VENTRICULAR ARRHYTHMOGENESIS IN DEVELOPING MYOCARDIAL INFARCTION IN THE PIG, WITH SPECIAL REFERENCE TO THE ROLE OF CYCLIC AMP

A thesis submitted

by

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to

my father and mother

DANIËL *and* CATHERINE MULLER

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ABSTRACT

Ventricular fibrillation accounts for the majority of deaths during acute myocardial infarction and thereby constitutes a major cardiological problem. Although the mechanism of ventricular fibrillation (VF) is undefined, cyclic AMP, the intracellular second messenger of sympathetic nervous activity has been implicated in the genesis of ventricular arrhythmias.

This study investigates

(a) the relationship between cyclic AMP and the incidence of ventricular arrhythmias in the anaesthetized open-chest pig subjected to acute regional myocardial ischaemia;

(b) the effect of pharmacological interventions which may either increase or decrease tissue cyclic AMP during acute regional myocardial ischaemia and relates these changes to the incidence of ventricular arrhythmias;

(c) the relative importance of site and size of the acutely ischaemic lesion on the incidence of ventricular arrhythmias;

(d) the severity and progression of ischaemia after coronary ligation at two different sites.

Coronary artery ligation, cyclic AMP and ventricular arrhythmias.

The anterior descending coronary artery was ligated at three different sites:

(a) ligation of the main stem (site 1) resulted in regional ischaemia of the anterior left ventricular wall, the interventricular septum and the anterior right ventricular wall. The left ventricular ischaemic lesion comprised about 15% of total left ventricular mass. A distinct phase of severe ventricular arrhythmias occurred approximately 10 to 30 minutes after ligation. Ventricular fibrillation occurred in 13 out

of 16 pigs. This highly unstable ventricular rhythm could be related to accumulation of cyclic AMP in the ischaemic zone, but was not linked to changes in tissue levels of adenosine triphosphate, phosphocreatine and lactate;

(b) ligation of two to four lateral branches (site 3) resulted in regional ischaemia confined the anterior left ventricular wall and comprised about 15% of total left ventricular mass. The size of this lesion was therefore identical to the lesion after main stem ligation (site 1) but differed in that the septum and apical regions were not involved. A low incidence of ventricular arrhythmias was evident and VF occurred in 5 out of 16 pigs only. At this time, no accumulation of cyclic AMP occurred in the ischaemic zone.

Regional left ventricular blood flow studies revealed that ischaemia after ligation at site 1 and site 3 was equally severe.

(c) Ligation of a single lateral branch (site 2) yielded a smaller ischaemic lesion, confined to the anterior left ventricular wall, comprising only about 7% of total left ventricular mass. The incidence of VF (viz. 2 out of 12 pigs) in this group was similar than after ligation of several lateral branches (site 3). Cyclic AMP in the ischaemic zone showed a small peak at 10 minutes only.

These results suggest

(a) a circumstantial association between accumulation of cyclic AMP and ventricular arrhythmias. Furthermore, when pigs from all three groups which developed VF were compared with those pigs without VF, it was evident that cyclic AMP only accumulated in pigs who encountered this arrhythmia.

(b) Site rather than size of an ischaemic lesion may be an important factor in the genesis of ventricular arrhythmias.

(c) Differences in the severity of ischaemia did not underlie the difference in the incidence of VF after ligation at site 1 and site 3.

Beta-adrenoceptor antagonism, cyclic AMP and ventricular arrhythmias after main stem ligation.

The cardioselective beta-antagonist metoprolol administered 30 minutes prior to ligation, reduced the incidence of ventricular premature systoles (VPS) and ventricular tachycardia (VT). In addition, metoprolol reduced the incidence of VF (viz. 2 out of 13 vs 13 out of 16 in controls). Two non-selective beta-antagonists sotalol and propranolol, administered in equipotent antagonistic doses, decreased the incidence of VPS and VT but failed to reduce the incidence of VF. All three beta-antagonists decreased tissue levels of cyclic AMP prior to ligation, but did not prevent the accumulation of cyclic AMP in the ischaemic zone after ligation. Failure of the beta-antagonists to reduce cyclic AMP in the ischaemic zone was probably due to a local increase in sympathetic activity, caused by several factors. Thus metoprolol was able to prevent VF even though tissue levels of cyclic AMP in the ischaemic zone was not reduced. Only metoprolol increased blood flow in the ischaemic zone. The reduction in the incidence of VF may be the consequence of preservation of ischaemic tissue.

Beta-adrenoceptor stimulation, cyclic AMP and ventricular arrhythmias after lateral branch ligation.

Infusion of isoproterenol after ligation resulted in a marked increase in the incidence of VT as well as VF (viz. 6 out of 7 pigs vs 2 out of 12 in controls). This highly unstable ventricular rhythm could be linked to increased tissue levels of cyclic AMP in the ischaemic and non-ischaemic zone.

Cyclic AMP, calcium ions and ventricular arrhythmias.

Cyclic AMP mediates the transmembrane influx of calcium ions, which may promote slow responses, generate after-depolarizations and underlie electrical uncoupling of ischaemic cells. These electrophysiological alterations have been implicated in the genesis of ventricular arrhythmias. We investigated the effect of the calcium channel blocking agent verapamil on isoproterenol-induced VF after lateral branch ligation as well as on spontaneous VF after main stem ligation.

Verapamil 0,9 mg/kg completely prevented isoproterenol-induced VF. This occurred even though tissue levels of cyclic AMP remained elevated. Atrio-ventricular block precluded the use of a similar dose after main stem ligation. However, verapamil 0,6 mg/kg failed to prevent spontaneous VF.

Conclusions

This study provides substantiating evidence for a link between cyclic AMP and ventricular arrhythmias.

a) An accumulation of cyclic AMP after ligation of the main stem could be related to a high incidence of VF.

b) Isoproterenol administration after ligation of a lateral branch resulted in an increase in tissue levels of cyclic AMP in ischaemic and non-ischaemic tissue. Concomitantly, the incidence of VF was sharply increased.

However, the elevation of cyclic AMP could not explain all patterns of ventricular arrhythmias.

a) Metoprolol reduced the incidence of VF after main stem ligation in the presence of an accumulation of tissue cyclic AMP in the ischaemic zone. However, metoprolol also decreased the severity of ischaemia by increasing

blood flow in the ischaemic zone. This effect could directly underlie the low incidence of VF in this group.

b) Verapamil reduced isoproterenol-induced VF despite increased tissue levels of cyclic AMP in the ischaemic and non-ischaemic zones. The cyclic AMP mediated transmembrane influx of calcium ions may therefore be an important factor.

Thus cyclic AMP may be one of the several factors in a chain of events in the genesis of ventricular arrhythmias.

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Beta-adrenoceptor antagonism, cyclic AMP and ventricular arrhythmias.

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Abbreviations

Cyclic AMP	adenosine 3',5'-cyclic monophosphate
VPS	ventricular premature systoles
VT	ventricular tachycardia
VF	ventricular fibrillation

CHAPTER 1

VENTRICULAR ARRHYTHMOGENESIS - AN OVERVIEW

I. The role of the sympathetic nervous system in ventricular arrhythmogenesis

Over the past 20 years, a plethora of clinical and experimental studies have implicated sympathetic stimulation as being of great importance in the genesis of ventricular arrhythmias. Some evidence does exist for opposing anti-arrhythmic influences mediated by parasympathetic stimulation. This dissertation will deal with the role of the sympathetic nervous system only.

A. Sympathetic stimulation and arrhythmias in the non-ischaemic heart.

Sympathetic neural stimulation decreased the ventricular fibrillation threshold in ventricles of the dog (Han 1969, Kliks *et al* 1975). Furthermore, stellate ganglion stimulation resulted in the development of VPS. (Han 1969, Verrier *et al* 1974).

B. Sympathetic stimulation and arrhythmias in the ischaemic heart.

Increased sympathetic nervous tone was found as early as 90 seconds after coronary artery ligation in cats (Malliani *et al* 1969) and may persist for as long as 12 days (Cha *et al* 1970). An enhanced adrenergic outflow was reflected by increased catecholamine levels in plasma and urine after the onset of experimental (Richardson *et al* 1960, Kelliher *et al* 1975) and human acute myocardial infarction (Nadeau and de Champlain 1979, Videback *et al* 1972).

A release of adrenaline or noradrenaline or both into the circulation preceded the onset of ventricular arrhythmias after coronary artery ligation in anaesthetized open-chest dogs (Staszewska-Barczak and Ceremuzynski 1968, Jewitt *et al* 1969).

Furthermore, the heart subjected to coronary artery ligation has shown hyperreactivity to the arrhythmogenic effects of sympathetic

stimulation (Cha *et al* 1970, Harris *et al* 1971). Substantiating evidence was produced by Schwartz *et al* (1976) who showed that stimulation of the left stellate ganglion resulted in a decreased ventricular fibrillation threshold of the ischaemic dog heart.

C. The importance of neural versus humoral catecholamines in the genesis of post-occlusion arrhythmias.

Controversy persists regarding the contributory roles of noradrenaline, released on sympathetic stimulation, versus adrenaline released from the adrenal medulla, in the genesis of arrhythmias after coronary occlusion.

Bilateral removal of the stellate ganglion or sympathetic chain ganglion or both, did not reduce the incidence of VF induced by coronary artery occlusion (Schaal *et al* 1969, Fowlis *et al* 1974). Opposite results have been reported by Skelton *et al* (1962). Ebert *et al* (1968) claim that denervation results in protection only, provided that complete depletion of all myocardial catecholamines had been accomplished before the onset of ischaemia.

However, Corr *et al* (1978) showed that catecholamines, independent on neural activation, may be important in the genesis of VF in the ischaemic heart.

D. Cyclic AMP, the second messenger in the receptor-effector chain, as marker of sympathetic activity.

The measurement of local adrenergic activity is problematic. The recording of sympathetic neural input will not account for regional differences, or for additional influences of circulating catecholamines.

Measurement of local catecholamines contained in the terminal pre-synaptic neuron, would include noradrenaline contained in extragranular stores, which may not be readily available to occupy the post-synaptic receptor.

Stimulation of the end-synaptic adrenergic receptor by catecholamines, activates the adenylate cyclase system in the cell membrane, which forms cyclic AMP from ATP (Drummond and Hemmings 1973, Sobel and Mayer 1973, Wollenberger 1975). When cyclic AMP is measured as an index of local sympathetic activity, it would reflect actual receptor occupation of all neural and humoral catecholamines. However, a further aspect to note is that enhanced adenylate cyclase activity may also result from stimulation of e.g. histamine and glucagon receptors.

E. Increased myocardial levels of cyclic AMP in setting of regional ischaemia.

Following the onset of ischaemia, myocardial tissue levels of cyclic AMP have been shown to increase in the dog (Wollenberger *et al* 1969, Dobson and Mayer 1973, Rabinowitz *et al* 1975, baboon and dog (Podzuweit *et al* 1978), cat (Corr *et al* 1978) and isolated rat heart (Lubbe *et al* 1978), and in man (Rabinowitz *et al* 1974, Strange *et al* 1974).

This increase may primarily be the result of enhanced sympathetic input to the myocardium. However, evidence exists that local events in the ischaemic myocardium may predispose to the accumulation of cyclic AMP. In isolated rat and rabbit hearts, devoid of centrally mediated sympathetic input, anoxia induced a release of one-quarter of the total cardiac catecholamines within 3 minutes. Tyramine administered 30 minutes prior to anoxia, prevented this effect (Wollenberger and Shahab 1965). These results strongly suggest a massive local mobilization of pre-synaptic noradrenaline. These authors later presented substantiating evidence for

this concept, obtained in the open-chest dog (Wollenberger *et al* 1967) and isolated perfused rat heart models of ischaemia (Shabab and Wollenberger 1967).

Several substances which accumulate in ischaemic tissue have been implicated in the liberation of noradrenaline from the axonal terminal, e.g. potassium, (Skinner *et al* 1975, Borda *et al* 1977) and lactate and other acid metabolites (Potter and Axelrod 1963).

The possibility that products of ischaemia could inhibit either re-uptake of catecholamines into the pre-synaptic terminal, or intracellular mono-amine oxidase, should also be considered. However, results to the contrary have been obtained, concerning re-uptake (Mathes and Gudbjarnason 1970).

Lysophosphoglycerides have been shown to accumulate in ischaemic tissue (Sobel *et al* 1978). Lysophosphatidyl choline increased adenylate cyclase activity by 30% in feline and rabbit broken cell preparations (Ahumada *et al* 1979). Cyclic AMP may, through a feedback mechanism, potentiate the accumulation of lysophosphoglycerides (Ahumada *et al* 1979) and could therefore stimulate its own formation.

Wollenberger (1972) postulated that the initial increase in tissue levels of cyclic AMP is due to an enhanced adenylate cyclase activity and that the maintenance of the elevated levels is, at least in part, due to the accumulation of acid products of glycolysis, causing a decrease in phosphodiesterase activity, which hydrolyses cyclic AMP to 5'-AMP. Mori (1976) demonstrated that in the dog with left anterior descending ligation, adenylate cyclase activity in ischaemic tissue was increased 15 to 60 minutes after occlusion, compared to non-ischaemic tissue. However, phosphodiesterase activity in the two zones showed no difference.

F. Cyclic AMP and arrhythmogenesis : The cyclic AMP hypothesis

In 1971 Ueda and Okumura suggested that that the arrhythmic action of some anaesthetic agents was due to their specific action of inhibiting phosphodiesterase activity, as found by Papp and Szekeres (1968). They postulated a possible role for cyclic AMP mediating the arrhythmogenic effects of catecholamines. This was endorsed by Rabinowitz *et al* in 1975.

A year later, Podzuweit, Lubbe and Opie (1976) formulated an hypothesis that the genesis of VF in the ischaemic heart may occur as a result of the accumulation of cyclic AMP in zones of ischaemia. This was based on findings that, in the anaesthetized open-chest baboon and dog, VF was preceded by a sharp increase in cyclic AMP in the ischaemic zone (Podzuweit *et al* 1978).

This hypothesis was later supported by the work of Corr *et al* (1978) who showed that increases of cyclic AMP in ischaemic tissue preceded the peak frequency of VPS in a feline preparation. More direct evidence came from Lubbe *et al* (1976, 1978) who showed that dibutyryl cyclic AMP administration lowered the ventricular fibrillation threshold of the isolated perfused rat heart. Podzuweit *et al* (1978), showed that an infusion of dibutyryl cyclic AMP at the edge of the infarct in the anaesthetized open-chest pig, provoked ventricular arrhythmias, during an otherwise "silent" phase.

Some electrophysiological mechanisms have been suggested to underlie the arrhythmogenic effects of cyclic AMP. This nucleotide has been strongly implicated in evoking "slow responses", which promote re-entry. Cyclic AMP induced automaticity in Purkinje fibres and provoked after depolarizations in otherwise non-automatic ventricular cells. The phenomena of re-entry and automaticity are thought to be prominently involved in ventricular arrhythmogenesis during developing myocardial infarction and are discussed in detail on pages 9 and 10.

II. POTASSIUM AND ARRHYTHMOGENESIS

In 1938, Dennis and Moore demonstrated an increase of over 50% in the levels of potassium in coronary venous blood within 9 minutes after coronary artery ligation in the cat. Harris *et al* (1954) produced supporting evidence and proposed that potassium was liberated from ischaemic tissue, mainly as a result of an energy deficit of the sodium-potassium pump in the cell membrane. Catecholamine release following coronary artery ligation has also been directly implicated in the accumulation of potassium (Ebert *et al* 1967).

In 1954 Harris postulated that potassium may be implicated in the genesis of ventricular arrhythmias, resulting from coronary artery ligation. This view was substantiated by the work of Dennis and Moore (1938) and Cherbakoff *et al* (1957). Regan *et al* (1967) showed a causal relationship between cumulative potassium loss and the incidence of ventricular arrhythmias within an hour of experimentally induced coronary artery thrombosis in the dog. Furthermore an infusion of isotonic potassium chloride into the left anterior descending coronary artery of the dog was followed by ventricular ectopic activity, leading to VT and VF (Ettinger *et al* 1973).

Depletion of cellular potassium cannot account for early arrhythmias following coronary artery occlusion, because it requires two to four hours to become evident (Jennings *et al* 1957). Increased extracell potassium has been suggested to play an important role (Johnson 1976, Holland and Brooks 1977). Hirche *et al* (1980) using potassium sensitive electrodes, showed that extracell potassium started to increase at about 10 seconds after ligation of the left anterior descending coronary artery in the pig, reaching a level of about 15 mEq/ℓ within 7 minutes.

These high levels coincided with an early phase of ventricular arrhythmias between 3 and 8 minutes. A second rise in extracell potassium levels to nearly 18 mEq/ℓ between 20 and 25 minutes occurred concomitantly with a second phase of arrhythmias.

The electrophysiological properties of increased extracell potassium levels and its possible role in ventricular arrhythmogenesis is discussed on page 10.

However, results suggesting no link between increased extracell potassium levels and the incidence of arrhythmias were presented by Wexler and Patt (1960) who failed to find a relationship between coronary sinus potassium levels and ectopic activity in the dog. Furthermore, Thomas *et al* (1970) showed a difference in the severity of arrhythmias in the dog with ischaemic lesions of similar size, but at different sites, while potassium liberation was similar in the two groups. Several clinical and experimental studies in which potassium loss during developing myocardial infarction was controlled in an attempt to prevent VF, were also not successful (Dixon *et al* 1965, Shinohara 1968, Arnott 1969).

III. SUBSTRATES AND ARRHYTHMIAS

A. Lactate

Lactate has been shown to decrease the action potential duration, accelerates phase 4 depolarization, and decreases the resting potential (Wissner 1974). These results may suggest that an accumulation of intracellular lactate may predispose to arrhythmias. However, no data to support this proposition has been published as yet.

B. Free fatty acids

Rowe and co-workers (1973) found that antilypolytic therapy reduced the incidence of VT in patients with acute myocardial infarction. In some clinical studies (Gupta *et al* 1969, Prakash *et al* 1972), high circulating concentrations could be associated with serious arrhythmias, but in others (Carlström and Christensson 1971, Hagenfeldt and Wester 1973) no such association could be found.

IV. ELECTROPHYSIOLOGICAL BASIS OF VENTRICULAR ARRHYTHMIAS AFTER CORONARY ARTERY OCCLUSION.

In the normal heart, maximal electrical asynchrony within the ventricular wall occurs during early repolarisation, about 30 milliseconds before the peak of the T-wave. This interval is referred to as the vulnerable period (Surawicz 1971). During this period, many cells are repolarised while others are relatively or even absolutely refractory. In the early stage after the onset of ischaemia in the ventricle, several electrophysiological parameters change in ischaemic tissue which could contribute to enhanced asynchrony throughout a larger portion of the cardiac cycle (Scherlag *et al* 1974, Williams *et al* 1974, Corr and Sobel 1977).

Specific changes to the normal myocardial action potential have been implicated in arrhythmogenesis (Hoffman and Cranefield 1964, Sano 1976). These include a decreased (less negative) resting potential, changes in the fast inward sodium channel, shortening of the duration of the action potential and an increased rate of spontaneous depolarization (Opie *et al* 1979).

A. Automaticity of Purkinje fibres

Conductive tissue is more resistant to the effects of anoxia than ordinary ventricular tissue, probably due to a relatively high glycogen content. This may be the result of a reduced glycolytic rate, as judged from decreased concentrations of glycolytic enzymes (Kübler *et al* 1972). Glycolytic function, which may have special importance in the electrical activity of the cell, is relatively prominent in conduction tissue. Purkinje fibres become damaged only at a later stage after the onset of myocardial ischaemia. When substrate supply to these cells is eventually altered, automaticity, especially in the presence of a decrease in resting potential, could be promoted (Opie *et al* 1979). When these Purkinje fibres eventually become necrotic, automaticity ceases (Julian 1980).

Cyclic AMP has been strongly implicated in provoking automaticity of Purkinje fibres. When cyclic AMP is introduced by inotophoresis into cardiac Purkinje fibres, the action potential is shortened and the rate of phase 4 depolarization is steeper. Similar results were obtained by using dibutyryl cyclic AMP (Kentera *et al* 1978).

Automaticity, being a cellular phenomenon, would be independent of the mass of ischaemic myocardium (Kaplinsky *et al* 1978) (compare re-entry).

B. The "slow response" and re-entry

When the fast inward sodium current which is responsible for the upstroke of the action potential is diminished or blocked, the newly evoken action potential shows a much slower rate of rise and velocity of conduction. These are known as "slow responses", possibly moving through a different channel. It is now generally accepted that

the calcium ion is predominantly responsible for this current (Opie *et al* 1979).

Following coronary artery occlusion, an optimal environment exists for the initiation of "slow responses" in zones of ischaemia. Increased extracell potassium (Johnson 1976, Hirche *et al* 1980) can diminish and later abolish the fast response, preventing the propagation of the action potential (Gettes 1975). Under these circumstances, accumulated cyclic AMP (Corr *et al* 1978, Podzuweit *et al* 1978) or catecholamines (Griffiths and Leung 1966) may evoke an action potential showing characteristics of the "slow response" (Reuter 1974), which underlie unidirectional block of impulse propagation (Cranefield 1975) resulting in re-entry.

In the periphery of the ischaemic zone, a large inhomogeneity of the severity of injury can be expected to result in large disparities in refractory periods and electrical activation. Together with local conduction block, re-entry may be strongly promoted (Han 1969, Scherlag *et al* 1974, Downar *et al* 1977). Re-entry was first described by Cranefield in 1975, p. 171:

> "A cardial impulse hits an island of depressed tissue and finds entry into the loop at one side and then slowly propagates through the island and hits the border where there is unidirectional block. If the adjacent normal tissue is ready to respond, the impulse emerges and produces an extrasystole."

C. THE INITIATION AND MAINTENANCE OF VENTRICULAR FIBRILLATION BY RE-ENTRY.

It is now generally accepted that arrhythmias in the early stage after coronary artery occlusion, are attributed to re-entry (Scherlag, 1974, Corday 1977, Corr and Sobel, 1977, Janse *et al* 1978). VF occurs most frequently during the first hour after coronary occlusion. A progressive delay of ventricular electrical activity at local epicardial sites has been demonstrated within minutes of coronary ligation (Scherlag 1974). When an impulse emerging from an island of delayed conduction in ischaemic tissue provokes excitation repetitively VT and VF result from a total disorganization of impulse propagation within the ventricles (Harris and Rojas 1943, Han 1969, Surawicz 1971, Zipes 1975).

Mechanisms that initiate VF may be quite different from those that sustain this arrhythmia. If conduction velocity remains slow because of the prematurity of activation and if the refractory period distal to the advancing wavefront is sufficiently brief, fibrillation will persist (Corr *et al* 1978).

Maintenance of VF is also dependent on a critical mass of myocardium, with altered electrophysiological properties (Moe and Abildskov 1959), i.e. a larger mass is required if conduction is not slow enough and refractoriness is not short enough to maintain re-excitation (Josephson *et al* 1979).

When a sufficient number of cells exhibits pronounced prolongation of refractory period or when conduction velocity of wavefronts is sufficiently enhanced, VF may terminate (Moe *et al* 1964).

CHAPTER II

THE EXPERIMENTAL MODEL AND GENERAL METHODOLOGY

The time interval between the unpredictable onset of acute myocardial infarction in man, and death, which usually occurs outside the hospital is seldom longer than sixty minutes (Kannel *et al* 1975, Julian 1979). VF is the major cause of sudden death (Jennings and Reimer 1974, Julian 1979). If these patients were to be under medical care, electrocardiographic and haemodynamic monitoring and observations of physiological response could surely elucidate possible mechanisms of this fatal arrhythmia. However, a study of biochemical changes in the myocardium, which may underlie the above to a great extent, would be of an invasive nature. An animal model in which coronary artery occlusion will persistently lead to VF within one hour, is therefore the most obvious alternative.

The model used in these studies is the anaesthetized open-chest pig with coronary artery ligation.

I. THE PIG AS EXPERIMENTAL ANIMAL

Conventionally, *in vivo* heart research has been done especially on the dog, but sometimes on the cat or baboon. Fundamental differences have been found between canine and human myocardial blood supply. After studying several animals, Lumb and Singletary (1962) concluded that the coronary artery distribution of the pig related best to that of man.

A. Coronary architecture

In the porcine heart, as in man, the right coronary artery dominates and is relatively long, (Blumgart *et al* 1950) giving rise to the posterior septal artery, with the anterior septal artery arising from the anterior descending. The diameter of the anterior septal artery rarely exceeds 30% of the diameter of the anterior descending.

(Lumb and Singletary 1962). In the dog, the anterior as well as the posterior septal arteries arise from the left coronary artery. The anterior septal artery is a very large vessel and the right coronary artery contributes very little to the normal blood supply of the mid part of the posterior wall. (Bartho and Gagnan 1964).

The pig shows less interanimal variation in coronary anatomy and distribution than the dog (Lumb and Hardy 1963).

B. Collateral circulation

Similarly to the normal, non-ischaemic human heart, the pig heart has minimal collaterals, located subendocardially and endomurally. (Fulton 1965, Jonson 1975). In contrast, collaterals in canine heart are more abundant (Eckstein 1954) and lie subepicardially (Schaper 1971). Many patients have a significant collateral network. However, this developed in response to regional ischaemia and is largely subendocardial in location (Schaper 1971).

Similar occlusion procedures produced primarily a subendocardial infarct in the dog, but a transmural infarct in the pig. In this regard, the pig infarct more closely resembles that in man (Fozzard 1975).

II. CRITIQUE OF THE MODEL

It must be accepted that no animal model will ever be ideal to study the physiology or pathology of man.

The main criticism for this model of acute myocardial infarction is that we are dealing with a healthy animal in which the

heart is assumed to be normal. In man, acute myocardial infarction is usually associated with, and the result of progressive impairment of coronary blood supply due to atherosclerosis, and the myocardium shows signs of pre-existing fibrous scarring and fatty infiltration (Verrier *et al* 1974, Oliver 1976). This becomes especially important when referring to the 'normal', non-ischaemic left ventricle after ligation. Some progress has been made developing an animal model in which spontaneous acute infarction would occur in the presence of severe coronary atherosclerosis. (Lee *et al* 1971).

III THE ANAESTHETIZED OPEN-CHEST PREPARATION

A. Advantages

1. Sequential myocardial tissue biopsies for biochemical analysis are easily obtainable.
2. The distribution of the larger coronary arteries is visible. Infarct size is therefore predictable to a great extent.
3. Dissection of the artery for the purpose of ligation can be performed, causing minimal damage to subjacent tissue, veins and nerves.
4. Johansson *et al* (1974) described a severe stress syndrome in pigs. Pale epicardial patches, an early symptom, would now be visible.
5. The exposed heart permits accurate epicardial electrographic monitoring.
6. This preparation facilitates catheterization of the heart.

B. Disadvantages

1. Anaesthesia per se unquestionably diminishes or obliterates the vicious circle of pain, anxiety, and neural and hormonal excitation described in man in the early stages of acute myocardial infarction. The mortality rate from experimental coronary occlusion in dogs was lower in anaesthetized animals (Manning *et al* 1939).
2. Barbiturate anaesthesia in the dog increased heart rate, decreased systemic pressure and decreased cardiac output, (Giles and Burch 1970) and may interfere with the ability of the sarcoplasmic reticulum to handle calcium (Legato 1969)
3. Rushmer (1954) found the left ventricle to empty almost completely during systole when the thorax is open, in contrast with some residual blood found in the closed chest animal and in man. He also described a temporary reduction in the size of the left ventricle after thoracotomy.
4. The open chest may initiate reflex mechanisms not present in man with acute myocardial infarction. Rabinowitz and co-workers (1975) showed that plasma cyclic AMP was elevated for 2 hours after thoracotomy in the dog.
5. Artificial respiration may decrease cardiac output and stroke volume in dogs (Morgan *et al* 1966).

IV. ANIMAL PREPARATION

Pigs (Large White crossed with Landrace) of either sex, weighing 27 to 33 kg. were studied. Due to severe stress cardiopathy described in pigs, (Johansson *et al* 1974) special care was taken to subject animals to minimal stress:

a. Animals were brought into the university animal house at least 8 days prior to the operation.

b. They were not fasted.

c. Pigs were not kept isolated in the pens.

Ketamine (Ketalar, Parke Davis) 10mg/kg was administered intramuscularly to immobilize the animals. Higher doses sometimes resulted in respiratory collapse. Atropine sulphate 0.3 mg was given simultaneously. The first doses of anaesthetic agents were administered into a vein on the ear. Later on, drugs were routinely given into a femoral vein. A femoral artery was cannulated and connected to a Statham P23Db transducer for monitoring blood pressure. Blood samples were also obtained from this artery. The animals were ventillated with room air through a cuffed endotracheal tube connected to a Harvard constant volume respirator (Harvard Apparatus Company, Dover, USA) at a rate of about 15 strokes per minute and a tidal volume of about 12 ml/kg body weight. Arterial blood pH, pCO_2 and pO_2 were monitored. pO_2 was controlled between 90 and 110 mmHg, and pH between 7.46 and 7.49.

In our studies a deep level of anaesthesia was maintained in order to control heart rate at about 120 beats per minute, to facilitate coronary artery dissection, biopsy taking and epicardial TQ-ST segment mapping and atrial catheterization. This rate is lower than in most other animal studies.

Each pig received thiopentone sodium (Intraval, Maybaker) 3 mg/kg and alcuronium chloride (Alloferin, Roche) 0,2 mg/kg intravenously about every 40 minutes.

Only when blood gases were stabilized and satisfactory and heart rate was well controlled, the heart was exposed by midsternal thoracotomy. Elevation of the apex renders the heart very irritable. The heart was therefore not cradled in the pericardium and the animal was tilted towards the right. The epicardium was moistened throughout the experiment by dripping on it saline 0,9% solution, kept at about 37°C. The maintenance of a rectal temperature of about 38°C was facilitated by covering the open chest and by heating the operating room.

V. CORONARY ARTERY DISSECTION AND LIGATION

The left anterior descending coronary artery was dissected free for about 5 mm at a point one-half to two-thirds the distance from the origin to its apical termination (main stem, site 1) or at the origin of a major lateral branch (small lateral, site 2) or at the origin of two to four adjacent lateral branches (large lateral, site 3) (fig. 2.1). Care was taken to remove covering layers only and not to damage vasculature or tissue. A linen thread was passed underneath and at least 15 minutes was then allowed for the heart to stabilize before ligation was performed by abrupt tightening of the ligature.

