THE MONOPHASIC ACTION POTENTIAL AS ~~A MEASURE~~ AN INDEX OF MYOCARDIAL ISCHAEMIA: STUDIES INCORPORATING MYOCARDIAL PERFUSION SCINTIGRAPHY.

By

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"I confess that altruistic and cynically selfish talk seem to me about equally unreal. With all humility, I think 'whatsoever thy hand findeth to do, do it with thy might,' infinitely more important than the vain attempt to love one's neighbour as one's self. If you want to hit a bird on the wing you must have all your will in focus, you must not be thinking about yourself, and equally, you must not be thinking about your neighbour; you must be living with your eye on that bird. Every achievement is a bird on the wing."

Oliver Wendell Holmes, Jr.

ABSTRACT

Malignant ventricular arrhythmias continue to be the major cause of death in relation to myocardial ischaemia. The basic electrophysiological alterations that accompany the onset of myocardial ischaemia have been extensively studied in *in vitro* preparations and animal studies. However, studies of myocardial ischaemia in man are limited by the difficulty in recording basic electrophysiological changes in the beating heart. Furthermore, such studies require production of controlled ischaemia and the ability to detect and verify such ischaemia during the electrophysiological study. This thesis has developed this technically complex approach.

The advent of the monophasic action potential (MAP) recording technique using pressure contact electrodes has allowed the study of myocardial cellular repolarization characteristics in human hearts during cardiac catheterisation. Also, advances in myocardial perfusion imaging techniques with the development of newer 99m technetium isonitrile analogues have made possible, the use of myocardial perfusion scintigraphy to document myocardial perfusion characteristics during interventions in the cardiac catheterisation laboratory. This thesis is based on a series of studies that have examined the use of MAPs to document typical early electrophysiological changes of myocardial ischaemia during experimental myocardial ischaemia and its use in combination with technetium 99m hexakis-2-methoxy-2 methylpropyl-isonitrile (99mTc- MIBI) myocardial perfusion scintigraphy to study myocardial ischaemia in patients during cardiac catheterisation.

An initial study in intact porcine hearts during transient coronary occlusion examined the ability of action potentials recorded by the MAP technique to register early changes of myocardial ischaemia from the ventricular endocardium. Recordings were made simultaneously from the endocardial (and epicardial) surfaces of the myocardium in 6 anaesthetised Landrace pigs (21 occlusions) and changes in MAP related to regional segment length alterations that occurred with the onset of myocardial ischaemia. Consistent shortening of the MAP duration in recordings from the endocardium qualitatively concordant in time with wall motion dyssynergy was documented during experimentally induced myocardial ischaemia.

A second study in 26 patients undergoing cardiac catheterisation used endocardial recordings of the MAP during atrial pacing to angina threshold. Ischaemic areas of myocardium were identified by injection of 99m Tc-MIBI at peak pacing stress. Recordings from the ischaemic endocardial regions showed a significantly greater shortening of action potential duration corrected for heart rate changes compared with the non-ischaemic areas. Sensitivity and specificity of various changes in MAP duration per unit change in heart rate for the detection of myocardial ischaemic were derived.

The third study used endocardial recordings of the MAP as a measure of myocardial ischaemia during dypiridamole myocardial perfusion imaging in patients with coronary artery disease. Coronary vasodilators such as dipyridamole and adenosine cause myocardial blood flow heterogeneity so that a radionuclide flow tracer can highlight areas of myocardium with a diminished coronary flow reserve. However, considerable controversy exists as to whether such flow heterogeneity is of sufficient magnitude to produce myocardial ischaemia. This issue was addressed using MAP recordings in 32 patients during dypiridamole infusion and injection of 99m

Tc-MIBI for subsequent perfusion scintigraphy. MAP duration changes indicative of myocardial ischaemia were seen in 18 of 20 recordings from areas with an abnormal perfusion pattern. The intensity of ischaemia was significantly greater in areas of myocardium dependant on angiographically evident collateralisation for myocardial viability.

Finally, the combination of MAP recordings and 99m Tc-MIBI perfusion scintigraphy was employed to study regional and potential arrhythmogenic effects of beta adrenoceptor stimulation in potentially ischaemic versus normal areas of human myocardium. MAPs were recorded simultaneously from the right and left ventricular endocardium in 14 patients (28 recording sites) during infusion of dobutamine. Perfusion at the recording site was assessed by the injection of 99m Tc-MIBI at peak doses of dobutamine. Action potential duration during dobutamine was compared to that during atrial pacing to identical pacing rates in the absence of dobutamine. In 21 recordings from normally perfused recordings, dobutamine produced a variable effect on the action potential duration over and above that produced by atrial pacing alone to identical pacing rates either lengthening or shortening the action potential duration. In the ischaemic zones, dobutamine invariably shortened the action potential duration. The data provide a possible mechanistic basis for the beneficial effects of beta adrenergic blockade in ischaemic heart disease.

A unique series of studies combining basic electrophysiological studies with a radionuclide imaging technique is presented. Validation and application of the two techniques to evaluate regional myocardial ischaemia and arrhythmogenic potential in the human heart are addressed.

TABLE OF CONTENTS

SECTION 3

SECTION 1

CHAPTER 1.1.

INTRODUCTION

Sudden cardiac death due to ventricular arrhythmia is a leading cause of death in association with coronary artery disease. (Lown B, 1978; Cobb et al 1980; Goldstein et al, 1981) The realisation that such deaths could be prevented by appropriate therapy of the cardiac arrhythmia has led to the development of intense interest in the electrophysiological alterations that accompany early myocardial ischaemia. Microelectrode and voltage clamp techniques have contributed considerably to our present understanding of basic cellular electrophysiology. Over the years, several *in vitro* studies and *in vivo* animal studies have explored the electrophysiological accompaniments of early myocardial ischaemia. (Lazzara et al, 1974; Downar et al, 1977; Lazzara et al, 1978; Hope et al, 1980; Janse and Wit, 1989) However, such studies in the human heart have hitherto been limited by the difficulty in studying basic electrophysiological changes in the beating human heart.

Direct extrapolation of findings in the isolated tissue and experimental animals to the intact human heart is limited by the inability to precisely simulate the complex environment of the ischaemic and infarcted human cardiac tissue. Additionally, the *in situ* heart is under the influence of various neuronal and humoral factors which are in a state of constant flux. That the mechanism of arrhythmogenesis and action of antiarrhythmic drugs vary in the ischaemic human heart was recently highlighted by the results of the Cardiac Arrhythmia Suppression Trial. (CAST Investigators, 1989) This multicentre trial evolved from the concept that suppression of ventricular ectopy, a recognized harbinger of more complex cardiac

arrhythmias, would result in reduced mortality in patients with coronary artery disease and impaired cardiac function. However, contrary to expectations, the mortality in patients given the antiarrhythmic drugs encainide or flecainide was significantly higher. These results emphasize the unpredictable and different effect of antiarrhythmic drugs in ischaemic and damaged human cardiac tissue from that in isolated tissue or animal studies which is where these drugs are primarily evaluated. It thus seems clear that studies in the human heart and in the context of myocardial ischaemia are needed.

Among methods potentially available for evaluation of cellular electrophysiological characteristics of the beating hearts, attempts to perform microelectrode studies deserve mention. (Woodbury and Brady, 1956; Czarnecka et al, 1973; Russel et al, 1977) Notwithstanding the difficulty of the approach in experimental preparations of the in situ beating heart, the technique cannot be applied to studies of the human heart. An alternative and only practical way is to apply the technique which has been available for over a century, namely recording of the monophasic action potential (MAP). The MAP signal obtained by local depolarization of the cells underlying the exploring electrode was validated by early investigators. Hoffman and colleagues in 1959, confirmed that the repolarisation phase of the transmembrane action potential coincides with that of the simultaneously recorded epicardial MAP signals. Recordings of the MAP from the human heart was first obtained in 1966 using suction electrodes. (Korsgren et al, 1966) Subsequent studies and developments saw the emergence of MAP recordings using the pressure contact electrodes (Shebatai et al, 1968; Olsson et al, 1971; Franz, 1983).

The advent of the MAP recording technique utilising pressure contact electrodes mounted on a conventional cardiac catheter has allowed the study of myocardial cellular depolarisation and repolarisation characteristics in the human heart during cardiac catheterisation. The primary use of the technique has been in the electrophysiological evaluation of antiarrhythmic drugs where its use has permitted assessment of these drugs in the human heart. (O'Donoghue and Platia, 1991) Until recently, exploration of antiarrhythmic drug effects on myocardial activation and repolarisation was derived from *in vitro* experiments in the basic electrophysiological laboratory. In addition, the MAP technique has opened up the possibility of exploring the basic electrophysiological effects of ischaemia in the *in vivo* human heart.

The MAP has been used to study myocardial ischaemia in experimental animal studies, (Franz et al, 1984; Dilly and Lab, 1987) and on the epicardium in humans during coronary artery bypass surgery. (Taggart et al, 1986) Myocardial ischaemia produces characteristic changes in the action potential of the affected cells. The ability of MAP recordings to reliably register characteristic changes of localised and early myocardial ischaemia has been repeatedly alluded to in the literature but no formal attempt to validate and apply the technique to studies of myocardial ischaemia in man has been made. The development of pressure contact electrodes mounted on conventional cardiac catheters has facilitated recordings of the MAP from the human endocardium for prolonged periods during cardiac catheterisation. (Franz, 1983; Olsson et al, 1986)

A further consideration in studies of myocardial ischaemia in man is the difficulty in provoking controlled ischaemia and the ability to monitor the areas made ischaemic. The conventional surface electrocardiogram can be

relatively insensitive to early myocardial ischaemia. (Battler et al, 1980) Additionally, it has a low sensitivity for the localisation of regional ischaemia. Wall motion abnormalities are early accompaniments of myocardial ischaemia. However, apart from echocardiography, currently available methods of detecting wall motion changes in human studies are cumbersome and not readily applicable in conjunction with cardiac catheterisation protocols. Radionuclide markers of myocardial ischaemia have a high sensitivity and specificity for detection of regional myocardial ischaemia. (Pitt and Strauss, 1976; Bailey et al, 1977; Okada et al, 1980) In recent years, several technetium-99m based myocardial perfusion markers have been developed. (Beller and Watson, 1991) One such agent namely, Tc-99m-hexakis-2-methoxy-2-methylpropyl-isonitrile known as Tc-99m-MIBI, Tc-99m-Sestamibi or RP-30 (DuPont Research Product) has gained wide acceptance with imaging qualities comparable to thallium 201 for myocardial perfusion studies. The radionuclide has the unique property of minimal myocardial washout such that imaging carried out up until 4 hours after injection of the isotope would represent perfusion characteristics at the time of injection. (Beller and Watson, 1991) This feature renders Tc-99m-MIBI ideal for use in with cardiac catheterisation procedures to monitor ischaemic areas produced during an intervention.

SCOPE OF THE PRESENT THESIS

This thesis describes a series of original studies utilising the MAP as a measure of myocardial ischaemia. In human studies, recordings of the MAP during interventions designed to provoke myocardial ischaemia were related to abnormalities on Tc-99m-MIBI perfusion scintigraphy which was performed simultaneously with the MAP recording procedure. In order to formulate a clear understanding of the MAP recording technique and perfusion scintigraphy, brief reviews of the pathophysiology and basic

cellular mechanisms in myocardial ischaemia, together with mechanisms of normal and abnormal blood flow in the human heart are presented. The methodology used in the studies namely MAP and Tc-99m-MIBI are discussed with reference to their validation and clinical use by previous investigators. The studies are then presented as papers and individually discussed.

CHAPTER 1.2

HISTORICAL PERSPECTIVE

1.2.1. Myocardial ischaemia

The link between coronary artery obstruction, malignant cardiac arrhythmias and sudden death was established early. As early as the 18th century, Heberden described the clinical syndrome of angina pectoris, syncope and sudden death. (Heberden, 1786) Herberden and other physicians of the time also observed that symptoms of myocardial ischaemia was often associated with an irregularity of the pulse possibly representing the earliest recognition of arrhythmia associated with myocardial ischaemia. However, the relationship between occlusive coronary artery disease and angina was not recognised until the autopsy study of Hunter in 1779. He described the presence of ossified coronary arteries in one of his patients who had had angina. Twenty years later, Parry described the state of the coronary arteries in autopsy studies in patients with angina: ".. coronaries may be so obstructed as to intercept the blood which should be the proper support of the muscular fibres of the heart". (Parry, 1799) Subsequent experimental studies by Erichsen in 1840 established the link between ischaemia and infarction. (Erichsen, 1841-42) He ligated the coronary artery of the dog and clearly demonstrated the cessation of cardiac muscular activity.

1.2.2. Electrophysiology of myocardial ischaemia

In fact, Erichsen's original observations during coronary occlusion makes early reference to ventricular fibrillation. He described the mode of cessation of ventricular activity following coronary occlusion as a" a slight tremulous motion". The early 20th century saw the development of the electrocardiogram. Many people were responsible for the evolution of the

electrocardiograph machine and the discipline of electrocardiography. (Lippmann, 1875; Lewis, 1925; Wilson, 1930) Einthoven however, receives the credit for the invention of the electrocardiograph. (Einthoven, 1941) Mention must be made of Frank Wilson who undertook the arduous task of translating electrocardiographic theory into clinical medicine. (Wilson, 1930) Around the time, several laboratory and clinical studies substantiated the relation between myocardial ischaemia and ventricular arrhythmias. (Porter, 1894; McWilliams, 1889; Lewis, 1909; Smith, 1918; Robinson, 1921) The association between myocardial ischaemia and propensity for ventricular fibrillation was addressed in 1940 by Wiggers and colleagues. He showed that myocardial ischaemia lowered fibrillation threshold and postulated that ectopic impulses would more readily induce ventricular fibrillation in the setting of ischaemia. (Wiggers et al, 1940)

In 1961, the technical ability to monitor continuous electrocardiography in active patients was developed by Holter. (Holter, 1961) Subsequent developments in Holter monitoring has permitted prolonged continuous monitoring of cardiac rhythms and allowed the elucidation of the close relation between arrhythmias and sudden cardiac death especially in the context of ischaemic heart disease. (Nikolic et al, 1982; Roelandt et al, 1984; Bayes deLuna et al, 1985) That at least the arrhythmic deaths resulting from ischaemic heart disease could be prevented accrued from the above observations. A surge in interest in the basic electrophysiology of myocardial ischaemia followed and a number of investigators reported the effects of ischaemia on the cardiac action potential. (Robinson, 1921; Kardesh et al, 1958; Samson and Scher, 1960; Prinzmetal et al, 1961).

1.2.3. Development of techniques to monitor basic cardiac electrophysiology

Early attempts at recording cardiac cellular electrical activity began with Burdon-Sanderson and Page who recorded action potentials from the frog heart by placing one electrode on the intact epicardial surface and the other on an injured site. (Burdon-Sanderson and Page, 1882) Phasic electrical activation during the cardiac cycle were recorded on a rotating drum. Burdon-Sanderson and Page called these recordings monophasic action currents. Seventy years later, Draper and Weidman reported the recording of the cardiac action potential by impaling a glass micropipette into a single cell. (Draper and Weidman, 1951) Further improvements in the microelectrode technology followed propelling a new era of basic cardiac electrophysiological studies. (Hoffman and Cranefield, 1960)

The original technique of recording monophasic injury potentials from the surface of the heart described by Burdon-Sanderson and Page however continued to generate interest. Although the microelectrode permitted accurate evaluation of intracellular electrical activity in isolated tissue, measurements in the intact beating heart was difficult. The possibility that similar information could be obtained from an electrode on the surface of the heart was therefore clearly attractive. Schütz introduced the suction electrode for recording of monophasic injury potentials wherein local myocardial injury or depolarisation was produced by the suction force. (Schütz, 1931) This method produced more stable signals of better quality. At this stage the developments in microelectrode techniques superseded the use of monophasic injury potentials briefly causing a lull in the progress of myocardial surface recordings of electrical potentials. In 1959, Hoffman and colleagues validated the monophasic injury potentials obtained by the suction technique against the intracellular potentials recorded using microelectrodes. (Hoffman et al, 1959) They documented the close similarity

between the action potentials obtained by the two methods and confirmed that the monophasic potentials obtained by the suction electrodes were a 'reliable index of the shape of the action potential during the entire phase of repolarization'. Additionally, when the shape of the action potential, as observed with the microelectrode, was changed by ions such as potassium or calcium, a similar change was observed in the potential recorded with the suction electrodes. Several later investigators have substantiated these observations confirming that localised potentials obtained from the myocardial surface by local injury or depolarisation are true representation of the intracellular action potentials in most respects and can be termed 'monophasic action potentials' (Franz et al, 1986; Ino et al, 1988).

Remarks

The historical notes presented above are based on Snellen H.A. "History of Cardiology", 1984. The older works maybe hard to find. However, they have been included in the reference list as a service and source of interest to the reader.

CHAPTER 1.3.

IMPORTANCE OF STUDIES OF MYOCARDIAL ISCHAEMIA

1.3.1. Scale of the problem and economic impact of coronary artery disease

Coronary artery disease remains the major cause of death in the Western World. In the United States alone, 5.4 million individuals are diagnosed as having coronary artery disease and over 550,000 deaths per year are attributable to coronary atherosclerosis. It is estimated that there are over 5 million survivors of myocardial infarction in the United States associated with a cost of approximately $8 billion annually. As a significant proportion of those affected are relatively young persons, the calculated annual economic loss is in the region of $60 billion. (Gotto and Farmer, 1988)

According to the Office of Health Economics, coronary heart disease caused 163,104 deaths (28% of total deaths for that year) in 1985 in England and Wales. (Office of Health Economics, 1987) More specifically, it was the major cause of premature deaths, especially for males (coronary artery disease caused 40% of all deaths in males aged between 45 and 65 years). The prevalence of coronary disease is highest in Scotland. The recent Scottish Heart Health Study of 10,359 men and women aged between 40 and 59 years elicited a history suggestive of angina in 5.5% of men and 3.9% of women. (Smith et al, 1990) The estimated cost to the National Health Service of the United Kingdom for the year 1985 was £390 million and can only have escalated since that estimate. Coronary artery disease is therefore of utmost importance as a public health problem.

1.3.2. Consequences of myocardial ischaemia

Although angina and myocardial infarction are the commonly recognised manifestation of coronary artery disease, sudden cardiac death may be its

first manifestation (it is the first manifestation in 20-25% of patients with coronary artery disease). Sudden cardiac death is estimated to be responsible for close to 400,000 deaths in the United States. (Lown, 1979) In 80% of patients who die suddenly from cardiac causes, coronary artery disease is present and the cause of death is commonly a malignant ventricular arrhythmia, either ventricular tachycardia or fibrillation. (Bayés de Luna et al, 1989) Although the majority of these patients have had previous myocardial infarctions, 20 to 30% merely have acute ischaemic episodes some of which may culminate in fresh infarcts. (Cobb et al, 1975; Goldstein et al, 1981) Other less common manifestation of myocardial ischaemia or infarction or both are left ventricular failure due to a chronic form of ischaemic cardiomyopathy and cardiac arrhythmias other than ventricular tachycardia and fibrillation. Irrespective of the mode of manifestation of the disease, it is associated with considerable morbidity and mortality. It is therefore clear that early detection of myocardial ischaemia is cardinal to the effective prevention of cardiac deaths. In particular, addressing the electrophysiological changes of early myocardial ischaemia is of paramount importance in the elucidation of mechanisms relating to fatal cardiac arrhythmias associated with ischaemic heart disease.

CHAPTER 1.4.

PATHOPHYSIOLOGY OF MYOCARDIAL ISCHAEMIA

1.4.1. Definition of ischaemia

Although ischaemia is conventionally described as a lack of blood in a particular tissue, the dynamic nature of the condition has to be taken into account. Myocardial ischaemia therefore represents an imbalance between myocardial demand for, and the vascular supply of oxygen, substrates and energy to the myocardial tissue. (Hearse, 1980) In addition to creating a relative deficit of materials required to sustain myocardial function, ischaemia also results in an insufficient capacity for removal of potentially toxic metabolites such as lactates, carbon dioxide and protons. The critical levels of flow and/or metabolic requirements which produce ischaemia is difficult to predict and is dependent on several factors. However the end result is often evident clinically as pain and consist of electrophysiologic and metabolic changes, cessation of contraction and sometimes heart failure. In most cases, the damage is reversible with restoration of flow. Progression to cell death and necrosis representing myocardial infarction results if adequate flow is not restored. The time period to the development of irreversible cell damage is variable in the human heart and is a function of several factors such as collateral flow and degree of myocardial hypertrophy.

It is important to realise that myocardial ischaemia cannot be described in absolute terms, since the blood flow and quantity of oxygen required to support the myocardium under one set of conditions may be different under another. In the human heart, a blood flow of 60 to 90 ml/min per 100 gm of myocardial tissue is required for adequate myocardial function under basal physiological conditions. However, when the mechanical activity of the heart is suppressed as in the use of beta blockers and metabolic activity

reduced as in hypothermic arrest during surgery, much lower blood flow rates (10 to 20 ml/min per 100 gm) may suffice to maintain myocardial viability. (Braunwald and Sobel, 1992)

1.4.2. Heterogeneity of myocardial damage from ischaemia

Ischaemic myocardial tissue evolves through a series of processes from reversible to irreversible damage, cell death and tissue necrosis. (Jennings and Ganote, 1974) The end stage of the sequence is myocardial infarction. An interesting feature of the process is the spatial heterogeneity of the process within any involved area of the myocardium. Varying conditions of workload, tissue perfusion and resistance to ischaemia may create severely ischaemic areas of myocardium interspersed with virtually normal areas. (Jennings and Ganote, 1976)

1.4.3. Sequence of electrophysiological and mechanical events in ischaemia

Despite the marked variability in ischaemic damage in any given myocardial tissue, contribution from several investigators (Braasch et al, 1968; Brachfield, 1974; Sobel, 1974; Opie, 1976; Hearse, 1980) have helped compile a remarkably detailed delineation of the sequence of events is myocardial ischaemia.

Recent reviews on the subject are provided by Allen and Orchard (1987) and Dresdner (1990). It is evident from these reviews that varying viewpoints exist on the exact sequence of events. Nevertheless, a 'best estimate' summary can be derived as follows:

<u>Events occurring within seconds of occlusion.</u>

The earliest consequence of impaired blood flow to the myocardial cell is a disturbance of the transmembrane ionic balances. (Case, 1971-72; Hillis and Braunwald, 1977) However, these changes are not immediately reflected in

the action potential recorded from the ischaemic cells. Reduction of mitochondrial activity and oxidative metabolism with concordant fall in ATP production immediately follow the ionic changes and a precipitous decline of contractile activity is seen. The precise reason for such immediate fall in contractile activity is not clear. There is rapid depletion of creatine phosphate stores with transfer of high energy phosphate to ADP in an effort to maintain ATP levels. Reduction in action potential amplitude and duration are recorded soon after (within a few seconds) although the exact mechanism underlying the changes are poorly understood. Extracellular potassium accumulates and intracellular sodium and chloride concentrations rise. Local cellular depolarisation can produce ST segment changes on the electrocardiogram as early as 30 seconds of onset of ischaemia. (Hillis and Braunwald, 1977) These events are followed by local release of catecholamines and stimulation of glycogenolysis. Intracellular acidosis develops and mitochondrial electron transport is reduced or halted. Fatty acid oxidation is replaced by glycogen utilisation. Accumulation of metabolites such as lactate and inorganic phosphates accrue.

Events within minutes of ischaemia.

Leakage of adenosine, inosine and other metabolites into the extracellular space produce local vasodilation which encourage collateral blood flow. There is increasing cellular acidosis with slowing of glycolytic metabolism followed by cellular swelling. There is marked alteration of ionic balances with cytoplasmic accumulation of calcium ions. Minor ultrastructural changes eg. mitochondrial swelling become apparent. If total ischaemia persist, structural changes in the myocyte progress and onset of irreversible changes and myocardial contracture will occur within variable period after the first hour of ischaemia.

1.4.4. Biochemical basis of changes in ischaemia

As mentioned above, the exact mechanism underlying the electrophysiological and mechanical changes that accompany the early phases of myocardial ischaemia are not clearly defined. These changes appear to be independent of ATP levels. (Jennings et al, 1981) There is leakage of potassium to the extracellular space even before and disproportionate to accumulation of sodium and chloride ions within the cell. The accumulation of the potassium ions in the extracellular space is often explained in the literature on the basis of loss of activity of the ATP dependant sodium-potassium pump. However, it is clear from Jennings work in dogs that ATP levels are relatively maintained during the early phases of ischemia. (Jennings et al, 1981) That the sodium-potassium pump operates in a near normal manner in the early phases of ischaemia was indirectly inferred from rate related alterations in potassium concentrations. (Weiss, 1982; Kléber, 1983) In ischaemic myocardium, the rise and fall of extracellular potassium in response to rate changes is essentially normal in the early phases and indirectly implies normal function of the sodium potassium pump. However, since the rate of pumping was not quantified in these studies, a decrease in pump activity may not have been detected. Another explanation for the increase in potassium efflux could be an increase in membrane permeability to anions caused by ischaemia. If this occurred, the membrane would depolarize and potassium would be distributed according to the Nernst formula. There are experimental results in keeping with this possibility which show that the loss of anion inorganic phosphate and lactate follows the same time course as the loss of potassium. (Mathur and Case, 1973)

1.4.5. Mechanism of electrophysiological changes in early ischaemia

Extracellular leakage of potassium maybe partially responsible for the changes observed on the action potential. Investigators in the late 70's suggested that the shortening of the action potential duration in early ischaemia may be due to a decrease in the plateau phase inward calcium current. (Carmeliet, 1978; Carmeliet, 1984) However, the main contradiction to this hypothesis is the fact that the time course of decrease in ATP and pH is not be sufficiently fast to account for the shortening of the action potential by means of their known effects on the calcium current.

A recent review of the mechanisms of action potential changes is provided by Janse and Wit. (1989) These authors stress the importance of the various factors associated with the ischaemic environment and which may cause changes in the action potential similar to that recorded from the ischaemic cells. The ischaemic surrounding of the cardiac muscle is comprised of several metabolites resulting from low flow. In addition to elevated extracellular potassium, there is hypoxia, elevation of pCO2, low pH, lack of substrates and accumulation of substances such as lysophosphoglycerides and catecholamines. Action potentials with the same characteristics as those recorded during ischaemia in vivo preparations have been documented in isolated preparations of ventricular muscle superfused with solutions containing several of the major ischaemic components. Each of these substances may have individual influence on membrane properties and the various combinations may exert changes that are not predictable from the individual action of each substance. It is therefore difficult to clarify the contributions from the various metabolites to the changes observed in the action potential during ischaemia. It is however generally recognized that hypoxia alone predominantly shortens the action potential duration with only small depolarization of the resting membrane potential. This may be

because extracellular potassium which is responsible for the partial depolarisation of the membrane potential is washed out by the perfusate in studies using hypoxic perfusates. (McDonald and MacLeod, 1973).

1.4.6. Contractile changes and its relation to electrophysiological activity

In experimental studies, wall motion abnormalities are recorded within a few beats of coronary occlusion and precede the changes registered on the action potential recorded from the ischaemic cells. (Lab and Woollard, 1980) This is rather surprising considering the fact that not only is the action potential the trigger for contraction, but its amplitude and duration exert some control over the magnitude of contraction. There is substantial experimental data to show that developed tension is a function of the duration and amplitude of the action potential. (Morad and Goldman, 1973) This obvious paradox is inadequately explained in the literature. The likely possibility is that in early ischaemia, fall in contractile activity may be less dependant on action potential characteristics. It is well recognised that the heart has virtually no stores of oxygen. This factor together with its high rate of energy expenditure results in sudden, striking decline of myocardial oxygen tension within seconds of coronary occlusion, coincident with the loss of contractile activity.

The actual mechanism of loss of contractile activity had not been clearly defined because of lack of suitable technology to directly measure calcium ion (Ca^{++}) delivery to the myocardial contractile proteins. Contraction is normally initiated by a rapid release of Ca^{++} from the sarcoplasmic membrane (the 'Ca^{++} transient') which in itself is dependent on a local critical concentration of Ca^{++} in the vicinity of the sarcoplasmic membrane. A recent study of myoplasmic Ca^{++} transients showed an abnormal and erratic increase in Ca^{++} transients in the acute phase of global ischaemia in

isolated perfused rabbit hearts. (Lee et al, 1988) Despite this, contraction in the ischaemic muscle falls. The implication therefore is that, rather than a fall in intracellular Ca^{++}, the sensitivity of the myofilaments to Ca^{++} is depressed during ischaemia. A suggested mechanism is that the fall in pH as occurs in ischaemia may reduce the sensitivity of the myofilaments to local Ca^{+} concentration by virtue of the H^{+} ions competing with Ca^{++} ions for the receptors on the troponin molecules. Thus actin-myosin interaction is impaired and contractility reduced. However, it seem unlikely that it is the major cause for the observed tension decline as the acidosis in the initial phase of ischaemia is too small and relatively slow to occur. (Allen and Orchard, 1987) Recent studies also indicate that local concentrations of inorganic phosphates (Pi) increase prior to the pH changes and thus may be the key factor in early contractile failure via its potent effect to decrease sensitivity of the myofilaments to Ca^{++}. (Lakatta and Maughan, 1990)

1.4.7. Pattern of ischaemic myocardial damage

At least partially because collateral flow increases from the deepest subendocardial layers to the subepicardium, the jeopardized myocardium within the area at risk dies in a transmural pattern beginning in the subendocardium. This progression of ischaemic damage has been termed the 'wave front' of ischaemia by Reimer et al. (1977) They demonstrated this phenomenon in dogs. Occlusion of the left circumflex coronary artery in open chested anaesthetised dogs for a period of 40 minutes followed by 2 to 4 days of reperfusion, resulted in subendocardial necrosis. As much as 40% of the transmural dimension of the myocardium was involved. With longer periods of obstruction to myocardial flow, greater percentages of the transmural dimensions were involved, 57% with 3 hour occlusions and 71% with 6 hour occlusions. Studies that followed by Forman et al have

confirmed a similar sequence of events in the human heart. (Forman et al, 1983)

The greater susceptibility of the subendocardium to ischaemia maybe attributed to the greater extracellular compressive forces during systole in the subendocardium compared to the subepicardium. Thus systolic blood flow is compromised in this region. In addition, the subendocardium is subject to greater wall stress and consequently, higher oxygen consumption. This combination of higher metabolic demand and greater resistance to flow results in a lower coronary vascular tone in the subendocardium than in the subepicardium. Consequently, the coronary vasodilatory reserve is limited in the endocardial layers and as perfusion is reduced, the subendocardial layers show signs of myocardial ischaemia before the more superficial myocardial layers. (Griggs et al, 1973)

CHAPTER 1.5.

