ARRHYTHMOGENIC POTENTIAL OF ALPHA-ADRENOCEPTOR STIMULATION IN THE RAT HEART

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by

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ABBREVIATIONS

mA = milliamps

msec = milliseconds

ATP = adenosine triphosphate

PCr = phosphocreatine

cAMP = cyclic adenosine monophosphate

cGMP = cyclic guanosine monophosphate

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ABSTRACT

A recent proposal is that the alpha$_1$-adrenoceptor may mediate the arrhythmogenic effect of catecholamines during acute myocardial ischaemia. The purpose of this thesis was to explore the role of alpha$_1$ and alpha$_2$-adrenoceptor stimulation on vulnerability to ventricular fibrillation in the normoxic rat ventricular myocardium and further to evaluate the possible underlying cellular mechanism.

The model used was the isolated perfused rat heart (Langendorff technique) in which ventricular fibrillation was electrically induced. The amount of current required to produce ventricular fibrillation was measured as the ventricular fibrillation threshold.

Alpha$_1$-adrenoceptor stimulation with methoxamine 10^{-6}M to 10^{-5}M increased the vulnerability to ventricular fibrillation. The arrhythmogenic effect of methoxamine could not be attributed to beta-adrenoceptor stimulation as it occurred in the setting of the beta-adrenoceptor antagonist agent, atenolol; furthermore no accumulation of cyclic AMP, the proposed arrhythmogenic second messenger of beta-adrenoceptor stimulation, occurred. Similarly no alteration in heart rate, coronary flow rate or myocardial high energy phosphate content accompanied the arrhythmogenic effect of methoxamine. The QT interval increased with alpha$_1$-adrenoceptor stimulation, this being an indirect index of prolongation of the action potential duration. The arrhythmogenic action of methoxamine was associated with a positive inotropic effect.

Prazosin 10^{-8}M (an alpha$_1$-adrenoceptor antagonist agent) produced a tenfold displacement to the right of the log concentration response curve of the positive inotropic effect of methoxamine. Prazosin 10^{-8}M prevented the methoxamine induced fall in ventricular fibrillation threshold. Alpha$_2$-adrenoceptor stimulation with B-HT 920 and B-HT 933 (azepexole), in the presence of the beta-adrenoceptor antagonist agent atenolol, did not alter the vulnerability to ventricular fibrillation. Alpha$_2$-adrenoceptor stimulation produced no alteration in heart rate, coronary flow rate or metabolic status.

We next explored the possible mechanism underlying the arrhythmogenic effect of methoxamine. Alpha$_1$-adrenoceptor stimulation enhances transsarcolemmal calcium ion influx and may induce sarcoplasmic reticulum calcium release. To assess the role of transsarcolemmal calcium movement in alpha$_1$-adrenoceptor mediated effects experiments were undertaken with nisoldipine and low extracellular calcium. To evaluate the role of sarcoplasmic reticulum calcium release, experiments were undertaken with ryanodine (an agent reputed to inhibit sarcoplasmic reticulum calcium release without effecting the slow inward current). Nisoldipine 10^{-8}M, reducing extracellular calcium (2.5 mM to 1.25 mM) and ryanodine 10^{-9}M to 10^{-8}M, prevented the arrhythmogenic and positive inotropic effect of methoxamine. Heart rate, metabolic status and cyclic AMP levels were unchanged with these procedures.

The mechanism underlying the arrhythmogenic action of alpha$_1$-adrenoceptor stimulation might be an increase in cytosolic calcium concentration. This increase may be secondary to (i) an enhanced transsarcolemmal calcium influx or (ii) an increase in the phasic release of calcium from the sarcoplasmic reticulum.

INTRODUCTION

The incidence of acute myocardial infarction and complications of cardiac morbidity and mortality in South Africa is reputed to be the highest in the world (360 deaths per 100,000 population in 1979). In the United Kingdom the Edinburgh community study shows that fifty percent of all deaths following acute myocardial infarction occur within two hours, the majority of which are sudden (Armstrong et al., 1972). Current experimental evidence suggests that ventricular fibrillation is the mechanism underlying sudden death. To reduce the high mortality rate following acute myocardial infarction requires the prevention of this early ventricular fibrillation; implicit in this concept is understanding the pathogenesis of ventricular fibrillation.

A) Catecholamines and ventricular fibrillation

Several lines of evidence indicate that enhanced adrenergic activity plays an important role in the genesis of ventricular fibrillation. Elevated plasma and urinary catecholamine levels follow myocardial infarction (Wollenberger and Shahab, 1965. Gauduel et al., 1979); circulating catecholamines facilitate the development of ventricular arrhythmias. Surgical denervation of the heart significantly reduces the incidence of ventricular fibrillation following coronary occlusion (Ebert et al., 1970). Similarly chemical denervation of the heart with 6-hydroxydopamine or reserpine protects against the development of ventricular fibrillation during both coronary occlusion and reperfusion (Sethi et al., 1973; Sheridan et al., 1980; Sommers and Jennings, 1972). These findings suggest that sympathetic stimulation is an important factor contributing to the genesis of

ventricular fibrillation during acute myocardial infarction. Enhanced adrenergic activity results in stimulation of both the alpha- and beta-adrenergic receptors.

B) The beta-adrenoceptor and ventricular fibrillation

It has generally been considered that the arrhythmogenic effect of catecholamines during acute myocardial ischaemia is mediated by beta-adrenoceptor stimulation (Lubbe et al., 1978). Cyclic AMP, the proposed intracellular second messenger of beta-receptor stimulation, has been linked to the genesis of ventricular fibrillation.

Evidence for this hypothesis is as follows:-

1) Elevated cyclic AMP levels have been demonstrated in the ischaemic myocardium of baboon heart prior to the onset of ventricular fibrillation (Podzuweit et al., 1978).
2) In the pig a relationship exists between post-ligation ventricular arrhythmias and the accumulation of myocardial cyclic AMP (Podzuweit and Lubbe, 1977).
3) In cats with regional myocardial ischaemia, ventricular arrhythmias are accompanied by increased myocardial cyclic AMP levels (Corr et al., 1978).
4) 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).
5) The shift of the dose-response curve to the right and the delay in the rise of tissue cyclic AMP during $beta_1$-adrenergic antagonism with atenolol, (Lubbe et al, 1978).

C) Possible electrophysiological alterations induced by cyclic AMP.

Cyclic AMP increases transsarcolemmal calcium influx by phosphorylation of the slow channel (Reuter 1974), this may induce ventricular arrhythmias by the following electrophysiological mechanisms,

1. Enhanced sarcolemmal calcium conductance may induce slow response action potentials in partially depolarized muscle fibres (Reuter, 1974), this predisposes to slow conduction and unidirectional conduction block thereby satisfying the requisites for the occurrence of re-entry.
2. Intracellular calcium ion overload may evoke delayed after depolarizations and thereby induce triggered automaticity.
3. Intracellular calcium ion overload may increase internal longitudinal resistance, cause uncoupling of cells and lead to ventricular fibrillation.

D) Effect of beta-adrenoceptor antagonist agents on ventricular fibrillation during acute myocardial ischaemia

1) In the dog beta-receptor antagonist agents, in general, prevent spontaneous ventricular fibrillation (Khan et al., 1972) and also inhibit the fall in ventricular fibrillation threshold. (Anderson et al, 1983).
2) In the rat (Thandroyen et al., 1983) and cat, (Sheridan et al., 1980) beta-receptor antagonists, in concentrations producing beta-receptor antagonism, do not prevent ventricular fibrillation. In concentrations higher

than that required to achieve beta-receptor antagonism some beta-antagonists prevent ventricular fibrillation (Thandroyen et al., 1983) possibly by a non specific method eg membrane stabilization action. (Barrett and Cullum, 1968).

E) Clinical efficacy of beta-adrenoceptor antagonist agents

The "beta-blocker heart attack clinical trials" tested whether regular administration of propranolol, (American beta-blocker heart attack trial research group, 1982) timolol (The Norwegian multicentre study group, 1981) and metoprolol (Herlitz et al, 1983) within 3 hours to 5 days of the onset of acute myocardial infarction, would result in a significant reduction in sudden death and cardiac mortality. All three trials showed a decrease in total cardiac mortality and sudden death: after 3 months in the metoprolol trial, 17 months in the timolol trial and 27 months in the propranolol trial. This suggests that long term treatment with beta-antagonist agents in patients surviving acute myocardial infarction reduces mortality. None of these clinical trials assess the effect of administration of beta-adrenoceptor antagonist agents within sixty minutes of the onset of acute myocardial infarction.

F) The alpha-adrenoceptor and ventricular fibrillation

A recent hypothesis is that the $alpha_1$-adrenoceptor may mediate the arrhythmogenic effect of catecholamines during acute myocardial ischaemia. Evidence supporting this hypothesis is as follows:

1) Alpha$_1$-adrenergic receptor number, as assessed by ligand binding with (^{3}H) prazosin, increased nearly two-fold in ischaemic cat myocardium within thirty minutes of coronary occlusion; a temporal association was evident with ventricular arrhythmias. (Corr et al., 1981). Receptor affinity (Kd) was not altered at any interval studied. The mechanism responsible for the increase in alpha$_1$-receptor number during ischaemia is not known; it has been postulated that alterations in membrane phospholipids in ischaemic tissue may influence the relative number of adrenergic receptors (Sobel et al., 1978).
2) Enhanced alpha-adrenergic responsiveness during reperfusion was demonstrated with the alpha$_1$-agonist methoxamine (Corr et al., 1981).
3) Prazosin, a specific alpha$_1$-adrenoceptor antagonist agent reduced premature ventricular complexes and ventricular fibrillation in the feline (Sheridan et al., 1980) and rat model (Thandroyen et al., 1983) of coronary artery ligation.
4) In the isolated perfused rat heart alpha$_1$ and also alpha$_2$-adrenoceptor antagonist agents prevented ventricular fibrillation during acute myocardial ischaemia. (Thandroyen et al., 1983).

Enhanced alpha-adrenergic responsiveness which occurs during acute myocardial ischaemia has been temporally related to the occurrence of ventricular arrhythmias. However the mechanism underlying the possible arrhythmogenic action is undefined.

G) Possible electrophysiological alterations induced by $alpha_1$-adrenoceptor stimulation

1) Delayed after-depolarizations (Vassalle and Musso, 1976).
2) Slow response type depolarizations (Miura et al., 1978).
3) Enhanced automaticity (Penkoske et al., 1978).
4) Increased idioventricular rate (Penkoske et al., 1978).

H) Clinical efficacy of $alpha_1$-adrenoceptor antagonist agents

The effect of specific $alpha_1$-adrenoceptor antagonist agents on sudden death and cardiac mortality has not been investigated during acute myocardial infarction in man. However Gould and coworkers in 1975 showed that the non specific alpha-adrenoceptor antagonist phentolamine did reduce the incidence of late onset ventricular premature beats during acute myocardial infarction (Gould et al., 1975).

I) Classification of alpha-adrenoceptors

Williams and Lefkowitz (1978) were the first to document the presence of myocardial alpha-adrenoceptors using radioligand binding techniques. Further experimentation with this technique suggested that two subtypes of alpha-adrenoceptors exist, these being distinguished on the basis of differential affinities of certain adrenergic ligands for each receptor subtype (Yamada et al., 1980. Williams et al., 1980). The two subtypes of alpha-adrenoceptors proposed were termed pre and postsynaptic receptors, the presynaptic alpha-adrenoceptor being located at

the level of the membranes of vesicles (storage sites of endogenous neurotransmitters). Excitation of the presynaptic alpha-adrenoceptor was postulated to inhibit the release of endogenous noradrenaline from the vesicles and to act as an inhibitory feedback mechanism. Stimulation of the postsynaptic alpha-adrenoceptor was postulated to mediate smooth muscle contraction (Van Zwieten and Timmermans., 1983).

The initial nomenclature proposed postsynaptic alpha-adrenoceptors as $alpha_1$-subtypes and presynaptic alpha-adrenoceptors as $alpha_2$-subtypes. However it was subsequently demonstrated that the preference for appropriate and selective agonists and antagonists of alpha-adrenoceptors did not run parallel to the pre- and post location of the receptors (Van Zwieten and Timmermans., 1983). Postsynaptic $alpha_2$-adrenoceptors exist which induce vasoconstriction in vivo (Van Meel et al., 1981).

More recent nomenclature classifies the alpha-adrenoceptor subtypes as follows:

(a) the terms pre- and postsynaptic refer solely to the anatomical position of receptors with respect to the synapse.

(b) $alpha_1$ and $alpha_2$ indicates the preference of the receptors for certain agonists and antagonists, this preference is not necessarily associated with a defined anatomical position of the receptor (Table 1).

Table 1

Agents	Receptor selectivity
Agonists	
Noradrenaline	$alpha_1$, $alpha_2$, $beta_1$
Adrenaline	$alpha_1$, $alpha_2$, $beta_1$, $beta_2$
B-HT 920	$alpha_2$
B-HT 933	$alpha_2$
Methoxamine	$alpha_1$
Clonidine	$alpha_2 > alpha_1$
Antagonists	
Phentolamine	$alpha_1$, $alpha_2$
Prazosin	$alpha_1$
Yohimbine	$alpha_2$
Rauwolscine	$alpha_2$

J) Alpha-adrenoceptors in the rat heart

Williams and co-workers investigated the binding characteristics of a ligand relatively selective to the $alpha_2$-adrenoceptor, (^3H) clonidine, as well as one selective to the $alpha_1$-adrenoceptor, (^3H) Prazosin, in rat heart homogenates.

The relatively selective alpha$_2$-ligand (^{3}H) clonidine, at concentrations up to 20 nM, demonstrated negligible binding whereas the alpha$_1$-specific ligand (^{3}H) prazosin saturated the binding sites. (Williams et al., 1980). These findings suggest that the alpha-adrenoceptors in the rat heart are predominantly of the alpha$_1$-subtype. Glossmann (Glossmann et al., 1980) also indentified only alpha$_1$-adrenoceptors in the rat myocardium.

Only one study provides evidence for the presence of alpha$_2$-adrenoceptor subtypes in the rat heart (Guicheney and Meyer, 1981). Thus alpha-adrenoceptors in the rat heart appear to be predominantly alpha$_1$-subtypes.