VI. BIOPSY TAKING

Biopsies were obtained by modification (Podzuweit *et al* 1978) of the technique described by Pool *et al* (1968) using a suction drill. The micro drill head has a diameter of 2 mm and penetrates the subepicardium to a depth of approximately 3 mm. Samples were expelled into liquid nitrogen within 3 seconds. This is well within the maximum

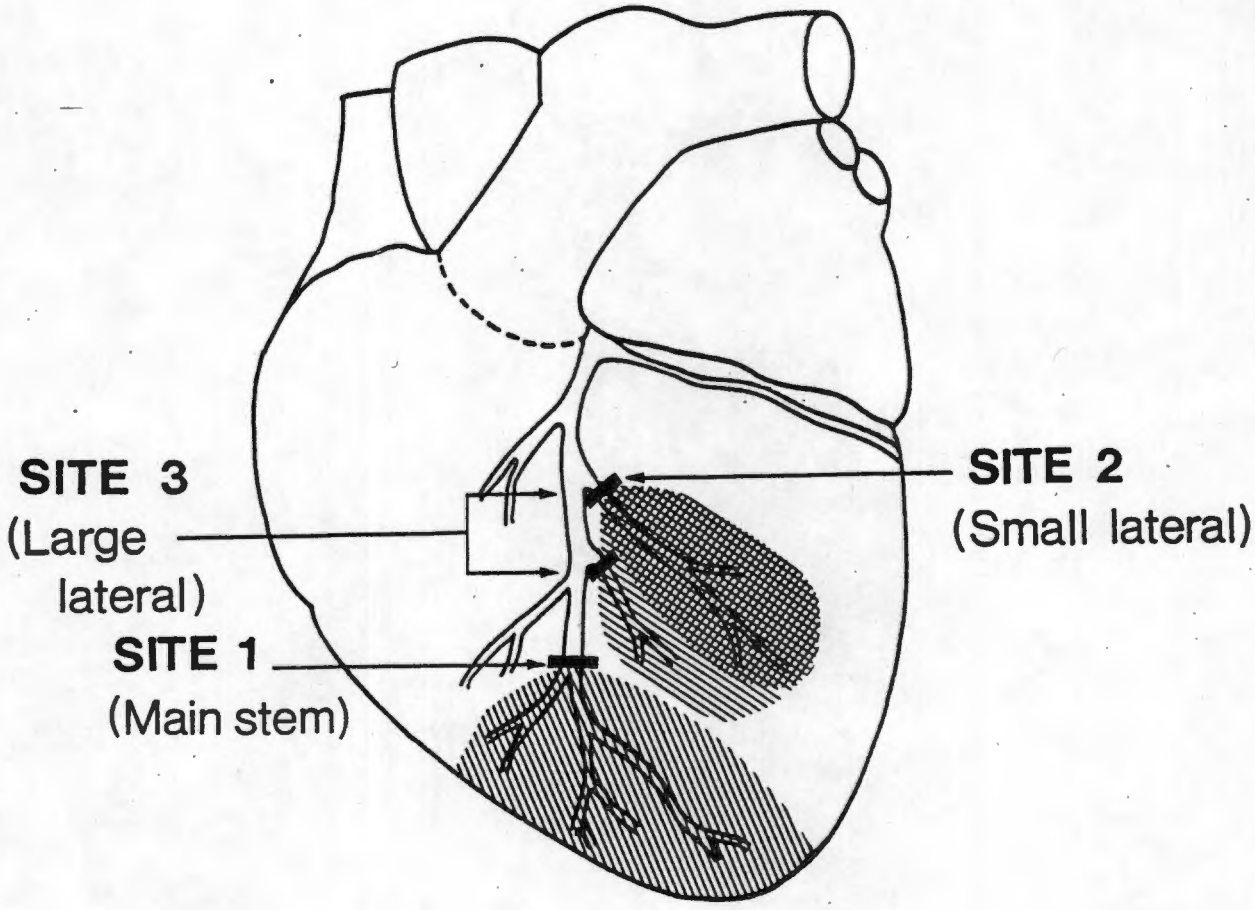


FIGURE 2.1

Different sites for ligation of the main left anterior descending coronary artery

delay of 30 seconds, suggested for preserving labile compounds in heart muscle (Allison *et al* 1978).

Using this drill biopsy technique, Podzuweit *et al* (1978) found basal values of cyclic AMP in dog ventricular muscle to be about 1,2 nanomoles/gram. When ventricular muscle samples were removed with pre-cooled tongs by Krause and Wollenberger (1980) values were as low as 0,43 to 0,73 nanomoles per gram. Because the difference in values of cyclic AMP found by the two groups have been questioned in the literature, we compared the outcome using both techniques in the pig. However, we found no difference in values of cyclic AMP, adenosine triphosphate, phosphocreatine and lactate in the 16 biopsies using either technique.

VII. DELINEATION OF THE ISCHAEMIC ZONE

At the end of the experiment, the zone of ischaemia of the intact functioning heart was delineated with the aid of a vital dye: Sulphan Blue 3 ml (B.P.C. 1954, Imperial Chemical Ind. Ltd., Macclesfield, Cheshire, U.K.) was mixed with saline (0,9%) 2 ml. The dye was rapidly injected into the left atrium. Vital tissue was stained dark blue. This cut-off line coincided with the line of visible decoloration and dyskinesis. The moment a sharp contrast was obtained, the heart was quickly excised and arrested in ice-cold sucrose 5% solution. The left ventricle was isolated and the ischaemic zone or "ischaemic myocardium at risk of infarction", (Darsee *et al* 1981) was dissected.

VIII. ASSESSMENT OF ARRHYTHMIAS

An Elema Mingograph-34 was used to record limb leads 1, and 2 or 3 electrocardiograms as well as arterial pressure at regular intervals

before, and continuously for 90 minutes after ligation, at a paper speed of 25 mm per sec. Arrhythmias were defined according to Katz (1977).

1. Ventricular premature systole: A widened QRS complex, bizarre in contour, not preceded by a P-wave and followed by a compensatory pause.
2. R-on-T: A ventricular premature systole occurring on the T-wave of the previous cycle.
3. Ventricular tachycardia: At least four consecutive uniform or multiform ventricular premature systoles. Runs to be interrupted by at least 6 normally conducted sinus beats.
4. Ventricular fibrillation: Chaotic, rapid undulations accompanied by an abrupt and complete disruption of arterial pressure. Runs to be interrupted by at least 6 normally conducted sinus beats.

Epicardial direct current shock (10 watt) was applied within 3 seconds after the onset of VF and repeated if necessary. If defibrillation did not occur within 90 seconds, this arrhythmia was accepted as fatal and the experiment was then terminated.

At the commencement of the study, an arbitrary scoring system was designed to enable us to present arrhythmias other than VF graphically.

Arrhythmias occurring during every 5 minute period for 90 minutes following ligation, were scored as follows:

Ventricular premature systoles	:	1 for every 5
R - on - T	:	2 for each one
Ventricular tachycardia	:	5 for each run lasting for 15 seconds or a fraction thereof.

IX. MEASUREMENT OF REGIONAL LEFT VENTRICULAR BLOOD FLOW

The earliest methods for measuring coronary flow were based on heat-exchange measurements, the so-called internal calorimetry (Grayson and Mendel, 1961). Driscol *et al* (1967) used electromagnetic flow-meters. None of these techniques measured flow to a specific portion of the left ventricle. Rb^{86} (Hershgold *et al* 1969) and I^{125} antipyrine clearance methods (Mueller *et al* 1974) measured flow per unit weight of myocardium. These methods often yield unreproducible results.

A. The microsphere technique for studying regional myocardial blood flow

The method is based on the principle that a non-diffusible indicator injected into the circulation, distributes according to blood flow during its first transit (Sapirstein 1956). Flow to any organ can be calculated in two ways.

1. Cardiac output method

   The cardiac output is measured and multiplied by the proportion of microspheres found in an organ, to the total number of microspheres injected, to give the flow that organ (Buckberg *et al* 1971).

2. Reference sample method

   Blood flow, RBF (reference blood collected at usually the femoral or carotid artery - millilitres per minute) and Cr (radioactivity of the collected blood) are measured. Since the method implies that the ratio of flow and radioactivity will be the same in all organs, myocardial flow MBF can now be determined if its radioactivity Cm is measured:

$$MBF = \frac{RBF \times Cm}{Cr}$$

(Makowski *et al* 1968, Domenech *et al* 1969).

We used the latter method for the first is prone to some error.

B. Materials and Methods

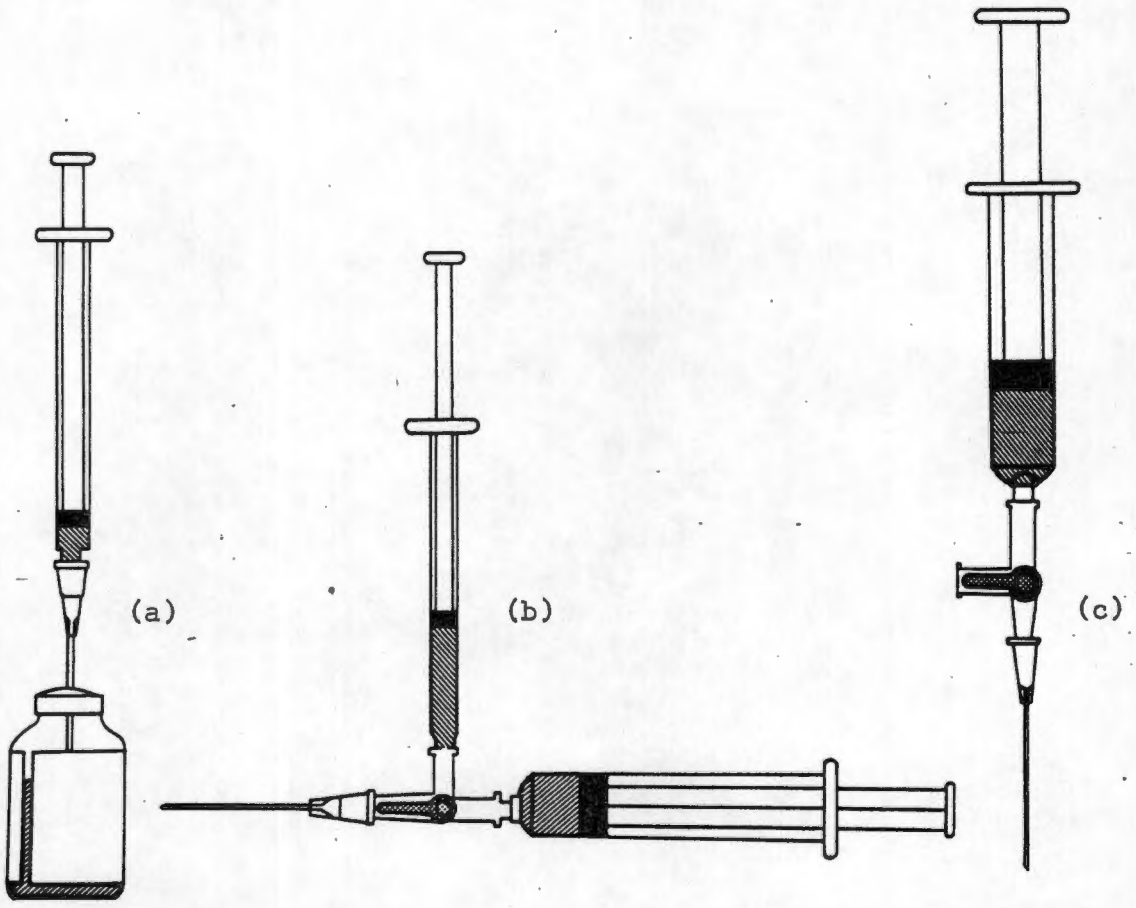
Carbonized microspheres 15 ± 5 μm (mean ± standard error of the mean) containing 1 mCi of each nuclide in 10 ml of 10% dextran were used.

An intravenous catheter needle (18G) was introduced through a purse-string suture in the left atrial wall, into the lumen. Sometimes this procedure resulted in atrial premature systoles. This could, however, always be overcome by repositioning the cannula.

For preparation of microsphere suspension, see illustration (Fig. 2.2).

A flow of about 20 drops per minute of saline 0.9% (containing 5 000 μ of heparin per 1 000 ml) to the atrium was maintained just prior to, during and for one minute after introduction of the microspheres. The spheres were administered over 10 seconds. A reference blood sample was collected at the femoral artery, starting simultaneously with the onset of administration and continued for 1,25 minutes, at a constant outflow of about 20 ml per minute. Following this collecting procedure, all microspheres were trapped. About 15 potassium hydroxide pellets were added to deproteinize the blood.

^{169}Yb labelled microspheres were introduced prior to dissection of the artery. At 20 minutes after ligation, ^{85}Sr and at 90 minutes,



(a) *0,1 ml microspheres withdrawn and then mixed with 0,9 ml saline.*

(b) *Microsphere suspension transferred to larger syringe containing more saline.*

(c) *Suspension ready for administration after thorough mixing.*

FIGURE 2.2

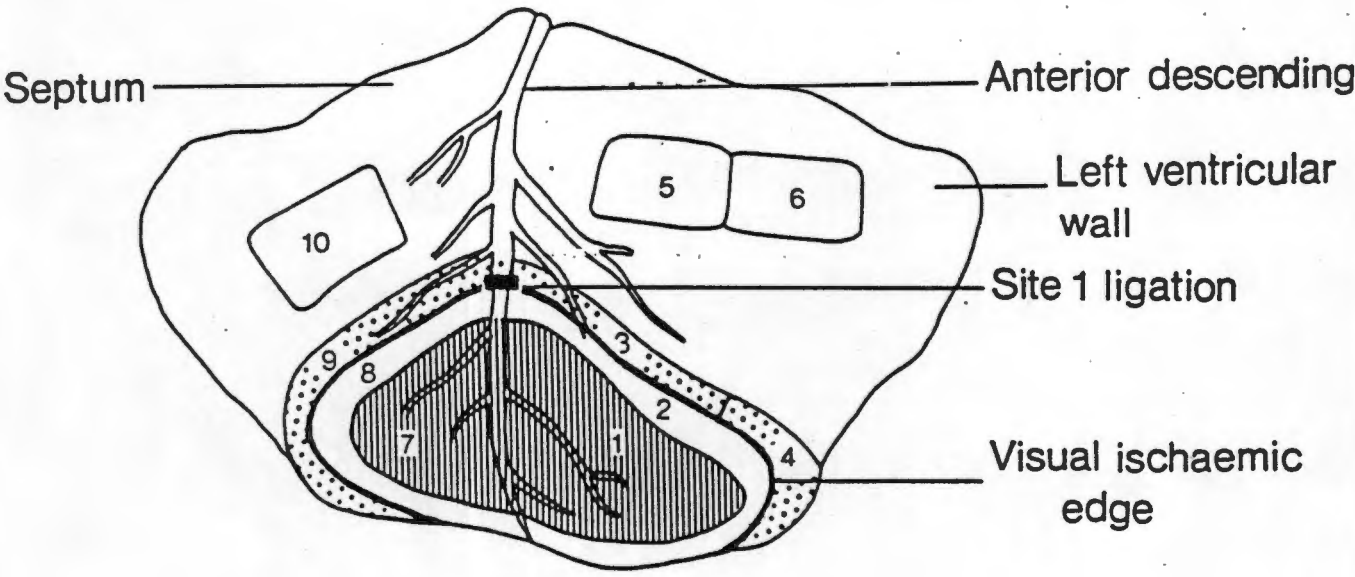
^{141}Ce microspheres were administered. If severe arrhythmias occurred during the time of administration, the results were not processed. At the end of the experiment, after introducing the vital dye (see page 19), the heart was excised and arrested in ice-cold dextrose 5% solution. The ventricle was isolated and freed of main blood vessels, papillary muscle and fat.

Samples for gamma-ray spectrometry were dissected as shown in Figure 2.3 and Figure 2.4. Each sample was divided into a subepicardial and subendocardial portion. Similarly, samples from the interventricular septum were divided into portions facing the left and right ventricle. The tissue samples were finely cut up in order to obtain random distribution of microspheres. The samples were then placed in special counting vials and the tissue weights determined.

Lubbe *et al* (1974) calibrated the variation of activity per unit weight of sample with sample size, using sample standard. It was found that samples varying with about 30% of the mean standard weight for a particular experiment, gave results within about 2.5% of the mean activity. Care was therefore taken to ensure that the weights of specimens from a single experiment were within these limits.

About 10 ml of haemolyzed blood containing either Yb, Sr or Ce was transferred to the counting vials and centrifuged at 2000 rpm for 5 minutes. The top 5 ml was then carefully removed and decanted and a new portion of 5 ml added. This was repeated until a final 5 ml containing all the spheres were left in the three counting vials.

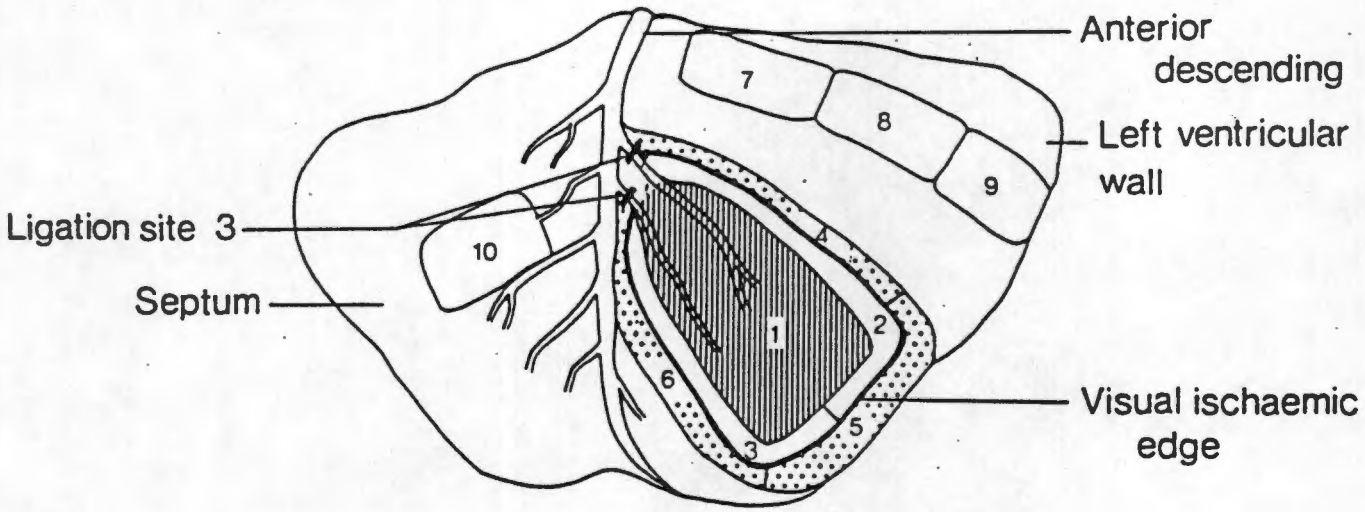
Gamma-ray spectrometry was performed within 60 days of the assay date of the microspheres. Radioactivity was assayed on a Ge-Li



Isolated left ventricle flattened out, showing tissue sampling for gamma-ray spectrometry after ligation at site 1

1,7	:	*mid-ischaemic zone*	
2,8	:	*peripheral-ischaemic zone*	*(within 6 mm of the visible edge)*
3,4,9	:	*peri-ischaemic zone*	*(within 6 mm of the visible edge)*
5,6,10	:	*non-ischaemic zone*	

FIGURE 2.3



Isolated left ventricle flattened out, showing tissue sampling for gamma-ray spectrometry after ligation at site 3

1	:	*mid-ischaemic*	
2,3	:	*peripheral-ischaemic zone*	*(within 6 mm of the visible edge)*
4,5,6	:	*peri-ischaemic zone*	*(within 5 mm of the visible edge)*
7,8,9,10	:	*non-ischaemic zone*	

After ligation at site 2 (single lateral branch) sampling was performed in a similar manner

FIGURE 2.4

detector (Princeton, Gammatec) at the Southern Universities Nuclear Institute, Faure, Cape. Gamma-ray energies up to 2 100 kev were recorded for 60 minutes per sample, stored in a 4 000 channel analyzer (Nuclear Data, ND 2400) and transferred to magnetic tape for processing on a Univac 1110 computer.

Absolute counting rates obtained in different experiments varied. The activity in different regions was, therefore, expressed as a percentage of the activity in the specific region prior to arterial dissection and ligation.

C. Critique of the method

1. Advantages

a. It permits simultaneous measurement to all regions of the heart.

b. Up to five differently labelled microspheres can be introduced into one animal without significant or consistent changes in aortic pressure or coronary flow (Domenech *et al* 1969).

c. Each animal can serve as its own control.

d. Less than 0.5% (usually under 0.1%) of total radioactivity in the heart was found in coronary venous blood (Domenech *et al* 1969). Two studies reported microsphere loss from ischaemic endocardium. However, coronary occlusion was maintained for longer than 24 hours (Capurro *et al* 1977, Jugdutt and Becker 1977).

e. Heart samples do not have to be processed immediately after administration of microspheres.

2. Disadvantages

a. Inadequate mixing prior to administration can lead to severe aggregation within the coronary bed. The introduction of our method of mixing greatly improved the distribution of microspheres. To suspend microspheres Tween-80 (polyoxyethylene sorbitan mono-oleate) 1% was added to each 10 ml. Millard *et al* (1977) reported severe cardiovascular side effects, using half the quantity (0.5%) of Tween-80. In our hands, only one incidence of VT and one of VF was induced after administration of microspheres containing 1% of Tween-80. However, when the concentration of Tween-80 was lowered to 0.2%, highly inadequate distribution of microspheres resulted in 6 out of 10 experiments.

b. The method allows the measurement of mean but not phasic flow.

c. Experiments have to be terminal to facilitate gamma-counting.

d. Preparation of samples for gamma-ray spectrometry involves much technical effort.

X. MEASUREMENT OF MECHANICAL FUNCTION OF THE LEFT VENTRICLE

For the purpose of this text, the term "mechanical function" refers to contractile activity and pressure development of the left ventricle.

A. Introduction

LV max dP/dt: This refers to the maximal rate of development of left ventricular pressure. This appears to be a reliable index of contractile activity (Wallace, 1963) and has been used widely in animal and clinical studies.

Physiological pressure measuring systems should meet three basic requirements: operational simplicity, stability and accuracy. When measuring the first derivative of ventricular pressure, the problem of damping in the recording system becomes of great importance. This can be dealt with best by using either a catheter tip transducer or to introduce a short rigid cannula connected to a transducer directly into the ventricular cavity through the wall. We found the second procedure to yield the most satisfactory results.

B. Methods

The anterior wall of the left ventricle, close to the apex was pierced, using a 14 gauge 5 cm semigrid Teflon needle (Deseret Angiocath, Deseret Pharmaceutical Co., Sandy, Utah, U.S.A.). The needle, directed towards the aortic valve, was connected directly to a Statham P23Db pressure transducer via a three-way stopcock. The transducer was secured horizontally in line with the right atrium. The catheter was frequently flushed with saline 0.9% (containing 5000 μ of heparin per 1000 ml) to prevent obstructions. The frequency-response of this system was assessed by determination of the damping ratio, as described by Wood (1950) and Marzagao (1974).

Left ventricular pressure, the first derivative of left ventricular pressure and a zero baseline were displayed continuously on the oscilloscope screen of an Electronics-for-Medicine multichannel recorder (E for M, White Plains, New York).

The transducer was calibrated against a 136 cm water column at the commencement of each experiment. Calibration of the differentiating channel was performed according to the procedure described in the manufacturer's operating manual.

Signals were recorded over at least 5 consecutive normal cardiac cycles at a paper speed of 50 and 200 mm per second, at the following times:

1. Before the administration of the last dose of anaesthetic agents before ligation and again two minutes prior to ligation, in order to establish possible changes in the parameters by these drugs.
2. At $2\frac{1}{2}$, 5, $7\frac{1}{2}$, 10, 15, 20, 30, 45, 60, 75 and 90 minutes after ligation.

XI. EPICARDIAL TQ-ST SEGMENT MAPPING OF THE LEFT VENTRICLE

We chose to measure the total TQ-ST deflection rather than the ST segment only, for this parameter was reported on in the majority of studies.

A. Recording electrodes

Two mercury-calomel electrodes, (Beckman Instruments, Inc. California, cat. 150665) with large porous glass membranes were used. One served as a reference electrode and was firmly secured onto a cotton swab on the pubic bone of the animal. A tapered cotton wick of 1 to $1\frac{1}{2}$ cm in length was attached to the second electrode. Throughout the mapping procedure, both electrodes were kept soaked in saline 0,9% solution at 37 ± 2°C, and the epicardium was repeatedly moistened with the same solution. Care was taken to keep the outside of the electrodes and the operator's hands dry, to prevent electrical interference. The electrodes were connected to the electrocardiograph via a 20 milli-volt current input. Calibration was obtained by applying a known voltage to the input, before

and after each experiment.

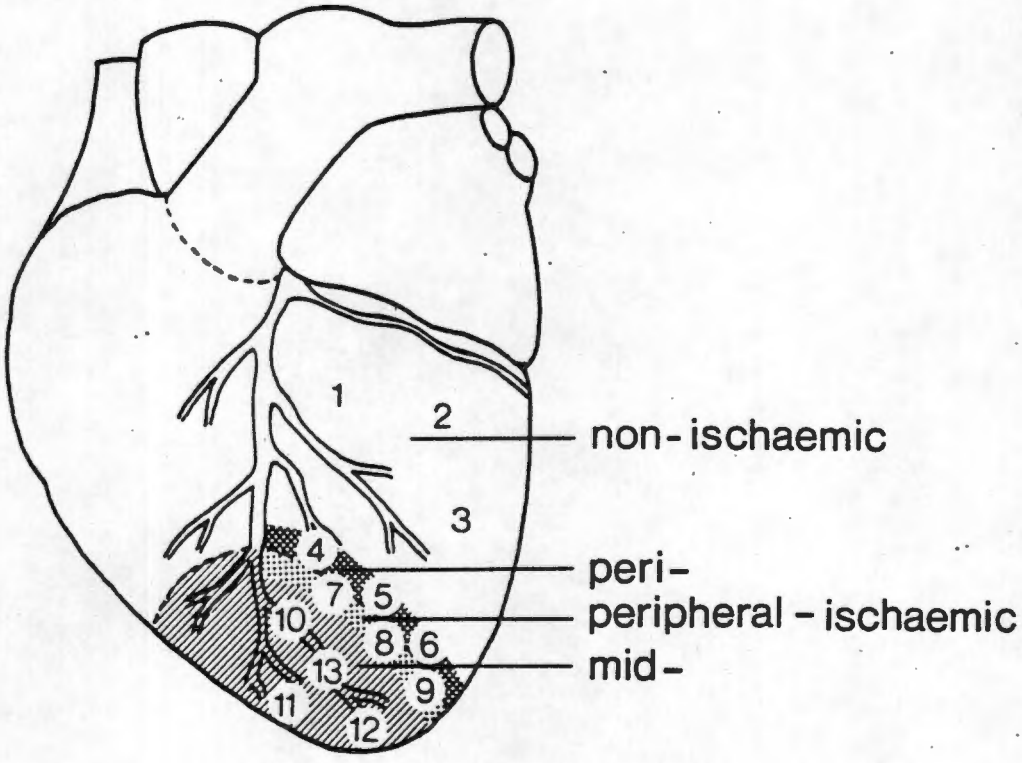
B. Myocardial topography

A map of the left anterior ventricular wall indicating superficial vasculature was constructed and 13 sites for mapping were selected according to the expected zone of ischaemia: 3 in a zone, remote from the ischaemic zone, 3 each in the peri- and peripheral zone (all sites within 5 mm of the visible border) and 4 in the mid-ischaemic zone. Figure 2.5 shows sites for mapping after main stem (site 1) ligation. After ligation of one (site 2) or more than one lateral branch (site 3) sites were selected in a similar manner. Slight shifting of border zone sites after ischaemia has set in, was sometimes necessary.

C. Mapping and measurement of the TQ-ST segment

Mapping was performed before dissection of the artery, as well as at 25, 45, 65 and 85 minutes after ligation. At least 4 normally conducted sinus beats were recorded at each site at a paper speed of 25 mm per second.

The TQ-ST deflection was measured from a point on the PQ segment immediately before the inscription of the R-wave to a point on the ST segment, 120 milliseconds later.



Sites for TQ-ST deflection mapping after ligation of the main stem of the left anterior descending coronary artery.

FIGURE 2.5

XII. TISSUE EXTRACTION AND BIOCHEMICAL ANALYSIS

The frozen biopsies weighing 15 to 20 mg were homogenized in a percussion mortar cooled in liquid nitrogen. Using an Ultra-Turax blender (Janke and Kunkel, Stanfen Brsg., West Germany) the tissue was extracted into ice-cold perchloric acid 5% w/v. The extracts were centrifuged and the supernatant neutralized and used for analysis.

A. Assay for adenosine 3',5'-cyclic monophosphate (cyclic AMP)

1. Introduction

Cyclic AMP 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 radio active compound A* and it binding to protein P, A will displace A* from the protein binding sites, in proportion to its concentration

$$P + \underset{\text{(labelled compound)}}{A^*} \longleftrightarrow P\,A^* \quad \text{(labelled complex)}$$

$$P + A \longleftrightarrow P\,A \quad \text{(unlabelled complex)}$$

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.

The assay kits contain TRIS (trishydroxymethylaminomethan)/EDTA (Ethylenedinitrilo tetra-acetic acid) buffer; purified bovine muscle protein, tritium-labelled cyclic AMP; cyclic AMP standard, charcoal adsorbent. (All in freeze-dried form).

2. Summary of the method

a. A sufficient number of microtubes (1,5 ml, Eppendorf, (2 for blanks, 4 for zero, two for each dilution of cyclic AMP standard and 2 for each unknown tissue sample) are placed into racks kept at 0°C in an ice-water bath.

b. Two tubes serve for determination of blank counts per minute due to background interference. 150 µl of buffer solution is pipetted into each.

c. Four tubes serve for determination of binding in the absence of cyclic AMP i.e., zero dose. 50 µl of buffer is pipetted into each.

d. Dilutions for constructing the standard curve is made to contain 4, 3, 2, 1.5, 1, 0.75, 0.5, 0.25 and 0.125 picomoles per 50 µl respectively. 50 µl of each of these dilutions is then pipetted into duplicate tubes.

e. 50 µl of each tissue extract to be analysed is pipetted into duplicate tubes.

f. 50 µl of labelled cyclic AMP is added to all assay tubes.

g. 100 µl of binding protein is added to all tubes except for the two tubes for determination of blank counts. For a summary of steps (b) to (g) see Table 2.1.

TABLE 2.1 SUMMARY OF STEPS (b) to (g).

	Buffer reagent	Dilution of standard solution	Tissue sample	Radio active cAMP	Binding protein
Charcoal blanks (2 tubes)	150	-	-	50	-
Zero dose (4 tubes)	50	-	-	50	100
0.125 to 4 picomole standards (2 tubes of each)	-	50	-	50	100
Unknown tissue samples (2 tubes of each	-	-	50	50	100

All volumes in microlitres

h. All tubes are vortex mixed for about 5 seconds.

i. The ice baths, containing the tubes are then incubated in a refrigerator at 2-4^{o}C for 18 hours.

j. At least 45 minutes before the end of the incubation period, the charcoal solution is placed into an ice bath and continuously stirred magnetically, for the remainder of the assay.

k. The assay tubes are then removed from the refrigerator and the ice is replenished if necessary.

l. 100 µl of charcoal suspension is added to each of a number of tubes that could be centrifuged simultaneously. The tubes are shaken briefly and centrifugation is started 3½ minutes after addition of charcoal to the last assay tube. Tubes are centrifuged at 3000 rpm for 2 minutes.

m. The tubes are immediately placed in the ice-water bath. 200 µl of the supernatant is removed without disturbing the charcoal pellet and transferred to 10 ml scintillant (Ready-Solv, Beckman) for beta counting.

n. Each tube is counted twice, each time for a period of at least 5 minutes in a Beckman liquid scintillation counter, model L660.

o. Counting efficiency is checked by adding 50 µl tritiated cyclic AMP to 10 ml scintillant, mixed with 1 ml water. The actual total activity is obtained by multiplying the counts for 50 µl by two-thirds. From this percentage bound at zero dose could also be determined.

3. Calculations

a. The average blank counts are subtracted from all other counts to correct for background interference.

b. Zero dose counts per minute (in the absence of unlabelled cyclic AMP) 'Co' is calculated by averaging counts per minute for the four 'zero' tubes.

c. Counts per minute (in the presence of standard or tissue sample unlabelled cyclic AMP) 'Cx': Counts per minute for each pair of standard dilutions as well as for each pair of tissue sample tubes are averaged.

d. The ratio $^{Co}/Cx$ for each standard dilution of cyclic AMP and for each unknown is now calculated.

e. A linear relationship is obtained when $^{Co}/Cx$ is plotted versus known concentrations of cyclic AMP. (Figure 2.6).

f. From $^{Co}/Cx$ calculated for each tissue sample, the concentration of cyclic AMP in picomole can be read off the graph. All calculations and plotting were done on a Tektronix-31 calculator system.

g. All tissue samples are assayed at least twice in duplicate. Corrections are then made for fresh tissue content and dilutions during the extracting procedure. A difference, not exceeding about 15% between final values of cyclic AMP (nanomole per gram fresh weight) is allowed for any one tissue sample.

