatomy of body water and electrolytes. Amer. J. Med. 27, 56.

Eisner, D.A. and Vaughan-Jones, R.D., 1983. Do calcium-activated potassium channels exist in the heart? Cell. Calcium 4, 371-386.

Erdmann, E., Philipp, G., Scholz, H., 1980. Cardiac glycoside receptor, Na^+/K^+ -ATPase activity and force of contraction in rat heart. Biochem. Pharmacol. 29, 3219-3229.

Fabiato, A., 1981. Myoplams free calcium concentration reached during the twitch of an intact isolated cardiac cell and during calcium-induced release of calcium from the sarcoplasmic reticulum of skinned cardiac cell from the adult rat or rabbit ventricle. J.

Gen. Physiol. 78, 457-497.

Fagbemi, O., 1985. The effects of combined administration of B-adrenoceptor antagonists and non-steroidal anti-inflammatory drugs on ligation-induced arrhythmias in rats. Br. J. Pharmacol. 85, 361-365.

Fiehn, W. and Hasselbach, W., 1970. The effect of phospholipase A on the calcium transport and the role of unsaturated fatty acids in ATPase activity of sarcoplasmic vesicles. Eur. J. Biochem, 13, 510-518.

Frank, J.S., Langer, G.A., Nudd, L.M. and Seraydarian, K., 1977. The myocardial cell surface, its histochemistry and the effect of sialic acid and calcium removal on its structure and cellular ion exchange. Circ. Res. 41, 702-714.

Fry, C.H. and Poole-Wilson, P.A., 1979. Electromechanical effects of pH in guinea-pig cardiac ventricular muscle. Proceedings of the Physiological Society, April 1979, c.60.

Gauduel, Y. and Duvelleroy, M.A., 1984. Role of oxygen radicals in cardiac injury due to reoxygenation. J. Mol. Cell Cardiol. 16, 459-470.

Gettes, L.S., 1981. Possible role of ionic changes in the appearance of arrhythmias. In: Pharmacology of antiarrhythmic agents. Ed. Szekeres, L. Pergamon : Oxford. England, pp. 41-64.

Grinwald, P.M. and Nayler, W.G., 1981. Calcium entry in the calcium paradox. J. Mol. Cell Cardiol. 13, 867-880.

Gross, R.W. and Sobel, B.E., 1982. Lysophosphatidylcholine metabolism in the rabbit heart: Characterization of metabolic pathways and partial purification of myocardial lyosphospholipase-transacylase. J. Biol. Chem. 257, 6702-6708.

Gross, R.W. and Sobel, B.E., 1982. Inhibition of myocardial lysophosphatidyl-

choline transcyclase by palmatyl carnitine: Implication for arrhythmogenesis. Trans. Assoc. Am. Physiciens. 94, 115-125.

Guarnieri, C., Flamigni, F., Rossoni-Caldarera, 1979. Glutathione peroxidase activity and release of glutathione from oxygen deficient perfused rat heart. Biochem. Biophys. Res. Communs. 89(2), 678-684.

Hall, E.R., 1973. The oxygen effect. In: Radiobiology for the radiobiologist. Ed. Hall, E.T., pp. 48-61, New york, Harper and Row .

Halliwell, B., Gulleridge, J.M.C., 1982. Oxygen radicals and tissue damage. Adv. Studies Heart Metab., 403-411.

Hamberg, M., Fredholm, B.B., 1976. Isomerization of prostaglandin H_2 into prostaglandin D_2 in the presence of serum albumin. Biochem. Biophys. Acta 431, 189-198.

Hammarström, S., 1983. Leukotrienes, Ann. Rev. Biochem 52, 355-377.

Hankin, J.H., Margen, S. and Goldsmith, N.F., 1970. Contribution of hard water to calcium and magnesium intake of adults. Am. J. Diet. Assoc. 56, 212-224.

Hano, J.E. and Harris, A.S., 1963. Effects of histamine and potassium release agents on ventricular rhythms before and after coronary occlusion. Am. Heart J. 65, 368.

Harris, A.S., Bisteni, A., Russel, R.A., Brigham, J.C. and Firestone, J.E., 1954. Excitatory factors in ventricular tachycardia resulting from myocardial ischaemia. Science 199, 200-203.

Harris, A.S., 1966. Potassium and experimental coronary occlusion. Am. Heart J. 71, 797-802.