K) Alpha-adrenergic mechanisms

i) Cyclic nucleotides (cAMP and cGMP)

Unlike beta-adrenoceptor stimulation, alpha-adrenoceptor stimulation is not associated with the accumulation of intracellular cyclic AMP. Methoxamine, the alpha$_1$-adrenoceptor agonist agent has been reported to exert a positive inotropic effect with no increase in myocardial cyclic AMP content (Endoh et al., 1975. Schümann et al., 1975). In another study methoxamine has been reported to decrease cyclic AMP levels independently of adenylate cyclase whereas alpha$_2$-adrenoceptor stimulation decreases cyclic AMP levels through the inhibition of adenylate cyclase (Schumann et al., 1975. Exton 1983. Hoffman et al., 1980. Tsai et al., 1979.). These studies imply that alpha-adrenergic responses occur independently of cyclic AMP accumulation.

A transient increase in cyclic GMP has been reported to follow alpha-adrenoceptor stimulation in several studies (Goldberg and Haddox, 1977. Amer and Byrne, 1975. Kunos et al., 1976. Watanabe and Besch, 1975). Other workers report no change in cyclic GMP concentration following alpha-adrenoceptor stimulation (Gardner and Allen, 1976, 1977). A change in cyclic GMP content does not appear to be a prerequisite for alpha-adrenoceptor mediated responses.

(ii) Calcium

Calcium ions have been proposed to play a crucial role in the mechanisms underlying alpha-adrenoceptor mediated responses.

(a) Vasculature

Vasoconstriction induced by stimulating vascular postsynaptic $alpha_2$-adrenoceptors can be reduced by use of calcium channel blocking agents through a non-competitive mechanism. Vasoconstriction evoked by stimulating vascular $alpha_1$-adrenoceptors are not influenced by calcium channel blocking agents (Van Zwieten et al., 1983). These findings suggest that $alpha_2$-adrenoceptor stimulation increases, whereas $alpha_1$-adrenoceptor does not effect, the inward calcium current in vasculature. Similar findings have been reported by Van Meel et al., 1980 and Muiro et al., 1978.

(b) Liver

Alpha$_1$-adrenoceptor stimulation may induce depolarization and the release of calcium ions from intracellular stores (Exton, 1981). In the adult rat liver, alpha$_1$-adrenoceptor responses are mediated by a calcium sensitive cascade triggered by a transient rise in cytosolic calcium (Exton, 1981).

(c) Heart

Alpha$_1$-adrenoceptor stimulation increases the slow inward current in ventricular muscle (Scholz, 1980). In a preliminary communication alpha$_1$-adrenoceptor stimulation has been demonstrated to phosphorylate a 15,000 MW protein localized to the sarcolemma, this possibly being the slow inward current regulatory mechanism (Lindemann and Wilson, 1984). Alpha$_1$-adrenoceptor stimulation does not phosphorylate the sarcoplasmic reticulum protein phospholamban. As in the liver, alpha$_1$-adrenoceptor stimulation may induce sarcoplasmic reticulum calcium release. Although very little is known about the mechanisms involved in alpha-adrenoceptor responses, available evidence strongly indicates that calcium may play a role as mediator.

L) Myocardial alpha-adrenoceptor responses

(i) Heart rate

Both positive and negative chronotropic changes have been reported following alpha-adrenoceptor stimulation (James et al., 1968, Rosen et al., 1977).

The sinus node does not respond to alpha-adrenoceptor stimulation, similarly no effect occurs on the pacemaker potassium current as found in purkinje fibres of cattle and sheep (Tsien, 1974. Hauswirth et al, 1976). Alpha-adrenoceptor stimulation does not appear to affect heart rate.

(ii) Left Ventricular Pressure

An $alpha_1$-adrenoceptor mediated positive inotropic effect has been observed in isolated heart preparations of rat, cat, guinea pig and also of man (Schümann et al., 1978. Endoh and Schümann, 1975a. Ledda et al., 1975. Mugelli et al., 1976).

Phenylephrine, an $alpha_1$-agonist agent, induces a positive inotropic response in the rat model (these experiments were conducted in the presence of propranolol, a beta-antagonist agent) (Osnes et al., 1975). Phentolamine (an $alpha_1$ and $alpha_2$-antagonist agent) antagonises the inotropic response of phenylephrine (Osnes et al., 1975). The positive inotropic profile of alpha-adrenoceptor stimulation differs from that of beta-adrenoceptor stimulation in the following aspects: Firstly there is no shortening of the time to peak tension. Secondly, the peak inotropic response is reached later (i.e. 240 seconds as opposed to 15 to 30 seconds). Thirdly, relaxation is not altered (Skomedal et al., 1977).

These qualitative differences between alpha and beta-adrenoceptor stimulation indicate the involvement of two different mechanisms of action. The mechanism underlying the alpha-adrenoceptor

mediated positive inotropic response is unknown; cAMP and sodium/potassium ATPase activity remain unchanged (Scholz, 1980. Endoh et al., 1975). It has been postulated that alpha-adrenoceptor effects may be mediated by increasing intracellular calcium ion concentration.

(iii) Coronary flow

Specific $alpha_1$-adrenoceptor stimulation with methoxamine induces coronary artery vasoconstriction in the rat model (Glomstein et al., 1967). In the presence of beta-adrenoceptor antagonism, coronary artery vasoconstriction follows sympathetic nerve activation. This vasoconstriction is prevented by alpha-adrenoceptor antagonist agents (Adam et al., 1970. Ek and Ablad, 1971, Feigl, 1975. Hamilton and Feigl, 1976).

HYPOTHESIS

The hypothesis addressed is that alpha-adrenoceptor mediated responses play a role in the genesis of ventricular fibrillation. The arrhythmogenic potential of $alpha_1$ and $alpha_2$-adrenoceptor stimulation in normoxic ventricular myocardium is undefined as is the possible underlying cellular mechanism.

This thesis explores the influence of $alpha_1$ and $alpha_2$-adrenoceptor stimulation on (1) vulnerability to ventricular fibrillation in the isolated perfused normoxic rat heart.

(2) coronary perfusion, mechanical function and myocardial metabolic status.

(3) transsarcolemmal calcium ion movement and sarcoplasmic reticulum calcium ion release.

CHAPTER 2

MODEL, MATERIALS AND METHODS

1) EXPERIMENTAL MODEL

A) Isolated perfused rat heart

The isolated Langendorff perfused rat heart was chosen as the model to investigate the role of alpha-adrenergic receptor stimulation on vulnerability to ventricular fibrillation.

B) Advantages of Model

1) In the intact in vivo animal model $alpha_1$-adrenoceptor stimulation causes peripheral vasoconstriction and a reflex bradycardia. The direct influence of $alpha_1$-adrenoceptor stimulation on the myocardium is therefore not able to be analysed. The isolated perfused heart preparation is independent of peripheral circulation, neuronal and hormonal influence. Hence analysis may be undertaken of the direct action of alpha-adrenoceptor stimulation on the ventricular myocardium.
2) Specific concentrations of alpha-adrenoceptor agonists and antagonists can be delivered to the myocardium by adding these agents to the perfusate. Hence concentration response curves of $alpha_1$ and $alpha_2$-adrenoceptor agonists/antagonists on vulnerability to ventricular fibrillation can be accurately

evaluated.

3) Metabolic and biochemical analysis can be measured and related to the effects of alpha-adrenoceptor stimulation/antagonism.

4) The arrhythmogenic action of alpha-adrenoceptor stimulation may be due to an increase in myocardial cell calcium concentration following cell membrane receptor stimulation. The role of calcium ions can be addressed by alteration of calcium ion concentration in the perfusate.

5) The arrhythmogenic action of alpha-adrenoceptor stimulation may be mediated by coronary vascular constriction causing focal myocardial ischaemia. This model enables one to accurately record coronary flow rates and hence relate alteration in coronary perfusion to vulnerability to ventricular fibrillation.

6) Catecholamine stimulation per se does not induce spontaneous ventricular tachycardia/ventricular fibrillation in the normoxic heart. The advantage of electrically induced ventricular fibrillation is that small changes in vulnerability to ventricular fibrillation can be measured.

7) Ventricular fibrillation induced by electrical current usually spontaneously reverts to sinus rhythm. Repeated measurements of ventricular fibrillation threshold can therefore be done on the same heart.

8) Conversion of ventricular fibrillation to sinus rhythm can be achieved by simple immersion of the heart in ice-cold Krebs-Henseleit buffer, this procedure causes minimal damage and is effective.

9) The model is not costly and rats are easily available.

C) Disadvantages of the Model

1) The perfusion fluid has no haemoglobin, hence coronary flow rates are unduly high.

2) Absence of autonomic neuronal innervation.

3) Due to the aortic retrograde perfusion, no volume work is performed by the heart.

4) No peripheral circulation exists.

5) Artificial induction of ventricular fibrillation (see below).

D) Ventricular Fibrillation Threshold

The artificial induction of ventricular fibrillation by delivery of electrical current to the myocardium is not necessarily

synonymous with spontaneous ventricular fibrillation. Hence the changes in vulnerability to ventricular fibrillation evoked by this technique cannot be extrapolated to alterations in spontaneous ventricular fibrillation.

Wiggers and co-workers (1940) proposed that vulnerability to ventricular fibrillation is a specific and inherent property of the myocardium, Dawes (1952) however, considered it to represent a measurement of myocardial excitability. More recently it has been proposed that alterations in ventricular fibrillation threshold may be accepted as a measure of the efficiency of an anti-arrhythmic agent (Bacaner 1968, Allen et al 1971, Gerstenslith, 1972).

2. MATERIALS

A) Animals

Male Long-Evans rats (240-310 g) were anaesthetised in a glass chamber with ether. The femoral vein was exposed by making an incision in the right inguinal region; 200 units of heparin was injected via the femoral vein. After 45 seconds the chest was opened via the diaphram and the heart was removed by incising the great vessels. The heart was immediately immersed in ice-cold Krebs-Henseleit solution to produce arrest.

B) Langendorff Perfusion system and the Perfusate

The incised edge of the aorta was mounted onto a metal cannula and perfused at 100 cm water pressure according to the Langendorff technique (described in 1895). The perfusion fluid

was Krebs-Henseleit solution consisting of the following: $NaHCO_3$ 25.0 mM, NaCl 118.5 mM, KCl 4,75 mM, KH_2PO_4 1,19 mM, $MgSO_4$ 1,19 mM and $CaCl_2$ 2.50 mM. The substrate was glucose 11 mM. This solution was aerated with 95% oxygen and 5% CO_2. The temperature was maintained at 37°C throughout the system by means of a water jacket around the glassware containing the perfusate. A Braun thermomix 1460 was used to maintain the water at a constant temperature. Perfusate pH ranged between 7.35 to 7.40 and pO_2 between 470 to 550 mmHg. Sodium and potassium levels were checked to be normal.

C) Electrocardiogram recordings

Continuous electrocardiograms were recorded from one electrode earthed on the metal aortic perfusion cannula and a second electrode inserted superficially into the right ventricular myocardium. The electrocardiogram was visually displayed on both an Electronics for medicine recorder as well as a Tetronix storage oscilloscope. The electrocardiogram with the square wave indicating the trigger was continuously displayed on the Electronics for medicine oscilloscope.

D) Electrical delivery system

The electrocardiogram potential was amplified by an EDTA 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 monitors the incoming synchronous pulse. After manual activation this Trigger box

delivers seven pulse beats; on the seventh beat it starts the horizontal sweep of the storage oscilloscope (Tetronix oscilloscope) whilst the eighth beat activates a Grass 588 stimulator (Grass Instrument Co., Massachusetts).

E) Stimulation of the left ventricle

Two thin platinum stimulating electrodes were superficially inserted into the left ventricle for delivery of electrical current to the myocardium. The electrodes were positioned horizontally in parallel 10 mm apart, one at the base of the heart (cathode), the other at the apex (anode). Damage to the coronary vessels was avoided by careful positioning of the stimulating electrodes.

3. METHODOLOGY

A) Ventricular fibrillation threshold

The train method was employed to obtain the ventricular fibrillation threshold. This involves the delivery of ten square wave pulses evenly distributed over the duration of 200 msec stimulation; delivery was commensed 10 msec after the trigger obtained from the initial pulse of the R-wave of the electrocardiogram. The duration of each pulse was 20 msec. consisting of 2 msec impulse and 18 msec delay between each pulse.

No difference in ventricular fibrillation threshold levels were found comparing the train method to the single stimulus method of electrical stimulation - this is based on experiments conducted

in the ischaemic heart laboratory/University of Cape Town.

Criteria for ventricular fibrillation

Ventricular fibrillation was diagnosed if six or more consecutive ventricular complexes showed total irregularity of morphology. Associated with this pattern was a profound lowering of the left ventricular pressure with loss of the left ventricular pressure wave thus displaying criteria required for ventricular fibrillation, that is mechanical failure associated with electrical abnormality.

The ventricular fibrillation threshold was obtained by stimulating the myocardium with an initial current of 3 mA, and progressively increasing the current in steps of 2 mA until ventricular fibrillation occurred. The ventricular fibrillation threshold was defined as the lowest current required to produce ventricular fibrillation on more than two occasions.

In this model ventricular fibrillation usually reverts spontaneously to sinus rhythm. However, if ventricular fibrillation persisted for longer than ten seconds the hearts were defibrillated by immersing them in ice-cold Krebs-Henseleit buffer.

B) Experimental protocol

After mounting the heart a stabilization period of 15 minutes was allowed. The heart rate, coronary flow rate and ventricular fibrillation threshold were then measured in each experiment.

Heart rates ranged between 240-360 beats/min. Coronary flow rates, expressed as ml/min were measured by collecting the coronary effluent in a graduated measuring tube over 1 minute. Control values varied from 5 to 12 ml/min. Control ventricular fibrillation threshold values were between 5 and 14 mA.

In those series where pharmacological agents were assessed the protocol was identical except that the agent was infused or perfused during the ten minute intervening period and thereafter throughout the experiment.

4. MECHANICAL FUNCTION

A) Methodology

Long-Evans rat hearts were mounted onto a metal cannula as previously described (Langendorff technique). In this series the hearts were paced at a rate of 330 beats/min., this was achieved by inserting a pacing electrode into the right ventricular myocardium. The second electrode was earthed on the metal aortic cannula. A voltage stimulating unit was used to deliver pacing pulses to the right ventricle, (pulse width 0,2msec, voltage 4-10 mA.) By pacing the hearts possible chronotropic influences on mechanical function were excluded.