4. Acquirement of maximal precision

This was obtained when:

a. Tissue samples were diluted if necessary, to contain levels of cyclic AMP within the range 0.5 to 4 picomoles per sample. This procedure yields a high scintillation counting rate and minimizes the possibility of non-specific interference.

b. The incubation period was controlled at 18 ± 1 hour.

c. The scintillation counting time per sample was extended to at least 10 minutes. (5 minutes twice.)

5. Special precautions

a. Non-reproducible pipetting appears to be the most important single factor leading to poor agreement between replicates. Reproducibility

of automatic micropipettes is checked regularly. Disposable pipette tips are used once only.

b. After reconstitution of the freeze-dried binding protein, the solution is subjected to one freeze-thaw cycle only.

c. The charcoal pellet forms more satisfactory when the temperature during centrifugation is maintained at maximally $4^{\circ}C$.

d. Chemiluminescence is minimized by placing vials into the scintillation counter, half-an-hour before counting is commenced.

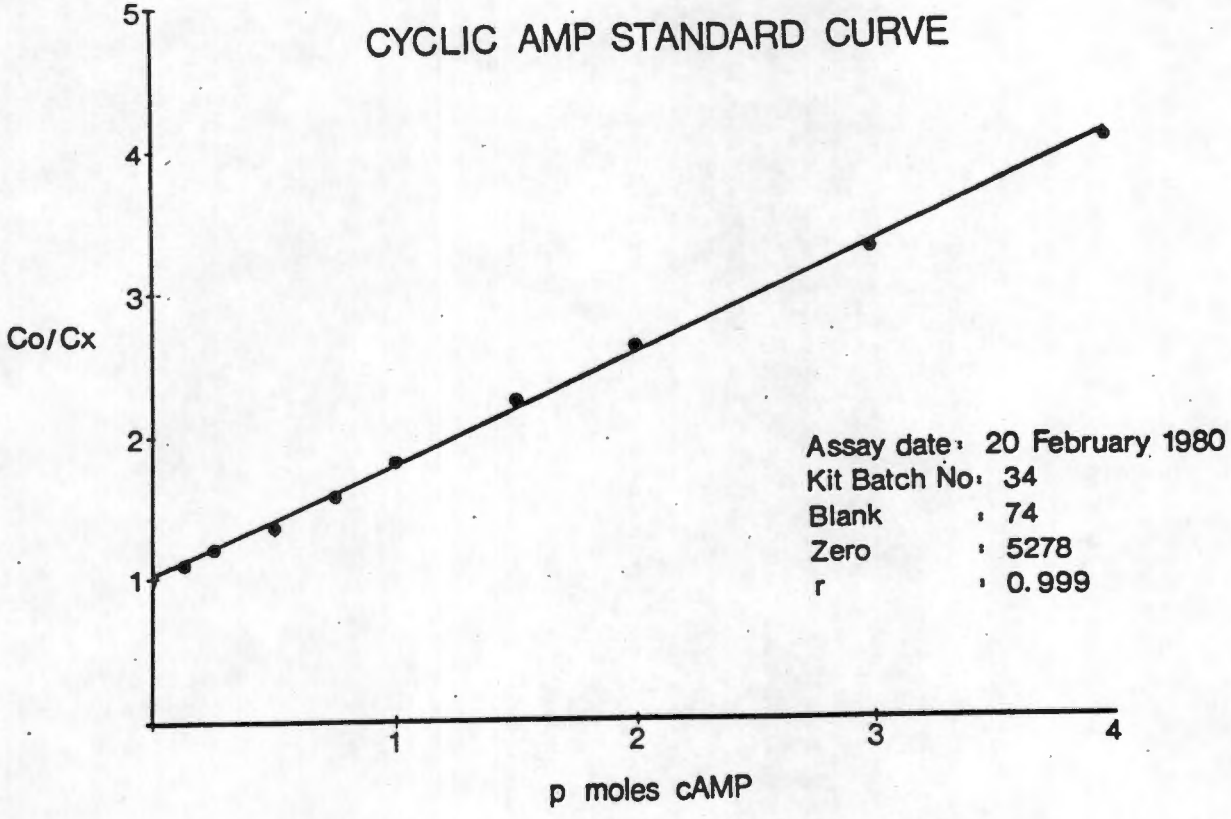

CYCLIC AMP STANDARD CURVE
Co/Cx
5
4
3
2
1
1
2
3
4
p moles cAMP
Assay date : 20 February 1980
Kit Batch No : 34
Blank : 74
Zero : 5278
r : 0.999

FIGURE 2.6

B. Assay for adenosine triphosphate (ATP) phosphocreatine (PCr) and lactate

All three compounds were assayed enzymatically by spectrophotometric methods. Standards, internal standards and blanks were included in every assay.

Adenosine triphosphate (Lampbrecht and Trautschold, 1974.)

The assay is based on the following reaction:

$$ATP + glucose \xrightleftharpoons{hexokinase} ADP + G6P$$

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

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 (pH7,5)	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	5.00 µl

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

Phosphocreatine (Lampbrecht and Trautschold, 1974)

The assay is based on the following reaction:

$$PCr + ADP \xrightleftharpoons[\text{phosphate kinase}]{\text{creatine}} ATP + \text{creatine}$$

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

phosphate kinase (10 mg/ml) is noted after at least 20 minutes were allowed for this reaction to take place. Creatine phosphate kinase is prepared fresh daily.

Lactate (Gutmann and Wahlefeld 1974)

The following reaction forms the basis of the assay:

$$\text{L - (+) - lactate} + \text{NAD} \xrightleftharpoons{\text{LDH}} \text{pyruvate} + \text{NADH} + \text{H}^+$$

5 µl LDH (10 mg/ml) is added to the following reaction mixture and the change in extinction brought about at 340 nm is read:

Buffer (hydrazine - glycine - EDTA - NaOH) pH 9.5	1.5 ml
NAD 1%	0.2 ml
H_2O dist.	1.1 ml
Sample	0.2 ml

XIII. EXPRESSION OF RESULTS

All tissue values are expressed in units per gram fresh weight and as means ± standard error of the mean.

XIV. STATISTICAL PROCEDURES

The following tests were used where appropriate:

Wilcoxon signed rank test; paired or unpaired Student's t test; Fisher's exact test (Feinstein 1977). Applying two tailed tests with allowance for unequal variances, P values lower than 0.05 were accepted to indicate significance.

CHAPTER 3

METABOLIC CHANGES AND VENTRICULAR ARRHYTHMIAS DURING 90 MINUTES FOLLOWING CORONARY ARTERY LIGATION: THE SIGNIFICANCE OF SIZE VERSUS SITE OF THE ISCHAEMIC LESION

I. AIM OF STUDY

Experiments in this chapter were undertaken to:

A. 1. - characterize arrhythmias occurring during the development of myocardial infarction in the pig.

2. - study changes in tissue levels of cyclic AMP, phosphocreatine, ATP and lactate in the ischaemic and non-ischaemic zones during this period.

3. - search for a possible correlation between periods of peak rhythm disturbances and fluctuations in tissue levels of these metabolites.

B. 1. - by varying the size and site of ischaemic lesions, establish the relative importance of these parameters on the incidence of ventricular arrhythmias and metabolic changes.

2. - determine any detrimental effects of site versus size of the ischaemic lesion on mechanical function of the left ventricle.

II. RESULTS

A. Metabolic changes and arrhythmias after ligation of the main stem of the left anterior descending coronary artery.

Ligation of the distal one-third of this artery resulted in a clearly demarcated zone of ischaemia. The ischaemic lesion in the left ventricle comprised 15,3 ± 0,6% of the total left ventricle mass, of which about 7,6% refers to the lesion in the left ventricular free wall and 7,6% to the lesion in the interventricular septum. The lesion in the right ventricle comprised 8,8% ± 0,4% of total right ventricular mass.

1. Ventricular arrhythmias during the first 90 minutes of anteroseptal ischaemia.

a. Time-course of ventricular premature systoles, ventricular tachycardia and ventricular fibrillation

In 15 out of 16 pigs, VPS began to occur 3 minutes after ligation and were multiform in contour. About 10 minutes later, VPS usually increased in frequency. Between about 35 and 65 minutes, only isolated VPS occurred. From about 65 to 90 minutes, VPS once more occurred at a higher frequency. However, the incidence during this period was lower than between 3 and 35 minutes.

Ventricular tachycardia occurred in 12 out of 16 animals, between about 10 to 30 minutes. After 30 minutes of ligation, VT never occurred.

Ventricular fibrillation was registered in 13 out of 16 pigs.

All episodes occurred between about 10 and 30 minutes, with mean time of onset at 23,9 ± 3,6 minutes after ligation. On four occasions, defibrillation could not be accomplished within 90 seconds. This arrhythmia was then accepted as fatal and the experiments were terminated.

b. Patterns of arrhythmias predisposing to ventricular fibrillation

In one pig, VF followed immediately after an isolated R-on-T beat. In eight pigs, VT developed into VF. In four pigs, VF was detached from VPS and runs of VT.

(See table 3.1 (top panel) and Figure 3.1 for a graphical presentation of arrhythmias occurring in 9 of these 16 pigs.)

2. Changes in tissue levels of cyclic AMP, ATP phosphocreatine and lactate during the first 90 minutes of anteroseptal ischaemia

Biochemical changes in the ischaemic and non-ischaemic left ventricle over the first 10 minutes were studied in a group of 10 pigs. Changes between 10 and 90 minutes were studied in another 9 pigs. This procedure was necessary due to the limited size of the ischaemic lesion in the left ventricular free wall, available for biopsy taking.

a. Tissue levels of cyclic AMP in ischaemic and non-ischaemic zones of the left ventricle. (Figures 3.1 and 3.2).

Between 0 and 5 minutes after ligation, tissue cyclic AMP in ischaemic and non-ischaemic zones was similar and did not differ from the pre-ligation control value of 0,94 ± 0,03 nmol/gram. At 5 minutes, cyclic AMP started to accumulate in ischaemic tissue,

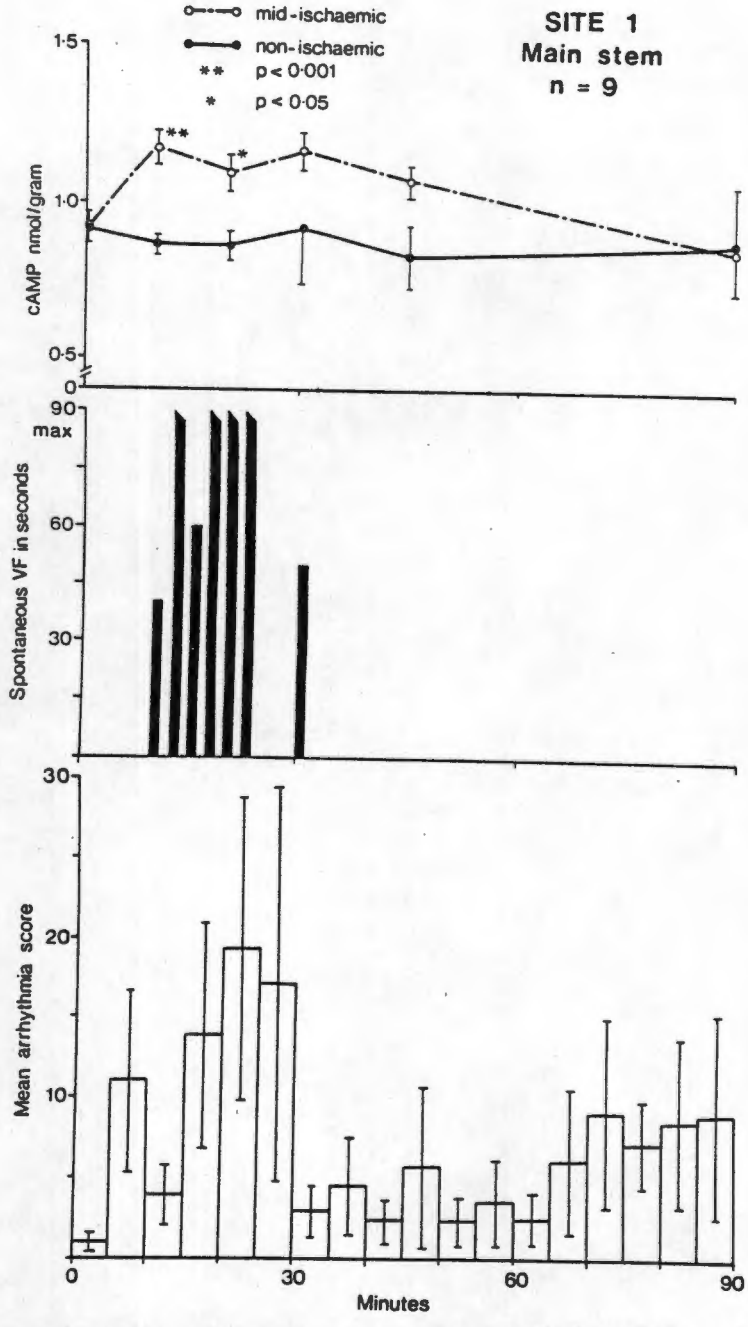
TABLE 3.1 ARRHYTHMIAS DURING 90 MINUTES FOLLOWING CORONARY ARTERY LIGATION

Site and size of ischaemic lesion	Pig No.	Ventricular Premature Systoles late	Ventricular Premature Systoles early (R-on-T)	Ventricular tachycardia (seconds)	Ventricular fibrillation (seconds)
	1	0	0	3	90 (max)$^{\Delta}$
	2	162	2	0	90 (max)
	3	16	2	0	0
	4	23	4	0	90 (max)
	5	128	6	30	0
	6	299	25	90	60
Site 1	7	101	0	60	90 (max)
(main stem)	8	688	0	65	42
15.3%±0.6%	9	246	13	106	50
	10	14	0	191	44
	11	279	0	513	82
(percentage	12	124	0	0	6
of left ventricular	13	235	0	121	0
mass)	14	108	0	153	50
	15	45	1	181	38
	16	70	0	150	9
		158.6±42.9	3.3±1.6	103.9±32.2	13 out of 16
	1	7	1	0	0
	2	2	0	0	0
	3	48	1	0	0
Site 2	4	8	0	0	0
(small	5	4	0	0	0
lateral branch)	6	171	13	90	21
6.9%±0.6%	7	3	0	0	0
	8	0	0	0	0
	9	36	0	0	0
	10	0	0	0	0
	11	36	1	0	90 (max)
	12	0	0	0	0
		26.3±14.0**	1.3±1.1	7.5±6.5**	2 out of 12†
	1	82	0	103	55
	2	57	0	26	8
	3	241	0	37	0
	4	45	0	10	10
	5	71	1	0	0
Site 3	6	137	0	66	0
(large	7	19	0	4	0
lateral branch)	8	95	0	88	90 (max)
13.4%±1.0%	9	107	0	6	0
	10	87	0	0	0
	11	231	0	3	0
	12	31	0	78	18
	13	122	1	0	0
	14	389	1	42	0
	15	214	0	0	0
	16	130	2	1	0
		128.6±24.2$^{\Delta}$	0.3±0.2	29.0±9.0*	5 out of 16††

* $p<0.05$ ** $p<0.01$ vs Site 1 $^{\Delta}$ $p < 0.005$ vs Site 2

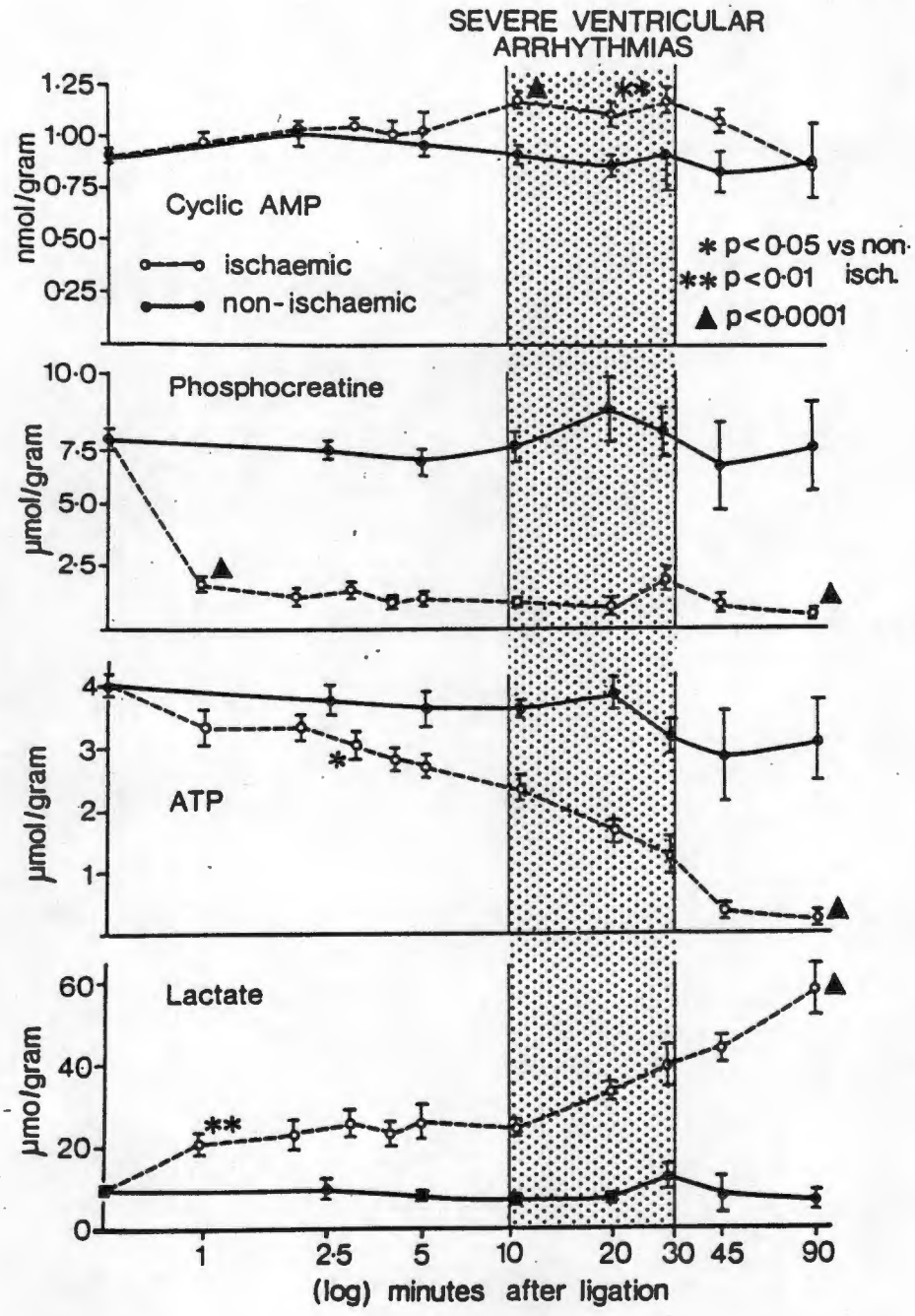
† $p<0.05$ †† $p<0.01$ vs Site 1 (Fisher's exact test, Feinstein 1977)

$^{\Delta}$ When direct current shock failed to restore normal rhythm within 90 seconds, the experiment was terminated.



Tissue levels of cyclic AMP, time of occurrence and duration of VF and scores for VPS and VT (page 20) during 90 minutes following ligation at site 1. Note the marked elevation of cyclic AMP in ischaemic tissue at the time of peak rhythm disturbances. Six out of 9 animals had at least one episode of VF between about 10 and 30 minutes. Four of these lasted for 90 seconds and were accepted as fatal. The experiments were therefore terminated (page 20). Peak scores for other arrhythmias were calculated between 5 and 30 minutes, with a second increase towards the end of the experiment. (For an extensive analysis of arrhythmias, in a larger group (16 pigs), see Table 3.1)

FIGURE 3.1



Within 1 minute of ligation, phosphocreatine abruptly decreased, while lactate accumulated in ischaemic tissue. Tissue levels of ATP in the ischaemic zone started to decrease at 3 minutes after ligation. During the period of severe arrhythmias, no fluctuations occurred in tissue levels of these metabolytes. However, a transient accumulation of cyclic AMP was confined to this period.

Phosphocreatine: All values between 1 and 90 min, $p < 0,0001$ (ischaemic vs non-ischaemic).

ATP: All values between 3 and 90 min, $p < 0,05$ to $p < 0,0001$.

Lactate: All values between 1 and 90 min, $p < 0,01$ to $p < 0,0001$.

FIGURE 3.2

reaching a peak of 1,17 ± 0,04 vs 0,91 ± 0,04 in the non-ischaemic zone ($p < 0,0001$, $n = 14$). At 30 minutes, tissue levels of cyclic AMP in the ischaemic zone started to decline, reaching pre-ligation levels at 90 minutes. Thus tissue levels of cyclic AMP in the ischaemic zone were elevated for at least 10 minutes, between 10 and 30 minutes after ligation, when compared to tissue levels in the non-ischaemic zone.

b. Tissue levels of ATP in ischaemic and non-ischaemic zones (Figure 3.2)

At 1 and 2 minutes after ligation, tissue levels of ATP in the ischaemic zone did not differ from the pre-ligation control value of 4,00 ± 0,17 µmol/gram. By 3 minutes, levels of ATP in ischaemic tissue declined to 3,05 ± 0,21 vs 3,76 ± 0,24 µmol/gram in the non-ischaemic zone ($p < 0,05$, $n = 10$). The preservation of ATP for 2 minutes after ligation was probably due to a phosphate transfer from phosphocreatine. While tissue levels of ATP in the non-ischaemic zone were maintained at pre-ligation values for the duration of the experiment, a further progressive decline occurred in the ischaemic zone, reaching a level of 1,23 ± 0,29 µmol/gram at 90 minutes ($n = 6$). Minimum tissue levels of ATP in the ischaemic zone were evident between 45 and 90 minutes.

c. Tissue levels of phosphocreatine in ischaemic and non-ischaemic zones (Figure 3.2).

At the time of the first biopsy, i.e. one minute after the onset of ischaemia, levels of phosphocreatine in ischaemic tissue had fallen from 7,46 ± 0,43 to 1,73 ± 0,24 µmol/gram ($p < 0,0001$, $n = 11$). This low level remained unchanged for the duration of the

experiment. Tissue levels of phosphocreatine in the non-ischaemic zone remained stable and were similar to the pre-ligation control value.

d. Tissue levels of lactate in ischaemic and non-ischaemic zones (Figure 3.2).

At the time of the first biopsy, i.e. 1 minute, lactate had already accumulated in ischaemic tissue, indicating the very early onset of anaerobiosis. Between one and 90 minutes, tissue levels of lactate in the ischaemic zone progressively increased from 20,83 ± 2,66 ($p < 0,01$ vs non-ischaemic value) to 58,07 ± 6,50 µmol/gram ($p < 0,0001$ vs non-ischaemic value, $n = 8$).

3. Changes in tissue levels of cyclic AMP, ATP, phosphocreatine, and lactate in relation to the incidence of ventricular arrhythmias after coronary ligation

In this study, a prime phase of ventricular arrhythmias was evident between 10 and 30 minutes after coronary ligation. A transient accumulation of tissue cyclic AMP in the ischaemic zone of the left ventricle was confined to this period. Although we could not show direct links between accumulated tissue cyclic AMP and arrhythmogenesis, these results do infer a strong relation.

On the other hand, distinct changes in tissue levels of ATP, phosphocreatine and lactate after ligation could be dissociated with the period of severe ventricular arrhythmias, for maximum or minimum levels did not occur during this phase. However, the importance of a progressive decrease in ischaemic tissue levels of ATP and an increase in levels

of lactate in arrhythmogenesis, cannot be excluded.

B. The significance of size and site of ischaemic lesion on metabolic changes and the incidence of ventricular arrhythmias

Ligation of the main stem of the left anterior descending coronary artery resulted in an ischaemic lesion, including a substantial portion of the left ventricular free wall, as well as the inter-ventricular septum. We now established the importance of (a) the site of the ischaemic lesion, by ligating two to four adjacent lateral branch of the anterior descending artery instead of the main stem, to yield ischaemic lesions of a similar size confined to the left ventricular free wall, excluding involvement of the interventricular septum and apical regions. (b) The size of an ischaemic lesion, by ligating a single lateral branch only.

1. Ventricular arrhythmias associated with large and small ischaemic lesions, confined to the left ventricular free wall

a. Ligation at site 3 (two to four lateral branches) (Table 3.1)

Ischaemic lesions in this group comprised 13,4 ± 1,0% of total left ventricular mass and therefore matched the size of the lesions after main stem ligation.

The time course of arrhythmias in this group was similar to those after main stem ligation. These pigs also showed a similar incidence of VPS. However, the overall duration of VT was shorter (29,0 ± 9,0, site 3 versus 103,9 ± 32,2, site 1, $p < 0,05$). Marked differences were also evident in the incidence of VF. Only 5 out of 16 animals developed VF, while this arrhythmia occurred in 13

out of 16 pigs with main stem ligation ($p < 0,01$). See Figure 3.3 for a graphical presentation of arrhythmias that occurred in 9 of these 16 pigs.

b. Ligation at site 2 (single lateral branch) (Table 3.1)

Ischaemic lesions in this group comprised 6,9 ± 0,6% of total left ventricular mass ($p < 0,005$ vs 13,4 ± 1,0% of large lateral lesions).

The time course of arrhythmias in this group was similar to those in pigs with a large lateral lesion. Although the incidence of VPS associated with the smaller anterolateral lesion was reduced, the overall duration of VT, as well as the incidence of VF were similar. See Figure 3.4 for a graphical presentation of arrhythmias occurring in 7 of these 12 pigs.

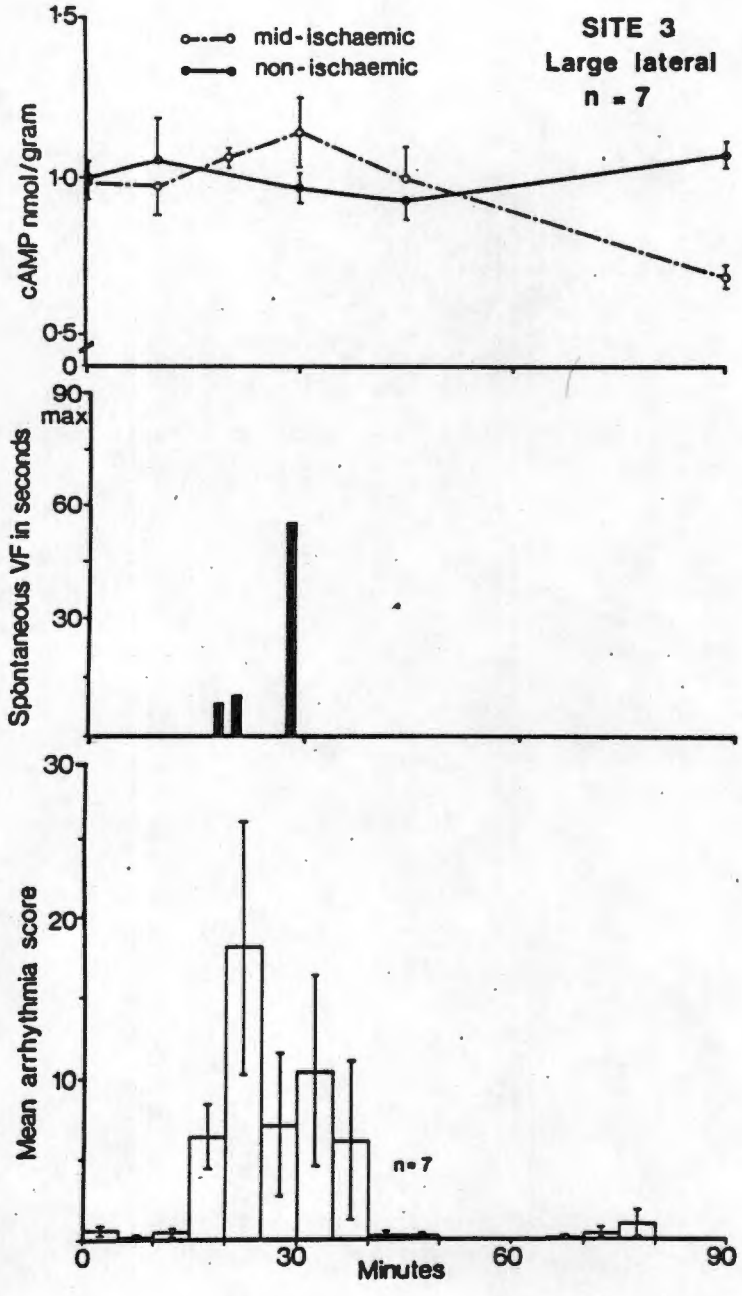
Thus, anteroseptal ischaemia was associated with a higher incidence of VT and VF than anterolateral lesions of a similar size. Smaller anteriolateral lesions were not associated with a lower incidence of VT and VF than large anterolateral lesions.

These results suggest that site of an ischaemic lesion rather than size may determine the incidence of severe ventricular arrhythmias.

2. Metabolic changes associated with large and small lesions in the left ventricular free wall

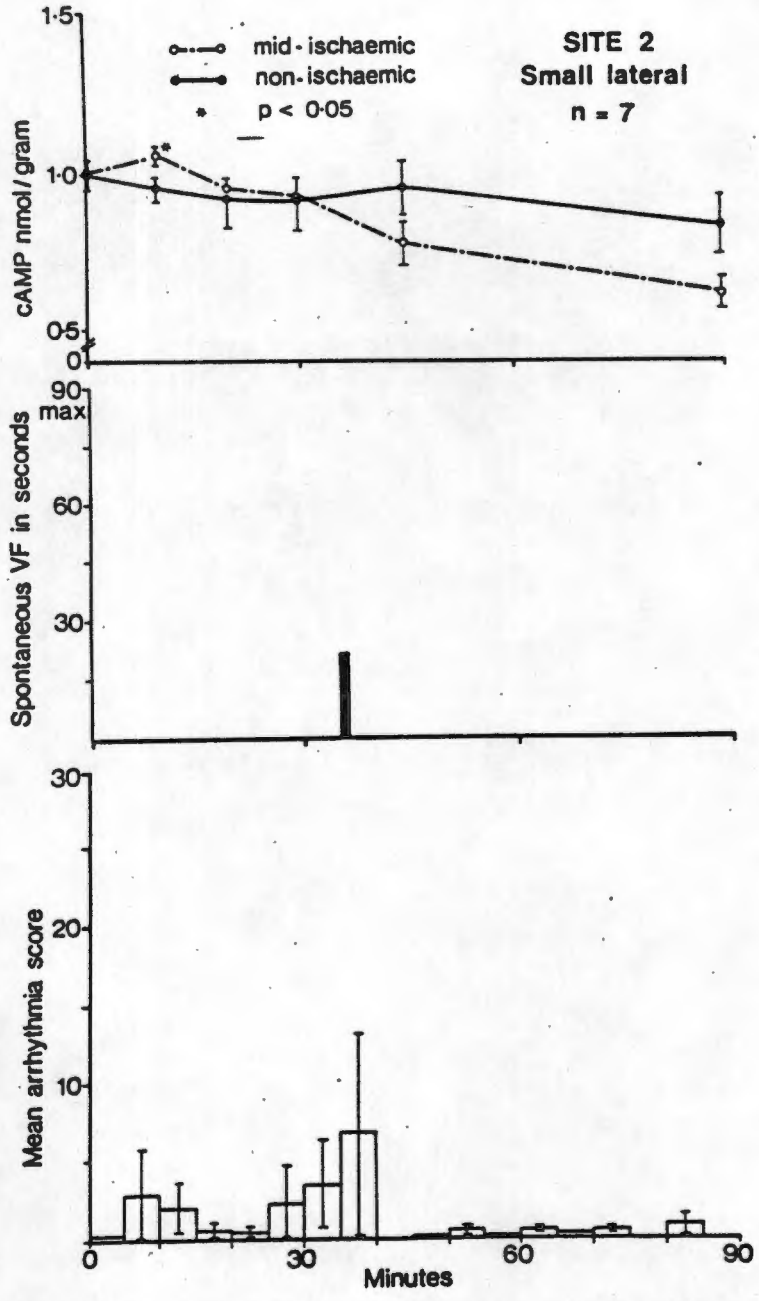
a. ATP, phosphocreatine and lactate

In table 3.2, changes in tissue levels of these metabolites in large and small anterolateral lesions are compared to levels in



Tissue levels of cyclic AMP, time of occurrence and duration of VF and scores for VPS and VT during 90 minutes after ligation at site 3. No elevation of cyclic AMP occurred in ischaemic tissue (compare Figure 3.1 and 3.2). Brief incidences of VF were observed in 3 out of the 7 pigs between 15 and 30 minutes). (For an extensive analysis of arrhythmias in a larger group of 16 pigs, see Table 3.1).

