

VENTRICULAR ARRHYTHMOGENESIS DURING ACUTE MYOCARDIAL ISCHEMIA

with special reference to the isolated Langendorff
perfused rat heart

A thesis submitted to the University of Cape Town
for the Degree of Doctor of Medicine

by

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To my parents

Barney and Ida

and my wife

Reka

for their love

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ABBREVIATIONS

VFT	=	ventricular fibrillation threshold
mA	=	milliamps
HR	=	heart rate
CF	=	coronary flow
CAL	=	coronary artery ligation
ATP	=	adenosine triphosphate
PCr	=	phosphocreatine
cAMP	=	cyclic adenosine monophosphate
dBcAMP	=	dibutyryl cyclic adenosine monophosphate

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ABSTRACTVENTRICULAR ARRHYTHMOGENESIS DURING ACUTE MYOCARDIAL ISCHEMIA

The mechanism of early ventricular fibrillation during acute myocardial ischemia is undefined. A current hypothesis is that the biochemical and metabolic sequelae of acute myocardial ischemia produce electrophysiological alterations in the ischemic myocardium, viz. slow response action potentials or depressed fast response action potentials which in turn facilitate the occurrence of reentry and hence ventricular fibrillation. A fundamental biochemical change during ischemia is extracellular potassium accumulation - such an alteration decreases the resting membrane potential of ischemic myocardial cells and also inactivates the fast inward sodium current. If there is mild or moderate extracellular potassium accumulation then the fast sodium current may only be partially inactivated and generation of an action potential - termed a depressed fast response - is dependent on reduced sodium inward current flowing through the existing fast channels. If there is severe extracellular potassium accumulation then the fast sodium current may be totally inactivated and generation of an action potential - termed a slow response - is dependent on factors promoting the transsarcolemmal calcium inward current. A metabolic consequence of ischemia, viz. enhanced adrenergic activity, promotes transsarcolemmal calcium influx and has therefore been linked to the development of slow response potentials. The intracellular expression of enhanced adrenergic activity is considered to be mediated by cyclic AMP, the reputed second messenger of beta receptor stimulation. This proposal is based on the circumstantial association between myocardial accumulation of cyclic AMP and the development of ventricular fibrillation and by the ability of

cyclic AMP to induce slow responses in partially depolarized fibres.

Slow responses and depressed fast responses are associated with slow rates of impulse propagation and have therefore been presumed to be responsible for the slow conduction and conduction block present in the ischemic myocardium. Slow conduction and conduction block predispose to the development of reentry which is currently considered to be the electrophysiological mechanism of early ventricular fibrillation. Electrophysiological mapping studies during acute myocardial ischemia link reentrant circuits to the development of ventricular fibrillation.

The problem of ventricular arrhythmogenesis during acute myocardial ischemia is approached by an analysis of: (a) the biochemical and metabolic factors reputed to produce alterations in the cardiac action potential; (b) the transsarcolemmal calcium current and the transsarcolemmal sodium current which underlie the development of slow responses and depressed fast responses respectively.

Model

The isolated Langendorff perfused rat heart was chosen as the model in which to study the problem of ventricular arrhythmogenesis in view of the following advantages: First, pharmacological agents can be added to the perfusion fluid and specific concentrations of such pharmacological agents can be attained in the myocardium. Secondly, alterations in the ionic milieu (eg. calcium ion) of the perfusion fluid is easily produced and the effect of such ionic alterations on ventricular arrhythmogenesis can be evaluated. Thirdly, the role of myocardial adrenergic receptor mediated influences on ventricular arrhythmogenesis can be determined. Fourthly, the role of metabolic and biochemical factors in ventricular arrhythmogenesis can be investigated.

Methodology

Ventricular fibrillation is induced by the train method of electrical

stimulation, electrical current being delivered by two stimulating electrodes across the surface of left ventricle during the T wave of the electrocardiogram i.e. during the vulnerable period. Ventricular fibrillation is defined as rapid, totally irregular ventricular rhythm for at least six beats and is accompanied by loss of left ventricular pressure. The lowest current required to induce ventricular fibrillation on two occasions is termed the ventricular fibrillation threshold.

Acute regional myocardial ischemia of the left ventricle is produced by ligation of the left main coronary artery. The duration of acute myocardial ischemia is 15 minutes because the trough of the ventricular fibrillation threshold is reached within 10 to 15 min of coronary artery ligation. The heart rate and total coronary flow rate are recorded throughout the experiment. Biochemical and metabolic profiles are analyzed in freeze clamped hearts 15 mins after coronary artery ligation, i.e. at the nadir of the ventricular fibrillation threshold.

Ventricular fibrillation induced by enhanced adrenergic stimulation

The mechanism of catecholamine-induced ventricular fibrillation is analysed in view of the possible link between enhanced adrenergic activity and ventricular arrhythmogenesis during acute myocardial ischemia. Cyclic AMP, the reputed intracellular second messenger of beta-adrenergic stimulation, has been proposed to play a major role in the genesis of ventricular fibrillation induced by adrenergic stimulation. It has been further proposed that cyclic AMP may mediate its arrhythmogenic effect by promoting transsarcolemmal calcium influx, thereby inducing calcium mediated electrophysiological alterations.

If this hypothesis is correct then it might be anticipated that:

- (i) beta-adrenoceptor antagonist agents should prevent adrenaline-induced ventricular fibrillation by inhibiting the accumulation of cyclic AMP;
- (ii) calcium antagonist procedures should prevent adrenaline-induced

ventricular fibrillation by inhibiting the transsarcolemmal calcium inward current.

Effect of beta adrenoceptor antagonist agents

Adrenaline 5×10^{-8} M to 5×10^{-7} M produced a concentration dependent increase in the vulnerability to ventricular fibrillation in the non-ischemic heart; such an effect was not associated with a temporal accumulation of myocardial cyclic AMP. Adrenaline 5×10^{-7} M produced the greatest increase in both vulnerability to ventricular fibrillation and accumulation of cyclic AMP.

Beta-adrenoceptor antagonism with atenolol 5×10^{-5} M inhibited the adrenaline 5×10^{-7} M induced vulnerability to ventricular fibrillation. Dibutyryl cyclic AMP, the analogue of cyclic AMP which penetrates the cell membrane more easily, enhanced the vulnerability to ventricular fibrillation. Beta-adrenoceptor antagonism with atenolol 5×10^{-5} M did not prevent the dibutyryl cyclic AMP induced ventricular fibrillation.

The beta-adrenergic receptor appears to play a major role in the genesis of ventricular fibrillation induced by adrenergic stimulation in the non-ischemic heart. Cyclic AMP, the proposed intracellular second messenger of beta-receptor stimulation may not mediate the arrhythmogenic effect of enhanced adrenergic stimulation; cyclic AMP accumulation may simply reflect an epi-phenomenon.

Effect of calcium antagonist procedures

Calcium channel antagonist agents, verapamil 1.5×10^{-7} M and nifedipine 10^{-6} M prevented the adrenaline 5×10^{-7} M induced vulnerability to ventricular fibrillation. That calcium ions mediate the adrenaline-induced ventricular fibrillation is suggested by the following arguments: First, a reduction in the extracellular calcium ion concentration prevented the adrenaline-induced ventricular fibrillation.

Secondly, 1(-) verapamil, the isomer of verapamil which inhibited the slow calcium inward current without altering the fast sodium inward current antagonized the catecholamine-induced ventricular fibrillation. Thirdly, nifedipine which does not interact with either α_1 or α_2 adrenoceptors (Motulsky et al, 1983), and is therefore a 'pure calcium antagonist', protected against adrenaline-induced ventricular fibrillation. Fourthly, protection was not due to preservation of high energy phosphate content in the myocardium. Fifthly, a clear dissociation could be shown between effects on coronary flow rate and on antiarrhythmic activity.

These findings suggest that calcium ions mediate the adrenaline-induced vulnerability to ventricular fibrillation. Calcium ions may act as intracellular second messenger in mediating the arrhythmogenic effect of catecholamine stimulation.

Ventricular fibrillation induced by acute myocardial ischemia

The role of the transsarcolemmal calcium current and the transsarcolemmal sodium current is investigated in the genesis of ventricular fibrillation during acute myocardial ischemia. This approach is based on the hypothesis that slow response action potentials or depressed fast response action potentials underlie the development of ventricular fibrillation during acute myocardial ischemia.

Slow response action potential

If calcium dependent slow responses underlie early ventricular fibrillation then:

- (1) Beta-adrenoceptor antagonist agents which prevent accumulation of cyclic AMP should inhibit the cyclic AMP induced slow inward calcium current and thereby prevent slow responses and ventricular fibrillation.
- (2) Alpha-adrenoceptor antagonist agents which decrease the

transsarcolemmal calcium inward current and possibly decrease intracellular calcium release should inhibit slow responses and thereby prevent ventricular fibrillation.

(3) Calcium channel antagonist agents, which decrease the transsarcolemmal calcium inward current, should inhibit the occurrence of slow responses and thereby prevent ventricular fibrillation.

Depressed fast response action potential

If depressed fast responses underlie early ventricular fibrillation then:

(1) Fast channel blocking agents which decrease the transsarcolemmal sodium inward current should inhibit depressed fast responses and thereby prevent ventricular fibrillation.

Effect of beta-adrenoceptor antagonist agents

During acute myocardial ischemia, beta-adrenoceptor antagonist agents, atenolol $5 \times 10^{-5} \text{ M}$ and metoprolol 10^{-5} M , in concentrations producing beta-receptor antagonism, are ineffective in preventing the enhanced vulnerability to ventricular fibrillation. In concentrations exceeding that required to produce beta-receptor antagonism, metoprolol 10^{-4} M but not atenolol 10^{-4} M prevents the enhanced vulnerability to ventricular fibrillation during acute myocardial ischemia. Further investigation using dl-propranolol $5 \times 10^{-6} \text{ M}$ and the d(+) isomer of propranolol $5 \times 10^{-6} \text{ M}$ show both agents to be equipotent in exhibiting ventricular antiarrhythmic activity. Protection against ventricular fibrillation could not be linked to reduction in heart rate, maintenance of coronary flow rate or preservation of metabolic status in the ischemic myocardium. The property common to dl-propranolol, d(+) propranolol, high concentrations of metoprolol and lacking in atenolol is 'membrane stabilizing activity'. The beta-adrenoceptor, as judged by the effects of beta-antagonist agents, does not appear to play a role in the genesis

of ventricular fibrillation during acute myocardial ischemia. The ventricular antiarrhythmic activity of certain beta-antagonist agents appears to be due to a non-specific effect, viz. 'membrane stabilizing activity'.

Role of cyclic AMP

During acute myocardial ischemia, a temporal relationship exists between accumulation of cyclic AMP in the ischemic myocardium and the development of ventricular fibrillation. It has therefore been proposed that cyclic AMP, the reputed intracellular second messenger of beta-receptor stimulation, plays a major role in the genesis of ventricular fibrillation during ischemia. The accumulation of cyclic AMP in the ischemic myocardium is not inhibited by selective beta-receptor antagonism, i.e. metoprolol 10^{-5} M, but rather by non-selective beta-receptor antagonism, i.e. metoprolol 10^{-4} M, calcium channel antagonism, i.e. verapamil 1.5×10^{-7} M, and fast channel inhibition, i.e. lignocaine 3×10^{-5} M. The reason for this puzzling and complex finding is unclear, but these findings indicate that generation of cyclic AMP in the ischemic myocardium may be dependent on factors other than beta-adrenoceptor stimulation.

Does cyclic AMP play a role in the genesis of ventricular fibrillation during acute myocardial ischemia? Reduction in the cyclic AMP content in the ischemic myocardium was, in certain situations, circumstantially associated with protection against ventricular fibrillation, but, in other instances, unassociated with protection against ventricular fibrillation. Ventricular antiarrhythmic activity could also occur despite no reduction in cyclic AMP content in the ischemic myocardium. The evidence presented indicates that cyclic AMP may be linked to ventricular fibrillation, but only in certain situations. Cyclic AMP is not the only factor nor the final messenger of ventricular fibrillation.

Effect of alpha adrenoceptor antagonist agents

Prazosin 10^{-5} M, an α_1 antagonist; yohimbine 10^{-6} M, an α_2 antagonist, and phentolamine 10^{-5} M, an α_1 and α_2 adrenoceptor antagonist agent, each completely prevented the enhanced vulnerability to ventricular fibrillation during acute regional myocardial ischemia in the absence or presence of adrenergic stimulation. Protection was not due to reduction in heart rate, maintenance of higher coronary flow rates, preservation of metabolic status in the ischemic myocardium or due to reduction in the cyclic AMP content in the ischemic myocardium. Do these findings indicate that α_1 and α_2 adrenergic receptor mediated influences play a role in the genesis of ventricular fibrillation during acute myocardial ischemia? The following arguments question the specificity of an α_1 or α_2 receptor mediated effect.

First, the protective concentration of prazosin (10^{-5} M) is 1000-fold higher than the dissociation constant of the myocardial α_1 receptor, whilst the protective concentration of yohimbine (10^{-6} M) is 100 and 1000-fold higher respectively than the initial and final dissociation constants of the myocardial α_2 adrenoceptor. Secondly, the protective concentration of prazosin, an α_1 antagonist, approximates that of yohimbine, an α_2 antagonist. Thirdly, the ventricular antiarrhythmic activity of both α_1 and α_2 receptor antagonist agents is difficult to reconcile on the basis of a specific receptor mediated event because of the disparate effect of α_1 and α_2 receptor mediated influences on calcium fluxes. Fourthly, endogenous myocardial catecholamine depletion by reserpine pre-treatment did not prevent the enhanced vulnerability to ventricular fibrillation during acute myocardial ischemia. Fifthly, the concentration of phentolamine which protects against ventricular

fibrillation also inhibits the fast sodium channel and prolongs the action potential duration. Finally, the concentration of prazosin which protects against ventricular fibrillation also prolongs the action potential duration. The evidence presented suggests that the ventricular antiarrhythmic effect is likely to be due to a non-specific effect, i.e. 'membrane stabilizing activity' or prolongation of the action potential duration rather than due to specific α_1 or α_2 receptor antagonism. However, two important aspects to note are that the anatomical distribution of myocardial α_1 and α_2 adrenoceptors is not clearly defined as is the regulation of extracellular and intracellular calcium ion movement by myocardial α_1 and α_2 receptors. Until these aspects have been defined, a role for the α_1 and α_2 adrenergic receptors cannot be excluded in the genesis of ventricular fibrillation during acute myocardial ischemia.

Effect of calcium antagonist procedures

During acute myocardial ischemia, calcium channel antagonist agents verapamil $1.5 \times 10^{-7} M$, nifedipine $10^{-6} M$ and diltiazem $5 \times 10^{-6} M$ attenuated the enhanced vulnerability to ventricular fibrillation in the absence and presence of adrenergic stimulation.

Protection was associated with reduction in heart rate, preservation of high energy phosphate, reduction in tissue cyclic AMP content and maintenance of higher total coronary flow rates; however, each of these factors could not account for ventricular antiarrhythmic activity. Redistribution of coronary flow from the non-ischemic to the ischemic myocardium, although unlikely, could not be excluded in the mechanism of protection. That the transsarcolemmal calcium inward current plays a role in the genesis of ventricular fibrillation is suggested by the following factors: First, reduction in extracellular calcium ion concentration from 2.5 mM to 1.0 mM partially prevented the enhanced

vulnerability to ventricular fibrillation during the acute myocardial ischemia. Secondly, 1(-) verapamil $1.5 \times 10^{-7} \text{ M}$, which inhibits the transsarcolemmal calcium inward current without alteration of the transsarcolemmal sodium inward current, partially prevented the vulnerability to ventricular fibrillation. Thirdly, nifedipine, shown not to interact with either α_1 or α_2 adrenergic receptors, attenuated the vulnerability to ventricular fibrillation. The common property shared by the above three procedures is inhibition of the transsarcolemmal calcium inward current. These findings suggest that the transsarcolemmal calcium current plays a role in the genesis of ventricular fibrillation during acute myocardial ischemia.

Effect of sodium channel antagonist procedures

The fast channel blocking agent lignocaine $3 \times 10^{-5} \text{ M}$ completely prevented the enhanced vulnerability to ventricular fibrillation during acute myocardial ischemia. Protection was not due to reduction in the heart rate, maintenance of higher coronary flow rates during acute myocardial ischemia or preservation of metabolic status in the ischemic myocardium. Lignocaine inhibits the fast sodium inward current but also produces an alteration in the time independent outward potassium current, such an action may be responsible for ventricular antiarrhythmic activity. Further investigation was therefore undertaken with tetrodotoxin, a specific inhibitor of the voltage dependent sodium inward current. Tetrodotoxin $3 \times 10^{-6} \text{ M}$ completely prevented the enhanced vulnerability to ventricular fibrillation during ischemia - this effect was not mediated by alteration of heart rate, maintenance of coronary flow, preservation of metabolic status or reduction in myocardial cyclic adenosine monophosphate content. These findings suggest that the transsarcolemmal sodium current plays a major role in the genesis of ventricular fibrillation during acute myocardial ischemia.

Conclusions:Ventricular fibrillation induced by enhanced adrenergic stimulation in the non-ischemic heart

1) Beta-adrenoceptor antagonist agents, in concentrations producing beta-receptor antagonism, prevent the adrenaline-induced vulnerability to ventricular fibrillation. The beta-adrenergic receptor is directly implicated in the genesis of ventricular fibrillation induced by enhanced adrenergic stimulation. Cyclic AMP, the proposed intracellular second messenger of beta-receptor stimulation, may not mediate the arrhythmogenic effect of enhanced adrenergic activity, but merely be an epi-phenomenon.

2) Calcium antagonist procedures prevent the adrenaline-induced vulnerability to ventricular fibrillation. Calcium ions appear to act as the intracellular second messenger in mediating the arrhythmogenic effect of enhanced adrenergic stimulation.

Ventricular fibrillation induced by acute regional myocardial ischemia

1) Beta-adrenoceptor antagonist agents, in concentrations producing beta-receptor antagonism, are ineffective in preventing the enhanced vulnerability to ventricular fibrillation. In concentrations higher than that required to produce beta-adrenoceptor antagonism, some but not all beta-antagonist agents prevent the enhanced vulnerability to ventricular fibrillation. The beta-adrenergic receptor does not appear to play a role in the genesis of ventricular fibrillation during acute myocardial ischemia. The ventricular antiarrhythmic activity of some beta-adrenoceptor antagonist agents appears to be due to a non-specific effect, eg. 'membrane stabilizing activity'.

2) The accumulation of cyclic AMP in the ischemic myocardium appears to be dependent on factors other than beta-receptor stimulation. Cyclic AMP

may be linked to ventricular fibrillation but only in certain situations; cyclic AMP is not the only factor nor the final messenger of ventricular fibrillation.

3) Alpha₁ and alpha₂ adrenoceptor antagonist agents prevent the enhanced vulnerability to ventricular fibrillation. The ventricular antiarrhythmic activity appears to be due to a non-specific effect, i.e. 'membrane stabilizing activity' or prolongation of action potential duration rather than due to specific alpha₁ or alpha₂ adrenoceptor antagonism. Before one can exclude a role for either the alpha₁ or alpha₂ receptor in ventricular arrhythmogenesis, the anatomical distribution of myocardial alpha₁ and alpha₂ adrenoceptors and their regulation of extracellular and intracellular calcium ion movement needs to be clearly defined.

4) Calcium channel antagonist agents and a reduction in extracellular calcium concentration attenuated the enhanced vulnerability to ventricular fibrillation. The transsarcolemmal calcium current appears to play a role in the genesis of ventricular fibrillation.

5) Fast channel blocking agents prevented the enhanced vulnerability to ventricular fibrillation. The transsarcolemmal sodium current appears to play a major role in the genesis of ventricular fibrillation.

CHAPTER 1HISTORICAL REVIEW AND EVOLUTION OF HYPOTHESIS UNDERLYINGVENTRICULAR ARRHYTHMOGENESIS1. HISTORICAL REVIEW[A] Characterization of ventricular arrhythmias

Ventricular arrhythmias following experimental coronary artery occlusion were first described in 1894 by Porter as "fibrillar contraction of the left ventricle". The pattern of ventricular arrhythmias following experimental coronary artery occlusion was characterized in 1909 by Lewis as ventricular extrasystoles, ventricular tachycardia and ventricular fibrillation. The size of ischemic myocardium was shown by Garrey in 1914 to be of critical importance in the genesis of ventricular fibrillation. Garrey illustrated that by successively cutting fibrillating ventricles into smaller and smaller sections, he could arrive at a section which did not allow fibrillation to occur. Thus the concept that reentry plays a role in the genesis of ventricular fibrillation was "born". The occurrence of ventricular extrasystoles, ventricular tachycardia and ventricular fibrillation following coronary artery occlusion in man was documented in 1921 by Robinson and Hermann.

[B] Sympathetic nervous stimulation and ventricular arrhythmogenesis

Between 1928 and 1934, evidence was presented showing that the sympathetic nervous system played a role in the genesis of ventricular arrhythmias in the normal heart and also during acute myocardial ischemia. Otto in 1927 and Hoff and Nahum in 1934 showed that

catecholamine stimulation evoked formation of ventricular extrasystoles, ventricular tachycardia and ventricular fibrillation in the normal heart, whilst MacEachern (1940) demonstrated that sympathetic neural denervation decreased the incidence of ventricular fibrillation following coronary artery occlusion.

[C] Vulnerable period and ventricular arrhythmogenesis

A major advance in our understanding of ventricular arrhythmogenesis was provided by the superb studies of Wiggers et al (1940) and Wegria et al (1941). Wiggers showed the following: First, ventricular fibrillation could be induced by electrical stimuli applied to the heart during the last 40 to 60 msec of systole and the first 20 msec of diastole. He defined this period which corresponds to the T wave of the electrocardiogram as the vulnerable period. Induced or spontaneous impulses applied during the vulnerable period could evoke ventricular fibrillation. Secondly, the development of acute myocardial ischemia was associated with an increase in the vulnerable period and an enhanced vulnerability to ventricular fibrillation. Thirdly, he proposed that spontaneous ventricular fibrillation could be explained by "release of two successive stimuli, the first inducing a premature contraction and the second inducing fibrillation because it falls on the vulnerable period".

[D] Early and late phase of ischemic ventricular arrhythmias

Harris and associates in their elegant studies in the 1940's and early 1950's illustrated two time-dependent phases of ischemic ventricular arrhythmias and proposed a biochemical basis for the genesis of ischemic ventricular fibrillation. (1) Harris et al (1943; 1950) noted that ventricular arrhythmias following coronary artery occlusion

occur in two phases: (a) an early or immediate phase within the first two hours with maximal incidence in the first 30 mins. These early ventricular arrhythmias frequently terminate in ventricular fibrillation. An intervening quiescent phase, 2-12 hours after coronary artery occlusion; (b) a late or delayed phase, 12 hours after coronary artery occlusion which results in ventricular premature beats but infrequently in ventricular fibrillation. Harris proposed that the electrophysiological mechanism underlying the two phases of ventricular arrhythmias may be different. (2) Harris and associates illustrated a temporal relationship between cellular potassium loss from the ischemic myocardium and occurrence of ischemic ventricular arrhythmias (1954) and also showed that infusion of potassium salts into local areas of the myocardium induced ventricular tachycardia and ventricular fibrillation (Harris et al, 1958). A biochemical basis for ventricular arrhythmogenesis was therefore proposed viz. that potassium ions were arrhythmogenic (Harris et al, 1958).

It was suggested that ventricular extrasystoles were generated from automatic foci in the ischemic myocardial cells bordering on the infarction zone. Harris attributed the excitability of ischemic cells to diastolic injury potential due to K^+ induced depolarization from ischemic cells.

2. ULTRASTRUCTURAL, ELECTROPHYSIOLOGICAL AND BIOCHEMICAL ALTERATIONS DURING ACUTE MYOCARDIAL ISCHEMIA

Over the past twenty years there has been delineation of the ultrastructural, electrophysiological and biochemical alterations in acute myocardial ischemia and correlation of these changes with the early and late phase ventricular arrhythmias. This thesis addresses the

mechanism of early ventricular arrhythmias during acute myocardial ischemia. First, I will review the ultrastructural, electrophysiological and biochemical alterations evident during acute myocardial ischemia in the early phase of ventricular arrhythmias; secondly, I will illustrate the proposed links between biochemical and electrophysiological alterations and early ventricular arrhythmias; thirdly, I will highlight the proposed electrophysiological mechanism of early ventricular arrhythmias and fourthly, I will define the approach undertaken in order to study the role of the metabolic and biochemical factors and transsarcolemmal Na^+ and Ca^{2+} ionic currents in the genesis of ventricular arrhythmias during acute myocardial ischemia.

[A] Ultrastructural alterations

Early changes:

Within minutes of the development of acute myocardial ischemia, ultrastructural alterations are evident in ventricular myocardial cells in the area of ischemia (Sobel, 1974; Jennings & Ganote, 1974). A temporal relationship exists between ultrastructural changes in ischemic ventricular myocardial cells, electrophysiological alterations and the occurrence of early ventricular arrhythmias (Wit & Bigger, 1975). It has, therefore, been proposed that the early ventricular arrhythmias may result from electrophysiological alterations in ultrastructurally abnormal ischemic ventricular myocardial cells (Wit & Bigger, 1975).

[B] Electrophysiological alterations

The onset of acute myocardial ischemia is associated with the development of electrophysiological alterations in the ischemic myocardium which are evident within 30 minutes of acute coronary artery occlusion, i.e. during the early phase of ventricular arrhythmias. Such

electrophysiological alterations have been linked to the occurrence of early ventricular arrhythmias.

(1) Resting membrane potential and phase 0

A decrease in resting membrane potential occurs within 7-10 mins of coronary artery occlusion and may reach -65 to -60 mV (Wit & Bigger, 1975; Downar et al, 1977; Kleber et al, 1978). The decrease in resting membrane potential (depolarization of the ventricular myocardial cell) results in partial or complete inactivation of the fast sodium channel (Weidemann, 1970; Cranefield et al, 1972). The question which is as yet unanswered is whether the upstroke (phase 0) of the depressed ischemic transmembrane action potential is dependent on reduced Na^+ influx through the existing fast channels (i.e. depressed fast response action potentials), or whether the upstroke is dependent on calcium ions flowing through the slow channel (calcium-dependent slow responses). In some studies the upstroke of the action potential show two components (Downar et al, 1977; Russell et al, 1977) suggesting that both Na^+ and Ca^{2+} ions may be responsible for generation of the upstroke of the depressed action potential. Depressed fast responses or slow responses are each associated with slow conduction and conduction block, predispose to reentry and each may thereby induce ventricular arrhythmias (Cranefield, 1975; Wit & Bigger, 1975).

(2) Transmembrane action potential

Within 2 min of coronary artery occlusion there is a decrease in both action potential amplitude and action potential duration (Downar et al, 1977; Kleber et al, 1978; Kardesh, 1977). The decrease in action potential duration is mainly due to shortening of the plateau phase (Downar et al, 1977; Kleber et al, 1978; Russell et al, 1977). Reduction in action potential duration predisposes to shortening of the relative and absolute refractory period and is thereby arrhythmogenic.

(3) Recovery of excitability of refractoriness

The absolute refractory period (i.e. threshold for excitation by applied stimuli) appears to shorten 2 min after ischemia (Brooks, 1960; Han & Moe, 1964; Levites et al, 1976; Niami et al, 1977). This alteration may be linked to shortening of the action potential duration and is theoretically arrhythmogenic.

During later (10 min after ischemia) or severe ischemia, the absolute refractory period appears to lengthen. This alteration appears to be due to development of post-repolarization refractoriness, i.e. the recovery of the ability of a cell to generate an upstroke of an action potential is delayed beyond completion of repolarization (El-Sheriff et al, 1974; Lazzara et al, 1975).

Gettes and Reuter (1974) showed that in cells with reduced resting membrane potentials recovery from inactivation of fast and slow inward currents is markedly delayed. Hence if adjacent ischemic myocardial cells have reduced resting membrane potentials, albeit at different levels, the recovery of cellular excitability is delayed and different, i.e. a dispersion of refractoriness may exist between adjacent ischemic cells.

Dispersion of refractoriness between ischemic cells or between ischemic and non-ischemic myocardial cells predispose to conduction block and hence reentry and is therefore arrhythmogenic.

(4) Conduction

A decrease in conduction velocity occurs such that fragmented electrical activity may persist in the ischemic myocardium for as long as 315 msec after the impulse has entered the area from the adjacent non-ischemic myocardium (Boineau & Cox, 1973). In addition, there is delayed activation of the ischemic myocardium, especially epicardial activation relative to the endocardium and development of conduction block.

[C] Possible electrophysiological mechanisms of early ventricular arrhythmias

(1) Reentry

The occurrence of slow conduction, conduction block and alteration in refractoriness in ischemic myocardial cells are conducive to the formation of reentrant circuits (Cranefield, 1975). It is considered that the ischemic action potential, be it a calcium dependent slow response or depressed fast response, is responsible for slow conduction, conduction block and hence reentry in the ischemic myocardium.