TECHNIQUES FOR THE DETECTION OF MYOCARDIAL ISCHAEMIA

Several non-invasive techniques have been developed over the years for the detection of myocardial ischaemia. These investigations aim to detect the various abnormalities in electrophysiological, biochemical and contractile parameters that are consequent upon the occurrence of myocardial ischaemia. They can be broadly classified as follows:

1.5.1. Detection of electrophysiological changes of ischaemia: The surface electrocardiogram

It must be recognized that the clinical electrocardiogram records the changing potential of an electrical field generated by the heart. It is an instant aggregate of the electrical activity of the cardiac cells and does not record directly the electrical activity of the source itself. Direct electrical activity of the heart can only be measured by an electrode in direct contact with the cardiac muscle. Despite this limitation, the ECG has remained the only practical means of documenting the electrical characteristics of the heart and it remains the gold standard for the detection of cardiac arrhythmias. In the detection of myocardial ischaemia, the surface ECG is usually recorded during an exercise protocol. The overall sensitivity of exercise electrocardiography for myocardial ischaemia is between 60 and 70%. (Fortuin and Weiss, 1977) Continuous ambulatory monitoring of the ST segments over prolonged periods has gained popularity in recent years to detect spontaneous episodes of ischaemia which may not be clinically evident (silent ischaemia).

1.5.2. Limitations of the surface ECG as a measure of ischaemia

As an index of myocardial ischaemia, the ECG has its limitations. Precordial ST segment mapping, while commonly used, is both imprecise and nonspecific. (Fozzard and DasGupta, 1976; Holland and Brooks, 1977) ST segment displacements do not directly measure the local electrophysiological changes in the ischaemic myocardium but rather the electrical gradient between ischaemic and normal myocardium. It follows therefore that no direct correlation of ST segment change with ischaemia should be expected. As the mechanism of ST segment change involve interaction between normal and abnormal tissue, any factor influencing membrane properties in *either* area will alter the ST segments, regardless of its influence on ischaemia. Fozzard and DasGupta in their excellent review of the theoretical considerations of the ST segment as an index of myocardial ischaemia, warn against the use of the ST segment as a direct measure of myocardial ischaemia. (Fozzard and DasGupta, 1976) Apart from the caveats mentioned above, it must be realized that the potential recorded by any ECG lead is determined by the sum of currents from all contributing area. Therefore, potentials propagating in opposite directions maybe subject to cancellation. For example, if endocardial injury lowers the ST segment, extension of the injury to the epicardial surface can return the the ST segment to normal or raise it. Similarly, if epicardial injury raises the ST segment, adjacent endocardial injury can normalize the ST segment. Further, an ischaemic area completely surrounded by normal tissue on all sides could be electrically silent, the vector sum of all currents being zero.

Often in clinical practice, changes in the surface ECG are considered in the context of the clinical picture and therefore provide a fair guide to the presence or absence of myocardial ischaemia. However, the problem arises when ST segment changes are used as a guide to evaluate ischaemic

responses in research protocols or experimental studies where effects of interventions on ischaemia are to be studied. The relative insensitivity of the surface electrocardiogram has been repeatedly implied in experimental and human studies. (Battler et al, 1980; Hauser et al, 1985; Taggart et al, 1989) In experiments examining the relationship between regional blood flow and ST segment changes, a poor correlation was demonstrated between the two parameters. (Smith et al, 1975; Kingaby et al, 1986) In human studies, Hauser et al examined the changes in wall motion and electrocardiographic indices during repeated coronary occlusion at coronary angioplasty. (Hauser et al, 1985) During angioplasty of 22 coronary stenoses in 18 patients, new or increased wall motion abnormalities were detected on echocardiography in 19 (86.4%) of the 22 procedures. ST segment changes were however observed in only 8 (36%) of the procedures and occurred on a mean of 10 seconds *later* than the wall motion changes. A similar study by Taggart et al during coronary angioplasty examined the relative ability of the surface ECG and unipolar epicardial electrogram recorded from the guide wire placed within the artery to be dilated. (Taggart et al, 1989) A relative paucity of ST segment changes on the surface ECG was again implied by these authors.

1.5.3. Techniques that characterize myocardial perfusion pattern

Studies of myocardial perfusion primarily involve the use of radionuclides. Although several radioactive particle have been used to study the coronary circulation, until recently thallium-201 has been the main agent used for studies of myocardial perfusion. In the last 5 years, several technetium based compounds have been developed. These agents will be discussed in detail later. That the sensitivity and specificity of myocardial perfusion scintigraphy are significantly greater than conventional exercise electrocardiography has been documented by several investigators and through results of multicentre trials. (Bailey et al, 1977; Ritchie et al, 1978;

Okada et al, 1980) Exercise myocardial perfusion scintigraphy is usually performed in conjunction with exercise electrocardiography. A focal defect in tracer uptake seen on the post exercise myocardial image suggests the presence of myocardial ischaemia or infarction. Ischaemia can be diagnosed if the initial defect found at exercise is no longer seen on re-imaging after several hours of rest (to observe the redistribution of thallium) or following a second injection at rest in the case of some of the newer technetium based tracers.

In patients unable to perform an adequate dynamic exercise test, non-exercise techniques using pharmacological agents have now emerged as attractive options. Specific coronary vasodilators such as dipyridamole and adenosine can be used to stress the coronary circulation and when used in conjunction with a radiotracer, has diagnostic ability comparable to imaging after conventional dynamic exercise testing. (Gould et al, 1978; Botvinik and Dae, 1991) Alternatively, myocardial stress and ischaemia can be provoked by use of inotropes such as dobutamine. (Mason et al, 1984; Pennell et al, 1991) Since the advent of these agents, the former techniques of atrial pacing stress, use of other beta adrenergic agonist (such as isoprenaline, dopamine and nor-epinephrine), hand grip and cold pressor tests have become less popular.

1.5.4. Methods to study wall motion abnormalities of myocardial ischaemia

Regional wall motion abnormalities appear early during myocardial stress in patients with coronary artery disease. The last 20 years have seen the evolution of several cardiac imaging techniques that can be used to detect these wall motion abnormalities. Of these, radionuclide ventriculography and echocardiography are of practical importance. Both these techniques have been been shown to be reliable in detection of wall motion

abnormalities and have been evaluated in conjunction with conventional dynamic exercise testing and with pharmacological agents (Wann et al, 1979; Crawford et al, 1984; Sinusas et al, 1984; Berthe et al, 1986). The technical difficulties encountered with performing echocardiography during exercise have retarded its widespread use. Further, the success of transthoracic echocardiography as an imaging modality is dependent on the presence of a satisfactory transthoracic window. In patients with obesity and chronic obstructive airways disease, adequate imaging can prove difficult. Transoesophageal echocardiography can overcome this problem albeit at the expense of the procedure being no longer non-invasive.

Other techniques which have been evaluated for detection regional wall motion abnormalities deserve mention and include the use of conventional computed tomography (CT) scanning with ECG gating, (Lackner and Thurn, 1981) fast or cine CT scanning, (Lipton et al, 1983) and magnetic resonance imaging. (Pennell et al, 1990) These techniques while demonstrating good correlation in diagnostic accuracy compared with conventional methodology, are expensive, require complex specialised equipment and have lower patient acceptability.

1.5.5. Characterisation of myocardial metabolism (Positron emission tomography)

A number of substance can be labelled with positron emitting radionuclides and can be used to study both myocardial perfusion and metabolism. The attraction of positron emission tomography (PET) lies in its ability to use positron emitting radionuclides such as oxygen-15, nitrogen-13 and carbon-11 which are ubiquitous in naturally occurring metabolic processes. Thus ^{15}O, ^{13}N and ^{11}C can be incorporated into radiopharmaceuticals that are true

metabolic substrates and consequently can be tailored to the investigation of selected metabolic pathways.

^{11}C-labeled palmitic acid combined with PET is used to study fatty acid metabolism. (Schelbert et al, 1982) Under normal aerobic conditions, most energy requirements of the heart are met by oxidation of free fatty acids. By observing the kinetics of ^{11}C, a non-invasive of assessment of regional myocardial free fatty acid metabolism can be made. Similarly, regional myocardial glucose uptake can be estimated by using ^{18}F 2-fluoro-2-deoxyglucose (FDG). Flourine -18 is a positron emitter with a half life of 2 hours. FDG exchanges across the the capillaries and cellular membranes in direct proportion to glucose transport. As the normal myocardium primarily metabolizes free fatty acids and has low glucose uptake, regions of ischaemia may show high uptake, indicating the primary reliance on glycolysis for energy in ischaemic myocardium.

The main limiting factor in the routine use of the PET scanner is its prohibitive cost in setting up and operation. Also, as a measure of myocardial ischaemia, PET scanning has little to offer over conventional myocardial perfusion scintigraphy. The main use of these tracers in conjunction with PET has been in studies of myocardial viability. (Tillish et al, 1986) Areas of apparently infarcted myocardial tissue may show active metabolism implying the presence of hibernating myocardium which is amenable to salvage by appropriate revascularisation techniques.

1.5.6. Cardiac catheterisation

To date, haemodynamic data and delineation of anatomy especially of the coronary circulation as obtained by cardiac catheterisation remains unsurpassed. The technique remains the 'gold standard' against which all

newer techniques must be validated. Its invasive nature which is associated with a significant morbidity and mortality poses cause for concern. However the nature of data obtained by cardiac catheterisation often cannot be provided by non-invasive techniques and the benefits frequently outweigh the risks. Although valiant attempts have been made to define coronary anatomy and blood flow using intravenous digital subtraction angiography and magnetic resonance imaging respectively, therapeutic decision making frequently requires coronary angiography.

In studies of myocardial ischaemia, it must be realised that coronary angiography provides anatomical information with little indication of myocardial perfusion physiology. This information can be indirectly surmised to an extent from left ventricular contraction pattern when combined with techniques that provoke myocardial stress. (Tobis et al, 1983) For adequate assessment of coronary blood flow physiology during rest and exercise, perfusion scintigraphy using a radiotracer such as thallium 201 or one of the newer technetium compounds offer the best results.

SECTION 2

CHAPTER 2.1.

METHODOLOGY I: THE MONOPHASIC ACTION POTENTIAL.

Recordings of monophasic action potentials were used a to measure cellular electrophysiological changes of myocardial ischaemia in the series of studies presented in this thesis. To fully understand the physiological basis and validity of this technique as measure of ischaemia, a brief review of the structure of the cardiac muscle, normal cardiac electrophysiology and alterations in the electrophysiological events that accompany myocardial ischaemia is necessary.

BASIC CARDIAC ELECTROPHYSIOLOGY

2.1.1. Introduction

Myocardial cells undergo single and sequential regenerative depolarisation and repolarisation, communicate with each other and propagate action potentials. In the last 30 years there has been a rapid growth in our understanding of the control of ionic flow in the normal and diseased heart. Similarly, the electrophysiological changes that accompany ischaemia and infarction has been extensively investigated in recent years. (Lazzara et al, 1974; Downar et al, 1977; Lazzara et al, 1978; Hope et al, 1980) Most of these studies have relied on experiments on isolated tissue. The acquisition of data from intact hearts with the same degree of accuracy as with isolated tissue is limited by currently available techniques. Rapidly changing electrophysiological conditions in the intact heart further hamper the accurate collection of data. Nevertheless, the abundant experimental data available in the literature provide information on the early cellular changes

that accompany ischaemia and act as substrates and triggers for important cardiac arrhythmias.

2.1.2. Structure of the cardiac cell in the context of electrophysiology

The biophysics of cardiac excitation and conduction was recently reviewed by Arnsdorf. (1990) The cardiac cell membrane is a thin lipid bilayer which serve to separate the aqueous phases inside and outside the cell. The phospholipid molecules of this bilayer have a hydrophobic and non-polar portion oriented towards the interior of the membrane (tail end) and a hydrophilic and polar portion (head end) oriented towards the internal or external aqueous environment of the cell. As the components of the lipid bilayer can be charged, it can act as a condensor or capacitor. The membrane has large glycoproteins some of which extend through the entire membrane connecting the internal and external aqueous phases and act as ion channels or pumps (eg. sodium-potassium ATPase pump). Some glycoprotein complexes penetrate only the outer cell membrane and may serve as receptor sites for neurotransmitters and hormones while others such as the adenyl cyclase system, protrude through the inner cell membrane. These membrane glycoproteins are responsible for most of the electrophysiological properties of the membrane.

2.1.3. Ion channels

The lipid bilayer membrane exhibits permeability to ions and water. The glycoproteins mentioned above control the permeability of the membrane by acting as channels. These glycoproteins form pores that permit ions to cross the membrane rapidly, thereby creating ion currents. A channel is often selective for certain ions ie. sodium channels or potassium channels. Opening of these channels depend on stimuli such as membrane voltage changes, chemical signals or mechanical deformation all of which probably

act by inducing conformational changes in the channel proteins. Some channels rectify, that is, they conduct ions more effectively in one direction than the other. The process of response of the ion channels to the various stimuli is termed gating. Channels maybe divided according to the mechanisms that determine their gating. Thus channels exist whose function may depend on voltage (voltage gated channels), time (time dependent channels) or the binding of molecules to receptors (ligand-gated channels).

2.1.4. Intercellular conduction

Intercellular communication between cardiac cells occurs via gap junctions, also called nexus. Gap junctions are specialized regions of intercalated discs that form the margin between adjacent cells. The intercalated discs are comprised of areas of strong adhesions between cells via the macula densa and fascia densa, and the gap junctions. The gap junctions are where the cells are in functional contact with each other. Whereas the areas of strong adhesions facilitate transfer of mechanical energy from one cell to the other, gap junctions provide areas of low resistance electrical coupling. Gap junctions not only permit longitudinal conduction between cells but by their presence on the lateral surface of the cells allow lateral or transverse conduction and therefore lateral uniformity and synchronization of cardiac contraction. (DeMello, 1982) Longitudinal conduction along cardiac cells has been likened to transmission along an electrical cable with similar resistance and current flow properties (linear cable theory). (Weidman, 1952) In the event of myocardial damage eg. ischaemia or infarction, the gap junction resistance increase mediated by increased intracellular calcium and help to seal off the effects of injured from uninjured cells. (Veenstra, 1990) Increased gap junction resistance leads to slowing of action potential propagation and consequent conduction delay or block.

The ability for transmission of impulses in longitudinal and transverse directions has been mentioned above. This dependance of electrophysiological parameters on direction of propagation is called 'anisotropy'. (Spach et al, 1981) Normal atrial and ventricular muscle is anisotropic. Anisotropy is ordinarily determined by fibre orientation such that conduction proceeds two to three times more rapidly along the long axis of fibres than transversely. Anisotropic conduction becomes important when considering arrhythmic mechanisms such as re-entry in diseased hearts. In ischaemic muscle for example, longitudinal conduction is more readily affected than transverse conduction. The resulting heterogeneity in the electrophysiological properties of adjacent cardiac fibres can create functional block and set the stage for re-entrant arrhythmias. (Dillon et al, 1988)

Finally, conduction in cardiac tissue is also possible by means of 'electrotonic' propagation between few adjacent cells. Local circuit current or electronic current flows whenever a voltage difference occurs between two sites within the myocardial syncitium. A true electrotonic potential is a response whose characteristics are dictated by the passive cable properties of the muscle and one in which the active membrane properties play no role. (Antzelevitch, 1990) In the normal heart, electrotonic interaction is important for the synchronization of impulse conduction. In poorly excitable regions as occurs with ischaemia, electrotonic propagation may 'conceal' in the tissue and make the tissue inexcitable to active conduction thereby creating an area of functional block. (Pressler, 1990)

2.1.5. The cardiac action potential

The cardiac action potential is considerably more complex than the nerve action potential. The beginning of each cardiac action potential resembles the beginning of the nerve action potential in that rapid activation of sodium channels give rise to a rapid, regenerative phase of depolarisation. However, the plateau phase of the cardiac action potential is paramount for effective synchronisation of activity throughout the ventricle. A detailed description of the various ionic channels that govern the time course of the ventricular action potential is beyond the scope of this thesis and the following subsections sets forth the basic understanding of the electrogenesis of the cardiac action potential.

2.1.6. Normal transmembrane ionic gradients

Normal resting and action potentials of the cardiac muscle cell are the result of transmembrane ionic gradients that are created by active ATP requiring pumps and by selective permeability of the cellular membrane to ions. In the resting state, the interior of the cell has about 150 mM potassium ions (K^+) and 10 mM sodium ions (Na^+). The resting membrane is primarily permeable to K^+, so the resting potential is a K^+ diffusion potential, established by the transmembrane gradient of K^+. With an extracellular K^+ level of about 5 mM, the resting potential would be about -90mV ie. the interior of the cell is negative to the exterior. When positive ions such as Na^+ move into the cell, an inward current flows tending to decrease negativity of the interior and to depolarise the cell. Conversely, when positive ions such as K^+ leave the cell, an outward current is produced and tend to make the interior of the cell more negative or to repolarise the cell. The action potential is generated by an increase in membrane permeability to Na^+, allowing Na^+ into the cell down its electrical and chemical gradient. At a threshold level of reduced intracellular negativity, cellular

depolarisation occurs. The depolarisation increases permeability to calcium (Ca^{++}) producing a second inward depolarising current. The process of depolarisation also causes a reduction in permeability to K^+ (the outward repolarising current) and delays repolarisation of the action potential for 250 to 350 ms (plateau phase of the action potential). Eventually K^+ permeability rises and Ca^{++} permeability falls leading to repolarisation.

The above description of the ionic mechanisms is a simplification of a rather complex and as yet incompletely understood process of membrane depolarisation and repolarisation. A number of inward and outward ionic currents currents have been described and operates differentially in muscle tissue and specialised conducting tissue. A recent report of the Task Force of the Working Group on Arrhythmias of the European Society of Cardiology (1991), has reviewed our present understanding of the various ionic currents. The various phases of the cardiac action potential are briefly described below (see figure 2.1.1).

2.1.7. Phases of the cardiac action potential

Phase 0 - Upstroke of the action potential:

The upstroke of the cardiac action potential is produced by the sudden increase in sarcolemmal membrane conductance to Na^+. An externally applied stimulus, or spontaneously generated local circuit current in advance of a propagating action potential, depolarises a sufficiently large area of membrane at a sufficiently rapid rate to open the Na^+ channels and depolarise the membrane further. When the membrane voltage reaches threshold, Na^+ rushes through specific ion channels into the cell down its electrochemical gradient depolarising the cell. The Na^+ channels then inactivate over 1 to 2 milliseconds. After they inactivate, the membrane must be repolarised before they can be reactivated.

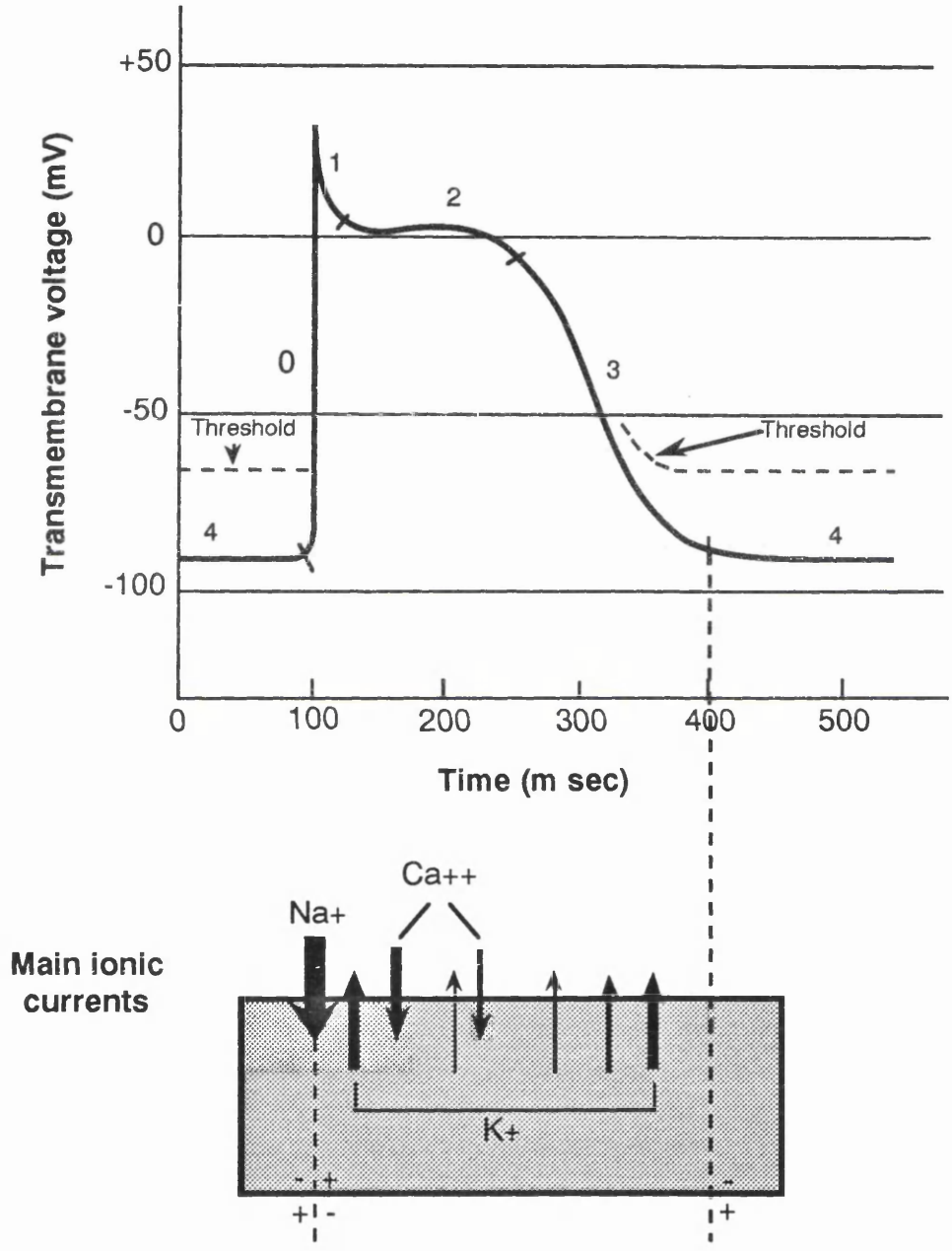

Figure 2.1.1: Action potential of a ventricular cell showing ionic currents involved during various phases and polarity changes in the cell membrane during depolarisation and repolarisation (see text for details).

The rate of depolarisation ie. the maximum rate of change of voltage over time (dV/dt_{max}) is an estimate of the rate and magnitude of Na^+ entry into the cell and is a determinant of the conduction velocity for the propagated action potential. A hypothetical *gate system model* has been suggested for the Na^+ conductance channel. (Hodgkin and Huxley, 1952) In this Hodgkin-Huxley model, sodium conductance is controlled by two parallel, gating processes, activation and inactivation. The activation process is controlled by three independent particles designated 'm' gates. For the channel to open, all three particles must be in the correct position. Inactivation of the channel is governed by the 'h' gate. Differential timings of the opening and inactivation of these gates is an important factor in the modulation of action potentials and refractoriness of premature beats.

The normal atrial, ventricular and His-Purkinje fibres have fast action potential upstrokes mediated via the Na^+ channel as described above. Action potentials in the normal sinus node and atrio-ventricular node on the other hand, have delayed upstroke with a reduced dV/dt_{max} and are called slow responses. This is because the upstrokes of the 'slow responses' are mediated by slow inward, predominantly Ca^{++} current rather than the fast Na^+ inward current.

Phase 1 repolarisation:

Repolarisation is not a simple reversal of the depolarisation process, The time course of repolarisation is much slower and involves a number of ionic currents some of which are different from those involved in depolarisation.

Following the rapid depolarisation phase 0, the membrane repolarises rapidly due to inactivation of the inward Na^+ current and activation of a

transient outward current. The transient outward current (I_{to}) is a K^+ current and consists of two components: one that is activated in a voltage dependent way, and one that is activated by and dependent on local rise of Ca^{++}. (Coraboeuf and Carmeliet, 1982) The presence and activity of the I_{to} varies in different species and even in the same species, between the epicardial and endocardial layers of the myocardium. (Antzelevitch et al, 1991)

The above currents return the membrane potential to near 0 mV, from which the plateau or phase 2 arises.

Phase 2 - Plateau phase:

During the plateau phase which lasts between 200 and 400 ms, membrane conductance to all ions fall to low levels. The transmembrane voltage remains near zero for more than 100 ms. The main current during this phase that influence the membrane potential is the slow inward current carried by Ca^{++} which essentially balance out minor outward currents during the phase. Two types of Ca^{++} channels have been described: T- and L-type. (Tsien et al, 1987) The role of the T-type in cardiac tissue is not clear. The L-type channel is prominent in the plateau phase and transports a current of large amplitude. Activation of the channel occurs at positive potentials. Inactivation which is voltage and Ca^{++} dependent is slow, and the time course of its inactivation is a major determinant of the rate of repolarisation during the plateau phase. A rapid increase in intracellular Ca^{++} as occurs with sympathetic stimulation stimulates the inactivation of the L-type channels and shortens the action potential duration. (Carmeliet E, 1990)

Intracellular Ca^{++} in the cardiac tissue is regulated by the Na^{+}-Ca^{++} exchanger which is an electrogenic pump. Depending on the membrane potential and the actual Na^{+} and Ca^{++} gradients, it can generate an outward or inward current. During the upstroke of the action potential the system may transiently transport Ca^{++} inward and Na^{+} outward. As intracelluar Ca^{++} rapidly rises and the transmembrane potential reverses, the system can transport Ca^{++} out of the cell in exchange for Na^{+}.

Another inward current of importance is a slowly inactivating Na^{+} current. (Coraboeuf et al, 1979) The inward Na^{+} current of the plateau phase is well developed in fibres that show a plateau at negative potentials, such as the Purkinje fibres. Inhibition of this current explains the shortening of the action potential in the presence of tetrodoxin or local anaesthetics which are potent Na^{+} channel blockers.

Main outward currents that maintain the plateau phase are a slowly inactivating component of the I_{to}, a Cl^{-} current, a slowly activating K^{+} current (I_k) and the Na^{+}, K^{+} - electrogenic pump current. The outward K^{+} current is slow to start but turns on in a time dependent manner and, when maximal, causes the cell to repolarise rapidly (phase 3).

Phase 3 repolarisation:

This portion is comprised mainly of the outward K^{+} current. There is time dependant inactivation of the slow inward currents and activation of the K^{+} rectifier, I_k, which results in an outward current so that extracellular movement of positive ions increases and the membrane potential shifts in a negative direction. As repolarisation continues, K^{+} conductance increases and these repolarisation changes self perpetuate in a regenerative manner.

The main K^+ current (I_k) during phase 3 repolarisation is carried through voltage gated channels with slow activation kinetics, giving it the name 'delayed rectifier'. There are probably several pharmacologically different I_k channels. (Walsh and Kass, 1988) $I_{k(ACh)}$ is K^+ current whose channel is activated by the muscarinic receptors and the $I_{k(ATP)}$ is a current carried through a metabolically regulated channel. The latter channel is blocked by ATP and is strongly activated during hypoxia. This effect of hypoxia on the $I_{k(ATP)}$ channel may contribute to the shortening of the action potential duration during ischaemia. (Noma, 1983)

Phase 4 - Resting membrane potential and diastolic depolarisation:

The resting membrane potential of the majority of the atrial and ventricular muscle remains quiescent throughout diastole. K^+ is the major ion determining the resting membrane potential. During diastole, the cell membrane is quite permeable to K^+ and relatively impermeable to Na^+. A negative potential varying between -50 to -95 mv (depending on the cell type) is maintained by the Na^+/K^+ pump which actively pumps Na^+ out of the cell against its electrochemical gradient and simultaneously pumps K^+ into the cell against its chemical gradient. This pump fueled by a Na^+/K^+ ATPase enzyme that hydrolyses ATP for energy, is bound to the myocyte membrane. This electrogenic pump generates a net outward movement of positive charges because it transports three Na^+ ions outward for two K^+ ions inward. The rate of Na^+/ K^+ pumping to maintain the same ionic gradient must increase as heart rate increases, since the cell gains a slight amount of Na^+ and looses a slight amount of K^+ with each depolarisation.

An inward rectifier K^+ current (I_{ki}) also plays a part in maintaining the resting membrane potential near the K^+ equilibrium potential. (Giles and

Imaizumi, 1988) The I_{ki} interacts with the Na^+/K^+ pump to regulate extracellular K^+.

2.1.8. Action potential in tissue capable of spontaneous discharge

In tissues capable of generating its own cardiac rhythm (ie. fibres in certain part of the atria, in the muscles of the mitral and tricuspid valves, His Purkinje fibres and in the sinus node and distal portion of the AV node), the diastolic membrane potential does not remain constant. Instead, there is spontaneous gradual depolarisation. If a propagating impulse does not depolarise the group of cells, it may reach threshold by itself and produce a spontaneous action potential. This property of spontaneously discharging cells is termed 'phase 4 diastolic depolarisation'. Phase 4 diastolic depolarisation leading to the initiation of an action potential results in automaticity. The automaticity of the sinus node is usually dominant in the normal heart and maintains normal sinus rhythm.

2.1.9. Normal response of the cardiac action potential to heart rate alterations

When heart rate increases, the action potential duration must shorten. This shortening is accomplished by increasing net outward current at earlier times. The increased outward current is due to at least two important factors that result in a net accumulation of K^+ extracellularly.

1. Sustained activation of the delayed rectifier (I_k):

At potentials negative to -50 mv during the diastolic interval, the I_k deactivates. If the diastolic interval is shortened sufficiently, all the I_k channels may not close during diastole and an increase in outward current will exist at the start of the next action potential. This sustained outward current will shorten the time period required for final repolarisation to begin resulting in shortening of the action potential duration. (Hauswirth et al, 1972)

2. Increase in Na^+/K^+ pump activity:

As the upstroke of the action potential is generated by the Na^+ entry through the tetradoxin sensitive channel, Na^+ influx will increase as the heart rate rises. This increase in Na^+ has to balanced by the Na^+/K^+ pump. As mentioned above, this pump extrudes three Na^+'s for each two K^+'s it returns to the cell. The net result is a outward current proportional to the rate of Na^+ transport. These pump currents associated with physiological increases in heart rate can significantly shorten action potential duration. (Gadsby and Cranefield, 1982)

Active K^+ influx and Na^+ efflux reach a new steady state after only one to two minutes. It therefore follows that adaptation of the action potential duration to a new steady state after an alteration in the heart rate can take upto 2 minutes. Action potential duration shortens abruptly when a premature extrastimulus is introduced. (Gettes, 1972) The relation of the amount of action potential shortening to the prematurity of an extrastimulus has been characterised as electrical restitution based on the time dependent restitution of membrane currents from the process of channel activation and inactivation. (Bass, 1975) Electrical restitution curves are constructed by pacing at steady state and interposing single beats at progressively shorter and longer coupling intervals in between trains of steady state pacing. Plotting electrical restitution curves during various interventions provides a means of studying the interrelation of rate changes to the other variables on the action potential duration (and hence, repolarisation). (Taggart et al, 1990)

ELECTROPHYSIOLOGY OF MYOCARDIAL ISCHAEMIA

2.1.10. Effect of ischaemia on transmembrane action potentials

Myocardial ischaemia produces profound effects on the cardiac action potential. Varying degrees of alteration of the resting membrane potential, inward and outward currents cause changes in the automaticity, refractoriness and conduction velocity of the cardiac action potential. These changes are important in the context of ventricular arrhythmias which arise as a consequence of myocardial ischaemia. The time course of ionic changes and possible mechanisms of changes in the action potential have been discussed in earlier sections.