FIGURE 3.3



Tissue levels of cyclic AMP, time of occurrence and duration of VF, and scores for VPS and VT (page 20) during 90 minutes following ligation at site 2. In contrast with figure 3.1, a transient and less prominent elevation of cyclic AMP occurred in ischaemic tissue at about 10 minutes after ligation. Only one animal had a brief episode of VF lasting for 22 seconds between 35 and 40 minutes. Scores for other arrhythmias were also greatly reduced. (For an extensive analysis of arrhythmias in a larger group of 12 pigs, see Table 3.1).

FIGURE 3.4

anteroseptal lesions. Tissue levels of phosphocreatine and ATP were largely similar in all three groups. Tissue levels of lactate in small anterolateral lesions were lower than in the other two groups.

These results provide further evidence for a dissociation between changes in tissue levels of phosphocreatine, ATP and lactate, and the incidence of severe ventricular arrhythmias.

b. Cyclic AMP (Figures 3.3 and 3.4)

A transient increase in tissue cyclic AMP in small anterolateral lesions was evident at 10 minutes after ligation only ($p < 0,05$ versus non-ischaemic tissue value). After ligation of more than one anterolateral branch, cyclic AMP tended to accumulate in ischaemic tissue at 30 minutes. However, tissue levels in the ischaemic and non-ischaemic zones were not significantly different.

c. Tissue levels of cyclic AMP in animals with ventricular fibrillation versus levels in animals with no episodes of fibrillation (Figure 3.5).

At the time of a temporary, however non-significant, elevation of tissue levels of cyclic AMP in large anterolateral lesions, 3 of the seven pigs encountered episodes of VF. These results prompted us to re-group all pigs according to the occurrence of VF. Twenty-three pigs were included in this study (site 1, 9: site 2, 7 and site 3, 7 pigs). For each pig, levels of cyclic AMP in ischaemic tissue for 90 minutes after ligation were compared to non-ischaemic tissue levels, by measuring trapezoidal areas under the two curves. A percentage difference was then calculated for each pig.

TABLE 3.2

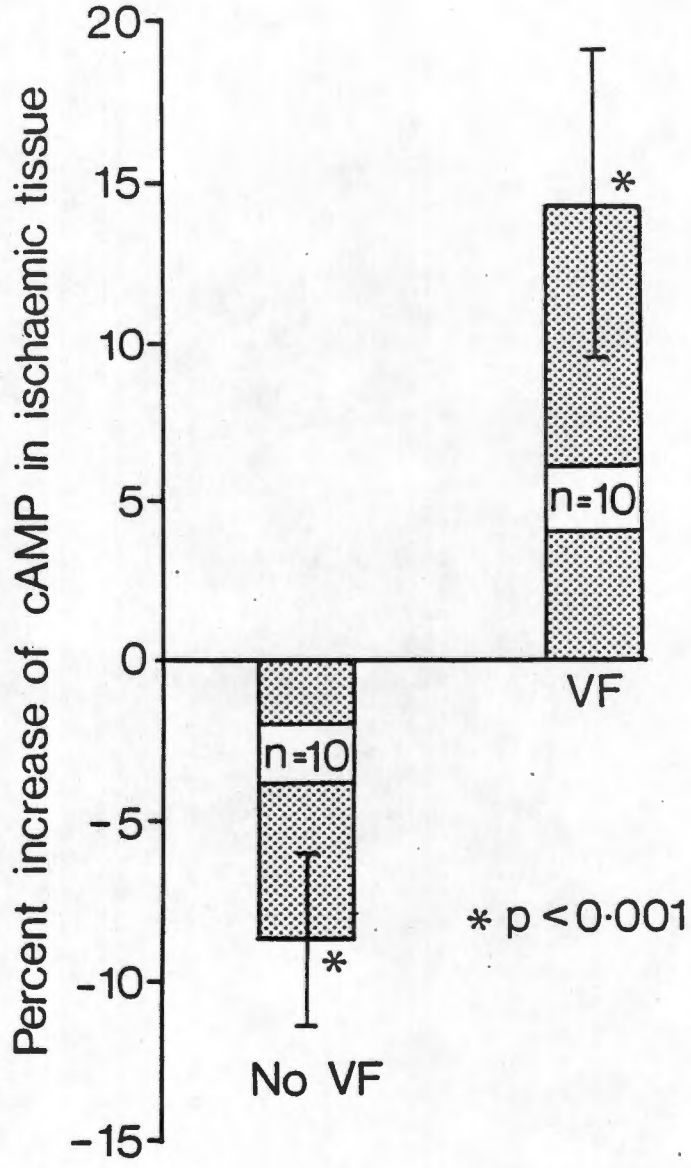
LEVELS OF ADENOSINE TRIPHOSPHATE (ATP), PHOSPHOCREATINE (PCr) AND LACTATE BEFORE LIGATION AND IN ISCHAEMIC TISSUE AFTER LIGATION

(μmol per gram)

		BEFORE LIGATION	MINUTES AFTER LIGATION			
			10	20	45	90
ATP	Site 1 (n=9)	3,72 ±0,31	2,33 ±0,20	1,66 ±0,15	0,39 ±0,08	0,25 ±0,11
	Site 2 (n=7)	3,27 ±0,33	1,92 ±0,25	1,39 ±0,17	0,72 ±0,38	0,62 ±0,32
	Site 3 (n=7)	3,83 ±0,36	2,64 ±0,35	1,63 ±0,29	0,47 ±0,06	0,50 ±0,16
PCr	Site 1	9,69 ±0,49	1,00 ±0,19	0,83 ±0,31	0,94 ±0,32	0,55 ±0,21
	Site 2	8,54 ±0,30	0,88 ±0,31	0,95 ±0,68	1,22 ±0,73	0,61 ±0,43
	Site 3	7,92 ±0,87	1,05 ±0,27	0,92 ±0,47	0,76 ±0,22	0,88 ±0,37
Lactate	Site 1	6,26 ±1,60	41,32* ±4,97	40,01 ±5,35	45,37 ±5,15	58,07* ±6,50
	Site 2	7,49 ±1,40	25,79*Δ ±1,94	34,44* ±2,39	32,45 ±9,97	30,19* ±7,47
	Site 3	9,55 ±1,39	46,52Δ ±5,03	48,38* ±4,79	53,12 ±6,97	51,02 ±7,28

* $p < 0,05$ Δ $p < 0,005$

Tissue levels of all three metabolites were largely similar, except that lactate accumulated to a lesser extent in small lateral lesions (site 2) than in large anteroseptal (site 1) and large anterolateral lesions (site 3).



In the ten pigs which developed VF, cAMP accumulated in ischaemic tissue. In contrast, in the other pigs with no episodes of VF, cAMP in ischaemic tissue was actually lower than in non-ischaemic tissue.

FIGURE 3.5

In the 10 pigs in which VF did occur, cyclic AMP in ischaemic tissue was increased by 14,3% + 4,8% compared to non-ischaemic tissue values. In the 10 pigs in which VF did not occur, cyclic AMP was actually 8,8% + 2,6% lower than non-ischaemic tissue values. In the remaining three pigs, VF was induced by taking a drill biopsy. These were excluded.

These results strongly emphasize that the accumulation of cyclic AMP in zones of ischaemia may be an important factor in the genesis of severe ventricular arrhythmias.

C. <u>The significance of site of an ischaemic lesion on mechanical function of the left ventricle</u> (Table 3.3).

Either the main stem or several anterolateral branches of this artery were ligated to determine the effect of an ischaemic lesion of a similar size, but at different sites, on mechanical function of the left ventricle.

Within 2,5 minutes of anteroseptal ischaemia (i.e. at the time of the first measurement), the maximal rate of pressure development of the left ventricle (dP/dt max) was decreased, compared to the pre-ligation control value. This low rate of pressure development was maintained up to 45 minutes after ligation. Anteroseptal ischaemia also resulted in an increased left ventricular end-diastolic pressure (LVEDP) within 5 minutes, which lasted for the duration of the experiment. Left ventricular systolic pressure (LVSP) remained unchanged.

When ischaemia was confined to the left ventricular wall, dP/dt

TABLE 3.3

EFFECT OF CORONARY LIGATION ON MECHANICAL FUNCTION OF THE LEFT VENTRICLE AND HEART RATE

MAIN STEM (SITE 1) n = 7, SIZE OF ISCHAEMIC LESION 15,6% + 0,7% (OF TOTAL LEFT VENTRICULAR MASS)

MIN. AFTER LIGATION	PRE-LIGATION	2,5	5	7,5	10	15	20	30	45	60	90
Heart rate (beats/min)	105 ±8	107 ±8	106 ±8	111 ±9	108 ±9	107 ±8	106 ±7	104 ±6	103 ±6	103 ±6	101 ±5
LVSP (mmHg)	93 ±2	92 ±2	93 ±4	95 ±2	96 ±3	92 ±2	91 ±3	93 ±3	97 ±1	96 ±2	96 ±1
LVEDP (mmHg)	3,1 ±0,6	5,3 ±0,3	6,2* ±0,7	7,5** ±0,7	6,3** ±0,6	6,4* ±0,4	6,4* ±0,4	6,3* ±0,4	5,7* ±0,6	5,9* ±0,6	5,3* ±0,5
LV dP/dt max (mmHg/sec)	2540 ±189	2338* ±165	2185* ±143	2311* ±150	2252* ±176	2222* ±118	2059** ±212	2128* ±174	2378* ±135	2389 ±159	2445 ±144

LARGE LATERAL (SITE 3) n = 8. SIZE OF ISCHAEMIC LESION 14,4% + 0,4%

Heart rate	122 ±8	122 ±8	122 ±8	121 ±8	122 ±8	117 ±7	120 ±6	120 ±6	122 ±6	119 ±6	119 ±5
LVSP	92 ±3	96 ±4	89 ±5	97 ±3	92 ±5	93 ±5	91 ±4	95 ±4	98 ±4	96 ±2	93 ±4
LVEDP	3,6 ±0,9	5,2 ±0,7	5,5 ±0,6	5,6 ±0,7	6,0 ±0,5	5,6 ±0,8	4,9 ±0,7	5,1 ±0,6	5,4 ±0,6	4,9 ±0,6	4,6 ±0,8
LV dP/dt max	2190 ±105	2069 ±144	2036 ±127	2164 ±158	2127 ±115	2166 ±132	2082 ±95	2224 ±114	2223 ±129	2262 ±120	2284 ±112

* $p < 0,05$ ** $p < 0,005$ compared to pre-ligation value

LVEDP = left ventricular end-diastolic pressure

LVSP = left ventricular systolic pressure

LV dP/dt = maximal rate of pressure development of left ventricle

max and LVSP were similar to pre-ligation values. LVEDP showed small but non-significant increases.

At the time of the first measurement after main stem ligation (i.e. $2\frac{1}{2}$ min) LVdP/dt max was already decreased. This was followed by an increase in LVEDP after 5 minutes of ischaemia. Large lateral ligation did not interfere with mechanical function of the left ventricle. In both groups, ligation did not change heart rate and LVSP.

III. DISCUSSION

This is the first study undertaken in the pig: (a) to demonstrate patterns of ventricular arrhythmias during the first 90 minutes after the onset of regional myocardial ischaemia in relation to changes in tissue levels of metabolites; (b) to investigate the importance of site and size of an ischaemic lesion on the incidence of ventricular arrhythmias and metabolic changes.

A. The character and time course of ventricular arrhythmias during developing myocardial infarction in the pig.

Ligation of the main stem of the left anterior descending coronary artery, at a point one-half of two-thirds from the origin to its apical termination resulted in ischaemia within the anterior left ventricular free wall, including the apical region, the inter-ventricular system and the right ventricle. This was followed by a distinct phase of severe ventricular arrhythmias between 10 and 30 minutes. Prior to 10 minutes, and between 30 and 90 minutes (i.e. end of our experiments) no severe arrhythmias occurred. This model offers excellent potential to study the effect of an intervention of arrhythmias associated with developing myocardial infarction.

Two studies in the dog and pig (Haase and Schiller 1969, Hirche *et al* 1980) have reported on an additional early phase of severe ventricular arrhythmias within the first 10 minutes. In the latter study in the pig, ischaemic involvement varied considerably between animals (8 to 26% of both ventricles). We suggest that the very early occurrence of VF was confined to those animals with larger ischaemic lesions. We have observed

that VF ensued shortly after higher ligation of the anterior descending, resulting in ischaemia which exceeds about 22% of both ventricles. The time course of severe ventricular arrhythmias in relation to the site of ligation of the main stem of the left anterior descending artery calls for further investigation.

When an ischaemic lesion of a similar size was confined to the left ventricular free wall excluding the apical regions, a lower incidence of VT and VF resulted. Thomas *et al* (1970) showed similar results in the dog. Durrer (1969) found the interventricular septum, anterior wall of the left ventricle and apical regions to be activated first after conduction of impulses through the atrioventricular node. Disruption of the majority of these early pathways of impulse propagation may be critical in arrhythmogenesis (Thomas *et al* 1970).

Reduction of the size of this anterolateral lesion did not result in a decreased incidence of VT and VF. These results are in contrast to the generally accepted link between size of an ischaemic lesion and the incidence of ventricular arrhythmias. (Kitchen *et al* 1977, Geltman *et al* 1979). In patients with acute myocardial infarction, the severity of ventricular arrhythmias could be related to the extent of myocardial injury, as assumed by creatine kinase curves (Roberts *et al* 1975).

Lown and co-workers (1975) introduced the concept of warning arrhythmias in patients and suggested that frequent VPS were predictors of VF. Contradictory clinical reports came from Lie *et al* 1975, and El Sherif *et al* 1976. We failed to show such a relationship in our animal model, for in 18 pigs, frequent VPS were followed by VF, but in another 22 pigs, VPS were not followed by VF.

B. Changes in tissue levels of high energy phosphates and lactate after coronary ligation, and ventricular arrhythmias

Residual tissue levels of phosphocreatine prior to ligation in the baboon and dog (Podzuweit *et al* 1977) appear to be higher than those found in the pig in our study (about 10 to 12 versus 8 µmol/gram). The abrupt fall in tissue levels of phosphocreatine in the ischaemic zone within 1 minute has also been shown in baboon and dog, as well as in the isolated perfused pig heart (Janse *et al* 1979).

Control tissue levels of ATP, as well as changes after ligation, were similar in the baboon and isolated perfused pig heart. However, ATP in ischaemic tissue in the dog was maintained at a higher level after ligation (about 3 versus less than 2 µmol/gram).

Tissue levels of lactate before ligation in our study, as well as in the isolated perfused pig heart were about 7, compared to about 2 µmol/gram in the dog and baboon. The progressive accumulation of lactate in zones of ischaemia was reported to be largely similar in all these studies.

Consistent to our results, Podzuweit *et al* (1977) failed to find an association between fluctuation in high energy phosphates and lactate, and ventricular fibrillation.

C. Substantiating evidence for a link between cyclic AMP accumulation in the myocardium and severe ventricular arrhythmias

Concomitantly with the distinct phase of severe ventricular arrhythmias between 10 and 30 minutes after ligation of the main stem of the anterior descending artery, cyclic AMP transiently accumulated in

ischaemic tissue. After ligation of a lateral branch or branches of this artery only, a lower incidence of VT and VF was recorded, while a very brief or no accumulation of cyclic AMP occurred in zones of ischaemia. Furthermore, when pigs were re-grouped according to the occurrence of VF, animals which encountered this arrhythmia showed a sharp increase of tissue cyclic AMP in zones of ischaemia, while no such accumulation occurred in pigs with no episodes of VF.

Our results are consistent to those found by Podzuweit *et al* (1977) who demonstrated an abrupt increase in tissue levels of cyclic AMP prior to the onset of VF in the baboon and dog. Similarly, Corr *et al* (1978) showed that maximal tissue levels of cyclic AMP preceded periods of peak frequency of premature ventricular complexes. However, these results suggest an indirect link only, between accumulated cyclic AMP in the myocardium, and ventricular arrhythmias. More direct evidence was provided by Lubbe *et al* (1976, 1978) and Podzuweit *et al* (1978) who demonstrated that ventricular arrhythmias could be evoked by the administration of dibutyryl cyclic AMP.

D. Magnitude of the rise in levels of cyclic AMP in ischaemic tissue

The most prominent increase in tissue levels of cyclic AMP in zones of ischaemia in our study, occurred 10 minutes after main stem ligation, but was only about 30% above control levels, prior to ligation. The phenomenon of compartmentation of cyclic AMP may be at work: above a critical tissue level, which exceeds the capacity of the heart cell to bind cyclic AMP, the free concentration within the cell increases rapidly for only a small increase in the total tissue content (Shimizu *et al* 1970, Corbin *et al* 1977, Terasaki and Brooker 1977). Drummond and Severson (1979)

suggested that localized increases of cyclic AMP that are too small to be measured by standard cyclic AMP assays, may be sufficient to activate a protein kinase and initiate a biological response. Experimental evidence substantiating this view was presented by Dobson and Mayer (1973) and Terasaki and Brooker (1977). In the baboon and dog (Podzuweit *et al* 1977) and cat (Corr *et al* 1978) larger increases in tissue cyclic AMP were found.

E. Late rise in tissue cyclic AMP, confined to the ischaemic lesion

In the dog and guinea pig, cyclic AMP in the myocardium was increased within 5 to 10 seconds of the onset of ischaemic (Wollenberger *et al* 1972). In our pig model, cyclic AMP started to accumulate in ischaemic tissue between 5 and 10 minutes after ligation. Our results do not exclude the possibility of a rise prior to one minute after ligation, although such a short-lived fluctuation seems rather unlikely.

Similar to our findings, Podzuweit *et al* (1977) described later rises of cyclic AMP, i.e. between 1 and 3 minutes after high ligation of the anterior descending in dogs, and only after 20 minutes of ligation of the anterior descending (distal third) in the baboon.

We found no increase in non-ischaemic tissue levels of cyclic AMP after ligation. In the cat model, Corr *et al* (1978) did find a rise in non-ischaemic tissue although this increase was less marked and of shorter duration than the rise in ischaemic tissue. Adrenaline liberation from the adrenal medulla may make a major contribution to increases of cyclic AMP in the non-ischaemic myocardium. Due to the relatively deep level of anaesthesia maintained in our experiments, a reflex liberation of adrenaline as described by Staszewska-Barczak (1971) may have been suppressed.

F. Effect of site of ischaemic lesion on mechanical function of the left ventricle

When the main stem of the anterior descending artery was ligated, an ischaemic lesion developed which involved a significant portion of the interventricular septum as well as the left ventricular free wall, including the apical regions. Left ventricular contractile activity was already decreased at the time of our first recording, i.e. at $2\frac{1}{2}$ minutes after the onset of ischaemia. This was followed by an increase in left ventricular end-diastolic pressure at 5 minutes after ligation. These changes persisted for at least 45 minutes. Brooks *et al* (1975) found similar changes in these parameters when measured at 5 and 60 minutes after main stem ligation in the pig. An ischaemic lesion of a similar size, not involving the septum and apical regions, resulted in minor changes in these parameters.

These results strongly support findings by Hood *et al* (1967), Soloff (1968) and Page *et al* (1971) that involvement of the apical regions contribute greatly to mechanical failure of the left ventricle. In addition, Corr *et al* (1976) found a major decrease in cardiac output after ligation of the left anterior descending coronary artery in cats, as opposed to minor decreases after right coronary artery, or circumflex, ligation.

G. A speculation on chains of events after the onset of ischaemia, leading to ventricular arrhythmias

The concept that compensatory mechanisms may come into play to maintain cardiac output in the presence of substantial infarction, was introduced by Hood *et al* (1967). These may include elevated left

ventricular filling and tachycardia (Hood *et al* 1967), and an increased inotropism subsequent to the release of endogenous catecholamines (Richardson 1963). In our experiments, ligation of the main stem of the anterior descending coronary artery was followed by a decrease in left ventricular contractile activity within 2,5 minutes. Between 5 and 10 minutes, cyclic AMP started to accumulate in ischaemic tissue and remained high for at least 10 minutes. On the contrary, ligation of several lateral branches of the anterior descending coronary artery resulted in lesions of a similar size but did not involve the interventricular septum and apical regions. In this group, left ventricular contractile activity was not altered after ligation, nor did cyclic AMP accumulate in ischaemic tissue.

A role for cyclic AMP has been well defined not only in physiological, but also in pathological chains of events (Sutherland *et al* 1968, Epstein *et al* 1970, Rutenburg *et al* 1971, Parker and Smith 1973). Ischaemia of the apical regions may trigger off a massive local liberation of catecholamines, with a resulting increase in tissue cyclic AMP, in an attempt to maintain contractility of the left ventricle to prevent life-threatening asystole. In addition, Mukherjee *et al* (1979) demonstrated an increase in beta-receptor density in ischaemic tissue within an hour of left anterior descending occlusion in the anaesthetized dog. But the accumulation of cyclic AMP in the ischaemic zone coincided with a period of severe ventricular arrhythmias, including VF. It has been shown that depletion of catecholamines, prior to coronary artery ligation in dogs, attenuated the incidence of VF but that all animals died within 36 hours, due to cardiac failure or asystole (Ebert *et al* 1970, Lown and Verrier 1976).

H. Proposed mechanisms of cyclic AMP induced ventricular arrhythmias

On the basis of distinct electrophysiological properties, cyclic AMP may be either directly implicated in the genesis of ventricular arrhythmias or at the least an important precipitating factor.

In ischaemic tissue, an increased extracell potassium concentration inhibits the fast channel (Opie *et al* 1979) and could totally block depolarization at high concentrations (Schneider and Sperelakis 1975). When dibutyryl cyclic AMP is added to such a preparation, a slow response results, which may underlie the development of re-entrant arrhythmias (Cranefield 1975).

Cyclic AMP may also contribute to arrhythmogenesis by promoting phase 4 depolarization of Purkinje fibres (Tsien 1973, Kentara *et al* 1978). In otherwise non-automatic cells, adrenergic stimulation of phosphodiesterase inhibition can provoke after-potentials and thereby cause automaticity (Lazara *et al* 1978).

Finally, the electrical uncoupling of cells in hypoxic muscle, possibly as a result of enhanced calcium influx, has been shown to be mediated by cyclic AMP (Wojtczak 1979). In zones of ischaemia, such a dissociation could be highly arrhythmogenic.

1. Ligation of the main stem of the anterior descending coronary artery yielded anteroseptal ischaemia including the apical regions. Ischaemia comprised about 15% of total left ventricular mass. A distinct phase of severe ventricular arrhythmias was evident between about 10 and 30 minutes after ligation. When 2 to 4 lateral branches of the anterior descending artery were ligated, an ischaemic lesion of a similar size resulted, but it was confined to the anterior left ventricular wall, excluding the apical regions. The incidence of VT and VF was lower in this group. Thus, the site rather than the size of an ischaemic lesion may be an important determinant of the incidence of ventricular arrhythmias.

2. When a single lateral branch of the anterior descending artery was ligated, the size of the ischaemic lesion was about 7%. The incidence of VT and VF in this group was similar to that in the group with ligation of several lateral branches (size of lesion about 15%). Thus, size of the ischaemic lesion may not be an important determinant of the incidence of ventricular arrhythmias.

3. Concomitantly with the phase demarcated by a high incidence of VT and VF after main stem ligation, cyclic AMP accumulated in ischaemic tissue. During the period of a lower incidence of VT and VF after ligation of one, or more than one, lateral branches, a very brief, or no accumulation of cyclic AMP was evident. Furthermore, when pigs were re-grouped according to the occurrence of VF, animals who encountered this arrhythmia showed a distinct increase of tissue cyclic AMP in zones of ischaemia, while no such accumulation was evident in pigs with no episodes of VF. These results provide substantiating evidence for a strong, however, indirect link between an accumulation of cyclic AMP

in the myocardium and severe ventricular arrhythmias.

4. No relation was found between changes in tissue levels of adenosine triphosphate, phosphocreatine and lactate, and periods of severe ventricular arrhythmias.

5. Ischaemia of the apical regions may contribute greatly to mechanical failure of the left ventricle.

CHAPTER 4

THE SEVERITY AND UNIFORMITY OF ISCHAEMIC INJURY AND THE PROGRESSION OF THE ISCHAEMIC PROCESS AFTER ANTEROSEPTAL AND ANTEROLATERAL LIGATION

I. AIM OF EXPERIMENTS

A. Severity of ischaemic injury

In the previous chapter, we showed that anteroseptal lesions were associated with a high incidence of severe ventricular arrhythmias and a marked impairment of left ventricular mechanical function. Anterolateral lesions of a similar size were associated with a lower incidence of arrhythmias while left ventricular function was not significantly impaired. In order to establish whether differences in the severity of ischaemia at the two sites did not underlie the above findings, regional left ventricular blood flow was measured.

B. Progression of the ischaemic process

In these experiments, measurement of regional left ventricular blood flow were done early as well as late in the experiment. In Chapter 3, changes in tissue levels of ATP, phosphocreatine and lactate were studied in the course of the experiment. From these results, the progression of the ischaemic process after anteroseptal and anterolateral ligation could be assessed.

C. The dimensions of the border zone in the pig

In experiments described in Chapter 3, biopsies to be analysed for ATP, phosphocreatine and lactate were taken from the mid-ischaemic and non-ischaemic zones only. In these studies, additional biopsies were taken from zones adjacent to the visible ischaemic edge. An analysis of regional differences in tissue levels of these metabolites would enable us to comment on the dimensions of the border zone between severely ischaemic and non-ischaemic tissue in the pig.

D. Is the magnitude of the TQ-ST segment deflection indicative of the severity of underlying ischaemia?

The magnitude of regional TQ-ST segment deflections in the epicardial extracellular electrogram was compared with blood flow in the underlying tissue.

II. LITERATURE REVIEW

A. The development of regional ischaemia and the "border zone" concept.

When coronary artery occlusion is maintained for 90 minutes (i.e. time of termination of our experiments) some portion of the acutely ischaemic tissue has already progressed to myocardial infarction (Jennings *et al* 1968, Schaper *et al* 1979), with further incremental increases in the size of the infarct during the next 24 hours (Reimer *et al* 1977).

Salvage of the ischaemic myocardium and the border zone concept (Braunwald and Maroko 1974, Braunwald 1976) have become household vocabulary to the contemporary cardiologist. However, two schools of thought have emerged concerning the character of this zone.

1. Studies in favour of a transitional zone blending imperceptibly with severely ischaemic and non-ischaemic tissue.

(a) Hearse *et al* 1977: They have showed the existence of a clearly definable zone characterized by maximal rates of change of blood flow, metabolism and electrophysiology in the dog with coronary artery occlusion. Although these authors did not provide direct

evidence, they favoured the concept that such a border zone could in principle consist of cells that are homogeneously injured but are injured less than the cells in the central area of the lesion.

(b) Cox *et al* 1968: A border zone in the dog heart was found surrounding severely ischaemic tissue; damage in this zone was confined to mitochondrial swelling.

Okun *et al* (1979) speculated on two possible arrangements of vessels that would conceivably account for such a border zone:

- Complex alternations of capillaries derived from both the occluded and non-occluded arteries would allow individual cells to be perfused by both circulations.
- A network of existing precapillary and capillary anastomoses between vessels from occluded and non-occluded arteries could provide sufficient blood flow at the border to maintain viability of ischaemic cells.

2. <u>Evidence for a zone consisting of a mixture of severely ischaemic and non-ischaemic cells</u>.

(a) As early as 1950/1952 Wiggers found that major coronary arterial branches terminate in an interdigitating pattern, which places finger-like projections of tissue normally perfused by one branch inside similar projections of tissue normally perfused by another branch (Wiggers 1950, Wiggers 1952). These findings were endorsed by work by Reimer *et al* 1977, Factor *et al* 1978, and also Harken *et al* 1978, with the aid of NADH fluorophotography. They showed that the distance between fluorescent ischaemic cells and

adjacent non-fluorescent tissue is less than 0,1 mm.

(b) Okun *et al* (1979) perfused normal canine coronary arteries separately with differently coloured silicone-rubber and showed that terminal homologous capillaries form loops, rather than anastomosing with heterologous capillaries, demonstrating an anatomical end-capillary bed.

(c) Hirzel *et al* 1977: In the dog with 24 hour infarction, a sharp demarcation was found between normal tissue and tissue in which creatine phosphokinase depletion was complete.

(d) Barlow *et al* 1977: Using fluorescence spectroscopy, they identified a zone that appeared as a patchwork of ischaemic and normally perfused tissue sharing sharp interfaces.

(e) Janse *et al* 1979: At the ischaemic edge, zones with normal glycogen content, interdigitated with zones completely depleted of glycogen.

Thus, evidence in favour of a zone consisting of a heterogeneous combination of normal and infarcted tissue (i.e. the "all-or-none" phenomenon would apply to any given cell) appears to outweigh the existence of a transitional zone in which all cells will be less injured than those in the mid-ischaemic zone, but more injured than those in the non-ischaemic zone.

B. Myocardial blood flow

1. Blood flow in the heart without regional ischaemia

In the non-ischaemic heart, the greater portion of blood goes to the left ventricle, including the septum, because of its greater muscle

mass and expenditure of energy (Domenech *et al* 1969). Perfusion through the left ventricle appears to be homogeneous, with endo- and epicardial flow equal, or endocardial flow somewhat higher (Bache *et al* 1977, Hoffman 1978).

About 85% of myocardial blood flow occurs during diastole because of extravascular compression and wall tension which develops during systole, especially in the deeper regions. The remaining 15% that does occur during systole is confined primarily to the epicardium (Hoffman 1978).

In the non-ischaemic heart, overall myocardial blood flow is linked closely to oxygen demand through a mechanism of autoregulation (Kloner *et al* 1976). Major determinants of oxygen demand are heart rate, development of myocardial wall tension and contractility (Braunwald 1971, Sonnenblick and Skelton 1971).

2. Blood flow in the heart with regional ischaemia

Determinants of myocardial flow in the heart with regional ischaemia are different, since blood flow has now been divorced from, and is less than oxygen demand. Consequently, flow becomes dependent on other factors, such as distribution and adequacy of collateral vessels, coronary perfusion pressure and intramyocardial tissue pressure. (James 1970).