Strong evidence for reentry has been the circumstantial association between ventricular arrhythmias (including ventricular tachycardia and ventricular fibrillation) and prolonged conduction delay in the ischemic myocardium (Janse et al, 1980; 1981). Moreover recent electrophysiological mapping experiments during acute myocardial ischemia indicate that macro-reentrant circuits play the major role in ventricular tachycardia, whereas micro-reentrant circuits are evident during ventricular fibrillation (Janse et al, 1981). Re-entry has, therefore, been proposed as the likely electrophysiological mechanism of early ventricular arrhythmias during acute myocardial ischemia.

(2) Injury current

An alternative hypothesis is that injury current flowing from ischemic to non-ischemic cells across the border zone may evoke ventricular arrhythmias by excitation of non-ischemic cells in the border zone or by generation of micro-reentrant circuits or triggered automaticity in the border zone (Katzung et al, 1975; Janse et al, 1981).

In diastole, the resting membrane potential of ischemic cells is reduced such that the intracellular compartment is positive to the intracellular compartment of normal cells. Intracellular current flows from ischemic to non-ischemic cells thereby generating current source in

the extracellular space of normal cells. (During systole current flow is opposite). If there is slow conduction in the ischemic myocardium and an area of inexcitability exists, such as the border zone, then current flow from ischemic cells to non-ischemic cells may reach non-ischemic cells at a time that they have repolarized. Hence excitation of normal cells or generation of micro-reentrant circuits in the border zone may occur and produce ventricular arrhythmias. Alternatively, current flow may induce formation of afterdepolarizations in the border zone which in turn may evoke triggered automaticity and ventricular arrhythmias (Cranefield, 1977).

[D] Biochemical and metabolic alterations

The onset of acute myocardial ischemia is associated with the development of metabolic and biochemical alterations in the ischemic myocardium within 30 min of the onset of ischemia, i.e. during the early phase of ventricular arrhythmias. These metabolic and biochemical alterations have been linked to the development of slow response action potentials or depressed fast response action potentials, i.e. abnormal action potentials presumed to be responsible for the slow conduction and conduction block present in the ischemic myocardium and held to underlie the development of reentry. Reentry is the likely electrophysiological mechanism of early ventricular arrhythmias. A biochemical basis for ventricular arrhythmogenesis has therefore been proposed.

I will first illustrate the biochemical and metabolic alterations evident during acute myocardial ischemia and then demonstrate their proposed link to electrophysiological alterations recorded in the ischemic myocardium.

(1) Energy depletion, lactate accumulation, metabolic and respiratory acidosis

In the acutely ischemic myocardium there is total or partial cessation in the extracellular delivery of oxygen and substrate, i.e. glucose, free fatty acid or lactate (Opie, 1978). High energy phosphate depletion occurs within 2 min of the onset of ischemia due to inhibition of O_2 dependent mitochondrial energy production. Anaerobic metabolism is stimulated in order to produce energy; consequently there occurs: (1) cellular accumulation of the end product of anaerobic metabolism - lactate; levels rise within 2 min with peak values evident 20 min after the onset of ischemia (Opie et al, 1973); (2) metabolic acidosis due to (a) proton formation generated during hydrolysis of ATP (Gevers, 1977), (b) poor tissue washout. A fall in pH occurs within 15 min of ischemia, pH 7.4 to 5.5 in the pig (Hirche, 1980) and 7.4 to 6.0 in the baboon (Bruyneel, 1972); (3) accumulation of pCO_2 and the development of respiratory acidosis (Khuri et al, 1975).

(2) Free fatty acid metabolism

Due to O_2 lack, there is cessation or reduction in myocardial mitochondrial metabolism of free fatty acids with accumulation of free fatty acids and their metabolites, i.e. acyl CoA and acyl carnitine. In addition the elevated levels of circulating catecholamines hydrolyse triglycerides in peripheral adipose tissue, initially increasing plasma free fatty acid levels and subsequently acyl CoA and acyl carnitine. The effect of accumulation of free fatty acids and metabolites, i.e. CoA and acyl carnitine are: (i) reduction in energy formation due to failure to metabolise free fatty acids as fuel (Opie, 1978); (ii) inhibition of energy dependent enzyme systems, eg. Na^+/K^+ ATPase, Ca^{2+} ATPase; (iii) accumulation of lysophosphoglycerides (Sobel et al, 1978) due to high levels of acyl CoA and acyl carnitine ^{and acidosis} /inhibiting enzymes responsible

for degradation of lysophosphoglycerides.

(3) Na⁺, K⁺ and Ca²⁺

Energy depletion and accumulation of lysophosphoglycerides in the ischemic myocardium each inhibit energy dependent processes, eg. Na⁺/K⁺ ATPase (Opie, 1979). This results in intracellular Na⁺ accumulation, cellular K⁺ loss and extracellular K⁺ accumulation. Extracellular K⁺ accumulation occurs after 1 min of ischemia, peak levels between 10 to 16 mM/l are evident within 20 min of ischemia. There is also intracellular Ca²⁺ accumulation due to (a) intracellular Na⁺ accumulation, (b) inhibition of Na⁺/Ca²⁺ exchanger and (c) inhibition of energy dependent Ca²⁺ ATPase.

(4) Catecholamines

Acute myocardial ischemia is associated with elevation in the circulating levels of catecholamines (Jewitt et al, 1969); an increase is evident within 30 secs and peak levels occur 40 min after coronary artery occlusion (Karlsberg et al, 1979). There is probably also local release of noradrenaline from the ischemic myocardium (Hirche, 1980), the level in the interneuronal cleft may reach 10⁻⁵ M within 60 min of coronary occlusion (Dietz et al, 1981).

Enhanced adrenergic activity results in stimulation of both the alpha and beta adrenergic receptors.

[E] Arrhythmogenic potential of the biochemical and metabolic changes

The development of acute myocardial ischemia is thus associated with accumulation of the following biochemical and metabolic factors in the ischemic myocardium during the early phase of ventricular arrhythmias: (1) extracellular K⁺ (2) noradrenaline (3) cyclic AMP (4) free fatty acid and activated long chain metabolites (5) lysophosphoglycerides (6) metabolic acidosis (7) respiratory acidosis (8) lactate. The

arrhythmogenic potential of these metabolic and biochemical alterations is now described.

(1) Potassium

The development of acute myocardial ischemia is associated with cellular potassium loss and extracellular K^+ accumulation (Thomas et al, 1970). Harris initially showed a temporal relationship between cellular K^+ loss from the ischemic myocardium and occurrence of ventricular arrhythmias (Harris et al, 1943). Subsequently several workers found that infusion of K^+ into the circulation resulted in ventricular arrhythmias and ventricular fibrillation. Potassium ions have therefore been implicated in ventricular arrhythmogenesis.

Arrhythmogenic potential

Extracellular K^+ accumulation decreases the resting membrane potential and thereby inactivates either partially or completely the fast sodium channel and predisposes to the development of depressed fast response action potentials or slow response action potentials, i.e. action potentials which predispose to reentrant ventricular arrhythmias.

(2) Catecholamines

The development of acute myocardial ischemia is associated with enhanced adrenergic activity.

(a) Beta adrenergic receptor stimulation and cyclic AMP

Cyclic AMP, the proposed intracellular second messenger of beta adrenoceptor stimulation, accumulates in the ischemic myocardium within 10 to 30 min of coronary artery ligation. Podzuweit and associates (1976) have proposed a hypothesis linking this accumulation of cyclic AMP to the development of serious ventricular arrhythmias, such as ventricular fibrillation. That cyclic AMP is an arrhythmogenic agent is supported by the evidence summarized as follows:

(I) Cyclic AMP accumulates in ischemic tissue before the onset of

- ventricular fibrillation in baboon, cat and pig models (Podzuweit, 1978);
- (II) the effects of isoproterenol in increasing tissue cyclic AMP and precipitating ventricular fibrillation in an otherwise stable pig heart model with a small lateral infarct (Muller, 1981);
- (III) the dose-response curve linking increases in tissue cyclic AMP with the decrease in the ventricular fibrillation threshold in the isolated rat heart model (Lubbe et al, 1976, 1978);
- (IV) the shift of the dose-effect curve to the right and the delay in the rise of tissue cyclic AMP during beta-1 adrenergic stimulation by the beta-1 adrenergic antagonist agent, atenolol (Lubbe et al, 1978);
- (V) the opposite effects of theophylline;
- (VI) the effect of dibutyryl cyclic AMP in precipitating spontaneous arrhythmias in the coronary ligated rat heart (Opie, 1979);
- (VII) the effect of coronary artery ligation in the rat heart in elevating tissue cyclic AMP and in decreasing the fibrillation threshold (Thandroyen, 1982);
- (VIII) the effect of infusions of dibutyryl cyclic AMP into the edge of the infarct in provoking arrhythmias (Podzuweit et al, 1978); and
- (IX) the electrophysiological properties of dibutyryl cyclic AMP especially in the presence of a high external K^+ (Opie et al, 1979).

Arrhythmogenic potential

The following effects of cyclic AMP may contribute to the development of ventricular arrhythmias.

- (I) In partially depolarized fibres cyclic AMP promotes formation of slow response action potentials by enhancing transsarcolemmal calcium influx (Schneider & Sperelakis, 1975; Vogel & Sperelakis, 1981).
- (II) Cyclic AMP may elicit the occurrence of afterdepolarizations in non-automatic cells (Lazzara et al, 1978a; Daries et al, 1981).
- (III) Cyclic AMP may induce electrical uncoupling of cells in hypoxic

muscle (Wojtczak, 1979).

(IV) Cyclic AMP may enhance phase 4 depolarization (Danilo et al, 1978).

(b) Alpha₁ adrenoceptor stimulation

Evidence that alpha₁ receptor stimulation plays a role in ventricular arrhythmogenesis is based on the following findings:

(I) Alpha₁ receptor number increase in the ischemic myocardium within 30 min of acute myocardial ischemia (Corr et al, 1981a) during the phase of early ventricular arrhythmias.

(II) An Alpha₁ adrenoceptor antagonist, prazosin, prevents the early ventricular arrhythmias in the feline model of coronary artery ligation (Sheridan et al, 1980).

Arrhythmogenic potential

The following effects of alpha₁ adrenergic stimulation may contribute to the development of ventricular arrhythmias:

(I) Alpha₁ stimulation may evoke formation of slow response action potentials in partially depolarized cells (Scholz, 1980).

(II) Alpha₁ stimulation may enhance idioventricular automaticity (Sheridan et al, 1980).

(3) Free fatty acids

Oliver et al (1968) illustrated that during acute myocardial infarction patients with the highest levels of plasma free fatty acids were more prone to develop severe ventricular arrhythmias. However, experimental investigation has failed to illustrate a direct arrhythmogenic role of free fatty acid in porcine (Most et al, 1977), canine (Opie et al, 1968) and rat (Didier et al, 1980) models subject to coronary artery ligation.

(4) Lysophosphoglycerides

A role for lysophosphoglycerides in the genesis of ventricular

arrhythmias is based on the following considerations: (a) tenfold lower concentrations of LPG than those calculated to occur in the ischemic myocardium (1.65 mM)(Gross et al, 1982) induce electrophysiological alterations in vitro similar to those recorded in the ischemic myocardium (Corr et al, 1979; Corr et al, 1981b); (b) lysophosphoglycerides induce ventricular arrhythmias in normoxic ventricular muscle (Man & Choy, 1982).

Arrhythmogenic potential

Lysophosphoglycerides may contribute to the development of ventricular arrhythmias by the following electrophysiological mechanisms (Corr et al, 1979, 1981b):

- (I) formation of slow response action potentials;
- (II) decreasing the action potential duration;
- (III) eliciting abnormal automaticity.

(5) Acidosis

Both metabolic (Opie, 1978) and respiratory acidosis (Khuri et al, 1975) occur in the ischemic myocardium. A direct relation between ventricular fibrillation and metabolic or respiratory acidosis has, as yet, not been defined.

Arrhythmogenic potential

- (I) Severe metabolic acidosis (pH 6.1) may induce slow responses in Purkinje fibres (Davis et al, 1976).
- (II) Respiratory acidosis in the presence of extracellular K^+ accumulation evokes formation of slow responses (Weiss & Shine, 1981).
- (III) H^+ ion accumulation increases internal longitudinal resistance and produces electrical uncoupling of cells (Wojtczak, 1979).

(6) Lactate

Acute myocardial ischemia results in anaerobic glycolysis and hence accumulation of end product, lactate. A relation between elevated lactate levels and ventricular fibrillation has not been shown in the pig

(Muller, 1981), baboon (Podzuweit et al, 1978) and rat (Thandroyen, 1983) models.

Arrhythmogenic potential

Lactate may decrease action potential duration and also enhance phase 4 depolarization (Wissner, 1974).

[F] Link between biochemical, metabolic and electrophysiological changes

Thus in the first 30 min of acute myocardial infarction there are marked metabolic and biochemical alterations which may induce the following electrophysiological changes: (1) decrease in action potential duration, (2) generation of slow response action potentials or possibly depressed fast response action potentials, (3) afterdepolarizations.

(1) Decrease in action potential duration

Elevation in free fatty acid and lactate levels and reduction in glycolytic and mitochondrial ATP production respectively in the ischemic myocardium each theoretically may shorten the action potential duration. Shortening of the action potential duration ^{may} decrease the absolute and effective refractory period and may therefore be arrhythmogenic. However, in experimental studies neither elevated lactate nor free fatty acids have been linked to the initiation of ventricular arrhythmias; however, glucose provision may possibly be antiarrhythmic. Current evidence therefore does not provide much support for the abovementioned mechanism in the genesis of early ventricular arrhythmias.

(2) Slow response action potential or depressed fast response action potential

The development of acute myocardial ischemia is associated with elevation of extracellular K^+ ion concentration. There is a putative role for K^+ in ventricular arrhythmogenesis viz. reduction in resting

membrane potential with partial or complete inactivation of the fast sodium channel. If there is partial inactivation the ischemic action potential is a depressed fast response, i.e. upstroke dependent on transsarcolemmal Na^+ inward current.

If there is complete inactivation of the Na^+ channel then there is a putative role for cyclic AMP, enhanced α_1 receptor stimulation in ventricular arrhythmogenesis viz. enhanced transsarcolemmal Ca^{2+} current with formation of slow responses. Both slow responses and depressed fast responses predispose to reentry which is the currently considered electrophysiological mechanism of early ventricular arrhythmias.

(3) Afterdepolarizations

Intracellular calcium overload may occur in the ischemic myocardium as a consequence of enhanced adrenergic stimulation, lysophosphoglyceride stimulation, or by inhibition of $\text{Na}^+-\text{Ca}^{2+}$ exchanger. Recently cytosolic calcium overload has been shown to induce a transient non-specific inward leak of Na^+ ions with resultant development of delayed afterdepolarizations (Kass et al, 1979; Colquhoun et al, 1981). Such delayed afterdepolarizations may in turn induce ventricular arrhythmias of the triggered automaticity type (Cranefield, 1977).

Theoretical considerations therefore suggest a further putative role for calcium ions, viz. evoking afterdepolarization induced triggered automaticity.

[G] Investigation of the hypothesis of ventricular arrhythmogenesis

The hypothesis assumed is that the metabolic and biochemical sequelae of acute myocardial ischemia induce slow response action potentials or depressed fast response action potentials which predispose

to reentry and hence ventricular fibrillation. This thesis investigates the role of (a) transsarcolemmal calcium and sodium ionic inward currents (held to underlie slow responses or depressed fast responses), (b) metabolic and biochemical factors in ventricular arrhythmogenesis during acute myocardial ischemia.

The isolated Langendorff perfused rat heart was chosen as the experimental model because of the advantages of being able to evaluate in depth the role of (a) transsarcolemmal sodium and calcium inward currents, (b) biochemical and metabolic factors and (c) adrenergic receptor mediated influences on ventricular arrhythmogenesis.

(1) Slow response action potential

If calcium dependent slow responses underlie early ventricular fibrillation then:

(I) Calcium antagonist agents which inhibit transsarcolemmal calcium inward current should prevent the occurrence of calcium dependent slow responses and thereby prevent early ventricular fibrillation.

(II) Beta-adrenoceptor antagonist agents which prevent accumulation of cyclic AMP, should inhibit the cyclic AMP-mediated slow inward calcium current and thereby prevent slow responses and early ventricular fibrillation.

(III) Alpha-adrenoceptor antagonist agents which prevent the transsarcolemmal calcium inward current and possibly alter intracellular calcium distribution, should inhibit slow responses and early ventricular fibrillation.

(2) Depressed fast responses

If depressed fast responses underlie early ventricular fibrillation then:

Fast channel blocking agents which inhibit the transsarcolemmal sodium inward current should prevent depressed fast responses and thereby inhibit the occurrence of ventricular fibrillation.

CHAPTER 2EXPERIMENTAL MODEL, MATERIAL AND METHODS1. EXPERIMENTAL MODEL[A] Isolated Langendorff perfused rat heart

The experimental model chosen to study the mechanism of ventricular fibrillation during acute myocardial ischemia was the isolated Langendorff perfused rat heart model. The experimental animal model devoid of atherosclerotic coronary artery disease, lacking in myocardial fibrosis, subject to acute coronary artery ligation and species specific does not therefore exactly mimic the pattern of acute myocardial infarction in man. Nevertheless, the principles involved, irrespective of the animal model used, appear relevant in order to gain further insight into the mechanism of ventricular arrhythmogenesis.

[B] Advantages of the model

1. Obviation of anaesthesia and therefore the anaesthetic induced changes in blood pressure, heart rate and cardiac output.
2. This model is devoid of hormonal and homeostatic reflexes operative in intact animals which may produce spontaneous and variable alterations in the vulnerability to ventricular fibrillation.
3. Ventricular fibrillation usually reverts spontaneously to sinus rhythm. Thus repeated measurements of ventricular fibrillation threshold can be undertaken in the same heart.
4. Electrical conversion of ventricular fibrillation to sinus rhythm is frequently unnecessary and therefore the damage caused

to the heart by application of large amount of electrical current is obviated.

5. The electrical current applied to the isolated rat heart is small, the mean ventricular fibrillation threshold value of 0,15 mJ required (Marzagao, 1974; Lubbe et al, 1975) is equivalent to 1000th of the mean value of 0,3 J required in the open chest dog model (Allen et al, 1971; Wolff et al, 1968). Thus small and subtle changes in the ventricular fibrillation threshold can be measured in this preparation, changes not possible in the intact large animal.
6. The rat is a small animal allowing for cheap accommodation and plentiful numbers.
7. The substrate supply of the heart can be maintained constant throughout the experiments. Moreover, hormones and pharmacological agents can be added to the perfusate and a specific concentration of an agent or hormone can be delivered to the myocardium.
8. Alteration of the ionic milieu (K^+ or Ca^{2+}) or substrate (glucose, free fatty acid) in the perfusate is easily achieved.
9. The biochemical and metabolic profile during acute myocardial ischemia can be investigated in depth and correlated with ventricular fibrillation.
10. The haemodynamic function of the heart can be correlated with ventricular fibrillation.

[C] Disadvantages of the model

1. The isolated heart is devoid of autonomic nervous control.
2. There is abnormally high coronary flow rate due to the absence of haemoglobin in the perfusate.

3. There is no peripheral circulation.
4. The retrograde aortic perfused heart performs no volume work.
5. The rat's action potential duration is narrow and does not vary with heart rate (Ypma, 1972). This is considered to be due to greater intracellular cycling of calcium.
6. As in any model, effects may be species specific.

[D] Previous use of this model studying electrically induced ventricular fibrillation

Marzagao (1974) showed that the isolated perfused rat heart was a suitable and stable model for study of the ventricular fibrillation threshold.

- (a) The vulnerability to ventricular fibrillation follows the trend described in large animal species.
- (b) The threshold values had a log normal distribution.
- (c) The energy requirements for induction of ventricular fibrillation were similar, notwithstanding difference in stimuli duration, viz. 2 or 10 msec respectively.
- (d) The heart rate, ventricular fibrillation threshold and left ventricular pressure did not change significantly over 3 hours.

[E] Criticism of the ventricular fibrillation threshold

Vulnerability to ventricular fibrillation is not necessarily synonymous with spontaneous ventricular fibrillation and therefore one cannot directly extrapolate changes in the vulnerability to fibrillation to changes in spontaneous ventricular fibrillation. Vulnerability to ventricular fibrillation was proposed by Wiggers and co-workers (1940) to be a specific and an inherent property of the myocardium whereas Dawes (1952) considered it to represent a measurement of myocardial

excitability. However, Bacaner (1968), Allen et al (1971) and Gerstenblith et al (1972) proposed that alterations in ventricular fibrillation threshold may be accepted as a measure of the efficacy of an antiarrhythmic agent. Previously Garrey (1914) proposed that the induction of ventricular fibrillation in myocardial tissue less than 1g was not possible, but subsequently Willebrands and co-workers (1973) were able to evoke ventricular fibrillation in the rat and mouse heart using tissue less than 1 gram in weight and size.

2. MATERIALS

[A] Animals

Long-Evans male rats (wt 250-350 g) were used for this study. They were anaesthetised in a glass chamber with ether, removed from the glass chamber, placed on a board, an incision made in the right groin to expose the femoral vein and 200 units of heparin injected via the femoral vein; after 30 seconds the chest was opened and the heart removed by cutting the great vessels. The heart was immediately placed in ice-cold Krebs-Henseleit solution to produce arrest.

[B] Perfusion system and perfusate

The aorta was mounted onto a cannula and perfused at 100 cm water pressure according to the technique devised by Langendorff in 1895. The time duration from opening of the chest to mounting of the aorta and perfusion of the heart ranged between 45 and 90 seconds. Perfusion fluid was Krebs-Henseleit solution, NaCl 118,5 mM, KCl 4,75 mM, CaCl₂ 2,50 mM, KH₂PO₄ 1,19 mM, Mg SO₄ 1,19 mM and NaHCO₃ 25,0 mM; substrate glucose 11 mM (Krebs & Henseleit, 1932).

This solution was aerated with 95% oxygen and 5% CO₂ and

maintained at a pH of 7.4. The temperature throughout the system and surrounding the heart was maintained constant at 37°C by way of a water jacket around the glassware containing the perfusion fluid. The water was maintained at 37°C by a thermostat apparatus, Braun Thermal Mix 2 apparatus from Germany. The pO_2 , pH, sodium and potassium concentrations were checked and were found to be within the normal limits. Measurements of the pH, pO_2 and pCO_2 were based on the method of Astrup using a microelectrode unit type E5021 Radiometer, Copenhagen.

[C] Electrocardiogram recording

Continuous electrocardiograms were recorded from one electrode on the metal aortic perfusion cannula and a 1 cm steel wire soldered to the thin flexible insulated copper wire inserted superficially into the free wall of the right ventricle. The electrocardiogram potential was amplified by an ETC 8 channel of the Electronics for Medicine Photographic Recorder.

The electrocardiogram with the square wave indicating the trigger was displayed continuously on the oscilloscope screen of the photographic recorder at a speed variable from 25-100 mm/sec and also displayed on the Electronics for Medicine Recorder.

[D] Electrical system

The electrocardiogram potential was amplified by an ETDA channel of the Electronics for Medicine Recorder. This channel has a tachometer and provides a synchronous output pulse. This output is coupled to a control box which counts the incoming synchronous pulse. After manual activation this trigger box delivers 7 pulse beats and on the seventh it starts the horizontal sweep of a storage oscilloscope (Type 549 Tektronix

storage oscilloscope), whilst the 8th beat activates a Grass S88 stimulator (Grass Instrument Co, Massachusetts). Once activated, the stimulator delivers a square wave pulse of pre-selected duration and delay to a constant current unit (Grass Constant Current Unit, model CCULA) which allows variation of the voltage according to the resistance between the electrodes enabling delivery of a controlled energy impulse. The voltage then goes to a stimulus isolation unit which assesses and enables accurate delivery of the proposed current to the heart.

[E] Left ventricular stimulation

Two thin platinum wire electrodes inserted for electrical stimulation were placed horizontal and parallel at the apex and base of the left ventricle respectively 10 mm apart. This distance was chosen on the basis of previous work (Marzagao, 1974). The electrode placement was such that no damage to coronary vessels occurred. The cathode was placed at the base just under the atrioventricular groove and the anode inserted at the apex.

3. METHODOLOGY

[A] Ventricular fibrillation threshold

Ventricular fibrillation threshold was obtained by the train method of electrical stimulation. This consists of delivery of 10 square wave pulses distributed evenly over the duration of 200 msec stimulation and commenced after a delay of 10 msec after the trigger obtained from the initial pulse of the R wave. Each pulse duration was 20 msec consisting of 2 msec impulse and 18 msec delay between the pulses. The train method differs from the single stimulus method for measurement of ventricular fibrillation threshold. The single stimulus method of

electrical stimulation involves input of a single pulse progressively scanning the T wave by increasing the delay from the triggering point of the QR wave. Experiments (in our laboratory) have demonstrated there to be no difference in ventricular fibrillation threshold levels obtained by either train or single stimulus method of electrical stimulation.

[B] Criteria for ventricular fibrillation and measurement of ventricular fibrillation threshold

Ventricular fibrillation was defined as repetitive irregular ventricular rhythm which persisted for more than 6 cycles. This pattern was associated with profound lowering of the left ventricular pressure with loss of the left ventricular pressure wave, thus fulfilling the criteria required for ventricular fibrillation, namely, electrical abnormality of the heart associated with mechanical failure. Ventricular fibrillation usually reverts spontaneously to sinus rhythm. If ventricular fibrillation persisted for longer than 15 sec hearts were defibrillated using ice-cold Krebs-Henseleit solution or if this failed, by DC electrical conversion. Ventricular fibrillation threshold value was expressed in mJoules as this is most representative because it incorporates the current, the voltage and stimulus duration permitting acceptable comparison of values obtained in same or different species, even when different stimulation voltages are required. The ventricular fibrillation threshold was estimated using an initial current of 4,0 mA and progressively increasing the current in steps of 2,0 mA until ventricular fibrillation occurred. The ventricular fibrillation threshold was defined as the lowest current required to produce ventricular fibrillation on more than 2 occasions. The ventricular fibrillation threshold values in control hearts ranged between 5 mA and 13 mA with the mean value approximately 9 mA.

[C] Protocol prior to coronary artery ligation

After mounting the heart, there was a stabilization period of 15 min after which the coronary flow rate, heart rate and ventricular fibrillation threshold was measured in each experiment. The coronary flow rate was measured by collecting the coronary effluent in a graduated measuring tube over 30 to 60 sec. Coronary flow rates for control hearts ranged from 6 to 13 ml/min depending on the size of the heart; the heart rate ranged between 200 to 300 beats/min. The ventricular fibrillation threshold levels were between 5 to 13 mA; hearts with thresholds above or below these levels were excluded from the study.

After an intervening period of 10 minutes, repeat coronary flow rates, heart rates and ventricular fibrillation threshold levels were measured.

In those series in which pharmacological agents were evaluated, the protocol was identical except that the agent was infused or perfused during the 10 min intervening period and subsequently throughout the experiment.

[D] Protocol during coronary artery ligation

Acute regional myocardial ischemia of the left ventricle was produced by ligation of the left main coronary artery within 2 mm of where it emerges below the left atrium (Kannengiesser et al, 1975). Successful ligation of the coronary artery was evident when there was reduction in the total coronary flow rate by at least 25% and a grey blue discoloration developed over the left ventricular free wall. Heart rate was recorded continuously and coronary flow rates measured two to four times during the 15 min period of ischemia. The ventricular fibrillation threshold was measured during the first 15 minute period of acute myocardial ischemia because the trough or nadir of the ventricular

fibrillation threshold is reached 10 to 15 minutes after coronary artery ligation. In a separate series of experiments, the ventricular fibrillation threshold was evaluated during the 15 minute period of acute regional myocardial ischemia with added adrenergic stimulation i.e. adrenaline 5×10^{-7} M. This series of experiments was undertaken because the development of acute myocardial ischemia in the in vivo situation is associated with (i) an increase in circulating level of catecholamines, principally adrenaline (peak concentration in man 5×10^{-9} M, (Jewitt et al, 1969)) and (ii) release of noradrenaline in the ischemic myocardium (peak concentration in animal 10^{-5} M (Dietz et al, 1981)). The chosen concentration of adrenaline viz 5×10^{-7} M therefore provides adequate adrenergic stimulation. At the end of the experiment, disulphan blue dye was introduced into the cannula immediately above the heart. This dye combines with oxygen, hence the non-ischemic myocardium is stained blue and the ischemic myocardium is unstained. The heart was then freeze-clamped with Wollenberger clamps (1960) pre-cooled in liquid nitrogen for biochemical analysis. Separation of the ischemic and non-ischemic tissue was then undertaken.

[E] Chemicals

All drugs were used in the form of racemic compounds, the exceptions being the isomers of verapamil and of propranolol.