The anterior wall of the left atrium was excised exposing the mitral valve. A 1mm diameter hollow plastic cannula with a flattened end was introduced into the left ventricular cavity via

the mitral valve. The introduced end of the cannula was passed through the apex of the left ventricular myocardium until the other flattened end rested against the endocardium of the left ventricle. The cannula was connected to a pressure transducer after being flushed with normal saline to remove any air trapped in the system. Left ventricular diastolic and systolic pressures were recorded by means of a Devices recording unit (type MX216). Before commencement and after completion of each experiment the recording unit was calibrated from 0 to 100 mmHg using a baumanometer. Left ventricular systolic pressures varied from 70 to 90 mmHg, mean 76.3 $\pm$ 1.86 mmHg; end diastolic pressures varied from 3 to 7 mmHg, mean 4.29 $\pm$ 0.23 mmHg.

B) Protocol

After mounting the heart a stabilization period of 15 minutes was allowed. The Devices recording speed for the duration of the experiment was 2.5 cm/min, however to accurately record left ventricular pressures this speed was increased to 2.5 cm/sec during certain recording times (0,5,30,60,120,180 and 240 seconds). Coronary flow rates were recorded intermittently during both the control and intervention periods.

To assess the effects of pharmacological agents on mechanical function the protocol was identical except that the agent was infused or perfused following the 15 minute control period and continued throughout the experiment.

C) Chemical agents

All the chemical agents were used in the form of racemic compounds. Compounds were prepared 4 hourly.

Drug	Form	Suppliers
Methoxamine Hydrochloride	Powder	Wellcome
B-HT 933	Powder	Boehringer
B-HT 920	Powder	Dr. Karl Thomas GMBH Biberach an der Riss
Prazosin	Powder	Pfizer, South Africa
Yohimbine Hydrochloride	Powder	Sigma, USA
Atenolol	Powder	ICI, South Africa
Nisoldipine	Powder	Bayer, United Kingdom
Ryanodine	Powder	Penick, U.S.A.

D) Statistical Methods

Results are expressed as the mean $\pm$ standard error of the mean for the number of the experiments. The number of experiments varied from 5 to 13 in each series. Probability (p) values were calculated using (a) students t test using two tailed values as corrected for unequal variances, and (b) analysis of variance. Probability values less than 0,05 indicated significant differences between mean values.

E) Biochemical analysis

The hearts to be biochemically analysed were freeze-clamped with Wollenberger clamps and freeze fried for $\pm$ 48 hours in an Edwards Modulyo freeze-drier. The freeze-fried hearts were then

homogenised in 6% percholoric 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:

$$\text{ATP} + \text{glucose} \xrightarrow[Mg_2+]{\text{hexokinase}} \text{ADP} + \text{G6P}$$

$$\text{G6P} + \text{NADP} \xrightarrow{\text{G6PDH}} \text{6PGA} + \text{NADPH} + H^+$$

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

$MgCl_2$ 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; H_2O 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:

$$PCr + ADP \xrightarrow[\text{Creatinine phosphokinase}]{Mg2+} ATP + \text{Creatine}$$

The further change in extinction at 340 nm on addition of 10 µl 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).

$$\text{Glucose} + ATP \xrightarrow{HK} G6P + ADP$$

$$G6P + G6PDH + NADP^{+} \rightarrow \text{6 phosphoglucono-lactone} + NADPH$$

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 and thus glycogen 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.

$P + A^* \rightleftharpoons P\,A^*$ (labelled complex)

(labelled compound)

$P + A \rightleftharpoons P\,A$ (unlabelled complex)

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. Co/Cx is plotted against concentration of cyclic AMP in the standard dilutions. Co: labelled nucleotide bound in the absence of unlabelled nucleotide. Cx: labelled nucleotide bound in the presence of a standard quantity of unlabelled nucleotide.

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

CHAPTER 3

THE ROLE OF ALPHA$_1$ AND ALPHA$_2$-ADRENOCEPTOR STIMULATION ON VULNERABILITY TO VENTRICULAR FIBRILLATION.

Results

A) Alpha$_1$-adrenoceptor stimulation in the normoxic ventricular myocardium

Methoxamine acts selectively on the alpha$_1$-adrenoceptor (Van Meel et al., 1980. Kobinger and Pichler, 1980); in high concentrations the beta-adrenoceptor may be stimulated (Benfey 1982). To ensure a pure alpha-adrenoceptor mediated effect experiments were undertaken in the presence of the selective beta$_1$-adrenoceptor antagonist atenolol 10^{-6}M. The log concentration response curve of methoxamine (Figure 1) demonstrates that methoxamine 10^{-7}M does not alter whereas methoxamine 10^{-6}M to 10^{-5}M increases vulnerability to ventricular fibrillation; the ventricular fibrillation thresholds being reduced from control values of 11.7 $\pm$ 0.6 mA and 8.7 $\pm$ 0.7 mA to 4.1 $\pm$ 1.2 mA ($p < 0.01$) and 3.8 $\pm$ 0.8 mA ($p < 0.01$) respectively.

The arrhythmogenic effect of methoxamine was not accompanied by alteration in the following,

(a) heart rate control values 267 $\pm$ 7 beats/min and 259 $\pm$ 19 beats/min, methoxamine 10^{-6}M and 10^{-5}M values 250 $\pm$ 6 beats/min and 290 $\pm$ 11 beats/min respectively. (p non significant)

(b) coronary flow rates control values 8.3 ± 0.5 ml/min and 7.2 ± 0.7 ml/min, methoxamine 10^{-6}M and 10^{-5}M values 8.5 ± 0.7 ml/min and 9.5 ± 0.8 ml/min respectively. (p non significant). (Table 1)

(c) metabolic status

(i) adenosine triphosphate, control value 3.8 ± 0.2 µmol/g, methoxamine 10^{-6}M and 10^{-5}M values 3.7 ± 0.4 µmol/g and 3.6 ± 0.2 µmol/g respectively.

(ii) lactate, control value 3.1 ± 0.4 µmol/g, methoxamine 10^{-6}M and 10^{-5}M values 2.8 ± 0.1 µmol/g and 3.6 ± 0.8 µmol/g respectively.

(iii) cyclic adenosine monophosphate, control value 0.44 ± 0.02 nmol/g, methoxamine 10^{-6}M value 0.43 ± 0.03 nmol/g (p non significant) (Table 2).

The QT interval remained unchanged with methoxamine 10^{-7}M but increased with higher concentrations, control values 79.6 ± 3.1 msec and 81.0 ± 3.8 msec, methoxamine 10^{-6}M and 10^{-5}M values 94.6 ± 4.4 msec ($p < 0.01$) and 97.3 ± 4.7 msec ($p < 0.01$) respectively (Table 1). $Alpha_1$-adrenoceptor stimulation with methoxamine 10^{-6}M and 10^{-5}M also produced a positive inotropic effect (Figure 2). Methoxamine 10^{-5}M increased the left ventricular systolic pressure from a control value of 80.8 ± 3.1 mmHg to 113.4 ± 2.5 mmHg. A characteristic three-phasic time course to the positive inotropic effect was evident. An initial small increase in left ventricular systolic pressure was noted after thirty seconds of adding methoxamine, this was followed by a second phase where the left ventricular systolic pressure plateau's or decreases momentarily before increasing slowly to a maximum effect at four minutes (Figure 2). No change in left ventricular end diastolic pressure was evident (results not shown). Accompanying the positive inotropic effect

was an increase in oxygen demand, control values 108.6 ± 9.7 µml/g/min and 125.0 ± 8.3 µml/g/min, methoxamine 10^{-6}M and 10^{-5}M values 165.6 ± 8.1 µml/g/min ($p < 0.01$) and 190.0 ± 14.5 µml/g/min ($p < 0.01$) respectively (Table 2).

B) Alpha$_2$-adrenoceptor stimulation in the normoxic ventricular myocardium

B-HT 920 and the more selective agent B-HT 933 are reputed to act selectively on the alpha$_2$-adrenoceptor (Van Zwieten and Timmermans, 1983. Kobinger and Pichler, 1980). The log concentration response curves of both B-HT 920 and B-HT 933 demonstrate no alteration in ventricular fibrillation threshold (figures 3 and 4). No positive inotropic effect was evident with B-HT 933 (Figures 5). No alteration in heart rate or coronary flow rate occurred with the addition of alpha$_2$-agonists (Tables 3 and 4).

C) Alpha$_1$-adrenoceptor antagonism with prazosin

Prazosin 10^{-8}M produces a tenfold displacement to the right of the log concentration response curve of the positive inotropic effect of methoxamine (Figure 6) indicating specific alpha$_1$-adrenoceptor antagonism. Prazosin 10^{-8}M prevents the enhanced vulnerability to ventricular fibrillation induced by methoxamine 10^{-6}M (Figure 7). The ventricular antiarrhythmic effect of prazosin was not accompanied by alteration in the following,

(a) heart rate, 243 ± 15 beats/min versus 256 ± 15 beats/min.

(b) coronary flow rates, 6.0 ± 6 ml/min versus 7.2 ± 0.4 ml/min

(c) metabolic status

(i) adenosine triphosphate, 3.9 ± 0.1 µmol/g versus 3.8 ± 0.2 µmol/g.

(ii) lactate, 3.4 ± 0.1 μmol/g versus 3.1 ± 0.4 μmol/g.

(iii) cyclic adenosine monophosphate, 0.47 ± 0.02 nmol/g versus 0.44 ± 0.02 nmol/g. (Tables 1 and 2)

DISCUSSION

A) Alpha$_1$-adrenoceptor stimulation

The findings presented in this study provide evidence that alpha$_1$-adrenoceptor stimulation is arrhythmogenic in the normoxic rat ventricular myocardium. This proposal is based on the following evidence. Firstly, specific alpha$_1$-adrenoceptor stimulation with methoxamine increases the vulnerability to ventricular fibrillation. This was accompanied by an increase in QT interval, an indirect index of an increase in action potential duration (a well described electrophysiological effect of alpha$_1$-adrenoceptor stimulation). Secondly alpha$_2$-adrenoceptor stimulation with B-HT 920 and B-HT 933 does not alter vulnerability to ventricular fibrillation. Thirdly the arrhythmogenic effect of methoxamine is not due to beta-adrenoceptor stimulation as the experiments were conducted in the presence of the selective beta-adrenoceptor antagonist agent atenolol. Furthermore there was no attendant evidence of beta-adrenoceptor stimulation as manifest by (i) no increase in myocardial cyclic AMP content and (ii) no positive chronotropic effect. Fourthly, the selective alpha$_1$-adrenoceptor antagonist agent prazosin 10^{-8}M (Kobinger and Pichler, 1980. Van Zwieten and Timmermans, 1983. Exton 1983) prevents the arrhythmogenic action of methoxamine. Selective alpha$_1$-adrenoceptor antagonism is evident by the finding that prazosin produces a tenfold displacement to the right of the log concentration response curve of the positive inotropic effect of methoxamine (Figure 6).

Prazosin also prevents spontaneous ventricular arrhythmias during acute regional myocardial ischaemia in the feline model (Sheridan et al., 1980). Fifthly, the arrhythmogenic action of methoxamine was unassociated with reduction in the coronary flow rate. Alpha-adrenoceptor stimulation may cause coronary vasoconstriction. The increase in metabolic rate secondary to the concomitant positive inotropic effect of methoxamine presumably accounts for the absence of changes in coronary perfusion rate. Sheridan and co-workers have demonstrated that the ventricular antiarrhythmic action of prazosin during acute regional myocardial ischaemia (and subsequent reperfusion of the ischaemic myocardium) cannot be ascribed to alteration in regional myocardial perfusion rate as measured by the miscrosphere method. (Sheridan et al., 1980). Finally, methoxamine 10^{-6}M to 10^{-5}M did not produce metabolic evidence of myocardial high energy phosphate depletion or accumulation of myocardial lactate, an index of anaerobic metabolism. The ability of prazosin to reverse the methoxamine induced fall in ventricular fibrillation threshold occurred independent of preservation of myocardial high energy phosphate stores or reduction in normal myocardial lactate content. Prazosin did not inhibit the methoxamine induced increase in QT interval, hence the arrhythmogenic action of methoxamine cannot be attributed to prolongation of the action potential duration.

The arrhythmogenic action of methoxamine was accompanied by an increase in left ventricular systolic pressure generation whilst relaxation was unaffected. The positive inotropic effect elicited by methoxamine can be attributed to pure alpha-adrenoceptor stimulation since (i) it occurred in the presence of the beta-adrenoceptor antagonist agent

atenolol and (ii) it was unassociated with an increase in myocardial cyclic adenosine monophosphate concentration. The positive inotropic effect of alpha-adrenoceptor stimulation differs from that evident with beta-adrenoceptor stimulation as evident from the following: firstly, time to peak left ventricular systolic pressure generation occurs after four minutes with alpha-adrenoceptor stimulation whereas with beta-adrenoceptor stimulation it occurs within 15 to 30 seconds. Secondly beta-adrenoceptor stimulation activates the relaxation phase of the myocardium, an effect not produced with alpha-adrenoceptor stimulation. Thirdly beta, but not alpha-adrenoceptor stimulation produces a positive chronotropic effect.

Methoxamine increases the slow calcium inward current and may potentially evoke the release of calcium from intracellular stores. Exton and co-workers have shown an increase in cytosolic calcium occurring within seconds of $alpha_1$-adrenoceptor stimulation in rat liver cells (Exton, 1981). A calcium ion mediated process may therefore be responsible for mediating (i) the arrhythmogenic action and (ii) the positive inotropic effect of $alpha_1$-adrenoceptor stimulation with methoxamine. This potential role of calcium in the arrhythmogenic action of methoxamine is further analysed in Chapter 4.