Hearse, D.J., Baker, J.E. and Humphrey, S.M., 1980. Verapamil and the calcium paradox. J. Mol. Cell. Cardiol. 12, 733-739.

Hearse, D.J., 1980. Cellular damage during myocardial ischaemia: metabolic changes leading to enzyme leakage. II In. Enzymes in cardiology:

Diagnosis and Research. Ed. Hearse, D.J. and De Leiris, J., John Wiley, Chichester, pp. 1-19.

Hearse, D.J. and Dennis, S.C., 1982. Myocardial ischaemia and metabolic changes associated with the gensis of ventricular arrhythmias. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press, London pp. 15-36.

Henquin, J.C., 1977. Tetraethylammonium potentiation of insulin release and inhibition of rubidium efflux in pancreatic islets. Biochem. Biophys. Comm. 77, 551-556.

Henry, P.D., Schuchleib, R., Borda, L.J., Roberts, R., Williamson, J.R. and Sobel, B.E., 1978. Effects of nifedipine on myocardial perfusion and ischaemic injury in dogs. Circ. Res. 43, 372-380.

Hess, M.L. and Manson, N.H., 1984. Molecular oxygen: Friend and foe. The role of the oxygen free radical system in the calcium paradox, the oxygen paradox and ischaemic/reperfusion injury. J. Mol. Cell. Cardiol. 16, 969-985.

Hess, M.L., Okabe, E., Ash, P. and Kontos, H.A., 1984. Free radical mediation of the effects of acidosis on calcium transport by cardiac sarcoplasmic reticulum in whole heart homogenates. Card. Res. 18, 149-157.

Higgins, T.J.C., Bailey, P.J. and Allsop, D., 1982. Interrelation between cellular metabolic status and susceptibility of heart cells to attack by phospholipase. J. Mol. Cell. Cardiol. 14, 645-654.

Higgs, G.A. and Vane, J.R., 1983. Inhibition of cyclo-oxygenase and lipoxygenase. Br. Med. Bull. 39(3), 265-270.

Hiraoka, M., Hirano, Y. and kawano, S., 1985. Mechanisms of after depolarizations and abnormal automaticity in mammalian ventricular muscles. J. Mol. Cell. Cardiol. 17(1), Abst. 34 of the 5th meeting of the Japanese section of the International

Society for Heart Research.

Hirche, H.J., Franz, C.H.R., Bös, L., Bissig, R., Lang, R. and Schramm, M., 1980. Myocardial extracellular potassium and H^+ increase and noradrenaline release as possible cause of early arrhythmias following acute coronary artery occlusion in pigs. J. Mol. Cell Cardiol. 12, 579-593.

Hirche, H.J., Friedrich, R., Kebbel, U., MacDonald, F. and Zylka, V., 1982. Early arrhythmias, myocardial extracellular potassium and pH. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press, London.

Honn, K.V. and Dunn, J.R., 1982. Nafazatrom (Bay g 6575) inhibition of tumor cell lipoxygenase activity and cellular proliferation. Febs Lett. 139(1), 65-68.

Imaizumi, Y. and Watanabe, M., 1981. The effect of tetraethylammonium chloride on potassium permeability in the smooth muscle cell membrane of canine trachea. J. Physiol. 316, 33-46.

Isenberg , G., 1977. Cardiac Purkinje Fibres: Calcium controls the potassium permeability via the conductants gK_1 and gK_2. Eur. J. Physiol. 371, 77-85.

Iseri, L.T., Freed, J., Bures, A.R., 1975. Magnesium deficiency and cardiac disorders. The Am. J. Med. 58, 837-846.

Janse, M.J., van Capelle, F.J.L., Morsink, H., Kléber, A.G., Wilms-Schopman, F., Cardinal, R., D'Alnoncourt, C.N. and Durrer, D., 1980. Flow of 'injury' current and patterns of excitation during early ventricular arrhythmias in acute regional myocardial ischaemia in isolated porcine and canine hearts. Circ. Res. 47, 151-165.

Johnson, C.J., Peterson, D.R., Smith, E.K., 1979. Myocardial tissue concentration of magnesium and potassium in men dying suddenly from ischaemic disease. Am. J. Clin. Nutr. 32, 967-970.

Johnston, K.M., MacLeod, B.A. and Walker,M.J.A., 1983. Effects of aspirin and prostacyclin on arrhythmias resulting from coronary artery ligation and on infarct size. Br. J. Pharmacol. 78, 029-037.