Myocardial blood flow studies, usually done in the dog, with a rich collateral network, revealed a severe reduction of blood flow in the centre of the ischaemic zone, increasing towards normal in the periphery (Maroko *et al* 1971, Sharma *et al* 1971). A greater subendocardial than subepicardial necrosis was usually evident (Griggs and Nakamura 1968, Salisbury *et al* 1963). Hyperaemia in the tissue surrounding the ischaemic

zone was found in some studies (Rees and Redding 1969) but not in others (Sharma *et al* 1971).

In the normal pig heart, as in man, very few or no pre-existing collaterals are found (page 13). It is therefore of great clinical importance to study the impairment of blood flow in the porcine heart with regional ischaemia. To our knowledge only Millard (1980) studied regional left ventricular blood flow quantitatively in the pig, although only changes in flow after ligation of several lateral branches of the left anterior descending artery were investigated.

C. The origin and interpretation of the TQ-ST segment deflection

"No other part of the electrocardiogram is subject to so many theories, interpretations and even more misinterpretations as the ST interval" (Shaefer and Haas 1962). Today, 20 years later, this may still hold true.

Due to the non-invasive nature of epicardial TQ-ST deflection measurements, this method has become very popular to study the severity of underlying ischaemia. However, some disagreement regarding the interpretation of these measurements has developed. (Flaherty 1976, Norris *et al* 1976, Ross 1976).

The displacement of the TQ-ST segment is the result of diastolic and systolic "injury currents" flowing between ischaemic and normal myocardium: due to the lower resting transmembrane potential in ischaemic cells during diastole, (Vincent *et al* 1976) a diastolic current of injury is expected to flow from the injured to the uninjured myocardium (Holland and Brooks 1977b) During systole, the transmembrane

potential of ischaemic cells becomes negative earlier than those of normal cells (Vincent *et al* 1976) presumably because of either incomplete depolarization or early repolarization or both (Wittig and Vaughan Williams 1976). These are reflected primarily in shortening of phase 2 and a decrease in overshoot (Kardesch *et al* 1958). The potential gradient between ischaemic and non-ischaemic tissue is now reversed and current flows in the opposite direction (Holland and Brooks 1977b).

Changes in the action potential underlying these "injury currents" have been attributed primarily to regional hyperkalemia produced by potassium leakage from injured cells (Holland and Brooks 1975). In agreement it was found that very small amounts of potassium infused into the coronary artery, simulated the electrocardiographic pattern of acute infarction by producing both ST segment elevations and baseline displacement (Logic *et al* 1968). However, Downar *et al* (1977) found a yet unidentified substance in blood draining ischaemic myocardium, causing more serious electrophysiological derangements than hyperkalemia. Identification of this substance will be of great importance for a better understanding of the electrophysiological principles of ST segment displacement.

It is generally accepted that the magnitude of the TQ-ST deflection basically depends on the strength of the "injury current". Spatial factors, especially the orientation of the recording electrode with respect to the geometry of the ischaemic boundary, have been shown to influence the magnitude of the deflection to a great extent (Holland and Brooks 1977 a).

It has also been realized that myocardial ischaemia is not the sole cause of the TQ-ST segment displacement. Secondary deviations could

be due to intraventricular conduction disturbances, pericarditis, scars and aneurysms, changes in wall thickness and arrhythmias (Surawicz 1977).

III. REGIONAL LEFT VENTRICULAR BLOOD FLOW AND EPICARDIAL TQ-ST SEGMENT STUDIES

A. Methods: (Fig. 4.1)

For the purpose of this study, the ischaemic zone was subdivided into a mid-ischaemic and peripheral ischaemic zone (close to the visible edge). The uninvolved portion of the left ventricle was subdivided into a peri-ischaemic (closer to the visible edge) and a non-ischaemic zone. (See Chapter 2 for a detailed description of methods.)

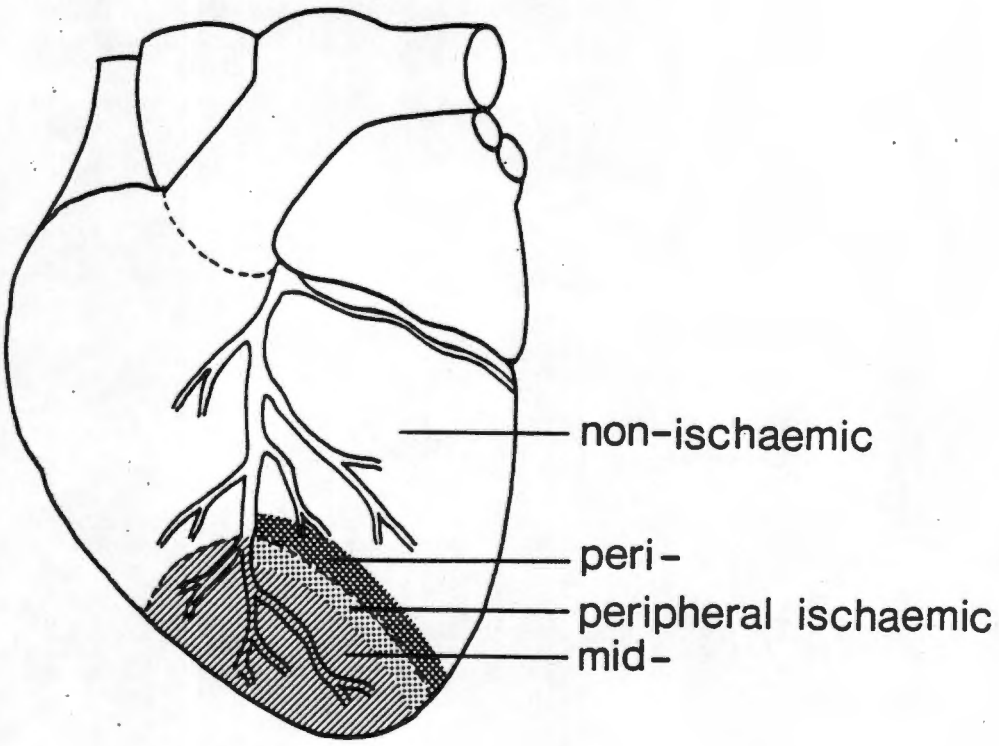
B. Results:

1. Size of ischaemic lesions:

Size of ischaemic lesion as defined by introducing a vital dye at the end of the experiment was: Site 1 (main stem, 6 pigs) 16.1 ± 0.7%; Site 3 (more than one lateral branch, 7 pigs) 15.2 ± 0.6% (percentage of total left ventricular mass). These were not different from size of the ischaemic lesion after ligation at the two sites in arrhythmia studies described in Chapter 3.

2. Left ventricular blood flow in the absence of regional ischaemia

Prior to arterial dissection and ligation, myocardial blood flow in the left ventricle was 1.21 ± 0.02 ml/gram.min^{-1} (n = 54). This is similar to the value found in pigs by Millard (1980) and in dogs. (Nakamura *et al* 1979).



Four zones preselected for the purpose of blood flow and epicardial electrographic studies

FIGURE 4.1

Flow in the anterior wall, apical regions and intraventricular septum was similar. Subepi- and subendocardial blood flow showed no differences. Similar results were found in the baboon and dog (Lubbe *et al* 1974). A lower subendocardial flow, as shown in some earlier studies (Brandi and McGregor 1969) would be unlikely in view of the greater oxygen demands that are normally present in the inner layers of the heart (Spotnitz *et al* 1966).

3. Blood flow in the left ventricle with regional ischaemia

In cases where subepicardial and subendocardial blood flow showed no differences, values were combined.

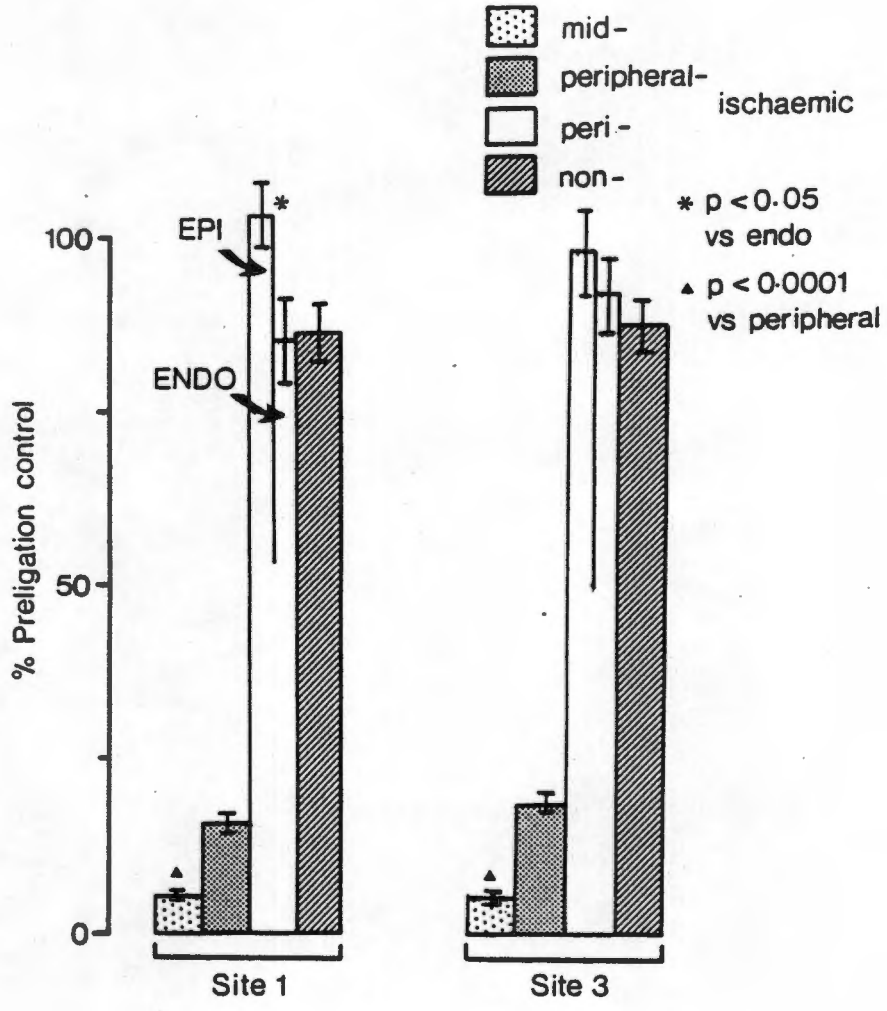
(a) Twenty minutes after ligation (Fig. 4.2):

i) Blood flow in the mid- and peripheral ischaemic zones

In the group with ligation at site 1 (main stem, 6 pigs) blood flow in the mid-ischaemic zone was 5.7% ± 0.7% (n = 28) of the pre-ligation control value and in the peripheral ischaemic zone, 16.2% ± 1.1% (n = 24, $p < 0.0001$). Blood flow in both the mid- and peripheral ischaemic zones after ligation at site 3 (more than one adjacent lateral branch, 7 pigs) showed similar values.

ii) Blood flow in the peri- and non-ischaemic zones

Ligation at site 1 resulted in a slight, non-significant decrease in blood flow in the non-ischaemic zone, compared to pre-ligation control values, with hyperaemia in favour of the subepicardium just outside the ischaemic edge i.e. in the peri-ischaemic zone. Endo/epicardial ratio in this zone was 0.83 ± 0.05.



Twenty minutes after ligation at the different sites, regional left ventricular blood flow was similar. Subepicardial hyperaemia was evident in the peri ischaemic zone after ligation at site 1.

FIGURE 4.2

(b) Ninety minutes after ligation: (Table 4.1)

At this stage, a further deterioration of blood flow occurred in the entire ischaemic zone after ligation at the two sites. No evidence of an increased blood flow in the peri-ischaemic zone was found as was the case at 20 minutes after ligation.

Arterial pressure in the two groups showed no differences at the time when myocardial blood flow was measured.

TABLE 4.1

Blood flow in ischaemic zones 20 and 90 minutes after ligation. (% pre-ligation control)

	Site 1 (main stem)		Site 3 (large lateral)	
	mid ischaemic	periph ischaemic	mid ischaemic	periph ischaemic
20 min	5,7 ±0,7	16,2 ±1,1	5,0 ±0,7	18,8 ±1,2
90 min	3,4** ±0,9	10,0** ±1,0	4,9 ±1,0	13,9* ±1,4

* $p < 0,05$ vs 20 min

** $p < 0,0005$ vs 20 min

Between 20 and 90 minutes after ligation marked reductions in blood flow were evident in both the middle and the periphery of the ischaemic lesions.

Conclusions

Using regional impairment of blood flow as an indicator of the severity of ischaemia, we conclude that, at the time of a difference in the incidence of ventricular arrhythmias and in the impairment of mechanical function associated with anteroseptal and large anterolateral lesion, i.e. at 20 minutes after ligation, ischaemia was equally severe. Between 20 and 90 minutes, a significant progression of ischaemia occurred in the two groups.

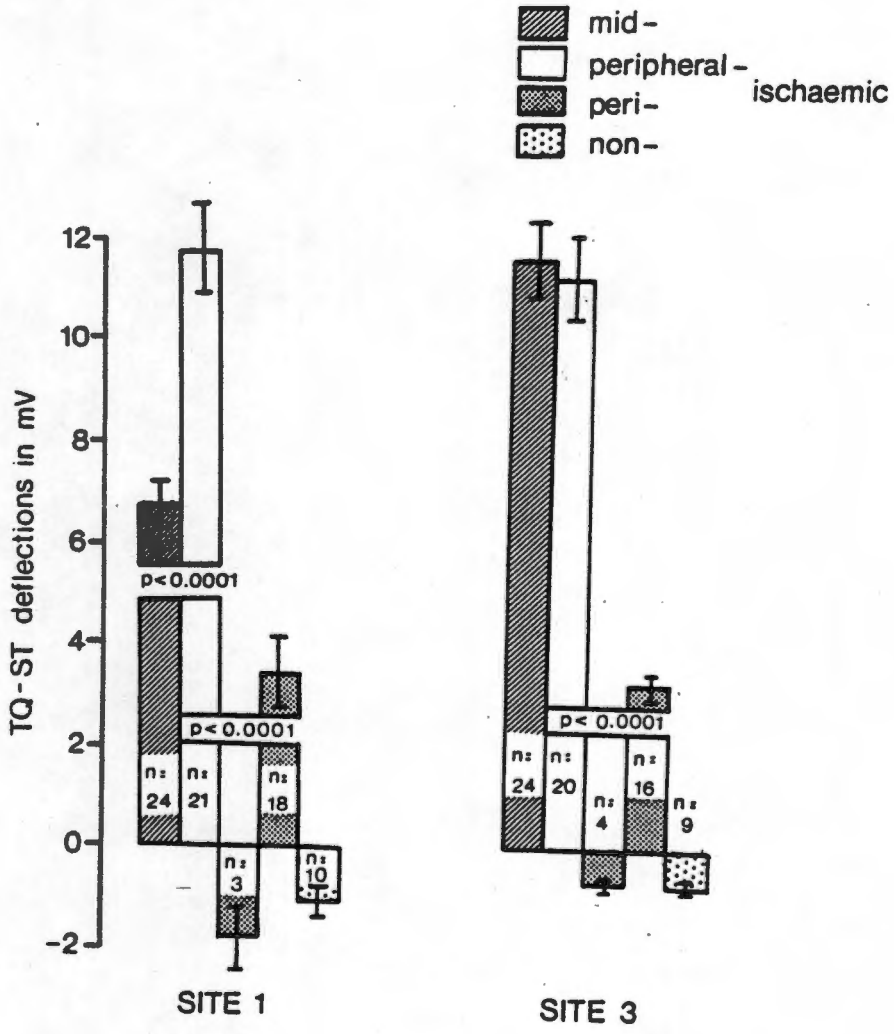
4. Regional differences in epicardial TQ-ST deflections recorded over the ischaemic left ventricle (Fig. 4.3)

(a) Deflections over the ischaemic zone: Deflections recorded over the periphery of anteroseptal lesions (within 5 mm of the visible edge) were of larger magnitude than those over the middle of the lesion. However, all deflections recorded over the middle and periphery of large anterolateral lesions were of a similar magnitude.

(b) Deflections over the non-ischaemic zone: In both groups, the extracellular electrogram over the peri-ischaemic zone (within 5 mm of the edge) showed mainly positive but sometimes also negative deflections. In the distant non-ischaemic zone, negative deflections were also occasionally recorded, but seldom exceeded 2 millivolts. Fig. 4.3 shows TQ-ST deflections 25 minutes after ligation only. The magnitude of these deflections in the different zones at 45, 65 and 85 minutes were largely similar.

Conclusions

The magnitude of TQ-ST segment deflections recorded over ischaemic lesions at different sites, but with equal reductions in



TQ-ST deflections 25 minutes after ligation. Note differences in the mid-ischaemic zone of the two groups.

FIGURE 4.3

blood flow showed differences.

The epicardial extracellular electrogram grossly over-estimates the ischaemic zone, for large deflections were recorded in the peri-ischaemic zone, where blood flow results did not indicate the presence of ischaemia.

IV. REGIONAL TISSUE METABOLITE STUDY

A. Methods (Fig. 4.4)

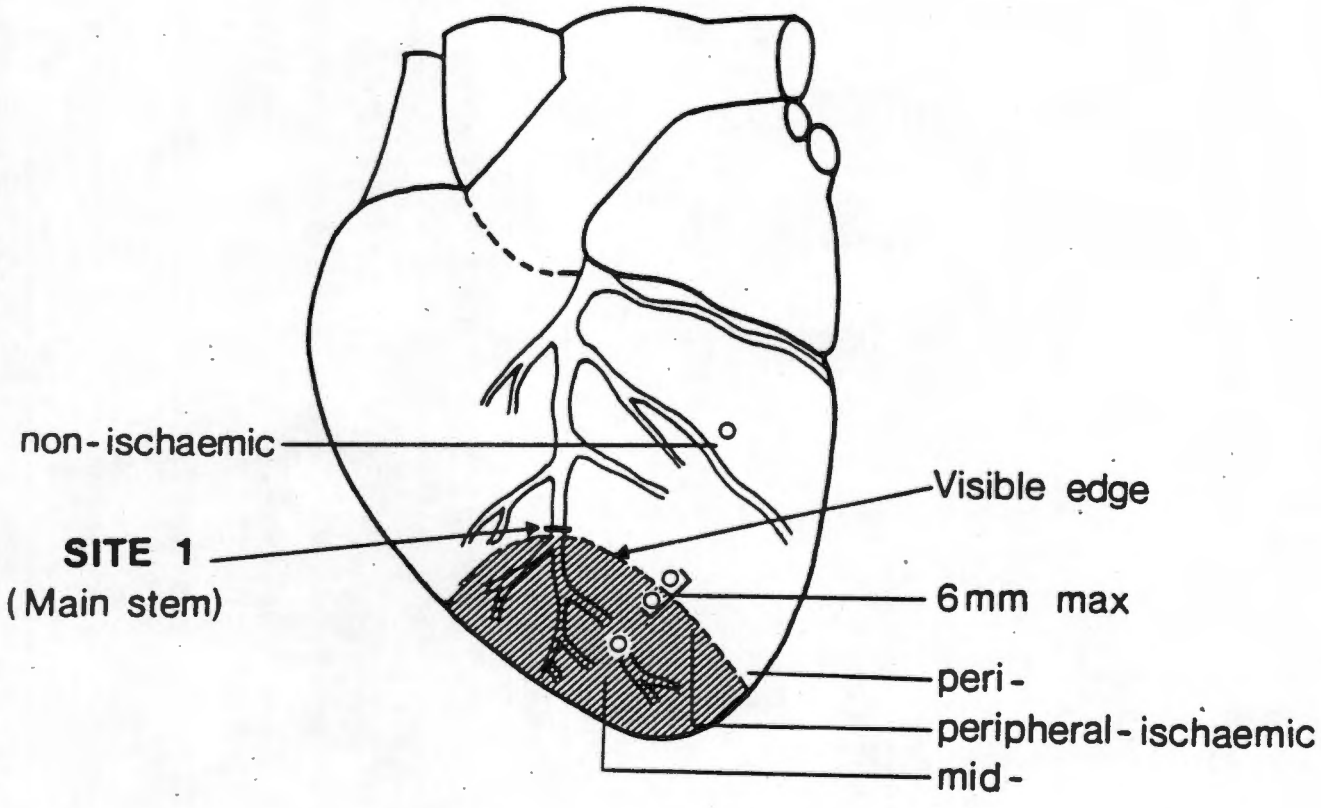
Ten and 90 minutes after ligation at site 1, four biopsies to be assayed for cyclic AMP, ATP, PCr and lactate were taken: one from the mid-ischaemic zone, one from the non-ischaemic zone and one each from the peripheral and peri-ischaemic zones, within a distance of maximally 3 mm from the visible edge (6 pigs).

B. Results (Fig 4.5)

At 10 as well as at 90 minutes tissue levels of all four metabolites, i.e. cyclic AMP, ATP, PCr and lactate, in the middle of the ischaemic zone were similar to those within 3 mm of the visible ischaemic edge. Within a distance of maximally 6 mm over the visible edge, peripheral ischaemic tissue levels were in sharp contrast to levels in the peri-ischaemic zone.

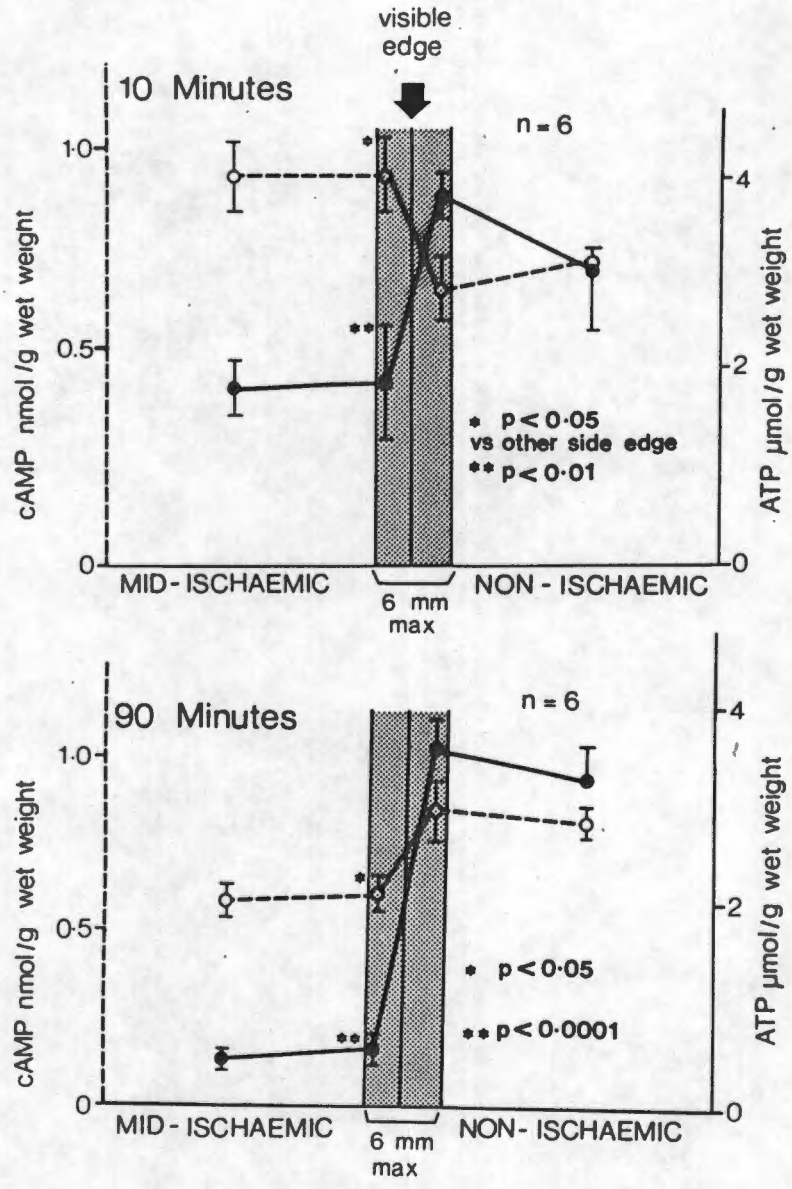
Conclusions

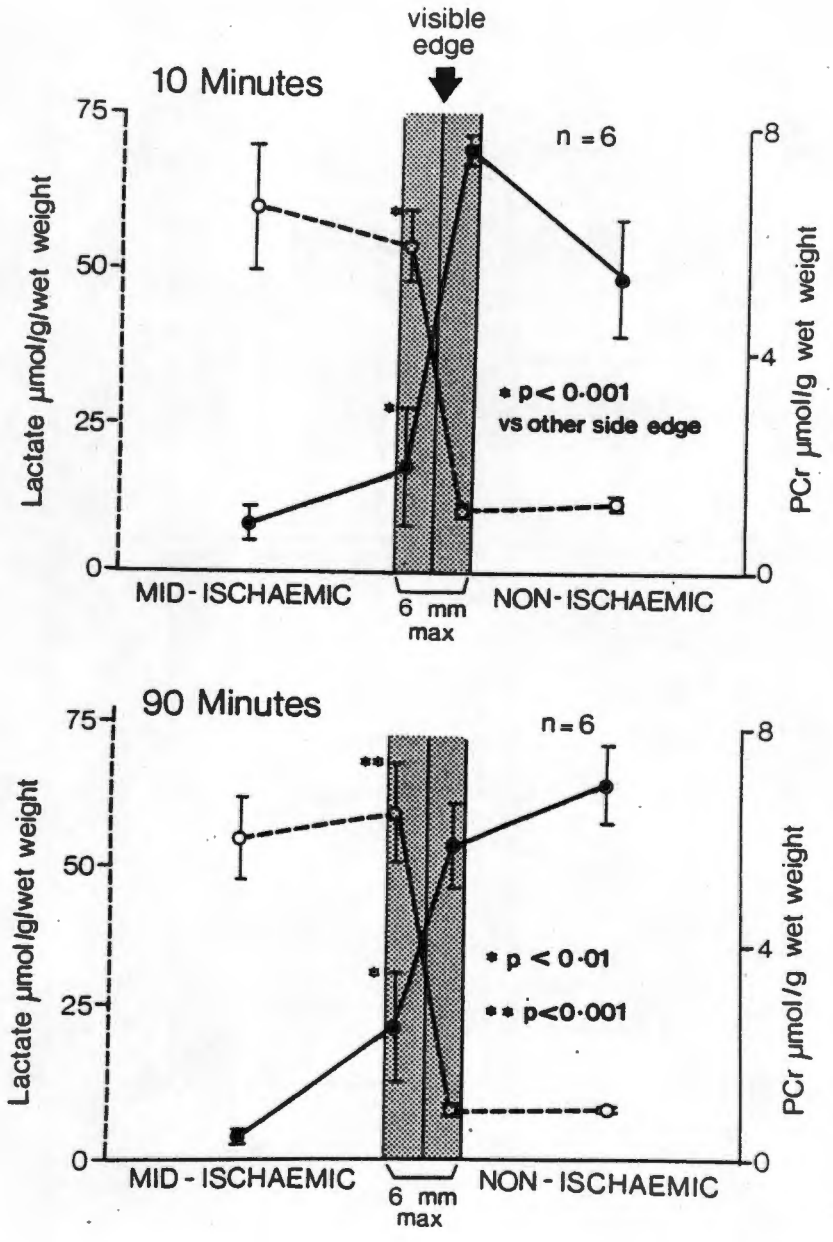
Metabolism in the zone of ischaemia was homogeneous. The lateral metabolic border could be expected to be confined to a zone of maximally 6 mm.



Sites of biopsies

FIGURE 4.4


visible edge
10 Minutes
n = 6
cAMP nmol/g wet weight
ATP µmol/g wet weight
1·0
0·5
0
4
2
0
* p < 0·05
vs other side edge
** p < 0·01
MID - ISCHAEMIC
6 mm max
NON - ISCHAEMIC
90 Minutes
n = 6
cAMP nmol/g wet weight
ATP µmol/g wet weight
1·0
0·5
0
4
2
0
* p < 0·05
** p < 0·0001
MID - ISCHAEMIC
6 mm max
NON - ISCHAEMIC



Tissue levels of cyclic AMP, adenosine triphosphate (ATP), phosphocreatine (PCr) and lactate in the mid-, peripheral-, peri- and non-ischaemic zone of anteroseptal lesions. Note sharp contrasts in levels within 6 mm over the visible edge.

FIGURE 4.5

V. DISCUSSION

A. Severity of ischaemia, ventricular arrhythmias, and impairment of mechanical function.

At 20 minutes after ligation, blood flow in the two lesions was reduced to a similar extent. The possibility that differences in the severity of ischaemia might have been the underlying cause of the differences in the incidence of severe ventricular arrhythmias and in the impairment of mechanical functions has therefore been excluded.

B. Marked progression of the ischaemic process during 90 minutes after coronary ligation in the pig.

It is generally accepted that a reduction in regional blood flow to less than 0,4 ml/gram. min^{-1} is indicative of ischaemic tissue being at risk to become necrotic (Darsee *et al* 1981). In our study, the mildest reduction in blood flow occurred in the periphery of the ischaemic lesion, with a residual flow of about 0,25 ml/gram. min^{-1} at 20 minutes after ligation. A marked further deterioration in blood flow was evident in the entire ischaemic zone between 20 and 90 minutes after ligation.

Janse *et al* (1980) showed that after a two-hour occlusion of the left anterior descending artery of the isolated pig heart, cells in the entire ischaemic lesion were irreversibly damaged.

These results, together with evidence of considerable metabolic deterioration during the first 90 minutes as described in Chapter 3, suggest that interventions to salvage the infarcting pig heart should be introduced at a very early stage after the onset of

ischaemia. Bearing in mind the similarities that do exist between the human and porcine heart, this suggestion may be clinically relevant.

C. The lateral ischaemic border zone in the pig.

1. Changes in blood flow over the ischaemic edge.

Although blood flow in the periphery of the ischaemic lesions was significantly higher than in the middle of the zones, flow increased over the visible edge from about 18% to 100% of the pre-ligation control value over a distance of maximally 12 mm. Due to limitations in our sampling method, abrupt changes over a shorter distance cannot be excluded. Millard *et al* (1980) introduced radioactive microspheres and, using a matrix method of sampling, demonstrated a precipitous margin of 2 mm in width between normally perfused and severely ischaemic myocardium in the pig during the first hour after ligation or more than one lateral branch.

2. Changes in tissue levels of ATP, phosphocreatine and lactate.

In anteroseptal lesions, tissue levels of ATP, phosphocreatine and lactate in the middle and periphery of the ischaemic lesion were similar, changing abruptly over a distance of maximally 6 mm over the edge. Janse *et al* (1980) described intermediate values for these metabolites over a broader zone in the "electrical border" of the isolated perfused pig heart with regional ischaemia. This "electrical border zone" was defined as that zone over which intermediate TQ-ST deflections were recorded. We have clearly shown that TQ-ST deflections overestimate the ischaemic zone, for we recorded intermediate deflections in the peri-ischaemic zone. This could explain the differences between their and our results.

3. Further investigations to define the lateral and transmural border zone in the pig.

Results from our study and also from others suggest that the border zone in the pig may be much narrower than in the dog; Hearse *et al* (1977) found gradients of metabolites and blood flow across a zone of 8 to 15 mm wide that spanned the visible ischaemic edge.

We have recently commenced a study to clearly define the lateral as well as the transmural dimensions of the border zone in the pig. A pre-cooled cutting matrix is delivered into the myocardium at high speed. (Hearse *et al* 1981.) Multiple smaller contiguous transmural biopsies could now be obtained simultaneously and then frozen *in situ*.