Kardesh and colleagues were the first to report on the effect of global ischaemia on the transmembrane potentials. (Kardesh et al, 1958) They reported a marked shortening of the action potential duration and a decrease in amplitude within a few minutes after occlusion in 17 isolated perfused rabbit hearts and 4 dog hearts. Similar results were subsequently reported during acute regional ischaemia in porcine and canine hearts. (Downar et al, 1977; Kléber 1978; Russell et al, 1979) A considerable number of recent publications have now established that ischaemia produces three main effects on the transmembrane action potentials: 1) The current carrier for excitation ie. the inward sodium current is altered in ischaemic cells. Maximum diastolic potential is reduced and there is a fall in the amplitude of the action potential. 2) The action potential duration is abbreviated early in ischaemia. 3) The upstroke velocity of the depolarisation phase of the action potential (V_{max}) is markedly reduced and conduction is slowed. (Cinca et al, 1980; Penny and Sheridan, 1983; Franz et al 1984; Dilly and Lab, 1987) These alterations in cell electrophysiology are the basis of the ECG changes and early ischaemic arrhythmias.

It is generally held that it is the defect of Na^+ channels that underlie the abnormal excitability of acute ischaemia. (Janse and Kléber, 1981) Slowing of conduction in ischaemic cells is by two mechanism: the decrease of inward Na^+ current and a tendency for the cells to uncouple from each other. The decrease in intracellular pH that accompany ischaemia increase the resistance of the gap junctions. Shortening of the action potential duration with ischaemia on the other hand is believed to be mediated via several factors as discussed in section 1.4.5. with both inward and outward currents being implicated. (Janse and Wit, 1989)

2.1.11. Translation of cellular electrophysiological changes to the clinical situation

ST segment *elevation* in the surface electrocardiogram represents the sum of TQ segment depression during diastole and ST segment elevation during systole; it arises from the potential difference that exist between ischaemic and non ischaemic tissues during these phases of cardiac cycle. During diastole, if we assume that normal cardiac tissue has a resting potential of -85 mV and that of ischaemic tissue directly under the electrocardiogram lead has a depolarised potential of -65mV, then an 'injury current' flows towards the healthy tissue and away from the electrocardiogram lead. This produces a negative TQ segment deflection. During systole, the healthy tissue maintains a plateau level of -10 mV, but the ischaemic tissue has a lower plateau at -30mV. Current flows toward the more negative potential in the ischaemic area, producing a positive deflection during the course of the ST segment of the surface electrocardiogram. (Sampson and Scher, 1960; Fozzard and Makielski, 1985) Similarly, the mechanism of ST segment *depression* during subendocardial ischaemia can be explained in the basis of relative differences in epicardial and endocardial action potentials and is shown in figure 2.1.2.

The polarity of the local T wave is dependent on the duration of the action potential and on the relative sequence of the repolarisation phase of the action potentials in the epicardial and endocardial layers or in the case of ischaemia, of repolarisation between ischaemic and non-ischaemic areas. (Taggart et al, 1990 b) In the normal myocardium, endocardial repolarisation occurs later than in the epicardium and the surface electrocardiogram would record a positive T wave polarity. Shortening of the endocardial action potential duration in ischaemia or a change in the sequence of repolarisation such that this normal relationship is reversed would register an inverted T wave in the recording over that area (see figure 2.1.2).

Onset of ventricular arrhythmias is frequently preceded by ischaemia. The normally long duration of the cardiac action potential provides a safe period during which re-excitation is impossible. The effect of ischaemia apart from increasing automaticity is to disperse in a patchy manner, the excitation, conduction velocity and action potential duration (and hence refractoriness of the myocardium) thereby providing a field for reentry circuits within areas of the myocardium.

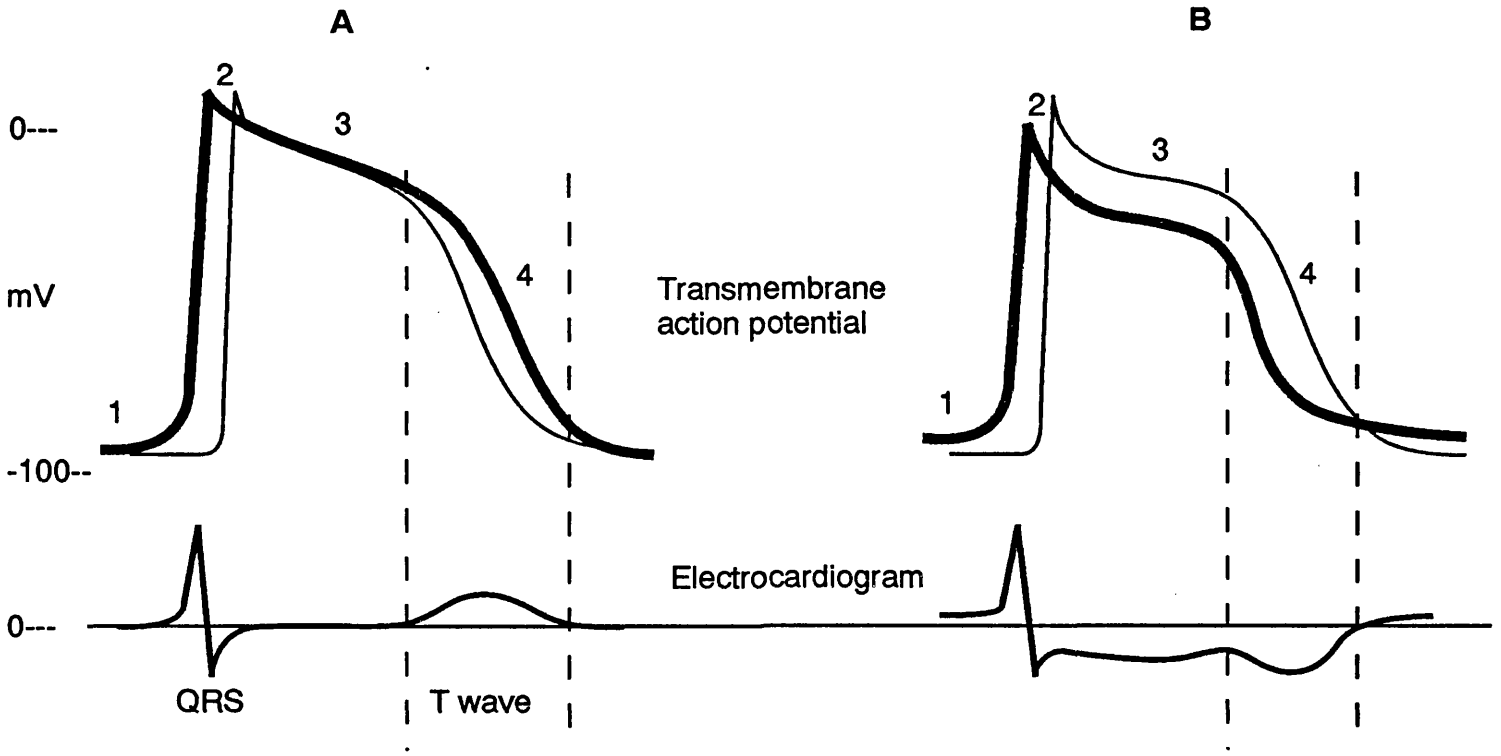

Figure 2.1.2: Relationship between transmembrane action potentials and surface electrocardiogram in a hypothetical example of subendocardial ischaemia. A) Action potentials from endocardium (thick line) and epicardium (thin line) are superimposed to demonstrate the differences in voltage at different points. The isoelectric portions of the ECG are derived from the portions of the action potentials where no potential differences occur (points 1 and 3). Inflections from the isoelectric baseline correspond to regions where differences in voltage are apparent ie. the QRS complex (point 2) and T wave (point 4). The polarity of the T wave follows that of the R wave because current flow during both depolarisation and repolarisation are in the same direction (endocardium to epicardium in this example). B) Changes encountered in subendocardial ischaemia. The ischaemic cell is partially depolarised and hence has a less negative diastolic membrane potential so that the TQ segment of the ECG registers elevation from the isoelectric point (point 1). The amplitude of the endocardial action potential is lower than that of the epicardial AP leading to ST segment depression (point 3). The action potential duration over the endocardium is shortened leading to reversal of polarity of the T wave (point 4). (Adapted from Taggart et al, 1990).

RECORDINGS OF THE CARDIAC ACTION POTENTIALS USING SURFACE ELECTRODES: MONOPHASIC ACTION POTENTIALS.

2.1.12. Development

Although the introduction of the glass microelectrode recording technique (Ling and Gerard, 1949) made it possible to study the electrophysiological activity of the cardiac cell, this technique was quite obviously unsuited to the beating heart. Studies in intact in situ hearts lends itself to developments based on Schütz's description of monophasic signals recorded with suction electrodes. (Schütz, 1931) The suction electrode technique recording of myocardial events had a gross configuration similar to that of the microelectrode technique. In order to distinguish the two types of records from each other, the suction electrode recording of a cardiac cycle was called the monophasic action potential (MAP) whilst the microelectrode technique was called an action potential (AP). These terminologies can be construed as misleading as both recordings possess a monophasic shape.

2.1.13. Genesis of the MAP

Unlike the micro-electrode which records from individual cells, the MAP is recorded with a catheter that has a tip diameter of approximately 1 mm and therefore cannot enter a single cardiac cell. The recordings are therefore extracellular and the exact nature of generation of the MAP has been debated. The mechanism of generation of the MAP has been discussed by many investigators (Schaefer et al, 1943; Cranefield et al, 1951; Hirsch et al, 1985; Olsson et al, 1989) but no quantitative analysis has yet been presented. Theoretical models have been suggested to relate quantitatively, the MAP to underlying myocardial electrical activities. (Hirsch et al, 1985)

Early investigators obtained monophasic action potentials by producing overt myocardial injury. (Schütz, 1931) Consequently, the contention was

that myocardial injury was an absolute requirement for the genesis of the MAP although it was recognized that the electromotive forces giving rise to the MAP arose from the uninjured subjacent tissue. (Schaefer et al, 1943; Cranefield et al 1951; Eyster and Gilson, 1946) However, it is now known that MAP can be signals can be produced by interventions that merely result in depolarisation of underlying cells without causing injury to the tissue. That such local depolarisation could be achieved by application of potassium chloride was shown by Dower and his colleagues and resulted in investigators of the time routinely employing local application of potassium for production of MAP signals. (Dower et al, 1962; Korsgren et al, 1966, Szekeres and Szurgent,.1974) The modern day use of the pressure contact electrodes lends further support to the fact myocardial *injury* is not an absolute requirement for the genesis of the MAP.

A hypothetical model inferred from data available in the current literature was recently presented by Franz. (Franz, 1991) Theoretically, the pressure contact or suction applied to produce the MAP signal depolarize the cells under the MAP electrode and make then electrically inert. However, the cells subjacent to the exploring electrode remain active and are capable of active depolarization and repolarization. Consequently, an electrical gradient is created between these normal cells and the depolarized cells beneath the electrode. Current flow between the two regions result during electrical systole and diastole and the electrical field thus created is proportionate to the strength of the current flow between the two regions. The MAP recording therefore essentially reflect the voltage time course of the normal cells that surround the volume of tissue depolarized by the exploring electrode.

2.1.14. Relationship between the transmembrane action potential and MAP: The relationship between the action potentials recorded by the microelectrode technique and the MAP has been analysed in detail by several investigators (Hoffman et al, 1958, Churney and Ohshima, 1963; Franz et al, 1986, Ino et al, 1988). Validation of the MAP recordings using the suction electrodes was first provided by Hoffman and colleagues in 1958. These investigators compared simultaneously recorded transmembrane action potentials signals obtained by microelectrode technique and the MAP using suction electrodes from isolated feline papillary muscle and in isolated perfused rabbit hearts. The MAP record was found to be a "reliable index of the time of arrival of excitation at the electrode and as a reliable index of the shape of the action potential during the entire phase of repolarization". When the shape of the transmembrane action potentials as observed by the microelectrode technique was altered by ions such as K^+ and Ca^{++}, the changes were accurately reflected in the potentials recorded by the suction electrodes. These findings were corroborated by Churney and Ohshima in 1963. Simultaneous recordings of the MAP and transmembrane action potentials from the *in situ* dog hearts again showed that the repolarization phases of the action potential signals obtained by both techniques were coincident.

Suction electrodes however posed problems with the stability of the recordings consequent upon irreversible injury and cellular uncoupling caused by the application of suction. (Holland and Arnsdorf, 1981) Also, the early suction electrodes registered small amplitude and were replete with artifacts. The tissue plug that is sucked into the suction tube has a high impedence and account for the low amplitude. Pressure contact electrodes surpassed these problems. MAPs obtained by pressure contact electrodes were validated against microelectrode recorded transmembrane action

potentials by Franz and coworkers in 1986. Simultaneous recordings of transmembrane action potentials and MAPs using sintered silver-silver chloride electrode tipped catheters were obtained from isolated rabbit heart septum preparations. Two hundred and ninety one simultaneous signals were digitized and analysed using a computer program for action potential duration measured at 30%, 60% and 90% repolarisations. A highly significant close correlation (r value = 0.98) was found for the various action potential durations recorded using the two techniques. A similar correlation was found when the total area bounded by the shape of the action potential and the horizontal line between the depolarization upstroke and repolarization downstroke at 30%, 60% and 90% repolarization confirming the close similarity in overall shape of the action potentials obtained by the two techniques. The investigators further tested the ability of the MAP to detect effects of perturbations in steady state on the action potential. The preparations were paced at different cycle lengths and the K^+ and Ca^{++} of the perfusate varied. Appropriate alterations in the action potential duration were coincidentally reflected in recordings obtained using both microelectrode and contact electrode techniques.

The MAP electrode has a tip diameter of approximately 1 mm and therefore must record the electrical activity from many cells surrounding the exploring electrode. It follows therefore that some differences exist between the action potential recorded from a single cell and the MAP:

1. The resting membrane potential of the MAP is always higher than that of the transmembrane action potential. Both the diastolic and action potential amplitude of the MAP signal is therefore always lower than that of the transmembrane action potential. The MAP typically records signals of

amplitude varying between 10 to 50 mV although much higher amplitudes are sometimes recorded.

2. Since the MAP is the net electrical activity from a number of cells under the electrode whose depolarizations occur sequentially, the upstroke of the MAP is of longer duration than that of a single cell. The V_{max} of the action potential from a single cell is much faster measuring 200 to 300 V/s in a canine ventricular cell (Draper and Weidmann, 1951) compared to 6.4 V/s on a MAP recording. (Franz et al, 1984) Also, the MAP upstroke maybe contaminated by the electrogram but this can be effectively reduced by the use of closely spaced bipolar electrodes.

3. The overshoot of MAP signal may be higher than that of the corresponding action potential. In almost all studies using the MAP, the systolic overshoot of the MAP signal over the zero potential has comprised one third of the total amplitude of the signal while the diastolic resting potential lies two third below the zero potential. This ratio appears to be independent of the absolute voltage of the MAP. In contrast, the transmembrane action potential when referenced to the zero potential is distributed with one fourth of the peak amplitude above the zero potential and the diastolic potential being three fourths below the zero line. (Hoffman, 1960) The offset of the MAP reference may be due to the fact that cells beneath the MAP tip electrode are depolarized to less than 0 mV.

Despite these disparities, the repolarization time course measured using the MAP is a true reflection of the transmembrane action potential and the duration of the action potential measured at periods between 30 and 90% repolarization remain a reliable measure of repolarization changes in the transmembrane action potentials for the cells in the immediate vicinity of

the MAP exploring electrode. In addition, the start of the rapid upstroke of the MAP coincides with the time of local excitation and has been employed as a measure of the time of local excitation. (Levine et al, 1986) However, the use of the MAP upstroke to determine local excitation and the speed of conduction is controversial and is discussed in section 2.1.17.

2.1.15. Recordings of MAP in man:

Korsgren et al in 1966 first described the technique for recording intracardiac MAPs in man via the transvenous route. Their technique involved the use of suction electrodes as well as local infusion of potassium to depolarise the cells underlying the electrode in the right atrium. The realisation that MAP recordings could be safely obtained from the human endocardium led to the refining of the suction electrodes by Olsson and colleagues who published a series of articles dealing with human right atrial and ventricular endocardial recordings (Olsson, 1971; Olsson et al, 1971; Olsson and Varnauskas, 1972) Local infusions of potassium were in fact unnecessary for the generation of adequate MAP signals and Olsson's group used close bipolar suction electrodes without potassium for their endocardial recordings. Suction electrodes however produced irreversible local cellular injury and thereby allowed recordings for only short periods of time.

The technique of using pressure contact electrodes for MAP recording in the human heart was described in 1980. (Miller et al, 1980; Franz et al, 1980) Bipolar catheters using silver/silver chloride non polarizable electrodes were developed to facilitate longer term recordings. (Franz, 1983) These catheters operate on the principle of pressure contact to induce depolarisation of underlying cells and produce MAP signals. Stable signals could be recorded for a period of upto 3 hours without the need for repositioning.

Whereas the above catheter designs were intended for intracardiac recordings of MAP, epicardial recordings have been carried out during cardiac surgery in man using hand held bipolar devices with non-polarizable electrodes mounted in acrylic frames. (Runnalls et al, 1987) MAP signals of good quality are easily obtained in this manner and have been used to study phenomenon of contraction-excitation feedback and adequacy of myocardial revascularisation during coronary artery bypass surgery. (Taggart et al, 1986; Taggart et al, 1988 b)

2.1.16. Technical aspects of MAP recording:

The MAP signal consists of both high frequency (upstroke) and low frequency (repolarisation phase and diastolic phase) components. The minimum high frequency response time for reliable MAP signals have not been determined but current recommendation is the use of a band width from 0 to 5,000 Hz. (Franz, 1991) DC coupled differential pre-amplification helps to boost the MAP amplitude and eliminate AC and other high frequency noise.

The MAP signal has a high signal to noise ratio and with appropriate equipment does not require low or high frequency filtering. As the diastolic resting potential of the MAP has a negative value, AC amplifiers tend to artefactually move the diastolic MAP potential upward and thereby mimicking phase 4 depolarisation. DC amplification is therefore preferred. The use of non-polarizable electrodes on the MAP catheter abets DC amplification.

Adequate electrical isolation from the patient is a pre-requisite in amplifiers used for the purpose.

2.1.17. Errors in MAP recordings:

The common error in the bipolar MAP recording technique is the influence of the mechanical activity of the heart. The ventricular MAP recording may be influenced by the mechanical activity of the atrium as well as the activity of the cardiac chamber from where the recordings are acquired. This potential limitation can unfavourably influence the interpretation of the MAP where pathoelectrophysiological phenomenon are anticipated at the time of the mechanical activity of the heart. Separation of the mechanical artifacts from genuine electrophysiological phenomenon is difficult.

Investigators have documented that the start of the rapid upstroke of the MAP coincides with the time of local excitation. (Levine, 1986) However, since the MAP is created from the electrical activity of a large number of cells, the upstroke of the MAP is of longer duration than that of the transmembrane action potential. During this 'slowed' depolarisation phase of the MAP, superimposition of the local electrogram will further influence the appearance of this phase. (Olsson et al, 1985) Interpretations of the variations in upstroke of the MAP are therefore not entirely reliable and most workers in the field use a tangent drawn to the fastest part of the upstroke to determine the onset of the MAP rather than the first point of deflection from the baseline. (Gettes, 1992)

2.1.18. Relation between the MAP and the electrocardiographic parameters:

On the surface electrocardiogram, the T wave represents myocardial repolarisation. The question may then be asked as to why the QT interval cannot be used as reliable measure of regional repolarisation. The action potential duration and QT interval measured on the surface ECG cannot be used interchangeably for several reasons. The QT interval is a measure of

the time from the start of ventricular depolarisation to the end of repolarisation. Although several studies have shown a close correlation between changes in the action potential duration and the QT interval, (Edvardsson and Olsson, 1981; Samuelsson and Harrison, 1981) the QT interval do not only reflect changes of myocardial repolarisation but also its depolarisation. Though a uniform prolongation of the repolarisation time may be detected as a prolonged QT interval, the QT itself represents the time from the first depolarisation to the final repolarisation in the axis of the selected leads. Furthermore, in diseased hearts where there is conduction delay and cellular uncoupling as with ischaemia, the QT interval may show a prolongation whereas the individual action potential duration will register shortening.

As the QT interval is an electrocardiographic summation of the individual action potential durations, it might conceal within itself a number of short action potential durations juxtaposed to long ones. (Vaughan Williams, 1982) The JT interval has therefore been suggested as a better surface marker of repolarisation time course. However, this interval is only an approximation of the repolarisation time since most cells have been partly repolarised before the QT is fully inscribed. A further difficulty with the use of the QT interval as a measure of time course of repolarisation is the actual measurement of the QT interval on the surface electrocardiogram. The onset of the QT interval is easily defined but identifying the end or the peak of the T wave is less reliable. Besides, the QT interval varies in different ECG leads and there is no unanimous agreement as to which lead should be used for QT measurement.

THE MONOPHASIC ACTION POTENTIAL AS A MEASURE OF ISCHAEMIA

2.1.19. Previous studies using the MAP as a measure of myocardial ischaemia:

In animal studies, the ability of the MAP signals to register early changes of myocardial ischaemia has been assessed by several investigators. (Franz et al, 1984; Dilly and Lab, 1987; Kingaby et al, 1986) Franz et al (1984) compared endocardial and epicardial MAP recordings with local ST segment changes on electrograms recorded from the close vicinity of the MAP recording site. These studies were performed during periods of transient coronary occlusion and during creation of transmural ischaemia/infarction in the dog. Ischaemia consistently produced characteristic changes in the MAP signal and these changes corresponded temporally to the ST/TQ segment changes in locally recorded electrograms. Maximal changes were apparent 4 to 5 minutes after coronary occlusion. Reperfusion resulted in rapid reversal of the MAP changes with pre-ischaemic control values being reached within 1 minute of reperfusion. Similar changes in the MAP were reproduced by local infusion of potassium. Kingaby et al (1986) compared the epicardial MAP and ST segment on the electrogram with regional blood flow during coronary occlusion in the pig. Changes in MAP were consistent and correlated better than ST segment alterations to decrements in blood flow which was measured in this study using microspheres.

The use of the MAP for the detection of myocardial ischaemia in humans has been limited to a few studies. Epicardial MAPs were recorded by Taggart et al (1986) during aorto-coronary bypass as a measure of completeness of myocardial revascularisation. During controlled occlusion of a saphenous vein graft supplying the area of MAP recording, changes characteristic of ischaemia were consistently produced in the MAP signal. Endocardial

recordings have been attempted in human studies recording from potentially ischaemic areas (Donaldson et al, 1983 b, 1984 a, 1984 b). However, these studies have not systematically produced controlled ischaemia nor validated the changes in endocardially recorded MAPs against an independent marker of ischaemia.

2.1.20. Changes in the MAP of myocardial ischaemia

Ischaemia induces several changes in the MAP signal. In animal studies, Franz et al documented in the MAP, all the changes of ischaemia that have been recognised in transmembrane action potential signals. (Franz, 1984) These included shortening of the action potential duration, loss of amplitude of the plateau phase and a reduction in the upstroke velocity (dV/dt_{max}) (see figure 2.1.3). Although all these changes can be documented relative to a pre-ischaemic situation in experimental studies, the MAP records a variable proportion of the intracellular action potential amplitude and upstroke velocity. Additionally, in the use of contact electrodes to record the MAP, alteration in the pressure of contact against the myocardium can depolarise variable number of cells and change the amplitude of the signal. Finally, there is a potential for the local electrogram to contaminate the upstroke of the MAP signal. These factors render measurements of the amplitude and dV/dt_{max} of the MAP signal relatively unreliable parameters. The duration of the MAP however, remain a reliable measure of the entire time course of repolarisation and registers shortening in response to ischaemia although on the epicardial surface in open chested animals, an initial prolongation has been reported. (Dilly and Lab, 1987) Kingaby et al (1986) showed that the degree of MAP

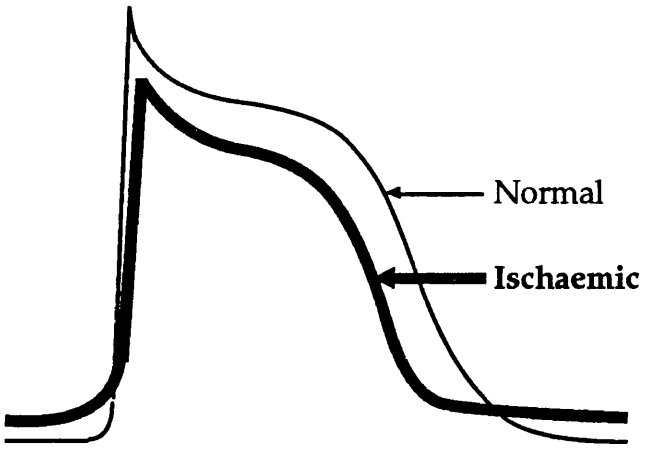

Figure 2.1.3: Typical changes in the monophasic action potential signal of ischaemia.

duration shortening correlated with the extent of reduction in regional myocardial blood flow.

2.1.21. Disadvantages of conventional markers of myocardial ischaemia

The most commonly used marker of myocardial ischaemia is the surface electrocardiogram. Although for practical purposes, the ECG provide a fair assessment of cardiac ischaemia, localisation of a region of myocardial ischaemia by epicardial or precordial ST segment mapping is fraught with problems. TQ and ST segment displacement do not directly reflect local electrophysiological changes in the ischaemic myocardium but the electrical gradient between ischaemic and non ischaemic myocardium an and can be influenced by any perturbation that affects membrane potential and shortening of the action potential duration in the ischaemic or non-ischaemic myocardium. These changes can also be caused by factors other than ischaemia such as electrolyte abnormalities, antiarrhythmic drugs and heart rate changes. (Franz, 1984) The surface ECG can therefore be both non-specific and insensitive to early ischaemia. Evidence to support this contention has been discussed in section 1.5.2. Furthermore, assessment of

the degree of regional ischaemia is further hampered by the fact that ST segment changes are influenced by the duration of ischaemia, declining progressively despite the development of irreversible myocardial ischaemia with time. The geometry of the ischaemic border, inhomogeneities in the volume conductor properties of the surrounding muscle and blood, and intraventricular conduction abnormalities further confound the interpretation between true TQ-ST segment shifts and reciprocal or secondary electrocardiographic changes. (Holland and Arnsdorf, 1977)

Biochemical markers of ischaemia such as myocardial lactate production, are not readily applicable to regional myocardial ischaemia. Positron emission tomography permits characterisation of myocardial metabolism and detection of altered metabolism associated with ischaemia and may evolve as a useful tool in this context. However, its high cost and requirement for cyclotron generated isotopes make it an impractical tool. Myocardial perfusion scintigraphy is an indirect measure of myocardial ischaemia with myocardial uptake of radiotracers depending primarily on regional blood flow rather than actual cellular dysfunction due to ischaemia. In addition, none of the above three methods can be applied for studying ischaemia that can be turned on and off in the course of a single study.

It is therefore desirable to have a methodology to directly estimate the location and severity of myocardial ischaemia.

2.1.22. Potential advantages of the MAP as a measure of ischaemia

Unlike the electrogram or electrocardiogram, the MAP registers changes in the immediate surroundings of the recording electrode and reflects cellular electrophysiological alterations in an area of approximately 5mm diameter. It is therefore an ideal tool for the study of localised ischaemia. It has been

shown to be more sensitive than the surface ECG for the detection of early myocardial ischaemia. (Donaldson et al, 1983 a, 1983 b; Taggart et, 1988) The MAP electrode can be moved from region to region to explore different areas of the myocardium. In a steady state situation, the duration of the MAP shortens in response to ischaemia and therefore permits quantification of ischaemia in terms of milliseconds of shortening. Finally, the MAP allows quantitation of ischaemia during several interventions in the course of a single study unlike the other methods mentioned above.

CHAPTER 2.2

METHODOLOGY II: MYOCARDIAL PERFUSION SCINTIGRAPHY

Scintigraphic imaging using Tc-99m-hexakis-2-methoxy-2-methylpropyl-isonitrile (Tc-99m-MIBI) was employed as an independent marker of regional myocardial perfusion in the series of studies presented in this thesis. To facilitate a clear understanding of the basis of myocardial perfusion studies, a brief review of human coronary artery physiology is presented.

CORONARY ARTERY PHYSIOLOGY

2.2.1. Basic Anatomy

Extramural arteries: The mammalian heart has two coronary arteries arising from the aorta: a left and right coronary artery. In subprimate species, the left main coronary artery divides into a septal, anterior descending and circumflex branches. In primates, instead of a single septal branch, the ventricular septum is supplied by multiple branches from the anterior and posterior descending branches. Also, in contrast to the subprimate species, the right coronary artery is usually dominant in man and swine; in addition to supplying most of the right ventricle, it also gives rise to the posterior descending artery, through which it supplies the posterior part of the ventricular septum and inferior wall of the left ventricle as well as the atrioventricular node. (Berne and Rubio, 1979)

Intramural arteries: These vessels arise almost at right angles from the large extramural ones and pass through the myocardium. Within the deeper layers of the myocardium, they branch dichotomously and get smaller. In human hearts they unite to form a subendocardial plexus in the trabeculae. (Fulton, 1965; Marcus, 1983)

Collateral circulation: Natural anastomoses between coronary arterial branches are known as collaterals which vary in size from 30 to 200μm in diameter. Depending on species, the site of these collaterals vary between the epicardium, within the myocardium and the subendocardial region; mainly epicardial in dogs and subendocardial in humans. In some species, eg. dogs, natural collaterals are plentiful whereas in others eg. swine and baboons, they are scarce. Under physiological conditions, collateral vessels have little or no flow in them and are collapsed with a diameter less than 40 μm. However, when myocardial perfusion is compromised, these collaterals enlarge to their full size and permit blood flow sufficient to maintain myocardial viability. Chronic ischaemia resulting from gradual narrowing of epicardial vessels, severe anaemia and exercise appear to enhance the development of collaterals. (Cohen et al, 1982; Scheel and Williams, 1985; Habib et al, 1991) Angiogenic biochemical factors have also been suggested as the stimuli to collateral formation. (Kurachi et al, 1985)

Capillaries: Most of the blood volume of the left ventricle reside in capillaries which are numerous with an average ratio of one capillary per muscle fibre. There is on average a capillary density of 3000 per square millimeter. Assuming a mean capillary diameter of 5-9 μm, the capillary blood volume occupy 10 to 20% of the left ventricular free wall myocardial volume at end diastole. (Hoffman and Ritman, 1987) Not all capillaries are perfused with blood; capillary recruitment is caused by hypoxia and its mediator substances.