Kalsner, S., 1983. The effect of magnesium deficiency and excess on bovine coronary artery tone and responses to agonists. Br . J. Pharmacol. 78, 629-638.

Kane, K.A., McDonald, F.M. and Parratt, J.R., 1979. Coronary artery ligation in anaesthetised rats as a model for the assessment of antidysrhythmic activity. Br. J. Pharmac. 66, 436.

Kane, K.A., McDonald, F.M. and Parratt, J.R., 1981. The anesthetized rat as a model investigating early post-ligation dysrhythmias. In: The rat electrocardiogram in pharmacology and toxicology. Pergamon Press, p. 235-238.

Karli, N.T., 1958. The effects of changes in ion concentration on ventricular fibrillation induced electrically. J. Physiol. 141, 366-376.

Karli, J.N., Karikas, G.A., Hatzipavlou, P.K., Levis, G.M. and Moulopoulos, S.N., 1979. The inhibition of Na^+ and K^+ stimulated ATPase activity of rabbit and dog heart sarcolemma by lysophosphatidylcholine. Life Sci. 24, 1869-1876.

Katz, A.M., 1982. Membrane-derived lipids and the pathogenesis of ischemic myocardium damage. J. Mol. Cell. Cardiol. 14, 627-632.

Kaumann, A.J. and Aramendia, P., 1968. Prevention of ventricular fibrillation induced by coronary ligation. J. Pharmacol. Exp. Thera. 164, 326-332.

Kirsch, U., Rodewald, G. and Kalmer, P., 1972. Induced ischaemic arrest: Clinical experiences with cardioplegia in open heart surgery. J. Thorac. Cardiovasc. Surg. 63, 121-130.

Kléber, A.G., 1983. Resting membrane potential, extracellular potassium activity, and intracellular sodium activity during acute global ischemia in isolated perfused guinea pig hearts. Circ. Res. 52, 442-450.

Kléber, A.G., 1984. Extracellular potassium accumulation in acute myocardial ischaemia. J. Mol. Cell. Cardiol. 16, 389-394.

Kuhel, E.A., Humes, J.L., Ham, E.A., 1980. Inflamation: The role of peroxidase-derived products. Adv. Prostaglandin. Thromboxane Res . 6, 77-86.

Kunze, D.L., 1977. Rate dependent change in extracellular potassium in rabbit atrium. Circ. Res. 41, 122-127.

Lab, M.J., 1982. Stress-strain related depolarization in the myocardium and arrhythmogensis in early ischaemia. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press,London, pp. 81-91.

Langer, G.A., 1984. Calcium at the sarcolemma. J. Mol. Cell. Cardiol. 16, 147-153.

Lee, Y. and Chang, S., 1977. Effect of lysolecithin on the structure and permeability of lecithin bilayer vesicles. Biochemistry 16, 1303-1309.

Lee, F.C. and Sobel, B.E., 1981. Phospholipid metabolism: myocardial phospholipase and acyltransferase. Fedn. Proc. Fedn. Am. Socs. Exp . Biol. 40, 1631.

Lin, C.I. and Vassalle, M., 1980. The antiarrhythmic effect of potassium and rubidium in strophanthidin toxicity. Euro. J. Pharmacol. 62, 1-15.

Linton, R.A.F., Lim,M., Band, D.M., 1982. Continuous intravascular monitoring of plasma potassium using potassium-selective electrode catheters. Crit. Care Med. 10(5), 337-340.

Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265-275.

Lubbe, W.F., Bricknell, O.L. and Marzago, C., 1975. Ventricular fibrillation threshold and vulnerable period in the isolated perfused rat heart. Cardiov. Res. 9, 613-620.

Lubbe, W.F., Daries, P.S. and Opie, L.H., 1978. Ventricular arrhythmias associated with coronary artery occlusion and reperfusion in the isolated perfused rat heart: a model for assessment of anti-fibrillatory action of antiarrhythmic agents. Card. Res. 12, 212-220.

Mann, R.Y.K. and Choy, P.C., 1982. Lysophosphatidylcholine causes cardiac arrhythmia. J. Mol. Cell. Cardiol. 14, 173-175.

McCord, J.M., 1985. Oxygen-derived free radicals in postischaemic tissue injury. The New England J.Med. Vol. 312(3), 159-163.

Meerson, F.Z., Kagan, V.E., Kozlov, Yu,P., Belkina, L.M., and Arkhipenko, Yu. V., 1982. The role of lipid peroxidation in pathogensis of ischaemic damage and the antioxidant protection of the heart. Basic Res. Cardiol. 77, 465-485.