B) <u>$Alpha_2$-adrenoceptor stimulation</u>

The log concentration response curves of B-HT 920 and B-HT 933 (azepexole) show that $alpha_2$-adrenoceptor stimulation does not increase vulnerability to ventricular fibrillation in the normoxic

rat ventricular myocardium (Figures 3 and 4). No alteration in

(i) left ventricular systolic pressure generation

(ii) oxygen demand

(iii) metabolic status or

(iv) coronary flow rate accompanied alpha$_2$-adrenoceptor stimulation. These findings question the presence of alpha$_2$-adrenoceptors in rat ventricular myocardium and argue against a functional role for alpha$_2$-adrenoceptors in rat ventricular myocardium. Moreover indirect support is provided for the concept that alpha-adrenoceptors of rat heart are predominantly of the alpha$_1$-subtype.

CONCLUSIONS

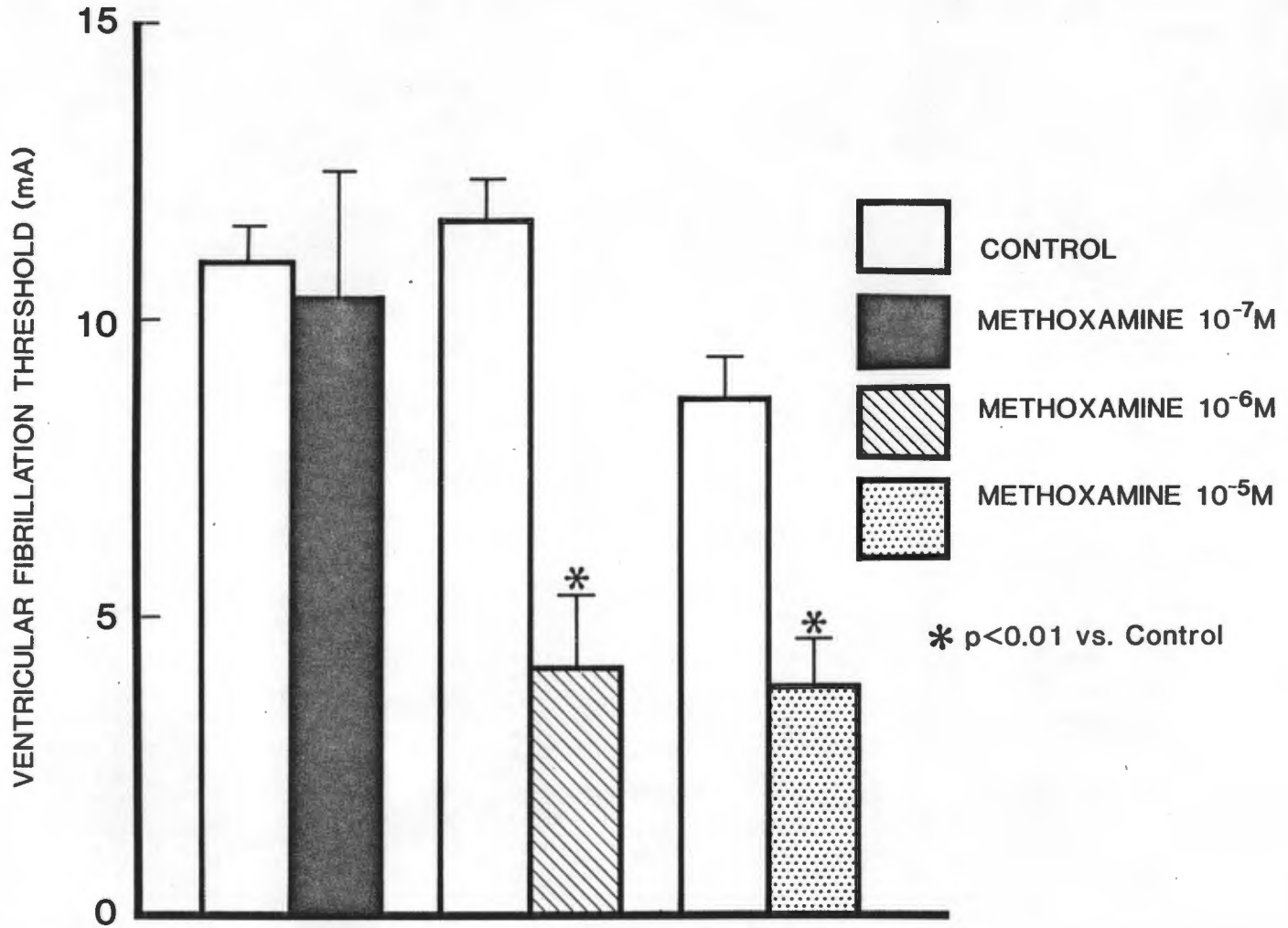
1) Specific alpha$_1$-adrenoceptor stimulation with methoxamine increases vulnerability to ventricular fibrillation in the normoxic rat ventricular myocardium; alpha$_2$-adrenoceptor stimulation is without effect.

2) The arrhythmogenic action of methoxamine occurs in the setting of beta-adrenoceptor antagonism and is not associated with myocardial accumulation of cyclic AMP (the proposed arrhythmogenic intracellular second messenger of beta-adrenoceptor stimulation). No alteration in heart rate or coronary flow rate occurred.

3) No depletion of myocardial high energy phosphate stores accompanied the arrhythmogenic action of methoxamine.

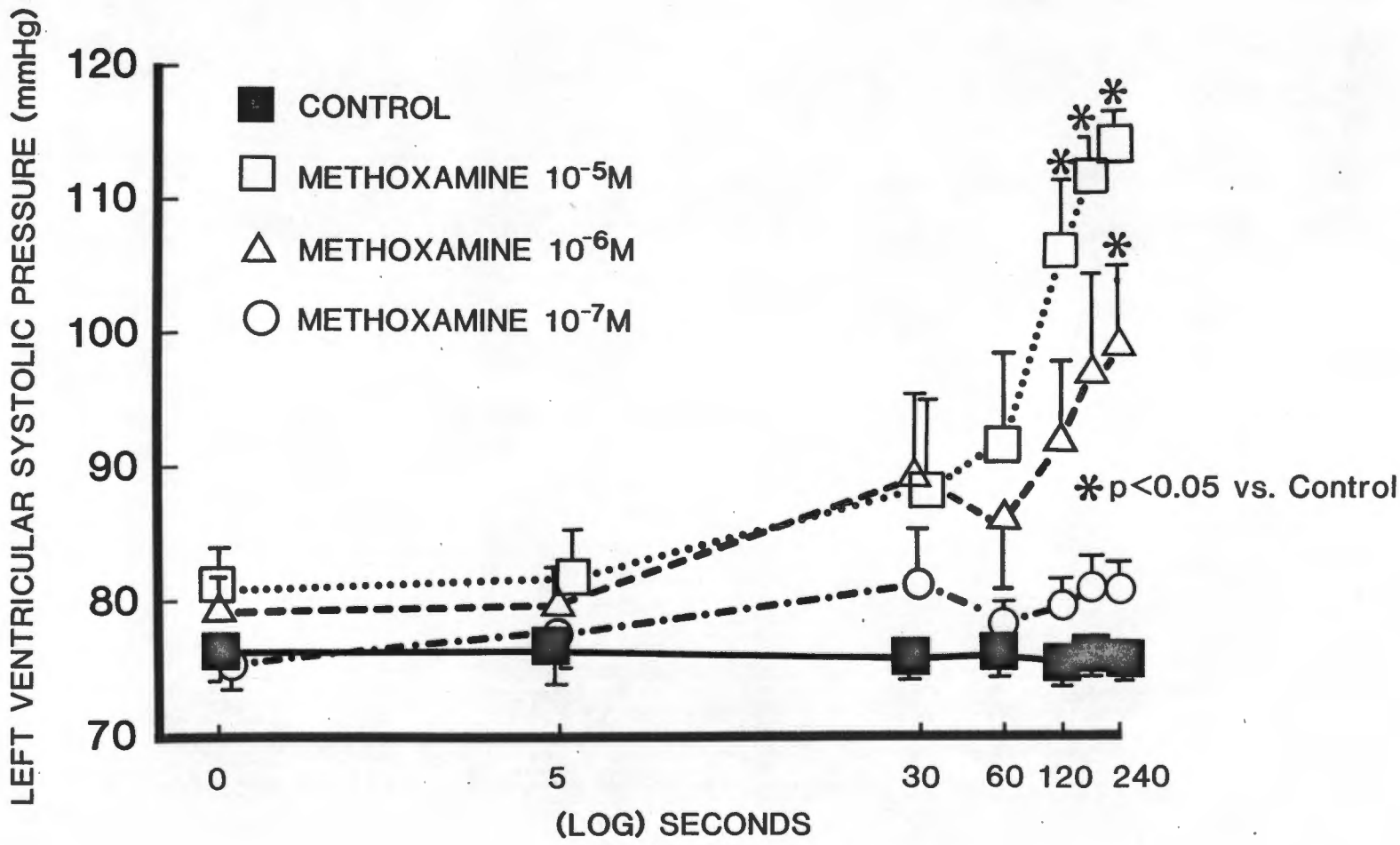
4) The arrhythmogenic action of methoxamine is associated with a positive inotropic effect with an increase in oxygen demand.

FIGURE 1 : Influence of alpha$_1$-adrenoceptor stimulation with methoxamine on ventricular fibrillation threshold.



all experiments undertaken with atenolol 10^{-6}M.

FIGURE 2 : Influence of alpha$_1$-adrenoceptor stimulation with methoxamine on mechanical function.



all experiments undertaken with atenolol 10^{-6}M.

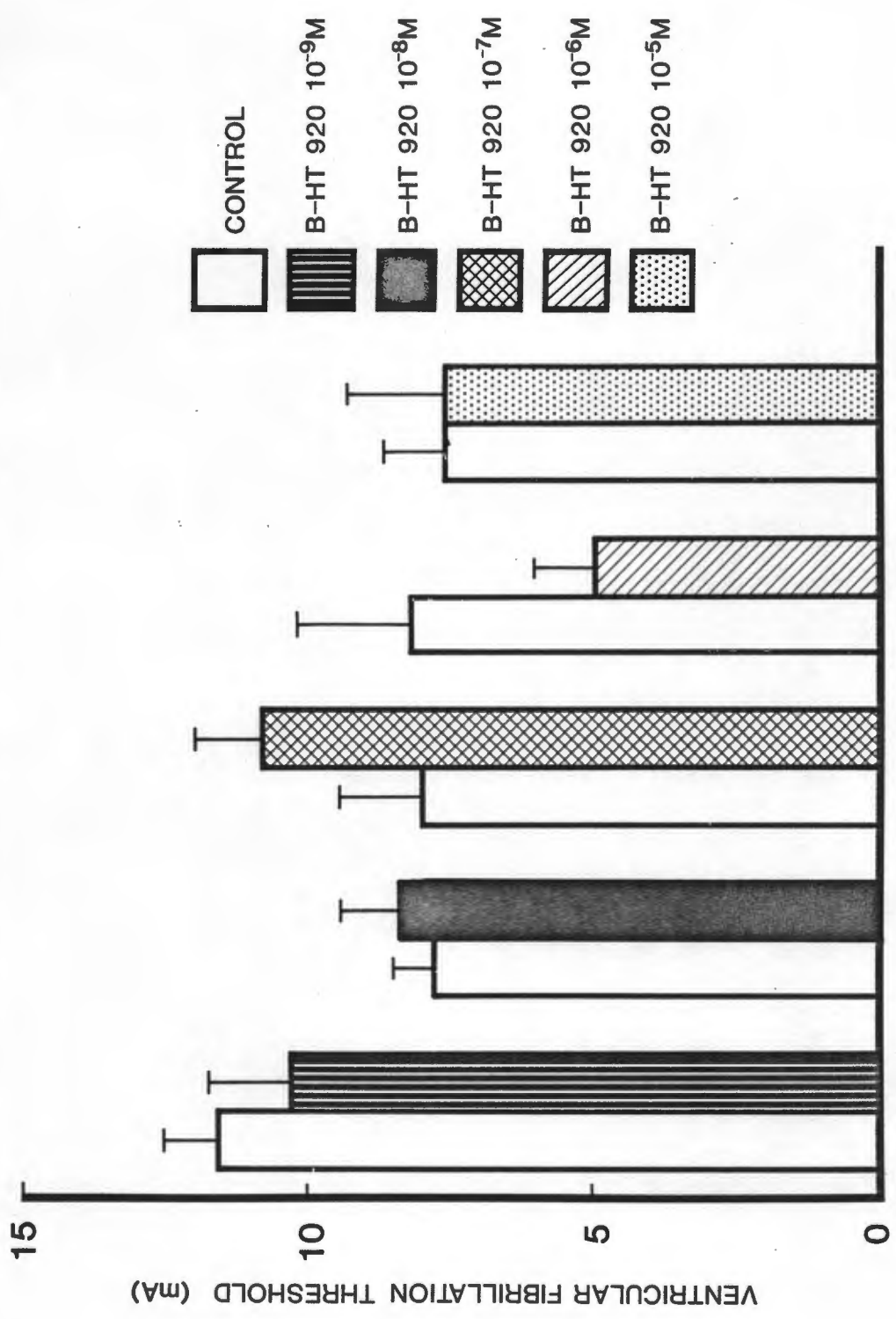


FIGURE 3 : Influence of alpha$_2$-adrenoceptor stimulation with B-HT 920 on ventricular fibrillation threshold.

all experiments undertaken with atenolol 10^{-6}M.