Veins: Blood entering the left anterior descending artery passes through the anterior interventricular veins to the great cardiac veins and thence to the coronary sinus and right atrium. Left circumflex artery blood returns

through a number of small veins directly into the coronary sinus. Most of the blood from the septum drain via the anterior cardiac veins or the posterior interventricular veins into the coronary sinus or even directly into the right ventricle. Right ventricular free wall drainage is mostly directly into the right atrium. Only about 60 to 80% of the total blood entering the left coronary artery emerges in the coronary sinus. (Nakazawa et al, 1978)

Nerves: The major coronary arteries are supplied by unmyelinated nerve fibres which end in varicocities on smooth muscle cells. Both acetyl choline and nor-epinephrine as well as other vasoactive agents have been identified in the different varicosities. Arterioles being the primary site of of blood flow regulation to the myocardium have the highest ratio of nerve endings to muscle.

2.2.2. Myocardial oxygen demand/consumption

Myocardial oxygen demand or consumption is the major factor influencing myocardial blood flow. Studies in isolated papillary muscle have examined oxygen consumption and its correlates ie.high-energy phosphate utilisation and heat production. (Parmley and Tyberg, 1976) During muscle contraction most of the energy is used in force generation (internal or contractile element work). Shortening (external work) uses about 10-15%; basal metabolism consisting mainly of protein synthesis and sarcolemmal Na^+/K^+ transport utilizes 15-20%; about 10% is taken up for activation energy relating to activity of the ATPase pump.

In studies on whole heart preparations, pressure work was found to have a greater influence on myocardial oxygen consumption than did volume work. (Sarnoff et al, 1958) Later studies highlighted the importance of peak wall tension, ventricular volume and wall thickness as determinants of

myocardial oxygen demand. (McDonald et al, 1966) The concept that pressure work is the main determinant of myocardial oxygen consumption lead to the use of indices such as tension-time index, peak systolic pressure X heart rate product or the triple product of systolic pressure, heart rate and duration of ejection as predictors of myocardial oxygen demand. Although these do not take into account wall stress, they often predict oxygen consumption well because they and wall stress are highly correlated.

2.2.3. Myocardial blood flow

Myocardial blood flow (F) which is synonymous with coronary flow is a function of pressure decrease and vascular resistance:

$$F = (P1-P2)/R$$

where P1 is the coronary arterial pressure (mm Hg), P2 is the back pressure on the other side of the coronary vascular bed (mm Hg), and R is the coronary vascular resistance (mm Hg/ ml/min). Coronary resistance has three components: 1. a viscous component indicated by resistance to flow in a fully dilated coronary bed during diastole (R1) 2. resistance offered by autoregulatory vascular smooth muscle and capillary pericyte constriction (R2), and 3. extravascular tissue pressure that compresses intramyocardial vessels during systole (R3). (Klocke, 1976) The influence of the three types of resistance on coronary flow is shown in figure 2.2.1.

If R2 and R3 are eliminated by maximally vasodilating the coronary arteries with adenosine during arrest in diastole, a linear increase in coronary flow will occur with increase in perfusion pressure (line A in figure 2.2.1). When the heart is allowed to beat, this linear relationship line is shifted downwards with a fall in the steepness of the slope (line B) as a result of the increased resistance that occurs during systole (extramural resistance - R3). Any increase in heart rate and consequently a smaller proportion of diastolic

time per minute will lower the total flow at any given perfusion pressure. (Domenech and Grich, 1976) Finally, when vascular tone is present, the pressure-flow relationship is altered due to autoregulation (line C).

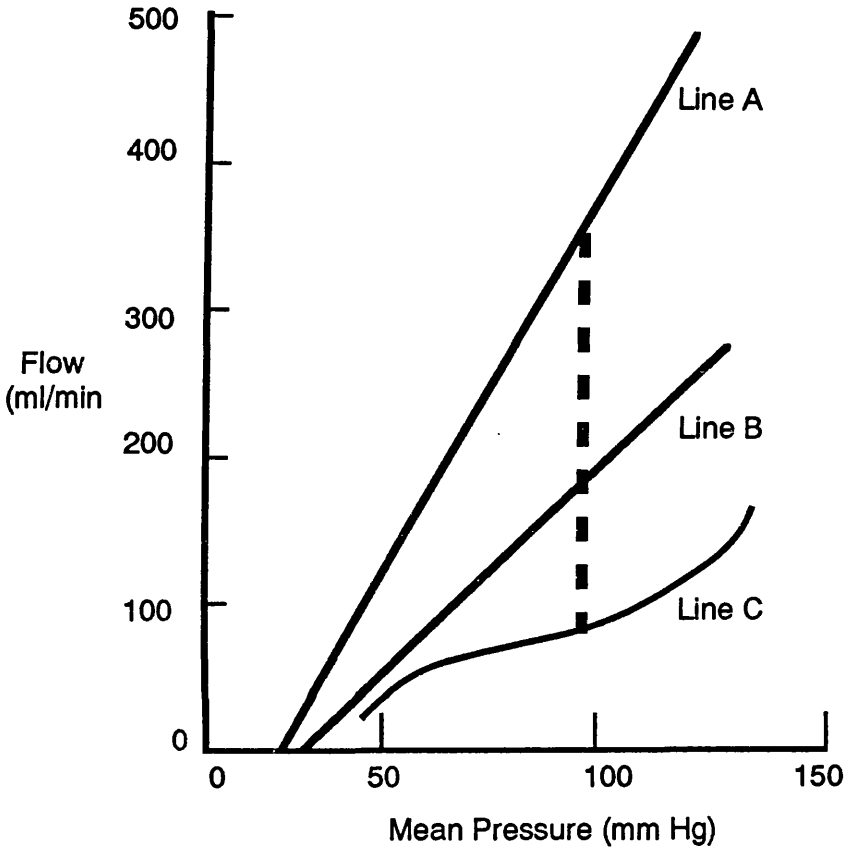

Figure 2.2.1: The relationship between coronary artery perfusion pressure and blood flow in the normal left ventricle during 1. maximal vasodilatation and diastolic cardiac arrest (Line A), 2. maximal vasodilatation in the beating heart (Line B), and 3. autoregulation (Line C). The dark dotted line is the coronary flow reserve. (Adapted from Hoffman, 1990).

Coronary autoregulation:

Over a wide range of coronary perfusion pressures (from about 70 to 130 mm Hg), the coronary vascular bed primarily at the arteriolar level, is able to autoregulate flow to maintain a constant level. (Hoffman and Spaan, 1990) This intrinsic property of the coronary vasculature has the obvious

advantage of protecting coronary flow from large fluctuations which might otherwise result from moderate changes in arterial pressure. Below the lower limit of the autoregulatory range, coronary flow decreases markedly when coronary perfusion pressure is decreased. Similarly, above the upper limit, coronary flow is markedly raised when perfusion pressure is raised. Within the autoregulatory range at any given pressure, coronary flow is lower during autoregulation than when the vessels are maximally dilated (figure 2.2.1).

Coronary flow reserve:

The difference between baseline levels of coronary flow when under autoregulation and that achieved by maximal vasodilatation as shown in figure 2.2.1 is termed the 'coronary flow or vascular reserve'. It is important to note from examining the figure that the coronary flow reserve is critically dependent on the actual perfusion pressure and positions of the line of autoregulation and the maximal vasodilation. (Hoffman, 1990) As the relation between mean pressure and maximal dilated flow is so steep, any change in perfusion pressure can significantly alter the coronary flow reserve. The coronary flow reserve would be greater at a higher perfusion pressure. Also, the lines of autoregulation and maximal vasodilation can be altered by various physiological and disease states. For example, the autoregulated resting flow can be elevated by anaemia or hypoxaemia, increase in myocardial oxygen demand due to exercise, tachycardia or thyrotoxicosis, and ventricular hypertrophy. Similarly, the line of maximal vasodilated flow becomes less steep with increased flow resistance (R1) due to fewer number of or narrowed vessels, increase in contractility and high viscosity as in polycythaemia.

The main consequence of a decreased coronary reserve is a limitation in the ability of the coronary vasculature to meet the extra demand of stress or exercise. As a result, myocardial oxygen supply will, at some stage, fail to meet demand and myocardial ischaemia will result.

2.2.4. Physiological basis for myocardial perfusion scintigraphy

Myocardial perfusion imaging with radiotracers is based predominantly on the principle that myocardial uptake and distribution of the radiotracer is proportionate to regional blood flow. It is also dependent on the viability of the myocardial tissue. Coronary blood flow (approximately 100 ml/min/100 g.) under normal resting conditions is uniform throughout the left ventricle. During period of increased oxygen demand, a normal coronary flow reserve permits augmentation by four to five times the resting flow rate.

In the presence of a normal coronary reserve therefore, myocardial uptake of the potassium analogue thallium or the newer technetium myocardial imaging agents would be uniform throughout the left ventricular myocardium. Coronary stenosis in an epicardial artery exceeding 50% of the diameter can impair coronary reserve and the ability to increase flow during stress. (Gould et al, 1974) Regions subtended by significantly diseased coronary arteries would therefore register attenuated radiotracer uptake in the face of myocardial stress. Localisation of regions with reduced coronary flow reserve is thereby achieved. As the myocardial uptake of the radiotracers utilized for myocardial imaging is also dependent on the viability of the myocardial tissue, ischaemic but viable muscle will demonstrate delayed uptake and clearance or 'filling in' with the radiotracer when coronary flow is restored to normal with rest. Infarcted areas of the

myocardium, on the other hand, fail to take up the radiotracer and show persistence of defects even at rest (see figure 2.2.2).

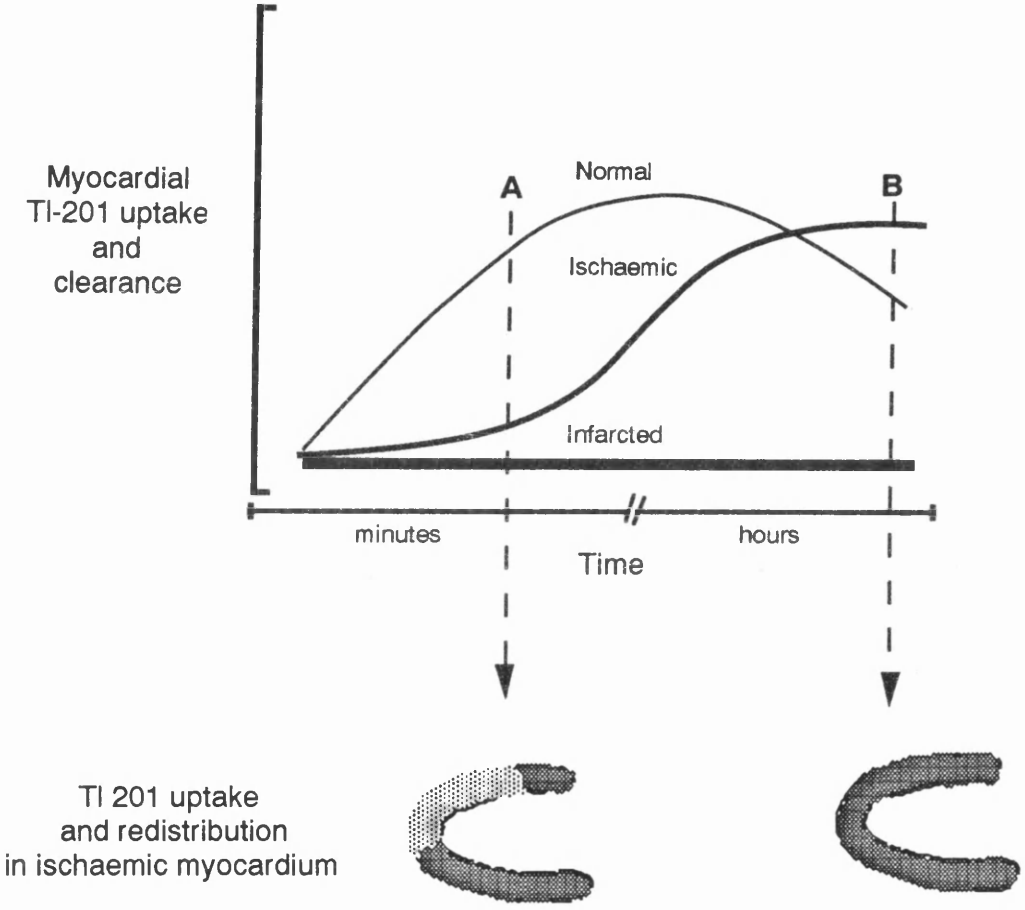

Figure 2.2.2: Principle of myocardial perfusion scintigraphy utilising thallium 201. In normal myocardium, initial thallium uptake is rapid, a linear relationship between thallium activity and blood flow maintained to a flow rate of approximately 2 - 3 litres per minute. In contrast, ischemic myocardial uptake of thallium is delayed. Imaging performed at this point in time (point A) will reveal reduced thallium uptake in the ischaemic segment of myocardium, in this case, the anterior wall. Myocardial clearance from normal myocardium begins almost immediately with significant redistribution occurring in 4 to 10 minutes. With reflow thallium uptake increases in the ischaemic areas. Also, clearance of existing thallium from the ischaemic area is slow. These processes combined with diminishing thallium activity in the normal myocardium produces the re-distribution image where the ischaemic segment appears to"fill in"(point B).

^{99m} TECHNETIUM HEXAKIS - 2 - METHOXY - ISOBUTYL - ISONITRILE (MIBI) FOR MYOCARDIAL PERFUSION SCINTIGRAPHY.

2.2.5. Technetium - 99m - labelled isonitriles

Over the last 20 years, thallium -201 has become established as the tracer for myocardial perfusion scintigraphy. However, as an imaging agent, it is less than ideal because of its low emission energy which results in tissue photon attenuation and image degradation. Technetium - 99m (^{99m}Tc) with its high emission energy of 140 keV is technically more suited to scintigraphic imaging. There is less tissue photon attenuation and consequently, better image resolution. Furthermore, the production of ^{99m}Tc by mobydenum-technetium generator ensures easy availability for patient use. Finally, the improved radiation dosimetry and much shorter half life of ^{99m}Tc (6 hours) compared to thallium - 201 (72 hours) permits the injection of 10 times as much radioactivity. These characteristics have earned ^{99m}Tc, the position of the 'work horse' of the modern nuclear laboratory. A myocardial perfusion agent labeled with ^{99m}Tc is therefore theoretically advantageous.

In the late 1980s, several isonitrile compounds labelled with ^{99m}Tc underwent clinical evaluation. Of these, 3 have been applied clinically. All these are lipophillic cationic ^{99m}Tc compounds with avid myocardial uptake. The first, Tc - 99m - t - butyl isonitrile (TBI), was suboptimal for myocardial imaging due to its prominent hepatic and pulmonary uptake (Campbell et al, 1986; Holman et al, 1984). Persistent liver uptake of TBI obscured imaging of perfusion defects in the inferior left ventricular wall. Additionally, pulmonary TBI behaved as a reservoir of the tracer so much so that subsequent washout of the tracer from the lungs could deliver significant amounts to the myocardium thereby altering the resulting perfusion scan from that corresponding to the initial injection and uptake of TBI.

(Benjamin Sia et al, 1986) The second tracer, Tc-99m-carboxyisopropyl isonitrile (CPI), had excellent myocardial uptake but showed progressive hepatic accumulation over time. (Holman et al, 1987) This tracer also had a relatively rapid myocardial washout rendering tomographic image acquisition unsatisfactory. The third, Tc - 99m - methoxy - isobutyl isonitrile, known generically as Tc - 99m- SESTAMIBI or MIBI has emerged as the isonitrile with the most favourable biological characteristic for myocardial perfusion imaging. (Okada et al, 1988) A further technetium compound that has favourable characteristics and has been approved for clinical use in the USA is a boronic acid adduct of technetium dioxamine namely, Tc-99m tebroxomine. (Rama et al, 1989) A number of newer technetium agents are expected. 'Myoview' (Amersham) a ^{99m}Tc cationic phosphine with no redistribution is currently undergoing evaluation. (Ell, 1992)

MYOCARDIAL KINETICS OF Tc-99m- MIBI

2.2.6. Myocardial uptake and retention

The exact mode of myocardial extraction of Tc-99m-MIBI is not clear. Being a lipophilic cation, cellular viability appears to be essential for uptake and retention of the radiopharmaceutical in the cell. Recent investigators have provided evidence to support this hypothesis. Beanlands et al (1990) studied the uptake of Tc-99m-MIBI in rat hearts during constant flow infusion of Tc-99m-MIBI. The hearts were treated with sodium cyanide to inhibit cytochrome c oxidase and a sarcolemmal membrane detergent Triton X-100. When corrected for potential changes in regional flow distribution, a significant reduction of myocardial uptake and accumulation with rapid clearance was seen in the damaged hearts compared to the control viable hearts. The authors concluded that accumulation and clearance kinetics of

Tc-99m-MIBI were significantly affected by cell viability, being dependent predominantly on sarcolemmal integrity.

Piwnica-Worms et al (1990) investigated the distribution of Tc-99m-MIBI in response to transmembrane potentials. Depolarisation of the cell membrane in chick myocardial cells produced marked reduction of uptake and retention of Tc-99m-MIBI. Additional depolarisation of the mitochondrial membrane potential further reduced net uptake of Tc-99m-MIBI to levels comparable to non-viable freeze thawed preparations. Hyperpolarisation of the mitochondrial membrane with the K^+/H^+ ionophore nigericin or the ATP synthetase inhibitor oligomycin significantly increased the net uptake and retention of Tc-99m-MIBI. These data indicated that the fundamental myocardial uptake mechanism of Tc-99m-MIBI involved passive distribution across plasma membrane and mitochondrial membranes and that at equilibrium, Tc-99m-MIBI is sequestrated within the mitochondria by its large negative transmembrane potentials.

2.2.7. Initial distribution

Following intravenous administration, the myocardial uptake of Tc-99m-MIBI in viable myocardium is proportional to myocardial blood flow similar to thallium-201. (Okada et al, 1988; Glover and Okada, 1990) In anaesthetised dogs undergoing partial occlusion of left circumflex coronary artery, Okada et (1988) showed a good correlation (r=0.92) between microsphere-determined myocardial blood flow and Tc-99m-MIBI distribution. A similar blood flow/tracer uptake relationship was demonstrated during dipyridamole infusion by Glover and Okada (1990). However, as with other diffusible indicators, Tc-99m-MIBI was shown to underestimate myocardial blood flow at flow rates greater than 2.0 ml/min/g (see figure 2.2.3). In low flow regions, the myocardial uptake of

Tc-99m-MIBI is higher relative to non-ischaemic uptake when compared to the microsphere-determined regional blood flow. This is probably due to increased extraction of diffusible indicator at low flows. (Canby et al, 1990). The phenomenon has also been observed with thallium-201.

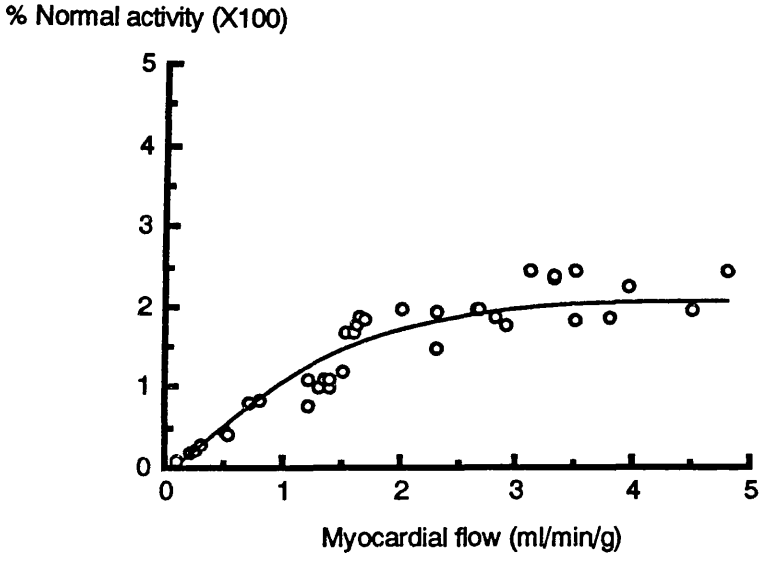

Figure 2.2.3: Scatterplot showing percentage of normal Tc-99m-MIBI activity distribution versus microsphere-determined myocardial blood flow after dipyridamole. Tc-MIBI distribution is linearly related to flow up until approximately 2.0 ml/min/g. At higher flow rates, Tc-MIBI distribution underestimates flow. (Adapted from Gover and Okada, 1990).

2.2.8. Comparison to Thallium 201

In studies comparing kinetics of Tc-99m-MIBI with thallium 201, the first pass myocardial extraction of Tc-99m-MIBI has been shown to be less than that for thallium 201. Leppo and Meerdink (1989) examined the myocardial transmicrovascular transport of Tc-99m-MIBI in blood perfused isolated rabbit heart model. They demonstrated an inverse relationship between coronary blood flow and fractional extraction of Tc-99m-MIBI. They showed that the mean peak value during the early plateau phase of extraction (E_{max}) for Tc-99m-MIBI was significantly less than the E_{max} for thallium 201 (0.39 vs

0.73) (see figure 2.2.4). The net myocardial extraction (E_{net}), an estimate of myocardial retention, averaged 0.41 for Tc-99m-MIBI and 0.57 for thallium 201. These lower first pass extraction characteristics of Tc-99m-MIBI compared to thallium 201 was confirmed by Marshall et (1990) who also concluded that the initial superiority of thallium 201 over Tc-99m-MIBI would disappear shortly after injection of the tracers because of the rapid washout characteristics of thallium 201.

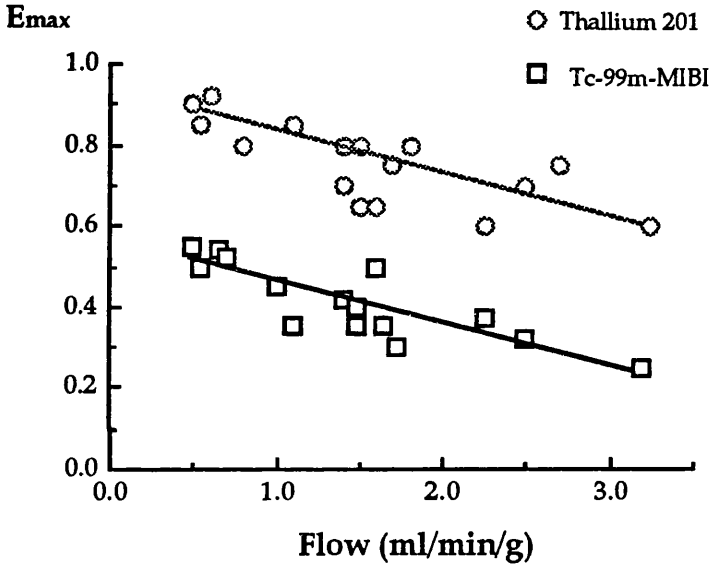

Figure 2.2.4: Maximum fractional extraction (Emax) versus flow (ml/min/g) for Tc-99m-MIBI and thallium 201 (Adapted from Leppo and Meerdink, 1989)

Using computer-based modelling techniques, Leppo et al (1989) computed the rates of transcapillary and cell membrane transport for the two tracers. Thallium 201 was also shown to have a higher transcapillary exchange rate than Tc-99m-MIBI. However, Tc-99m-MIBI had a higher parenchymal cell permeability and higher volume of distribution. This characteristic of Tc-99m-MIBI tends to offset the disadvantage of its reduced transcapillary exchange compared to thallium. The net overall effect of these differences

in the kinetics between the two tracers is therefore insignificant when the two agents are used for perfusion imaging in vivo.

Uptake of Tc-99m-MIBI and thallium has also been studied in stunned myocardium and in myocardium with flow low enough to produce dysfunction but not cellular necrosis. (Sinusas et al, 1989; Sinusas et al, 1990) Ischemia which produces profound systolic dysfunction does not affect Tc-99m-MIBI or thallium-201 uptake as long as myocardial cells are viable. Tc-99m-MIBI is extracted as long as the cell membrane integrity is maintained and blood flow persists. This characteristic would suggest that, like thallium-201, Tc-99m-MIBI is also ideal as an agent for the detection of myocardial viability is severely ischaemic or stunned myocardium.

2.2.9. Myocardial clearance of Tc-99m-MIBI

Okada et al (1988) studied clearance of Tc-99m-MIBI in dogs subjected to circumflex coronary artery stenoses. Myocardial activity of the tracer was continuously monitored with miniature implantable radiation detectors for 4 hours after administration of the radionuclide. The 4 hour fractional clearance of the tracer was minimal and equivalent from both normal and ischaemic zones (0.15±0.05 vs 0.15±0.07). These data suggest that there is no significant redistribution of Tc-99m-MIBI over a 4 hour period.

Li et al (1990) compared Tc-99m-MIBI redistribution with thallium-201 after transient myocardial ischaemia in anaesthetised dogs. Tc-99m-MIBI and thallium-201 were injected after 1 minute of left anterior descending artery occlusion. After 6 minutes reflow was established and serial tomographic imaging obtained. There was a slight but definite and significant filling in of the previously ischemic area after 3 hours of reperfusion. This was in sharp contrast to the substantial delayed redistribution seen as expected with

thallium 201. Thus it appears that contrary to early belief, Tc-99m-MIBI does undergo some redistribution although to a considerably lesser extent and more slowly than thallium-201. This reduced delayed redistribution with Tc-99m-MIBI maybe due to its long retention time combined with the low blood level of Tc-99m-MIBI resulting in very little of the tracer repository available for any reaccumulation.

2.2.10. Clinical use of Tc-99m-MIBI

The easy availability of technetium from a generator coupled with the high 140 keV energy with less scatter and attenuation heralded great promise for the technetium compounds as a myocardial imaging agent. In clinical studies however, the technetium compounds have not proved to be any better than thallium-201 in the diagnostic sensitivity and specificity for coronary artery disease. This maybe because, the earlier studies have utilized thallium imaging protocols and that the acquisition and analysis protocols for Tc-99m-MIBI have not yet been optimised. The greatest advantage of Tc-99m-MIBI as a perfusion scintigraphic agent lies in the ability to uncouple the time of injection from the time of imaging so that injection and imaging need not be performed in nearby locations. This property based on the fact that the radiopharmaceutical undergoes minimal washout from ischaemic myocardium, for the first time permits the documentation of perfusion abnormalities following an intervention in the cardiac catheter laboratory or in the coronary care unit. (John et al, 1991; 1992 a; 1992 c; 1992 d)

2.2.11. Sensitivity and specificity for detection of coronary artery disease

In studies using both planar and tomographic imaging, the diagnostic sensitivity and specificity of 99mTc MIBI for detection of coronary artery disease has been shown to be comparable to thallium 201. After initial

planar studies established the safety of Tc-99m-MIBI in humans, (Wackers et al, 1989, Taillefer et al, 1988 a) extensive clinical information was generated on the clinical utility of Tc-99m-MIBI and SPET through the North American Multicentre Clinical trials (Maddahi et al, 1990) and studies by several investigators. (Kahn et al, 1989; Kiat et al, 1989; Iskandrian et al, 1989)

In the Phase III Multicenter North American Clinical Trial, 22 centers in the United States and 2 in Canada participated in an open label study designed to compare Tc-99m sestamibi with Tl-201 imaging and coronary angiography. All patients underwent maximal exercise Tl-201 and Tc-99m sestamibi SPET studies separated by an average of 4 days. Two day rest and exercise Tc-99m sestamibi SPET studies were performed. Coronary angiography was performed within an average of 16 days. The preliminary results of the SPET studies were reported by Maddahi et al (1990). Of the 278 patients in the study, 39 had normal coronary arteries, 153 had angiographically documented coronary artery disease, and 86 had less than 5% likelihood of coronary artery disease. When examined for the presence of a stress defect, interpretation of the Tl-201 and Tc-99m-MIBI images were concordant in 92% of the 278 patients. Among 6,677 segments assessed, the agreement was 92%. When studies were categorized to take into account fixed defects, there was a total concordance noted in 83%. In assessing angiographically documented coronary artery disease, the overall sensitivity for detecting >50% stenosis in the 153 patients were 89% for Tc-99m-MIBI and 90% for Tl-201.

Thus the available data suggest that Tc-99m-MIBI is comparable in its diagnostic efficacy to Tl-201. As mentioned above, these trials used acquisition parameters that were optimised for Tl-201 and improved

accuracy is to be expected with Tc-99m-MIBI when ideal acquisition and analysis parameters are worked out.

TECHNICAL ASPECTS OF TOMOGRAPHIC MYOCARDIAL PERFUSION SCINTIGRAPHY USING TC-99m-MIBI

2.2.13. Acquisition protocols

The low washout characteristics of Tc-99m-MIBI necessitates two separate injections for stress and rest images. A number of acquisition protocols for imaging with the agent have been explored. (Garcia et al, 1990) In the series of studies presented in this thesis, a two day, two dose protocol has been employed whereby a dose of 350 to 400 mBq of Tc-99m-MIBI was administered in conjunction with the 'stress' intervention the first day. The 'rest' images were acquired the following day after a second dose of 350 to 400 MBq. The early trials comparing Tc-99m-MIBI with thallium-201 for diagnosis of coronary artery disease were done using such two day two dose protocols.

In order to minimise hospital stay and limit the total duration of the study, protocols have been developed where stress and rest imaging are performed on the same day. Taillefer et al (1988 b) demonstrated that the rest and stress Tc-99m-MIBI imaging could be performed on the same day where a third of the total approved dose of the agent was administered for the rest study, and two thirds of the total dose administered four hours later for the stress study. A delay of 4 hours between studies allow the radioactivity from the rest study to decay to approximately 30% before the stress study is begun. Using this protocol, the group demonstrated excellent agreement between for the presence and type of defects defects between Tc-99m-MIBI and thallium-201 planar imaging. In a subsequent study with SPET imaging, rest/stress and stress/rest protocols were compared in 18 patients. (Taillefer et al, 1989)

Although, overall diagnostic accuracy was excellent for both protocols, the rest/stress technique identified 7% more segments that were ischaemic that were diagnosed as scar by the stress/rest technique. Thus the rest/stress sequence appears to be more effective for defining the presence of reversible abnormalities. The apparent discrepancy may be due to the high residual background activity from an initial stress study causing an uneven background when the subsequent rest study is performed resulting in an overestimation of the frequency of fixed defects. Overall, the results from the investigators provide evidence that same day protocols are practical with Tc-99m-MIBI. Nevertheless, because of myocardial background from the first injection, these same day protocols are likely to be less than ideal in terms of contrast (rest/stress) or reversibility (stress/rest) compared with the 2 day protocols.

2.2.14. Acquisition and reconstruction parameters

The minimal washout characteristics of Tc-99m-MIBI allows longer acquisition times with Tc-99m-MIBI compared with thallium-201. Whereas acquisition with thallium-201 has to be limited to 15 minutes permitting acquisition of 32 X 30 second images over a 180° arc of rotation, acquisition with Tc-99m-MIBI can be carried out over a longer period of time without the danger of significant data contamination due to redistribution. Data can be acquired over a 360 degree rotation permitting 64 X 30 second projections.