D. Changes in regional left ventricular blood flow after coronary artery ligation.

1. Blood flow in the ischaemic zones.

Most studies did not differentiate between mid- and peripheral ischaemic zones. A reduction between 25% and 55% was shown in zones of ischaemia. All these investigations were done in dogs (Regan *et al* 1969, Becker *et al* 1971). Becker *et al* (1972b) did distinguish between central infarct zone and infarct edge in the dog and found a residual blood flow of about 50% in the former, and 85% in the latter zone. Hearse *et al* (1977) found a residual blood flow of less than 15% in the centre of the ischaemic zone and between 20 and 50% in the peripheral ischaemic zone in dogs.

Lubbe *et al* (1974) described a more drastic reduction in the

baboon with residual flow values of about 25% of control in the periphery and about 10% in the centre of the ischaemic zone, 10 minutes after ligation of the left anterior descending coronary artery. According to our observations, ligation of this artery in the pig, results in a more severe reduction of coronary flow in the periphery (about 80%) as well as in the mid-ischaemic zone (about 92%) than in the dog or baboon.

We found equally severe reductions in blood flow after ligation of more than one lateral branch of the anterior descending (site 3) with an ischaemic lesion of a similar size. These results on lateral ligations are similar to those found by Millard (1980) in the pig.

Our results showed no differences in the severity of subendo- versus subepicardial ischaemia as defined by reductions in blood flow. Griggs and Nakamura (1968) showed more severe subendocardial than subepicardial necrosis in the dog.

2. Blood flow in the non-ischaemic zones.

Controversy persists concerning the existence and, if so, the significance of a hyperperfused zone bordering acutely ischaemic myocardium (Rivas *et al* 1976, Hirzel, 1977, Janse *et al* 1980).

Ligation of the main stem of the anterior descending was followed by a transient hyperaemia, confined to the subepicardium. This is in agreement with results from dogs (Hearse *et al* 1977, Ribeiro 1980). Blood flow may be directed away from the subendocardium due to existence of some collaterals in the subepicardium (Ribeiro 1980).

Hyperaemia may be the result of (a) a reflex vasodilation. (Becker *et al* 1973). (b) A fall in extravascular pressure in the non-ischaemic zone, which allows a passive increase in arterial inflow (Herzberg *et al* 1966), or (c) vasodilator metabolites, i.e. adenosine and prostaglandins from the ischaemic zone, (Driscol and Ekstein, 1967).

3. Blood flow gradients and arrhythmias.

Lubbe *et al* (1974) related steeper gradients of blood flow over the ischaemic edge after coronary ligation in the baboon, to a higher incidence of VF. In our experiments, main stem and large lateral ligations resulted in equally steep gradients of blood flow at the visible edge, but arrhythmias in the former group were more severe. However, this distinct period of severe rhythm disturbances, about 20 minutes after ligation, occurred at the time of a transient hyperaemia. This hyperaemia, confined to the subepicardium, may have increased the inhomogeneity of electrical activity and metabolism at the ischaemic edge and may have been a contributing factor in arrhythmogenesis.

E. The epicardial TQ-ST segment deflection as an indicator of ischaemia.

1. Relation between the magnitude of the deflection and local blood flow in zones of ischaemia.

Despite clear differences in blood flow in the middle and periphery of anterolateral lesions, the TQ-ST deflections recorded over the two zones showed no differences in magnitude. Furthermore, after main stem ligation, deflections over the middle of the lesion were much smaller than those recorded over the periphery, where ischaemia was less severe.

Thus, no simple relation exists between the severity of underlying ischaemia, as defined by regional blood flow and the magnitude of the epicardial TQ-ST deflection.

2. Interpretation of deflections outside the ischaemic zone.

The registration of positive TQ-ST deflections outside the visible ischaemic edge, although of smaller magnitude than inside, clearly shows that the epicardial extracellular electrogram overestimates the zone of ischaemic involvement. Small negative deflections, (< 2 millivolts) which were sometimes recorded over the non-ischaemic zone, probably reflect influences of the ischaemic boundary.

3. The significance of spatial factors.

Extensive studies by Holland and Brooks (1975, 1977a, 1977b) suggest that the magnitude and polarity of epicardial TQ-ST deflections are determined by:

(a) the position of the recording electrode with respect to the ischaemic boundary(ies);
(b) the area of ischaemic involvement;
(c) the transmural shape of the ischaemic zone;
(d) the thickness of the wall at the boundary(ies).

4. Effect of conduction delay.

Conduction delay associated with severe ischaemia should be considered as a possible cause of a decrease in the magnitude of deflections, especially in the middle of ischaemic lesions. (Muller *et al* 1978).

VI. SUMMARY AND CONCLUSIONS, CHAPTER 4

1. The difference in the incidence of ventricular arrhythmias after anteroseptal and anterolateral ligation (Chapter 3) was not due to differences in the severity of ischaemia, for a similar impairment of blood flow was evident in the two lesions. A transient hyperaemia, confined to the subepicardium in the peri-ischaemic zone at the time of rhythm disturbances after anteroseptal ligation, could have contributed to arrhythmogenesis.

2. Severe ischaemia was evident within 20 minutes of ligation of the main stem or more than one lateral branch of the left anterior descending coronary artery, with a further marked deterioration within 90 minutes of ligation. Interventions to salvage the infarcting pig myocardium should therefore be introduced at a very early stage.

3. Sharp changes in tissue levels of metabolites, and rates of blood flow within a short distance over the visible cyanotic edge, suggest that the border zone in the pig is much narrower than in the dog.

4. The magnitude of epicardial TQ-ST segment deflections is not a direct indicator of the severity of underlying ischaemia, as assessed by regional blood flow measurements.

CHAPTER 5

BETA-ADRENOCEPTOR ANTAGONISM, CYCLIC AMP AND VENTRICULAR ARRHYTHMIAS

I. BETA-ADRENOCEPTOR ANTAGONISM IN DEVELOPING MYOCARDIAL INFARCTION

Since the introduction of the beta-adrenoceptor antagonists about 17 years ago, very few studies have been conducted to establish the anti-arrhythmic value of these compounds during developing myocardial infarction. Table 5.1 summarizes some results

II. AIM OF EXPERIMENTS.

a. When the main stem of the left anterior descending coronary artery was ligated, a transient accumulation of cyclic AMP occurred coincident with a distinct period of severe ventricular arrhythmias, including VF (Chapter 3). Theoretically, the introduction of competitive antagonism at the beta-receptor should decrease the formation of cyclic AMP by the adenylate cyclase system. We therefore measured tissue levels of cyclic AMP after the administration of a beta-antagonist and investigated whether such an intervention would prevent ventricular arrhythmias following coronary artery ligation.

b. In the studies cited in Table 5.1, cardioselective beta-antagonists always provided protection against the development of ventricular arrhythmias. However, not all authors considered cardioselectivity to be related to anti-arrhythmic effects. We studied the effect of metoprolol, a cardio-selective beta-antagonist in comparison with that of sotalol and propranolol, both non-selective antagonists. The anti-arrhythmic value of metoprolol shortly after coronary artery ligation has not as yet been established.

c. We studied regional left ventricular blood flow after the administration of propranolol, sotalol or metoprolol,and ligation.

TABLE 5.1

BETA-ANTAGONIST	MODEL	DOSE	ANTI-ARRHYTHMIC EFFECT	SUGGESTED MECHANISM/ REASON FOR FAILURE	REFERENCE
d-Propranolol Δ	Conscious dog, circumflex ligation	5 minutes prior to ligation 0,1mg/kg IV	Decrease in mortality rate (within 2 hours of ligation) ($p<0,001$ vs untreated animals)	Protection result of beta-antagonism and not due to local anaesthetic effect. Myocardial selectivity not a precondition for protection	Khan *et al* 1972
		1,0 mg/kg IV	None		
Sotalol	"	0,2 mg/kg IV	Decrease in mortality ($p<0,005$)		
		3,2 mg/kg IV	Decrease in mortality ($p<0,005$)		
d-Propranolol Δ	"	0,07 mg/kg IV	None		
Sotalol & d-Propranolol Δ	"	0,2 + 0,07 mg/kg IV	Decrease in mortality ($p<0,005$)		
Practolol *†	"	1,4 mg/kg IV	Decrease in mortality ($p<0,025$)		
Propranolol Δ	Anaesthetized dog, anterior descending and septal artery ligation	Before ligation 0,5 mg/kg IV	No protection against premature systoles (VPS), ventricular tachycardia (VT) or VF	Myocardial selectivity although ISA may be important to protect against development of VF	Pearle *et al* 1978
Practolol *†	"	1,5-2,5 mg/kg IV	Protected against VF (occurring within 30 minutes of ligation) but not against VPS or VT		
Propranolol Δ	Anaesthetized cat, anterior descending ligation	20 minutes before ligation 0,75 mg/kg IV	No protection against VF, but period of VPS occurrence shorter	Cardiac sympathetic neural activity not important or that not all adrenergic synapses were blocked	Corr & Gillis 1975
Atenolol *	Anaesthetized dog, circumflex ligation	Oral pretreatment for 5 days: 2-10 mg daily	Protected against VF	Preventing local release of catecholamines in ischaemic myocardium	Menken *et al* 1979
Propranolol Δ	Anaesthetized dog, circumflex ligation	10 minutes before ligation 0,08 mg/kg IV	LOW dose protected against VF but HIGH dose did not	Anti-arrhythmic effect not related to beta-blocking action. High dose may result in greater loss of sympathetic activity which may lead to a higher degree of depression of contractility.	Pentecost & Austen 1966
		0,2 mg/kg IV			
Sotalol	Anaesthetized dog, anterior descending ligation	10 minutes before ligation 0,5 mg/kg IV	LOW dose did not protect while HIGH dose offered protection against VF	Protective effect due to prolongation of action potential duration	Kaumann & Aramendia 1968
		10,0 mg/kg IV			
Metoprolol *	Conscious dog, anterior descending ligation	18-24 hours after ligation 5 mg/kg IV	Both protected against arrhythmias, 18-24 hours after ligation	Membrane activity may be more important than beta-blocking activity	Sivam & Seth 1978
Propranolol Δ		3 mg/kg IV			
Propranolol Δ	Anaesthetized cat, anterior descending ligation	15 minutes before ligation 0,75 mg/kg IV	Protected against VPS but not VF	Beta-blockade does not attenuate all efferent traffic to the heart	Corr *et al* 1978

* = Myocardial selectivity

Δ = Membrane stabilizing activity

† = Intrinsic sympathetic activity

Marshall and Parratt (1976) showed that propranolol, in contrast to practolol (cardioselective beta-antagonist) decreased blood flow in ischaemic regions after coronary artery ligation in dogs.

d. Left ventricular contractile activity and pressure development were measured, for if beta-antagonism prove to be anti-arrhythmic, the cardiosuppressant effects of the specific antagonist will have to be clearly defined.

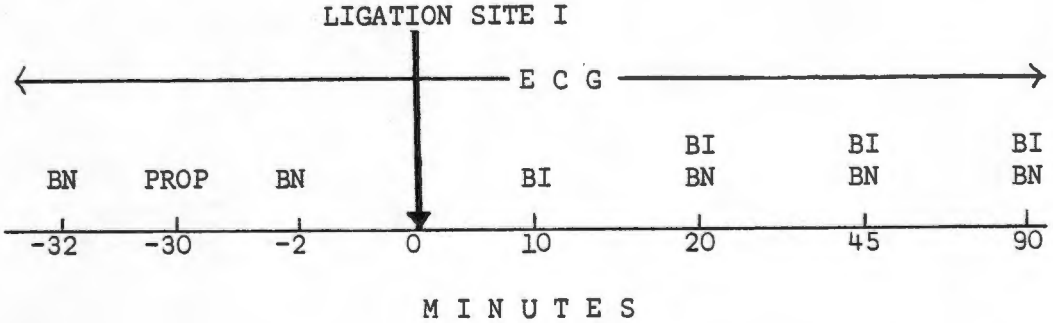
e. Evidence exists for the preservation of ATP (Obeid *et al* 1976) and a decrease in lactate production (Mueller *et al* 1974) in zones of ischaemia after the administration of a beta-antagonist. We assayed sequential biopsies taken from the ischaemic and non-ischaemic left ventricle for high energy phosphates and lactate.

III. METHODS AND RESULTS

A. Effect of propranolol a non-selective beta-antagonist on tissue levels of cyclic AMP and arrhythmias.

Propranolol HCl (Imperial Chemical Industries) was used. The powder was dissolved in water for injection. Propranolol 1,5 mg/kg was administered 30 minutes prior to ligation. This does was decided on, for it reduced heart rate by about 30%, lasting for about 2½ hours

Protocol: (9 pigs)



ECG = limb leads 1 and 2 or 3 registered continuously at paper speed of 25 mm/sec.

PROP = propranolol 1,5 mg/kg

BN = biopsy from non-ischaemic left ventricle (6 pigs only)

BI = biospy from ischaemic left ventricle (6 pigs only)

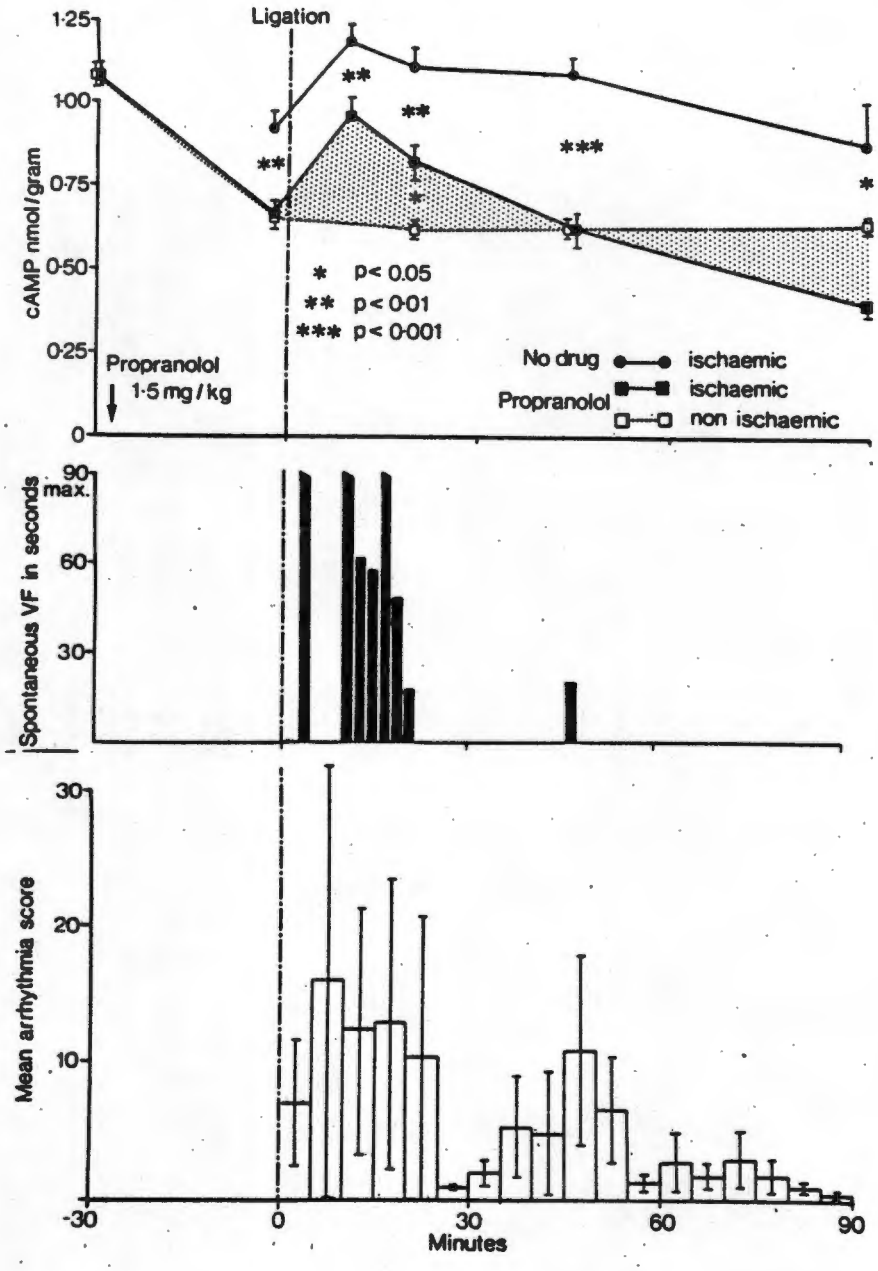
A similar protocol was followed for metoprolol and sotalol.

Results: (Fig. 5.1 and Table 5.2) Propranolol administration significantly reduced tissue levels of cyclic AMP in the normal left ventricle prior to ligation, but did not prevent the transient accumulation of cyclic AMP in ischaemic tissue after ligation, as in control animals.

Propronolol administration reduced the incidence of VPS and VT. However, the incidence of VF was similar to that in control animals, as 8 out of 9 pigs encountered this arrhythmia.

B. Effect of metoprolol, a cardioselective beta-antagonist, on tissue levels of cyclic AMP and arrhythmias.

Metoprolol tartrate (Ciba-Geigy) was used. The powder was dissolved in water for injection and administered in a dose of 10 mg/kg, 30 minutes prior to ligation to 13 pigs. This does was repeated 10 minutes after ligation because the heart rate started to approach pre-administration levels at this stage.



Tissue levels of cyclic AMP, time of occurrence and duration of VF, and scores for VPS and VT in 6 of the 9 pigs pre-treated with propranolol. Despite beta-antagonism cyclic AMP accumulated in ischaemic tissue and the incidence of VF remained high as in untreated animals (Fig. 3.2)

FIGURE 5.1

TABLE 5.2 ARRHYTHMIAS DURING 90 MINUTES FOLLOWING CORONARY ARTERY LIGATION

Drug and size of ischaemic lesion	Pig No.	Premature Systoles: Ventricular late	Premature Systoles: early (R-on-T)	Ventricular tachycardia (seconds)	Ventricular fibrillation (seconds)
	1	0	0	3	90 (max) Δ
	2	162	2	0	90 (max)
	3	16	2	0	0
	4	23	4	0	90 (max)
	5	128	6	30	0
	6	299	25	90	60
No drug	7	101	0	60	90 (max)
	8	688	0	65	42
15,3%±0,6%	9	246	13	106	50
(Percentage of total left ventricular mass)	10	14	0	191	44
	11	279	0	513	82
	12	124	0	0	6
	13	235	0	121	0
	14	108	0	153	50
	15	45	1	181	38
	16	70	0	150	9
		158,6±42,9	3,3±1,6	103,9±32,2	13 out of 16
	1	17	0	0	7
	2	10	1	0	18
Sotalol	3	100	7	1	12
(10 mg/kg)	4	20	1	0	18
	5	35	0	0	14
15,0%±0,7%	6	39	0	0	25
		36,8±13,4*	1,5±1,1	0,2±0,2**	6 out of 6
	1	5	0	0	0
	2	94	0	0	0
	3	17	0	121	0
	4	11	0	0	0
	5	10	0	0	0
Metoprolol	6	8	0	0	0
(10 mg/kg)	7	10	0	2	4
16,4%±0,6%	8	85	0	0	0
	9	40	7	13	7
	10	130	0	0	0
	11	237	0	0	0
	12	15	0	0	0
	13	83	0	0	0
		57,3*	0,5	9,7*	
		±18,9	±0,5	±9,3	2 out of 13***
	1	13	0	8	90 (max)
	2	31	0	0	0
	3	106	0	2	90 (max)
	4	104	44	16	57
Propranolol	5	0	0	5	90 (max)
(1,5 mg/kg)	6	59	2	6	34
14,9%±0,7%	7	15	0	0	42
	8	153	0	0	67
	9	65	0	160	90 (max)
		60,7*	5,1	21,9*	
		±17,2	±4,9	±17,4	8 out of 9

* $p < 0,05$, ** $p < 0,01$, *** $p < 0,001$, all vs no drug

Δ When direct current shock failed to restore normal rhythm within 90 seconds, the experiment was terminated.

Results: (Fig. 5.2 and Table 5.2) Metoprolol administration resulted in a reduction of tissue levels of cyclic AMP prior to ligation but the accumulation of cyclic AMP in ischaemic tissue after ligation could not be prevented, as was the case with propranolol. Metoprolol significantly reduced the incidence of VPS and VT. In addition, metoprolol reduced the incidence of VF, compared to untreated pigs.

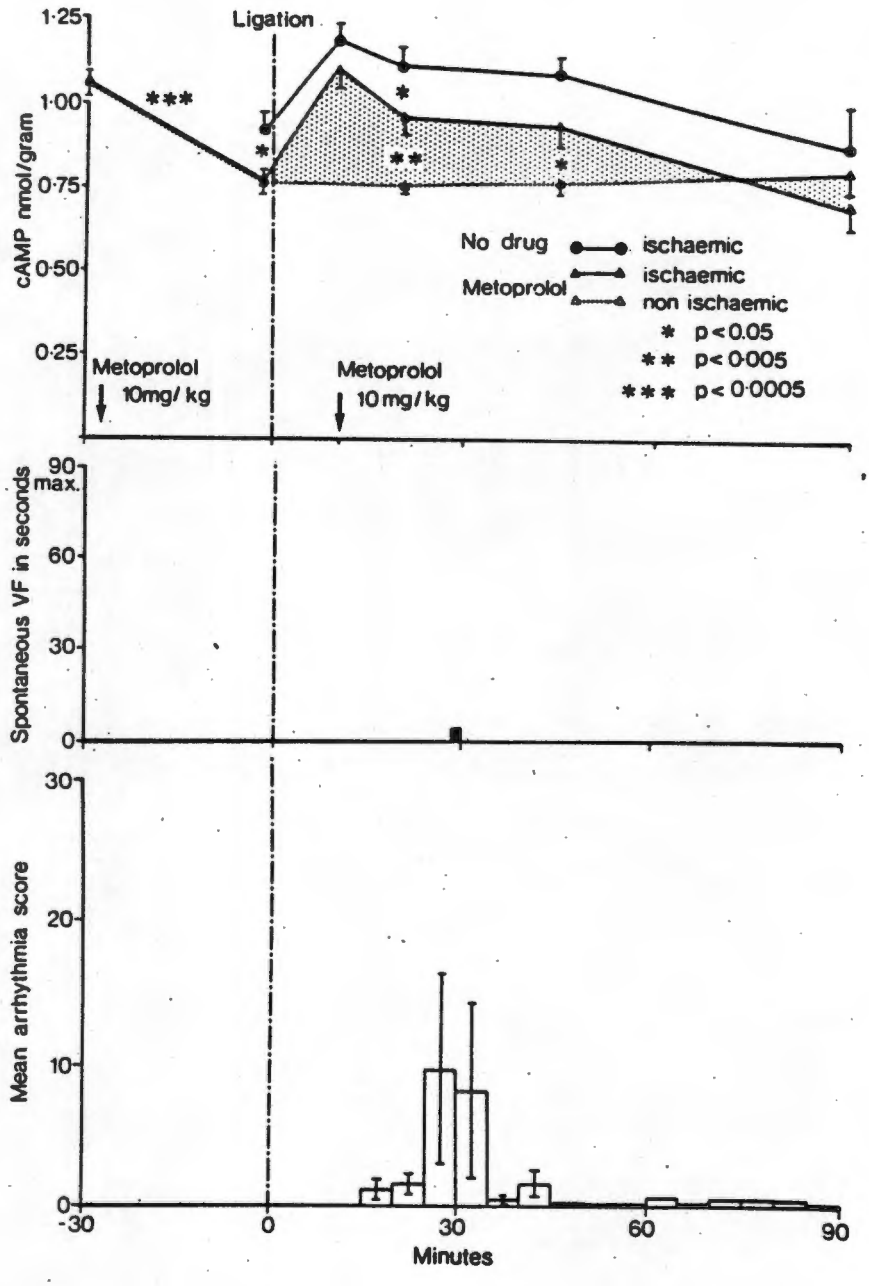
Thus, propranolol and metoprolol failed to prevent the accumulation of cyclic AMP after ligation. Both drugs reduced the incidence of VPS and VT. However, only metoprolol prevented the development of VF.

C. Dose-effect curves

The reason for metoprolol preventing VF, while propranolol did not, could have been due to unequal potency of the two competitive antagonists to occupy myocardial beta-receptors in the biophase, in the doses administered. To test this, we undertook dose-effect studies. These experiments were undertaken in open-chest animals in order to account for possible effects of thoracotomy. The coronary artery was not ligated.

1. The effect of increasing doses of a beta-agonist on heart rate (control curve, Fig. 5.3).

Isoproterenol HCl (ampoules, containing 0,2 mg/ml, Winthrop), diluted with water for injection, was administered over 90 seconds. Doses ranged from 0,465 to 46,5 µg. A minimum period of 30 minutes was allowed after heart rate had returned to basal levels, before an incremental dose was administered (4 pigs). At higher doses, a plateau in the heart rate effect was reached, as expected, indicating maximal receptor occupation by the agonist.



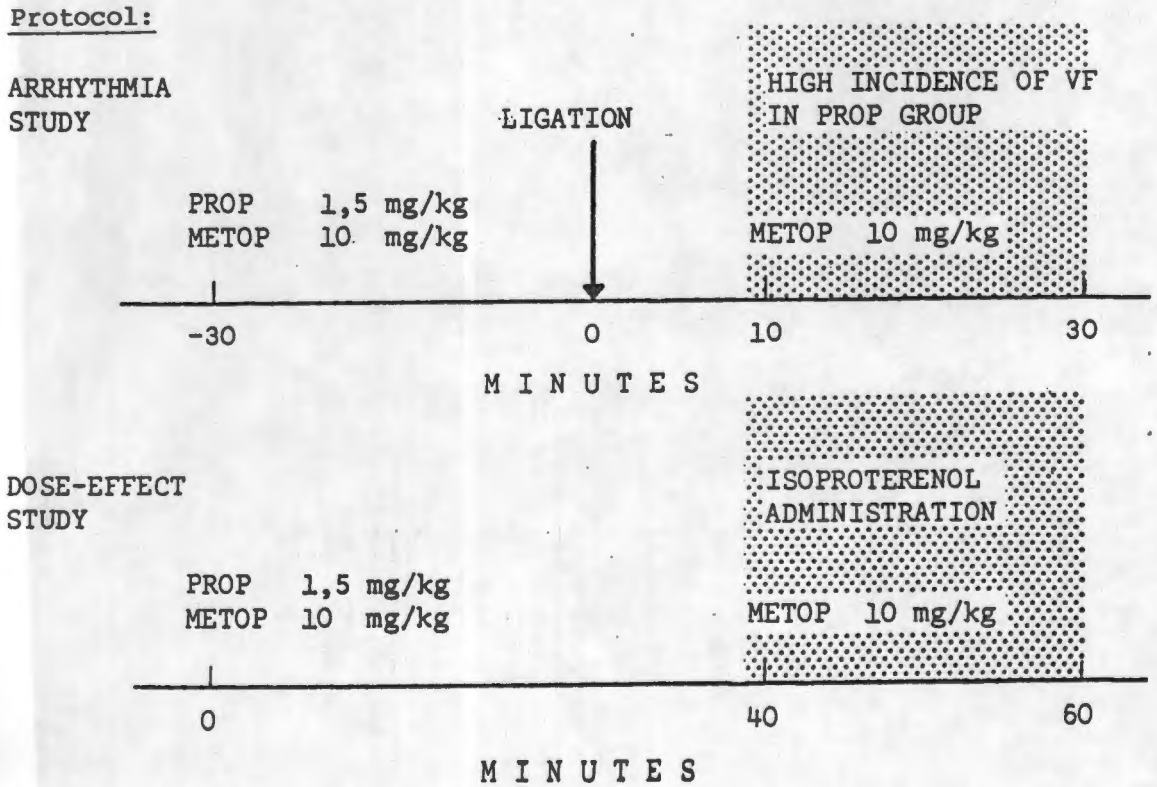
Tissue levels of cyclic AMP and arrhythmias in 8 of the 13 pigs treated with metoprolol. The accumulation of cyclic AMP in ischaemic tissue after ligation could not be prevented. However, the incidence of VPS, VT as well as VF was greatly reduced compared to untreated animals (Fig. 3.2).

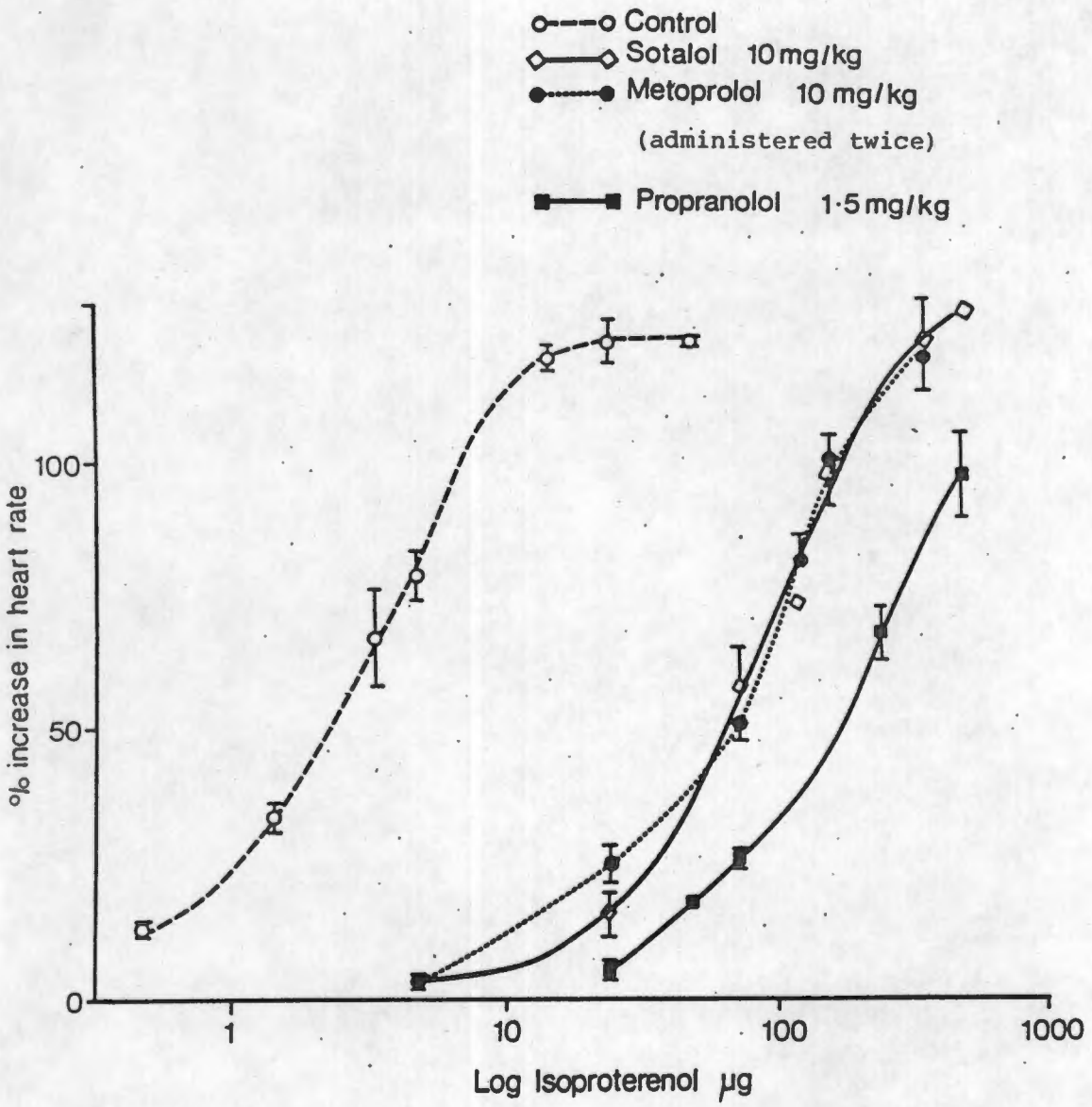
FIGURE 5.2

2. Dose-effect curves after administration of metoprolol and propranolol (Fig. 5.3)

In the preceding arrhythmia study, propranolol and metoprolol were administered 30 minutes prior to ligation. A second dose of metoprolol was given 10 minutes after ligation. Between about 10 and 30 minutes after ligation, a high incidence of VF was evident in the propranolol group, while only two episodes of VF were recorded in the metoprolol group.