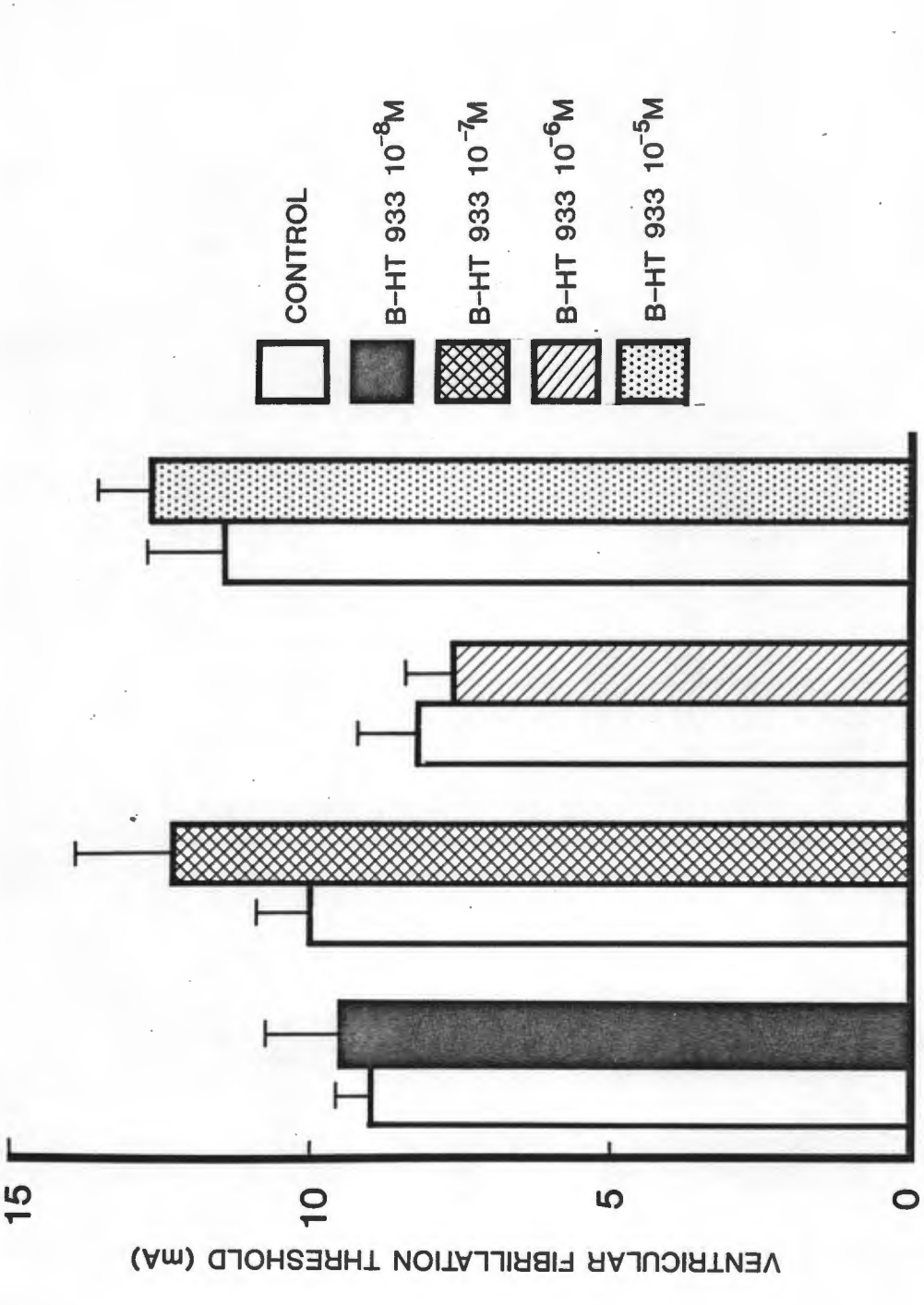
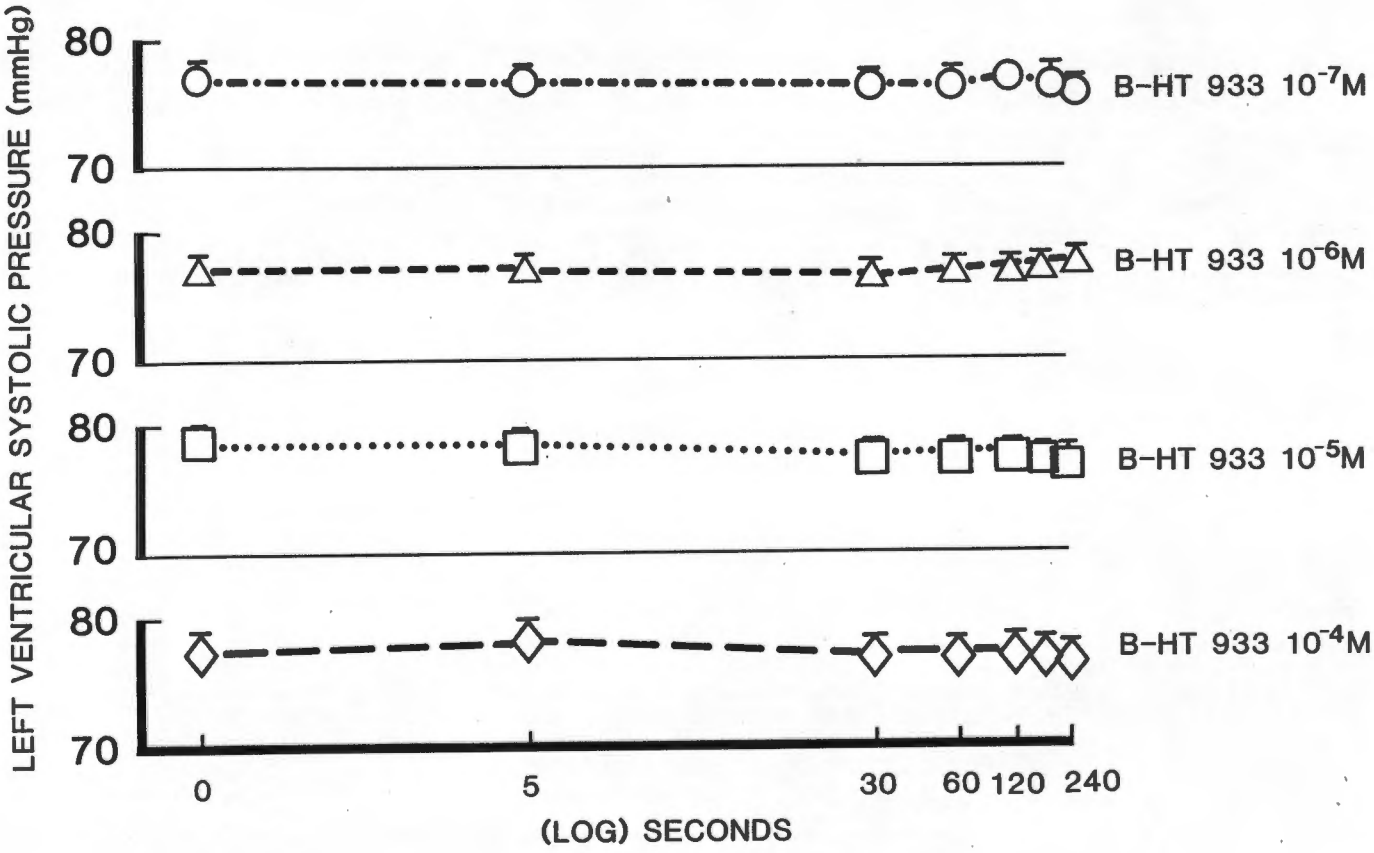


FIGURE 4 : Influence of alpha$_2$-adrenoceptor stimulation with B-HT 933 on ventricular fibrillation threshold.

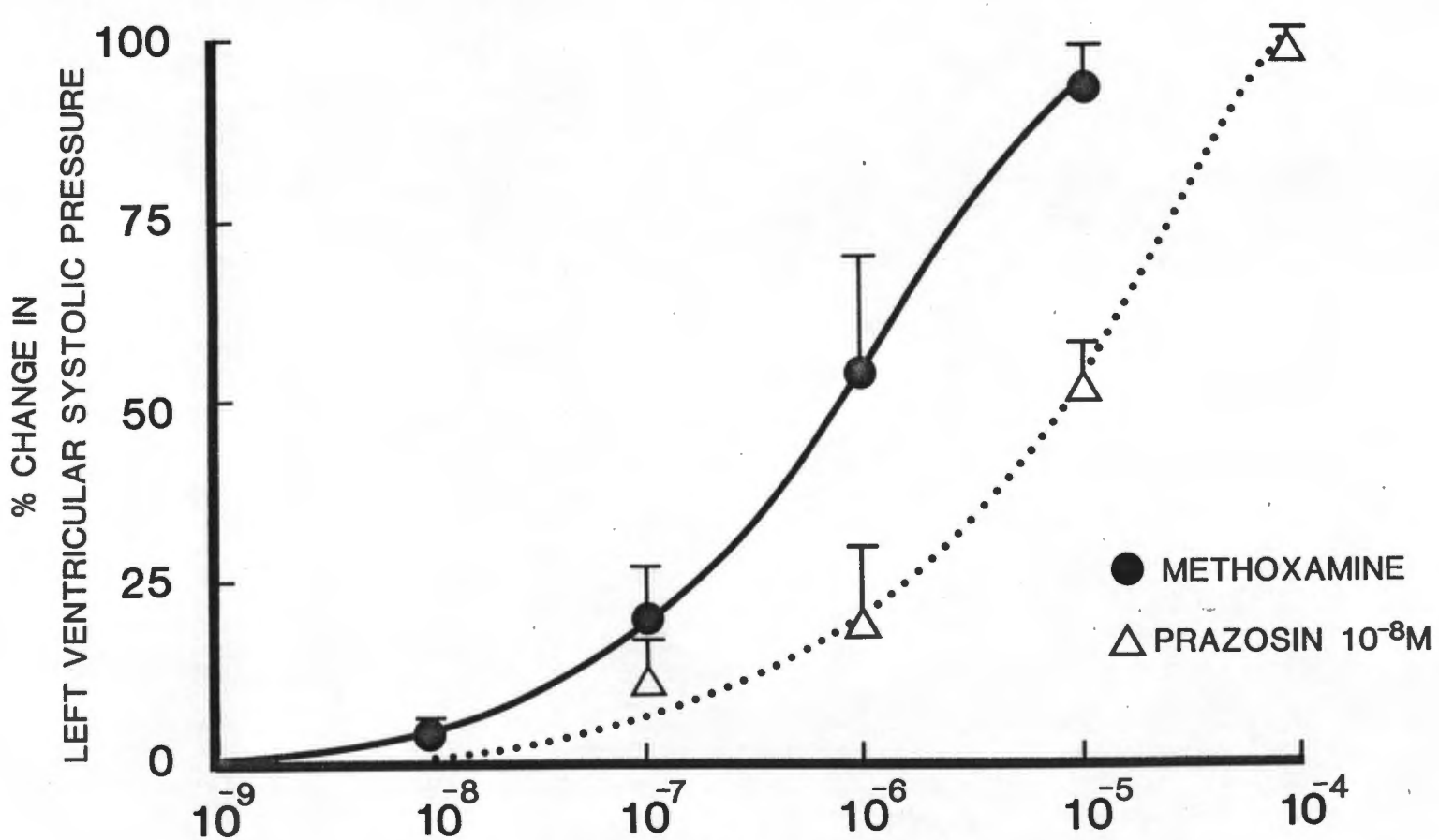
all experiments undertaken with atenolol 10^{-6}M.

FIGURE 5 : Influence of alpha$_2$-adrenoceptor stimulation with B-HT 933 on mechanical function.



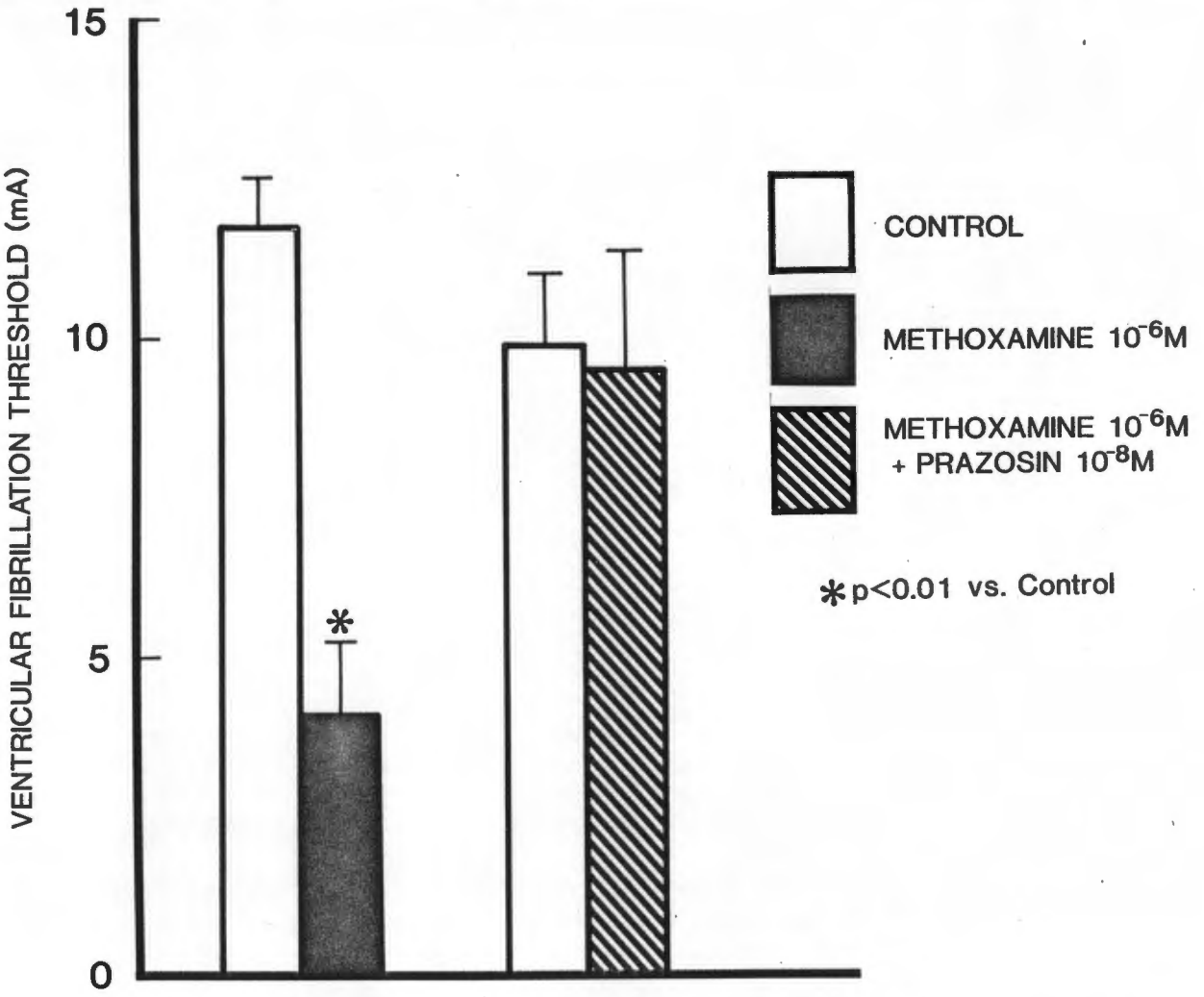
all experiments undertaken with atenolol 10^{-6}M.