Details of acquisition and reconstruction parameters used for SPET imaging in the studies presented in this thesis are as follows:

Equipment:

IGE 400 AC Starcam Gamma Camera/Computer system

Acquisition:	
Projections	64 X 30 seconds
Arc of rotation	360 degrees
Collimator	Low energy high resolution (parallel hole)
Photopeak	140 keV (20%, 3% offset)
Reconstruction:	
Filter	Ramp Hanning (0.75 cycles/pixel)
Attenuation correction	Yes (μ = 0.12 cm^2)
Slices	1 pixel thick
Display:	Short axis, vertical long axis, horizontal long axis.

SECTION 3

SUMMARY AND CONTRIBUTION OF PAPERS

Animal study

Paper I

Title: Simultaneous endocardial and epicardial recordings of the ventricular monophasic action potentials during early myocardial ischaemia and reflow in the intact porcine heart: Relation to regional wall motion.

Summary: Endocardial and epicardial action potentials were recorded during transient coronary occlusions in intact open chested anaesthetised pig heart. Changes observed in the MAPs during experimentally induced myocardial ischaemia were correlated with regional segmental wall motion abnormalities recorded using mechanical transducers.

Contributions of the paper are:

1. Demonstration of a definite and consistent relationship between changes in the monophasic action potentials recorded from ischaemic areas and development of regional wall motion abnormalities.

2. The demonstration of disparate changes in the monophasic action potential between the epicardial and endocardial surfaces in the early phases of myocardial ischaemia.

3. A consistent and uniform shortening of the endocardial monophasic action potential duration with the onset of myocardial ischaemia.

4. A transient dissociation between the epicardial MAP changes which show prolongation of duration in the first 30 seconds and the wall motion activity which decline precipitously within a few beats of the onset of ischaemia.

Human studies

Paper II

Title: Endocardial monophasic action potentials for the detection of myocardial ischaemia in man: A study using atrial pacing stress and myocardial perfusion scintigraphy.

Summary: Endocardial recordings of the monophasic action potentials from ischaemic and non-ischaemic areas of the left or right ventricle or both during atrial pacing to angina threshold in 26 patients. Changes in the steady state MAP duration of myocardial ischaemia were shown to correspond to regional myocardial perfusion characteristics as detected by perfusion scintigraphy using Tc-99m-MIBI.

Contributions of the paper are:

1. Validation of endocardial monophasic action potentials for the detection of myocardial ischaemia against an established technique such as myocardial perfusion scintigraphy.

2. Derivation of the sensitivity and specificity of changes in the action potential duration per unit change in heart rate for the detection of myocardial ischaemia. These values are the first reference values for the use of the monophasic action potentials as a measure of ischaemia in the face of a changing cycle length.

3. The demonstration of the practicality of combining two separate techniques (MAP recording with Tc-99m-MIBI imaging) in the cardiac catheter laboratory to study simultaneously basic electrophysiological parameters and perfusion characteristics.

4. The use of Tc-99m-MIBI for the detection of myocardial ischaemia with with atrial pacing stress

Paper III

Title: Vasodilator myocardial perfusion imaging: Demonstration of local electrophysiological changes of ischaemia using endocardial recordings of the monophasic action potential.

Summary: Application of endocardial recordings of the monophasic action potentials for the detection of regional myocardial ischaemia in the controversy of dipyridamole induced scintigraphic perfusion defects in 32 patients. Evidence for regional myocardial ischaemia induced by selective coronary vasodilatation is presented with significant differences in the extent of ischaemia in collateralised and non-collateralised areas of myocardium.

Contributions of this paper are:

1. The demonstration of a degree of myocardial ischaemia as an almost invariable accompaniment of coronary vasodilation in the presence of significant epicardial coronary stenoses.

2. The clear demonstration of a significantly greater degree of myocardial ischaemia in areas where myocardial viability was dependent on angiographically visible collaterals.

3. The practicality of the use of Tc-99m-MIBI with dipyridamole stress for the detection of significant coronary artery disease.

4. The first study of its kind to utilize endocardial monophasic action potentials to explore the presence or absence of regional myocardial ischaemia with dipyridamole.

Paper IV

Title: Direct effect of dobutamine on the action potential duration in ischaemic compared to normal areas in the human ventricle.

Summary: A study designed to explore the effects of beta adrenergic stimulation on the action potential duration and by inference, repolarisation in normal and potentially ischaemic areas of the human right and left ventricles. Whereas beta stimulation produced variable effects on the action potential duration in the normal areas, in potentially ischaemic areas identified by Tc-99m-MIBI perfusion scintigraphy, the action potential duration consistently shortened. A significant change in the dispersion of action potential duration was induced by dobutamine in ischaemic areas creating arrhythmogenic substrates.

Contributions of the paper are:

1. Demonstration of potentially arrhythmogenic effects of beta adrenergic stimulation on action potential duration in ischaemic compared to normal

human myocardium. By inference, a mechanistic basis is provided for the beneficial effect of beta blocking drugs to reduce mortality from sudden cardiac death.

2. The use of Tc-99m-MIBI in conjunction with dobutamine stress in the cardiac catheterisation laboratory

3. The first study of the effects of beta stimulation on the action potentials in the human heart.

CHAPTER 3.2.

SIMULTANEOUS ENDOCARDIAL AND EPICARDIAL RECORDINGS OF THE VENTRICULAR MONOPHASIC ACTION POTENTIALS DURING EARLY MYOCARDIAL ISCHAEMIA AND REFLOW IN THE INTACT PORCINE HEART: RELATION TO REGIONAL WALL MOTION

3.2.1. ABSTRACT

Background and Objectives: The monophasic action potential signal recorded from the myocardial surface has been shown to be sensitive to early myocardial ischaemia. However, its use has been primarily limited to the epicardial surface. Differences in the action potential characteristics and responses exist between endocardial and epicardial layers. I have therefore studied the effect of early myocardial ischaemia on simultaneously recorded endocardial and epicardial monophasic action potentials during experimental coronary occlusions each lasting 3 minutes in anaesthetised pigs. These changes were related to alterations in segmental wall motion.

Results: On the epicardial surface, monophasic action potential duration measured at 70% repolarisation showed an initial prolongation from a mean ± SEM of 280±7.5 msec to 290±7.7 msec ($p<0.001$) at 40 seconds following coronary occlusion. Thereafter, epicardial action potential duration shortened until reflow was established (mean shortening = 39.6±4.2 msec; $p<0.0001$ compared to preocclusion values). In contrast, monophasic action potentials recorded simultaneously from the endocardial surface consistently registered a shortening of the action potential duration without any initial prolongation. Significant shortening from 277.7±11.6 msec to 270±12.2 msec ($p<0.001$) was apparent at 30 seconds of ischaemia with progressive shortening of action potential duration to 231.1±9.8 msec at 3 minutes. Onset of decline of segmental shortening preceded monophasic

action potential changes. Systolic shortening expressed as a fraction of the total segment length fell to 60% of control value 10 seconds following coronary occlusion; $p< 0.001$. Recovery of action potential duration during reflow in both epicardial and endocardial recordings were simultaneous and concordant with resolution of wall motion changes.

Conclusions: These results confirm the reliability of endocardially recorded monophasic action potential signals as a measure of myocardial ischaemia. The disparate response of the epicardial and endocardial action potential duration in the early phases of myocardial ischaemia creates a gradient in action potential duration (and by inference, repolarisation) within the ventricular wall and may contribute to re-entrant arrhythmias seen in the initial few minutes of ischaemia.

3.2.2. INTRODUCTION

Cardiac muscle action potentials measured using the monophasic action potential (MAP) recording technique are sensitive to changes in the cellular electrophysiological properties of the ischaemic myocardium. (Russell et al, 1979; Kingaby et al, 1986; Dilly and Lab, 1987) These recordings have been used to specifically localise the ischaemic zone in both animal and human studies. (Franz et al, 1984; Taggart et al, 1986). However, their use has been primarily limited to the epicardial surface of the heart where early myocardial ischaemia has been reported to demonstrate a variable effect on the action potential duration. There is a paucity of data on ischaemic changes in the MAP recordings obtained from the endocardium. Whereas, the duration of the MAP recorded from the epicardium has been shown to register an initial lengthening before shortening, it is not known whether such changes occur in recordings from the endocardium. (Daniel et al, 1978; Dilly and Lab, 1987) In recent years, considerable differences in the electrophysiological responses of endocardial and epicardial layers of the

myocardium to various perturbations such as ischaemia, rate changes and drug actions have been described in isolated heart and tissue preparations. (Gilmour and Zipes, 1980; Kimura et al, 1986; Antzelevitch, 1991) To my knowledge, there have been no studies that have systematically examined the responses of simultaneously recorded MAPs from the endocardium and epicardium in the *in situ* heart to experimentally induced myocardial ischaemia.

Abnormal wall motion is known to occur within a few beats of onset of experimental ischaemia. (Tennant and Wiggers, 1935; Katz, 1973) The temporal relationship between wall motion changes and alterations in the electrical properties of the epicardium has been addressed in studies using the duration of the MAP and localised segment lengths. (Lab and Woollard, 1978) Again, there is no data at present for the precise relationship between endocardial changes and the development of regional wall motion changes during myocardial ischaemia.

In the present study, I have recorded MAPs simultaneously from the endocardium and epicardium during experimentally induced regional ischaemia in the pig heart. These measurements were combined with segment length measurements from the epicardial surface. The purposes of the study were 1: to document changes in endocardial recordings of the MAP during transient coronary occlusions, 2: to relate these changes to regional wall motion abnormalities, and 3: to define any differential response in MAPs recorded simultaneously from the endocardium and epicardium to the early phases ischaemia.

3.2.3. MATERIALS AND METHODS

Experimental preparation

The study conforms with the United Kingdom Home Office regulations on the care and use of laboratory animals. Six Landrace pigs of either sex weighing between 23 and 26 kgs were pre-medicated with intramuscular azaperone (8 mg/kg) and anaesthetised with intravenous methohexitane sodium. The pigs were ventilated with positive pressure and anaesthesia maintained with 1% halothane carried in a 1:1 oxygen nitrogen mixture. Ventilation was controlled to maintain arterial pH between 7.35 to 7.4 and PCO2 between 35 to 40 mm Hg. Core temperature was maintained between 37° and 38°C by a heating element incorporated into the operating table. The left common carotid artery was cannulated with an 8F rigid polyethylene catheter which was placed in the aorta to within 1cm of the aortic valve. Systemic pressure was monitored via a Statham P50 pressure transducer. An intravenous cannula was introduced into the right jugular vein for infusion of 0.9% saline solution. The anterior chest wall was removed at the costochondral junctions. The pericardium was opened and the heart supported in a pericardial cradle. An 8F micromanometer tipped cannula (Gaeltec Ltd; model S9b) was inserted into the left ventricle through an apical stab incision for continuous monitoring of left ventricular pressure. A Dexon suture snare was placed around the mid left anterior descending artery or one or two of the proximal diagonal branches. Tightening of the snare enabled production of an area of myocardial ischaemia approximately 2 cm X 3 cm.

Epicardial MAP recordings

MAPs were recorded from the epicardium by means of custom made suction operated devices. (Lab and Woollard, 1978) Briefly, these device consists of a hollow perspex tube of 2.5 mm diameter through which suction is applied

for attachment to the epicardium. A thin silver/silver chloride wire passes through the wall of the tube and makes contact with the epicardium directly. This wire forms one input to a differential amplifier while the other input is derived from a second, external, silver/silver chloride wire electrode in contact with a circular sponge glued outside the wall of the suction tube. This sponge when soaked with saline forms a wick for stable electrical contact with the epicardium. Using these suction and wick electrodes, MAP recordings were obtained from localised areas of the epicardium.

Endocardial MAP recordings

For endocardial recordings, pressure contact electrodes mounted on a hand held catheter was used. The catheter body consisted of an suitably shaped insulated stiff metallic wire 10 cm long and with an external diameter of 2.6 mm. The contact electrode consisted of a silver/silver chloride pellet mounted at the tip and the indifferent electrode was a second pellet mounted on the side of the catheter 5 mm from the tip and flush with the wall of the catheter. The catheter electrode was introduced into the left ventricular cavity by direct puncture and the tip positioned in a desired location confirmed by palpation. Apposition of the electrode against the endocardial surface resulted in MAP signals of 30 to 50 mV amplitude.

Segment length measurements

A custom made tripodal device previously described by Lab and Woollard (1978) was used as a segment length transducer. In brief, the tripod device weighing approximately 2 grams is attached to the epicardial surface by vacuum through its legs. The apices of the triangle subtended by the tripod are on a radius of 3-5 mm. Strain gauges applied to each leg detected movement which were calibrated in units of length. Changes in segment length are calculated from summating the motion along three axes at 120° to

each other. During placement, the tripod is rotated on the heart until maximum displacement is obtained from one of the legs thereby ensuring that the axis of maximum shortening remain parallel to that leg. The length changes detected by the tripodal device was related to the left ventricular pressure to construct pressure-length loops as described by Tyberg et al. (1974) (see figure 3.2.1.).

Signal processing

The MAP signals were pre-amplified using a high impedence DC amplifier (Watco Ltd). The signals obtained were displayed on a Devices M19 heated stylus chart recorder and a Tektronix 5103 N oscilloscope during the experiment. The data were stored on magnetic tape by a TEAC XR-50L cassette tape recorder for off line analysis.

Study Protocol

All recordings were made during steady state right atrial pacing at a rate set at 20% above the resting heart rates to overcome any reflex tachycardia induced by onset of ischaemia. After a 30 minute period for stabilisation, the suture around the chosen segment of the left coronary artery was briefly tightened to identify the potential area of ischaemia evidenced by the development of regional dysynergy and cyanosis. Tripodal segment length transducers were applied by suction over the identified area of potential ischaemia and an area for control recordings remote from the ischaemic area. Suction electrodes for MAP recordings were placed beneath the tripodal devices in the area spanned by the legs of the device without causing hindrance to the free movement of the tripod. The endocardial electrode was introduced through a left ventricular posterior stab incision and positioned opposite the epicardial electrode in the area to be made ischaemic. Multiple recordings of simultaneous epicardial and endocardial

recordings were made during coronary occlusions lasting 3 minutes each with periods in between to allow complete recovery of regional and global cardiac function and return of electrophysiological parameters to baseline.

Data Analysis

Monophasic action potential signals obtained during steady state atrial pacing were used for analysis. Data obtained where heart rate control was lost were not included in analysis. Only MAP recordings with a uniform shape in five consecutive beats and which resembled the accepted configuration for transmembrane action potentials were accepted for analysis. MAP duration was measured at 70% repolarisation.

The outputs from the legs of the tripod segment length transducer were fed into an analogue circuit for on-line continuous arithmetic summation to produce a 'summed segment length' which was used to define the regional segment length changes. (Lab and Woollard, 1978) The excursion of the tripod however depend on several factors as previously described. (Dean and Lab, 1989) Thus although the device was calibrated, measurements were expressed as percent shortening of the maximum segment length. The vertical distances between points L1, L2 and L3 shown in figure 3.2.2. were used to derive percentage shortening:

$$\text{Percentage shortening} = \frac{\text{Distance between L1 and L3}}{\text{Distance between L1 and L2}} \times 100$$

Statistical comparisons of data between control and post intervention were made using paired Student's t test. Values are expressed as means ± SEM. A p value of <0.05 was accepted as significant.

3.2.4. RESULTS

Recordings during a total of 21 transient occlusions each lasting 3 minutes were obtained during the experiments. MAP recordings of quality suitable for analysis were available from the epicardial surface alone (n=8) or simultaneously from the epicardium and endocardium (n = 13). Steady state atrial pacing cycle lengths for the experiments ranged between 600 and 467 msecs (median cycle length = 500 msec).

Epicardial changes during ischaemia

On the epicardial surface the action potential duration in recordings from the ischaemic area consistently lengthened in the initial period following coronary occlusion. This initial lengthening which reached peak values at 40 seconds following occlusion was apparent in epicardial ischaemic area recordings during 19 of the 21 occlusions. During one occlusion there was no change and in another, there was shortening of the MAP duration. Mean values of MAP duration in the pre-occlusion phase was 280±7.5 msec and 40 seconds post occlusion 290±7.7 msec ($p<0.001$). No significant alteration was observed in recordings from the non-ischaemic areas of the epicardium, the corresponding values being 282.9±6.4 msec and 283.0±6.4 msec (p=NS). After the initial 40 seconds, progressive shortening of the MAP duration in recordings from the ischaemic area was evident until release of the occlusion (mean shortening = 39.6±4.2 msec; $p<0.0001$ compared to pre-occlusion values).

Typical example of changes in MAP duration on the epicardium is shown in figure 3.2.3 together with segment length alteration. Lengthening of the ischaemic area epicardial MAP duration is evident in the first minute following occlusion. Thereafter, shortening of the MAP duration occurs. Shortening below the pre-occlusion value is seen only after 60 seconds into

ischaemia. Segment length changes precede the onset of MAP duration changes with dyskinetic systolic lengthening of the ischaemic segment apparent by 10 seconds following coronary occlusion. This is clearly demonstrated in the derived pressure-length loop shown below the MAP and segment length signals in figure 3.2.3. In this example, the ischaemic zone epicardial MAPs also display other features described with ischaemia namely, fragmentation and delay of the upstroke and loss of amplitude of the signal.

The relationship between ischaemic segment length changes and the loss of diastolic and systolic amplitude of the MAP signals is shown in figure 3.2.4. Onset of dyskinetic systolic bulging evident on the segment length recorded from the ischaemic zone precedes the loss of amplitude on the epicardial MAPs but with progressive ischaemia, changes are synchronous in both MAP and segment length recordings. With reflow, resolution of segment length abnormalities are temporally matched to recovery of MAP amplitude.

Endocardial changes

In contrast to the epicardial recordings, endocardial action potential duration consistently shortened following coronary occlusion without the initial lengthening of MAP duration that occurred on the epicardium. Shortening of the action potential duration was seen at 30 seconds into ischaemia in the endocardial recordings during 11 of the 13 occlusions. Mean values for MAP duration in the pre-occlusion period, 10, 20 and 30 seconds following occlusion were 277.7 ± 11.6 msec, 276.5 ± 12.1 msec (p = NS compared to pre-occlusion), 274.4 ± 12.2 msec (p = NS compared to pre-occlusion), and 270.6 ± 12.2 msec ($p < 0.001$ compared to pre-occlusion) respectively. Progressive shortening of MAP duration occurred until release of coronary occlusion at

3 minutes (mean shortening 48.4 ± 6.3 msecs; $p < 0.0001$ compared to pre-occlusion values).

MAP duration shortening was seen in 12 of the 13 recordings 30 seconds following coronary occlusion. Adequate MAP signals from the endocardial surface could be maintained in only 10 of the occlusions 60 seconds into the occlusion. All these 10 recordings registered MAP shortening. The extent of shortening registered during the individual coronary occlusions is shown in figure 3.2.5.

Figure 3.2.6 depicts changes in MAP signals in simultaneous endocardial and epicardial recordings. Whereas the epicardial MAP signal shows lengthening of the repolarisation phase duration in the first minute of ischaemia, endocardial MAP duration shortens without the initial prolongation. In this example, recovery of action potential duration is earlier in the endocardial recordings. Recordings of segment length from the ischaemic area directly over the endocardial MAP electrode demonstrate systolic lengthening concurrent with the endocardial MAP duration shortening.

Pooled data

Figure 3.2.7 shows the pooled data for mean changes in the MAP duration for recordings from epicardial non-ischaemic area, epicardial ischaemic area and endocardial ischaemic area. MAP duration is normalised to control values and shown as a percentage of the control values. The epicardial normal zone recordings show no change in MAP duration throughout the entire 4 minutes of recording. In recordings from the ischaemic areas, the disparate response of the epicardium and endocardium in the first minute of ischaemia is apparent. Initial lengthening MAP duration on epicardium

reaches maximum values at 40 seconds into ischaemia and begins to fall below control values only after a minute of ischaemia.

In the lower panel of figure 3.2.7, segment length data is shown as the fraction of peak systolic shortening in relation to maximal segment length. Significant loss of systolic contraction combined with overall lengthening of the ischaemic segment occurs as early as 10 seconds following occlusion. These changes are apparent before significant changes appear in the MAPs recorded from the same area (shortening in the case of endocardial MAPs or initial lengthening in the case of epicardial MAPs). Recovery of MAP duration and segment length shortening occur concurrently.

3.2.5. DISCUSSION

Recordings of the monophasic action potential (MAP) offer a convenient means of measuring the myocardial repolarisation time course in the intact *in situ* heart. Changes in the action potential duration are characteristic of early myocardial ischaemia. Although MAPs recorded from the epicardium have been utilised as measure of myocardial ischaemia, its use on the endocardium has not been fully evaluated. The present study utilized simultaneous MAP recordings from the endocardial and epicardial surfaces of an area of myocardium during the first three minutes of myocardial ischaemia and early phases of reflow. The changes produced in the MAPs were compared to segmental wall motion recorded from the same area using mechanical segment length transducers. Epicardial MAPs showed a prolongation of action potential duration during the first minute of ischaemia consistent with previous studies in open chested animals. Thereafter, progressive shortening of the MAP duration occurred. On the endocardial surface, shortening of the MAP duration was apparent without the initial lengthening seen on the epicardial surface. Changes in segmental

motion was more rapid in onset and precipitous compared to MAP duration changes although during reflow, recovery of both mechanical and electrophysiological parameter showed a close temporal relationship (figure 3.2.7).

Fundamental differences in epicardial and endocardial action potential configuration and response to rate adaptation, drug effects and ischaemia have been described in isolated tissue. (Kimura et al, 1990; Litovsky and Antzelevitch, 1989; Krishnan and Antzelevitch, 1991) These differences have been attributed to the presence of a prominent transient outward current (I_{to}) in the epicardium but a relatively weak I_{to} in the endocardium. (Litovsky and Antzelevitch, 1988; Furukawa et al, 1990) The intact heart however remain under the influence of the various hormonal and neural impulses that accompany myocardial ischaemia. (Malliani et al, 1969; Schömig et al, 1991) Responses of the myocardial cells under these conditions may therefore vary from that documented in isolated tissue. The responses to the endocardial and epicardial layers of the myocardium to various perturbations in steady state in the *in vivo* heart has received little attention primarily because simultaneous recording of the epicardial and endocardial action potentials in the intact beating heart is difficult to obtain using the microelectrode technique. The monophasic action potentials using suction electrodes or contact electrodes has been shown to closely mimic transmembrane action potentials although of lower amplitude and upstroke velocity. (Hoffman et al, 1959; Franz et al, 1986; Ino et al, 1988) The action potential duration is a reliable measure of the repolarisation period of the cells beneath the exploring electrode. The present study using simultaneous recordings of the MAPs from the epicardial and endocardial surfaces show a clear disparity in response of the epicardium and endocardium to myocardial ischaemia in its early phase.

The finding of initial prolongation in the epicardial action potential duration has been repeatedly documented in previous studies. (Daniel et al, 1978; Dilly and Lab, 1987) In isolated perfused hearts and open chested animal studies, this initial prolongation has been ascribed to possible epicardial cooling that accompany loss of regional blood flow. Prolongation of the action potential duration on the epicardium has been demonstrated by the application of cooled (10° C) saline drops at the site of recording. (Lab and Woollard, 1978) However, direct measurement of temperatures from the epicardium following coronary occlusion has shown only a 0.5° C fall in temperature. Therefore other possible mechanisms have to be considered: One mechanism maybe the influence of the I_{to} on epicardial repolarisation. The greater activity of the I_{to} on the epicardial surface is responsible for a 'spike and dome' pattern to phase 1 of myocardial repolarisation. The magnitude of this spike and dome has a positive correlation with action potential duration in isolated tissue studies. (Litovsky and Antzelevitch, 1989) Under ischaemic conditions, loss of the spike and dome which occurs at low heart rates can be reversed by pacing at faster heart rates. (Antzelevitch et al, 1990) It is possible therefore, that at the heart rates used in the present study, an increased activity of I_{to} on the epicardial surface could have accentuated the plateau phase briefly before other ischaemic factors overwhelmed this effect and shortened the action potential duration. (Antzelevitch, 1992) A second mechanism may be the effect of a bimodal pattern of pH alteration that has been documented in the early phases of ischaemia. Allen et al (1985) have observed a transient initial alkalosis in the first minute of ischaemia before anaerobic glycolysis sets in with lactate production and acidosis. It is possible that this transient alkalosis may play a part in the observed initial prolongation of action potential duration. A third consideration is the influence of mechano-electrical feedback.

Myocardial stretch/strain can influence the action potential duration. (Lab, 1982 a, 1982 b; Lab and Dean, 1989) The dyskinetic myocardial segment lengthening observed early in ischaemia may well exert an influence on the action potential duration and more so on the epicardium due to its greater circumferential segment length alteration. Whatever the mechanism might be, the phenomenon of initial prolongation of epicardial action potential duration has been documented in several studies in the whole heart and is likely to operate in closed chest situation. Temperature decrements on the epicardium can occur even in closed chest models of ischaemia, since the normal blood flow serves to warm the muscle. (Janse and Wit, 1989)

In contrast to the changes observed on the epicardial surface, monophasic action potentials on the endocardium registered consistent shortening of the duration following onset of ischaemia. These ischaemia related changes in action potential duration were detected during 12 of the 13 coronary occlusions 30 seconds into ischaemia. With progressive ischaemia MAP shortening became apparent in all recordings. These findings are in keeping with the observations of Franz et al who in intact dog hearts demonstrated marked shortening of the monophasic action potential within 30 seconds of occlusion of the regional coronary artery. (Franz et al, 1984) Similar observations were recorded by Donaldson et al (1983 a) who measured paced evoked responses and MAP duration from the endocardial surface of beagles. However, other studies have found less dramatic effects on endocardially recorded monophasic action potentials. (Taggart et al, 1988) The disparity may be partly explained by species differences and experimental conditions. A further and more important consideration is the problem of accurate localisation of the MAP electrode in the ischaemic area. It is characteristic of the MAP to record from very localised areas and any recording outside the ischaemic area or in border zones may register

attenuated effects. Great care was taken to ensure placement of the endocardial MAP electrode in the centre of the potentially ischaemic area in the present study so as to enable detection of early changes undiluted by possible volume conducter effects of the blood surrounding the indifferent electrode of the endocardial MAP catheter (see section 3.3.5. for detailed discussion). (John et al, 1991)

The observed inhomogeneity of epicardial and endocardial responses to ischaemia alters the normal gradient in action potential duration that exist between the two layers of the myocardium. Such transmural dispersion in repolarisation although theoretically alluded to in isolated tissue experiments (Antzelevitch, 1991) has not been previously documented in the *in situ* heart. The dispersion of repolarisation demonstrated in the early phases of ischaemia in the present study implies heterogenous recovery of excitability within the ventricular wall although in the ischaemic situation, post repolarisation refractoriness can occur and the repolarisation time course may not directly correlate with refractoriness. (Downar et al, 1977) However, for variable periods of the very early phases of ischaemia, these changes occur concomitantly and may contribute as substrates for phase 1a re-entrant tachyarrhythmia that is commonly seen in the first few minutes of ischaemia. (Janse and Wit, 1989)

The finding of early mechanical dysfunction in this study is in concert with previous observations. Although transmembrane ionic balances are the first to be disrupted by ischaemia, mechanical decline in regional function following coronary occlusion is well known to precede changes in action potential. (Tennant and Wiggers, 1935; Kardesh et al, 1958; Katz, 1973; Allen and Orchard, 1987) Regional cyanosis and contractile abnormalities appear as early as 5 seconds following coronary occlusion. (Hearse and Dennis, 1982)

The mechanism for such rapid deterioration of mechanical activity is probably related to an acute alteration of the sensitivity of contractile proteins to intracellular ionic calcium (see section 1.4.6). (Allen and Orchard, 1987; Lakatta and Maughan, 1990) Mechanical factors such as loss of perfusion pressure in the myocardial segment can also lead to a loss of developed tension. Accumulation of extracellular potassium, lactate and other components of the ischaemic environment follow and produce characteristic changes in the action potential namely, loss of amplitude, reduction of upstroke velocity and shortening of the action potential duration. (Janse and Wit, 1989) Abbreviation of the action potential duration leads to reduced calcium entry into the cell and further depression of contractile function. The results of the present study also imply an ischaemia related transient electro-mechanical dissociation in that, despite prolongation of the action potential duration on the epicardium, mechanical activity falls precipitously. This phenomenon would be in keeping with the concept that acute ischaemic failure has a multifactorial basis some of which dominate over electrical activation factors.

3.2.6. CONCLUSION

In a study designed to examine the electro-mechanical responses to the early phase of myocardial ischaemia in the intact heart in situ, simultaneous recordings of epicardial and endocardial MAPs together with regional wall motion activity were made during brief periods of coronary occlusion. I have shown that endocardial recordings of the MAP consistently register shortening of the duration during experimentally induced ischaemia. These changes are temporally related to wall motion changes although the rapidity of the development of MAP duration changes lag behind the onset of ischaemic wall motion dyssynergy in this model of total regional ischaemia. A clear disparity in epicardial and endocardial responses to ischaemia has

also been demonstrated in the first 40 seconds following coronary occlusion with the epicardial layer showing a transient prolongation of action potential duration. Such modification of the repolarisation time course within the ventricular wall disrupts the normal orderly sequence of recovery of excitability and may act as substrate for re-entrant arrhythmias in the early phases of myocardial ischaemia.