We compared the beta-antagonistic potency of propranolol and metoprolol during a period that would coincide with this period of arrhythmias. Isoproterenol (46,5 to 465 µg) was therefore administered as described in paragraph 1 between about 40 and 60 minutes after propranolol 1,5 mg/kg was given (3 pigs) and directly after the second dose of metoprolol, following the first dose by 40 minutes (3 pigs).





Log dose-effect curves for isoproterenol on heart rate in the absence and presence of beta-antagonism.

FIGURE 5.3

Results: Propranolol 1,5 mg/kg resulted in a much further shift of the dose-effect curve to the right, indicating that it was more potent to occupy beta-receptors in the biophase than metoprolol (10 mg/kg given twice).

D. Motives for studies undertaken with sotalol

Following from the dose-effect curve results, it appears that failure of propranolol (1,5 mg/kg) to prevent the development of VF may have been the result of too high a beta-antagonistic dose.

In contrast to metoprolol, propranolol possesses potent membrane stabilizing activity, which could have resulted in cardiosuppressant effects, which may have contributed to the development of VF. Furthermore, propranolol exhibits non-selective beta-antagonism, while metoprolol preferentially antagonises cardiac beta-receptors.

Sotalol is a non-selective beta-antagonist without membrane stabilizing activity. Major differences between propranolol, metoprolol and sotalol are summarized:

	Cardioselective	Membrane Activity
Propranolol	No	Yes
Metoprolol	Yes	Little
Sotalol	No	None

Dose-effect investigations with sotalol revealed that a single dose of 10 mg/kg resulted in a similar shift of the dose-effect curve, to that after metoprolol administration (Fig. 5.3).

E. Effect of sotalol, a non-selective beta-antagonist, on tissue levels of cyclic AMP and arrhythmias (Fig. 5.4 and Table 5.2).

Sotalol HCl powder (Bristol Myers) was dissolved in water for injection and administered in a dose of 10 mg/kg, 30 minutes prior to ligation (6 pigs).

Following sotalol administration, tissue levels of cyclic AMP showed similar patterns to those after metoprolol or propranolol administration.

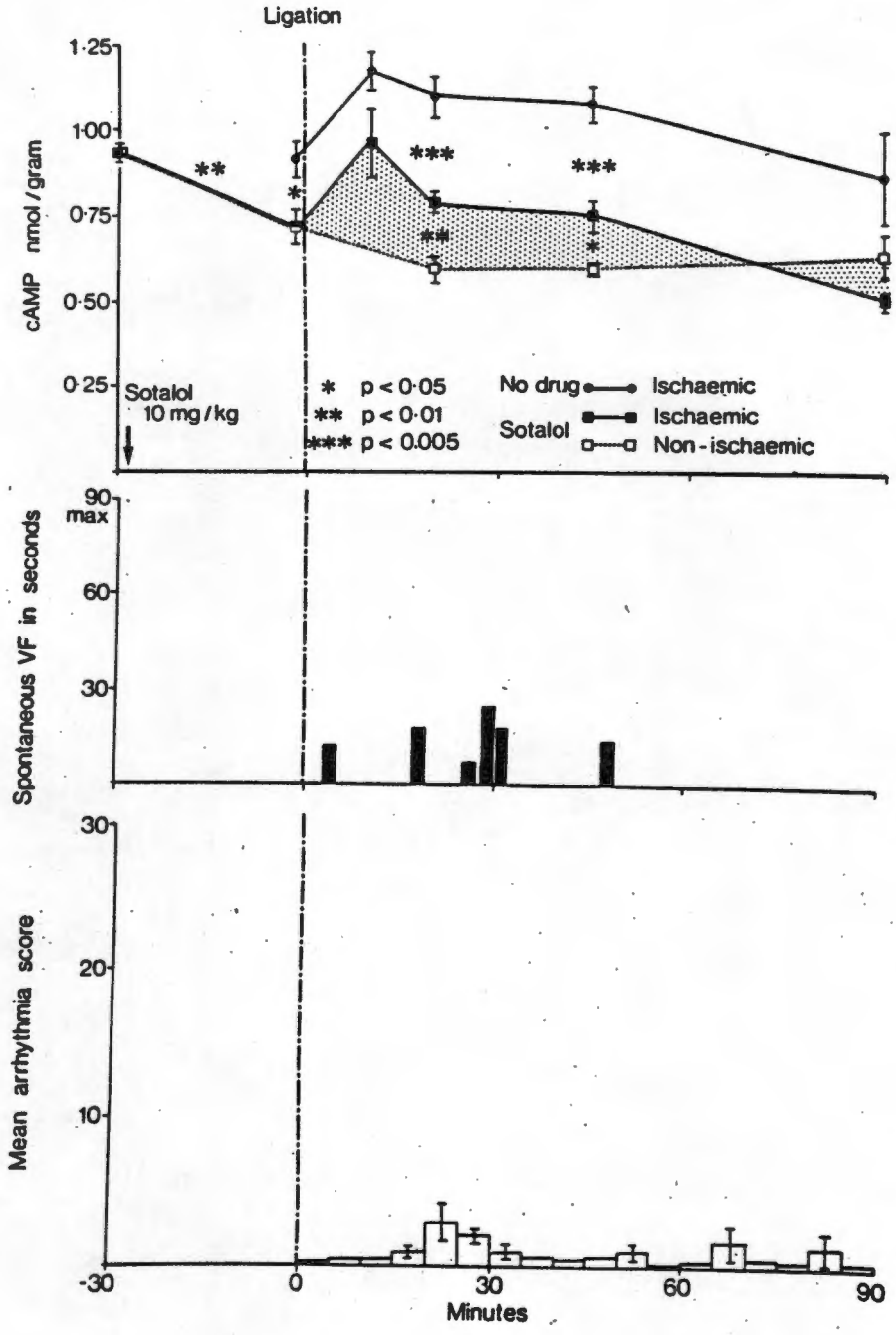
Sotalol, like propranolol, reduced the incidence of VPS and VT but did not prevent VF.

It could be argued that the administration of a second dose of metoprolol 10 minutes after ligation (i.e. at the beginning of the period of arrhythmias) was essential to prevent VF. We therefore administered a second dose of sotalol 10 mg/kg 10 minutes after ligation (4 pigs). However, the intervening dose still did not prevent the development of VF.

Thus the non-selective beta-antagonists propranolol and sotalol (without membrane activity) reduced the incidence of VPS and VT, but the incidence of VF remained high as in untreated pigs. The cardioselective beta-antagonist metoprolol prevented VPS, VT as well as VF. Therefore cardioselectivity appears to be important to prevent VF.

F. Differences in residual levels of tissue cyclic AMP and the incidence of ventricular fibrillation in the propranolol, metoprolol and sotalol group (Table 5.3)

At 20 and 45 minutes after ligation, tissue levels of cyclic AMP in ischaemic as well as in non-ischaemic tissue of the metoprolol group



Effect of sotalol on tissue levels of cyclic AMP and arrhythmias in 6 pigs. Sotalol did not prevent the accumulation of cyclic AMP in ischaemic tissue after ligation. The incidence of VF was similar to that in untreated pigs. However, the incidence of VPS and VT was reduced.

FIGURE 5.4

TABLE 5.3

Tissue levels of cyclic AMP in ischaemic and non-ischaemic zones after the administration of a beta-antagonist and ligation

		MINUTES AFTER LIGATION			
		10	20	45	90
metoprolol (n = 8)	ischaemic	1,09 ±0,05	0,94 ±0,04	0,93 ±0,06	0,69 ±0,07
	non-ischaemic	-	0,73 ±0,01	0,76 ±0,03	0,79 ±0,06
propranolol (n = 6)	ischaemic	0,96 ±0,05	0,81* ±0,02	0,61* ±0,05	0,39* ±0,03
	non-ischaemic	-	0,61* ±0,03	0,62* ±0,03	0,62 ±0,02
sotalol (n = 6)	ischaemic	0,96 ±0,10	0,79* ±0,03	0,75* ±0,04	0,50 ±0,03
	non-ischaemic	-	0,60* ±0,04	0,60** ±0,01	0,63 ±0,06

* $p < 0,05$ vs metoprolol

** $p < 0,001$ vs metoprolol

were higher than in the sotalol or propranolol group. At this time, a lower incidence of VF was evident in the metoprolol group. These results may indicate that a critical reduction in tissue levels of cyclic AMP after ligation is essential to prevent the development of VF.

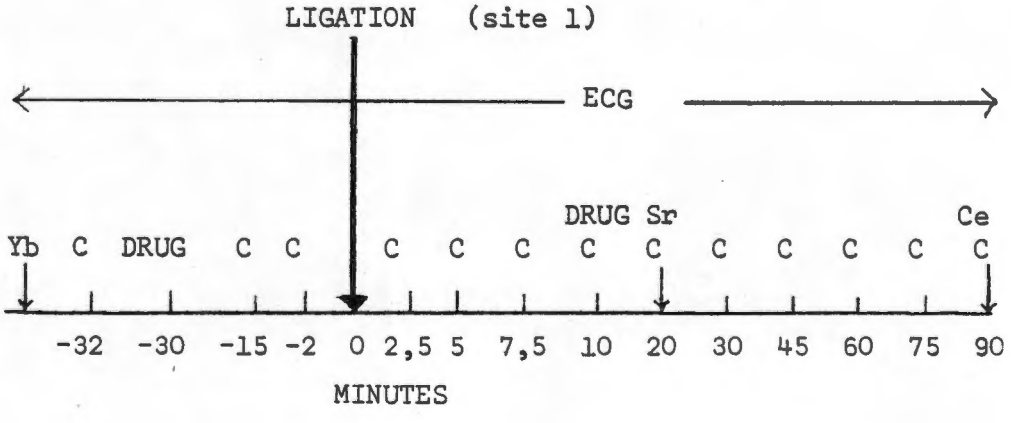
G. Tissue levels of ATP, phosphocreatine and lactate in the ischaemic zone, after administration of propranolol, metoprolol and sotalol. (Table 5.4).

During the period of arrhythmias, (i.e. 10 and 20 minutes after ligation), tissue levels of ATP and PCr in the metoprolol group were higher than in the propranolol group. Tissue levels in the sotalol group showed intermediate values.

Tissue levels of lactate were similar in the three groups.

H. Effect of propranolol, metoprolol and sotalol on regional myocardial blood flow, and mechanical function of the ischaemic left ventricle.

1. Experimental protocol



ECG = limb leads 1 and 2 or 3 registered continuously at paper speed of 25 mm/sec.

Yb = Yterbium microspheres

Sr = Strontium microspheres

Ce = Cerium microspheres

C = Measurement of left ventricular contractile activity, - systolic, and - end diastolic pressure.

DRUG = administration of propranolol, metoprolol or sotalol, in doses as described earlier.

(See Chapter 2 for details on methodology)

2. Results

a. Influence of beta-adrenoceptor antagonism on regional left ventricular blood flow (Fig. 5.5)

In all animals pretreated with a beta-antagonist, blood flow in subendo- and subepicardial portions of the left ventricle with regional ischaemic was similar, as was the case prior to drug administration and ligation.

1. Twenty minutes after ligation

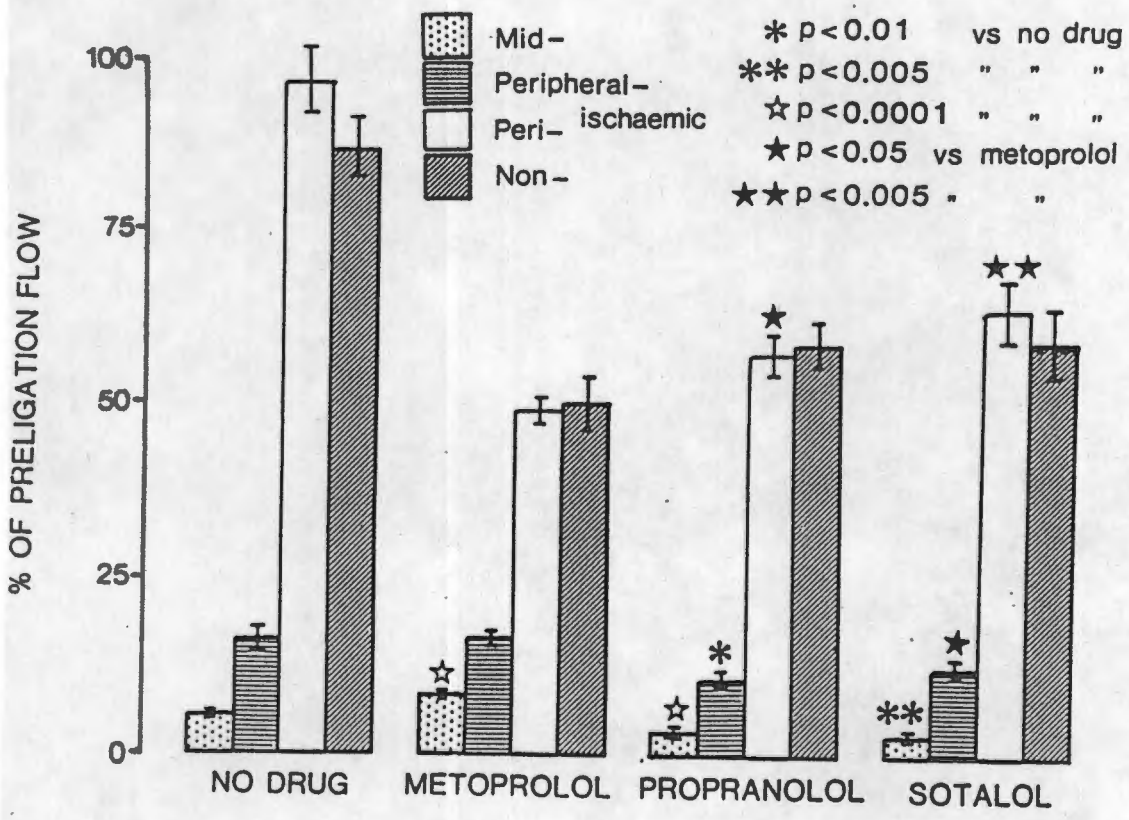
a. Mid- and peripheral ischaemic zones

Metoprolol administration increased blood flow in the middle of the ischaemic lesion to 8,6 ± 0,6% (n = 31) (percentage of pre-ligation control value) versus 5,7 ± 0,1% (n = 28; $p < 0,0001$) in untreated animals. On the contrary, both propranolol and sotalol administration reduced blood flow in the mid-ischaemic zone. Residual flow in the propranolol group was 3,6 ± 0,4% of the pre-ligation control

TABLE 5.4 TISSUE LEVELS OF ADENOSINE TRIPHOSPHATE (ATP), PHOSPHOCREATINE (PCr) AND LACTATE AFTER THE ADMINISTRATION OF PROPRANOLOL, METOPROLOL and SOTALOL.

				Ischaemic Zone			
		Before drug	Before ligation	MINUTES AFTER LIGATION 10	20	45	90
Propranolol (n = 6)	ATP	3,35 ± 0,23	3,97 ± 0,28	2,09* ± 0,21	1,22* ± 0,21	0,61 ± 0,17	0,67 ± 0,21
	PCr	7,01 ± 0,76	7,06 ± 0,72	0,68* ± 0,21	1,11 ± 0,20	0,80 ± 0,21	2,21 ± 1,00
	Lactate	11,74 ± 4,50	12,29 ± 3,86	27,11 ± 3,90	38,80 ± 2,59	44,52 ± 5,85	37,43 ± 4,12
Metoprolol (n = 8)	ATP	3,77 ± 0,26	4,07 ± 0,20	2,86 ± 0,22	2,42 ± 0,38	0,79 ± 0,17	1,06 ± 0,41
	PCr	6,61 ± 0,64	6,25 ± 0,74	2,35 ± 0,58	1,86 ± 0,59	1,09 ± 0,41	1,44 ± 0,38
	Lactate	9,60 ± 3,62	9,96 ± 2,98	30,73 ± 6,03	41,77 ± 6.19	43,25 ± 7,29	47,15 ± 8,59
Sotalol (n = 6)	ATP	3,89 ± 0,25	3,97 ± 0,32	2,23 ± 0,21	1,68 ± 0,37	0,41 ± 0,06	0,19 ± 0,06
	PCr	7,23 ± 0,39	7,36 ± 0,45	2,13 ± 1,16	1,40 ± 1,00	1,08 ± 0,55	0,74 ± 0,60
	Lactate	8,12 ± 1,68	9,25 ± 2,35	31,77 ± 2,67	44,67 ± 5,07	49,53 ± 4,83	47,86 ± 9,24

* $p < 0,05$ vs metoprolol



Twenty minutes after ligation, blood flow in the mid-ischaemic zone of the metoprolol group was higher, while blood flow in the propranolol and sotalol groups was lower than in untreated animals. Propranolol administration also decreased blood flow in the periphery of the ischaemic lesion.

FIGURE 5.5

value (n = 16; $p < 0,0001$) and in the sotalol group 3,2 ± 0,6% (n = 19; $p < 0,005$, both vs untreated animals).

Blood flow in the periphery of the ischaemic lesions in the metoprolol and sotalol groups was similar to that in untreated pigs, while propranolol administration decreased blood flow to 10,9 ± 1,0% (n = 12) versus 16,2 ± 1,7% (n = 28; $p < 0,01$) in untreated animals.

b. Peri- and non-ischaemic zones

In the peri-ischaemic zone (i.e. just outside the visible edge) blood flow in the propranolol group was 57,0 ± 3,0% (n = 18) of the pre-ligation value, and in the sotalol group 63,3 ± 4,2% (n = 18). However, in the metoprolol group blood flow was 49,0 ± 1,9% (n = 38). Blood flow in the metoprolol group was lower than in both the propranolol ($P<0.05$, and sotalol groups ($P<0.005$).

All three beta-antagonists caused a reduction of about 50% in blood flow in non-ischaemic zones.

2. Differences in regional left ventricular blood flow 20 and 90 minutes after ligation (Table 5.5).

a. Mid- and peripheral ischaemic zones

A further deterioration of blood flow was evident 90 minutes after ligation in animals pretreated with beta-antagonists, as was the case in untreated pigs.

b. Peri- and non-ischaemic zones

In the sotalol group and in untreated pigs, blood flow at 90 minutes was similar to that at 20 minutes. In the metoprolol group, blood flow at 90 minutes was higher. In the propranolol group, values

TABLE 5.5

Blood flow in the ischaemic and non-ischaemic left ventricle 20 and 90 minutes after ligation (percentage of preligation control)

		20 min		90 min
No drug	mid	5,7 ± 0,1	*	3,4 ± 0,9
	periph isch	16,2 ± 1,7	*	10,0 ± 1,4
	peri	96,7 ± 4,8		87,1 ± 5,0
	non	87,1 ± 4,3		89,1 ± 4,8
Metoprolol	mid	8,6 ± 0,6	***	3,2 ± 0,2
	periph isch	16,5 ± 1,0	***	7,6 ± 0,7
	peri	49,0 ± 1,9	**	60,6 ± 3,2
	non	49,9 ± 3,9	*	64,5 ± 3,6
Propranolol	mid	3,6 ± 0,4	**	1,9 ± 0,1
	periph isch	10,9 ± 1,0		10,2 ± 0,7
	peri	57,0 ± 3,0	*	48,9 ± 2,5
	non	58,5 ± 3,2	*	50,1 ± 2,0
Sotalol	mid	3,2 ± 0,6		1,7 ± 0,5
	periph isch	12,5 ± 1,2		9,7 ± 1,1
	peri	63,3 ± 4,2		68,1 ± 4,7
	non	58,8 ± 4,8		65,2 ± 8,3

* $p < 0,05$ 20 versus 90 minutes

** $p < 0,005$

*** $p < 0,0001$

at 90 minutes were lower than those at 20 minutes.

3. Redistribution of blood flow by metoprolol (Table 5.5)

Blood flow in the mid- and peripheral ischaemic zones in the metoprolol group was lower at 90 minutes than at 20 minutes. In contrast, blood flow in the peri- and non-ischaemic zones was higher at 90 minutes than at 20 minutes.

Metoprolol administration may have resulted in a temporary redistribution of blood flow in favour of the ischaemic myocardium 20 minutes after ligation. The low incidence of VF at this time in the metoprolol group may have been related to this effect.

b. Effect of metoprolol and sotalol on mechanical function of the left ventricle (propranolol was not studied). (Table 5.6)

In untreated animals, main stem ligation was followed by a decrease in maximal rate of pressure development of the left ventricle (dP/dt max) by about 20% and an increase in left ventricular end diastolic pressure (LVEDP) by about 60% (Chapter 3). After the administration of metoprolol or sotalol, but prior to ligation, a sharper decrease in dP/dt max occurred (about 65%) but similar increases occurred in LVEDP than after ligation in untreated animals. Coronary ligation did not result in further changes in these parameters. However, the second dose of metoprolol given 10 minutes after ligation, resulted in a further increase in LVEDP.

The administration of metoprolol and sotalol did not have any effect on left ventricular systolic pressure.

TABLE 5.6

Effect of beta-antagonism and ligation on mechanical function of the left ventricle, and heart rate.

METOPROLOL (n = 5). Size of ischaemic lesion 15,9%±0,8%

	MINUTES BEFORE LIGATION				MINUTES AFTER LIGATION										
	-32 Pre-drug		-15	-2	2,5	5	7,5	10	15	20	30	45	60	75	90
HR	108 ±5		83** ±5	86 ±7	86 ±7	86 ±7	86 ±7	86 ±7	81 ±7	75 ±6	78 ±4	78 ±6	80 ±6	84 ±7	82 ±7
LVSP	108 ±6		107 ±5	99 ±3	100 ±4	102 ±4	100 ±4	100 ±5	92 ±7	84 ±4	90 ±1	97 ±4	96 ±4	98 ±6	102 ±1
LVEDP	4,7 ±0,6		8,5* ±0,9	8,2** ±0,6	9,7 ±1,2	10,0 ±1,2	10,0 ±0,7	10,7 ±1,2	12,0 ±1,7	13,5++ ±0,5	12,7+ ±0,8	12,5+ ±1,0	11,7 ±1,1	11,5 ±1,3	11,7 ±1,4
LVdP/dt (max)	2397 ±72		1277** ±92	1556* ±100	1431 ±126	1507 ±88	1492 ±99	1388 ±216	938 ±189	771 ±83	1012 ±110	1267 ±101	1336 ±143	1385 ±167	1383 ±149
					SOTALOL (n = 5). Size of ischaemic lesion 15,4%±0,6%										
HR	105 ±2		68*** ±2+	66** ±3†	65† ±3	65† ±3	66† ±5	66† ±4	66 ±4	66 ±4	67 ±4	66 ±4	66 ±3	67† ±3	68† ±4
LVSP	99 ±4		93 ±1	98 ±3	93 ±2	93 ±6	99 ±6	98 ±4	97 ±6	100 ±7	106† ±4	93 ±4	106 ±7	111 ±4	108 ±4
LVEDP	4,2 ±0,7		7,2 ±1,0	7,6* ±1,4	9,2 ±0,8	10,6 ±0,5	12,5 ±2,5	10,4 ±1,5	9,4 ±0,9	10,2 ±1,5	9,6 ±1,8	7,8 ±1,0	7,8 ±1,0	7,2 ±0,5	7,0 ±0,6
LVdP/dt (max)	2247 ±89		1410** ±72	1359** ±104	1306 ±96	1182 ±108	1227 ±125	1225 ±110	1210 ±125	1265 ±111	1387 ±84	1293 ±105	1359 ±154	1413 ±142	1393 ±182

DRUG ADMINISTRATION

LIGATION

* p < 0,05
** p < 0,01 vs pre-drug
*** p < 0,001

\+ p < 0,05
++ p < 0,01 vs pre-ligation
† p < 0,05 vs metoprolol

HR = heart rate
LVSP = left ventricular systolic pressure
LVEDP = left ventricular end diastolic pressure
LVdP/dt (max) = maximal rate of pressure development of the left ventricle

Thus the administration of both beta-antagonists resulted in a considerable deterioration of mechanical function of the left ventricle. The question now remains whether decreasing the dose of metoprolol would have less deleterious effects on mechanical function and, if so, whether the marked protection against the development of VF by the agent would be retained.

c. Effect of beta-antagonism on heart rate before and after ligation

Although sotalol and propranolol initially slowed the heart more than metoprolol, similar rates were registered between 15 and 60 minutes after ligation, i.e. during the time of rhythm disturbances.

d. Q-T interval prolongation

The Q-T interval in the pig measured by us is 0,39 ± 0,02 seconds (n = 6). This falls well within the normal range for humans (0,35 to 0,44 seconds in the adult; Estes 1974).

Fifteen minutes after the commencement of sotalol administration, this interval was increased to 0,46 ± 0,02 seconds ($p < 0,005$) after a correction was made for the decreased heart rate. This prolongation was maintained for the duration of the experiment. No such effect was found after metoprolol administration. This interval was not measured in the propranolol pretreated group.

IV. DISCUSSION

A. Effect of beta-adrenoceptor antagonism on the accumulation of tissue cyclic AMP and the incidence of ventricular arrhythmias.

1. Tissue levels of cyclic AMP after the administration of a beta-antagonist.

Ligation of the main stem of the left anterior descending coronary artery is followed by an accumulation of cyclic AMP in ischaemic tissue, occurring concomitantly with a period of severe ventricular arrhythmias, including VF. When the beta-adrenoceptor antagonist propranolol, metoprolol or sotalol was administered 30 minutes prior to coronary ligation, tissue levels of cyclic AMP in the left ventricle were sharply reduced. However, despite this low level prior to ligation, tissue levels of cyclic AMP in the ischaemic zone early after ligation were higher than in non-ischaemic tissue, following similar patterns than in untreated animals.

Possible reasons for the persistent accumulation of cyclic AMP after ligation

a. The competitive antagonism was surmountable due to:

   (1) An enhanced sympathetic activity confined to the ischaemic lesion, as a result of a local release of noradrenaline from storage sites in the end-neuron (Wollenberger and Shahab 1965, Wollenberger *et al* 1967).

   (2) Liberation of noradrenaline from the end-neuron by products of ischaemia e.g. potassium (Skinner *et al* 1975, Borda *et al* 1977) and lactate and other acid metabolites (Potter and Axelrod 1963).

b. The emergence of beta-receptors under conditions of ischaemia

(Mukherjee *et al* 1979).

c. Enhancement of adenylate cyclase activity (Mori 1976) by lysophosphoglycerides (Sobel *et al* 1978) or via receptors other than those belonging to the beta-adrenergic type.

2. Beta-antagonism and the incidence of ventricular arrhythmias after coronary ligation

Despite a persistent accumulation of tissue levels of cyclic AMP in the ischaemic zone in propranolol, metoprolol and sotalol pre-treated animals, the incidence of VF differed between groups. In the propranolol and sotalol groups, the incidence of VF was as high as in untreated animals. When metoprolol was administered 30 minutes prior to ligation, the incidence of VF was greatly reduced.

All three beta-antagonists reduced the incidence of VPS as well as VT after ligation.

Two questions arise from these results:

a. Is cyclic AMP implicated in the genesis of ventricular arrhythmias?

The dissociation of accumulated cyclic AMP in zones of ischaemia and the genesis of VF in the metoprolol group may imply that cyclic AMP is not arrhythmogenic and that the links described in Chapter 3 were coincidental. However, it should be considered that, for a critical tissue level of cyclic AMP to be arrhythmogenic, other factors must be present:

Increased extracell potassium (Hirche *et al* 1980) may abolish fast responses in zones of ischaemia. Accumulated cyclic AMP under these conditions, may evoke "slow responses" (Reuter 1974) which have been

strongly implicated in arrhythmogenesis (Chapter 1). Metroprolol administration could have prevented the accumulation of extracell potassium in the ischaemic zone.

Cyclic AMP may only be an intermediate messenger in an arrhythmogenic chain of events. The cyclic AMP mediated influx of calcium ions may be prominently involved in ventricular arrhythmogenesis.

b. Are different mechanisms involved in the genesis of ventricular tachycardia and ventricular fibrillation?

The development of "slow responses" is thought to underlie both the development of VT and VF. However, these results showing that sotalol and propranolol protected the ischaemic myocardium from developing VT, but not VF, raise the possibility that the genesis of these arrhythmias may involve different mechanisms.

3. A critical reduction in tissue levels of cyclic AMP - an important prerequisite to prevent ventricular fibrillation after ligation.

After the administration of metoprolol, higher residual tissue levels of cyclic AMP were evident in ischaemic and non-ischaemic tissue, than in sotalol and propranolol pretreated animals. These differences were most prominent at the time of a low incidence of VF in the metoprolol group and a higher incidence of VF in the other two groups.

These results suggest that a critical level of sympathetic activity in ischaemic, but more likely in non-ischaemic zones, as indicated by local tissue levels of cyclic AMP may be important to

prevent the genesis of VF. This may be the reason why a low dose of propranolol in the study of Pentecost and Austin (1966, Table 5.1) protected against the development of VF, while a high dose did not. Similarly, Khan *et al* (1972, Table 5.1) found propranolol 0,1 mg/kg IV to prevent VF, while 1,0 mg/kg IV did not prevent the genesis of VF. It appears that protection against VF never occurred, once a dose of propranolol as high as 0,2 mg/kg was used.

Pentecost and Austin attributed the ineffectiveness of the high dose to a severe suppression of mechanical function. In our experiments, mechanical function was equally suppressed by metoprolol and sotalol and could therefore not account for the difference in the incidence of VF in the two groups.

B. <u>The validity of constructing dose-chronotropic effect curves to indicate adrenolytic potency of an antagonist in the end-synapse in ventricular tissue</u>

Sotalol 10 mg/kg administered once (p 104) and metoprolol 10 mg/kg administered twice (p 102) were equipotent in shifting the dose-effect curve for isoproterenol on heart rate to the right. When administered in the same doses and during the same time course as above in the cyclic AMP/arrhythmia studies, they were not equally potent occupying the end-synaptic receptors in the left ventricular tissue as judged by residual tissue levels of cyclic AMP. This was especially evident at 20 and 45 minutes after ligation.

Furthermore, sotalol and propranolol administration resulted in similar residual tissue levels of cyclic AMP after ligation, but showed dissimilar shifts of the dose-effect curve. Therefore, if tissue levels of cyclic AMP could be held to indicate activity at the level of the beta-adrenoceptor, our results indicate that the ability of an antagonist to block isoproterenol-induced chronotropic response does not accurately reflect its end-synaptic adrenolytic potency in the left

ventricle.

C. Electrophysiological properties of propranolol, metoprolol and sotalol

According to our results, the classification of propranolol, metoprolol and sotalol as Class II antiarrhythmic agents according to Vaughan-Williams (1975) is still valid, for all three showed anti-arrhythmic activity which could be associated with an adrenolytic action. Sotalol prolongs the Q-T interval and was therefore also classified as a Class III anti-arrhythmic agent (Singh and Vaughan-Williams (1970)). In our study, Q-T interval prolongation also followed sotalol administration. Metoprolol and propranolol did not exhibit this property. However, no distinct anti-arrhythmic effect could be ascribed to sotalol in our experiments, for propranolol, metoprolol and sotalol reduced the incidence of VPS and VT. However, only metoprolol decreased the incidence of VF. Metoprolol may possess some electrophysiologic property (e.g. inhibiting the slow inward calcium channel) which could underlie the marked anti-fibrillatory effect.