Meesmann, W., 1982. The possible role of the sympathetic nervous system in the gensis of early post-ischaemia arrhythmias. In: Early arrhythmias resulting from myocardial ischaemia Ed. Parratt, J.R., Macmillan Press, London, pp. 139-152.

Mest, H.J., Taube, Ch., Förster, W., Metsä-Ketelä, T. and Vapaatalo, H., 1981. Influence of cardiac rhythm disturbances and anti-arrhythmic drugs on the efflux of PGE, $PGF_{2\alpha}$, Cyclic AMP and Cyclic GMP in canine coronary sinus blood. Prost.Med. 7, 1-13.

Moschos, C.B., Haider, B., De La Cruz, C., Lyons, M.M. and Reagan, T.J., 1978. Antiarrhythmic effects of aspirin during non-thrombotic coronary occlusion. Circ. 57(4), 681-684.

Moschos, C.B., Haider, B., Escobinas, A.J., Gandhi, A. and Regan, T.J. 1980. Chronic use of aspirin versus indomethacin during non-thrombotic myocardial ischaemia. Am. Heart J. 100, 647-652.

Müller, B., Wilsmann, K., 1982. Effects of the optical isomers of D600 on cardiovascular parameters and on arrhytymias caused by aconitine and coronary artery ligation in anaesthetized rats. J. Cardiov. Pharmacol. 4, 615-621.

Myerbrug, R.J., Gelbard, H., Nilsson, K., Sung, R.J., Moraiss, A.R., Bassett, A.L., 1977. Long term electrophysiological abnormalities resulting from experimental myocardial infarction in cats. Circ. Res. 41, 73-84.

Nakanishi, T., Nishioka, K., Jarmakani, J.M., 1982. Mechanisms of tissue calcium gain during reoxygenation after hypoxia in rabbit myocardium. Am. J. Physiol. 242, H437-H449.

Nayler, W.G., Grau, A., Slade, A., 1976. A protective effect of verapamil on hypoxic heart muscle. Cardiovasc. Res. 10, 650-662.

Nayler, W.G., 1981. The role of calcium in the ischaemic myocardium. Am. J. Pathol. 102, 262-270.

Nayler, W.G., Sturrock, W.J., 1984. An inhibitory effect of verapamil and diltiazem on the release of noradrenaline from ischaemic and reperfused hearts. J. Mol. Cell. Cardiol. 16, 331-344.

Needleman, P., Marshall, G.R., and Sobel,B.E., 1975. Hormone interactions in the isolated rabbit heart synthesis and coronary vasomotor effects of prostaglandins, angiotensin and bradykinin. Circ. Res. 37, 802-808.

Needleman, P., 1976. The synthesis and function of prostaglandins in the heart. Fed. Proc. 35, 2376-3381.

Needleman, P. and Kaley, G., 1978. Cardiac and coronary prostaglandin synthesis and function. The New England J. Med. Vol. 298(20), 1122-1128.

Nordin, B.E.C., 1976. Plasma calcium and plasma magnesium homeostasis. In: Calcium, phosphate and magnesium metabolism. Ed. Nordin, B.E.C., pp. 208-216, Edinburgh, Churchill,Livingstone.

Oliver, M.F., 1982. Sudden cardiac death - An overview. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press, London, pp. 1-13.

Opie, L.H., 1978. Effects of regional ischaemia on metabolism of glucose and fatty acids. Circulation Res. 38(Suppl.) 52-74.

Opie, L.H., Nathan, D., and Lubbe, W.F., 1979. Biochemical aspects of arrhythmogenesis and ventricular fibrillation. Am. J. Cardiol. 43, 131-148.

Osman, F.H., Ammar, E.M., Afifi, A.M. and Ahmed, N.M., 1976. Potentiation by lithium and protection by rubidium of digitalis intoxication. Jap. J. Exp. Med. 46-1.

Page, E. and Polimeni, P.I., 1973. Magnesium in heart muscle. Circ. Res. 33, 367-374.

Patterson, E., Eller, B.T. and Lucchesi, B.R., 1983. Effects of diltiazem upon experimental ventricular dysrhythmias. The J. Pharmacol. Exper. Therap. 225(1), 224-233.

Piper, P., 1983. Pharmacology of leukotrienes. Br. Med. Bull. 39(3), 255-259.