												-----Reflow-----			
No.	Pretie	10s	20s	30s	40s	50s	60s	90s	120s	150s	180s	190s	200s	210s	240s
1.	260	259	257	256	256	256	256	256	258	257	258	258	257	257	258
2.	261	261	261	262	260	260	261	260	261	261	261	261	261	263	264
3.	265	265	265	265	265	265	265	265	265	265	265	265	260	260	270
4.	280	280	280	280	280	275	280	280	280	285	285	285	285	287	290
5.	315	315	315	315	315	315									
6.	290	285	290	290	290	290	290								
7.	237	239	238	241	241	240	242	241	243	244	244	245	244	244	247
8.	281	280	281	280	281	280	281	282	282	282	282	281	281	282	281
9.	265	265	265	265	265	265	265	265	265	265	265	265	267	267	270
10.	269	269	274	274	275	273	273	267	266	270	266	262	261	263	266
11.	292	298	298	300	297	290									
12.	266	266	265	265	265	266	266	267	268	268	269	271	271	269	272
13.	237	235	236	234	235	236	235	235	236	235					
14.	251	250	252	253	252	251	249	252	251	252	251	250	249	252	251
15.	230	231	230	230	230	230	232	230	230						
16.	300	297	298	298	297	299	300	299	301	304	305	303	306	308	307
17.	320	320	320	320	320	320	320	320	320	320	320	320	320	324	325
18.	300	300	300	300	300	300									
19.	325	320	325	325	325	320	325	325	330	325	325	325	325	325	330
20.	320	319	320	321	322	320	321	320	320	320	319	321	320	320	320
21.	325	325	325	325	325	325	325	325	325	325	325	325	325	325	325

*Table 3.2.1, A: Monophasic action potential duration (measured at 70% repolarisation) for recordings from the **epicardial control** area during 3 minutes of coronary occlusion and the first minute of reflow.*

												----Reflow----------			
No.	Pretie	10s	20s	30s	40s	50s	60s	90s	120s	150s	180s	190s	200s	210s	240s
1.	258	262	265	266	264	259	253	242	233	223	215	238	242	244	250
2.	260	262	267	270	270	268	260	245	236	231	220	230	236	244	250
3.	270	270	275	285	275	260	250	245	235	225	215	230	235	235	270
4.	264	264	265	269	272	273	263	252	236	221	214	220	227	230	252
5.	327	330	332	328	323	313	309	295	280	274					
6.	285	286	287	288	286	285	280	265							
7.	264	274	272	274	276	276	274	269	245	227	221	226	231	237	247
8.	288	290	294	295	295	291	287	275	265	255	240	246	251	265	279
9.	279	285	289	290	288	283	275	271	264	258	250	250	255	259	270
10.	265	265	270	270	265	260	255	245							
11.	260	265	270	280	285	260									
12.	260	260	264	268	271	273	269	260	256	250	244	243	244	250	263
13.	234	233	238	241	242	249	248	236	226	220	217	230	234	234	232
14.	244	246	247	251	252	253	255	250	246	238	220	230	237	239	242
15.	215	220	220	225	228	230	220	210	200						
16.	320	323	325	319	310	302	294	285	272	248	238	272	276	287	310
17.	340	340	340	350	370	365	360	345	330	320	305	310	315	320	340
18.	340	340	360	355	355	355									
19.	300	300	300	300	300	300	295	295	285	275	275	295	295	300	305
20.	312	311	314	316	322	314	312	301	282	280	265	265	273	271	270
21.	310	310	310	310	315	315	310	300	295	290	280	285	320	320	320

*Table 3.2.1, B: Monophasic action potential duration (measured at 70% repolarisation) for recordings from the **epicardial ischaemic** area during 3 minutes of coronary occlusion and the first minute of reflow.*

												---Reflow----------			
No.	Pretie	10s	20s	30s	40s	50s	60s	90s	120s	150s	180s	190s	200s	210s	240s
1.	235	230	225	225	220	215	215	215	215	215	210	215	225	230	235
3.	280	280	285	270	270	255	245	240	230	230	225				
6.	300	300	305	295											
8.	320	320	325	315	315	310	300	295	290	280	280	295	295	300	310
9.	290	290	290	295	280	280	275	270	260	235	220	230	270	280	290
11.	275	274	272	271	264	260	255	245							
12.	250	250	250	245	245	240	235	230	220	210	205	230	230	235	
13.	240	240	240	235	230	220	215	210	205	200	200	210	215	225	
14.	235	230	225	215	225	190									
15.	205	200	195	195	180										
16.	340	340	336	329	324	321	314	302	290	260	257	260	269	280	300
20.	320	320	319	316	316	313	300	290	255	250	240	270	269	282	306
21.	320	320	300	310	300	300	290	290	280	280	280	290	310	310	320

*Table 3.2.1, C: Monophasic action potential duration (measured at 70% repolarisation) for recordings from the **endocardial ischaemic** area during 3 minutes of coronary occlusion and the first minute of reflow.*

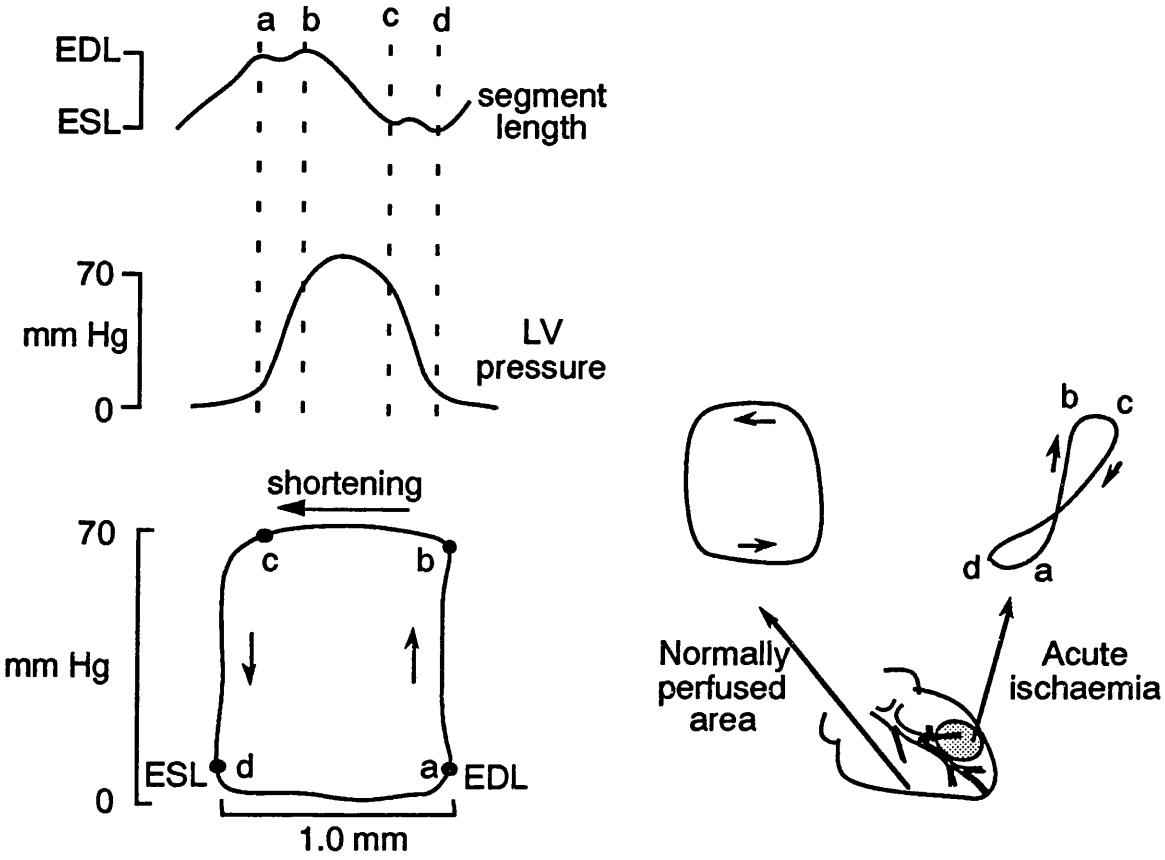

Figure 3.2.1: Left panel shows the pressure-length loop generated by plotting left ventricular pressure (y axis) and length of myocardial segment (x axis). End systolic length (ESL) was defined as the segmental length at the point of return of aortic flow to 0, and end diastolic length (EDL), defined by the initial upslope of the first derivative of the left ventricular pressure (dp/dt). Points a, b, c and d represent isovolumic contraction, onset of ejection, isovolumic relaxation, and ventricular filling respectively. The right panel shows the effects of ischaemia on the pressure-length loop. During isovolumic contraction (a to b), there is lengthening of the ischaemic segment causing the loop to lean over to the right. During systole, dyskinetic lengthening (b to c) occurs. Post systolic shortening is superimposed on isovolumic relaxation (c to d) resulting in a figure of eight configuration to the loop. (Reproduced with kind permission of Dr. M.J. Lab, Department of Physiology, Charing Cross Hospital and Medical School, London).

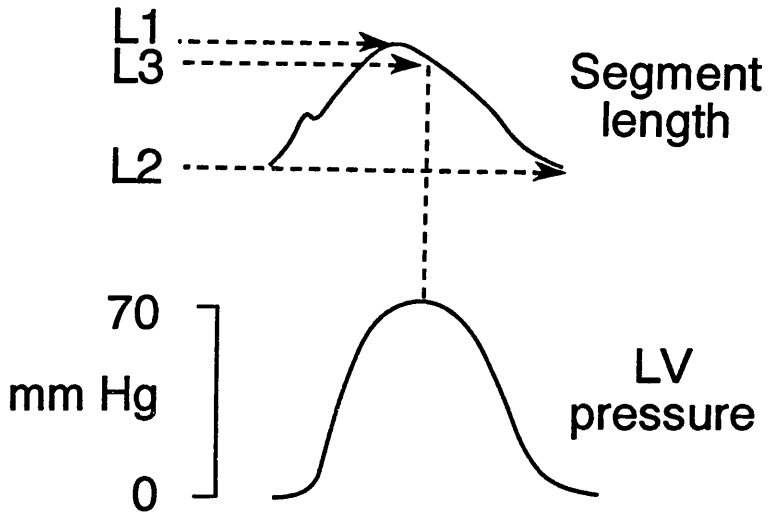

Figure 3.2.2: Measurement of systolic shortening of segment length as a function of total length. Peak systolic shortening (measured between L1 and L3) was expressed as a percentage of total segment length (measured between L1 and L2).

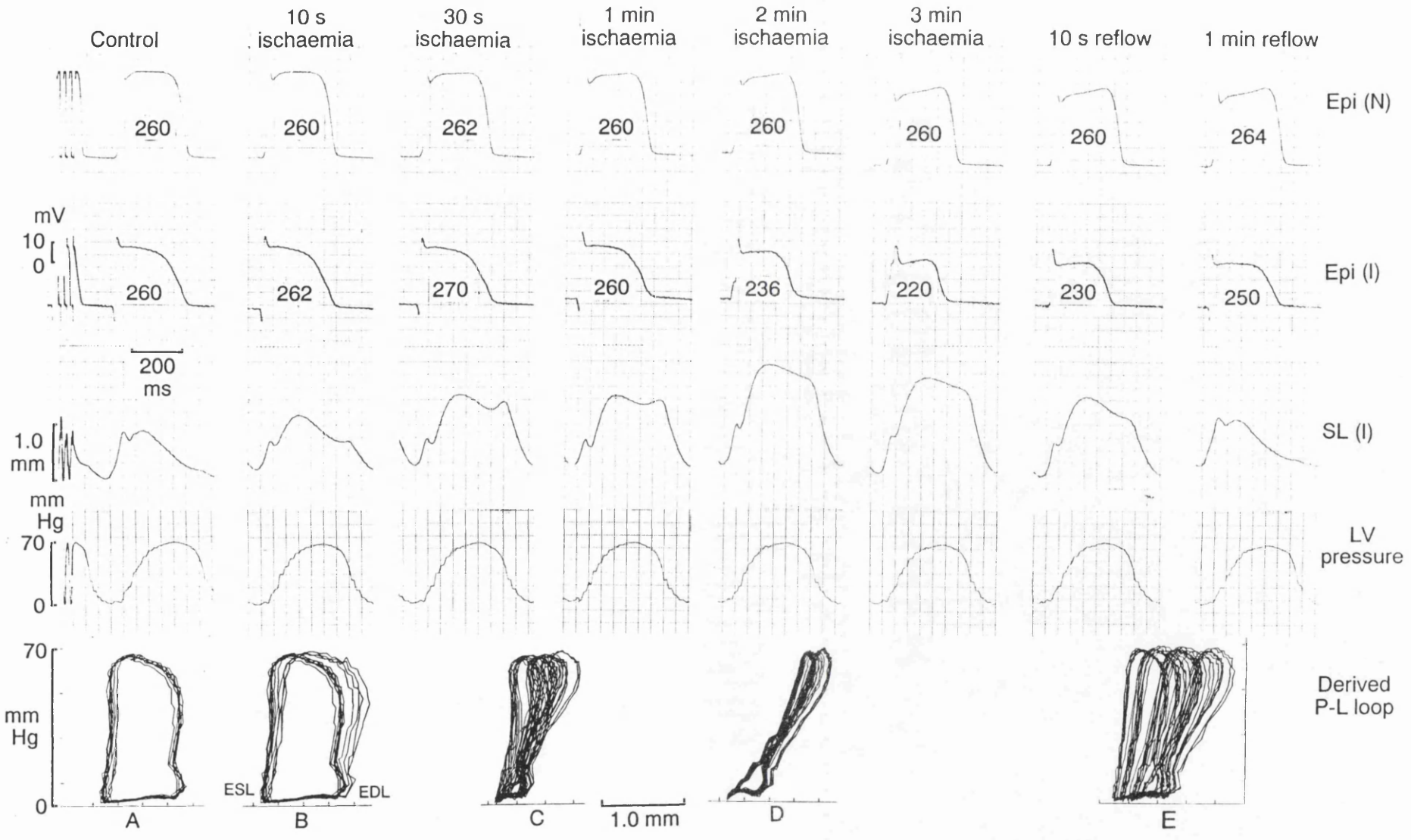
Control
10 s ischaemia
30 s ischaemia
1 min ischaemia
2 min ischaemia
3 min ischaemia
10 s reflow
1 min reflow
Epi (N)
260
260
262
260
260
260
260
264
mV
10
0
Epi (I)
260
262
270
260
236
220
230
250
200 ms
1.0 mm
SL (I)
mm Hg
70
0
LV pressure
70
mm Hg
0
ESL
EDL
A
B
C
1.0 mm
D
E
Derived P-L loop

***Figure** 3.2.3: Typical changes in steady state epicardial monophasic action potentials (MAPs) and regional segment segment length together with derived pressure length loops during coronary occlusion and reflow. A transient prolongation of ischaemic zone MAP (Epi - I) is seen at 30 seconds into ischaemia. Thereafter, MAP duration shortens, the upstroke becomes delayed and fragmented, and there is loss of amplitude of the signal. These changes reverse during reflow. The epicardial normal zone MAPs (Epi - N) essentially remain unchanged. Pressure length (P - L) loops derived from the ischaemic segment length (SL - I) and left ventricular (LV) pressure are shown in the bottom panel. Systolic lengthening becomes apparent 10 seconds into ischaemia and causes the loop to lean to the right (P - L loop B). As ischemia progress, there is loss of volume of the loop with systolic lengthening producing a figure of 8 pattern (P - L loops C and D). During reflow with return of normal wall motion activity, the loop swings back from right to left and regains volume (P -L loop E).*
(Basic cycle length = 500 msec).
***ESL**, end systolic length; **EDL**, end diastolic length.*

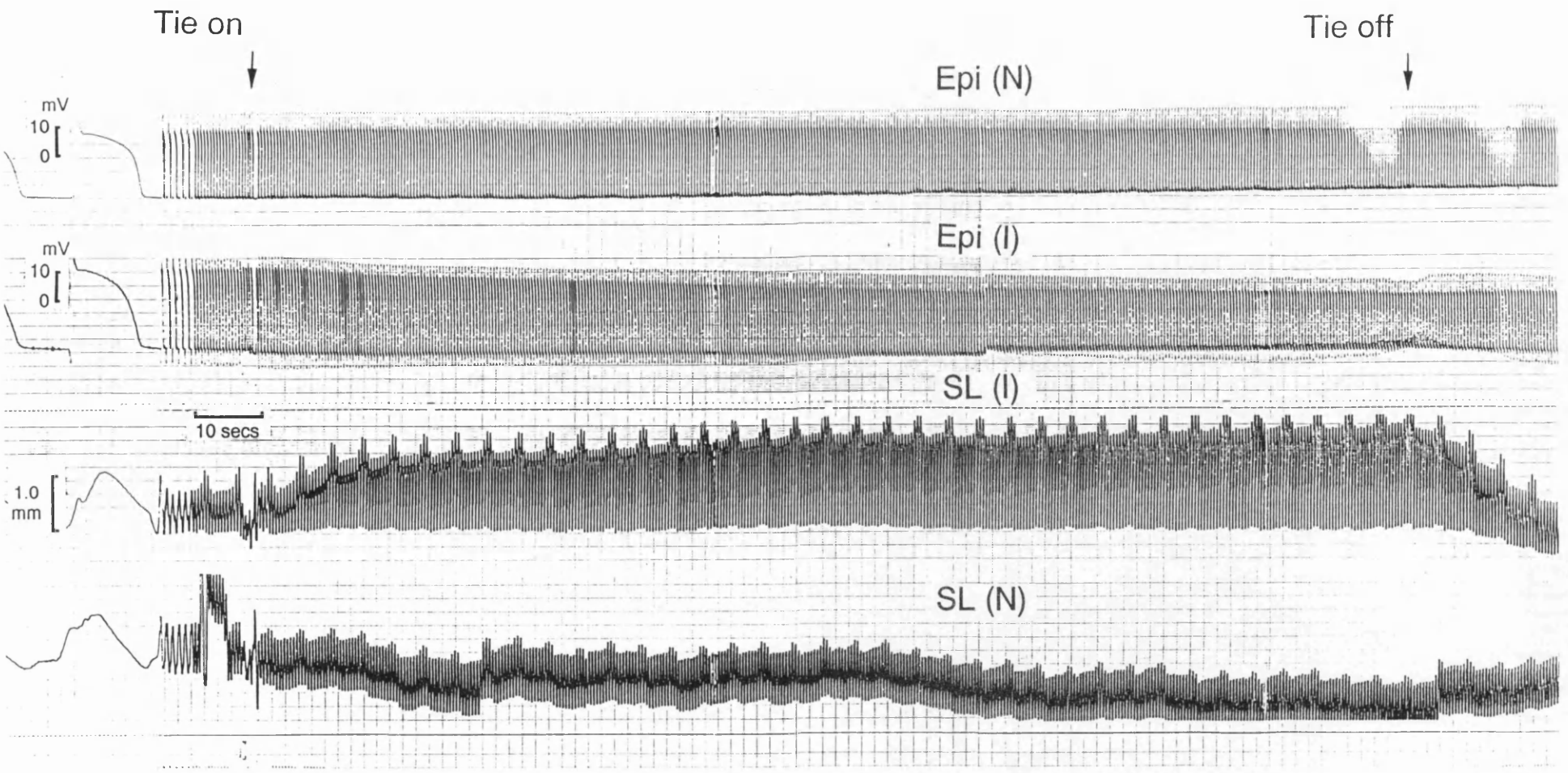
Tie on
Tie off
Epi (N)
mV
10
0
Epi (I)
mV
10
0
SL (I)
10 secs
1.0
mm
SL (N)

Figure 3.2.4: Relationship between epicardial monophasic action potentials (MAPs) and regional wall motion alterations for ischaemic and non-ischaemic areas. Epicardial MAPs from the ischaemic area (Epi - I) show progressive diminution of signal amplitude and loss of diastolic potential following coronary occlusion. Changes in segment length in the ischaemic area (SL - I) manifest as systolic lengthening of the ischaemic segment precede the MAP changes. Recovery of both MAP and wall motion changes are concordant during reflow. MAP and segment length recordings from the non-ischaemic ares (Epi - N and SL - N respectively) show no alteration.

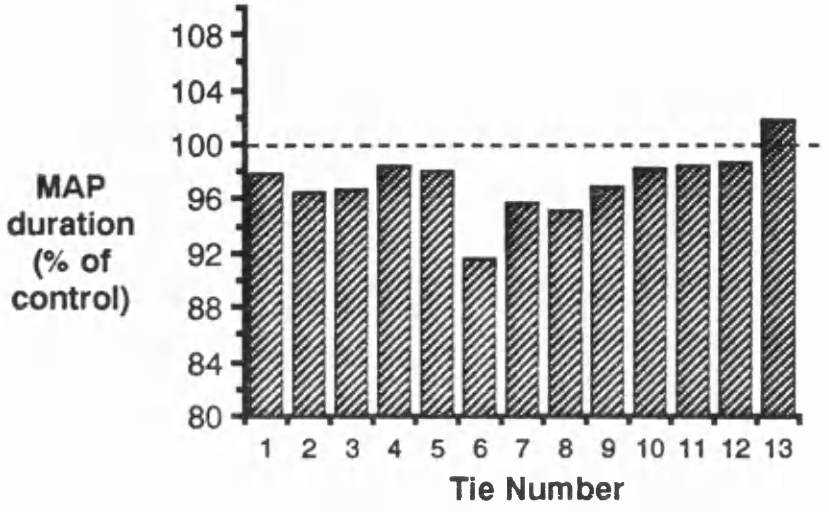

Figure 3.2.5, A: Extent of shortening of action potential duration observed for each of 13 recordings from the endocardium 30 seconds following coronary occlusion. Compared to preocclusion (control) values, shortening is seen in 12 of the 13 recordings.

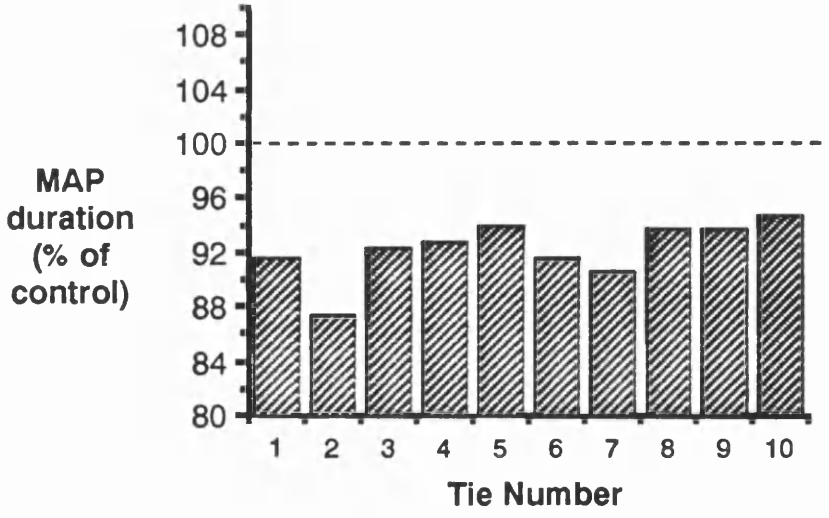

Figure 3.2.5, B: Extent of shortening of action potential duration observed for each of 10 recordings from the endocardium 60 seconds following coronary occlusion. Compared to preocclusion (control) values, shortening is seen in all 10 recordings.

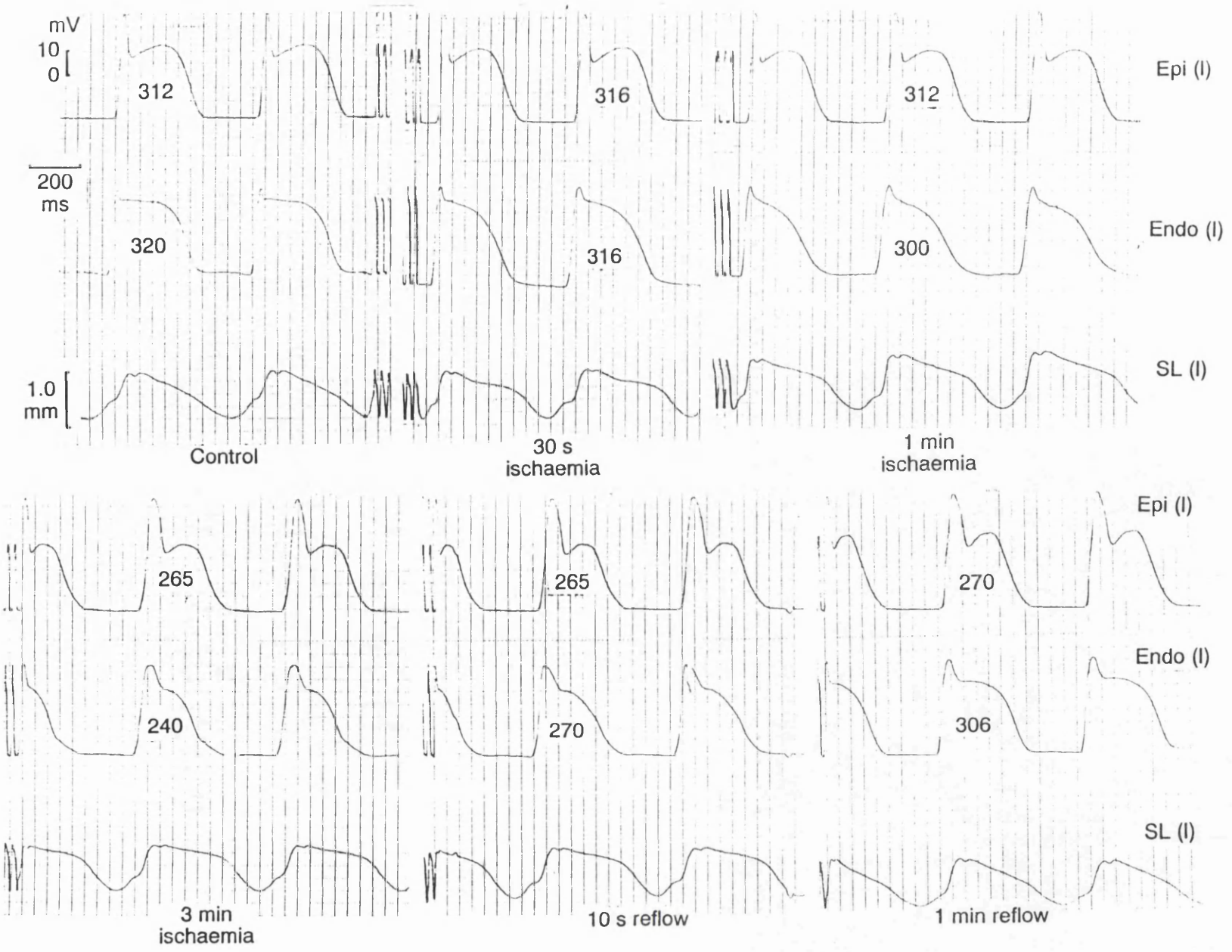
mV
10
0
200
ms
1.0
mm
312
316
312
320
316
300
Epi (I)
Endo (I)
SL (I)
Control
30 s
ischaemia
1 min
ischaemia
265
265
270
240
270
306
Epi (I)
Endo (I)
SL (I)
3 min
ischaemia
10 s reflow
1 min reflow

Figure 3.2.6: Simultaneous recordings of steady state epicardial (Epi (I)) and endocardial (Endo (I)) monophasic action potentials (MAPs) from the ischaemic territory. The epicardial MAP registers an initial prolongation of MAP duration whereas endocardial recordings shorten without the initial lengthening. In this example recovery of MAP duration is faster in the endocardial recordings. SL (I), segment length from ischaemic zone. (Basic cycle length = 500 msec).

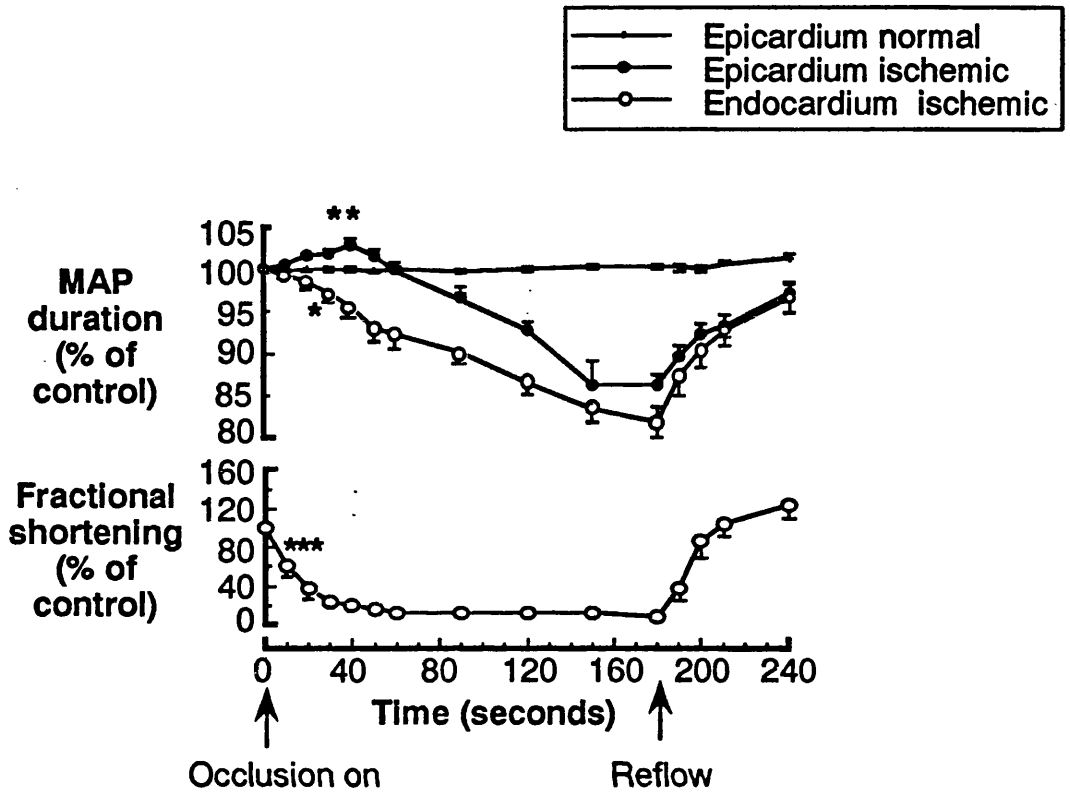

*Figure 3.2.7: Pooled data for changes in the MAP duration during coronary occlusion and reflow for the three recordings zones normalised to control values (top panel of graph). Corresponding mean values for changes in shortening of segment length normalised to control values is shown in the botton panel of the graph. The epicardial normal zone recordings show no change in MAP duration throughout the entire 4 minutes of recording. Epicardial ischaemic zone recordings register an initial prolongation which peaks at 40 seconds (**$p < 0.001$ compared to preocclusion). Thereafter shortening of MAP duration occurs with values below control seen only at 60 seconds of ischaemia. On the endocardial surface, MAP duration shortens without the intial prolongation reaching significance at 30 seconds (*$p<0.001$ compared to preocclusion). Loss of segment length shortening is rapid with fall in segment length shortening to 60% of control by 10 seconds (***$p < 0.001$). Recovery of both MAP duration and segment length activity are concordant.*

CHAPTER 3.3.

ENDOCARDIAL MONOPHASIC ACTION POTENTIAL RECORDINGS FOR THE DETECTION OF MYOCARDIAL ISCHAEMIA IN MAN - A STUDY USING ATRIAL PACING STRESS AND MYOCARDIAL PERFUSION SCINITGRAPHY

3.3.1. ABSTRACT

In a study designed to appraise the use of monophasic action potentials (MAPs) to detect myocardial ischaemia in human endocardial recordings, changes in steady state MAP duration were compared in recordings between normal and ischaemic areas of myocardium identified by the use of a radionuclide tracer simultaneously with the MAP recording procedure. Single site recordings were made from the left and/or right ventricular endocardium in 26 patients (32 recording sites) during atrial pacing to angina threshold. Pacing was maintained for 2 minutes at each increment in heart rate and MAPs recorded at the end of each 2 minute period. Perfusion defects produced by atrial pacing stress were detected using technetium 99^{m} hexakis-2-methoxy-2-methylpropyl-isonitrile injected at peak pacing stress. In 18 recordings from normally perfused areas of endocardium, MAP duration at 70% and 90% repolarisation shortened by a mean (±SEM) of 20.9 (0.9) ms and 22.0 (1.1) ms respectively for every 100 ms change in cycle length. This is in keeping with the effect of cycle length changes on the action potential duration. The extent of shortening was significantly greater ($p<0.01$) for 14 recordings from ischaemic areas being 32.0 (2.3) and 33.8 (2.6) ms respectively, indicating the additional effect of localised myocardial ischaemia. A range of values of action potential duration (measured at 70% repolarisation) shortening in unit time was analysed for sensitivity and specificity for the detection of

ischaemia. A value of 25 ms/100 ms change in cycle length provided the optimum compromise with 83% sensitivity and specificity.