Drug	Form	Obtained from
verapamil hydrochloride	solution	Knoll, West Germany
d(+), l(-) isomers of verapamil hydrochloride	powder	Dr W G Nayler, Australia
nifedipine hydrochloride	powder	Bayer Chemicals, West Germany
diltiazem hydrochloride	powder	Tanabe Seikayu, Tokyo
metoprolol tartrate	powder	Ciba Geigy, South Africa
atenolol	powder	ICI, South Africa
propranolol	powder	ICI, South Africa
d(+) isomer of propranolol	powder	ICI, South Africa
prazosin	powder	Pfizer, South Africa
yohimbine hydrochloride	powder	Sigma, USA
phentolamine mesylate	solution	Boots, South Africa
reserpine	solution	Ciba, South Africa
lignocaine hydrochloride	solution	Keatings, South Africa
tetrodotoxin	powder	Sigma, USA
adrenaline	powder	Taeber & Corsen, S Africa

[F] Statistical methods

Results are expressed as mean \pm standard error of the mean for the number of the experiments. The number (n) of hearts in each series ranged from 5 to 16. Probability [p] values were calculated with either (a) one way or two way analysis of variance or (b) students t test (paired or unpaired) using two-tailed values corrected for unequal variances. Probability values less than 0,05 indicated significant difference between mean values.

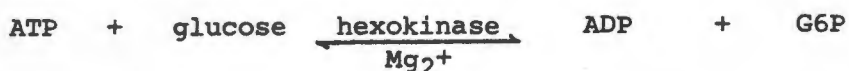
[G] Biochemical analysis

Hearts were freeze-clamped with Wollenberger clamps and freeze dried for 48 hours in an Edwards Modulyo freeze-drier. The freeze-dried hearts, weighing approximately 100 mg were homogenised in 6% perchloric acid with an Ultra-turrax homogeniser. The extracts were centrifuged and the supernatant neutralised with tris/KOH buffer to pH 7,0 and used for analysis.

Adenosine triphosphate (ATP), phosphocreatine (PCr) were assayed in neutralised extracts. All compounds were assayed enzymatically by spectro-photometric methods. Standards, internal standards and blanks were included in every assay.

1. Adenosine triphosphate (Lampbrecht and Trautschold, 1974)

The assay is based on the following reaction:



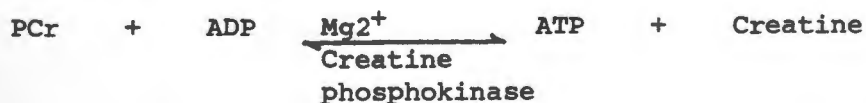
The change in extinction at 340 nm was noted on the addition of hexokinase 5 μ l (10 mg/ml) to 0,2 ml tissue extract, mixed with 2,8 ml assay medium, containing:

MgCl₂ 0,10 ml; tris buffer 0,2M (pH 7,5) 1,00 ml; NADP 1% w/v 0,10 ml; glucose 100 mM 0,05 ml; H₂O dist 1,55 ml; G6PDH 1 mg/ml 0,005 ml.

0,05 ml ADP (10mM) is then added to provide sufficient ADP for subsequent assay of PCr and any further change in extinction is noted.

2. Phosphocreatine (Lampbrecht and Trautschold, 1974)

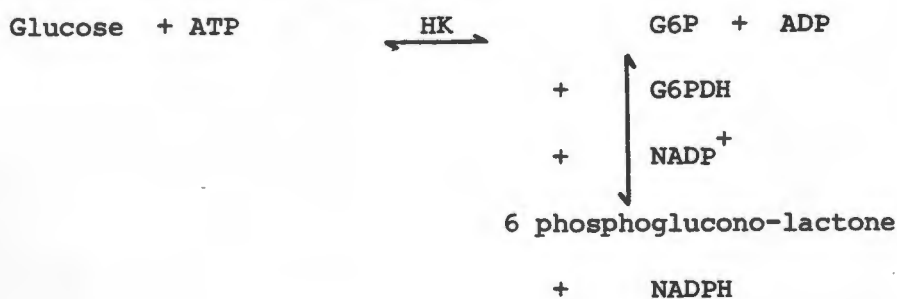
The assay is based on the following reaction:



The further change in extinction at 340 nm (on addition of 10 μ l creatine phosphate kinase (10 mg/ml) after 30 min is proportional to the amount of PCr present.

3. Glycogen (Good et al, 1933)

The glycogen level was determined by the method described by Good et al (1931). The tissue is first digested with 40% KOH and heat. The glycogen in the digested tissue was precipitated with cold ethanol. The glycogen is hydrolysed to its glucose components with 2N HCl and heat (96°C). The extract was then neutralised and assayed enzymatically by a spectrophotometric method. Standards, internal standards and blanks were included in every assay. The assay is based on the following reaction using hexokinase (HK) and glucose 6 phosphate dehydrogenase (G6PDH).

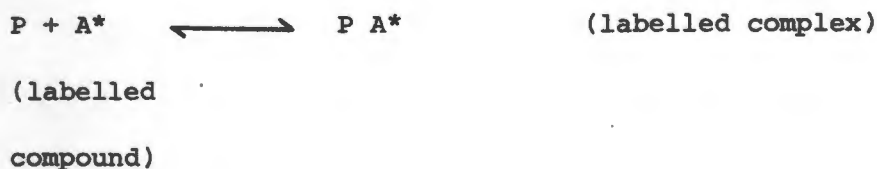


The change in extinction at 340 nm was noted at the addition of HK and G6PDH to tissue extract and 2,8 ml of assay medium. The increase in NADPH measured, is proportional to the amount of glucose present thus glycogen is originally present.

4. Cyclic AMP

Cyclic AMP (cAMP) was measured by the method described by Tovey et al (1974). This competitive protein binding assay is based on the following principle:

If a stable compound A is introduced into a system which contains a constant amount of radioactive compound A* and is binding to protein P, A will displace A* from the protein binding sites, in proportion to its concentration.





The amount of radioactivity bound in the labelled complex decreases as the amount of unlabelled compound is increased. Cyclic AMP in the sample and a fixed quantity of tritium labelled cyclic AMP compete for binding to a protein with high specificity and affinity for cyclic AMP. The amount of labelled cyclic AMP - protein complex formed is inversely proportional to the amount of unlabelled cyclic AMP in the sample. The unbound nucleotide is removed by a precipitation reaction using activated charcoal. A supernatant, containing the bound nucleotide is obtained by centrifugation and removed for scintillation counting. A standard curve is constructed. C_0/C_x is plotted against concentration of cyclic AMP in the standard dilutions. C_0 : labelled nucleotide bound in the absence of unlabelled nucleotide. C_x : labelled nucleotide bound in the presence of a standard quantity of unlabelled nucleotide.

Assay kit contains: Tris/EDTA buffer; purified bovine muscle protein; ($8\text{-}^3\text{H}$) cyclic AMP; cyclic AMP standard; charcoal absorbent. (All in freeze-dried form; the Radiochemical Centre, Amersham, England).

CHAPTER 3CATECHOLAMINE INDUCED VENTRICULAR FIBRILLATION1. RATIONALE FOR INVESTIGATION OF CATECHOLAMINE INDUCED VENTRICULAR FIBRILLATION

Several lines of evidence link enhanced adrenergic activity to the genesis of ventricular fibrillation during acute myocardial infarction (Ceremuzynski et al, 1969; Jewitt et al, 1969). Adrenaline induces myocardial electrophysiological alterations similar to those present during acute myocardial infarction (Gilmour & Zipes, 1980) and also increases the myocardial vulnerability to ventricular fibrillation (Lubbe et al, 1978).

A logical approach in our understanding of ventricular arrhythmogenesis during acute myocardial infarction is to first define the mechanism of catecholamine-induced ventricular fibrillation. Lubbe and co-workers (1978) proposed that cyclic AMP, the reputed intracellular second messenger of beta-receptor stimulation, mediates the arrhythmogenic effect of adrenergic stimulation. Cyclic AMP promotes calcium ion influx during the slow phase of the action potential (Schneider & Sperelakis, 1975; Vogel & Sperelakis, 1981); hence a further implication has been that calcium ions play a role in the genesis of ventricular fibrillation, possibly by induction of abnormal slow responses or by afterdepolarizations.

If this hypothesis is correct then it might be anticipated that:

- 1) beta-adrenoceptor antagonist agents should prevent catecholamine induced ventricular fibrillation by inhibiting the accumulation of cyclic AMP;

2) calcium antagonist procedures should prevent catecholamine induced ventricular fibrillation by inhibiting the transsarcolemmal calcium ion influx.

I therefore investigated the effect of beta-adrenoceptor antagonist procedures and calcium antagonist procedures on adrenaline induced ventricular fibrillation in the non-ligated heart.

2. RESULTS

[A] Control series

In 8 control hearts the mean heart rate was 250 ± 10 beats/min, the average coronary flow was 9.0 ± 0.5 ml/min and the ventricular fibrillation threshold was 10.0 ± 0.3 mA. Biochemical analysis revealed the following metabolic status: adenosine triphosphate 3.9 ± 0.2 $\mu\text{mol/g}$, phosphocreatine 4.5 ± 0.6 $\mu\text{mol/g}$ and cyclic AMP 0.44 ± 0.02 nmol/g.

[B] Concentration response of adrenaline (Table 3-1)

The effect of adrenaline 5×10^{-8} M to 5×10^{-7} M on heart rate, coronary flow rate, ventricular fibrillation threshold, cyclic AMP and high energy phosphate is depicted in Table 3-1. Adrenaline caused a concentration dependent increase in both the coronary flow rate and heart rate; the maximum increment in the coronary flow occurred with 5×10^{-7} M. Adrenaline 5×10^{-8} M to 5×10^{-7} M evoked a concentration dependent reduction in the ventricular fibrillation threshold and a corresponding increment in the tissue levels of cyclic adenosine monophosphate.

According to the dose response curve of adrenaline 5×10^{-8} M to 5×10^{-7} M, adrenaline 5×10^{-7} M caused (i) the greatest reduction in ventricular fibrillation threshold $10.0 \pm 0,3$ to $3.1 \pm 0,8$ mA

(p < 0.0001), (ii) maximum increase in the tissue level of cyclic AMP, 0.44 ± 0.02 to 0.91 ± 0.03 nmol/g (p < 0.001), (iii) depletion of ATP content 3.9 ± 0.2 to 3.1 ± 0.3 μ mol/g (p < 0.05) and (iv) loss of myocardial glycogen stores 14 ± 2 to 2.0 ± 0.2 μ mol glucose equivalent/g (p < 0.001)(Fig 3-2).

[C] Effect of beta-adrenoceptor antagonism on adrenaline-induced changes

Atenolol 5×10^{-5} M produced a greater than 10-fold shift to the right of the log concentration effect curves for isoproterenol on heart rate thereby indicating effective beta-adrenoceptor antagonism (Fig 3-1). Atenolol 5×10^{-5} M inhibited the increase in heart rate and coronary flow rate and also prevented the fall in the ventricular fibrillation threshold during adrenaline 5×10^{-7} M stimulation (Table 3-2); in addition atenolol 3.8×10^{-5} M inhibited the adrenaline induced cyclic AMP accumulation (Lubbe et al, 1978).

[D] Effect of beta-adrenoceptor antagonism on dibutyryl cyclic AMP induced changes (Table 3-3)

Dibutyryl cyclic AMP 1.5×10^{-4} M, the analogue of cyclic AMP which enters the cell more easily, decreased the ventricular fibrillation threshold from 7.7 ± 1.3 to 3.4 ± 1.0 mA (p < 0.02) and increased the coronary flow rates 9.7 ± 0.5 to 19.1 ± 1.6 mls/min (p < 0.02). Beta-adrenoceptor antagonism with atenolol 5×10^{-5} M did not prevent the dibutyryl cyclic AMP induced fall in the ventricular fibrillation threshold, 2.9 ± 0.7 vs 3.4 ± 1.0 mA (NS) nor did it alter the coronary flow rate, 17.8 ± 1.3 vs 19.1 ± 1.6 mls/min (NS).

[E] Effect of calcium channel antagonist agents on adrenaline induced changes (Table 3-4)

Both verapamil and nifedipine were subject to a dose response curve to assess the effect in producing coronary artery vasodilation and slowing of the heart rate. Verapamil $1.5 \times 10^{-7} \text{ M}$ and nifedipine 10^{-6} M produced maximum coronary artery vasodilation and induced bradycardia, thereby manifesting effective calcium antagonism. Nifedipine attenuated the positive chronotropic effect of adrenaline $5 \times 10^{-7} \text{ M}$ by approximately 15% but did not enhance the increase in coronary flow induced by adrenaline. Verapamil $1.5 \times 10^{-7} \text{ M}$, nifedipine 10^{-6} M completely prevented the adrenaline-induced vulnerability to ventricular fibrillation: 10.9 ± 1.4 ($p < 0.05$), 8.7 ± 1.4 ($p < 0.05$) respectively versus adrenaline $5 \times 10^{-7} \text{ M}$, $3.1 \pm 0.8 \text{ mA}$.

To further delineate the mechanism of the ventricular antiarrhythmic activity of verapamil, investigation was undertaken with l(-) and d(+) verapamil isomers.

[F] Effect of l(-) and d(+) isomers of verapamil on

(i) Guinea-pig papillary muscle (Tables 3-5, 3-6)

l(-) verapamil decreased the action potential duration (at 90% repolarization of the normal transmembrane action potential) and also antagonised calcium dependent slow response action potentials in the isolated superfused guinea-pig papillary muscle. d(+) verapamil produced no alteration of action potential duration of the transmembrane action potential or of slow response action potential. These findings indicate that l(-) verapamil is quantitatively the active isomer in inhibiting the slow calcium inward current.

(ii) Rat heart (Figure 3-2)

Both l(-) and d(+) verapamil $1.5 \times 10^{-7} \text{ M}$ inhibited the

adrenaline $5 \times 10^{-7} \text{M}$ induced increment in the heart rate response: 328 ± 11 , 333 ± 13 versus 370 ± 6 beats/min ($p < 0.05$, $p < 0.05$), but left the enhanced coronary flow rates unchanged: 18.0 ± 0.3 versus 18.4 ± 0.4 ml/min. l(-) Verapamil but not d(+) verapamil prevented the adrenaline-induced reduction in the ventricular fibrillation threshold: l(-) verapamil 10.8 ± 1.0 mA ($p < 0.01$), d(+) verapamil 2.0 ± 0.5 mA (NS) versus adrenaline $5 \times 10^{-7} \text{M}$ 3.1 ± 0.8 mA. Both isomers of verapamil ameliorated the adrenaline-induced depletion of tissue ATP levels, but were ineffective in preventing the marked breakdown of myocardial glycogen stores. In keeping with the latter finding, neither l(-) nor d(+) verapamil prevented the adrenaline-induced accumulation of cyclic AMP, l(-) verapamil 0.94 ± 0.04 nmol/g, d(+) verapamil 0.93 ± 0.05 nmol/g, both values NS versus adrenaline $5 \times 10^{-7} \text{M}$, 0.91 ± 0.03 nmol/g.

[G] Effect of reduction in extracellular Ca^{2+} concentration on adrenaline induced changes (Table 3-4)

To further clarify the role of the transsarcolemmal calcium current in adrenaline induced ventricular fibrillation, the effect of reduction in extracellular calcium concentration was analysed. Reduction in perfusate Ca^{2+} ion concentration from 2.5 mM to 1.25 mM or to 1.00 mM completely prevented the adrenaline induced fall in ventricular fibrillation threshold. Control (perfusate Ca^{2+} 2.5 mM) 10.0 ± 0.3 mA; adrenaline $5 \times 10^{-7} \text{M}$ + perfusate Ca^{2+} 2.5 mM, 3.1 ± 0.8 mA; adrenaline $5 \times 10^{-7} \text{M}$ + perfusate Ca^{2+} 1.25 mM, 18.6 ± 3.9 mA; adrenaline $5 \times 10^{-7} \text{M}$ + perfusate Ca^{2+} 1.0 mM, 27.3 ± 5.1 mA. Coronary flow rates and heart rates are depicted in Table 3-4.

[H] Effect of calcium channel antagonist agents on dibutyryl cyclic AMP induced changes (Table 3-7)

The next question to address was whether calcium antagonists were effective in preventing the enhanced vulnerability to ventricular fibrillation induced by cyclic AMP, the reputed intracellular second messenger of beta-adrenergic stimulation. Dibutyryl cyclic AMP 1.5×10^{-4} M, the analogue of cyclic AMP which penetrates the cell membrane more easily, decreased the ventricular fibrillation threshold from 7.7 ± 1.3 to 3.4 ± 1.0 mA ($p < 0.02$), increased the coronary flow rates from 9.7 ± 0.5 to 19.1 ± 1.6 ml/min ($p < 0.02$) but did not produce any alteration in the heart rate. Both verapamil 1.5×10^{-7} M and nifedipine 10^{-6} M prevented the dBcAMP induced fall in the ventricular fibrillation threshold, but each agent did not prevent the dBcAMP induced increase in coronary flow rates. Both nifedipine and verapamil reduced heart rate.

3. DISCUSSION

[A] Electrophysiological effects of adrenaline

Adrenaline in supraphysiological concentrations induces, in the normoxic rat myocardium, electrophysiological alterations which predispose to ventricular arrhythmias. These electrophysiological alterations include: (i) reduced resting membrane potential, action potential amplitude and duration; (ii) conduction disturbances such as conduction delay, unidirectional block, summation and inhibition and (iii) abnormal automatic activity including triggered sustained rhythmic activity (Gilmour & Zipes, 1980).

[B] Adrenaline, beta-adrenoceptor, cyclic AMP and ventricular fibrillation

The arrhythmogenic effect of adrenaline is shown by its ability to enhance the vulnerability to ventricular fibrillation in the isolated non-ligated rat heart. The adrenaline induced vulnerability to ventricular fibrillation has been previously linked to accumulation of myocardial cyclic adenosine monophosphate (Lubbe et al, 1976; Lubbe et al, 1978). The evidence of the study does not show a correlation between reduction in ventricular fibrillation threshold and accumulation of myocardial cyclic AMP. Specifically, adrenaline 5×10^{-8} M markedly increased cyclic AMP content but did not alter the vulnerability to ventricular fibrillation. Cyclic AMP accumulation may therefore reflect an epi-phenomenon. Beta-adrenoceptor antagonism with atenolol prevents the adrenaline induced fall in ventricular fibrillation threshold. Dibutyryl cyclic AMP, the analogue of cyclic AMP which penetrates the cell membrane more easily, enhances the vulnerability to ventricular fibrillation; this arrhythmogenic effect is not inhibited by beta-adrenoceptor antagonism. The beta-adrenoceptor is therefore implicated in the genesis of ventricular fibrillation induced by enhanced adrenergic stimulation. The arrhythmogenic effect of adrenergic stimulation may not be mediated by cyclic AMP, notwithstanding the ability of dibutyryl cyclic AMP to induce ventricular fibrillation.

[C] Cyclic AMP, calcium antagonist procedures and ventricular fibrillation

Calcium channel antagonist agents and a reduction in extracellular calcium concentration completely prevented the adrenaline-induced vulnerability to ventricular fibrillation. This effect occurred even though there was no reduction in the myocardial

content of cyclic AMP. This finding further dissociates changes in cyclic AMP from alterations in vulnerability to ventricular fibrillation and indicates that cyclic AMP is not the final messenger of ventricular fibrillation. However, a further interpretation is that calcium antagonists may mediate protection against ventricular fibrillation at a site distal to the beta-adrenoceptor-adenylate cyclase-cyclic AMP chain of events. Support for this inference stems from the finding that both verapamil and nifedipine were able completely to prevent the enhanced vulnerability to ventricular fibrillation evoked by the cAMP analogue, dibutyryl cyclic AMP.

Are these findings compatible with the proposed hypothesis that the transsarcolemmal calcium current plays a major role in the genesis of catecholamine-induced ventricular fibrillation? The above data do not provide direct proof for this hypothesis because calcium antagonist agents not only inhibit transsarcolemmal calcium influx but also produce a variety of effects, each of which may be responsible for ventricular antiarrhythmic activity. (i) Coronary artery vasodilation (Fanburg et al, 1964); (ii) preservation of metabolic status (Weber, 1959); (iii) α_1 and α_2 adrenoceptor antagonist activity (Nayler et al, 1982) and (iv) sodium channel antagonist activity (Bayer et al, 1975).

[D] Calcium and ventricular fibrillation

That calcium ions mediate catecholamine induced ventricular fibrillation is suggested by the following arguments: First, a reduction in the calcium ion concentration in the perfusion fluid completely prevented the adrenaline induced vulnerability to ventricular fibrillation. Secondly, 1(-) verapamil, the isomer of verapamil which inhibited the slow calcium channel without altering the fast sodium channel, antagonised the catecholamine induced vulnerability to

ventricular fibrillation. Thirdly, nifedipine which does not interact with either α_1 or α_2 adrenoceptors (Motulsky et al, 1983) and is therefore a 'pure calcium antagonist' protected against the adrenaline induced ventricular fibrillation. Fourthly, protection was not due to preservation of myocardial high energy phosphate content or glycogen stores. Fifthly, a clear dissociation could be shown between the effects on coronary flow rates and on antiarrhythmic activity.

These findings suggest that calcium ions mediate catecholamine induced ventricular fibrillation.

4. CONCLUSIONS

1. Beta-adrenoceptor antagonist agents completely prevent the adrenaline induced vulnerability to ventricular fibrillation. The beta-adrenoceptor is directly implicated in the genesis of such ventricular fibrillation. Cyclic AMP, the proposed intracellular second messenger of beta-receptor stimulation, does not appear to mediate the arrhythmogenic effect of enhanced adrenergic activity, rather cyclic AMP accumulation may reflect an epi-phenomenon.

2. Calcium antagonist procedures completely prevent the adrenaline-induced vulnerability to ventricular fibrillation. Calcium ions may act as the intracellular second messenger in mediating the ventricular arrhythmogenic effect of enhanced adrenergic stimulation.

Figure 3-1

LOG CONCENTRATION EFFECT CURVES FOR ISOPROTERENOL
ON HEART RATE IN THE ABSENCE OR PRESENCE OF ATENOLOL

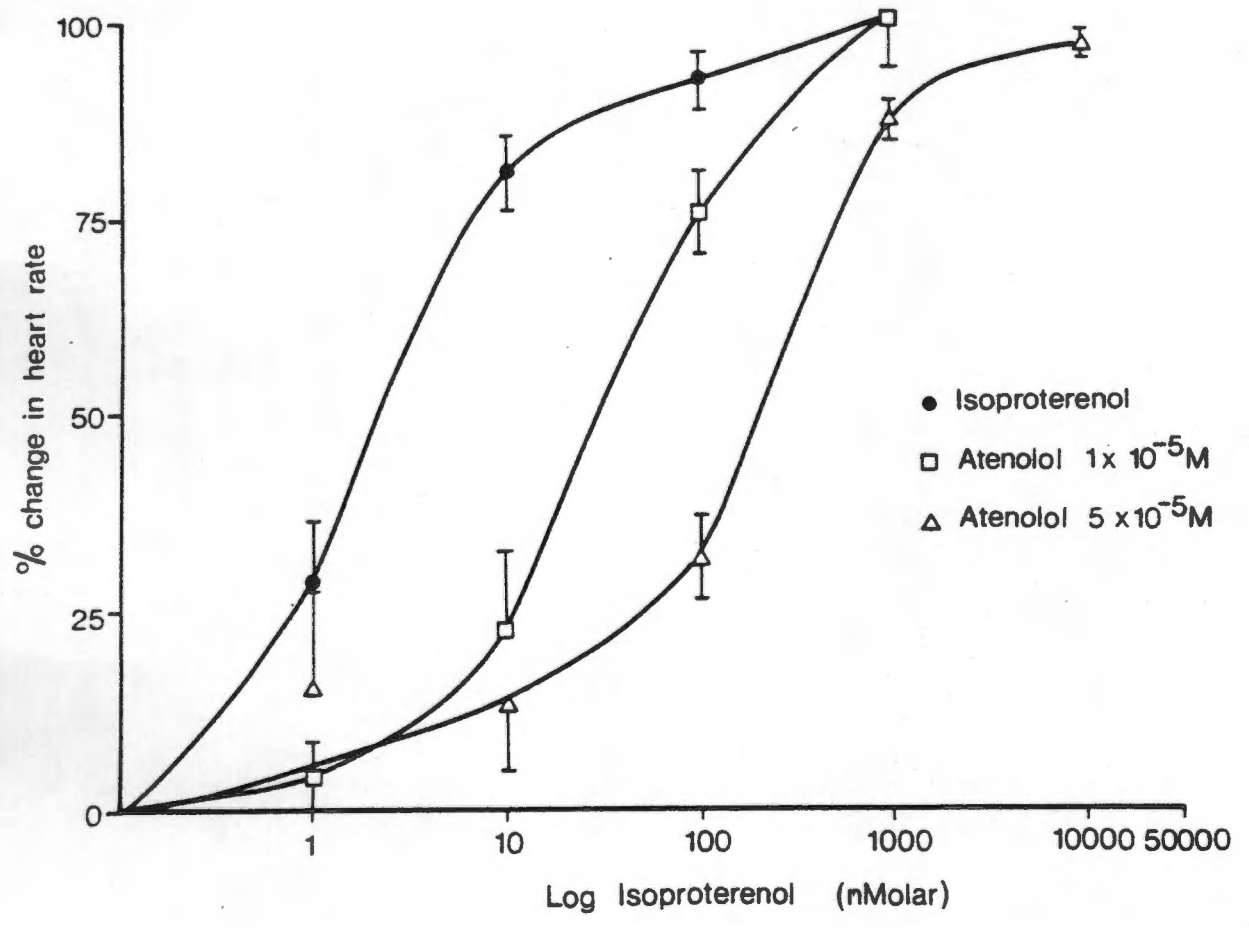


Figure 3-2

EFFECT OF d(+) AND l(-) ISOMERS OF VERAPAMIL
 1.5×10^{-7} M ON ADRENALINE INDUCED CHANGES IN
 THE NON-LIGATED HEART

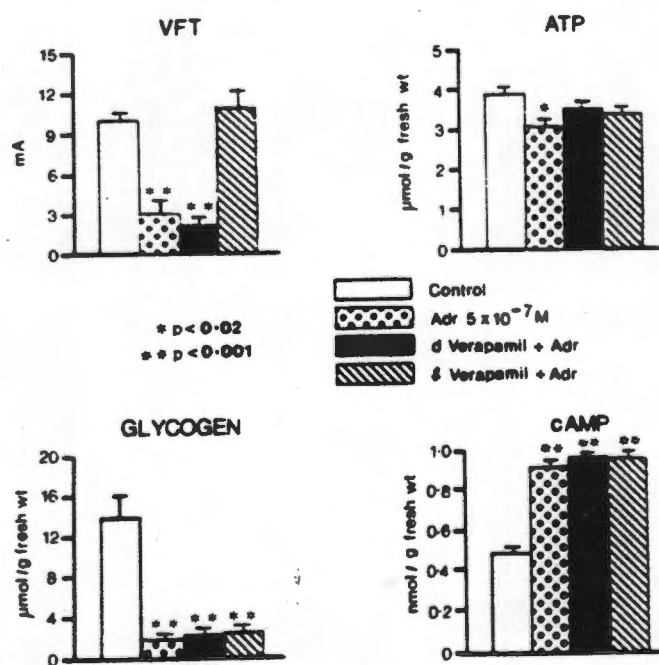


Table 3-1

Adrenaline-mediated changes in the non-ligated rat heart

	Control	Adrenaline		
		5x10 ⁻⁸ M	10 ⁻⁷ M	5 x 10 ⁻⁷ M
Heart rate (beats/min)	250 <u>+10</u>	346** <u>+12</u>	350** <u>+14</u>	370** <u>+6</u>
Coronary flow (ml/min)	9.0 <u>+0.5</u>	17,8** <u>+0,5</u>	16,7** <u>+0,5</u>	17.4** <u>+0.4</u>
VFT (mA)	10.0 <u>+0.3</u>	8,4 <u>+1,3</u>	2,8** <u>+0,9</u>	3.1** <u>+0.8</u>
ATP (umol/g fresh weight)	3.9 <u>+0.2</u>	-	3,6 <u>+0,2</u>	3.1* <u>+0.3</u>
cAMP (nmol/g fresh weight)	0.44 <u>+0.02</u>	0,73** <u>+0,05</u>	0,77** <u>+0,02</u>	0.91** <u>+0.03</u>

Number of hearts = 6-15 in each series

p values: adrenaline vs control in each instance

* p < 0.05

**p < 0.001

Table 3-2

Effect of beta-adrenoceptor antagonist - atenolol -
on adrenaline induced changes in the non-ligated
rat heart

	Control	Adrenaline $5 \times 10^{-7}M$	Atenolol $5 \times 10^{-5}M$ + Adrenaline $5 \times 10^{-7}M$
n	8	15	7
Heart rate (beats/min)	250 <u>+10</u>	370** <u>+6</u>	295* <u>+9</u>
Coronary flow (ml/min)	9.0 <u>+0.5</u>	17.4** <u>+0.4</u>	10,7 <u>+0,5</u>
VFT (mA)	10.0 <u>+0.3</u>	3.1* <u>+0.8</u>	8,9 <u>+0,4</u>

n = number of hearts

Values represent Mean + SEM

p values adrenaline or atenolol + adrenaline vs control

* p < 0,05

**p < 0,001

Table 3-3

Effect of atenolol $5 \times 10^{-5}M$ on dibutyryl cyclic
AMP $1.5 \times 10^{-4}M$ induced changes

<u>Control</u>			<u>dBcAMP $1.5 \times 10^{-4}M$</u>		
<u>VFT₁</u>	<u>HR₁</u>	<u>CF₁</u>	<u>VFT₂</u>	<u>HR₂</u>	<u>CF₂</u>
(mA)	(beats/min)	(mls/min)	(mA)	(beats/min)	(mls/min)
7.7	268	9.7	3,4*	258	19.1*
<u>+1.3</u>	<u>+13</u>	<u>+0.5</u>	<u>+1.0</u>	<u>+13</u>	<u>+1.6</u>

<u>Control</u>			<u>dBcAMP $1.5 \times 10^{-4}M$ + atenolol $5 \times 10^{-5}M$</u>		
<u>VFT₁</u>	<u>HR₁</u>	<u>CF₁</u>	<u>VFT₂</u>	<u>HR₂</u>	<u>CF₂</u>
(mA)	(beats/min)	(mls/min)	(mA)	(beats/min)	(mls/min)
8.5	288	8.8	2,9*	265	17.8*
<u>+0.8</u>	<u>+12</u>	<u>+0.4</u>	<u>+0.7</u>	<u>+13</u>	<u>+1.3</u>

Values represent Mean \pm SEM

p < 0.02 paired t-test 2 vs 1 in each instance

Table 3-4
Effect of verapamil, nifedipine and lowered extracellular calcium
on adrenaline-mediated changes in the non-ligated rat heart

		Adrenaline $5 \times 10^{-7}M$						
		+						
	Control	Adrenaline $5 \times 10^{-7}M$	Verapamil $1.5 \times 10^{-7}M$	Nifedipine $1 \times 10^{-6}M$	Reduced Ca^{2+} 1.25mM	Reduced Ca^{2+} 1.0mM		
Number of hearts (n)	8	15	7	6	7	8		
Heart rate (beats/min)	250 +10	370** +6	250 paced	322* +17	369* +11	364* +7		
Coronary flow (ml/min)	9.0 +0.5	17.4* +0.4	16.0* +0.9	17.7* +0.6	14.4* +0.7	13.5* +0.6		
VFT (mA)	10.0 +0.3	3.1* +0.8	10.9 +1.4	8.7 +1.4	18.6 +3.9	27.3* +5.1		

Ca^{2+} concentration 2.5mM unless otherwise indicated
p values V or N or reduced Ca^{2+} plus adrenaline $5 \times 10^{-7}M$ versus control. Also adrenaline alone versus Control.
*p < 0.02 **p < 0.0001

Table 3-5 Effect of d(+) and l(-) isomers of verapamil on the transmembrane action potential of the guinea-pig papillary muscle

	Control n=5	l(-) verapamil 1.5 x 10 ⁻⁷ M n=4	Control n=6	d(+) verapamil 1.5 x 10 ⁻⁷ M n=5
Action potential amplitude (mV)	107 ± 1.54	NS - 100 ± 3.17	103 ± 1.24	NS - 103 ± 1.85
Action potential duration at 90% repolarization (msec)	148 ± 6.91	<0.01 - 110 ± 6.39	133 ± 10.09	NS - 146 ± 12.11
dV/dt of phase 0 of action potential (Vsec)	209 ± 21.60	NS - 236 ± 18.50	220 ± 21.80	NS - 193 ± 15.00

Values represent Mean ± SEM

NS = Not significant

Results represent effect of isomers of verapamil 30 minutes after addition.