Podzuweit, T., Dalby, A.J., Cherry, G., and Opie, L.H., 1978. Tissue levels of cyclic AMP in ischaemic and non ischaemic myocardium following coronary artery ligation. J. Mol. Cell. Cardiol. 10, 81-94.

Podzuweit,T., 1982. Early arrhythmias resulting from acute myocardial ischaemia; possible role of cyclic AMP. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press, London, pp. 171-198.

Poole-Wilson, P.A., Harding, D., Bourdillon, P. and Fleetwood, G., 1982. Mechanism of myocardial protection through calcium blockade. In: Protection of tissue against hypoxia. Ed. Wauquier *et al.*, Janseen Research Foundation, Amsterdam, pp. 351-364.

Prozialeck, W.C. and Weiss, B., 1982. Inhibition of calmodulin by phenothiazines and related drugs: structure-activity relationship. J. Pharmacol. Exp. Therap. 222(3), 509-516.

Rao, P.S., Cohen, M.V. and Mueller, H.S., 1983. Production of free radicals and lipid peroxides in early experimental myocardial ischaemia. J. Mol. Cell. Cardiol. 15, 713-716.

Rau, E.E., Shine, K.I. and Langer, G.A., 1977. Potassium exchange and mechanical performance in anoxic mammalian myocardium. Am. J. Physiol. 232(1), H85-H94.

Reuter, H., 1967. The dependence of slow inward current in Purkinje fibres on the extracellular calcium concentration. J. Physiol. 192, 479-492.

Riemersma, R.A., 1982. Myocardial catecholamine release in acute myocardial ischaemia; relationship to cardiac arrhythmias. In: Early arrhythmias resulting from myocardial ischaemia. Ed. Parratt, J.R., Macmillan Press, London, pp. 125-138.

Robertson, J.D., 1959. The ultrastructure of cell membranes and their derivatives. Biochem. Soc. Symp. 16, 3-43.

Rowe, G.T., Manson, N.H., Caplan, M., and Hess, M.L., 1983. Hydrogen peroxide and hydroxyl radical mediation of activated leukocyte depression of cardiac sarcoplasmic reticulum. Circ. Res. 53, 584-591.

Roy, R.S. and McCord, J.M., 1983. Superoxide and ischaemia conversion of xanthine dehydrogenase to xanthine oxidase. In: Greenwald, R., Cohen, G., Eds. Oxyradicals and their scavenger system. Vol. 2, Cellular and Molecular Aspects. New York. Elsevier Science, pp. 145-153.

Schaper, W., 1984. Experimental infarcts and the microcirculation. In: Therapeutic approaches to myocardial infarct limitation. Ed. Hearse, D.J. and Yellon, D.M., Raven Press, New York, pp. 79-90.

Schwartz, A.B., 1978. Potassium-related cardiac arrhythmias and their treatment. Angiology 29(3), 194-205.

Sedlis, S.P., Corr, P.B., Sobel, B.E. and Ahumada, G.G., 1983. Lysophosphatidylcholine potentiates calcium accumulation in rat cardiac myocytes. Am. J. Physiol. 244, H32-H38.

Seelig, M.S. and Heggtveit, H.A., 1974. Magnesium interrelationships in ischaemic heart disease (a review). Am. J. Clin. Nutr. 27, 59-79.

Shigenobu, K. and Sperelakis, N., 1972. Calcium current channels induced by catecholamines in chick embryonic hearts whose fast sodium channels are blocked by tetrodotoxin or elevated potassium. Circ. Res. 31(12), 932-952.

Shimoni, Y., Raz, S. and Gotsman, M.S., 1984. Two potentially arrhythmogenic mechanisms of adrenaline action in cardiac muscle. J. Mol. Cell. Cardiol. 16, 471-478.

Shine, K.I. and Douglas, A.M., 1974. Magnesium effects on ionic exchange and mechanical function in rat ventricle. Am. J. Physiol. 227, 317-324.

Shine, K.I., 1979. Myocardial effects of magnesium. Am. J. Physiol. 237, H413-H423.

Shine, K.I. and Douglas, A.M., 1983. Low calcium reperfusion of ischemic myocardium. J. Mol. Cell Cardiol. 15, 251-260.

Skou, J.C., 1957. The influence of some cations on adenosine triphosphate from peripheral nerves. Biochem. Biophys. Acta 23, 394.

Smith, H.J., Singh, B.N., Nisbet, H.D. and Norris, R.M. , 1975. Effects of verapamil on infarct size following experimental coronary occlusion. Cardiovasc. Res. 9, 569-578.