These results support the applicability of the endocardially recorded MAPs for the detection of ischaemia. Such methodology may provide a means of assessing therapeutic interventions aimed at the early phase of ischaemia.

3.3.2. INTRODUCTION

The earliest changes of myocardial ischaemia are localised to small areas and as such may be absent or delayed on the electrocardiogram. (Battler et al, 1980; Taggart et al, 1989) It is to this early phase of ischaemia that therapeutic strategy should ideally be directed in order to reduce the likelihood of ventricular arrhythmias which tend to develop as the ischaemic injury increases with time. The increasing use of catheter directed therapeutic interventions such as coronary angioplasty where several successive coronary artery occlusions are performed, provides an ideal model for the study of early phases of ischaemia and the effect of any therapeutic modalities. However, at present, techniques to monitor and quantify such transient ischaemic changes are limited.

I have examined the use of the steady state recordings of the monophasic action potential (MAP) from endocardial surfaces as an index of ischaemia. MAP signals can be conveniently recorded from the ventricular endocardium using an electrode tipped catheter similar to a conventional cardiac catheter. (Franz et al, 1986; Olsson et al, 1985) Gentle apposition of the the electrode tip to the endocardial surface results in local depolarization creating a 'window' to enable an intracellular potential to be recorded from the group of cells underlying the electrode. The resultant action potential signal is attenuated in comparison to the intracellular

action potentials but its duration mirrors that of the intracellular recordings. (Hoffman et al, 1959; Ino et al, 1988) Ischaemia induces shortening of the action potential duration primarily mediated by accumulation of extracellular potassium, local hypoxia and acidosis. (Downar et al, 1977; Janse and Wit, 1989) Measurement of the MAP duration therefore enables quantification of ischaemia expressed in terms of milliseconds of shortening.

Although the MAP has been used as a measure of ischaemia on the epicardial surface in both animal and human studies, its use in human endocardial recordings in this context has yet to be fully validated. (Franz et al, 1984; Kingaby et al 1986; Taggart et al, 1986; John et al, 1992) The aim of the present study was to provide such validation using myocardial perfusion scintigraphy as an independent marker for regional myocardial ischaemia. An incremental atrial pacing protocol was used to provoke ischaemia. As the increase in heart rate on its own shortens the MAP duration, the study was designed to compare recordings between areas of normal and abnormal perfusion in which the effect of heart rate was common to both.

3.3.3. PATIENTS AND METHODS

Summary

Recordings were made of MAP from the left and/or right ventricular endocardium during incremental atrial pacing to angina. At peak pacing rate, technetium 99^{m} hexakis-2-methoxy-2-methylpropyl-isonitrile ($Tc99^{m}$-MIBI) was administered for subsequent scintigraphic imaging. The position of the MAP recording catheter in the ventricle was documented by biplane cinematography. Changes in the action potential duration were then related to the perfusion characteristics in the area of recording.

Patients

Twenty six patients undergoing routine cardiac catheterisation for investigation of chest pain were selected at random for the study. Patients with unstable angina, greater than mild left main stem stenosis, atrial fibrillation or significant conduction defects were excluded from the study. There were 16 female and 10 male patients with an age range of 39 to 73 (median 59 years). One patient had undergone coronary bypass surgery previously and another, coronary angioplasty and were being re-investigated for recurrence of angina. The hospital ethical committee gave approval for this study.

Electrophysiological measurements

Endocardial MAP recordings:

MAPs were recorded with purpose built bipolar pressure contact catheters mounted with silver/silver chloride electrodes (Cordis (UK) Ltd); size 7 French). The exploring electrode (1.0 mm in diameter) was situated at the tip. The reference electrode (1.0 mm in diameter) was situated 5 mm proximal to the tip and was flush with the wall of the catheter. Contact of the exploring electrode with the endocardium results in MAP signal of usually 20 - 40 mV amplitude.

Signal processing:

Signals were fed into a Gould isolated preamplifier (model 11 - 5407 - 58) to provide patient isolation and then into a DC Gould universal amplifier (model 13 - 4615 - 58). The amplifiers were set to give an output of 1 V for 40 mV input with frequency response of 300 Hz. The signals were displayed on a Simonsen and Weel monitor (Model MTS 102). A Gould Instruments chart recorder (model 3400 30-V8 404 - 12) was used for hard copy recordings

of the action potential signals and routine electrocardiogram at a paper speed of 125 mm/s. The action potential signals were calibrated with a direct current millivolt source (Time Electronics model 404 S).

Myocardial scintigraphy

Technetium 99m hexakis-2-methoxy-2-methylpropyl-isonitrile (Tc-99m-MIBI) was used for myocardial perfusion scintigraphy with single photon emission tomography (SPET) employing a two day, two dose protocol. Images were acquired with a single detector rotating gamma camera/computer system (IGE 400 AC - Starcam) equipped with a low-energy, high-resolution collimator. The acquisition protocol used the following parameters: 20% energy window with a 3% offset around 140 keV technetium 99m photopeak; sixty four 30 second views acquired over a 360° circular arc of rotation commencing at 45° right anterior oblique; data acquisition and storage on a 128 X 128 word matrix; pre-filtering of acquired data with a Hanning filter (0.75 cycles/cm) and transformation to a 64 X 64 word matrix. A backprojection reconstruction algorithm was used to obtain transaxial sections (one pixel thick). These reconstructed tomographic slices were then reoriented in the short axis, horizontal long and vertical long axes for display and visual analysis.

Procedure

Patients were fasted for 4 to 6 hours. Routine left ventricular and coronary angiography was performed via the femoral route using the Judkin's technique and employing a non-ionic angiographic dye (Omnipaque 350, Nycomed (UK) Ltd.). This was followed by positioning of MAP catheters in the left and/or right ventricular endocardium. In the left ventricle the curve of the catheter led to the catheter tip being located in the lateral or postero-lateral wall in the majority of patients. In the right ventricle, it was

usually possible to locate the catheter tip on the right ventricular side of the inter-ventricular septum in an area that would be expected to be supplied by the left anterior descending artery.

For atrial pacing, a bipolar 6 French temporary pacing lead (Cordis) was positioned in the right atrial appendage or at the junction of the right atrium and superior vena cava. Atrial pacing was commenced at 10 beats above the patient's resting heart rate and incremented by 10 beats until the patient developed angina, lost 1:1 atrio-ventricular conduction or achieved a heart rate of 150 beats per minute. If loss of 1:1 atrio-ventricular conduction occurred at low heart rates, 0.6 mg of atropine was administered intravenously. Each pacing train was maintained for 2 minutes to obtain steady state action potential recordings.

At peak paced heart rate, 370 MBq of $Tc99^{m}$-MIBI was administered intravenously. To ensure adequate myocardial distribution of the tracer during pacing induced ischaemia, pacing was continued for 2 minutes after the tracer injection. In patients who developed angina, there was prompt resolution of symptoms and ECG changes on cessation of pacing. The patient was returned to the ward and encouraged to eat a light fatty meal to facilitate hepatic clearance of the isotope. Myocardial perfusion imaging with SPET was carried out between one and two hours after injection of the tracer. Rest imaging was performed 24 hours later with the patient fasting for 4 hours and after a second dose of 370 MBq of $Tc99^{m}$-MIBI as for the stress imaging protocol.

Analysis of data

Significant coronary artery disease was defined as greater than 50% stenosis in a major epicardial vessel. Coronary angiographic data was interpreted by

visual inspection and degree of stenosis classed as mild (representing < 50% stenosis), moderate (50% to 70% stenosis) or severe (> 70% stenosis).

Myocardial perfusion images were interpreted by one observer who was blinded to the angiographic and MAP data. In case of equivocal results, a consensus opinion was accepted. Scintigrams were read as normal if they were without defect in the initial set or abnormal if there were reversible or fixed defects. Myocardial segments with diminished $Tc99^{m}$-MIBI uptake without reversal of defect on the rest study were considered 'fixed defects'. Segments with partial reversal as evidenced by incomplete filling of the initial perfusion defect, were considered partially ischaemic and those segments with complete reversal of defect were considered ischaemic. Defect localization was related to coronary distribution according to the standard anatomic relationship where anterior and septal abnormalities were considered to be in the territory of the left anterior descending artery, lateral or postero-lateral defects in the territory of the left circumflex artery and inferior abnormalities in the territory of the right coronary artery. Apical defects could relate to the territory of any major coronary artery. (Borges-Neto et al, 1988)

The duration of the MAP was measured at both 90% and 70% repolarisation. Figure 3.3.1 shows the method of measurement of MAP duration at 70% and 90% repolarisation. The amplitude of the MAP signal is measured in mV as the distance between the diastolic baseline and the highest part of the plateau of repolarisation. The duration is measured in milliseconds with reference to the total amplitude of the signal. Any alteration in the amplitude of the signal therefore has little influence on the duration measurements as both values would change proportionately. The terminal portion of the action potential was defined by drawing a tangent to

the fastest part of the downstroke of the baseline. (Autenrieth et al, 1975 a) This method eliminated the possibility of including afterdepolarisations in the measurements which would have produced long duration action potentials at 90% repolarisation. In addition, this technique of measurement also overcomes possible difficulties in in measuring action potentials exhibiting a continuous decline of phase IV. Steady state values of action potential duration at 70% and 90% repolarisation obtained at the end of each 2 minute pacing train have been employed for data analysis.

The position of the MAP catheter in the ventricle was documented at the time of cardiac catheterisation by biplane cinematography and subsequently related to the perfusion abnormalities on the perfusion scan. Recordings from myocardial areas reported to have a normal perfusion pattern was designated group A and those from areas of abnormal perfusion, Group B.

Due to the wide inter-individual variation in the heart rates at which pacing was commenced and terminated, changes in steady state MAP duration are expressed corrected for every 100 ms change in cycle length. This normalization is based on the observation that the relationship between MAP duration and cycle length is linear over the range of cycle lengths employed in the study (see appendix). (John et al, 1992a) Statistical analyses of comparison between groups A and B were made using the Mann-Whitney U test. Pooled data for each group are expressed as means with the standard error of the mean.

3.3.4. RESULTS

General characteristics

Of the twenty six patients studied, 23 had significant coronary artery stenoses. Ten patients had single vessel disease, 11 had two vessel disease

and 2 had triple vessel disease on angiography. Two patients had normal coronary arteries and the third had no significant residual lesions following coronary angioplasty 9 months earlier. Details of patients in the study are given in table 1. Among 38 coronary arteries with significant stenoses, 33 had related perfusion abnormalities on scintigraphy thus yielding a per vessel sensitivity of 86.8%. When fixed defects due to previous myocardial infarction were excluded, the sensitivity fell to 83.9%.

Recording sites

Initial studies were confined to monophasic action potential recordings from the left ventricle (n=19) and later studies used recordings from either both ventricles (n=6) or right ventricle only (n=1). In all, 32 recordings were obtained from 26 patients. Of these, 18 recordings were assessed to be from areas corresponding to normal perfusion pattern on the scintigram (group A) and 14 from areas of the ventricle corresponding to areas on perfusion scan with hypoperfusion patterns indicating myocardial ischaemia (group B).

Recordings from non ischaemic areas

An example of of a recording from a non-ischaemic zone is shown in figure 3.3.2. The action potential duration decreases with incremental pacing consistent with cycle length related changes. The perfusion images (figure 3.3.2a) obtained following incremental pacing from a cycle length of 667 ms to 500 ms (heart rate 90 to 120 beats per minute) show normal myocardial perfusion pattern. The duration of the MAP measured at 70% repolarization recorded from the lateral wall of the left ventricle shortens in a decremental fashion from 248 ms to 216 ms in keeping with shortening of the steady state interbeat interval (figure 3.3.2b).

Simultaneous recording from ischaemic and non ischaemic areas

Steady state MAP recordings obtained simultaneously from ischaemic and non ischaemic zones during incremental pacing demonstrate shortening of the action potential duration but those from the ischaemic zone shorten to a greater extent. Typical examples of MAP signals from areas of ventricle relating to normal and ischemic areas are shown in figure 3.3.3. Figure 3.3.3a shows SPET images of a patient with a tight main left circumflex artery stenosis. At peak pacing at a cycle length of 500 ms (heart rate 120 beats per minute) she developed angina. Stress images show perfusion defects in the postero-lateral left ventricular wall which reperfuses during rest. Monophasic action potentials were recorded simultaneously from the left ventricular postero-lateral wall and the right ventricular septum (figure 3.3.3b). Approximate recording positions represented on the scintigram are indicated by arrows in figure 3.3.3a. The steady state action potential duration over the right ventricular septum (normally perfused area) shortens by 40 ms in keeping with the decrement in cycle length (increase in heart rate). In recordings from the left ventricle (area of abnormal perfusion), the action potential duration shortens in excess of 100 ms indicating the additive effect of ischaemia.

'Field of view' of action potential recordings

Figure 3.3.4 illustrates the very localised nature of the action potential recordings. Patient 10 had significant left anterior and right coronary artery disease producing anterior and inferior reversible perfusion defects on the perfusion scan. The postero-lateral wall from where the action potentials were recorded had a normal perfusion pattern. The 70% and 90% steady state action potential duration shortens by 32 and 28 ms respectively over a 150 ms alteration in cycle length representing a 21.3 ms and 18.6 ms change

respectively when normalized for 100 ms change in cycle length - a shortening that is compatible with heart rate effect only.

Analysis of pooled data

Table 2 shows a comparison of MAP duration shortening for recordings from group A (normal zone recordings) and group B (ischaemic zone recordings). When action potential duration was normalized for cycle length changes, recordings from normally perfused areas showed a mean shortening of action potential duration measured at 70% repolarisation of 20.9 (0.9) ms per 100 ms change in cycle length compared to 32.0 (2.3) ms for recordings from ischaemic areas ($p< 0.01$). The corresponding values for action potential duration measured at 90% repolarisation were 22.0 (1.1) ms and 33.8 (2.6) ms respectively ($p< 0.01$) (See figure 3.3.5).

Sensitivity and specificity of values of action potential duration

In order to test the applicability of this data to assessing the presence or absence of ischaemia in any individual patient, I have calculated the sensitivity and specificity of a range of action potential duration (measured at

70% repolarisation) changes per unit time (figure 3.3.6). For example, for an action potential duration shortening of 18 ms/100 ms shortening of cycle length, the sensitivity is 100% but the specificity is low at 22%. On the other hand, for a value of 44 ms/100 ms change in cycle length, although the specificity is 100 %, the sensitivity is low at 14%. The optimum value for action potential duration (at 70% repolarisation) that represent ischaemia in the face of a changing cycle length as in a standard pacing protocol is a value of 25ms/ 100ms decrement of the cycle length. Above this value, the specificity rises but the sensitivity falls.

3.3.5. DISCUSSION

This study provides evidence that MAPs recorded from the endocardial surface is a reliable means of detecting regional myocardial ischaemia. Myocardial ischaemia was induced by incremental atrial pacing. Regional myocardial perfusion was documented simultaneously with the MAP recording procedure using $Tc99^{m}$-MIBI. Changes in the MAP duration were compared in areas with normal and abnormal perfusion patterns. In the normally perfused areas, MAP duration shortened as function of cycle length shortening (increasing heart rate). In the hypoperfused areas, MAP duration shortening was significantly greater indicating the additional effect of ischaemia. The data was also analysed in terms of sensitivity and specificity for ischaemia during the standard incremental pacing protocol used in this study. On this basis, a value of action potential duration (measured at 70% repolarisation) shortening of 25 ms/100 ms change in cycle length provides the optimum sensitivity coupled with optimum specificity for the detection of ischaemia.

Much of the data on electrophysiological changes that accompany ischaemia have been gathered using micro-electrode recordings of intracellular action potentials in isolated tissue. (Janse and Kléber, 1981; Kléber, 1983; Fozzard and Makielski, 1985) In vivo studies in animals and humans have employed the MAP which is now well established as a reliable representation of the intracellular action potential although of lower amplitude and upstroke velocity. (Hoffman et al, 1959; Olsson, 1985; Ino T et al, 1988) Both animal and human epicardial studies have shown the duration of the MAP to be a sensitive index of early localised myocardial ischemia. (Franz et al, 1984; Kingaby et al, 1986; Taggart et al, 1988) The more recent development of silver/silver chloride tipped pressure contact electrodes has enabled the acquisition of stable endocardial MAP signals

over prolonged periods of time. (Franz, 1983; Franz et al, 1986) Such electrodes are now commercially available and are primarily used in evaluation of drug effects on repolarization time course and in studies of rate adaptation changes. (Franz et al, 1988; Platia et al, 1988; Seed et al, 1987; Taggart et al, 1990 b) I have explored the use of this technique in the documentation of regional myocardial ischaemia in human endocardial studies in view of its potential application in quantification of ischaemia in conjunction with cardiac catheterisation procedures.

The MAP technique has a number of advantages over conventional methods for detection of ischaemia such as the ECG or myocardial perfusion scintigraphy. One of the attractions of the technique is the ability to record early ischaemic changes from localised areas of the endocardium. Recordings made close to or in contact with the myocardium such as the MAP have the advantage that a relatively small area of underlying ischaemia subtends a large angle to the recording electrode. Such an electrode registers a greater effect than a more distant electrode ie. the ECG to which an ischaemic zone of similar size would subtend a smaller angle. (Holland and Arnsdorf, 1977) Another reason for the higher sensitivity of the MAP compared to the ECG is that the latter is a global summation of the action potentials throughout the heart. As such, many of these action potentials which propagate in opposite directions are subject to cancellation and may not register any influence on the electrocardiogram.

The MAP allows repeated measures of changes during sequential therapeutic interventions in the course of a single study unlike perfusion scintigraphic studies which only allow single interventions to be quantified at a time. The primary usefulness of the MAP as measure of ischemia is however, likely to be in a research capacity. The technique would be ideally

suited to the evaluation of measures directed at altering the severity of ischemic responses. The model for such transient ischaemia by controlled coronary occlusions would be the patient undergoing percutaneous coronary angioplasty. The heart rate in such situations can be held constant and any change apparent in the duration of steady state MAP signals would therefore be predominantly a measure of ischaemia.

One disadvantage of such localised endocardial recordings is that it is necessary for the catheter electrode to be in contact with the ischaemic area. The development of steerable MAP catheters should minimise this problem. A possible uncertainty with endocardial recordings is the influence of the intra-cavitary blood acting as a volume conductor. In both epicardial and endocardial recordings, the contact electrode is directly apposed to the myocardium. For epicardial recordings, the design of the recording equipment is such that the indifferent electrode makes contact with the epicardium at a distance of 1 to 2 mm from the contact electrode through a small cotton wick or saline soaked sponge. (Runnalls et al, 1987) For endocardial recordings, the indifferent electrode which is situated 5 mm proximal to the tip electrode makes contact with the endocardium via the intra-cavitary blood which behaves as a volume conductor. The indifferent electrodes could therefore pick up repolarization characteristics from distant areas of myocardium diluting the information from the contact electrode. This phenomenon is a theoretical limitation in endocardial studies but previous work by Taggart et al. (1988) has shown that although this mechanism altered the electrogram recorded from the indifferent electrode, it had no significant effect on the MAP signals from the area of ischaemia.

The action potential duration is not only dependant on the immediate preceding cycle length but also on the cycle lengths of a number of beats beforehand. In the present study, observations were made at the end of a 2 minute period following alteration in the pacing rate. Following an abrupt change in heart rate, ideally a period of 3 minutes should be allowed at each paced rate for complete adaptation to a new steady state. (Seed et al, 1987) However, the majority of the adaptive process is complete in a period of about 90 seconds. (Franz et al, 1988) In order to reduce the total duration of the protocol, I recorded my observations at the end of 2 minutes and consider it unlikely that this has induced a significant error in the results.

Other factors such as antiarrhythmic drugs and autonomic effects may also influence the duration of the MAP duration. None of the patients in the study were receiving medications which are conventionally considered to influence directly, the ventricular action potential duration. (Vaughan Williams, 1984) The procedure itself and the initiation of angina may have enhanced the sympathetic activity in some patients. Catecholamines have been shown to exert a variable effect on the ventricular action potential duration eliciting either lengthening, (Mitchell et al, 1986) shortening, (Autenrieth et al, 1975 b) or a biphasic response. (Kass and Wiegers, 1984) These disparities probably relate to the differences in the steady state heart rate, dose, and speed of administration employed in the various studies (see discussion in section 3.5.5). (Taggart et al, 1990) The majority of patients in the present study were receiving beta blockers (without class 3 action) which would be expected to minimise these effects.

Ischaemia has been shown to have several effects on the action potential namely, shortening of the duration, loss of amplitude and diastolic potential, and reduction in maximum upstroke velocity. (Fozzard and

Makielski, 1985) These changes tend to go hand in hand. As the monophasic action potential records a variable proportion of the amplitude and upstroke velocity characteristics of the intracellular action potential, I have confined my measurements to the duration of the MAP as an index of ischaemia.

A relatively new perfusion marker namely $Tc99^{m}$-MIBI was employed in this study for perfusion imaging. Unlike thallium 201, this tracer has the advantage of minimal myocardial washout permitting administration to the patient at a time and site remote from where imaging is carried out. (Okada et al, 1988) This feature renders $Tc99^{m}$-MIBI ideal for use with cardiac catheterisation procedures. Imaging carried out 1 to 2 hours after administration of the tracer would reveal perfusion characteristics representative of that during the time of injection, in this case of that during the MAP recording procedure. The agent has not been previously employed with atrial pacing stress to detect myocardial ischaemia. The present study utilising $Tc99^{m}$-MIBI in conjunction with cardiac catheterisation during atrial pacing stress had shown diagnostic sensitivity comparable to conventional myocardial perfusion scintigraphy.

3.3.6. CONCLUSION

In order to validate the use of endocardial monophasic action potential (MAP) recordings for the detection of regional myocardial ischaemia, an incremental pacing protocol was used in combination with radionuclide perfusion imaging. In the non ischaemic zones (areas of normal perfusion pattern) the MAP duration shortened as a function of the decrement in cycle length (increased heart rate). In recordings from ischaemic zones (area of hypoperfusion), the MAP duration shortened by an additional 11 ms per 100 ms change in cycle length. Values of action potential duration

shortening in unit time that are representative of an ischaemic effect as opposed to a rate effect have been established and appraised them in terms of sensitivity and specificity. These data confirm the applicability of endocardial MAP recordings for the detection and quantification of endocardial ischaemia. Such methodology would provide a convenient means of evaluating transient ischaemic responses and therapeutic interventions on the early phases of ischaemia which maybe silent clinically and absent electrocardiographically.

Table 3.3.1: Details of patients in the study:

Patient Number	Age Sex	Previous MI	Drugs	Coronary artery anatomy LAD	LCX	RCA	Atrial pacing Min	Max	Perfusion scan	MAP catheter position	Group
1	55,F	Nil	Nil	Normal	Normal	Normal	80	130	Normal	Postero-lateral wall of LV	A (LV)
2	65,F	Nil	Nifedip.	Normal	Normal	Severe	100	120	Inferior RPD	Inferior wall of LV	B (LV)
3	68,F	Posterior	Atenolol Nifedip.	Mild	Occluded	Moderate	90	120	Lateral wall partial RPD	Lateral wall of LV	B (LV)
4	45, F	Nil	Nil	Normal	Normal	Normal	90	120	Normal	Postero-lateral wall of LV	A (LV)
5	61, M	Nil	Atenolol Nifedip.	Occluded	Mild	Occluded	80	100	Anterior and inferior RPD	Lateral wall of LV	A (LV)
6	42, M	Nil	Metoprolol	Normal	Severe	Normal	90	140	Infero-lateral RPD	Infero-lateral wall of LV	B (LV)
7	67, F	Anterior	Metoprolol Nifedip.	Occluded	Normal	Occluded	80	120	Antero-septal partial RPD; Inferior RPD	Anterior wall of LV	B (LV)
8	46, F	Inferior	Atenolol	Mild	Normal	Severe	90	140	Inferior FD	Anterior wall of LV	A (LV)
9	71, F	Nil	Metoprolol Diltiazem	Severe	Moderate	Severe	70	90	Anterior RPD	Anterior wall of LV	B (LV)
10	52, M	Nil	Atenolol	Severe	Normal	Occluded	80	100	Anterior and inferior RPD	Lateral wall of LV	A (LV)
11	61, M	Inferior	Metoprolol	Mild	Normal	Occluded	80	120	Inferior FD	Lateral wall of LV	A (LV)
12	59, F	Inferior	Oxprenolol Nifedip.	Normal	Mild	Normal (Previous PTCA)	70	130	Inferior FD	Lateral wall of LV	A (LV)
13	73, F	Inferior	Diltiazem	Severe	Mild	Moderate	90	140	Anterior and inferior RPD	Postero-lateral wall of LV	A (LV)
14	59, F	Inferior	Atenolol Nifedip.	Moderate	Normal	Occluded	80	120	Inferior FD Anterior RPD	Postero-lateral wall of LV	A (LV)

15	70, F	Nil	Metoprolol Nifedip.	Occluded	Moderate	Severe	80	100	Anterior partial RPD;	Anterior wall of LV; Apex of RV Inferior RPD	B (LV) A (RV)
16	58, M	Nil	Atenolol Nifedip.	Occluded	Severe	Normal	90	110	Anterior and Lateral RPD	Lateral wall of LV	B (LV)
17	55, M	Nil	Atenolol	Severe	Mild	Normal	90	120	Anterior RPD	Apex of RV	A (RV)
18	56, M	Nil	Nil	Severe	Patent graft	Patent graft	120	150	Anterior RPD	Anterior wall of LV	B (LV)
19	64, M	Anterior	Timolol	Occluded	Mild	Normal	90	110	Anterior FD Partial RPD of septum	Postero-lateral wall of LV; Septum of RV	A (LV) B (RV)
20	51, M	Posterior	Atenolol Nifedip.	Normal	Occluded	Severe	90	120	Lateral partial RPD	Postero-lateral wall of LV	B (LV)
21	59, F	Posterior	Atenolol Nifedip.	Mild	Severe	Moderate	90	130	Lateral partial RPD	Postero-lateral wall of LV; Septum of RV	B (LV) A (RV)
22	44, F	Inferior	Diltiazem	Normal	Normal	Severe	82	132	Inferior FD	Postero-lateral wall of LV; Septum of RV	A (LV) A (RV)
23	49, M	Nil	Atenolol	Occluded	Occluded	Mild	90	110	Anterior and Lateral RPD	Lateral wall of LV	B (LV)
24	39, F	Nil	Nil	Severe	Normal	Normal	100	140	Anterior RPD (Septum normal)	Postero-lateral wall of LV; Septum of RV	A (LV) A (RV)
25	62, F	Nil	Atenolol Nifedip.	Normal	Severe	Normal	80	120	Infero-lateral RPD	Postero-lateral wall of LV; Septum of RV	B (LV) A (RV)
26	59, F	Inferior	Metoprolol	Mild	Moderate	Occluded	80	120	Inferior FD; Lateral RPD	Postero-lateral wall of LV	B (LV)

Key to abbreviations: MI = myocardial infarction; Nifedip = nifedipine; RPD = reversible perfusion defect; FD = fixed defect; LAD = left anterior descending; LCX = left circumflex artery; RCA = right coronary artery; PTCA = percutaneous transluminal coronary angioplasty; MAP = monophasic action potential; LV = left ventricle; RV = right ventricle.
Group A = Recording from area of normal perfusion.
Group B = Recording from area of abnormal perfusion.

Table 3.3.2:. A, Maximum and minimum cycle lengths and change in steady state action potential duration per 100 ms change in cycle length.

GROUP A

Patient No. (recording site)	Max CL (ms)	Min CL (ms)	ΔCL (ms)	Δ APD		ΔAPD per 100ms Δ CL	
				70%	90%	70%	90%
1 (LV)	750	462	288	64	64	22.2	22.2
4 (LV)	667	500	167	32	32	19.2	19.2
5 (LV)	750	600	150	40	40	26.7	26.7
8 (LV)	667	429	238	52	60	21.8	25.2
10 (LV)	750	600	150	32	28	21.3	18.6
11 (LV)	750	500	250	52	60	20.8	24
12 (LV)	857	462	395	92	92	23.3	23.3
13 (LV)	667	429	238	48	60	20.2	25.2
14 (LV)	750	500	250	44	36	17.6	14.4
15 (RV)	750	600	150	24	20	16.0	10.6
17 (RV)	667	500	167	28	36	16.7	21.5
19 (LV)	667	545	122	20	28	16.4	22.9
21 (RV)	667	462	205	36	36	17.6	17.6
22 (LV)	728	456	272	72	76	26.4	27.9
22 (RV)	728	456	272	72	76	26.4	27.9
24 (LV)	600	429	171	40	44	23.4	25.7
24 (RV)	600	429	171	40	44	23.4	25.7
25 (RV)	750	500	250	40	44	16.0	17.6

Table 3.3.2. B, Maximum and minimum cycle lengths and change in steady state action potential duration per 100 ms change in cycle length.

GROUP B

Patient No. (recording site)	Max CL (ms)	Min CL (ms)	ΔCL (ms)	Δ APD		ΔAPD per 100 ms ΔCL	
				70%	90%	70%	90%
2 (LV)	600	500	100	32	32	32	32
3 (LV)	667	500	167	56	56	33.5	33.5
6 (LV)	667	429	238	60	68	25.2	25.2
7 (LV)	750	500	250	80	84	32	33.6
9 (LV)	857	667	190	48	48	25.2	25.2
15 (LV)	750	600	150	40	48	26.6	32
16 (LV)	667	545	122	32	36	26.2	29.5
18 (LV)	500	400	100	36	36	36	36
19 (RV)	667	545	122	68	76	55.7	62.3
20 (LV)	667	500	167	52	56	31.1	33.5
21 (LV)	667	462	205	60	60	29.3	29.3
23 (LV)	667	545	122	28	28	22.9	22.9
25 (LV)	750	500	250	108	112	43.2	44.8
26 (LV)	750	500	250	72	76	28.8	30.4

Key to Abbreviations: CL = cycle length; APD = action potential duration; LV = left ventricle; RV = right ventricle.