Table 3-6 Effect of d(+) and l(-) isomers of verapamil $1.5 \times 10^{-7}M$ on slow response action potentials of guinea-pig papillary muscle

	<u>Slow response amplitude</u> (mV)	<u>Slow response duration</u> <u>at 90% repolarization</u> (msec)
Control (n=12)	69 ± 3.33	101 ± 4.43
Without drug 20 min (n=4)	76 ± 3.43	110 ± 4.08
l(-) verapamil 20 min (n=4)	35 ± 6.99	48 ± 7.82
d(+) verapamil 20 min (n=4)	70 ± 2.64	101 ± 4.27

p < 0.01 (between Without drug and l(-) verapamil for both amplitude and duration)
 p < 0.01 (between Control and l(-) verapamil for both amplitude and duration)
 p < 0.01 (between Without drug and d(+) verapamil for duration)

Slow response action potential:

18 mM K^+ depolarized guinea-pig papillary muscle plus $10^{-6}M$ isoproterenol.

Stimulation rate 30/min.

Variable stimulus voltage.

Values represent Mean ± SEM

Table 3-7 Effect of verapamil $1.5 \times 10^{-7}M$ and nifedipine $10^{-6}M$
on dibutyryl cyclic AMP induced changes in the isolated
non-ligated rat heart

<u>Control</u>			<u>dBcAMP $1.5 \times 10^{-4}M$</u>		
<u>VFT₁</u>	<u>HR₁</u>	<u>CF₁</u>	<u>VFT₂</u>	<u>HR₂</u>	<u>CF₂</u>
(mA)	(beats/min)	(mls/min)	(mA)	(beats/min)	(mls/min)
7.7	268	9.7	3.4**	258	19.1**
<u>+1.3</u>	<u>+13</u>	<u>+0.5</u>	<u>+1.0</u>	<u>+13</u>	<u>+1.6</u>

<u>Control</u>			<u>dBcAMP $1.5 \times 10^{-4}M$</u> <u>+ verapamil $1.5 \times 10^{-7}M$</u>		
<u>VFT₁</u>	<u>HR₁</u>	<u>CF₁</u>	<u>VFT₂</u>	<u>HR₂</u>	<u>CF₂</u>
(mA)	(beats/min)	(mls/min)	(mA)	(beats/min)	(mls/min)
13.0	256	9.7	12.2	204*	17.4**
<u>+1.0</u>	<u>+15</u>	<u>+0.3</u>	<u>+1.2</u>	<u>+19</u>	<u>+0.9</u>

<u>Control</u>			<u>dBcAMP $1.5 \times 10^{-4}M$</u> <u>+ nifedipine $10^{-6}M$</u>		
<u>VFT₁</u>	<u>HR₁</u>	<u>CF₁</u>	<u>VFT₂</u>	<u>HR₂</u>	<u>CF₂</u>
(mA)	(beats/min)	(mls/min)	(mA)	(beats/min)	(mls/min)
8.7	256	8.0	6.0	208*	14.2**
<u>+1.2</u>	<u>+9</u>	<u>+0.4</u>	<u>+1.0</u>	<u>+13</u>	<u>+0.6</u>

Values represent Mean + SEM

* p < 0.05 paired t-test 2 vs 1 in each instance

** p < 0.02

CHAPTER 4BETA-ADRENOCEPTOR ANTAGONIST AGENTS AND VULNERABILITY TO
VENTRICULAR FIBRILLATION DURING ACUTE REGIONAL
MYOCARDIAL ISCHEMIA1. PROPOSED ROLE OF BETA ADRENERGIC RECEPTOR AND CYCLIC AMP IN
VENTRICULAR FIBRILLATION

Enhanced adrenergic activity increases the vulnerability to ventricular fibrillation in the non-ischemic rat heart (Lubbe et al, 1978). Opie and co-workers (1981) have provided evidence that cyclic AMP, the intracellular second messenger of beta-receptor stimulation, mediates the arrhythmogenic effect of enhanced adrenergic activity in the non-ischemic rat heart. The development of acute myocardial ischemia is associated with an increase of the circulating levels of noradrenaline and adrenaline (Ceremuzynski et al, 1969), probable release of noradrenaline from the ischemic myocardium (Hirche et al, 1980) and accumulation of cyclic AMP (Podzuweit et al, 1978; Corr et al, 1978) in the ischemic myocardium. Podzuweit and co-workers (1978) illustrated a temporal relationship between cyclic AMP accumulation in the ischemic myocardium and onset of ventricular fibrillation during acute myocardial ischemia. It has, therefore, been argued that cyclic AMP mediates the arrhythmogenic effect of enhanced adrenergic activity during acute myocardial ischemia. It has been further proposed that in ischemic myocardial cells, partially depolarized by extracellular potassium accumulation, cyclic AMP may act as an arrhythmogenic agent by promoting the transsarcolemmal calcium inward current, thereby predisposing to slow response action potentials and reentrant ventricular arrhythmias. If

this hypothesis is correct, then beta-adrenoceptor antagonist agents should prevent cyclic AMP accumulation, indirectly inhibit transsarcolemmal calcium ion influx and thereby prevent the occurrence of slow response action potentials and ventricular arrhythmias. I therefore investigated the effect of beta-adrenoceptor antagonist agents on the vulnerability to ventricular fibrillation during acute myocardial ischemia.

2. RESULTS

[A] Coronary artery ligation (Tables 4-1, 4-2, 4-3)

Ligation of the left main coronary artery decreased the heart rate, 243 ± 10 to 199 ± 14 beats/min ($p < 0.02$) and also the total coronary flow rate 7.8 ± 0.5 to 3.9 ± 0.5 ml/min ($p < 0.01$). There was a marked fall in the ventricular fibrillation threshold, the trough being reached 10 to 15 min after coronary artery ligation. VFT values: preligation 10.0 ± 0.3 mA, 15 min coronary artery ligation 2.0 ± 0.3 mA ($p < 0.01$). At the nadir of the ventricular fibrillation threshold (i.e. 15 min after coronary artery ligation) depletion of adenosine triphosphate, phosphocreatine and glycogen was evident in the ischemic myocardium. In addition, there was accumulation of lactate and cyclic AMP in the ischemic myocardium. In a separate series of experiments, the simultaneous infusion of adrenaline 5×10^{-7} M during left main coronary artery ligation increased the heart rate and total coronary flow rate ($p < 0.01$ in each case) and further enhanced the vulnerability to ventricular fibrillation during the period of acute myocardial ischemia; VFT value 15 min CAL + adrenaline 5×10^{-7} M being 0.6 ± 0.1 mA.

[B] Atenolol

Atenolol 5×10^{-5} M produced a greater than 10-fold shift to the right of the log concentration effect curves for isoproterenol on heart rate thereby indicating effective beta-adrenoceptor antagonism (Fig 4-1).

Atenolol 5×10^{-5} M to 10^{-4} M, however, did not prevent the fall in ventricular fibrillation threshold during acute myocardial ischemia (Fig 4-2) nor did it alter the heart rate or coronary flow rate during this period (Table 4-1). Moreover, atenolol did not prevent the depletion of adenosine triphosphate or phosphocreatine or inhibit the accumulation of cyclic AMP in the ischemic myocardium (Lubbe et al, 1981).

Atenolol 5×10^{-5} M maintained higher ventricular fibrillation threshold levels during the first ten minutes of acute myocardial ischemia with concomitant adrenaline 5×10^{-7} M stimulation; however, at 15 min (i.e. the period of maximum vulnerability to ventricular fibrillation) protection was not evident (Fig 4-3).

[C] Metoprolol

Metoprolol 10^{-6} M and 10^{-5} M produced a greater than 10-fold shift to the right of the log concentration effect curves for isoproterenol on heart rate thereby indicating effective beta-adrenoceptor antagonism (Fig 4-4). Metoprolol 10^{-5} M, however, did not prevent the fall in ventricular fibrillation threshold during acute myocardial ischemia (Fig 4-5), nor did it produce any alteration in the coronary flow rate or heart rate during this period (Table 4-1). Moreover, metoprolol 10^{-5} M did not prevent the depletion of ATP or PCR in the ischemic myocardium (Table 4-2) or inhibit the accumulation of cyclic AMP in the ischemic myocardium (Table 4-3). However, a 10-fold higher concentration, metoprolol 10^{-4} M, increased the ventricular

fibrillation threshold before coronary artery ligation, and also maintained higher ventricular fibrillation threshold levels during acute myocardial ischemia (Fig 4-5). This protective effect was associated with lower tissue cyclic AMP levels both in the non-ischemic and ischemic myocardium (Table 4-3). A reduction in heart rate was evident before and after coronary artery ligation; total coronary flow rates were no different from the control series subject to coronary artery ligation (Table 4-1).

[D] dl-Propranolol

dl-Propranolol, $5 \times 10^{-6} \text{M}$ attenuated the fall in the ventricular fibrillation threshold during acute myocardial ischemia (Fig 4-6). The ventricular antiarrhythmic effect of propranolol could not be linked to alteration either in the coronary flow rate or heart rate during acute myocardial ischemia (Table 4-1). In work emanating from this laboratory, Lubbe and co-workers (1981) showed that dl-propranolol $8 \times 10^{-6} \text{M}$ and $1.6 \times 10^{-5} \text{M}$ exhibited ventricular antiarrhythmic activity but did not preserve adenosine triphosphate or phosphocreatine levels in the ischemic myocardium. However, dl-propranolol $8 \times 10^{-6} \text{M}$ and $1.6 \times 10^{-5} \text{M}$ reduced cyclic AMP levels in the ischemic myocardium.

[E] d(+) Propranolol

The effect of dl-propranolol on the fall in ventricular fibrillation threshold after coronary artery ligation was further investigated using the d(+) isomer of propranolol. d(+) Propranolol $5 \times 10^{-6} \text{M}$ attenuated the fall in the ventricular fibrillation threshold during acute myocardial ischemia (Fig 4-6). The ventricular antiarrhythmic action of d(+) propranolol could be linked to reduction in cyclic AMP in the ischemic myocardium (Lubbe et al, 1981).

3. DISCUSSION

[A] Beta receptor antagonism with atenolol, metoprolol and propranolol

Beta-adrenoceptor antagonism with atenolol $5 \times 10^{-5} \text{ M}$ and metoprolol 10^{-5} M was ineffective in preventing the fall in ventricular fibrillation threshold during acute myocardial ischemia. Beta-adrenoceptor antagonism with atenolol $5 \times 10^{-5} \text{ M}$ attenuated the fall in the ventricular fibrillation threshold during the first 10 min of acute myocardial ischemia with concomitant adrenergic stimulation; however, this protective effect was not maintained at 15 min, i.e. the time of maximum vulnerability to ventricular fibrillation. In concentrations higher than that required to produce beta-adrenoceptor antagonism, metoprolol 10^{-4} M but not atenolol 10^{-4} M prevented the fall in the ventricular fibrillation threshold during acute myocardial ischemia. Ventricular antiarrhythmic activity was not due to reduction in heart rate, maintenance of total coronary flow rates or preservation of metabolic status (Lubbe et al, 1981). Further investigation utilizing the d(+) isomer of propranolol $5 \times 10^{-6} \text{ M}$ revealed this isomer to be equipotent to dl-propranolol $5 \times 10^{-6} \text{ M}$ in exhibiting ventricular antiarrhythmic activity during acute myocardial ischemia.

[B] 'Membrane stabilizing activity' and ventricular fibrillation

The beta-adrenoceptor antagonising activity of d(+) isomer of propranolol is approximately 50 times less than that of the l(-) isomer of propranolol, while the d(+) analogue possesses primarily 'membrane stabilizing activity' (Barrett and Cullum, 1968). Iansmith and co-workers (1983) have recently shown that at 10^{-6} M , dl propranolol and d(+) propranolol both exhibit 'membrane stabilizing activity'. The

equivalent effects of dl and the d(+) analogue of propranolol and of high concentrations of metoprolol on the ventricular fibrillation threshold can therefore best be explained by their common property - 'membrane-stabilizing activity'. In agreement with this concept, atenolol which was without effect on the ischemia-induced fall in the ventricular fibrillation threshold lacks 'membrane-stabilizing activity'.

[C] Beta adrenergic receptor and ventricular fibrillation

The beta-adrenergic receptor, as judged by the effects of beta-adrenoceptor antagonist agents, does not appear to play a role in the genesis of ventricular fibrillation during acute myocardial ischemia. Support for this proposal stems from the finding that beta-adrenoceptor antagonist agents, in concentrations evoking beta-receptor antagonism, do not prevent spontaneous ventricular fibrillation during acute myocardial ischemia in the cat (Corr et al, 1978) and in the pig (Muller, 1981). Moreover, in concentrations higher than that required to achieve beta-receptor antagonism, only certain beta-antagonists - metoprolol but not sotalol - prevent ventricular fibrillation in the pig (Muller, 1981). In the dog, beta-adrenoceptor antagonists, in general, prevent the occurrence of spontaneous ventricular fibrillation (Khan et al, 1972; Pearle et al, 1978; Menken et al, 1979) or attenuate the fall in ventricular fibrillation threshold (Anderson et al, 1983). However, the mechanism of the ventricular antiarrhythmic activity has been attributed to several factors: (i) beta-adrenoceptor antagonism (Khan et al, 1972; Andersen et al, 1983); (ii) 'membrane stabilizing activity' (Sivam and Seth, 1978); (iii) prolongation of the action potential duration (Kaufman and Aramendia, 1968). The prevailing experimental evidence suggests that the beta-adrenoceptor does not play a role in the genesis of ventricular

fibrillation during acute myocardial ischemia in the rat, pig and cat models. In contrast, in the dog, there is evidence linking the beta-adrenoceptor to the genesis of ventricular fibrillation during acute myocardial ischemia. This disparate effect of beta-adrenoceptor antagonist agents on ventricular antiarrhythmic activity may reflect species difference, there being a higher number of beta-adrenergic receptors in the canine myocardium.

Beta-adrenoceptor antagonist agents in concentrations producing beta-receptor antagonism decrease the incidence of ventricular extrasystoles and/or ventricular tachycardia during acute myocardial ischemia in the cat (Corr et al, 1978) and pig models (Muller et al, 1981); the ability of atenolol to exhibit ventricular antiarrhythmic activity during the early phase of acute myocardial ischemia plus adrenergic stimulation in the rat probably reflects a similar potential. The beta-adrenergic receptor may, therefore, play a role in the genesis of ventricular extrasystoles and ventricular tachycardia during acute myocardial ischemia.

[D] Beta adrenergic receptor and cyclic AMP accumulation

The beta-adrenergic receptor does not appear to be responsible for the accumulation of cyclic AMP in the ischemic myocardium. This proposal is based on (i) the failure of extremely high concentrations of atenolol to inhibit the rise of cyclic AMP in the ischemic myocardium of the rat heart; (ii) the inability of high concentrations of beta antagonists, metoprolol, sotalol and propranolol to prevent the accumulation of cyclic AMP in the ischemic myocardium of the pig heart (Muller, 1981); (iii) the ability of both active l(-) and relatively inactive d(+) isomers of propranolol to prevent accumulation of cyclic AMP in the ischemic myocardium of the rat heart. What then

causes the accumulation of cyclic AMP in the ischemic myocardium? The answer to this question is unknown but factors incriminated include lysophosphoglycerides, ^(Ahumada et al 1979) histamine or fall in pH inhibiting phosphodiesterase activity.

[F] Cyclic AMP and ventricular fibrillation

The next question to address is whether the intracellular level of cyclic AMP, which is elevated in acute myocardial ischemia, plays a role in the genesis of ischemic ventricular fibrillation. Our findings with beta-adrenoceptor antagonist agents are in agreement with the concept that the intracellular level of cyclic AMP may regulate the vulnerability to ventricular fibrillation; thus atenolol neither reduced cyclic AMP accumulation nor prevented the fall in the ventricular fibrillation threshold, whilst metoprolol and propranolol attenuated both the rise in cyclic AMP and also fall in ventricular fibrillation threshold. Thus, although the beta-adrenergic receptor may not be directly responsible for the rise in cyclic AMP in the ischemic myocardium, regulation of the ischemic tissue cyclic AMP level may be linked to alteration in vulnerability to ventricular fibrillation with beta-adrenoceptor antagonist agents.

4. CONCLUSIONS

1. The beta-adrenergic receptor does not appear to play a role in the genesis of ventricular fibrillation during acute myocardial ischemia.
2. The protective effect of beta-adrenoceptor antagonist agents appears to be due to a non-specific effect, eg. 'membrane-stabilizing activity' rather than due to specific beta-adrenoceptor antagonism.
3. The beta-adrenergic receptor does not appear to be responsible for

generation of cyclic AMP in the ischemic myocardium.

4. Changes of intracellular cyclic AMP content in the ischemic myocardium can be linked to alteration in the vulnerability to ventricular fibrillation produced by beta-adrenoceptor antagonists.

Figure 4-1

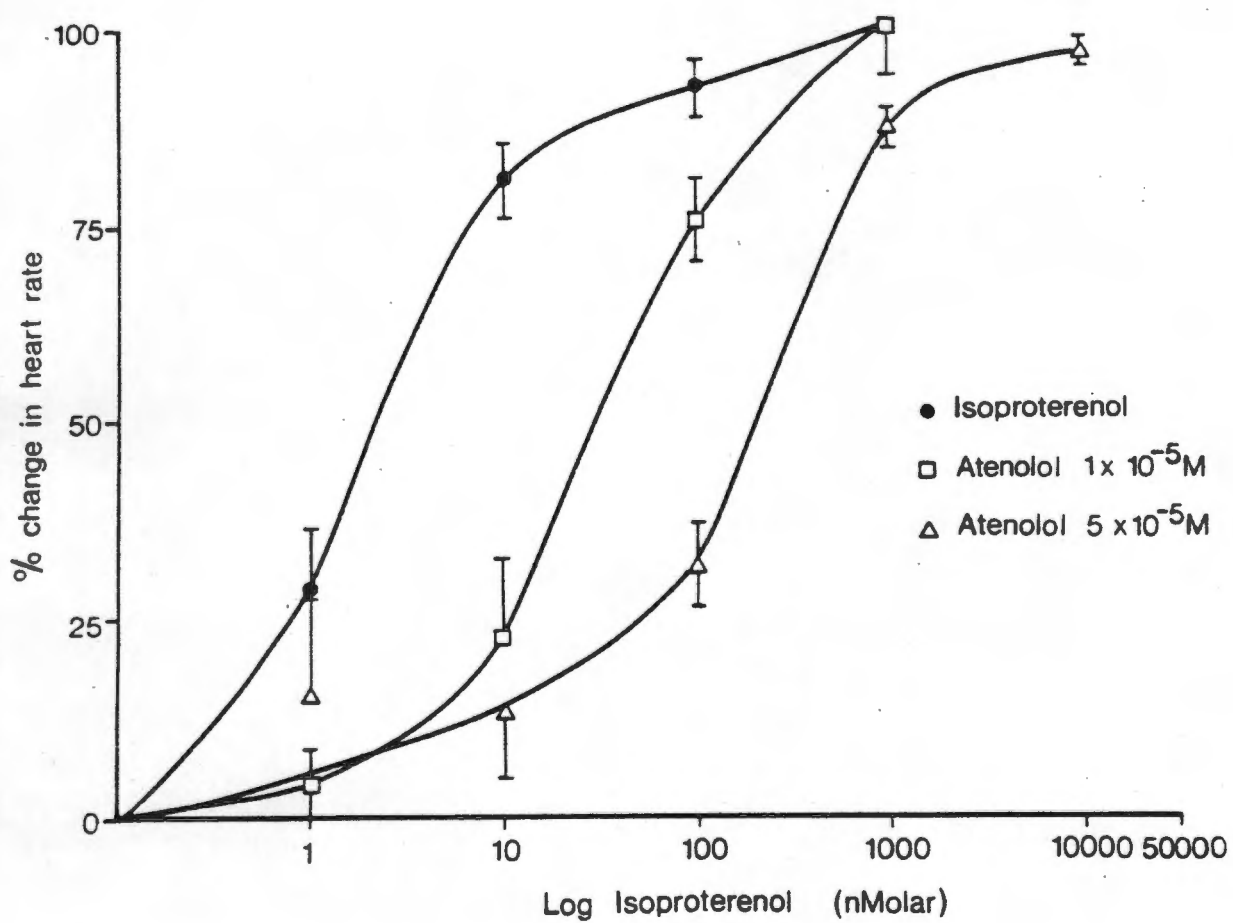
LOG CONCENTRATION EFFECT CURVES FOR ISOPROTERENOL
ON HEART RATE IN THE ABSENCE OR PRESENCE OF ATENOLOL