FIGURE 6 : Influence of prazosin 10^{-8}M on the log concentration response curve of the positive inotropic effect of methoxamine.



all experiments undertaken with atenolol 10^{-6}M.

FIGURE 7 : The alpha$_1$-adrenoceptor antagonist agent prazosin 10^{-8}M prevents the methoxamine 10^{-6}M induced fall in ventricular fibrillation threshold.



all experiments undertaken with atenolol 10^{-6}M.

Table 1 Influence of alpha$_1$-adrenoceptor stimulation with methoxamine on coronary flow, heart rate, QT interval and ventricular fibrillation threshold

	Coronary flow (ml/min)		Heart rate (beats/min)		QT interval (msec)		Ventricular fibrillation threshold (mA)	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug
Control	8.5 ± 0.3	7.5 ± 0.3	248 ± 6	228 ± 7	78.3 ± 3.6	80.0 ± 3.0	9.7 ± 0.8	9.0 ± 1.1
Methoxamine 10^{-7}M	7.5 ± 0.4	6.4 ± 0.5	269 ± 10	251 ± 10	71.3 ± 1.9	80.0 ± 4.3	11.0 ± 0.6	10.3 ± 2.3
Methoxamine 10^{-6}M	8.3 ± 0.5	8.5 ± 0.7	267 ± 7	250 ± 6	79.6 ± 3.1	94.6 ± 4.4*	11.7 ± 0.6	4.1 ± 1.2*
Methoxamine 10^{-5}M	7.2 ± 0.7	9.5 ± 0.8	259 ± 19	290 ± 11	81.0 ± 3.8	97.3 ± 4.7*	8.7 ± 0.7	3.8 ± 0.8*
Methoxamine 10^{-6}M plus Prazosin 10^{-8}M	7.2 ± 0.4	6.0 ± 0.6	256 ± 15	243 ± 15	77.3 ± 2.4	91.3 ± 3.8*	9.8 ± 1.3	9.5 ± 1.8

Results expressed as mean ± SEM
Number of experiments = 6-13 in each series
All experiments undertaken with atenolol 10^{-6}M
*$p<0.01$ versus control

Table 2 Influence of alpha$_1$-adrenoceptor stimulation with methoxamine on myocardial oxygen demand and metabolic status

	Myocardial Oxygen demand (µml/g/min)		ATP (µmol/g)	Lactate (µmol/g)	PCr (µmol/g)	cAMP (nmol/g)
	Control	Drug				
Control	111.0 ± 9.1	122.6 ± 9.8	3.8 ± 0.2	3.1 ± 0.4	3.4 ± 0.2	0.44 ± 0.02
Methoxamine 10^{-7}M	-	-	4.0 ± 0.1	2.7 ± 0.1	3.7 ± 0.2	0.37 ± 0.02
Methoxamine 10^{-6}M	108.6 ± 9.7	165.6 ± 8.1*	3.7 ± 0.4	2.8 ± 0.1	3.9 ± 0.3	0.43 ± 0.03
Methoxamine 10^{-5}M	125.0 ± 8.3	190.0 ± 14.5*	3.6 ± 0.2	3.6 ± 0.8	3.6 ± 0.6	-
Methoxamine 10^{-6}M plus Prazosin 10^{-8}M	-	-	3.9 ± 0.1	3.4 ± 0.1	3.3 ± 0.3	0.47 ± 0.02

Results expressed as mean ± SEM
Number of experiments = 6-13 in each series
All experiments undertaken with atenolol 10^{-6}M
*$p<0.01$ versus control

Table 3 Influence of alpha$_2$-adrenoceptor stimulation with B-HT 920 on ventricular fibrillation threshold, heart rate and coronary flow

	Ventricular fibrillation threshold (mA)		Heart rate (beats/min)		Coronary flow (ml/min)	
	Control	Drug	Control	Drug	Control	Drug
Control	9.7 ± 0.8	9.0 ± 1.1	248 ± 6	228 ± 7	8.5 ± 0.3	7.5 ± 0.3
B-HT 920 10^{-8}M	7.8 ± 0.7	8.4 ± 1.0	245 ± 31	213 ± 24	7.6 ± 1.0	6.1 ± 0.6
B-HT 920 10^{-7}M	8.0 ± 1.2	10.8 ± 1.2	242 ± 14	211 ± 13	7.2 ± 0.4	5.3 ± 0.4
B-HT 920 10^{-6}M	8.2 ± 2.0	5.0 ± 1.1	251 ± 13	211 ± 18	8.2 ± 0.8	6.5 ± 1.0
B-HT 920 10^{-5}M	7.6 ± 1.1	7.6 ± 1.7	219 ± 3	197 ± 8	7.2 ± 0.7	5.5 ± 0.4

Results expressed as mean ± SEM
Number of experiments = 6-13 in each series
All experiments undertaken with atenolol 10^{-6}M

Table 4 Influence of alpha$_2$-adrenoceptor stimulation with B-HT 933 on ventricular fibrillation threshold, heart rate and coronary flow

	Ventricular fibrillation threshold (mA)		Heart rate (beats/min)		Coronary flow (ml/min)	
	Control	Drug	Control	Drug	Control	Drug
Control	9.7 ± 0.8	9.0 ± 1.1	248 ± 6	228 ± 7	8.5 ± 0.3	7.5 ± 0.3
B-HT 933 10^{-8}M	7.3 ± 0.7	9.5 ± 1.3	271 ± 8	243 ± 7	10.7 ± 0.5	9.4 ± 0.6
B-HT 933 10^{-7}M	10.0 ± 0.9	12.3 ± 1.6	261 ± 8	239 ± 10	10.4 ± 0.4	10.0 ± 0.5
B-HT 933 10^{-6}M	8.2 ± 1.1	7.6 ± 0.9	274 ± 9	258 ± 17	11.8 ± 0.3	11.7 ± 0.6
B-HT 933 10^{-5}M	11.4 ± 1.3	12.6 ± 0.9	276 ± 15	216 ± 11	10.8 ± 0.5	8.3 ± 0.7

Results expressed as mean ± SEM
Number of experiments = 6-13 in each series
All experiments undertaken with atenolol 10^{-6}M

CHAPTER 4

THE POSSIBLE ROLE OF TRANSSARCOLEMMAL CALCIUM MOVEMENT AND SARCOPLASMIC RETICULUM CALCIUM RELEASE IN THE GENESIS OF ALPHA$_1$-ADRENOCEPTOR MEDIATED RESPONSES.

Results

A) Transsarcolemmal calcium ion movement

The possible role of transsarcolemmal calcium movement in alpha$_1$-adrenoceptor mediated responses was explored by two procedures (1) nisoldipine and (2) lowering extracellular calcium ion concentration.

1) Nisoldipine 10^{-8}M prevents

(i) the methoxamine 10^{-6}M induced fall in ventricular fibrillation threshold, (Figure 8) and

(ii) the positive inotropic effect of methoxamine 10^{-6}M to 10^{-5}M (Figure 9).

The ventricular antiarrhythmic effect was associated with

(a) an increase in coronary flow rate, 14.3 $\pm$ 0.6 ml/min versus 9.8 $\pm$ 0.8 ml/min ($p < 0.01$) and

(b) inhibition of the increase in QT interval; control to methoxamine 10^{-6}M values 79.6 $\pm$ 3.1 msec to 94.6 $\pm$ 4.4 msec ($p < 0.01$) respectively; control to methoxamine 10^{-6}M plus nisoldipine 10^{-8}M values, 76.0 $\pm$ 4.7 msec to 88.7 $\pm$ 2.2 msec ($p = 0.06$) respectively (Table 5).

There was no alteration in the following:

(i) Heart rate 244 ± 14 beats/min versus 280 ± 23 beats/min (p non significant)

(ii) Metabolic status

- Adenosine triphosphate 4.1 ± 0.1 μmol/g versus 3.8 ± 0.2 μmol/g (p non significant).
- lactate 3.3 ± 0.5 μmol/g versus 3.1 ± 0.4 μmol/g (p non significant)
- cyclic adenosine monophosphate 0.42 ± 0.02 nmol/g versus 0.44 ± 0.02 nmol/g. (p non significant).(Tables 5 and 6).

2) Lowering extracellular calcium inhibits the methoxamine induced fall in ventricular fibrillation threshold: methoxamine 10^{-6}M plus calcium 2.5 mM value 4.1 ± 1.2 mA, methoxamine 10^{-6}M plus calcium 1.25 mM value 14.1 ± 2.5 mA ($p < 0.01$) (Figure 8).

The protective effect of lowering extracellular calcium was unassociated with alteration in the following:

(i) coronary flow rate, 11.8 ± 0.6 ml/min versus 11.3 ± 0.3 ml/min or

(ii) heart rate 266 ± 7 beats/min versus 280 ± 12 beats/min. (p non significant)

Reducing extracellular calcium inhibits the methoxamine 10^{-6}M induced increase in

(i) left ventricular systolic pressure, 85.3 ± 3.5 mmHg versus 113.4 ± 2.5 mmHg (Figure 9) and

(ii) oxygen demand, 140.5 ± 9.4 μml/g/min versus 165.6 ± 8.1 μml/g/min (Tables 5 and 6).

B) Sarcoplasmic reticulum calcium release

The possible role of sarcoplasmic reticulum calcium ion release in $alpha_1$-adrenoceptor mediated responses was explored using ryanodine. Ryanodine 10^{-9}M inhibits the methoxamine induced fall in ventricular fibrillation threshold: methoxamine 10^{-6}M value 4.1 $\pm$ 1.2 mA, methoxamine 10^{-6}M plus ryanodine 10^{-9}M value, 14.2 $\pm$2.4 mA (Figure 10). Ryanodine 10^{-9}M and 10^{-8}M inhibits the positive inotropic effect of methoxamine 10^{-6}M and 10^{-5}M respectively (Figure 11, Table 7). The protective effect of ryanodine could not be attributed to improvement of or alteration in the following:

(i) coronary flow rate 10.9 $\pm$ 0.4 ml/min versus 11.0 $\pm$ 0.3 ml/min (p non significant).

(ii) heart rate 268 $\pm$ 11 beats/min versus 268 $\pm$ 15 beats/min (p non significant).

(iii) QT interval Ryanodine 10^{-9}M does not prevent the increase in QT interval; control to methoxamine 10^{-6}M values 79.6 $\pm$ 3.1 msec to 94.6 $\pm$ 4.4 msec ($p < 0.01$) respectively; control to methoxamine 10^{-6}M plus ryanodine 10^{-9}M values 78.7 $\pm$ 3.4 msec to 94.7 $\pm$ 2.0 msec ($p < 0.01$) respectively.

(iv) metabolic status - adenosine triphosphate, 4.3 $\pm$ 0.1 µmol/g versus 3.8 $\pm$ 0.2 µmol/g.
- lactate, 2.9 $\pm$ 0.1 µmol/g versus 3.1 $\pm$ 0.4 µmol/g (p non significant). (Tables 5 and 6).

DISCUSSION:

Alpha$_1$-adrenoceptor stimulation increases transsarcolemmal calcium ion influx by phosphorylation of a 15000 MW sarcolemmal protein in the rat heart (Lindemann and Wilson, 1984). It may also induce the release of calcium from the sarcoplasmic reticulum in rat liver (Exton, 1981). Enhanced transsarcolemmal calcium influx or sarcoplasmic reticulum calcium release may result in an increase in intracellular free calcium ion concentration (Figure 12). Increased transsarcolemmal calcium influx may induce slow response action potentials (Reuter, 1974) whereas enhanced sarcoplasmic reticulum calcium release may evoke depolarizing after potentials (Reuter, 1974). Each of these electrophysiological alterations are considered to predispose to ventricular fibrillation.

The arrhythmogenic effect of methoxamine was associated with a positive inotropic effect (compare figures 1 and 2). An increase in force of contraction is ultimately due to an increase in cytosolic calcium ion concentration or increase in sensitivity of myofilaments to calcium or both. Hence it is possible that the arrhythmogenic action of alpha$_1$-adrenoceptor stimulation was associated with an increase in cytosolic calcium.

The possible role of transsarcolemmal calcium ion influx was explored by use of (1) nisoldipine and (2) lowering extracellular calcium ion concentration. Nisoldipine, a dihydropyridine derivative inhibits the voltage dependent influx of calcium (Henry et al., 1980). Nisoldipine binds with the nitrendipine binding site in cardiac sarcolemma (Murphy and Snyder, 1982), reputedly at the calcium channel or a site

functionally related to this channel (Sarmiento et al., 1983). It is relatively selective since it does not bind with the sodium channel (Henry et al., 1980), has no affinity for the $alpha_1$-adrenoceptor (Motulsky et al., 1983) and leaves the outward potassium current unaltered (Kass 1982). Decreasing the extracellular calcium ion concentration reduces the driving force for calcium since calcium ions are considered to depolarize the cell membrane.

Both nisoldipine and low extracellular calcium ion concentration prevented the methoxamine induced fall in ventricular fibrillation threshold. The protective effect afforded by nisoldipine was associated with an increase in myocardial perfusion rate. It is unlikely that the ventricular antiarrhythmic effect of nisoldipine was due to an improvement in myocardial perfusion as

(1) the arrhythmogenic and positive inotropic effect of methoxamine was not accompanied by a decrease in myocardial perfusion rate.

(2) lowering extracellular calcium concentration inhibited the arrhythmogenic and positive inotropic effect of methoxamine without improvement in myocardial perfusion rate.

(3) the protective effect of prazosin was not associated with an increase in myocardial perfusion rate.

The protective effect of nisoldipine and lowered extracellular calcium ion concentration could not be ascribed to alteration in QT interval, reduction in heart rate or improvement in myocardial metabolic status.

Ryanodine was used to explore the possible role of sarcoplasmic reticulum calcium ion release in the genesis of ventricular fibrillation induced by $alpha_1$-adrenoceptor stimulation.

Ryanodine is considered to inhibit the phasic release of calcium ions from the sarcoplasmic reticulum but does not alter the transsarcolemmal calcium ion influx. Evidence for this proposal is as follows:

(1) the negative inotropic response to ryanodine in ventricular myocardium correlates with the species dependence on sarcoplasmic reticulum calcium release for contractile development (Sutko and Willerson, 1980).