D. Preservation of high energy phosphates during beta-antagonism, and arrhythmias

At the time of a low incidence of VF in the metoprolol group, tissue levels of adenosine triphosphate and phosphocreatine were higher than in the propranolol group (high incidence of VF). However, in the sotalol group, in which the incidence of VF was also high, intermediate values of high energy phosphates were evident.

Further investigations will have to be done to enable us to comment on possible anti-arrhythmic effects mediated by the preservation of high energy phosphate compounds.

E. Effect of propranolol, metoprolol and sotalol on regional left ventricular blood flow after ligation

1. The classification of coronary beta-receptors

Metoprolol preferentially antagonises myocardial beta-adrenoceptors, while propranolol and sotalol antagonise $beta_1$ as well as $beta_2$ receptors. When an attempt is made to explain differences in regional left ventricular blood flow after the administration of these antagonists, the dispute on the classification of beta-receptors in the coronary bed needs attention.

Evidence in favour of the classification of cardiac vascular receptors as belonging to the $beta_1$ sub-type has been presented by Baron (1972) and Johansson (1973). On the contrary, most *in vivo* studies showed that vascular receptors belong to the $beta_2$-subtype (Mark *et al* 1972, Gross and Feigl 1975) or that they are at least different from myocardial $beta_1$ receptors (Adam *et al* 1970, Ross and Jorgensen, 1970). From this follows that the term myocardial selectivity, rather than cardio-selectivity should be used (Bussman 1970).

2. Blood flow in the non-ischaemic zone

Propranolol and sotalol caused about a 40% decrease in blood flow in the non-ischaemic zone, compared to untreated animals, while metoprolol administration resulted in a slightly further decrease. This is in general agreement with previous studies with propranolol (Stein *et al* 1967, Pitt and Craven 1970, Becker *et al* 1972a) and is held to be basically the result of decreased contractility and heart rate, and subsequently a decreased oxygen demand.

In clinical practice, the significance of cardioselectivity remains a matter of dispute, especially when high doses of cardioselective drugs are administered. In the non-ischaemic zone, where blood flow was high, relatively high concentrations of metoprolol could be expected to reach the biophase to occupy $beta_1$ but also vascular $beta_2$ receptors, as would be the case with propranolol and sotalol. This would then result in an unopposed alpha-receptor mediated vasoconstriction. This sequence of events could have contributed to the decrease in flow in the non-ischaemic zone.

3. Blood flow in the ischaemic zone

In the mid-ischaemic zone, metoprolol increased blood flow, compared to untreated animals. On the contrary, propranolol and sotalol decreased blood flow in this zone. Because relatively lower concentrations of these antagonists could be expected to be present in the mid-ischaemic zone, metoprolol may now preferentially antagonise $beta_1$ receptors. $Beta_2$ receptors in the coronary bed would then be left unopposed to maintain normal vascular tone. Our results suggest significant adrenergic activity in zones of ischaemia, as reflected by local tissue levels of cyclic AMP, despite beta-antagonism. Localised noradrenaline would be available to facilitate vasoconstriction via unopposed alpha receptors in the presence of $beta_2$ receptor antagonism by propranolol and sotalol. Marshall and Parratt (1978) actually measured an active increase in resistance to blood flow in ischaemic zones after propranolol, but not after practolol (cardioselective beta-antagonist) administration.

This decrease in the severity of ischaemia as indicated by an increase in blood flow in the ischaemic zone after metoprolol administration may directly underlie the low incidence of VF in this group.

SUMMARY AND CONCLUSIONS, CHAPTER 5

1. The beta-adrenoceptor antagonists metoprolol, propranolol and sotalol could not prevent the accumulation of cyclic AMP in the ischaemic zone, following main stem ligation. The reasons for this may be that the competitive antagonism was probably surmountable. The emergence of beta-receptors in the ischaemic zone, or enhancement of adenylate cyclase activity should also be considered to underlie this phenomenon.

2. These results question the link between cyclic AMP and arrhythmias, for metoprolol decreased the incidence of VF in the presence of accumulated cyclic AMP in the ischaemic zone. However, cyclic AMP may be an intermediate messenger in an arrhythmogenic chain of events.

3. A decrease in the severity of ischaemia, as indicated by an increase in blood flow in the ischaemic zone in the metoprolol group may underlie the low incidence of VF in this group. The cardioselective properties of metoprolol are suggested to be directly related to this beneficial effect on blood flow.

4. Sotalol and propranolol decreased the incidence of VPS and VT but failed to decrease the incidence of VF. These results suggest that the mechanisms which underlie VT and VF may differ.

5. A critical level of local adrenergic activity in the left ventricle after ligation and beta-antagonism may maintain an undisturbed ventricular rhythm.

6. Dose-chronotropic response studies with beta-antagonists may not accurately predict the potency of these antagonists to occupy beta-receptors left ventricular tissue.

CHAPTER 6

CATECHOLAMINE STIMULATION, CYCLIC AMP AND VENTRICULAR ARRHYTHMIAS. THE EFFECT OF CALCIUM CHANNEL INHIBITION ON CATECHOLAMINE-INDUCED AND SPONTANEOUS VENTRICULAR ARRHYTHMIAS FOLLOWING CORONARY ARTERY LIGATION

I. AIM OF EXPERIMENTS

a. Would enhanced tissue levels of cyclic AMP during catecholamine stimulation increase the incidence of ventricular arrhythmias?

In Chapter 3, we presented strong, however indirect evidence that an accumulation of cyclic AMP in the ischaemic myocardium is linked to the genesis of ventricular arrhythmias following coronary artery ligation. If this holds true, a further increase in tissue cyclic AMP, by introducing beta-receptor stimulation should enhance its pathological effects, resulting in a higher incidence of ventricular arrhythmias.

Ligation of a single lateral branch of the anterior descending coronary artery provided a suitable model to test this proposition, for minimal changes in cyclic AMP after ligation were associated with minor rhythm disturbances. (Chapter 3).

b. Effect of the calcium channel blocking agent verapamil on catecholamine induced ventricular arrhythmias

Electrophysiological studies showed that the calcium ion may be implicated as a further messenger of cyclic AMP mediated ventricular arrhythmias. It could be expected that elevated tissue levels of cyclic AMP during catecholamine stimulation would enhance calcium ion influx. To assess the role of the calcium ion in ventricular arrhythmogenesis, we investigated the effect of the calcium channel blocking agent verapamil on arrhythmias following anterolateral ligation and during catecholamine stimulation.

c. Effect of verapamil on spontaneous ventricular arrhythmias following coronary artery ligation

Several animal studies reported significant protection by calcium channel blocking agents against VF following coronary artery ligation. In the pig, ligation of the main stem of the anterior descending coronary artery resulted in a high incidence of VF. (Chapt. 3)

We tested the effect of verapamil in this model.

II. METHODS AND RESULTS

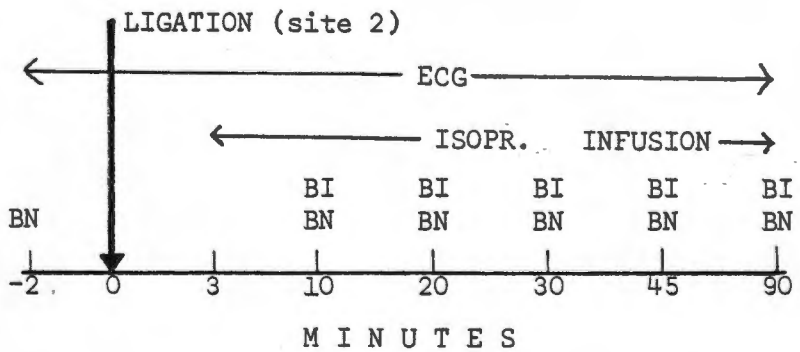
A. Tissue levels of cyclic AMP and arrhythmias following antero-lateral ligation and catecholamine stimulation. The effect of the addition of verapamil

1. Methods

a. Group 1 (12 pigs) Pigs with ligation of a single anterolateral branch of the left descending coronary artery (site 2, Chapter 3) served as controls.

b. Group 2 (7 pigs) Three minutes after ligation of a single anterolateral branch isoproterenol infusion (0,5 µg/kg min^{-1}) was started and continued up to the end of the experiment. Isoproterenol HCl (ampoules, containing 0,2 mg/ml, Winthrop) was used.

Protocol:



ECG = limb leads 1 and 2 or 3, registered continuously at paper speed of 25 mm/sec.

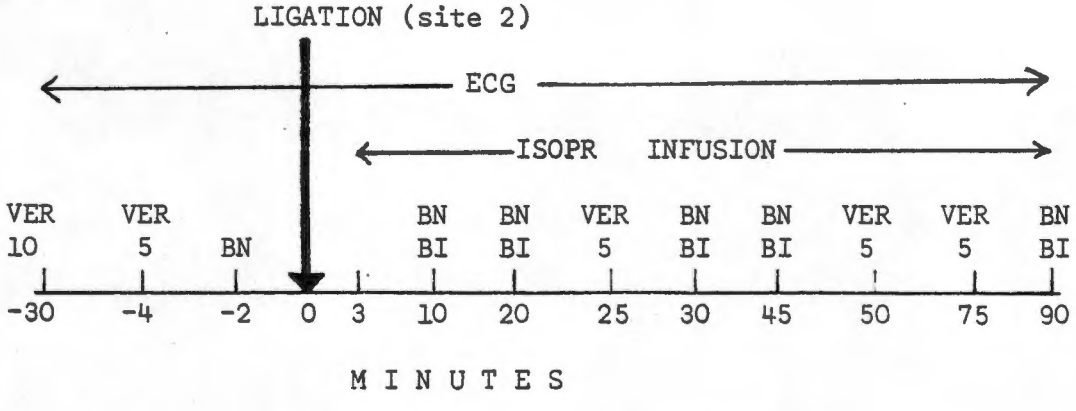
ISOPR = isoproterenol 0,5 µg/kg min^{-1}

BN = biopsy from non-ischaemic left ventricle

BI = biopsy from ischaemic left ventricle

c. Group 3 (6 pigs): Verapamil 0,9 mg/kg was administered in divided doses. Isoproterenol was infused as in group 2. Verapamil (ampoules containing 5 mg/2ml, Knoll) was used

Protocol:



ECG = limb leads 1 and 2 or 3, registered continuously

ISOPR = isoproterenol 0,5 µg/kg min^{-1}

BN = biopsy from non-ischaemic left ventricle

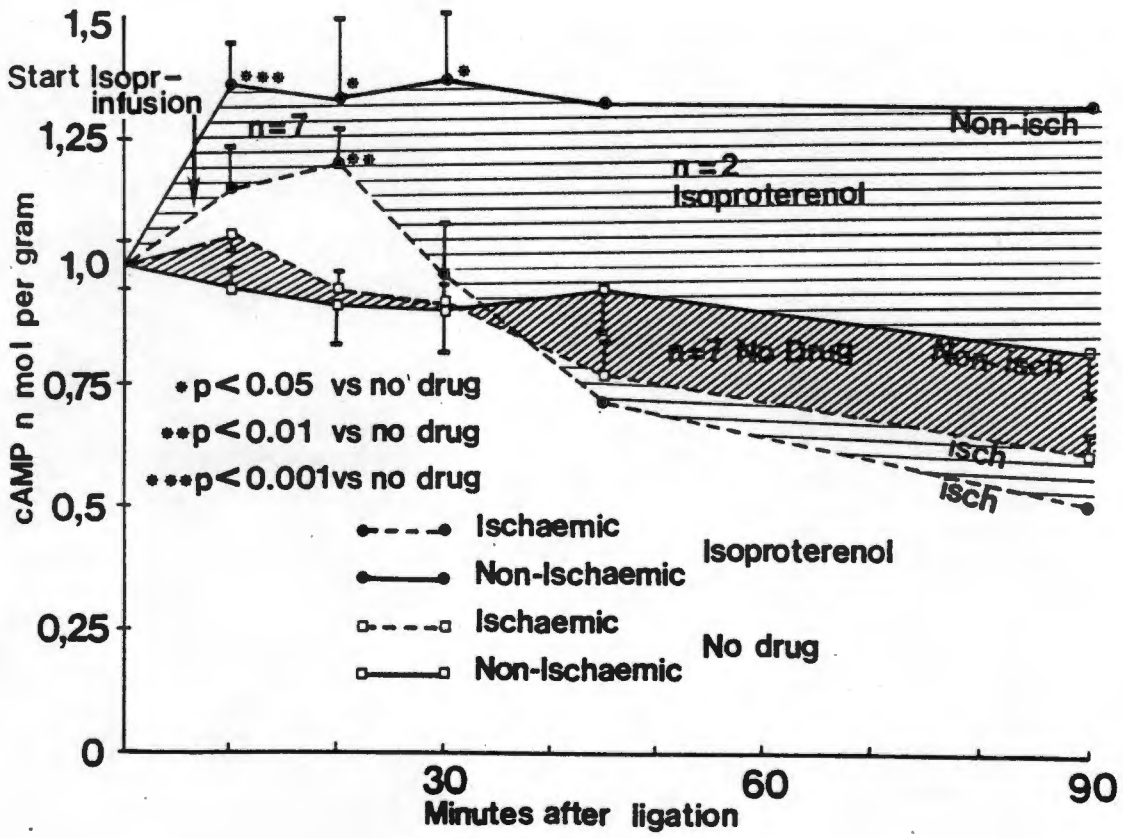
BI = biopsy from ischaemic left ventricle

VER = verapamil 10 mg or 5 mg, administered over 5 minutes.

2. Results

a. Tissue levels of cyclic AMP (Fig. 6.1)

Group 1: A transient increase in tissue levels of cyclic AMP in ischaemic tissue was evident 10 minutes after ligation.



Tissue levels of cyclic AMP after ligation at site 2, in the absence and presence of isoproterenol infusion.

FIGURE 6.1

Group 2: During isoproterenol administration, at the time of the first biopsy, (i.e. 10 minutes after ligation) levels of cyclic AMP in non-ischaemic tissue were sharply increased, compared to Group 1. In ischaemic tissue only a transient elevation of cyclic AMP occurred during 30 minutes following ligation.

Group 3: (not included in figure). The addition of verapamil in the presence of isoproterenol stimulation left high levels of cyclic AMP in ischaemic and non-ischaemic tissue unchanged.

b. Ventricular arrhythmias (Table 6.1)

Group 1: After ligation of an anterolateral branch, ventricular rhythm was largely stable, with a single episode of VF in 2 out of 12 pigs only.

Group 2: Within about 3 minutes after the onset of isoproterenol infusion, a regular continuous VT was precipitated in 6 out of 7 pigs. Seventeen episodes of VF occurred in 6 pigs and only two animals out of 7 survived the full duration of the experiment. The incidence of VPS did not differ from that from Group 1.

Group 3: Verapamil completely prevented VF associated with coronary artery ligation and isoproterenol infusion. The duration of VT also appeared to be decreased, however no significant difference could be shown. The incidence of VPS was similar to that in the other groups.

c. Tissue levels of adenosine triphosphate and phosphocreatine (Table 6.2)

Adenosine triphosphate. Levels of ATP in ischaemic tissue during isoproterenol administration in group 2 did not differ from those in

TABLE 6.1 ARRHYTHMIAS DURING 90 MINUTES FOLLOWING CORONARY ARTERY LIGATION

Drug and size of ischaemic lesion	Pig No.	Ventricular premature systoles late	Ventricular premature systoles early (R-on-T)	Ventricular tachycardia (seconds)	Ventricular fibrillation (seconds)
No Drug	1	7	1	0	0
	2	2	0	0	0
	3	48	1	0	0
6.9%	4	8	0	0	0
± 0.6%	5	4	0	0	0
(percentage of total left ventricular mass)	6	171	13	0	21
	7	3	0	0	0
	8	0	0	90	0
	9	36	0	0	0
	10	0	0	0	0
	11	36	1	0	90 (max)
	12	0	0	0	0
		23.4 ± 14.0	1.3 ± 1.1	7.5 ± 7.5	2 out of 12
	1	55	0	0	90 (max)
Isoproterenol	2	20	0	4260	48, 9, 60
8.5%	3	3	2	2820	18, 10, 28, 90 (max
± 1.6%	4	0	4	780	0
	5	0	0	1680	17, 13, 90 (max)
	6	0	1	1380	17, 21, 90 (max)
	7	10	5	3760	10, 30, 90 (max)
		12.5 ± 7.6	1.7 ± 0.7	2148.6 ± 625.9	6 out of 7 $^{\Delta}$
	1	75	1	120	0
	2	88	0	1885	0
Isoproterenol	3	1270	3	900	0
+	4	10	4	180	0
Verapamil	5	77	0	120	0
8.4%	6	43	0	1031	0
± 0.7%		260.5 ± 202.2	0.8 ± 0.6	706.0 ± 288.5	0 out of 6 $^{\Delta\Delta}$

$^{\Delta}$ $p < 0.01$ vs No Drug (Fisher's exact test)

$^{\Delta\Delta}$ $p < 0.005$ vs Isoproterenol (Fisher's exact test)

group 1. However, isoproterenol reduced tissue levels of ATP in the non-ischaemic zone. Note that the administration of verapamil prior to ligation in group 3 resulted in higher tissue levels of ATP than in group 1 and group 2. After ligation, verapamil maintained these higher levels in non-ischaemic tissue for 90 minutes after ligation amd preserved ATP in ischaemic tissue for at least 20 minutes, compared to both groups 1 and 2.

Phosphocreatine: Verapamil prevented the fall in PCr in non-ischaemic tissue during isoproterenol administration. Phosphocreatine levels in ischaemic tissue was similar in the three groups.

d. Arterial pressure and heart rate

Group 1: Ligation of the anterolateral branch of the left anterior descending coronary artery produced no alteration in both the systolic and diastolic aortic pressures.

Group 2: Isoproterenol infusion reduced the systolic pressure from 106 ± 6 to 47 ± 4 mmHg ($p < 0.01$) and the diastolic pressure from 65 ± 4 to 26 ± 3 mmHg ($p < 0.005$) within 5 minutes of ligation. isoproterenol increased the heart rate from 115 ± 7 to 175 ± 7 beats/min ($p < 0.01$).

Group 3: Verapamil administered prior to ligation decreased the systolic pressure from 108 ± 6 to 81 ± 5 mmHg ($p < 0.05$) and diastolic pressure from 66 ± 4 to 40 ± 5 mmHg ($p < 0.005$). During isoproterenol infusion there was no further reduction in either the systolic (78 ± 4 mmHg) or diastolic (36 ± 5 mmHg) pressure. Verapamil did not prevent the increase in heart rate by isoproterenol.

TABLE 6.2

TISSUE LEVELS OF ADENOSINE TRIPHOSPHATE AND PHOSPHOCREATINE BEFORE AND AFTER ANTEROLATERAL LIGATION

		Before ligation	Minutes after ligation	10	30
Group 1 No drug (n=12)	ATP	3.27 ±0.33	isch	1.92$^{\Delta\Delta}$ ±0.25	0.79 ±0.28
			non-isch	3.37$^{\Delta}$ ±0.13	2.98$^{\Delta}$ ±0.25
	PCr	8.54 ±0.30	isch	0.88 ±0.31	2.31 ±0.56
			non-isch	7.12 ±0.36	6.02* ±0.96
Group 2 Isoproterenol (n=7)	ATP	3.55 ±0.19	isch	2.38$^{\Delta}$ ±0.20	1.15 ±0.43
			non-isch	2.68$^{\Delta}$ ±0.43	1.52$^{\Delta}$ ±0.39
	PCr	7.16 ±0.98	isch	1.69 ±0.44	2.30 ±1.69
			non-isch	3.51* ±0.78	1.93$^{\Delta}$ ±1.05
Group 3 Isoproterenol + verapamil (n=8)	ATP	4.72** ±0.15	isch	4.18 ±0.29	1.31 ±0.71
			non-isch	4.56 ±0,25	5.27 ±0.57
	PCr	8.45 ±0.31	isch	2.72 ±0.03	2.08 ±1.57
			non-isch	7.05 ±0.74	8.32 ±0.54

* $p < 0.05$ vs isoprot. + verapamil

$^{\Delta}$ $p < 0.005$

$^{\Delta\Delta}$ $p < 0.0001$

isch = ischaemic non-isch = non-ischaemic

B. Effect of verapamil on spontaneous ventricular arrhythmias and tissue levels of cyclic AMP, and on high energy phosphates following ligation of the main stem of the left anterior descending coronary artery (6 pigs)

Verapamil 0.9 mg/kg was administered in divided doses, i.e. 0.2 mg 30 minutes prior to ligation and 0.1 mg/kg 5 minutes before and at 25, 50 and 75 minutes after ligation. This lower dose regime was used, because the higher dose used during isoproterenol stimulation resulted in second and third degree atrioventricular block (3 pigs). However, verapamil administered at this lower dose showed no anti-arrhythmic activity, for the incidence of all ventricular arrhythmias was as high as in the control group (Chapter 3). Whether higher doses of verapamil would decrease the incidence of arrhythmias after main stem ligation, but during electrical pacing, remains to be investigated.

Tissue levels of cyclic AMP and also of ATP and PCr after verapamil administration were similar than in the group with main stem ligation only.

Verapamil caused a transient decrease in arterial pressure, up to about 10 minutes after ligation.

III. DISCUSSION

A. Further evidence for the implication of cyclic AMP in ventricular arrhythmogenesis

Ligation of an anterolateral branch of the left descending coronary artery is followed by a transient peak in tissue levels of cyclic AMP in the ischaemic zone and was associated with a relatively

stable heart rhythm. Beta-adrenergic stimulation by isoproterenol infusion after ligation in our model increased tissue levels of cyclic AMP especially in the non-ischaemic zone. Simultaneously, the incidence of severe ventricular arrhythmias, i.e. VT and VF, was sharply increased. These results provide substantiating evidence for a link between an accumulation in myocardial tissue levels of cyclic AMP and ventricular arrhythmias.

B. Catcholamine induced cellular damage and arrhythmias

Rona *et al* (1959) showed that large doses of isoproterenol produced myocardial lesions similar to those that followed coronary artery ligation. Maroko *et al* (1971) administered isoproterenol in similar doses used by us to dogs with coronary ligation and demonstrated an increase in the zone of ischaemic injury, as indicated by creatine phosphokinase levels and local ST segment elevation.

This aggravation of ischaemic injury is thought to be primarily the result of an increased oxygen demand subsequent to an increase in heart rate and contractility. Other secondary factors are also recognised: (a) Due to $beta_2$ medicated peripheral vasodilation, systemic hypotension as found in our study, could render the myocardium underperfused (Rona *et al* 1959). (b) We found a depletion of ATP in non-ischaemic tissue during isoproterenol administration. Similar results were found by Fleckenstein *et al* (1974). ATP has been shown to be of primary importance to maintain cell integrity.

An increase in the size of these lateral lesions per se during isoproterenol administration could not have accounted for the severe

tachyarrhythmias that resulted, for a presumably more substantial increase of an anterolateral lesion from about 7% to about 16% (Chapter 3) resulted in an increase in the incidence of VPS only, leaving the evidence of VT and VF unaltered (Compare Table 3.1 to Table 6.1).

C. Calcium and ventricular arrhythmogenesis

Podzuweit, Lubbe and Opie (1976) postulated that accumulation of intracellular cyclic AMP could mediate slow channel induction, thereby predisposing to the development of slow response action potentials. Slow responses are considered to underlie re-entrant ventricular arrhythmias. If this hypothesis is correct then the further mediator of ventricular fibrillation is the calcium ion since slow responses are calcium mediated. Cyclic AMP and hence calcium have been implicated in the generation of after depolarizations in non-automatic cells (Lazzara *et al* 1978). Such after depolarizations may provoke automaticity and thereby evoke ventricular arrhythmias (Imanishi *et al* 1976). Enhanced calcium influx has been implicated in cyclic AMP induced electrical uncoupling of cells in hypoxic muscle (Wojtczak, 1979). In zones of myocardial ischemia, such an association could be highly arrhythmogenic.

D. Calcium channel blocking agents and catecholamine induced ventricular arrhythmias after coronary artery ligation.

In this study the calcium channel blocking agent verapamil afforded substantial protection against catecholamine induced VF. Earlier studies in dogs (Schmidt and Hanna 1967, Brooks *et al* 1980) showed similar results.

1. Locus of adrenolytic action

The work of Nayler and Szeto (1972) and Fleckenstein (1971) indicate that verapamil is not a competitive antagonist at the level of the beta receptor. We have shown that verapamil prevented catecholamine induced VF in the absence of any reduction in tissue levels of cyclic AMP. These results provide strong evidence that verapamil mediates protection at a site distal to the beta receptor-adenylate cyclase chain of events.

To further elucidate the exact locus of action, my colleague Dr. Francis Thandroyen undertook studies with the *d*- and *l*-isomers of verapamil. In equimolar concentrations, both isomers are almost equipotent in evoking coronary artery vasodilation; however *l*-verapamil is 10 to 15 times more potent than *d*-verapamil in evoking negative dromotropism and inotropism respectively (Satoh *et al* 1980),. In the isolated Langendorff-perfused rat heart subjected to main left coronary artery ligation, *l*- but not *d*-verapamil attenuated the adrenaline induced fall in the ventricular fibrillation threshold (Thandroyen, Muller and Opie 1981, in preparation). These results suggest that protection occurred as a result of inhibition of transmembrane calcium influx.

2. Mechanisms of the anti-arrhythmic effects of verapamil

Calcium channel inhibition may result in several sequelae, each with the propensity to mediate protection against ventricular arrhythmias.

a. Direct electrophysiological effects

Studies in our laboratory on the isolated guinea-pig papillary muscle have revealed that calcium channel blocking agents may

prevent slow response action potentials evoked by dibutyryl cyclic AMP and suppress after-depolarizations and automaticity (Daries *et al* 1981 - in preparation).

b. Preservation of high energy phosphates

Catecholamine stimulation during myocardial ischaemia promotes transmembrane calcium influx and thereby increases cellular energy utilization via energy dependent sequestration of calcium by the sarcoplasmic reticulum (Fanburg *et al* 1972) and activation of myofibrillar ATPase (Weber 1972). In addition, catecholamine stimulation may cause energy transfer block between phosphocreatine and ATP (Opie and Horak 1981). These processes may explain the reduced ATP levels evident in the non-ischaemic myocardium during myocardial ischaemia and catecholamine stimulation.

Calcium channel blocking agents may preserve high energy phosphate content by decreasing transmembrane calcium influx (Reimer *et al* 1977) and enhancing glycolytic flux (Weishaar *et al* 1979). In our studies, protection against catecholamine induced VF was associated with the preservation of ATP and PCr in especially the non-ischaemic myocardium. Preservation of ATP has been proposed to maintain both cell integrity (Opie and Bricknell 1979) and electrical stability of the heart (McDonald *et al* 1971, Cheneval *et al* 1972). Whether the preservation of high energy phosphate is a factor of importance in protecting against catecholamine induced VF or merely a circumstantial finding cannot be ascertained. However, tissue levels of ATP and PCr in the group which received isoproterenol and verapamil after ligation, were higher than in the group with anterolateral ligation only, while the incidence of VF in the two groups was similar. These results suggest a dissociation

between high tissue levels of high energy phosphates and anti-arrhythmic effects.

E. Effect of verapamil on spontaneous ventricular arrhythmias following coronary artery ligation.

The development of atrioventricular conduction block precluded the use of the same dose of verapamil (0.9 mg/kg) which afforded protection against isoproterenol induced VF. The effect of higher doses during electrical pacing remains to be established. Kaumann and Aramendia (1968) described the complete prevention of the development of VF after ligation of the left anterior descending artery in the dog, by giving verapamil 0.79 mg/kg, 10 minutes prior to ligation. Atrio-ventricular conduction disturbances did occur, however it was of a transient nature.

F. Status of cyclic AMP and calcium in the genesis of ventricular arrhythmias

Results from this chapter further indicated that the accumulation of cyclic AMP after coronary artery ligation could not explain all patterns of ventricular arrhythmias. In this chapter, the calcium channel blocking agent verapamil has been shown to prevent VF in the presence of increased tissue levels of cyclic AMP in ischaemic and non-ischaemic tissue. These results suggest that the cyclic AMP mediated entry of calcium may be prominently involved in the genesis of ventricular arrhythmias.

SUMMARY AND CONCLUSIONS

1. Ligation of a single lateral branch of the anterior descending artery (site 2) was followed by a low incidence of VF and other ventricular arrhythmias. Cyclic AMP did not accumulate in the ischaemic zone, except for a small peak at 10 minutes after ligation. Isoproterenol infusion after ligation at site 2, sharply increased the incidence of VT and VF, which could be associated with an increase in tissue levels of cyclic AMP. These results provide substantiating evidence for a link between cyclic AMP and ventricular arrhythmias.

2. The calcium channel blocking agent verapamil (0.9 mg/kg) completely prevented isoproterenol-induced VF. This anti arrhythmic effect occurred despite increased tissue levels of cyclic AMP. The inhibition of calcium ion influx by verapamil may have prevented the cyclic AMP mediated development of slow responses and after depolarizations, which have been implicated in the genesis of ventricular arrhythmias.

2. Verapamil administered in a dose of 0.6 mg/kg failed to prevent spontaneous VF following ligation at site 1. Atrio-ventricular block precluded the use of a higher dose.

CONCLUSIONS ON POSSIBLE MECHANISMS OF VENTRICULAR ARRHYTHMOGENESIS

This study provides substantiating evidence for a link between cyclic AMP and ventricular arrhythmias for

a) a transient accumulation of tissue cyclic AMP in the ischaemic zone after main stem ligation was associated with a high incidence of VPS, VT and VF.

b) Cyclic AMP did not accumulate after lateral branch ligation and the incidence of VT and VF was lower.

c) When all pigs were regrouped according to the occurrence of VF, pigs who encountered this arrhythmia, showed a sharp increase in tissue levels of cyclic AMP in the ischaemic zone while no such accumulation occurred in pigs with no VF.

d) Isoproterenol infusion after lateral branch ligation increased tissue levels of cyclic AMP and increased the incidence of VT and VF.

However, accumulated cyclic AMP in the ischaemic zone could not be reconciled with all patterns of ventricular arrhythmias, for

a) the calcium channel blocking agent verapamil prevented isoproterenol-induced VF despite increased tissue levels of cyclic AMP in the ischaemic and non-ischaemic zone.

These results suggest that cyclic AMP may be an intermediate messenger in an arrhythmogenic chain of events of which the trans-membrane influx of calcium ions may be an important further messenger.

b) The beta-antagonist metoprolol reduced the incidence of VF in the presence of an accumulation of cyclic AMP in the ischaemic zone. Regional blood flow studies revealed that metoprolol increased blood flow in the ischaemic zone. This effect could directly underlie the low incidence of VF in this group. However, metoprolol may have exerted anti-arrhythmic activity by

1. Preventing the accumulation of extracellular potassium in the ischaemic zone and thereby precluded the cyclic AMP mediated development of slow responses which predispose to ventricular arrhythmias.
2. Metoprolol may possess calcium channel inhibitory properties.

Ischaemia of the interventricular septum and apical regions may have contributed to the development of ventricular arrhythmias after main stem ligation. When ischaemia was rendered less severe by metoprolol administration , the incidence of ventricular arrhythmias was lower.

Sotalol and propranolol administration decreased the incidence of VPS and VT, but failed to decrease the incidence of VF. Furthermore, verapamil decreased the incidence of VF but did not reduce the incidence of VT. It is generally accepted that the development of slow responses may result in re-entry, which in turn could lead to tachyarrhythmias, including VT and VF. However, these results raise the possibility that the genesis of VT and VF may involve different mechanisms.

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