Figure 4-2

EFFECT OF ATENOLOL ON VENTRICULAR FIBRILLATION

THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

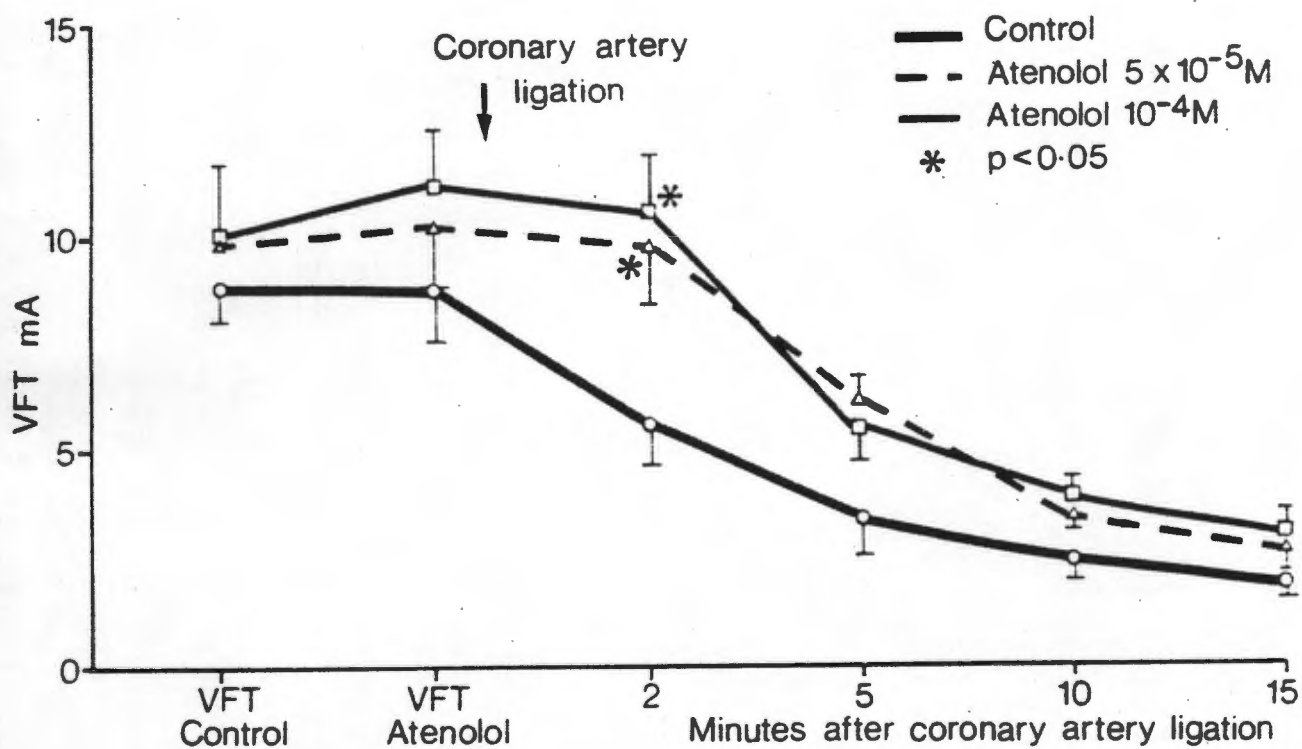


Figure 4-3

EFFECT OF ATENOLOL ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA
PLUS ADRENERGIC STIMULATION

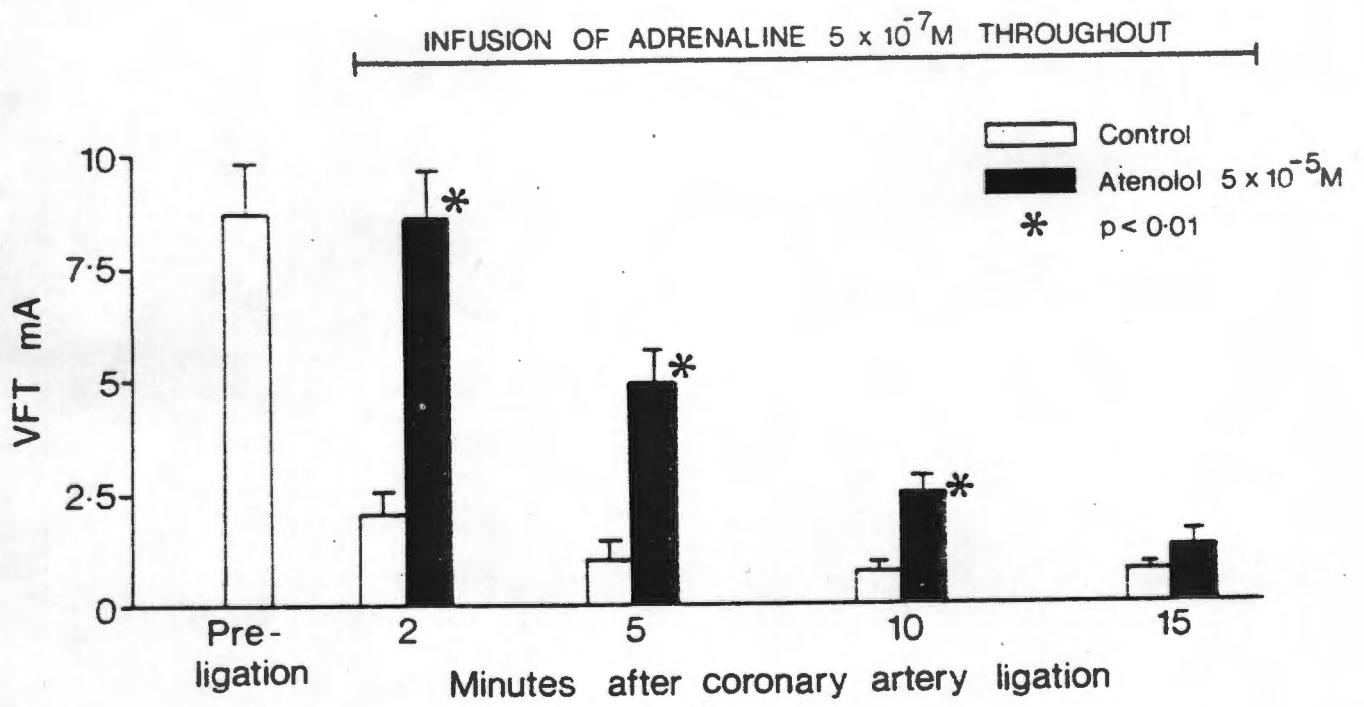


Figure 4-4

LOG CONCENTRATION EFFECT CURVES FOR ISOPROTERENOL
ON HEART RATE IN THE ABSENCE OR PRESENCE OF
METOPROLOL

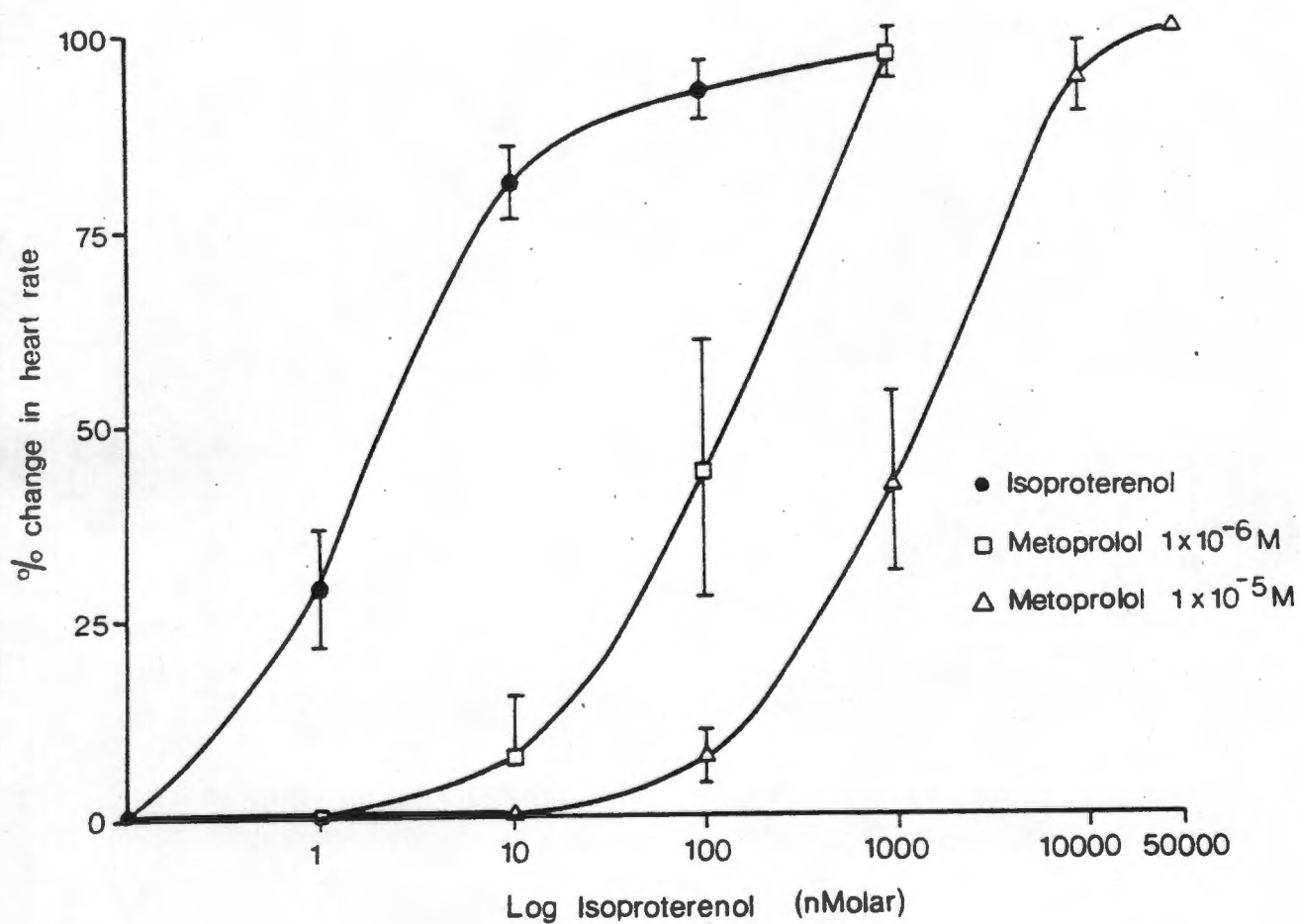


Figure 4-5

EFFECT OF METOPROLOL ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

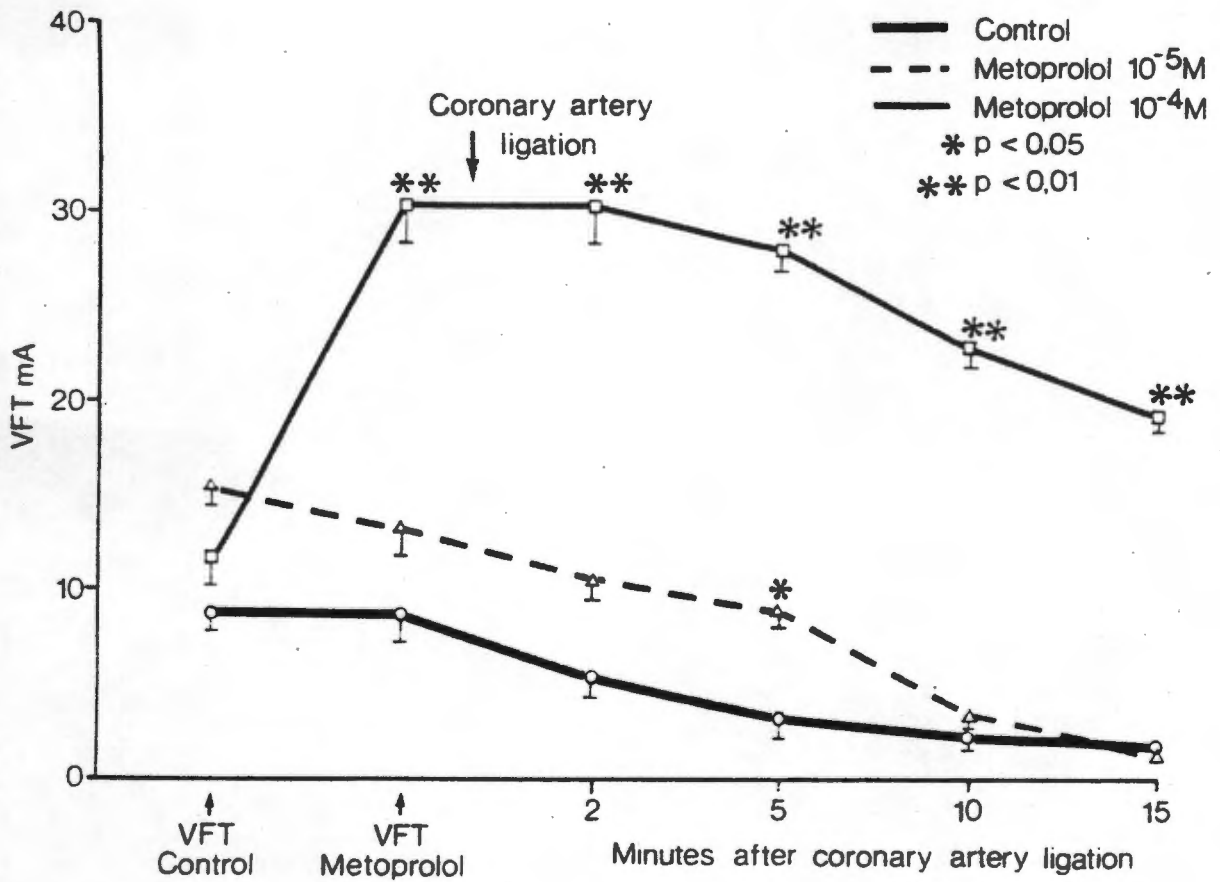


Figure 4-6

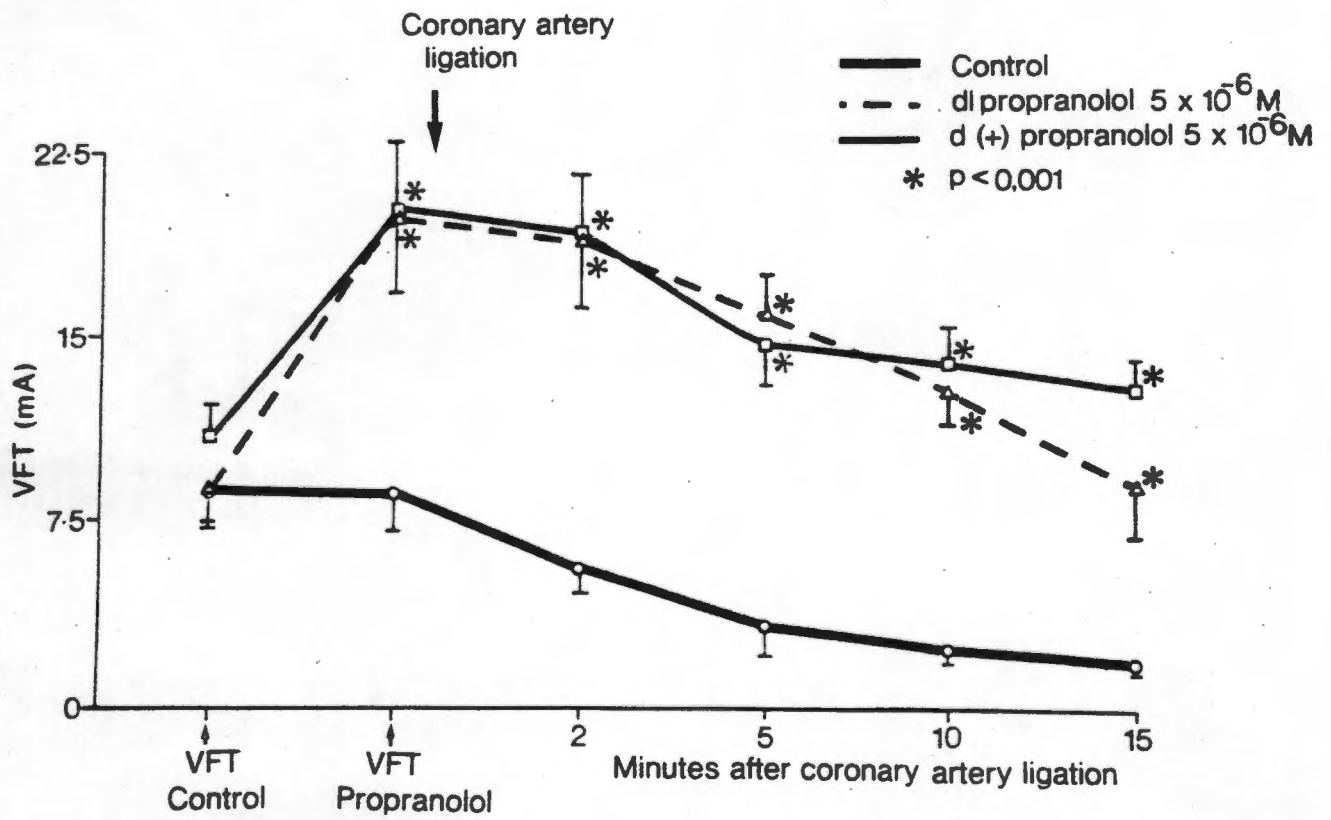
EFFECT OF PROPRANOLOL ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

Table 4-1

Effect of beta-adrenoceptor antagonist agents on heart rate, total coronary flow rate and ventricular fibrillation threshold after coronary artery ligation

	<u>Mean heart rate</u> (beats/min)	<u>Mean total coronary flow</u> (mls/min)	<u>VFT 15' CAL</u> (mA)
1. Non-ligated heart	243 ± 10	7.8 ± 0.5	10.0 ± 0.3
2. Coronary artery ligation (CAL)	199 ± 14	3.9 ± 0.5	2.0 ± 0.3
p vs 1	<0.02	<0.01	<0.01
3. CAL + atenolol 5 x 10 ⁻⁵ M	208 ± 7	3.7 ± 0.5	2.6 ± 0.5
p vs 1	<0.05	<0.01	<0.01
p vs 2	NS	NS	NS
4. CAL + atenolol 10 ⁻⁴ M	200 ± 8	3.9 ± 0.4	3.0 ± 0.5
p vs 1	<0.05	<0.01	<0.01
p vs 2	NS	NS	NS
5. CAL + metoprolol 10 ⁻⁵ M	192 ± 14	3.9 ± 0.4	1.7 ± 0.2
p vs 1	<0.01	<0.01	<0.01
p vs 2	NS	NS	NS
6. CAL + metoprolol 10 ⁻⁴ M	129 ± 6	3.0 ± 0.3	19.3 ± 0.7
p vs 1	<0.001	<0.01	<0.01
p vs 2	<0.001	NS	<0.01
7. CAL + dl propranolol 5 x 10 ⁻⁶ M	200 ± 8	4.1 ± 0.3	9.0 ± 2.0
p vs 1	<0.05	<0.01	NS
p vs 2	NS	NS	<0.01
8. CAL + d(+) propranolol 5 x 10 ⁻⁶ M	204 ± 10	4.2 ± 0.4	12.8 ± 1.3
p vs 1	<0.05	<0.01	NS
p vs 2	NS	NS	<0.01

Number of hearts = 5-12

Values represent Mean ± SEM

Table 4-2 Effect of beta-adrenoceptor antagonist agents on tissue high energy phosphate, glycogen and lactate levels 15 min after coronary artery ligation

	Non-ischemic myocardium			Ischemic myocardium				
	ATP	PCr	Glycogen	Lactate	ATP	PCr	Glycogen	Lactate
	[————— μmol/g —————]			[————— μmol/g —————]				
1. Coronary artery ligation (CAL)	4.07 +0.17	4.33 +0.28	10.09 +0.67	1.56 +0.36	2.07 +0.23	1.54 +0.21	2.54 +0.67	16.41 +2.33
2. CAL + metoprolol 10 ⁻⁵ M	3.69 +0.30	4.11 +0.41	7.61 +1.93	3.11 +0.59	1.57 +0.17	0.94 +0.10	1.49 +0.47	16.15 +2.1
3. CAL + dl propranolol 1.6 x 10 ⁻⁵ M	3.61 +0.20	4.10 +0.40	-	-	1.70 +0.13	1.40 +0.10	-	-

Number of hearts = 6-12
 Values represent Mean ± SEM
 Data in 3 from Lubbe et al (1981)

Table 4-3

Effect of beta-adrenoceptor antagonist agents on
tissue cyclic AMP and ventricular fibrillation
threshold levels 15 minutes after coronary artery
ligation

	Cyclic AMP		VFT 15' CAL (mA)
	<u>Non-ischemic myocardium</u>	<u>Ischemic myocardium</u> (nmol/g)	
1. Non-ligated heart	0.44 ± 0.02		10.0 ± 0.3
2. Coronary artery ligation (CAL)	0.54 ± 0.02	0.71 ± 0.03	2.0 ± 0.3
p vs 1	< 0.01	<0.001	<0.01
3. CAL + metoprolol 10 ⁻⁵ M	0.50 ± 0.04	0.61 ± 0.05	1.7 ± 0.2
p vs 1	NS	<0.01	<0.01
p vs 2	NS	NS	NS
4. CAL + metoprolol 10 ⁻⁴ M	0.39 ± 0.03	0.38 ± 0.01	19.3 ± 0.7
p vs 1	NS	<0.05	<0.01
p vs 2	<0.001	<0.001	<0.01
5. CAL	0.45 ± 0.01	0.60 ± 0.03	1.6 ± 0.2
6. CAL + dl propranolol 1.6 x 10 ⁻⁵ M	0.46 ± 0.02	0.47 ± 0.03	7.7 ± 1.1
p vs 5	NS	<0.01	<0.01
7. CAL + l(-) propranolol 1.6 x 10 ⁻⁵ M	0.40 ± 0.02	0.47 ± 0.02	7.0 ± 1.3
p vs 5	NS	<0.01	<0.01
8. CAL + d(+) propranolol 1.6 x 10 ⁻⁵ M	0.41 ± 0.02	0.44 ± 0.02	11.5 ± 2.8
p vs 5	NS	<0.01	<0.01

Number of hearts = 5-12

Values represent Mean ± SEM

NB: 5 to 8, data from Lubbe et al (1981)

CHAPTER 5ALPHA-ADRENOCEPTOR ANTAGONIST AGENTS AND VULNERABILITY TO VENTRICULAR
FIBRILLATION DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA1. PROPOSED ROLE OF ALPHA ADRENERGIC RECEPTOR IN VENTRICULAR
FIBRILLATION

Enhanced adrenergic activity has been linked to the development of ventricular arrhythmias during acute myocardial ischemia. Endogenous catecholamines, adrenaline and noradrenaline, stimulate myocardial α_1 and α_2 adrenergic receptors. A temporal association exists between an increase in α_1 receptor number in the ischemic myocardium and the occurrence of ventricular arrhythmias during acute regional myocardial ischemia in the cat (Sheridan et al, 1980; Corr and Crafford, 1982). The α_1 adrenergic receptor has therefore been implicated in ventricular arrhythmogenesis.

α_1 adrenergic stimulation enhances the transsarcolemmal calcium inward current (Bruckner and Scholz, 1980; Scholz 1980) and may cause sarcoplasmic reticulum calcium release (Niedergerke and Page, 1981). Hence, in ischemic myocardial cells partially depolarized by extracellular potassium accumulation, enhanced α_1 adrenergic stimulation may induce calcium dependent slow response action potentials and thereby predispose to reentrant ventricular arrhythmias. If this hypothesis is correct then α_1 adrenoceptor antagonism should inhibit transsarcolemmal calcium ion influx and thereby prevent slow responses and ventricular arrhythmias. α_2 adrenoceptors are also present in the myocardium (Guicheney et al, 1978) but their role, if any, in ventricular arrhythmogenesis is undefined. The purpose of this study

was to evaluate the role of α_1 and α_2 adrenoceptor mediated influences on ventricular arrhythmogenesis. I therefore investigated the effect of α_1 and α_2 adrenoceptor antagonist agents on vulnerability to ventricular fibrillation during acute myocardial ischemia.

2. RESULTS

[A] Prazosin, Yohimbine and Phentolamine

Prazosin (α_1 antagonist), yohimbine (α_2 antagonist), and phentolamine (α_1 and α_2 antagonist) prevented the fall in the ventricular fibrillation threshold during acute regional myocardial ischemia (Figs 5-1, 5-2, 5-3). Complete protection against electrically induced fibrillation was evident with prazosin 10^{-5} M, yohimbine 10^{-6} M and phentolamine 10^{-5} M. More recent experiments illustrate that prazosin 10^{-5} M and yohimbine 10^{-6} M attenuate the fall in the ventricular fibrillation threshold during acute myocardial ischemia and simultaneous adrenergic stimulation (Fig 5-4).

[B] Heart rate and coronary flow rate pattern

Ventricular antiarrhythmic activity occurred despite the presence of similar or lower total coronary flow rates than the control series subject to coronary artery ligation (Table 5-1). Protection against ventricular fibrillation was not associated with a significant reduction in heart rate (Table 5-1).

[C] Metabolic status

Prazosin 10^{-5} M, yohimbine 10^{-6} M and phentolamine 10^{-5} M,

concentrations which exhibited anti-fibrillatory activity, did not preserve tissue content of adenosine triphosphate, phosphocreatine or glycogen in the ischemic myocardium (Table 5-2). Prazosin 10^{-5} M and yohimbine 10^{-6} M did not reduce the tissue levels of either lactate or cyclic AMP in the ischemic myocardium (Table 5-3).

[D] Reserpine pre-treatment

Reserpine 1.5 mg and 5.0 mg administered intraperitoneally 48 and 24 hours respectively prior to sacrifice of the rat depletes myocardial stores of catecholamines (Gaudel et al, 1979). Further evidence of catecholamine depletion was the failure of tyramine (an agent which releases myocardial norepinephrine stores) to increase either the heart rate or coronary flow rate. Prior reserpinization did not prevent the fall in ventricular fibrillation threshold during acute myocardial ischemia (Table 5-4).

[E] Electrophysiological action of the α_1 adrenoceptor antagonist agent, prazosin

In the isolated superfused guinea-pig papillary muscle, prazosin 10^{-5} M increased the action potential duration at 90% repolarization but did not alter the dV/dt (fast channel conductance) of the transmembrane action potential. Prazosin 10^{-6} M produced no alteration of the transmembrane action potential (Table 5-5).

3. DISCUSSION

[A] Alpha-adrenoceptor antagonist agents and ventricular antiarrhythmic activity

Prazosin an α_1 antagonist; yohimbine an α_2

antagonist and phentolamine an α_1 and α_2 antagonist agent each completely prevented the enhanced vulnerability to ventricular fibrillation during acute regional myocardial ischemia in the absence and presence of adrenergic stimulation. These findings complement, in part, the recent study of Sheridan and co-workers (1980) in which prazosin and phentolamine decreased the incidence of ventricular arrhythmias during acute regional myocardial ischemia in the cat; however, a new finding is that an α_2 adrenoceptor antagonist agent, yohimbine, displays ventricular antiarrhythmic activity.

[B] Heart rate, coronary flow and ventricular antiarrhythmic activity

The protective effect of α_1 and α_2 adrenoceptor antagonist agents against ventricular fibrillation was not due to reduction of heart rate or preservation of total coronary flow rates. Ventricular antiarrhythmic activity could occur even in the presence of extremely low coronary flow rates during ischemia, thereby arguing against the concept that redistribution of coronary flow was responsible for protection. It has also been shown in cat model of acute regional myocardial ischemia that the antiarrhythmic effect of α_1 antagonist agents is unrelated to preservation of or redistribution of coronary flow (Sheridan et al, 1980).

[C] Metabolic profile and ventricular antiarrhythmic activity

Ventricular antiarrhythmic activity was not associated with preservation of energy status in the ischemic myocardium, thereby excluding a metabolic factor in the mechanism of protection. Furthermore, ventricular antiarrhythmic activity was not dependent on reduction of the cyclic AMP level in the ischemic myocardium. Alteration

in the vulnerability to ventricular fibrillation may therefore occur independently of change in cyclic AMP content. Thus although cyclic AMP may be linked to ventricular fibrillation in certain situations, it is not the only factor involved in the genesis of ventricular fibrillation nor the final messenger of ventricular fibrillation.

[D] Alpha₁ and alpha₂ adrenergic receptor mediated influences and ventricular fibrillation

Do both alpha₁ and alpha₂ adrenergic receptor mediated influences play a role in the genesis of ventricular fibrillation during acute myocardial ischemia? A mechanism specific to the alpha₁ or alpha₂ receptor is questioned on the basis of the following evidence:

(i) Reputed effects of alpha₁ and of alpha₂ adrenoceptor antagonism

Alpha₁ myocardial receptors are located post-synaptically, their activation increases transsarcolemmal calcium ion influx and possibly also releases calcium from intracellular organelles. Alpha₁ receptor antagonism therefore inhibits transsarcolemmal calcium influx - such an effect may account for ventricular antiarrhythmic activity. Alpha₂ myocardial receptors are considered to be located presynaptically where they mediate feedback inhibition of noradrenaline release - theoretically decreasing the transsarcolemmal calcium ion influx. Alpha₂ receptor antagonism therefore increases transsarcolemmal calcium influx - such an effect should evoke ventricular arrhythmias rather than induce suppression. The ventricular antiarrhythmic effect of both alpha₁ and alpha₂ antagonist agents is therefore difficult to reconcile on the basis of a specific receptor mediated event because of the disparate effect of alpha₁ and alpha₂ receptor mediated influences on calcium fluxes.

Recent evidence that in vascular smooth muscle, α_2 adrenoreceptors are also located post-synaptically and enhance transsarcolemmal calcium ion influx (Timmermans and Van Zwieten, 1982) may explain a unitary mechanism of protection for α_2 antagonist agents and α_1 antagonist agents, viz. inhibition of transsarcolemmal calcium ion influx. However, myocardial post-synaptic α_2 receptors regulating the transsarcolemmal calcium inward current have, as yet, not been characterized. The anatomical distribution of myocardial α_1 and α_2 adrenoreceptors and their role in regulating extracellular and intracellular calcium ion movement needs to be clarified in order to understand more fully the ventricular antiarrhythmic effect of α_1 and of α_2 antagonist agents.

(ii) Specificity of concentrations exhibiting ventricular antiarrhythmic activity

First, the protective concentration of prazosin (10^{-5} M) is at least 1000-fold higher than the reported dissociation constant of α_1 adrenergic receptor in the rat myocardium, 10^{-10} M (Skomedal et al, 1981), whilst the protective concentration of yohimbine (10^{-6} M) is at least a 1000-fold higher than the initial dissociation constant of α_2 receptor, 10^{-10} M (Guicheney et al, 1978) and 100-fold higher than the final dissociation constant of the α_2 receptor in the rat myocardium, 10^{-8} M (Guicheney et al, 1978). Secondly, the protective concentration of prazosin, an α_1 antagonist, approximates that of yohimbine, an α_2 antagonist, thus decreasing the likelihood of a specific α_1 or α_2 receptor mediated mechanism. Thirdly, if α_1 and α_2 receptor mediated influences play a major role in the genesis of ventricular fibrillation then myocardial catecholamine depletion should prevent the enhanced vulnerability to ventricular

fibrillation. However, myocardial catecholamine depletion by reserpine pre-treatment did not prevent the enhanced vulnerability to ventricular fibrillation induced by acute myocardial ischemia. Fourthly, alpha adrenergic stimulation lengthens the action potential duration, an effect reversed by alpha-adrenoceptor antagonism (Rosen et al, 1977). In this study the ventricular antiarrhythmic effect of prazosin was associated with prolongation and not shortening of the action potential duration. Fifthly, the concentration of phentolamine which protects against ventricular fibrillation decreases the automaticity and membrane responsiveness and increases action potential duration and effective refractory period in canine Purkinje fibres (Rosen et al, 1971).

The findings presented in the rat suggest that the protective effect of alpha antagonist agents is unlikely to be due to specific α_1 or α_2 receptor antagonism but rather due to a non-specific effect, viz. 'membrane-stabilizing activity' or prolongation of action potential duration. Preliminary work in the dog model of acute regional myocardial ischemia suggests that the alpha adrenergic receptor does not play a role in the genesis of ventricular arrhythmias (Stewart et al, 1980). In contrast, in the cat the α_1 adrenoceptor has been linked to ventricular arrhythmogenesis - however, the role of the α_2 adrenoceptor has not been evaluated (Corr and Crafford, 1982). The ventricular antiarrhythmic effect of α_1 antagonist agents in the cat has been linked to reduction in cellular calcium concentration (Sharma et al, 1983).

Thus the precise role of the α_1 and α_2 adrenergic receptors in the genesis of ventricular fibrillation is unclear at present. Investigation of the anatomical distribution of myocardial α_1 and α_2 receptors, their regulation of extracellular and intracellular calcium ion movement and their relation to ventricular arrhythmogenesis is required in several experimental models.

4. CONCLUSIONS

1. Alpha₁ and alpha₂ adrenoceptor antagonist agents completely prevent the enhanced vulnerability to ventricular fibrillation during acute myocardial ischemia.
2. The ventricular antiarrhythmic activity is not due to reduction of heart rate, maintenance of coronary flow rates, preservation of metabolic status or reduction of cyclic AMP content in the ischemic myocardium.
3. The ventricular antiarrhythmic activity of alpha antagonist agents appear to be due to a non-specific effect, viz. 'membrane-stabilizing activity' or prolongation of the action potential duration.
3. The alpha adrenergic receptor does not appear to be implicated in the genesis of ventricular fibrillation during acute myocardial ischemia - however, further investigation is required before one can exclude a role for either the alpha₁ or alpha₂ receptor in ventricular arrhythmogenesis.