(2) ryanodine prevents the contractile response to paired electrical stimulation which is thought to be mediated by enhanced sarcoplasmic reticulum calcium release (Sutko et al., 1979. Sutko and Willerson, 1980).

(3) ryanodine allows the treppe effect (positive inotropy in response to increase in heart rate) but inhibits the post rest contractile response (considered to be dependent on sarcoplasmic reticulum calcium ion release). (Frank and Sleator, 1975. Sutko and Willerson, 1980).

(4) ryanodine abolishes the aequorin signals evoked by calcium induced calcium release in skinned ventricular myocytes (A. Fabiato in Sutko and Kenyon, 1983).

(5) ryanodine abolishes after contractions and prevents afterpotentials, electrophysiological alterations presumed to result from oscillatory release of calcium from the sarcoplasmic reticulum (Sutko and Kenyon, 1983).

(6) ryanodine may potentiate calcium uptake in isolated cardiac sarcoplasmic reticulum preparations (Jones et al., 1979).

(7) ryanodine 10^{-6}M does not inhibit the slow inward calcium current (Sutko and Kenyon, 1983).

(8) according to two calcium dependent processes, phosphorylase A activity and resting tension, ryanodine decreases intracell calcium activity in rat ventricular myocardium (Sutko and Kenyon, 1983).

Ryanodine prevented the arrhythmogenic effect of methoxamine. This protective effect could not be ascribed to alteration in QT interval, reduction in heart rate, improvement in coronary flow rate or preservation of metabolic status.

In this isolated rat heart preparation nisoldipine, reduced extracellular calcium, and ryanodine each prevented the arrhythmogenic action of methoxamine. All three procedures may have a common end effect of reducing intracellular free calcium ion concentration. Consistent with this hypothesis was the finding that all three procedures prevented the methoxamine induced increase in left ventricular systolic pressure. Thus reduction in intracellular free calcium ion concentration may be the possible mechanism underlying the antiarrhythmic activity. This suggests that calcium ion influx across the sarcolemma or the phasic release of calcium ions from the sarcoplasmic reticulum may play an important role in the genesis of ventricular fibrillation following $alpha_1$-adrenoceptor stimulation.

Indirect support for the above hypothesis stems from the findings of:

1. Exton and co-workers who demonstrated an increase in cytosolic calcium occurring within one second of $alpha_1$-adrenoceptor stimulation in rat liver cells (Exton 1981).
2. Corr and co-workers, who showed enhanced $alpha_1$-adrenoceptor responsiveness upon reperfusion of the acutely ischaemic cat myocardium (Corr et al., 1981).

3. Sharma and co-workers, who reported that alpha$_1$-antagonist agents (prazosin amongst others) prevent the accumulation of intracellular calcium 10 minutes after reperfusion of the acutely ischaemic cat myocardium (Sharma et al., 1983).

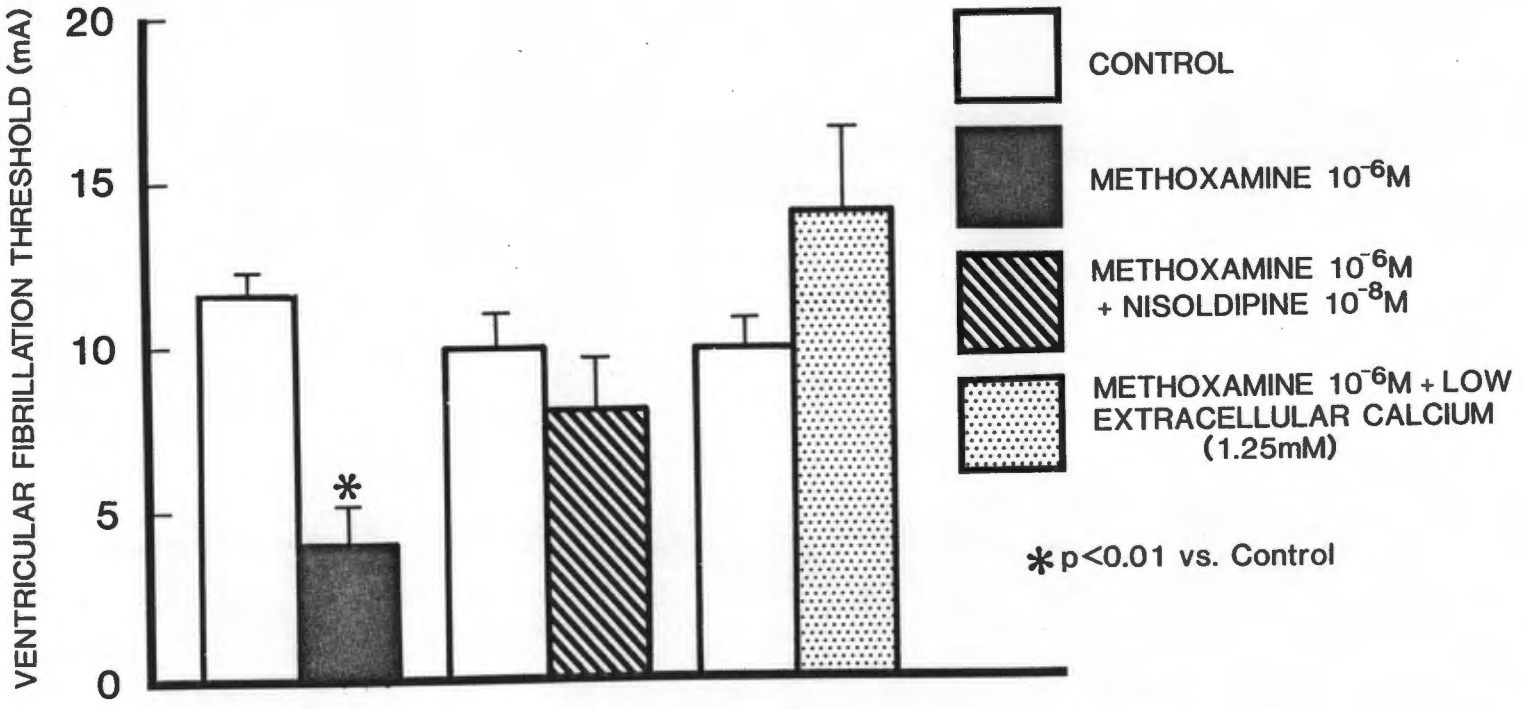
CONCLUSIONS

Alpha$_1$-adrenoceptor stimulation enhances the vulnerability to ventricular fibrillation in the isolated perfused rat heart. This sequence is inhibited by procedures thought to decrease cytosolic calcium concentration. The possible mechanism underlying alpha$_1$-adrenoceptor mediated responses may be an increase in cytosolic calcium ion concentration secondary to either

(i) an enhanced transsarcolemmal calcium ion influx or

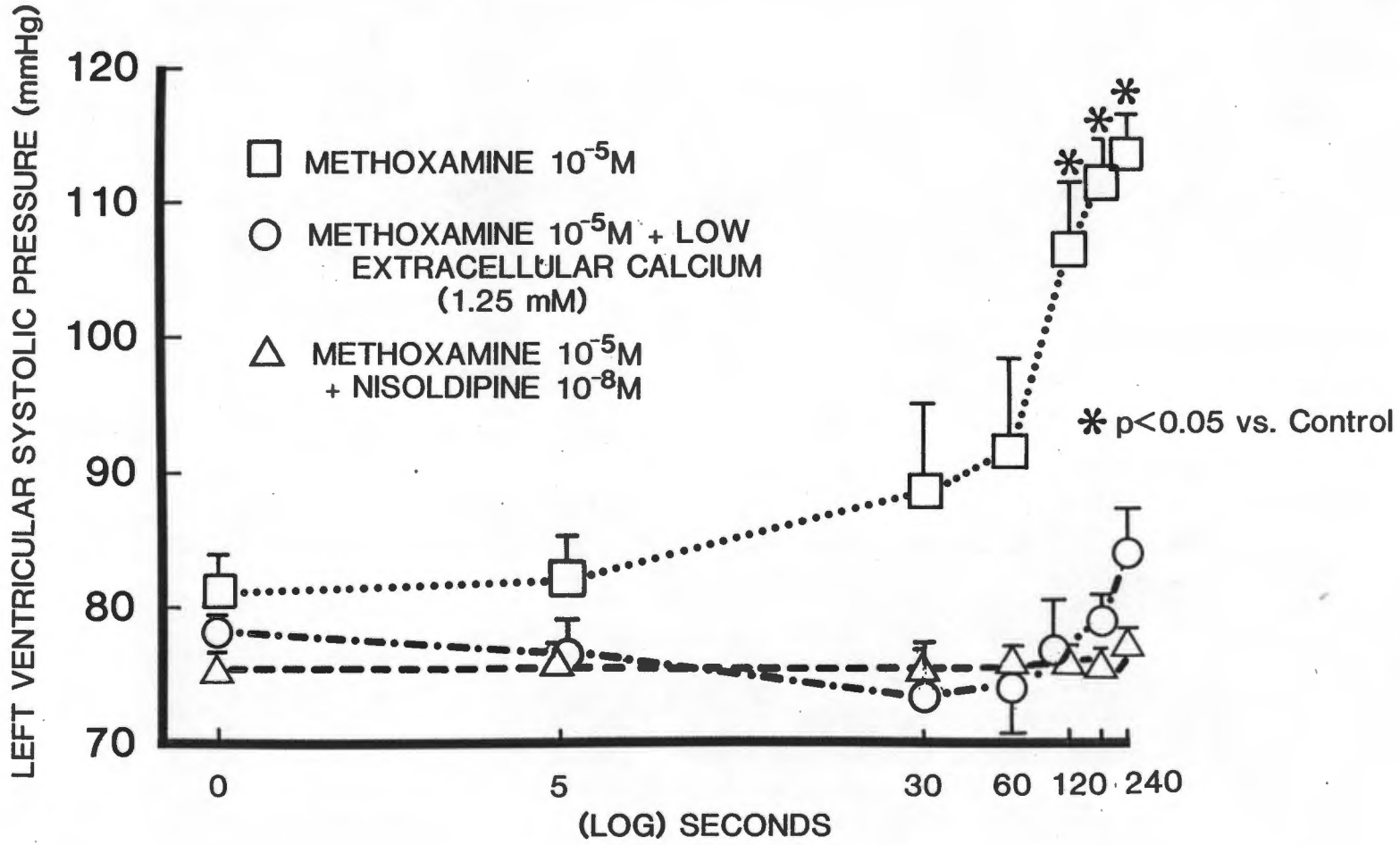
(ii) an increase in the phasic release of calcium ions from the sarcoplasmic reticulum.

FIGURE 8 : Nisoldipine 10^{-8}M and low extracellular calcium (1.25 mM) prevent the methoxamine 10^{-6}M induced fall in ventricular fibrillation threshold.



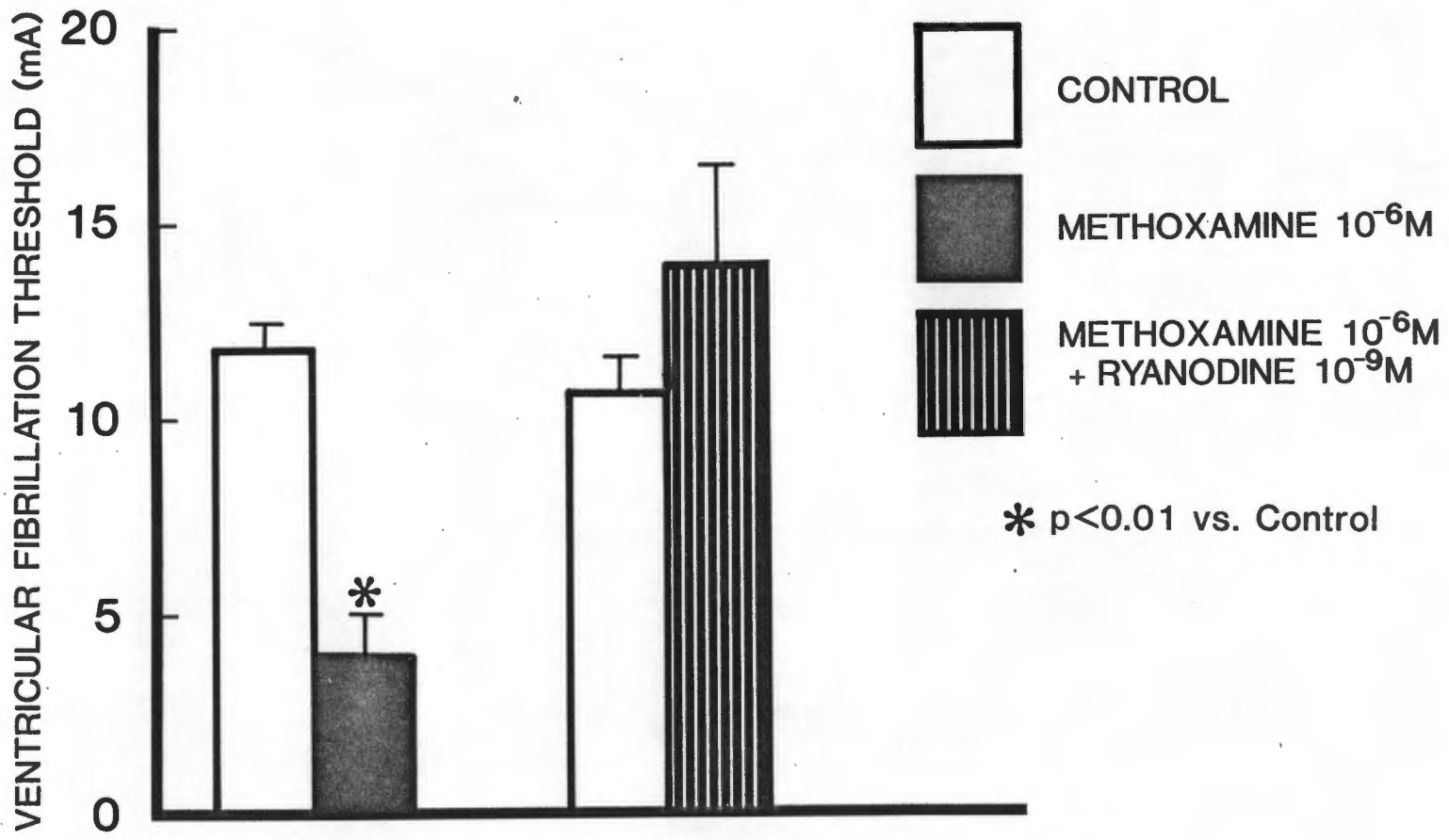
all experiments undertaken with atenolol 10^{-6}M.