Sobel, B.E., Corr, P.B., Robison, A.K., Goldstein, R.A., Witowski, F.X. and Klein, M.S., 1978. Accumulation of lysophosphoglycerides with arrhythmogenic properties in ischaemic myocardium. J. Clin. Inv. 62, 546-553.

Spear, J.F., 1981. Relation between the scalar electrocardiagram and cellular electrophysiology of the rat heart. In: The rat electrocardiogram in pharmacology and toxicology. Pergamon Press, pp. 29-40, Eds. Budden, R., Detweiler, D. and Zbinden, G.

Sperelakis, N. 1984. Properties of calcium-dependent slow action potentials: Their possible role in arrhythmias. In; Calcium antagonists and cardiovascular disease. Ed. L.H. Opie, New York.pp.227-291

Steenbergen, C., Deleeuw,G., Rich, T. and Williamson, J.R., 1977. Effects of acidosis and ischemia on contractility and intracellular pH of rat heart. Circ. Res. 41(6), 849-858.

Steenbergen, C. and Jennings, R.B., 1984. Relationship between lysophospholipid accumulation and plasma membrane injury during total *in vitro* ischaemia in dog heart. J. Mol. Cell. Cardiol. 16, 605-621.

Szelenyi, I., 1973. Magnesium and its significance in cardiovascular and gastrointestinal disorders. World Rev. Nutr. Diet. 17, 189-224.

Thomas, M.J., Mehl, K.S., Prayor, W.A., 1978. The role of superoxide anion in the xanthin oxidation of linoleic acid. Biochem. Biophys. Res. Commun. 83, 927-932.

Thuillez, C., Maury, M. and Giudicelli, J.F., 1983. Differential effects of verapamil and diltiazem on regional blood flow and function in the canine normal and ischemic myocardium. J. Card. Pharmacol. 5, 19-27.

Tomita, T. and Yamamoto, T., 1971. Effects of removing the external potassium on the smooth muscle of guinea-pig taena coli. J. Physiol. 212, 851-868.

Tsien, R.W., 1976. Possible effects of hydrogen ions in ischaemic myocardium.Circ.Res. 53 (3), 1-14.

van der Vusse, G.J. and Reneman, R.S., 1985. Pharmacological intervention in acute myocardial ischaemia and reperfusion. TIPS, 76-79.

Vleugels, A., Vereecke, J. and Carmeliet, E., 1980. Ionic currents during hypoxia in voltage clamped cat ventricular muscle. Circ. Res. 47, 501-508.

Watanabe, A.M. and Besch, H.R.B., 1974. Cyclic AMP modulation of slow calcium influx channels in guinea-pig hearts. Circ. Res. 35, 316-324.

Weglicki, W.B., 1980. Degradation of phospholipids of myocardial membranes. In: Degradative processes in heart and skeletal muscle Ed. Wildenthal, Elsevier, North-Holland Biochem. Press. pp. 377-387.

Weiss, J. and Shine, K.I., 1982a. Potassium accumulation and electrophysiological alterations during early myocardial ischaemia. Am. J. Physiol. 243, H318-H327.

Weiss, J. and Shine, K.I. 1982. Extracellular potassium accumulation during myocardial ischaemia in isolated rabbit heart. Am. J. Physiol. 242, H619-H628.

Weltzien, H.U., 1979. Cytolytic and membrane-perturbing properties of lysophosphatidylcholine. Biochemica et Biophysica Acta 559, 259-287.

Winkler, A.W., Hoff, H.E. and Smith, P.K., 1938. Electrocardiographic changes and concentration of potassium and serum following intravenous injection of potassium chloride. Am. J. Physiol. 124, 478.

Woodward, B., 1981. A model of ventricular fibrillation in the isolated rat heart. J. Pharmacol. Method 6, 219-231.

Woodward, B. and Zakaria, M.N.H., 1985. Effect of some free radical

scavengers on reperfusion induced arrhythmias in the isolated rat heart. J. Mol. Cell. Cardiol. 17, 485-493.

Yates, J.C. and Dhalla, N.S., 1975. Structural and functional changes associated with failure and recovery of hearts after perfusion with calcium-free medium. J. Mol. Cell. Cardiol. 7, 91-103.

Zimmerman, A.N.E. and Hülsmann, W.C., 1966. Paradoxical influence of calcium ions on the permeability of the cell membranes of the isolated rat heart. Nature 211, 646-647.