Figure 5-1

EFFECT OF PRAZOSIN ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

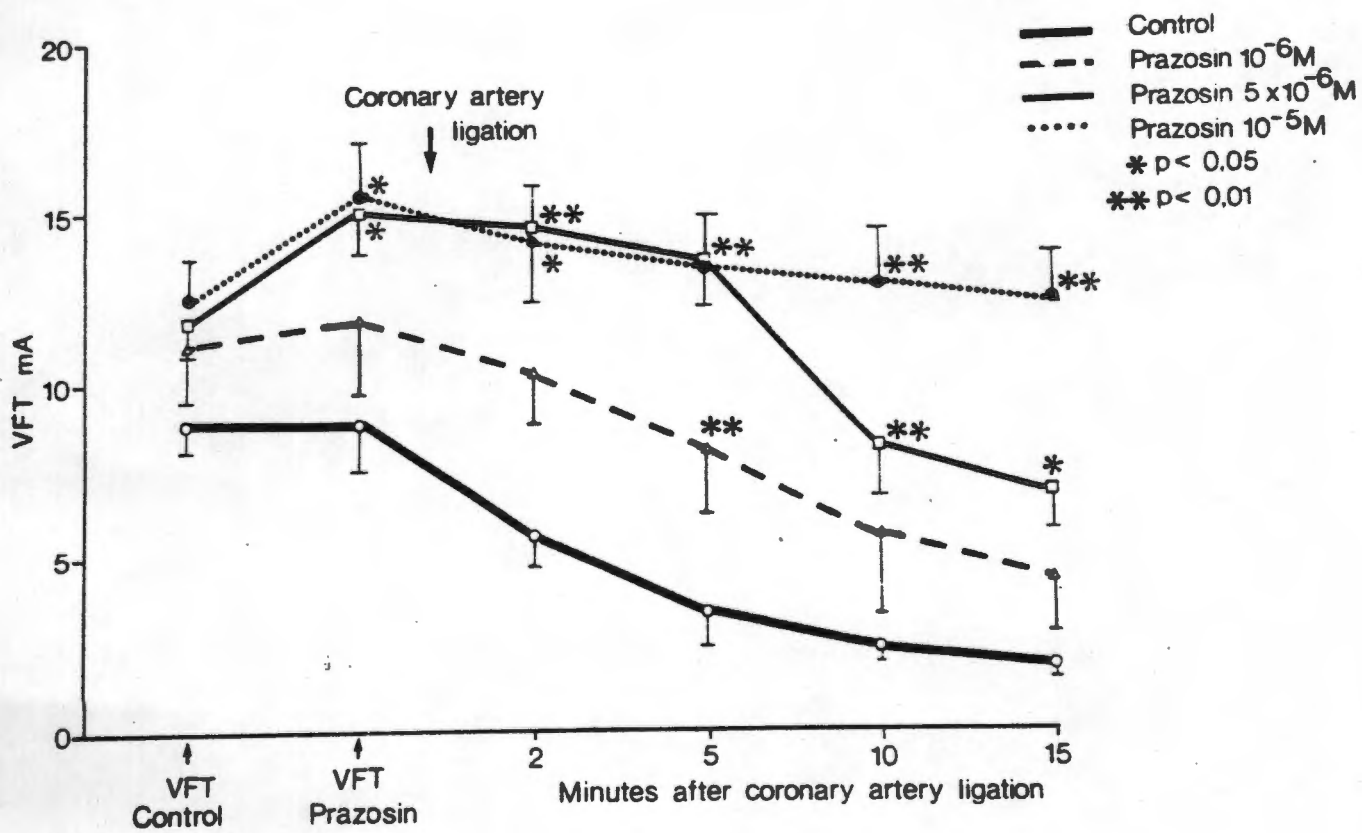


Figure 5-2

EFFECT OF YOHIMBINE ON VENTRICULAR FIBRILLATION

THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

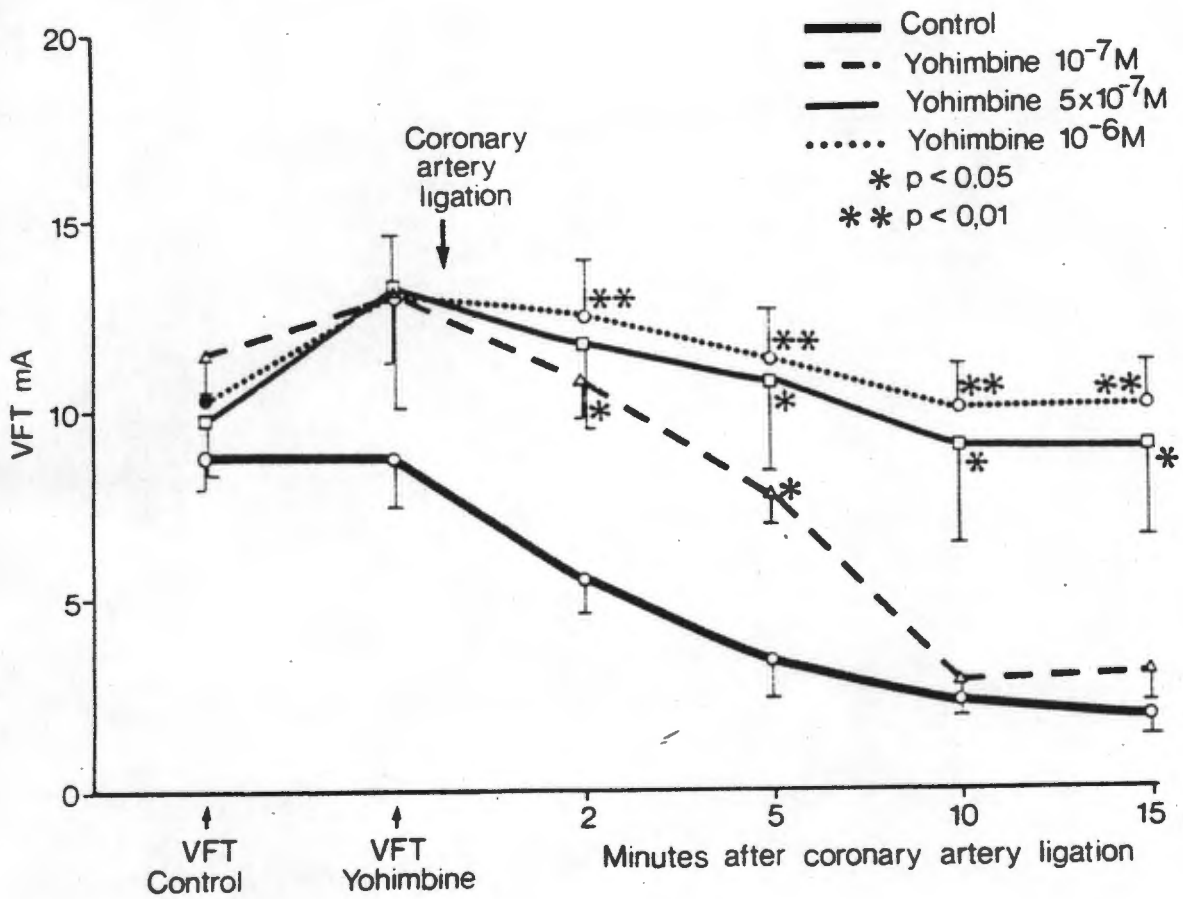


Figure 5-3

EFFECT OF PHENTOLAMINE ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

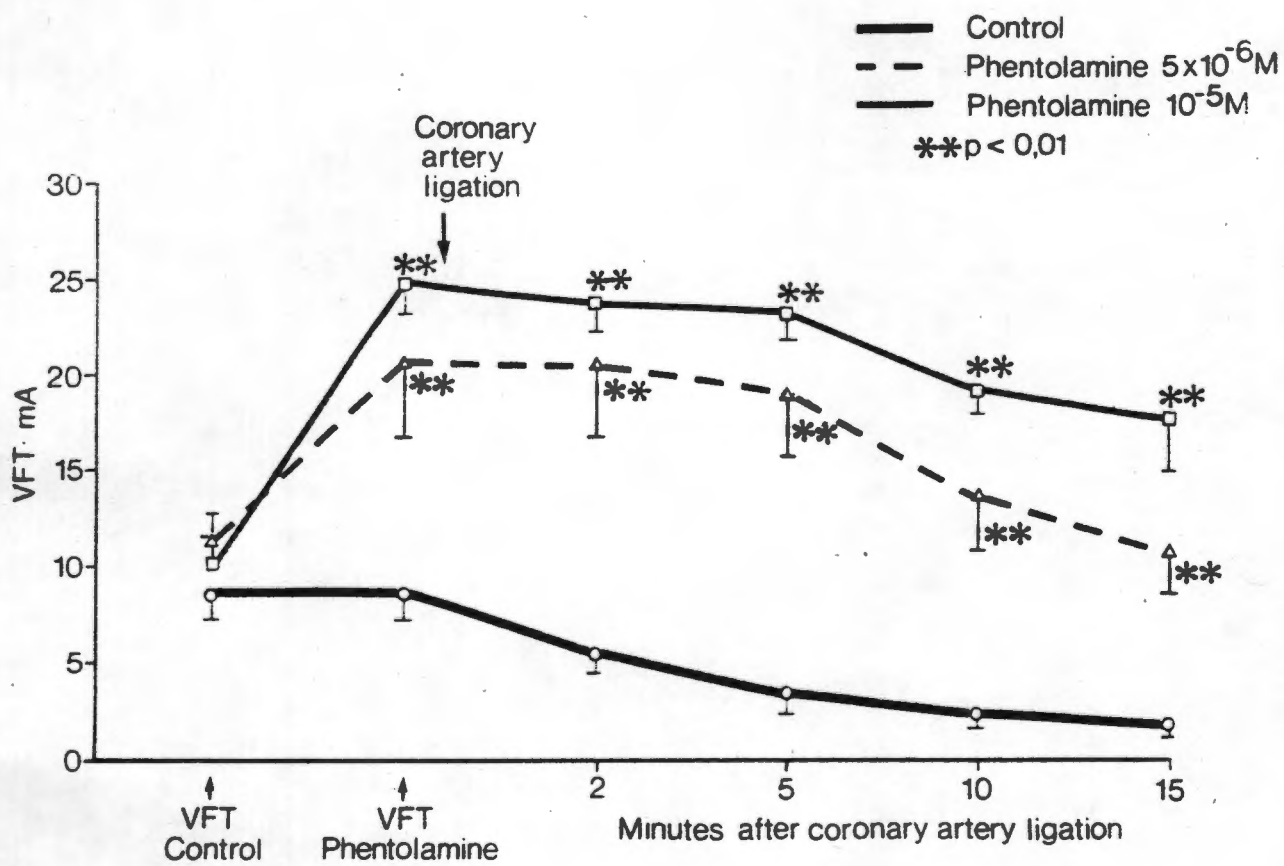


Table 5-1

Effect of alpha-1 and alpha-2 adrenoceptor antagonist agents on heart rate, total coronary flow rate before and after coronary artery ligation

	<u>Non-ligated heart</u>		<u>Coronary artery ligation</u>	
	CF (ml/min)	HR (beats/min)	CF (ml/min)	HR (beats/min)
1. Control	7.8 <u>+0.5</u>	243 <u>+10</u>	3.9 <u>+0.5</u>	199 <u>+14</u>
2. Prazosin 10 ⁻⁵ M	8.4 <u>+0.4</u>	266 <u>+6</u>	2.0 <u>+0.1</u>	156 <u>+7</u>
p vs 1	NS	NS	< 0.05	< 0.05
3. Prazosin 5 x 10 ⁻⁶ M	8.3 <u>+0.5</u>	266 <u>+13</u>	2.8 <u>+0.2</u>	174 <u>+8</u>
p vs 1	NS	NS	NS	NS
4. Yohimbine 10 ⁻⁶ M	9.4 <u>+0.4</u>	268 <u>+7</u>	3.2 <u>+0.2</u>	179 <u>+10</u>
p vs 1	NS	NS	NS	NS
5. Yohimbine 5 x 10 ⁻⁷ M	7.6 <u>+0.4</u>	252 <u>+11</u>	2.8 <u>+0.3</u>	197 <u>+18</u>
p vs 1	NS	NS	NS	NS
6. Yohimbine 10 ⁻⁷ M	9.4 <u>+0.3</u>	287 <u>+8</u>	3.6 <u>+0.3</u>	221 <u>+15</u>
p vs 1	NS	NS	NS	NS
7. Phentolamine 5 x 10 ⁻⁶ M	7.9 <u>+0.2</u>	274 <u>+17</u>	3.3 <u>+0.3</u>	195 <u>+4</u>
p vs 1	NS	NS	NS	NS

Values represent Mean \pm SEM

Table 5-2 Effect of alpha₁ and alpha₂ adrenoceptor antagonist agents on tissue high energy phosphate, glycogen and lactate levels 15 min after coronary artery ligation

	Non-ischemic myocardium			Ischemic myocardium			
	ATP	PCr	Glycogen	ATP	PCr	Glycogen	Lactate
	μmol/g			μmol/g			
Coronary artery ligation (CAL)	4.03 +0.12	4.50 +0.30	10.09 +0.67	1.96 +0.17	1.67 +0.23	2.54 +0.67	16.41 +2.33
CAL + prazosin 10 ⁻⁵ M	4.16 +0.12	5.00 +0.27	7.86 +1.89	1.95 +0.21	1.56 +0.14	1.73 +0.33	12.04 +1.98
CAL + yohimbine 10 ⁻⁶ M	4.07 +0.17	4.22 +0.25	8.31 +1.13	1.73 +0.21	1.27 +0.19	3.49 +0.75	11.19 +0.94
CAL + phentolamine 10 ⁻⁵ M	3.95 +0.25	4.52 +0.57	6.73* +0.79	1.38 +0.25	1.22 +0.31	1.36 +0.32	12.11 +1.58

Number of hearts = 6-12
 Values represent Mean ± SEM
 p vs CAL
 * p < 0.01

Table 5-3

Effect of alpha-1 and alpha-2 adrenoceptor antagonist agents on tissue cyclic AMP and ventricular fibrillation threshold levels 15 minutes after coronary artery ligation

	Cyclic AMP		VFT 15' CAL (mA)
	Non-ischemic myocardium (nmol/g)	Ischemic myocardium	
Non-ligated heart	0.44 ± 0.02	-	10.0 ± 0.3
Coronary artery ligation (CAL)	0.54 ± 0.02	0.71 ± 0.03	2.0 ± 0.3
p vs 1	<0.01	<0.001	<0.01
CAL + prazosin 10 ⁻⁵ M	0.64 ± 0.05	0.72 ± 0.02	14.9 ± 1.4*
p vs 1	< 0.01	<0.001	NS
p vs 2	< 0.05	NS	<0.01
CAL + yohimbine 10 ⁻⁶ M	0.61 ± 0.02	0.64 ± 0.03	10.5 ± 1.5*
p vs 1	<0.01	<0.001	NS
p vs 2	<0.05	NS	<0.01

* p < 0.01

Number of hearts = 6-12

Values represent Mean ± SEM

Table 5-4

Effect of reserpine pre-treatment on ventricular
fibrillation threshold level 15 min after coronary
artery ligation

	<u>VFT preligation</u> (mA)	<u>VFT 15' CAL</u> (mA)
1. Coronary artery ligation (CAL) (n = 8)	8.0 \pm 0.4	1.9 \pm 0.3
2. Reserpine pre-treatment + CAL (n = 7)	7.6 \pm 0.3	1.7 \pm 0.2
p vs 1		

Values represent Mean \pm SEM

Table 5-5

Electrophysiologic action of prazosin on guinea-pig
ventricular myocardium

	<u>Action potential duration</u> <u>90% repolarization</u> (msec)	<u>dV/dt</u> (v/sec)
1. Control (n = 3)	158 ± 4	250 ± 35
2. Prazosin 10 ⁻⁶ M (n = 3)	159 ± 7	250 ± 35
p vs 1	NS	NS
3. Control (n = 4)	151 ± 3	290 ± 14
4. Prazosin 10 ⁻⁵ M (n = 4)	181 ± 4	310 ± 22
p vs 3	<0.02	NS

Values represent Mean ± SEM, 40 min after addition of prazosin

CHAPTER 6CALCIUM-ANTAGONIST PROCEDURES AND VULNERABILITY TO VENTRICULAR
FIBRILLATION DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA1. PROPOSED ROLE OF CALCIUM IONS IN VENTRICULAR FIBRILLATION

The development of acute myocardial ischemia is associated with a rapid increase of extracellular potassium (Harris, 1954; Hirche et al, 1980), and an accumulation of cyclic AMP in the ischemic myocardium (Podzuweit et al, 1978). In this environment, elevated levels of cyclic AMP may promote formation of slow response action potentials (Reuter, 1974; Gettes, 1975; Vogel and Sperelakis, 1981). Recently an increase in α_1 adrenergic receptor number (Corr et al, 1981a) has been demonstrated in the ischemic myocardium; enhanced α_1 receptor stimulation may theoretically also induce formation of slow response action potentials (Bruckner and Scholz, 1980; Scholz, 1980).

Current electrophysiological evidence indicates that the calcium dependent slow response action potential is one possible mechanism which may be responsible for reentry and hence generation of early ventricular arrhythmias during acute myocardial ischemia (Cranefield, 1975; Wit and Bigger, 1975).

If this hypothesis relating to the electrophysiological mechanism of early ventricular arrhythmias is correct, then calcium antagonist procedures which inhibit transsarcolemmal calcium inward current (Fleckenstein, 1971) should also antagonise calcium dependent slow response action potentials and thereby protect against early ventricular arrhythmias.

The present investigation was, therefore, designed to study the

effect of calcium channel antagonist agents and reduction of extracellular calcium on the vulnerability to ventricular fibrillation during acute regional myocardial ischemia.

2. RESULTS

[A] Verapamil

Verapamil increased the coronary flow rates, verapamil $1.5 \times 10^{-7}M$, 9.1 ± 1.1 to 14.9 ± 1.0 ($p < 0.005$), $6 \times 10^{-8}M$, 9.2 ± 0.5 to 12.2 ± 0.7 ml/min ($p < 0.005$) respectively and decreased the heart rate, verapamil $1.5 \times 10^{-7}M$, 226 ± 11 to 195 ± 4 ($p < 0.05$), $6 \times 10^{-8}M$, 224 ± 2 to 196 ± 1 beats/min ($p < 0.001$) respectively. Atrioventricular conduction block occurred when concentrations above $1.5 \times 10^{-7}M$ were used.

After coronary artery ligation the total (ischemic and non-ischemic) coronary flow rates fell, verapamil $1.5 \times 10^{-7}M$, 9.3 ± 1.3 ($p < 0.0001$); $6 \times 10^{-8}M$, 7.5 ± 0.5 ml/min ($p < 0.001$) respectively, but were elevated in comparison to total coronary flow rates (3.9 ± 0.5 ml/min) of control coronary artery ligated hearts (Table 6-1).

Verapamil $1.5 \times 10^{-7}M$ did not elevate the ventricular fibrillation threshold in the non-ligated heart but maintained higher ventricular fibrillation threshold values during the phase of acute regional myocardial ischemia (Fig 6-1). In a separate series of hearts paced at a heart rate (230 beats/min) similar to the control series, verapamil $1.5 \times 10^{-7}M$ maintained higher ventricular fibrillation threshold levels during acute regional myocardial ischemia, thus excluding slow heart rate as the factor mediating protection against ventricular fibrillation (Fig 6-1). Investigation with d(+) and l(-) optimal isomers of verapamil $1.5 \times 10^{-7}M$ (both series paced at

230 beats/min) showed that d(+) verapamil $1.5 \times 10^{-7} \text{M}$ was as effective as l(-) verapamil $1.5 \times 10^{-7} \text{M}$ in attenuating the fall in the ventricular fibrillation threshold during myocardial ischemia (Fig 6-2).

dl verapamil $1.5 \times 10^{-7} \text{M}$ attenuated the fall in the ventricular fibrillation threshold during acute myocardial ischemia with concomitant adrenaline $5 \times 10^{-7} \text{M}$ stimulation (Fig 6-6). Investigation with the optical isomers of verapamil showed that l(-) verapamil but not d(+) verapamil $1.5 \times 10^{-7} \text{M}$ attenuated the fall in the ventricular fibrillation threshold during ischemia plus adrenaline $5 \times 10^{-7} \text{M}$ stimulation (Fig 6-7).

[B] Nifedipine

Nifedipine 10^{-7}M to 10^{-6}M produced a non-concentration dependent increase in the coronary flow rates prior to ligation and preserved coronary flow rates during acute myocardial ischemia (Table 6-1). Nifedipine $1 \times 10^{-6} \text{M}$ was the highest concentration which could be used without production of spontaneous atrioventricular dissociation.

In non-ligated hearts, nifedipine $1 \times 10^{-6} \text{M}$ elevated the ventricular fibrillation threshold 10.8 ± 1.3 to $16.5 \pm 2.2 \text{ mA}$ ($p < 0.05$). Nifedipine $1 \times 10^{-6} \text{M}$ was associated with higher ventricular fibrillation threshold values throughout the 15 min period of acute myocardial ischemia whereas at two lower concentrations, i.e. $5 \times 10^{-7} \text{M}$ and $1 \times 10^{-7} \text{M}$ this was not evident (Fig 6-3).

Nifedipine $1 \times 10^{-6} \text{M}$ also attenuated the fall in ventricular fibrillation threshold during acute myocardial ischemia plus adrenaline $5 \times 10^{-7} \text{M}$ stimulation (Fig 6-6).

[C] Diltiazem

Diltiazem $5 \times 10^{-6} \text{M}$, $5 \times 10^{-7} \text{M}$ and $1 \times 10^{-7} \text{M}$ increased coronary flow rates in similar fashion to verapamil and nifedipine (Table 6-1). At concentrations higher than $1 \times 10^{-6} \text{M}$ complete atrioventricular dissociation occurred. Diltiazem 10^{-7}M to $5 \times 10^{-6} \text{M}$ did not increase the ventricular fibrillation threshold levels in non-ligated hearts at all concentrations tested.

The series of hearts perfused with diltiazem $5 \times 10^{-6} \text{M}$ and paced at a heart rate of 230 beats/min exhibited a similar trend to the verapamil $1.5 \times 10^{-7} \text{M}$ and nifedipine $1 \times 10^{-6} \text{M}$ series in being able to maintain higher ventricular fibrillation threshold values during the 15 min period of acute myocardial ischemia (Fig 6-4). Unlike nifedipine, diltiazem in lower concentrations, $5 \times 10^{-7} \text{M}$ and $1 \times 10^{-7} \text{M}$, displayed a trend towards higher ventricular fibrillation threshold values in the first 10 min following coronary artery ligation but at 15 min this was not evident (Fig 6-4).

During acute myocardial ischemia with adrenaline $5 \times 10^{-7} \text{M}$ stimulation, diltiazem $5 \times 10^{-6} \text{M}$ partially prevented the fall in the ventricular fibrillation threshold (Fig 6-6).

[D] Reduction in perfusate Ca^{2+} concentration

Reduction in the perfusate calcium ion concentration from 2.5 mM to 1.0 mM was not associated with preservation of coronary flow rate or reduction in heart rate during acute myocardial ischemia (Table 6-1). Reduction in the extracellular calcium ion concentration was, however, associated with higher ventricular fibrillation threshold levels during acute myocardial ischemia in the absence or presence of adrenaline $5 \times 10^{-7} \text{M}$ stimulation (Figs 6-5, 6-8).

[E] High energy phosphates

The tissue measurements of adenosine triphosphate and phosphocreatine in both the non-ischemic and ischemic myocardium in the control series and in hearts subjected to calcium-antagonist procedures are shown in Table 6-2.

Calcium channel antagonist agents, in general, maintained higher ATP levels in the ischemic myocardium. However, reduction in perfusate Ca^{2+} concentration from 2.5 mM to 1.0 mM was not associated with preservation of ATP in the ischemic myocardium. Both decreased and increased vulnerability to ventricular fibrillation (compare nifedipine series) were associated with preservation of ATP in the ischemic myocardium. Moreover protection against ventricular fibrillation could occur in the absence of preservation of ATP in the ischemic myocardium, viz verapamil 1.5×10^{-7} M (paced series) and reduction in perfusate calcium (1.0 mM series).

Calcium channel antagonist agents but not a lowered extracellular Ca^{2+} ion concentration displayed a trend towards preservation of phosphocreatine levels in the ischemic myocardium. However no association was evident between myocardial phosphocreatine levels and vulnerability to ventricular fibrillation.

[F] Cyclic AMP (Table 6-3)

In the control non-ligated rat heart the cyclic AMP content was 0.44 ± 0.02 nmol/g fresh weight. In the control coronary artery ligated rat heart, the cyclic AMP content in the non-ischemic myocardium was 0.54 ± 0.02 and in the ischemic myocardium 0.71 ± 0.03 nmol/g fresh weight respectively. The cyclic AMP level in the non-ischemic myocardium was elevated in comparison to the level in the non-ligated heart ($p < 0.01$). In addition, there was an accumulation of cyclic AMP in the ischemic

myocardium relative to the non-ischemic myocardium ($p < 0.001$). This increase in cyclic AMP in the ischemic myocardium has been considered to play a major role in ventricular arrhythmogenesis.

Calcium channel antagonist agents reduced cyclic AMP levels in the ischemic myocardium, nifedipine 10^{-6} M, 0.56 ± 0.02 ($p < 0.02$), diltiazem 5×10^{-7} M, 0.53 ± 0.04 ($p < 0.01$) vs control 0.71 ± 0.03 nmol/g fresh weight. Nifedipine 5×10^{-7} M reversed the cyclic AMP content in the ischemic zone to the level found in the control non-ligated heart. Reduction in perfusate calcium concentration decreased cyclic AMP levels in both the non-ischemic and ischemic myocardium to values comparable to that found in the control non-ligated rat heart.

Lower cyclic AMP levels in the ischemic myocardium were associated in some series with protection and in other series with no protection against ventricular fibrillation. Although there was an absolute reduction in the cyclic AMP content in the ischemic zone in all series subjected calcium antagonist procedures, the cyclic AMP levels in these zones were always higher than the values present in the non-ischemic myocardium. Thus a relative accumulation of cyclic AMP still occurred in the ischemic zone.

3. DISCUSSION

Verapamil 1.5×10^{-7} M, nifedipine 10^{-6} M, diltiazem 5×10^{-6} M and a reduction in the extracellular Ca^{2+} ion concentration attenuated the fall in the ventricular fibrillation threshold during acute myocardial ischemia in the absence and presence of adrenergic stimulation. Recent studies in the dog (Brooks et al, 1980; Clusin et al, 1982), rat (Fagbemi and Parratt, 1981) and pig (Muller et al, 1984)

complement our findings that calcium channel antagonist agents exhibit ventricular antiarrhythmic activity during acute myocardial ischemia. The exact mechanism whereby protection against ventricular fibrillation occurs appears complex in view of the heterogeneity of effects produced by calcium antagonist procedures, viz. (i) slowing of the heart rate; (ii) coronary artery vasodilation; (iii) preservation of myocardial high energy phosphate content; (iv) reduction in tissue cyclic AMP content; (v) inhibition of transsarcolemmal calcium influx and (vi) non-specific effect .

[A] Heart rate

The ventricular antiarrhythmic effect of calcium antagonist procedures occurred independently of the heart rate. First, reduction in perfusate calcium concentration attenuated the fall in the ventricular fibrillation threshold despite no alteration in the heart rate. Secondly, hearts perfused with verapamil and paced at a heart rate similar to the control series maintained higher ventricular fibrillation threshold values during acute myocardial ischemia.

[B] Coronary artery vasodilation

Maintenance of higher coronary flow rates or redistribution of coronary flow via coronary artery vasodilation is one possible mechanism whereby calcium antagonist procedures may exhibit ventricular antiarrhythmic activity (Ross and Jorgensen, 1967; Haas and Hartfeldes, 1962).

However, preservation of total coronary flow rates during acute myocardial ischemia was associated in some series with protection whilst in other series with lack of protection against ventricular fibrillation. Moreover reduction in perfusate calcium concentration

exhibited antifibrillatory activity despite no alteration in total coronary flow rates. Since intramyocardial coronary flow redistribution was not measured, this factor cannot be excluded in the mechanism of protection. However, the following arguments suggest that this was unlikely to be the factor mediating protection. First, a lowered extracellular calcium concentration exhibited ventricular antiarrhythmic activity but did not alter total coronary flow rates; reduction in extracellular calcium concentration has not been shown to cause redistribution of coronary flow (Clusin et al, 1982). Secondly, in two other series, the antifibrillatory effect of verapamil and diltiazem could not be ascribed to coronary artery vasodilation or redistribution of coronary flow (Sherman et al, 1981; Clusin et al, 1982).

[C] High energy phosphates

Procedures which inhibit cardiac metabolism may preserve high energy phosphate content (Weber, 1959). Preservation of energy status may maintain cell membrane integrity and also prevent the decrease in action potential duration; two factors which may account for ventricular antiarrhythmic action.

Calcium channel antagonist agents demonstrated a trend towards preservation of ATP in the ischemic myocardium. However, high ATP levels were associated with both decreased and increased vulnerability to ventricular fibrillation (compare nifedipine series). Furthermore, protection against ventricular fibrillation could occur in the absence of preservation of ATP in the ischemic myocardium (reduced perfusate calcium series and paced verapamil 1.5×10^{-7} M series). Tissue levels of PCR did not correlate with antifibrillatory activity. Thus attenuation of the fall in the ventricular fibrillation threshold occurred independently of preservation of energy status.

[D] Cyclic AMP

Podzuweit et al (1976) proposed that accumulation of cyclic AMP in the ischemic myocardium may play a major role in the genesis of ventricular fibrillation. The present study demonstrates that all calcium antagonist procedures reduced levels of cyclic AMP in both the non-ischemic and ischemic myocardium. Reduced cyclic AMP content in the ischemic myocardium was associated with both protection and lack of protection against electrically induced ventricular fibrillation.