FIGURE 9 : Nisoldipine 10^{-8}M and low extracellular calcium (1.25 mM) prevent the methoxamine 10^{-5}M induced positive inotropic effect.



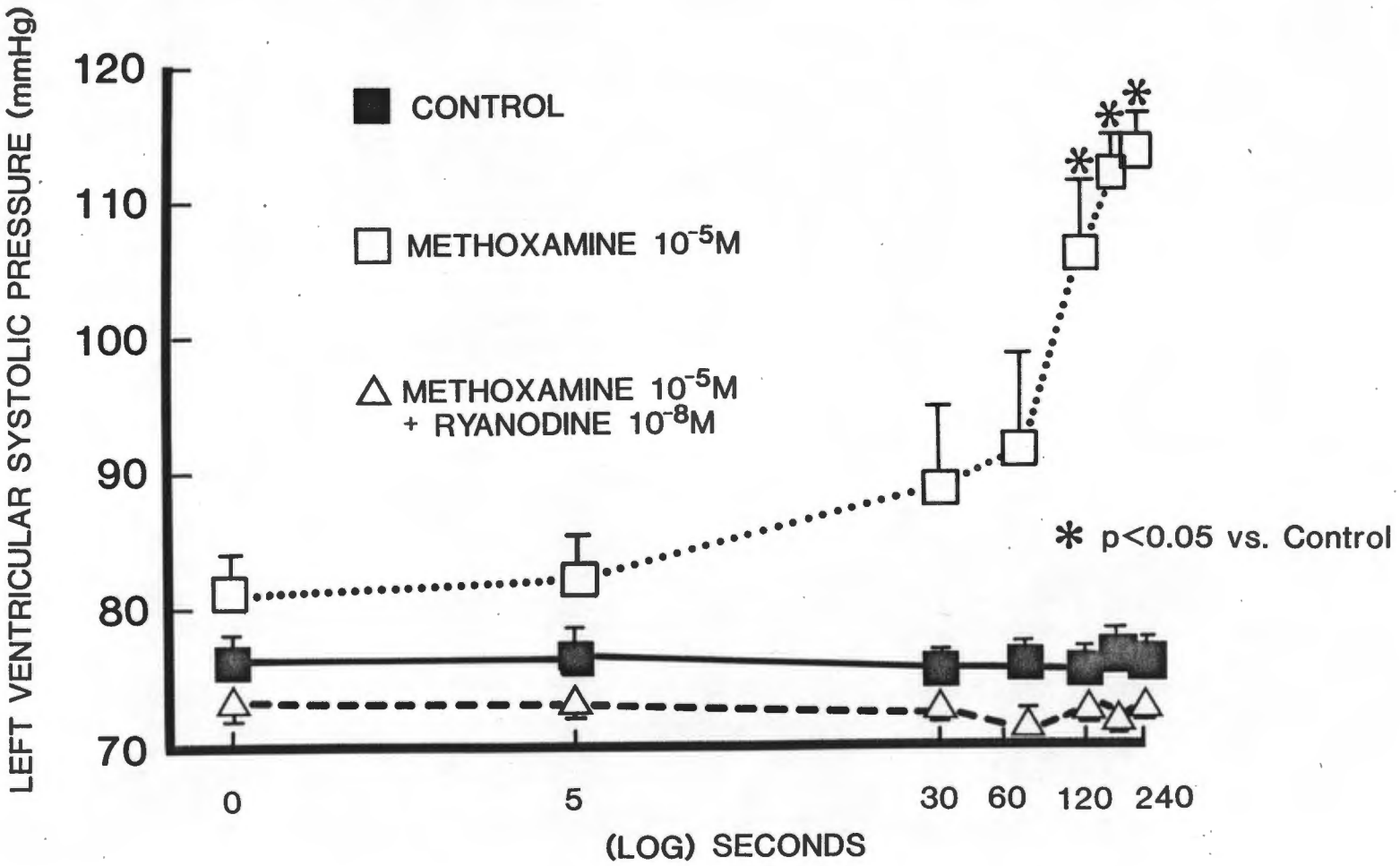
all experiments undertaken with atenolol 10^{-6}M.

FIGURE 10 : Ryanodine 10^{-9}M prevents the methoxamine 10^{-6}M induced fall in ventricular fibrillation threshold.



all experiments undertaken with atenolol 10^{-6}M.

FIGURE 11 : Ryanodine 10^{-8}M prevents the methoxamine 10^{-5}M induced positive inotropic effect.



all experiments undertaken with atenolol 10^{-6}M.

FIGURE 12 : Alpha$_1$-adrenoceptor stimulation increases cytosolic calcium concentration.

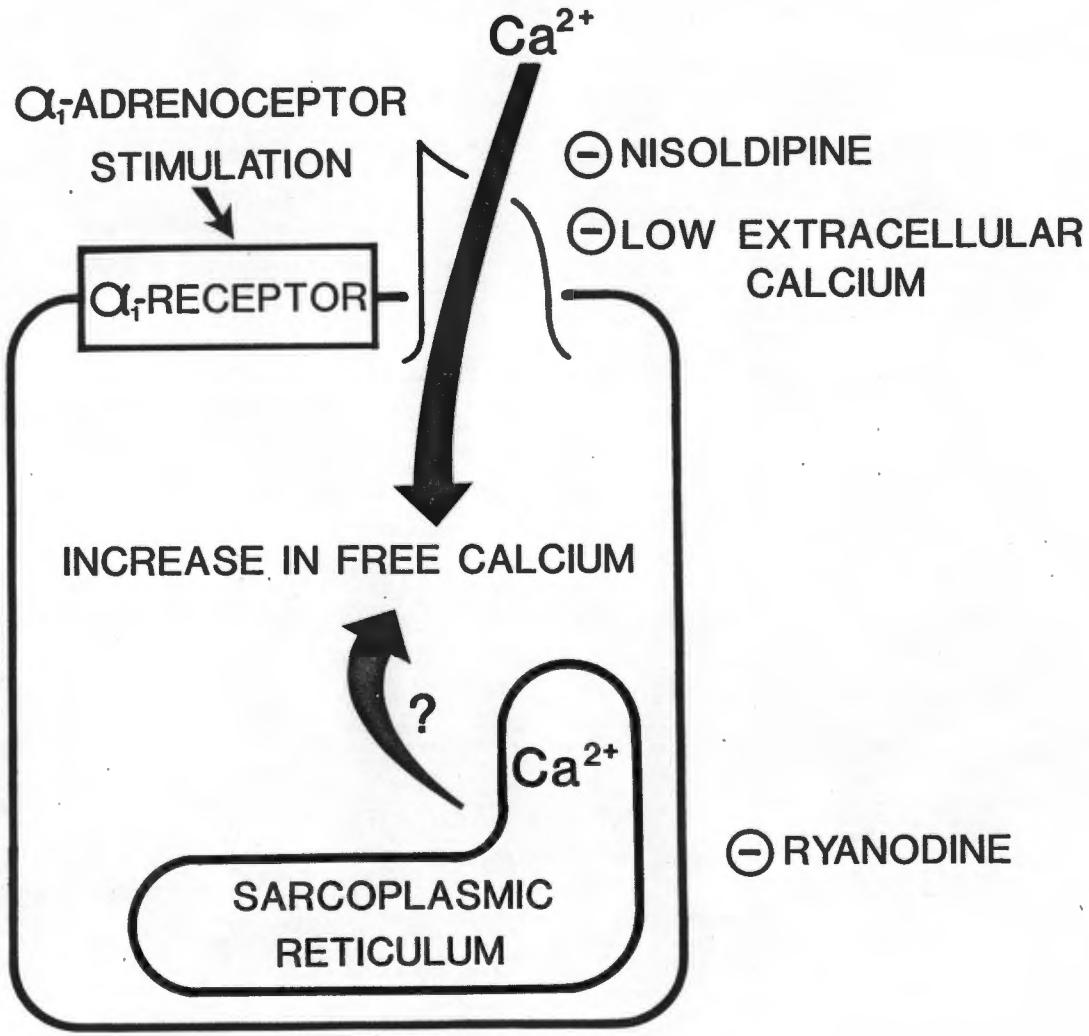


Table 5 Influence of nisoldipine, low calcium (1.25 mM) and ryanodine on the methoxamine-induced alterations in ventricular fibrillation threshold, heart rate and coronary flow

	Ventricular fibrillation threshold (mA)		Heart rate (beats/min)		Coronary flow rate (ml/min)		QT interval (msec)	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug
Control	9.7 ± 0.8	9.0 ± 1.1	248 ± 6	228 ± 7	8.5 ± 0.3	7.5 ± 0.3	78.3 ± 3.6	80.0 ± 3.0
Methoxamine 10^{-6}M	11.7 ± 0.6	4.1 ± 1.2*	267 ± 7	250 ± 6	8.3 ± 0.5	8.5 ± 0.7	79.6 ± 3.1	94.6 ± 4.4*
Methoxamine 10^{-6}M plus Nisoldipine 10^{-8}M	10.0 ± 1.1	8.2 ± 1.6	280 ± 23	244 ± 14	9.8 ± 0.8	14.3 ± 0.6*	76.0 ± 4.7	88.7 ± 2.2
Methoxamine 10^{-6}M plus Low Ca^{2+} (1.25mM)	9.8 ± 0.7	14.1 ± 2.5	280 ± 12	266 ± 7	11.3 ± 0.3	11.8 ± 0.6	-	-
Methoxamine 10^{-6}M plus Ryanodine 10^{-9}M	10.7 ± 1.0	14.2 ± 2.4	268 ± 15	268 ± 11	11.0 ± 0.3	10.9 ± 0.4	78.7 ± 3.4	94.7 ± 2.0*

Results expressed as mean ± SEM
Number of experiments = 6-13 in each series
All experiments undertaken with atenolol 10^{-6}M
*$p<0.01$ versus control

Table 6 Influence of nisoldipine, low calcium (1.25 mM) and ryanodine on the methoxamine-induced alterations in myocardial oxygen demand and metabolic status

	Ventricular fibrillation (mA)		cAMP (nmol/g)	ATP (µmol/g)	Lactate (µmol/g)	Oxygen demand (µml/g/min)	
	Control	Drug				Control	Drug
Control	9.7 ± 0.8	9.0 ± 1.1	0.44 ± 0.02	3.8 ± 0.2	3.1 ± 0.4	111.0 ± 9.1	122.6 ± 9.8
Methoxamine 10^{-6}M	11.7 ± 0.6	4.1 ± 1.2*	0.43 ± 0.03	4.0 ± 0.1	2.8 ± 0.1	108.6 ± 9.7	165.6 ± 8.1*
Methoxamine 10^{-6}M plus Nisoldipine 10^{-8}M	10.0 ± 1.1	8.2 ± 1.6	0.42 ± 0.02	4.1 ± 0.1	3.3 ± 0.5	-	-
Methoxamine 10^{-6}M plus Low Ca^{2+} (1.25mM)	9.8 ± 0.7	14.1 ± 2.5	-	-	-	124.2 ± 7.3	140.5 ± 9.4
Methoxamine 10^{-6}M plus Ryanodine 10^{-8}M	10.7 ± 1.0	14.2 ± 2.4	-	4.3 ± 0.1	2.9 ± 0.1	-	-

Results expressed as mean ± SEM
Number of experiments = 5-13 in each series
All experiments undertaken with atenolol 10^{-6}M
*$p<0.01$ versus control

Table 7 Influence of ryanodine $10^{-9}M$ on the methoxamine $10^{-6}M$ induced positive inotropic effect

	Left ventricular systolic pressure (mmHg)		
	0 minutes		4 minutes
Control	76.3 ± 1.2		75.7 ± 1.2
Methoxamine $10^{-6}M$	79.0 ± 2.8	$p<0.05$	95.4 ± 6.0
Methoxamine $10^{-6}M$ plus Ryanodine $10^{-9}M$	75.4 ± 0.7	p-NS	81.2 ± 4.3

Results expressed as mean ± SEM
Number of experiments = 5-6 in each series
All experiments undertaken with atenolol $10^{-6}M$
Paired t-test used

CHAPTER 5

CONCLUSIONS

1) Alpha$_1$-adrenoceptor stimulation with methoxamine enhanced the vulnerability to ventricular fibrillation in the normoxic rat ventricular myocardium; alpha$_2$-adrenoceptor stimulation with B-HT 920 and B-HT 933 was without effect.

2) The arrythmogenic action of methoxamine was accompanied by the following:
   (a) an increase in QT interval (an indirect index of prolongation of the action potential duration).
   (b) an increase in oxygen demand and
   (c) a positive inotropic effect with a characteristic triphasic response.

3) The alpha$_1$-adrenoceptor mediated responses occurred in the presence of the beta-adrenoceptor antagonist agent atenolol, and were not associated with an increase in myocardial cyclic AMP concentration. There was no alteration in heart rate, coronary flow rate or metabolic status.

4) Specific alpha$_1$-adrenoceptor antagonism with prazosin prevented both the enhanced vulnerability to ventricular fibrillation and the positive inotropic effect of methoxamine.

5) Nisoldipine and reduced extracellular calcium, procedures considered to inhibit transsarcolemmal calcium ion influx, prevented the arrhythmogenic and positive inotropic effect of methoxamine.

Ryanodine, an agent which is reputed to inhibit sarcoplasmic reticulum calcium ion release, also prevented the arrhythmogenic and positive inotropic effect of methoxamine. These interventions are considered to have a common end effect in reducing intracellular calcium ion concentration.

6) The possible mechanism underlying the arrhythmogenic action of $alpha_1$-adrenoceptor stimulation might therefore be an increase in cytosolic calcium ion concentration. This increase in cytosolic calcium ion concentration may be secondary to an enhanced transsarcolemmal calcium ion influx or an increase in the phasic release of calcium ions from the sarcoplasmic reticulum.

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