If cyclic AMP is held to be an arrhythmogenic agent, why are reduced levels in the ischemic myocardium not associated with protection? The exact reason for this complex finding is unclear, but there are two possible explanations:

1. Although calcium antagonist procedures reduced the cyclic AMP content in both the non-ischemic and ischemic myocardium, the cyclic AMP level in the ischemic zone was significantly higher than that found in the non-ischemic zone in all series. Specifically, nifedipine $5 \times 10^{-7} M$ reduced the cyclic AMP level in ischemic zone to normal levels, but was not associated with protection. However, this cyclic AMP level in the ischemic zone was still elevated relative to the non-ischemic myocardium. Thus the relative accumulation of cyclic AMP in the ischemic myocardium rather than the absolute level in the ischemic zone may be of importance in the genesis of ventricular fibrillation.
2. The alternative possibility is that cyclic AMP does not play a role in the genesis of ventricular fibrillation during acute myocardial ischemia.

[E] Calcium channel antagonist agents - alpha-adrenoceptor interaction

Verapamil has been shown to interact with both α_1 and

alpha₂ adrenoceptors (Nayler et al, 1982). Hence it has been suggested that the ventricular antiarrhythmic activity of verapamil may not be due to inhibition of the transsarcolemmal calcium inward current but rather due to alpha-adrenoceptor antagonism. Nifedipine does not interact with either alpha₁ or alpha₂ adrenergic receptors (Motulsky et al, 1983); hence it can be termed a relatively 'pure calcium antagonist'. That nifedipine antagonised the enhanced vulnerability to ventricular fibrillation during acute myocardial ischemia is strong, albeit indirect, evidence that the protective effect is due to inhibition of transsarcolemmal calcium inward current and not to either alpha₁ or alpha₂-adrenoceptor antagonism.

[F] Optical isomers of verapamil

To further define the mechanism of protection of calcium antagonists and to analyse the role of the transsarcolemmal calcium current in ventricular arrhythmogenesis, investigation was undertaken with the d(+) and l(-) isomers of verapamil. Electrophysiological studies in the superfused guinea-pig papillary muscle showed that blockade of the slow channel calcium current was achieved by l(-) but not d(+) verapamil; both isomers did not affect the fast sodium channel. Recent evidence suggests that qualitatively both l(-) and d(+) verapamil block the slow calcium channel; however quantitatively l(-) verapamil is more potent than d(+) verapamil. Both isomers, reputedly, only inhibit the fast sodium channel when used in concentrations above 3×10^{-6} M (Gloor and Urthaler, 1983).

In the isolated rat heart, both l(-) and d(+) verapamil 1.5×10^{-7} M partly attenuated the fall in the ventricular fibrillation threshold during acute myocardial ischemia. During acute myocardial ischemia with concomitant adrenergic stimulation, l(-) but not

d(+) verapamil attenuated the enhanced vulnerability to ventricular fibrillation. The antifibrillatory effect of verapamil appears specific to the calcium channel during the setting of myocardial ischemia and adrenergic stimulation.

[G] Reduction in extracellular calcium ion concentration

More direct evidence that the transsarcolemmal calcium inward current plays a role in ventricular arrhythmogenesis stems from the finding that a reduction in perfusate calcium concentration decreased the vulnerability to ventricular fibrillation during acute myocardial ischemia. Further support for an arrhythmogenic role of calcium arises from the data of Clusin and co-workers (1982) that reduction in serum ionized calcium by infusion of Na citrate mimics the effect of diltiazem in prolonging the latency period to ventricular fibrillation in the dog model of global left ventricular ischemia.

The findings presented in this chapter indicate that the transsarcolemmal calcium current plays a role in the genesis of ventricular fibrillation during acute myocardial ischemia. That calcium antagonist procedures only partially prevent the fall in the ventricular fibrillation threshold suggest that the transsarcolemmal calcium current may not be the only factor involved in the genesis of ventricular fibrillation.

4. CONCLUSIONS

1. Calcium channel antagonist agents and a reduction in the extracellular calcium concentration attenuated the fall in ventricular fibrillation threshold during acute myocardial ischemia both in the absence and presence of adrenergic stimulation.

2. Protection was not due to maintenance of total coronary flow, reduction in heart rate, preservation of energy status or reduction in cyclic AMP content.
3. Redistribution of coronary flow from non-ischemic to ischemic myocardium, although unlikely, could not be excluded in the mechanism of protection.
4. The transsarcolemmal calcium inward current appears to play a role in the genesis of ventricular fibrillation during acute myocardial ischemia.

Figure 6-1

EFFECT OF VERAPAMIL ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

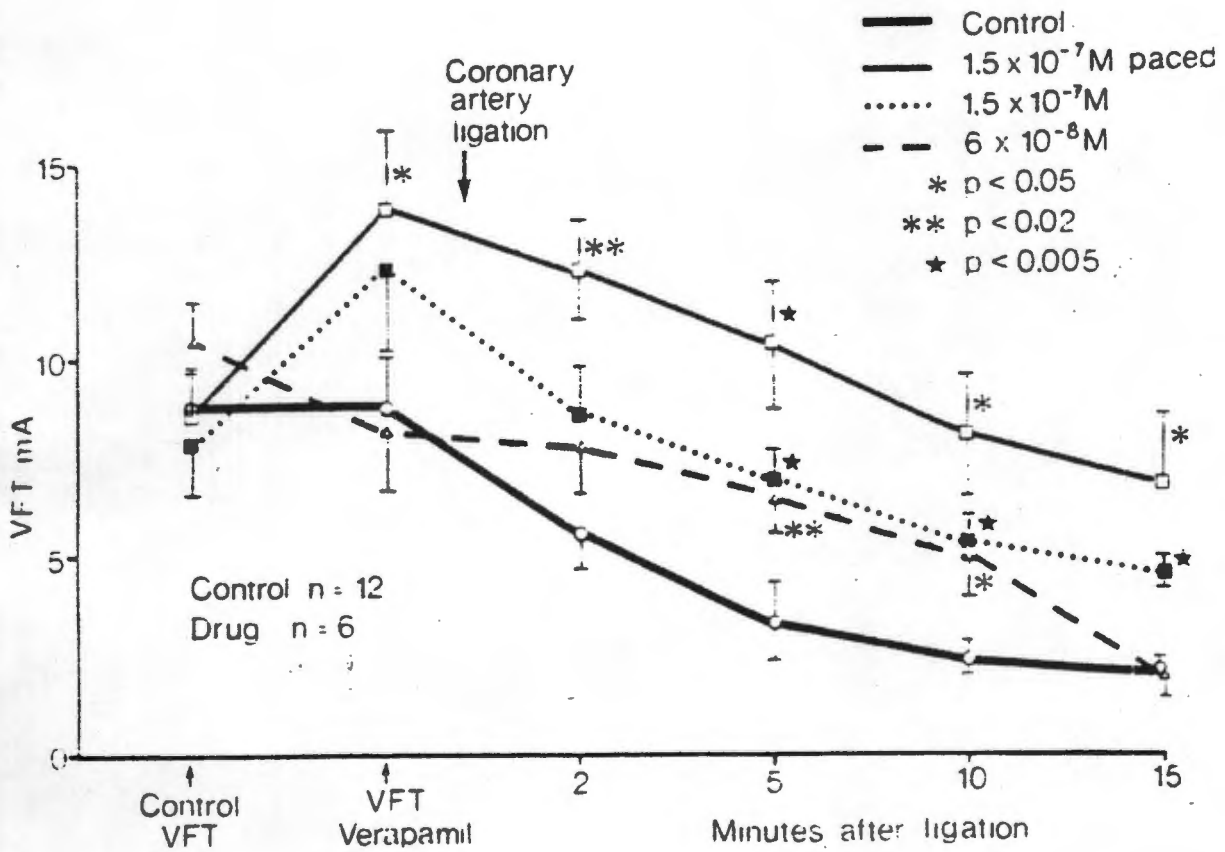


Figure 6-2

EFFECT OF dl VERAPAMIL, d(+) VERAPAMIL, l(-) VERAPAMIL
ON VENTRICULAR FIBRILLATION THRESHOLD DURING ACUTE
REGIONAL MYOCARDIAL ISCHEMIA

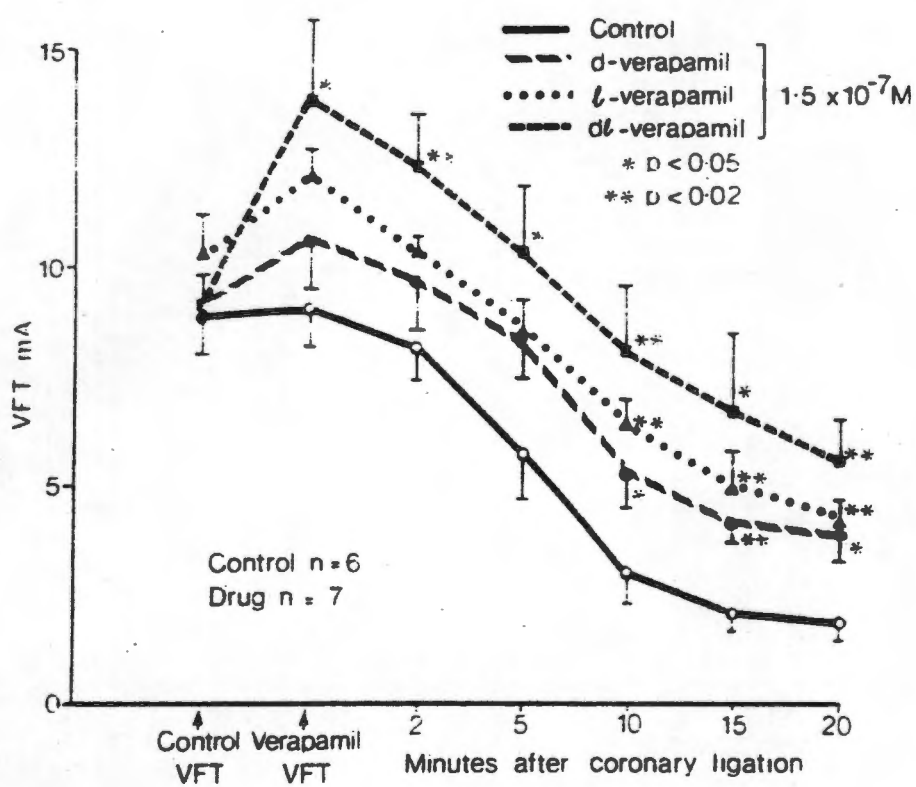


Figure 6-3

EFFECT OF NIFEDIPINE ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

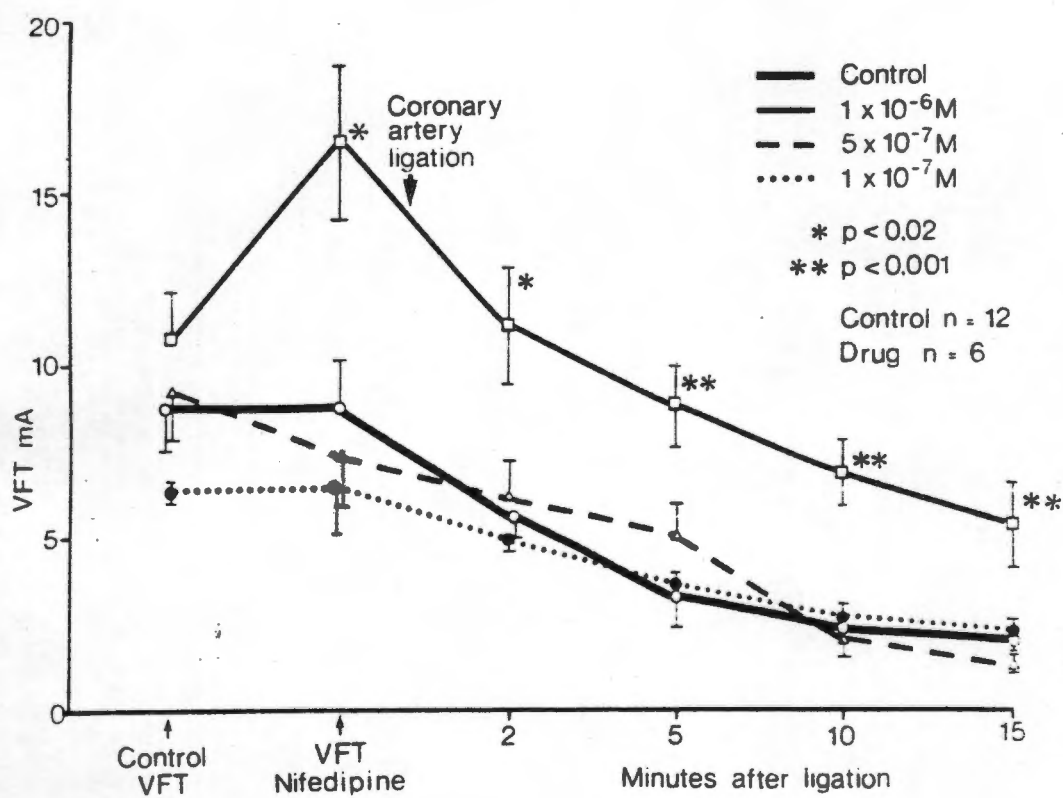


Figure 6-4

EFFECT OF DILTIAZEM ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

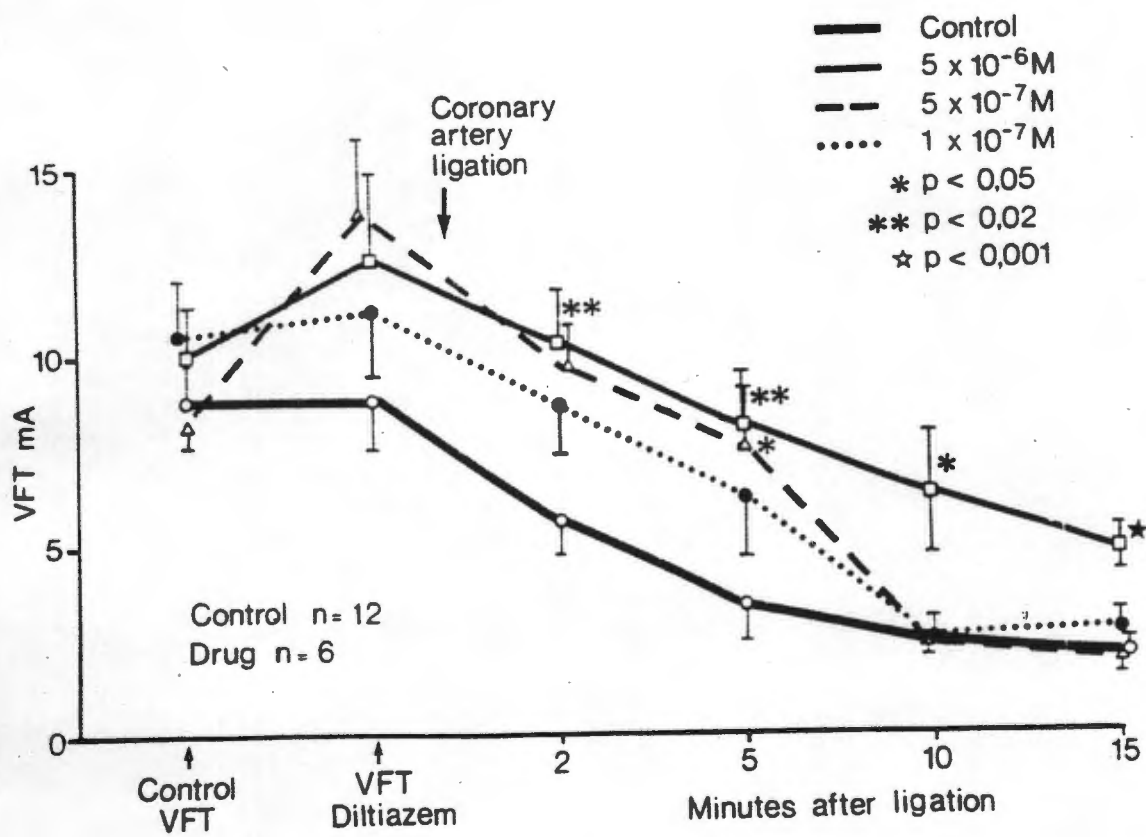


Figure 6-5

EFFECT OF REDUCTION OF EXTRACELLULAR CALCIUM
CONCENTRATION ON VENTRICULAR FIBRILLATION THRESHOLD
DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

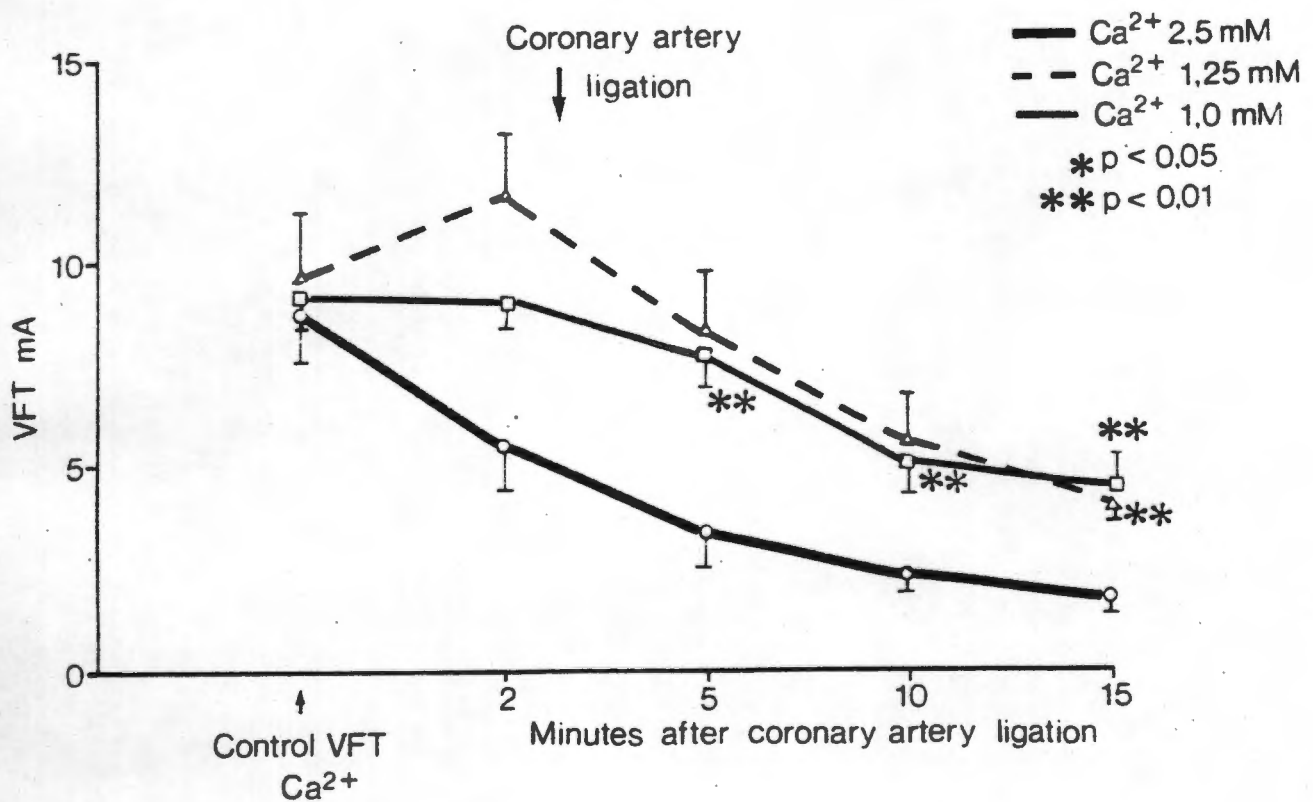


Figure 6-6

EFFECT OF CALCIUM ANTAGONIST AGENTS ON VENTRICULAR
FIBRILLATION THRESHOLD DURING ACUTE REGIONAL
MYOCARDIAL ISCHEMIA PLUS ADRENERGIC STIMULATION

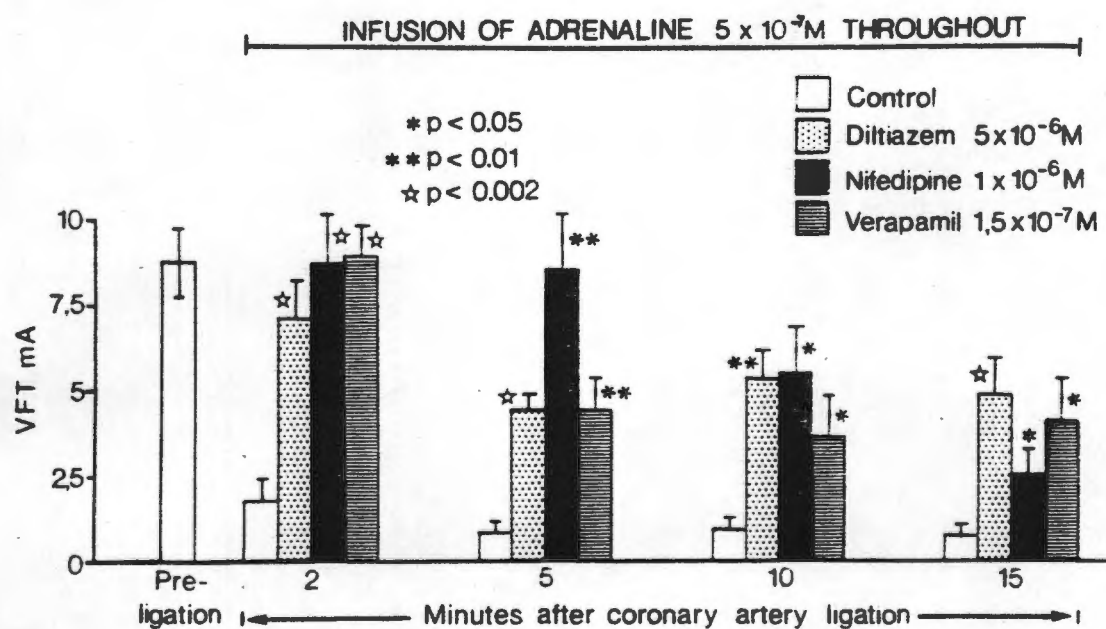


Figure 6-7

EFFECT OF d(+) AND l(-) VERAPAMIL ON VENTRICULAR
FIBRILLATION THRESHOLD DURING ACUTE REGIONAL
MYOCARDIAL ISCHEMIA PLUS ADRENERGIC STIMULATION

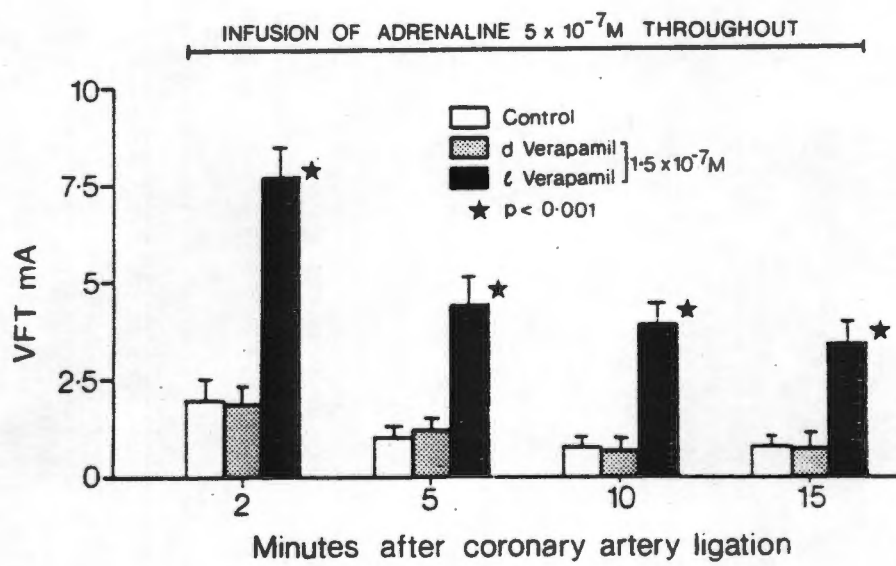


Figure 6-8

EFFECT OF REDUCTION OF EXTRACELLULAR CALCIUM ION
CONCENTRATION ON VENTRICULAR FIBRILLATION THRESHOLD
DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA PLUS
ADRENERGIC STIMULATION

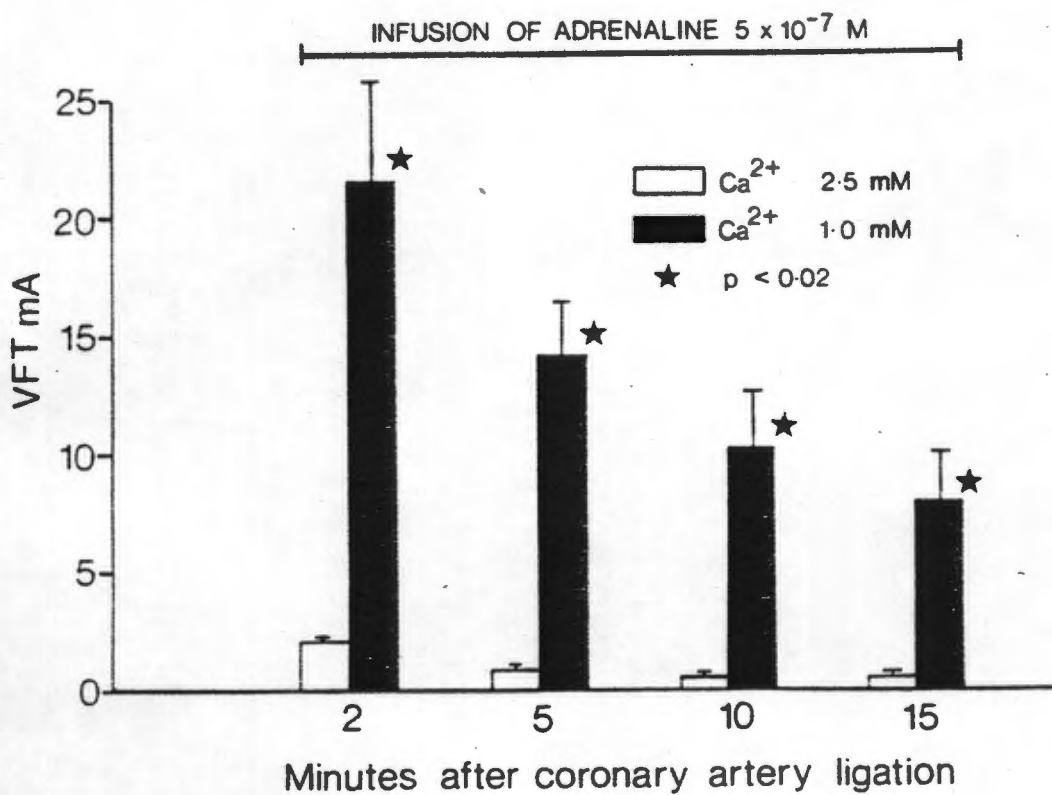


Table 6-1

Effect of calcium channel antagonist agents and reduction in extracellular calcium on heart rate, total coronary flow rate and ventricular fibrillation threshold after coronary artery ligation

<u>Procedure</u>	<u>Mean heart rate</u> (beats/min)	<u>Mean coronary flow</u> (ml/min)	<u>VFT 15' CAL</u> (mA)
Control (non-ligated heart)	243 ± 10	7.8 ± 0.5	10.0 ± 0.3
Coronary artery ligation (CAL)	199 ± 14	3.9 ± 0.5	2.0 ± 0.3
CAL + Verapamil 1.5 x 10 ⁻⁷ M	250 (paced)	9.3 ± 1.3**	6.7 ± 1.8*
CAL + 1(-)verapamil 1.5 x 10 ⁻⁷ M	230 (paced)	8.0 ± 0.5**	4.9 ± 0.8*
CAL + nifedipine 1 x 10 ⁻⁶ M	185 ± 8	9.0 ± 0.8**	4.7 ± 0.9*
		NS	p<0.001
CAL + nifedipine 5 x 10 ⁻⁷ M	201 ± 8	8.0 ± 0.4**	1.3 ± 0.2
CAL + nifedipine 1 x 10 ⁻⁷ M	194 ± 9	8.0 ± 0.7**	1.8 ± 0.4
CAL + diltiazem 5 x 10 ⁻⁶ M	230 (paced)	10.1 ± 0.3**	4.8 ± 0.5**
		p<0.02	p<0.001
CAL + diltiazem 5 x 10 ⁻⁷ M	183 ± 14	7.9 ± 0.5**	1.9 ± 0.6
CAL + lowered perfusate Ca ²⁺ (1.0mM instead of 2.5mM)	221 ± 7	4.0 ± 0.2	4.3 ± 0.7*

*p < 0.02 **p < 0.001

Number of hearts = 6-12

Values represent Mean ± SEM

p values vs coronary artery ligation

In addition, p values nifedipine 1 x 10⁻⁶M vs nifedipine 5 x 10⁻⁷M
and diltiazem 5 x 10⁻⁶M vs diltiazem 5 x 10⁻⁷M

Table 6-2

Effect of calcium channel antagonist agents and reduction in extracellular calcium on tissue high energy phosphate and ventricular fibrillation threshold levels 15 minutes after coronary artery ligation

<u>Procedure</u>	<u>Non-ischemic myocardium</u>		<u>Ischemic myocardium</u>		<u>VFT</u>
	<u>($\mu\text{mol/g}$)</u>		<u>($\mu\text{mol/g}$)</u>		<u>(mA)</u>
	<u>ATP</u>	<u>PCr</u>	<u>ATP</u>	<u>PCr</u>	
Coronary artery ligation	3.90 <u>+0.23</u>	4.49 <u>+0.61</u>	1.91 <u>+0.28</u>	1.77 <u>+0.39</u>	2.0 <u>+0.3</u>
Verapamil $1.5 \times 10^{-7}\text{M}$ paced	3.19 <u>+0.31</u>	4.81 <u>+0.66</u>	2.85 <u>+0.45</u>	2.87 <u>+0.98</u>	6.7** <u>+1.8</u>
Verapamil $1.5 \times 10^{-7}\text{M}$ unpaced	5.27** <u>+0.12</u>	5.66 <u>+0.37</u>	4.70*** <u>+0.54</u>	3.98** <u>+0.92</u>	4.5** <u>+0.4</u>
Nifedipine $1 \times 10^{-6}\text{M}$	3.51 <u>+0.05</u>	4.28 <u>+0.05</u>	2.74** <u>+0.13</u>	2.13 <u>+0.21</u>	4.7** <u>+0.9</u>
Nifedipine $5 \times 10^{-7}\text{M}$	4.36 <u>+0.29</u>	5.46 <u>+0.69</u>	2.84* <u>+0.20</u>	3.04 <u>+0.35</u>	1.3 <u>+0.2</u>
Diltiazem $5 \times 10^{-7}\text{M}$	3.54 <u>+0.21</u>	4.07 <u>+0.16</u>	2.39 <u>+0.28</u>	2.52 <u>+0.34</u>	1.9 <u>+0.6</u>
Diltiazem $5 \times 10^{-6}\text{M}$	3.63 <u>+0.09</u>	3.35 <u>+0.10</u>	2.37 <u>+0.13</u>	1.61 <u>+0.13</u>	4.8** <u>+0.5</u>
Lowered perfusate Ca^{2+} (1.0mM instead of 2.5mM)	3.47 <u>+0.21</u>	3.99 <u>+0.24</u>	1.51 <u>+0.20</u>	1.03 <u>+0.09</u>	4.3** <u>+0.7</u>

*p < 0.05 **p < 0.02 ***p < 0.001

p value vs coronary artery ligation

Values represent Mean \pm SEM

Table 6-3

Effect of calcium channel antagonist agents and
reduction in extracellular calcium on tissue cAMP
and ventricular fibrillation threshold levels
15 minutes after coronary artery ligation

<u>Procedure</u>	<u>Cyclic AMP</u>			<u>VFT</u> (mA)
	<u>Non-ischemic myocardium</u>		<u>Ischemic myocardium</u>	
	(nmol/g fresh wt)		(nmol/g fresh wt)	
1. Control (non-ligated heart)	0.44 ± 0.02			10.0 ± 0.3
2. Coronary artery ligation (CAL)	0.54 ± 0.02	<0.001	0.71 ± 0.03	2.0 ± 0.3
p vs 1	<0.01		<0.001	<0.01
3. Verapamil 1.5x10 ⁻⁷ M unpaced	0.39 ± 0.03	<0.02	0.55 ± 0.05	4.5 ± 0.4
p vs 1	NS		NS	<0.01
p vs 2	<0.001		<0.01	<0.01
4. Nifedipine 1x10 ⁻⁶ M	0.41 ± 0.05	<0.05	0.56 ± 0.02	4.7 ± 0.9
p vs 1	NS		<0.01	<0.01
p vs 2	<0.01		<0.01	<0.02
5. Nifedipine 5x10 ⁻⁷ M	0.32 ± 0.03	<0.02	0.46 ± 0.04	1.3 ± 0.2
p vs 1	<0.01		NS	<0.01
p vs 2	<0.001		<0.005	NS
6. Diltiazem 5x10 ⁻⁷ M	0.42 ± 0.03	<0.05	0.53 ± 0.04	1.9 ± 0.6
p vs 1	NS		NS	<0.01
p vs 2	<0.01		<0.01	NS
7. Diltiazem 5x10 ⁻⁶ M	0.44 ± 0.02	<0.005	0.55 ± 0.02	4.8 ± 0.5
p vs 1	NS		<0.01	<0.01
p vs 2	<0.01		<0.01	<0.01
8. Lowered perfusate Ca ²⁺ (1.0mM instead of 2.5mM)	0.36 ± 0.03	<0.01	0.46 ± 0.02	4.3 ± 0.7
p vs 1	NS		NS	<0.01
p vs 2	<0.001		<0.001	<0.02

n = 5 to 12

Values represent Mean ± SEM

CHAPTER 7FAST CHANNEL BLOCKING AGENTS AND VULNERABILITY TO VENTRICULAR
FIBRILLATION DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA1. PROPOSED ROLE OF SODIUM IONS IN VENTRICULAR FIBRILLATION

The development of acute myocardial ischemia is associated with accumulation of extracellular potassium ion concentration in the ischemic myocardium, peak recorded values reaching 16.3 mM (Hirche et al, 1980). This degree of extracellular potassium accumulation has been shown to decrease the resting membrane potential of cardiac cells to values approximating -60 mV. It has been presumed that at such resting membrane potentials, the fast sodium channel is inactivated and the upstroke of the action potential is generated by enhanced calcium inward current evoked by adrenergic stimulation. Such action potentials, termed slow responses, are considered to be responsible for slow conduction in the ischemic myocardium and also the development of early ventricular fibrillation.

However, to date there is no conclusive evidence that the initial upstroke of the ischemic action potential is due to the slow inward calcium current. Recent evidence shows that at resting membrane potentials of -57 mV to -60 mV, the fast sodium channel even though it is almost completely inactivated, plays a role in generation of the initial upstroke of the action potential (Arita et al, 1983). Such action potentials, termed depressed fast responses are associated with conduction velocity slow enough to maintain reentrant circuits (Arita et al, 1983). Depressed fast responses may therefore be responsible for the slow conduction in the ischemic myocardium and also the development of early ventricular fibrillation.

If this hypothesis is correct then fast channel blocking agents which inhibit the transsarcolemmal sodium inward current should inhibit the occurrence of depressed fast responses and thereby prevent early ventricular fibrillation. I, therefore, investigated the effect of fast channel blocking agents, lignocaine and tetrodotoxin, on vulnerability to ventricular fibrillation during acute myocardial ischemia.

2. RESULTS

[A] Lignocaine

Lignocaine 3×10^{-5} M and 5×10^{-5} M elevated the ventricular fibrillation threshold, reduced heart rate and decreased coronary flow rates respectively prior to coronary artery ligation. During acute myocardial ischemia lignocaine 3×10^{-5} M to 5×10^{-5} M but not 10^{-5} M maintained higher ventricular fibrillation threshold levels than the control series subject to coronary artery ligation; in fact complete ventricular antiarrhythmic activity was evident (Fig 7-1). Protection was associated with heart rates and total coronary flow rates similar to that of the control series (Table 7-1); moreover preservation of metabolic status in the ischemic myocardium was not evident (Table 7-2). However, ventricular antiarrhythmic activity was accompanied with reduction in cyclic AMP content in both the non-ischemic and ischemic myocardium (Table 7-3).

[B] Tetrodotoxin

Tetrodotoxin 5×10^{-7} M and 3×10^{-6} M reduced the heart rate prior to coronary artery ligation: 296 ± 15 to 237 ± 11 ($p < 0.05$); 272 ± 9 to 206 ± 7 ($p < 0.05$) but did not alter coronary flow rate prior to coronary artery ligation: 8.8 ± 0.6 to 7.6 ± 0.4 (NS); 9.0 ± 0.3 to 8.1

+ 0.9 (NS). Tetrodotoxin $5 \times 10^{-7} \text{M}$ and $3 \times 10^{-6} \text{M}$ did not elevate ventricular fibrillation threshold levels prior to coronary artery ligation. Tetrodotoxin $3 \times 10^{-6} \text{M}$ but not $5 \times 10^{-7} \text{M}$ completely prevented the fall in ventricular fibrillation threshold during the entire period of acute myocardial ischemia (Fig 7-2). Protection against ventricular fibrillation was associated with total coronary flow rates and heart rates similar to the control series subject to coronary artery ligation (Table 7-1). Ventricular antiarrhythmic activity was not accompanied by preservation of adenosine triphosphate, phosphocreatine or glycogen content in the ischemic myocardium (Table 7-2); moreover there was no reduction in the lactate or cyclic AMP content in the ischemic myocardium (Table 7-3).

3. DISCUSSION

[A] Fast channel blocking agents and ventricular antiarrhythmic activity

Lignocaine $3 \times 10^{-5} \text{M}$ and tetrodotoxin $3 \times 10^{-6} \text{M}$ each completely prevented the fall in the ventricular fibrillation threshold during acute regional myocardial ischemia. Cardinal and associates (1981) showed that lignocaine $2 \times 10^{-5} \text{M}$ prevents spontaneous ventricular fibrillation in the ischemic porcine heart, whilst Borer et al (1976) illustrated that in concentrations of $5 \times 10^{-6} \text{M}$ to $3 \times 10^{-5} \text{M}$ lignocaine reduces the incidence of ischemic ventricular fibrillation in the dog.

Protection against ventricular fibrillation was not due to reduction in heart rate or dependent on preservation of total coronary flow rate during acute myocardial ischemia. Moreover, ventricular antiarrhythmic activity was not the result of preservation of high energy

phosphate stores in the ischemic myocardium, thereby excluding a metabolic factor in the mechanism of protection. Lignocaine but not tetrodotoxin decreased cyclic adenosine monophosphate content in the ischemic myocardium. Thus reduction in cyclic AMP content in the ischemic myocardium is not a prerequisite for protection against ventricular fibrillation.

[B] Transsarcolemmal sodium, intracellular sodium and ventricular antiarrhythmic activity

Lignocaine reduces the transsarcolemmal inward sodium current in cardiac muscle (Colatsky, 1982; Bean et al, 1981) and also decreases the intracellular sodium activity of sheep heart Purkinje fibres (Deitmer and Ellis, 1980; Eisner et al, 1983). The latter action of lignocaine has been ascribed to reduction in transsarcolemmal sodium influx which in turn allows the sodium potassium pump to extrude more sodium than that which leaks in; hence reduction in intracellular sodium activity occurs. Do these findings, therefore, indicate that the transsarcolemmal sodium inward current plays a major role in ventricular arrhythmogenesis during acute regional myocardial ischemia? A factor which questions this hypothesis is that lignocaine not only affects the fast sodium channel, but also produces an increase in the time independent outward potassium current; ^(Arnsdorf and Bigger 1972) such an effect may account for ventricular antiarrhythmic activity. Further investigation was, therefore, undertaken with tetrodotoxin, a specific inhibitor of the voltage dependent fast sodium channel (Blankenship, 1976; Coraboeuf and Vasort, 1968)). Tetrodotoxin $3 \times 10^{-6}M$ completely prevented the fall in the ventricular fibrillation threshold during acute myocardial ischemia. At approximately this concentration in sheep heart Purkinje fibres, tetrodotoxin inhibited the transsarcolemmal inward sodium current and reduced the intracellular

sodium activity by 40% (Deitmer and Ellis, 1980). The findings with lignocaine and tetrodotoxin provide strong evidence that the transsarcolemmal sodium inward current plays a major role in the genesis of ventricular fibrillation.

[C] Mechanism of ventricular antiarrhythmic action

The antiarrhythmic action of lignocaine and tetrodotoxin is probably due to a decrease of the transsarcolemmal inward sodium current with reduction in the excitability of cardiac tissue. Such an effect would inhibit depressed fast responses and thereby prevent reentrant circuits critical to the genesis of ventricular fibrillation. Experimental evidence favouring this concept include the following: (1) Lazzara and associates (1978b) illustrated that lignocaine and tetrodotoxin abolished propagating depressed action potentials and extinguished very slow conduction of both ischemic and hypoxic canine ventricular myocardial fibres; (2) Kupersmith et al (1975) showed that lignocaine selectively depressed conduction in the ischemic myocardium; (3) Arita and co-workers (1983) showed that lignocaine and tetrodotoxin decreased conduction velocity of depressed fast responses and produced complete conduction block and inexcitability of cardiac tissue; (4) Lignocaine and tetrodotoxin abolish depressed fast responses but do not alter slow response action potentials (Brennan et al, 1978).

An alternative explanation for the antiarrhythmic action is inhibition of the arrhythmogenic transient non-specific inward current which predisposes to afterdepolarizations and triggered automaticity. Both lignocaine and tetrodotoxin, by decreasing the inward sodium current, reduce the intracellular sodium and thereby the intracellular calcium ion concentration; the latter effect inhibits the arrhythmogenic transient non-specific inward current.

Although the antiarrhythmic action of lignocaine and tetrodotoxin may be mediated by either one of the two above mechanisms, the critical factor to both is inhibition of the transsarcolemmal sodium inward current.

4. CONCLUSIONS

1. Lignocaine and tetrodotoxin each completely prevented the fall in the ventricular fibrillation threshold during acute myocardial ischemia.
2. Protection was not due to reduction in heart rate, coronary artery vasodilation, preservation of energy status or reduction in myocardial cyclic adenosine monophosphate.
3. Ventricular antiarrhythmic activity appears to be due to inhibition of the transsarcolemmal sodium inward current.

Figure 7-1

EFFECT OF LIGNOCAINE ON VENTRICULAR FIBRILLATION

THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

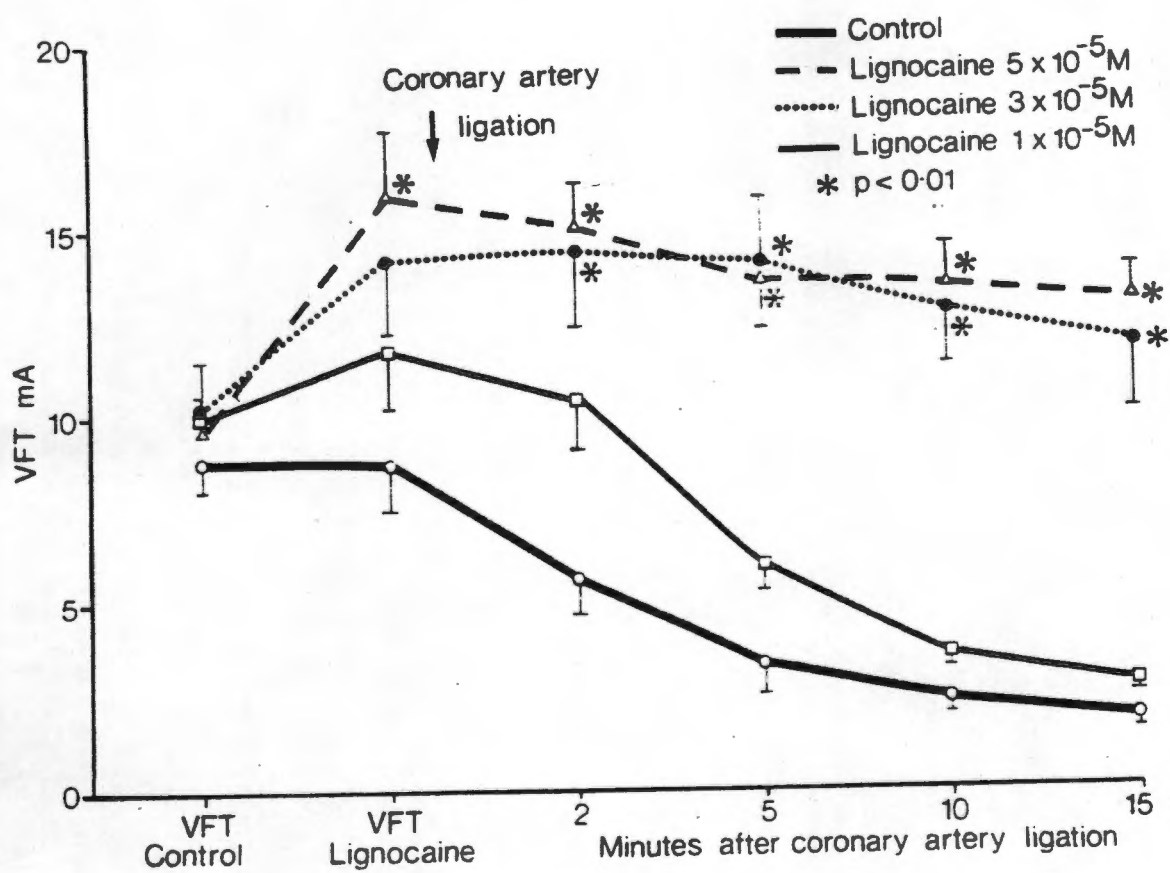


Figure 7-2

EFFECT OF TETRODOTOXIN ON VENTRICULAR FIBRILLATION
THRESHOLD DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

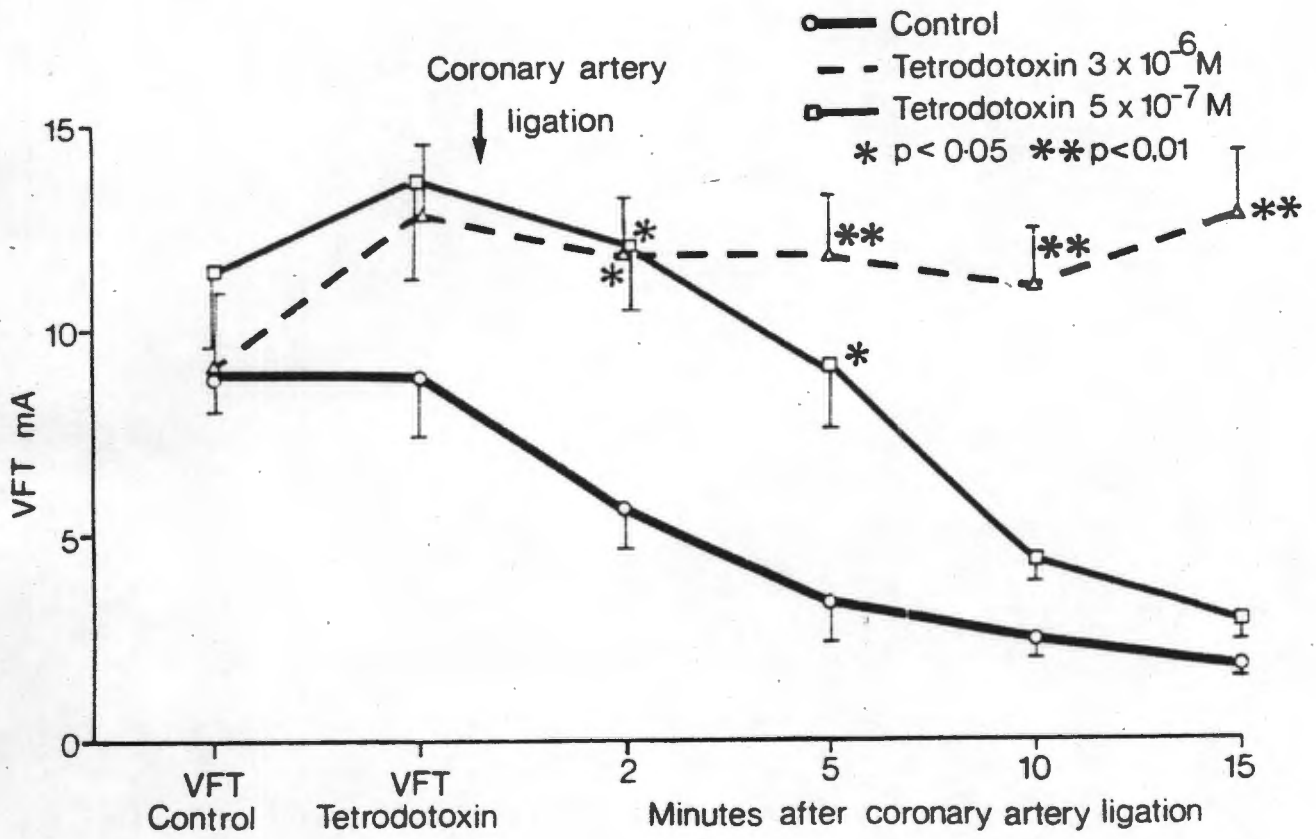


Table 7-1

Effect of fast channel blocking agents on heart rate, total coronary flow rate and ventricular fibrillation threshold after coronary artery ligation

<u>Procedure.</u>	<u>Mean heart rate</u> (beats/min)	<u>Mean total coronary flow</u> (mls/min)	<u>VFT 15' CAL</u> (mA)
1. Non-ligated heart	243 \pm 10	7.8 \pm 0.5	10.0 \pm 0.3
2. Coronary artery ligation (CAL) p vs 1	199 \pm 14 <0.02	3.9 \pm 0.5 <0.01	2.0 \pm 0.3 <0.01
3. CAL + lignocaine 10 ⁻⁶ M p vs 1 p vs 2	172 \pm 5 <0.01 NS	3.9 \pm 0.3 <0.01 NS	2.8 \pm 0.4 <0.01 NS
4. CAL + lignocaine 5 x 10 ⁻⁵ M p vs 1 p vs 2	207 \pm 8 <0.05 NS	4.9 \pm 0.3 <0.05 NS	13.0 \pm 1.0 NS <0.01
5. CAL + tetrodotoxin 5 x 10 ⁻⁷ M p vs 1 p vs 2	199 \pm 4 <0.01 NS	4.0 \pm 0.3 <0.01 NS	3.0 \pm 0.4 <0.01 NS
6. CAL + tetrodotoxin 3 x 10 ⁻⁶ M p vs 1 p vs 2	200 \pm 10 <0.02 NS	4.3 \pm 0.4 <0.01 NS	12.7 \pm 1.5 NS <0.01

Number of hearts = 5-12

Values represent Mean \pm SEM

Table 7-2

Effect of fast channel blocking agents on tissue
metabolic status 15 minutes after coronary artery
ligation

	<u>ATP</u>	<u>Ischemic myocardium</u>		
		<u>PCr</u>	<u>Glycogen</u>	<u>Lactate</u>
	[————— $\mu\text{mol/g}$ fresh wt —————]			
1. Coronary artery ligation (CAL)(n=12)	2.07 <u>+0.23</u>	1.54 <u>+0.21</u>	2.54 <u>+0.67</u>	16.41 <u>+2.33</u>
2. CAL + tetrodotoxin $3 \times 10^{-6}\text{M}$ (n=5)	0.78 <u>+0.10</u>	0.90 <u>+0.14</u>	2.48 <u>+0.29</u>	15.11 <u>+2.14</u>
p vs 1	< 0.01	NS	NS	NS
3. CAL + lignocaine $3 \times 10^{-5}\text{M}$ (n=5)	0.62 <u>+0.17</u>	0.80 <u>+0.12</u>	-	11.49 <u>+0.86</u>
p vs 1	<0.01	<0.01	-	NS

Values represent Mean + SEM

Table 7-3

Effect of fast channel blocking agents on tissue cyclic AMP and ventricular fibrillation threshold levels 15 minutes after coronary artery ligation

<u>Intervention</u>	<u>Cyclic AMP</u>		<u>VFT 15' CAL</u> (mA)
	<u>Non-ischemic</u> <u>myocardium</u> (nmol/g fresh wt)	<u>Ischemic</u> <u>myocardium</u>	
Non-ligated heart	0.44 ± 0.02		10.0 ± 0.3
Coronary artery ligation (CAL)	0.54 ± 0.02	0.71 ± 0.03	2.0 ± 0.3
p vs 1	< 0.01	< 0.001	< 0.01
CAL + lignocaine 3 x 10 ⁻⁵ M	0.33 ± 0.02	0.50 ± 0.02	11.8 ± 1.8
p vs 1	< 0.01	NS	NS
p vs 2	< 0.001	< 0.01	< 0.01
CAL + lignocaine 5 x 10 ⁻⁵ M	0.34 ± 0.02	0.45 ± 0.02	13.0 ± 1.0
p vs 1	< 0.01	NS	NS
p vs 2	< 0.001	< 0.01	< 0.01
CAL + tetrodotoxin 3 x 10 ⁻⁶ M	0.54 ± 0.03	0.63 ± 0.03	12.7 ± 1.5
p vs 1	< 0.02	< 0.001	NS
p vs 2	NS	NS	< 0.01

Number of hearts = 5-12

Values represent Mean ± SEM

CHAPTER 8CONCLUSIONS

[A] VENTRICULAR FIBRILLATION INDUCED BY ENHANCED ADRENERGIC STIMULATION IN THE NON-ISCHEMIC HEART

(1) Beta-adrenoceptor antagonist agents, in concentrations producing beta-receptor antagonism, prevent the adrenaline-induced vulnerability to ventricular fibrillation. The beta-adrenergic receptor is directly implicated in the genesis of ventricular fibrillation induced by enhanced adrenergic stimulation. Cyclic AMP, the proposed intracellular second messenger of beta-receptor stimulation may not mediate the arrhythmogenic effect of enhanced adrenergic activity, but simply be an epi-phenomenon.

(2) Calcium antagonist procedures prevent the adrenaline-induced vulnerability to ventricular fibrillation. Calcium ions appear to act as the intracellular second messenger in mediating the arrhythmogenic effect of enhanced adrenergic stimulation.

[B] VENTRICULAR FIBRILLATION INDUCED BY ACUTE REGIONAL MYOCARDIAL ISCHEMIA

(1) Beta-adrenoceptor antagonist agents, in concentrations producing beta-receptor antagonism, are ineffective in preventing the enhanced vulnerability to ventricular fibrillation. In concentrations higher than that required to produce beta-adrenoceptor antagonism, some but not all beta-antagonist agents prevent the enhanced vulnerability to

ventricular fibrillation. The beta-adrenergic receptor does not appear to play a role in the genesis of ventricular fibrillation during acute myocardial ischemia. The ventricular antiarrhythmic activity of some beta-adrenoceptor antagonist agents appears to be due to a non-specific effect, eg. 'membrane stabilizing activity'.

(2) The accumulation of cyclic AMP in the ischemic myocardium appears to be dependent on factors other than beta-receptor stimulation. Cyclic AMP may be linked to ventricular fibrillation but only in certain situations; cyclic AMP is not the only factor nor the final messenger of ventricular fibrillation.

(3) Alpha₁ and alpha₂ adrenoceptor antagonist agents prevent the enhanced vulnerability to ventricular fibrillation. The ventricular antiarrhythmic activity appears to be due to a non-specific effect, i.e. 'membrane stabilizing activity' or prolongation of action potential duration rather than due to specific alpha₁ or alpha₂ receptor antagonism. Before one can exclude a role for either the alpha₁ or alpha₂ receptor in ventricular arrhythmogenesis, the anatomical distribution of myocardial alpha₁ and alpha₂ adrenoceptors and their regulation of extracellular and intracellular calcium ion movement needs to be clearly defined.

(4) Calcium channel antagonist agents and a reduction in extracellular calcium concentration attenuated the enhanced vulnerability to ventricular fibrillation. The transsarcolemmal calcium current appears to play a role in the genesis of ventricular fibrillation.

(5) Fast channel blocking agents prevented the enhanced vulnerability to ventricular fibrillation. The transsarcolemmal sodium current appears to play a major role in the genesis of ventricular fibrillation.

[C] POSSIBLE ELECTROPHYSIOLOGICAL MECHANISM OF VENTRICULAR
FIBRILLATION DURING ACUTE REGIONAL MYOCARDIAL ISCHEMIA

This thesis does not address the electrophysiological mechanism of ventricular fibrillation, hence an answer to this question is not evident. Nevertheless, based on the findings that the transsarcolemmal calcium and sodium ionic currents both appear to play a role in the genesis of ventricular fibrillation, inferences as to the possible electrophysiological mechanism of ventricular fibrillation can be drawn.

(1) A likely inference is that slow response action potentials and depressed fast response action potentials may both co-exist in the ischemic myocardium and each may predispose to ventricular fibrillation. Is there any evidence to support this hypothesis? Calcium channel antagonist agents suppress depressed action potentials and automaticity in some fibres whereas fast channel blocking agents suppress depressed action potentials and automaticity in other fibres of diseased human ventricular myocardium, resected for control of malignant ventricular arrhythmias. Both slow responses and depressed fast responses have been proposed to participate in abnormal impulse generation and propagation and to modulate ventricular arrhythmias in diseased human ventricular myocardium (Gilmour et al, 1983).

(2) Inhibition of the transsarcolemmal calcium current decreases intracellular calcium concentration, whereas inhibition of the transsarcolemmal sodium current decreases intracellular sodium and thereby the intracellular calcium concentration. The ventricular antiarrhythmic effect of both calcium antagonist procedures and fast channel blocking agents may therefore be explicable on the basis of reduction in cytosolic calcium concentration. Cytosolic calcium overload (which occurs during acute myocardial ischemia) predisposes to

afterdepolarizations and may thereby induce triggered automaticity and ventricular arrhythmias. Afterdepolarization induced triggered automaticity is therefore an alternative electrophysiological mechanism of ventricular fibrillation.

Future investigation should therefore evaluate:

- (i) the exact nature of the electrophysiological alteration which evokes ventricular fibrillation, i.e. slow response action potential, depressed fast response action potential or afterdepolarization, and
- (ii) define the role of intracellular sodium ions and calcium ions in the genesis of ventricular fibrillation.

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