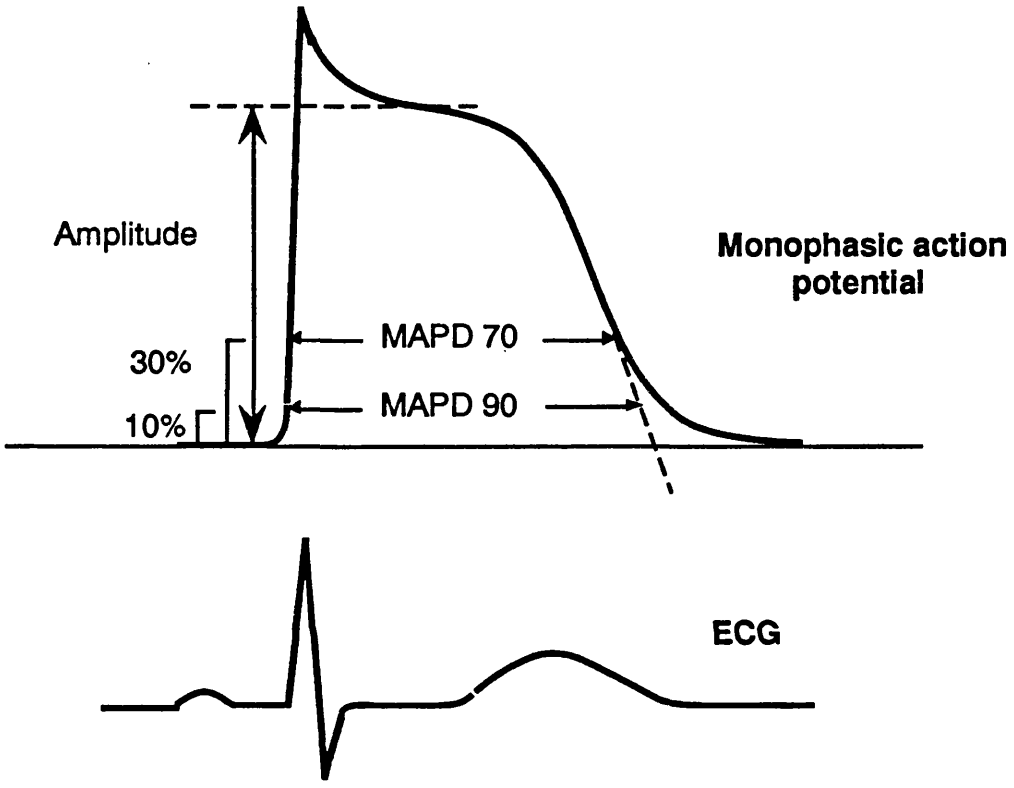

Figure 3.3.1: Typical monophasic action potential (MAP) signal is shown together with the ECG to demonstrate the time relationship with the QRST of the ECG. The amplitude of the MAP in mV is the distance between the diastolic baseline and the highest point of the plateau phase of repolarisation. MAP duration (MAPD) is measured in msec at 70% and 90% repolarisation with reference to the total amplitude of the MAP signal. A tangent was drawn to the fastest part of the downstroke to define the terminal portion of the MAP signal.

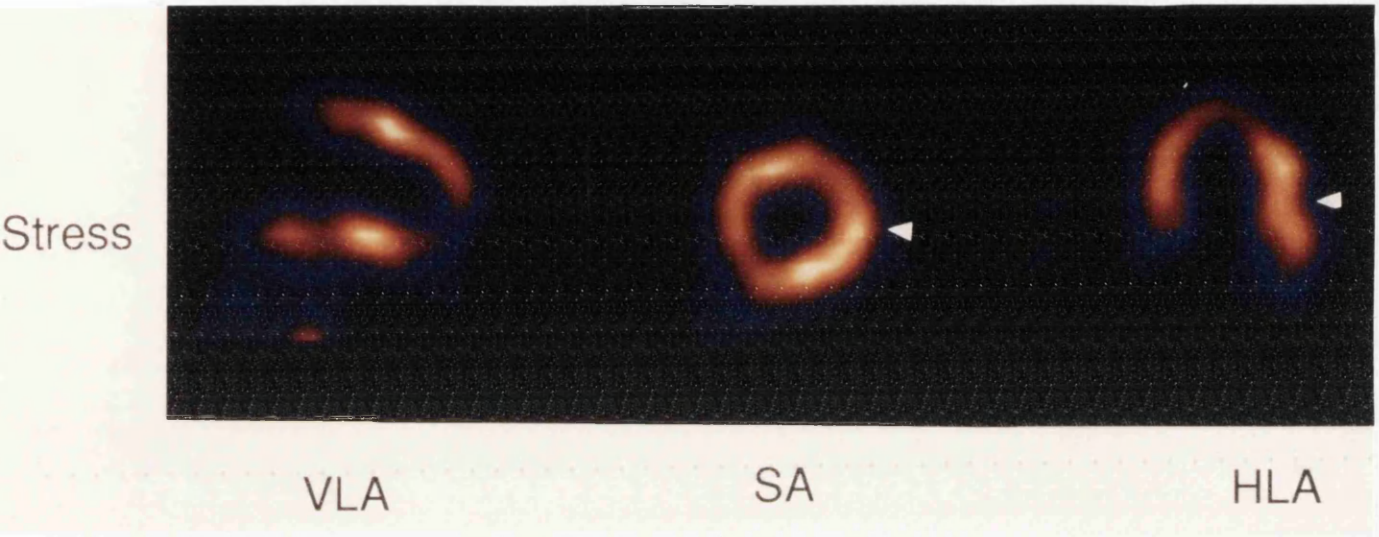

***Figure** 3.3.2. A, Perfusion images (SPET) of patient 4 with normal coronary arteries demonstrating a normal perfusion pattern following incremental pacing from a cycle length of 667 ms to 500 ms (heart rate 90 to 120 beats per minute).*

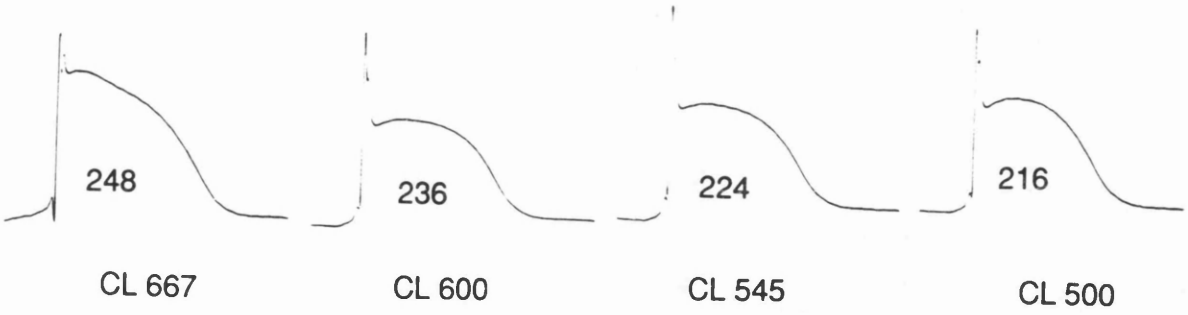

***Figure** 3.3.2. B, Monophasic action potentials (MAPs) recorded from the lateral wall of the left ventricle (approximate recording position represented on scintigram indicated by arrow) demonstrates shortening of the duration (MAP duration measured at 70% repolarisation in msec is shown as number within the figures) as a function of cycle length changes.*

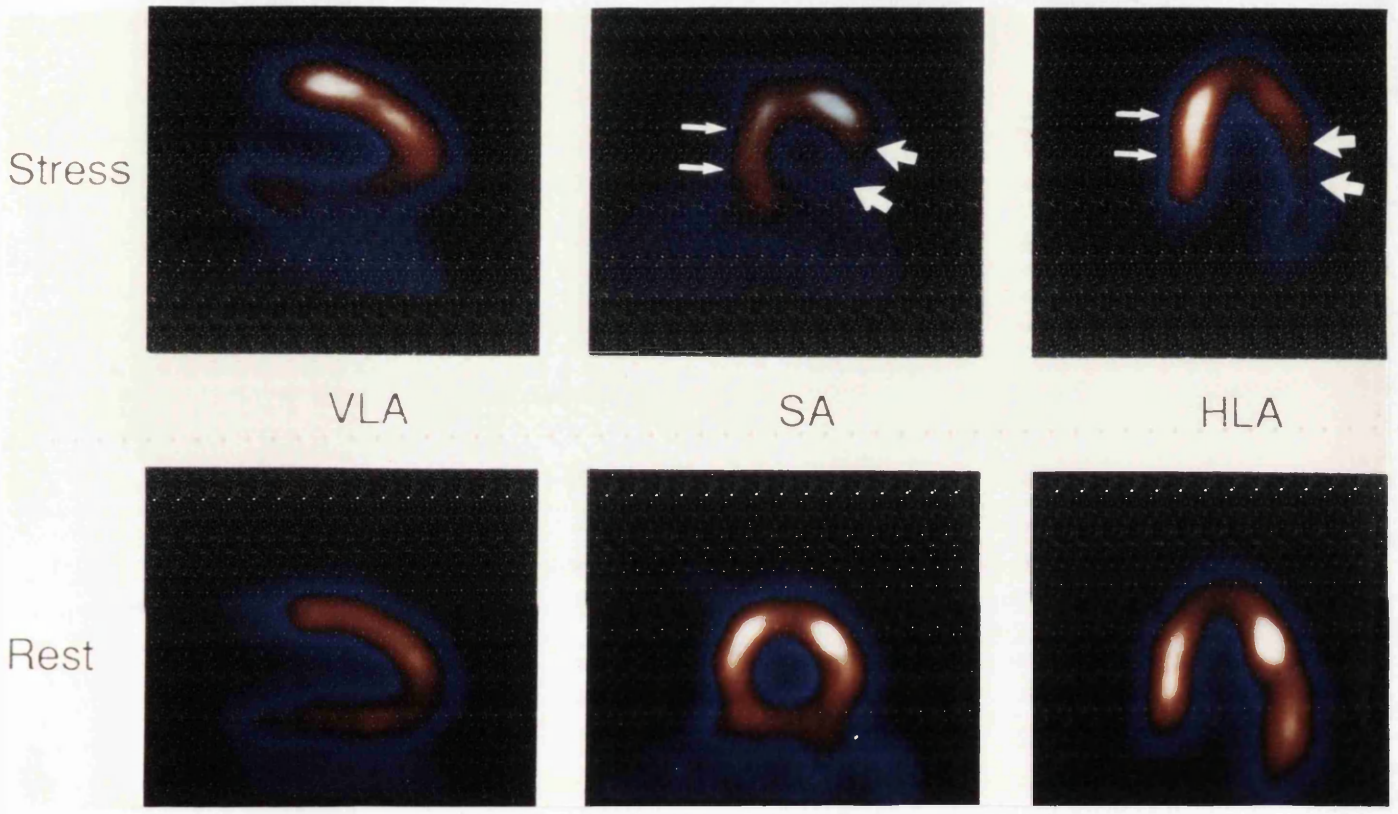

Figure 3.3.3. ***A,*** *Perfusion images (SPET) of patient No. 25 with tight left main circumflex artery stenosis. Incremental atrial pacing between cycle lengths of 750 and 500 msec (heart rate 80 to 120 beats per minute) produced angina and perfusion deficits in the postero-lateral wall which reperfuses at rest.*

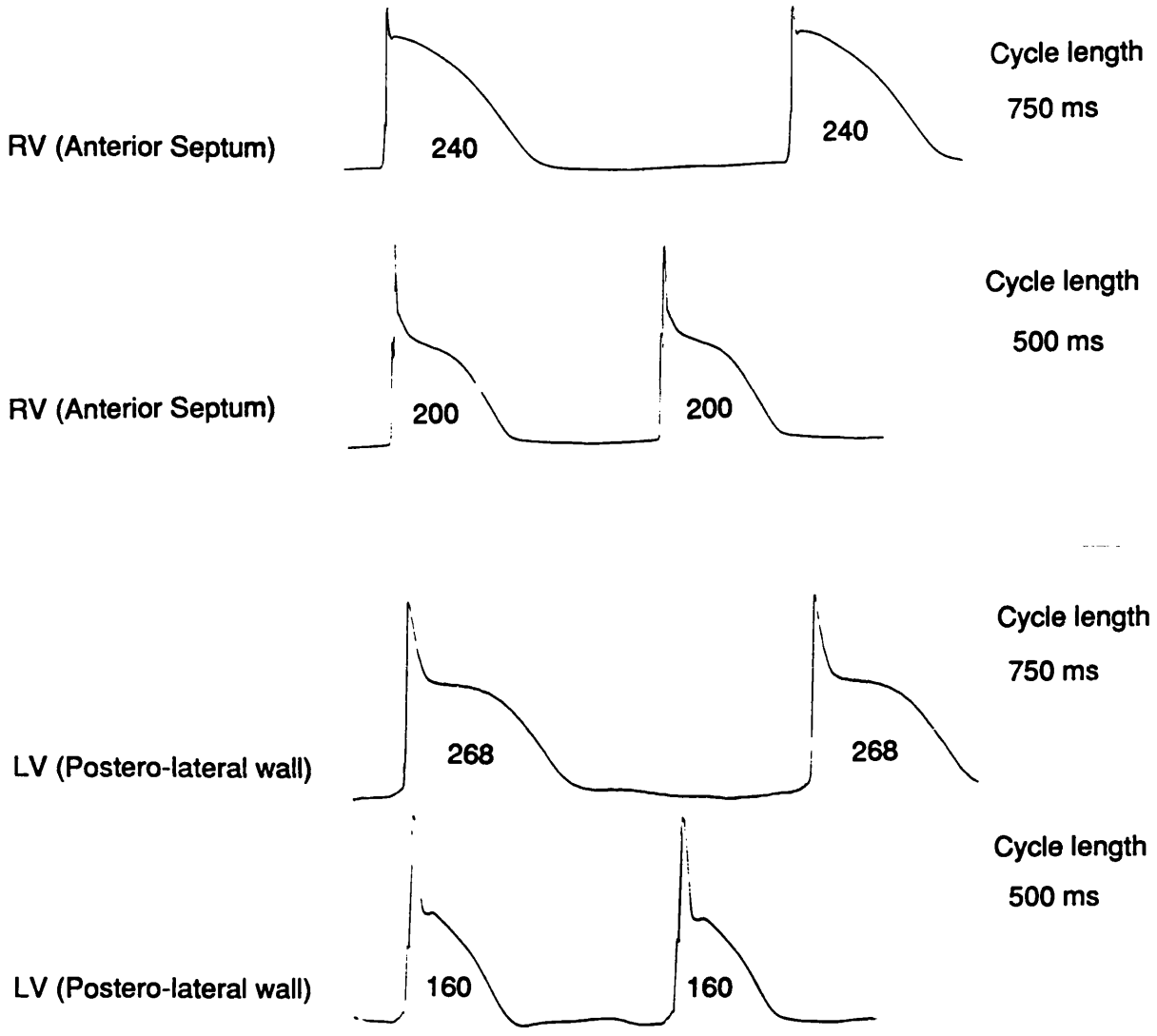

Figure 3.3.3. B, Monophasic action potential (MAP) signals were recorded simultaneously from the right ventricular septum (top panel) and left ventricular postero-lateral wall (bottom panel). Approximate recording position represented on the scintigram are indicated by thin arrows for the right ventricular site and thick arrows for the left ventricular site in figure 3.3.3. A. Steady state duration (MAP duration measured at 70% repolarisation in msec is shown as number within figures) shortens by 40 ms over the normally perfused right ventricular septum whereas a shortening of 108 ms occurs over the abnormally perfused left ventricular wall.

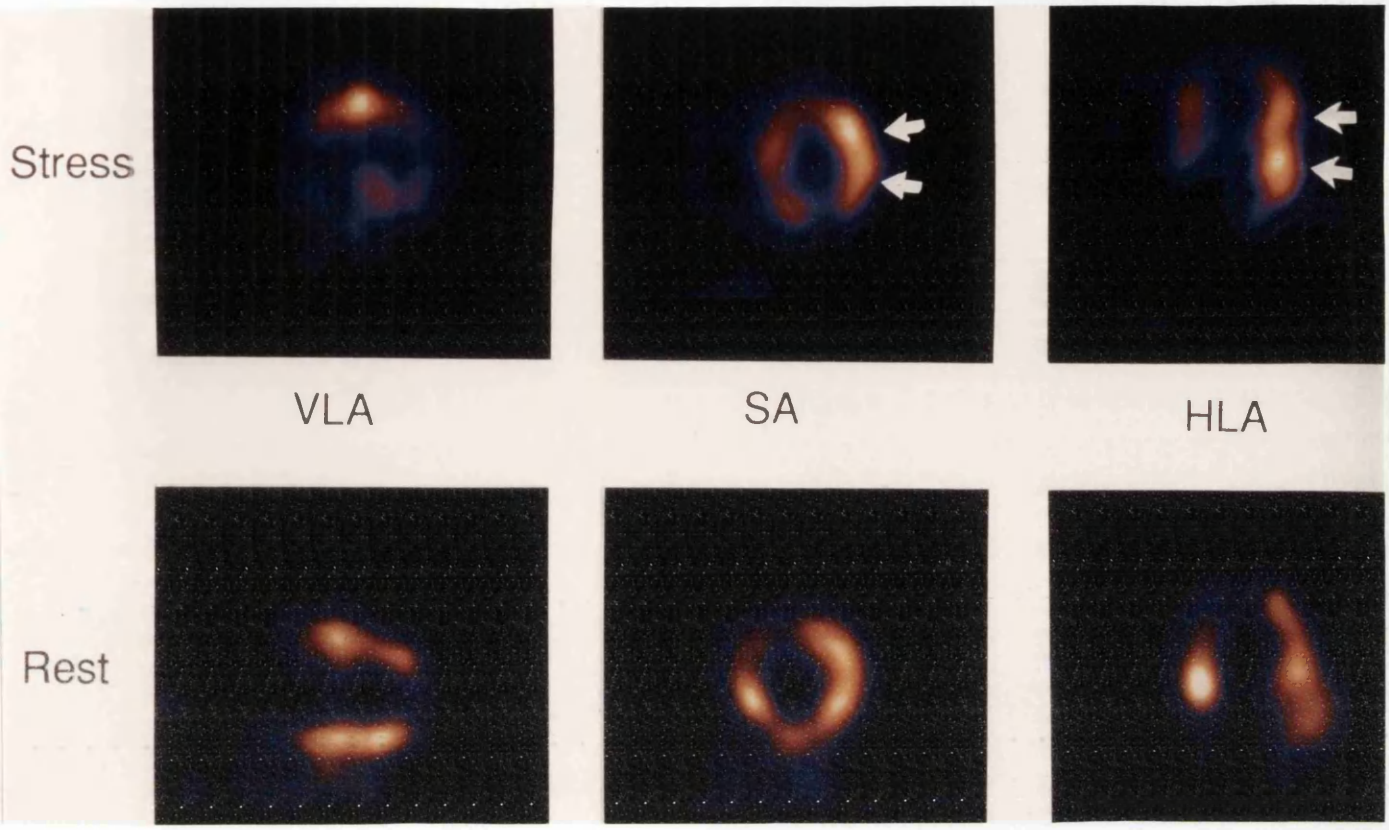

Figure 3.3.4. A, Perfusion images (SPET) of patient 10 with tight left anterior descending and right coronary artery stenoses. Reversible perfusion defects are demonstrated in both anterior and inferior left ventricular wall. The lateral wall remains normally perfused.

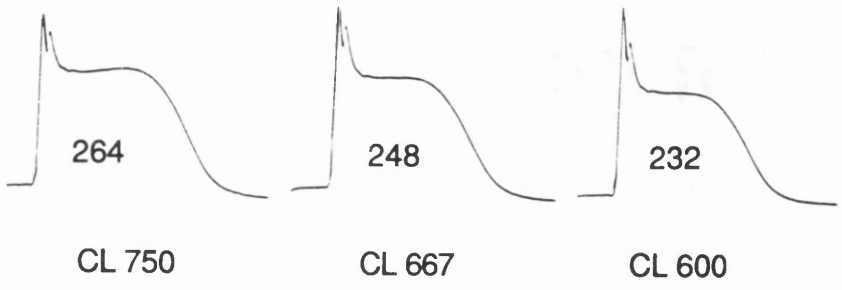

Figure 3.3.4. B, Monophasic action potentials (MAPs) recorded from the lateral wall of the LV (approximate recording position represented on the scintigram is indicated by arrows in figure 3.3.4.A) demonstrated shortening of duration in keeping with cycle length related changes only. MAP duration measured at 70% repolarisation in msec is shown as number within the figures.

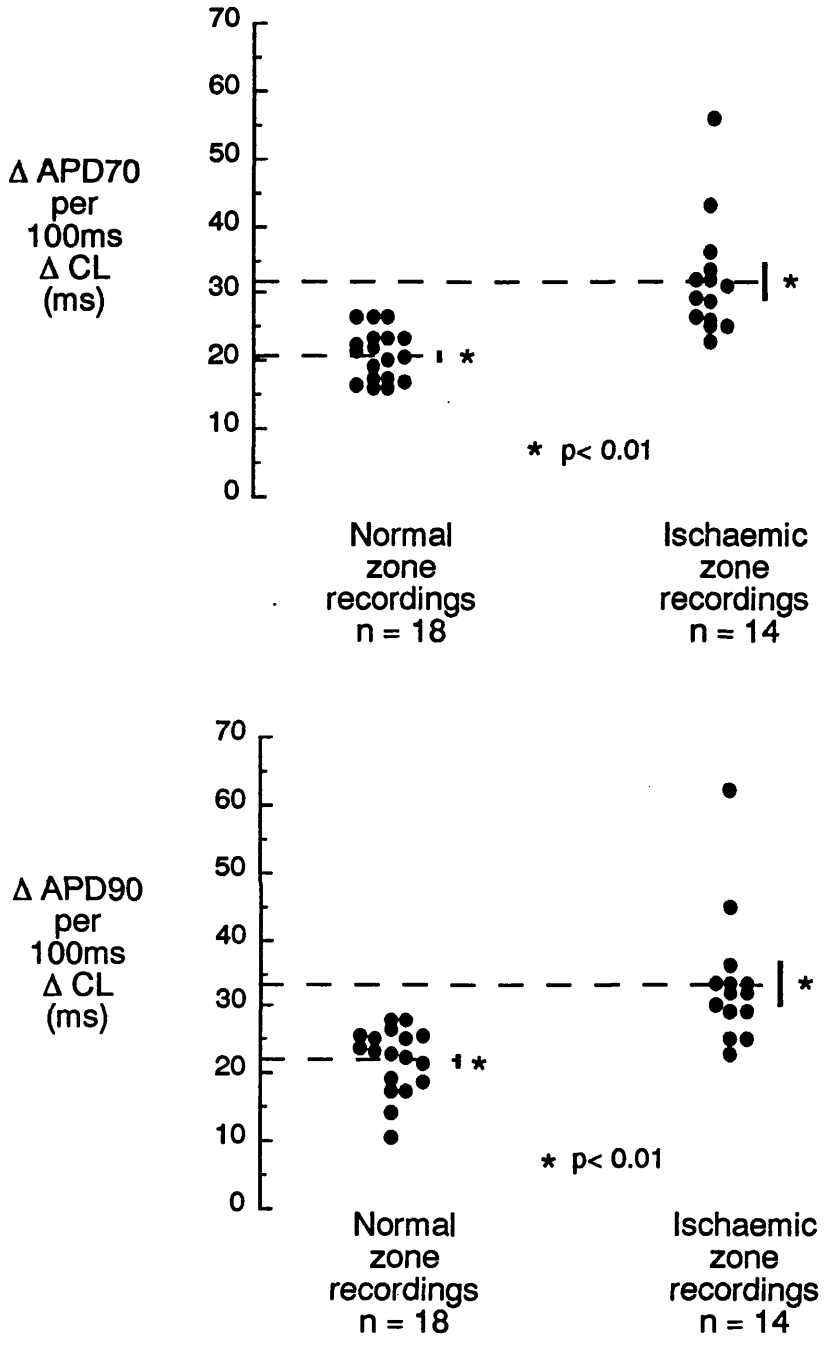

*Figure 3.3.5: Pooled data of changes in action potential duration per 100 ms change in cycle length (CL) for recordings from normal and ischaemic zones. Horizontal broken lines represent the mean value and the vertical solid bars, the standard error of the mean. **APD70,** action potential duration measured at 70% repolarisation. **APD90,** action potential duration measured at 90% repolarisation.*

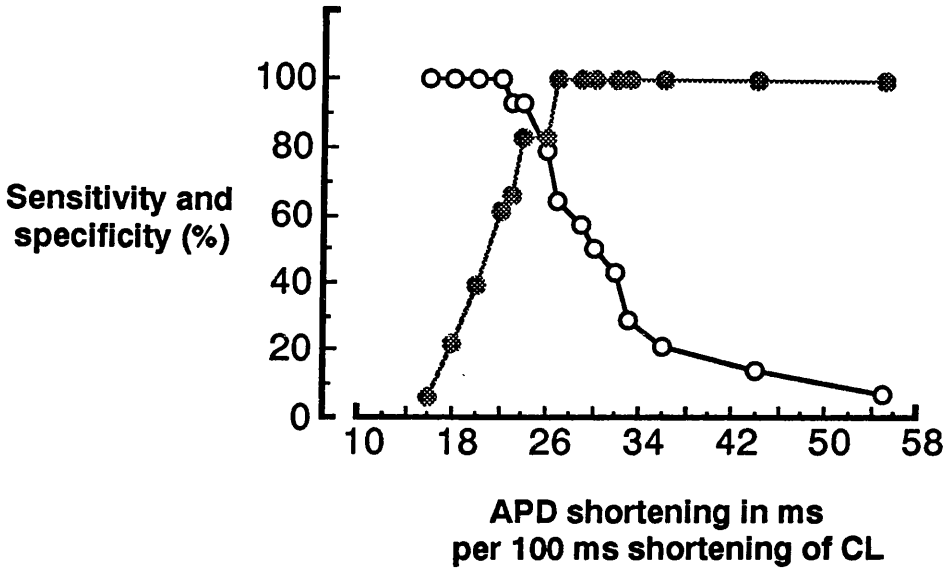

Figure 3.3.6: Sensitivity and specificity for the detection of ischaemia for a range of action potential duration (measured at 70% repolarisation) shortening between 16 and 55 ms/100 ms shortening of cycle length. Optimum compromise occurs where the line for sensitivity (open circles) and the line for specificity (shaded circles) cross at 25 ms. APD, action potential duration; Cl, cycle length.

CHAPTER 3.4.

VASODILATOR MYOCARDIAL PERFUSION IMAGING: DEMONSTRATION OF LOCAL ELECTROPHYSIOLOGICAL CHANGES OF ISCHAEMIA USING ENDOCARDIAL RECORDINGS OF THE MONOPHASIC ACTION POTENTIAL

3.4.1. ABSTRACT

Objective: Controversy exists as to the incidence and severity of myocardial ischaemia provoked in the course of perfusion scintigraphy using coronary vasodilators. I addressed this question using endocardial recordings of steady state monophasic action potentials as an independent marker of early localised myocardial ischaemia.

Patients: Thirty one patients undergoing routine cardiac catheterisation for investigation of chest pain were studied.

Setting: The studies were carried out at the Middlesex Hospital, London, a tertiary cardiac referral centre.

Design : Single site monophasic action potential recordings were obtained from the left and/or right ventricle (50 recording sites) during i.v. infusion of dipyridamole (0.015 mg/kg/min X 4 minutes). Heart rate was held constant with atrial pacing at 20% above the patient's resting rate. Technetium 99m hexakis-2-methoxy-2-methylpropyl-isonitrile (MIBI) was administered 4 minutes after dipyridamole and single photon emission tomographic imaging performed an hour later. Rest images were obtained the following day (two day, two dose protocol). Based on the scintigraphic perfusion characteristics and coronary anatomical data for the action potential recording site, recordings were grouped into: group 1 - recordings from areas with a normal perfusion pattern (n = 30), group 2 - recordings from areas with a perfusion defect and subtended by significantly narrowed

coronary arteries without obvious angiographic collateral supply (n = 10), and group 3 - recordings from areas with a perfusion defect and subtended by occluded arteries with angiographically evident collaterals from adjacent vessels (n = 10).

Results: Changes in the monophasic action potential duration indicative of ischaemia ie. shortening of steady state action potential duration, occurred in 18 of the 20 recordings from areas of abnormal perfusion. Peak changes were apparent at 8 minutes from the start of the dipyridamole infusion. Mean (SEM) changes for action potential duration alteration between control and peak effect at 8 minutes were 253.1 (4.9) ms to 254.3 (5.3) ms for group 1 (p = NS), 262.4 (5.5) ms to 249.2 (5.2) ms for group 2 ($p< 0.001$) and 244.4 (4.4) ms to 220.4 (4.0) ms for group 3 ($p< 0.0001$). These changes were significantly different between the three groups ($p< 0.01$). ST segment changes on the surface electrocardiogram were seen in only 8 patients, all with collateralised areas of viable myocardium.

Conclusions: These data provide strong evidence for the presence of myocardial ischaemia in regions of reversible perfusion defects induced by dipyridamole. In addition, this study shows that such ischaemia is of greater intensity and more likely to be clinically apparent when myocardial viability is dependant on collateral circulation.

3.4.2. INTRODUCTION AND BACKGROUND

Myocardial perfusion scintigraphy utilising vasodilators such as dipyridamole or adenosine is in widespread use as a diagnostic tool. (Gould et al, 1978; Boucher et al, 1985; Verani et al, 1990) The underlying pathophysiological mechanisms however, remain incompletely defined. Both these agents dilate coronary arteries and produce substantial increments in coronary blood flow. (Strauss and Pitt, 1977; Sorensen et al, 1985) In coronary vascular beds distal to a flow limiting stenosis,

autoregulatory mechanisms operate producing maximal vasodilatation under resting conditions and thereby limiting coronary flow reserve. (Klocke, 1987) Dipyridamole for example, would therefore induce a preferential dilatation in the vascular bed subtended by normal arteries but exert minimal effects on those distal to a stenotic artery. (Sorensen et al, 1985) Regional differences in blood flow thus created would be sufficient to allow heterogenous distribution and uptake of radiotracers producing scintigraphic images within which areas of low tracer uptake are conventionally interpreted as representing ischaemia. However, unlike with exercise perfusion scintigraphy, pharmacological vasodilatation produce increase in coronary supply in excess of demand and areas of relative hypoperfusion may not necessarily be ischaemic. Furthermore, in clinical use, overt manifestations of myocardial ischaemia are infrequent with dipyridamole. (Beller, 1989; Ranhosky et al, 1990) Such observation has led to the suggestion that dipyridamole caused little, if any, myocardial ischaemia.

Development of transient regional left ventricular wall motion asynergy is specific for the presence of myocardial ischaemia. (Beller and Gibson, 1987) This occurs in 50 to 60% of patients with significant coronary artery disease given dipyridamole and is evidence that myocardial ischaemia is at least partly a function of pharmacological coronary vasodilatation. (Picano et al, 1985; Pennel et al 1990) Although previous reports have explored the occurrence of ischaemia, (Flemeng et al, 1974; Keltz et al, 1987) and have forwarded possible physiological mechanisms, (Picano, 1989) the true incidence of ischaemia with dipyridamole remain undefined. The definition of ischaemia or otherwise with vasodilator perfusion imaging is important as the technique is gaining popularity and dipyridamole has been licensed for the purpose. Moreover, the ability of dipyridamole to produce

perfusion deficits in the region of mild to moderate coronary stenosis (Josephson et al, 1982) renders necessary the documentation of the pathophysiological importance of such lesions. Accordingly, I have used an electrophysiological marker of localised ischaemia namely, endocardial recordings of monophasic action potentials in order to document the presence or absence of ischaemia in areas of dipyridamole induced perfusion deficits. The duration of the monophasic action potentials measured under steady state conditions registers early changes of localised myocardial ischaemia, shortening in response to ischaemia. (Franz et al, 1984; Taggart et al, 1986; John et al, 1991) Recordings were made from the left and/or right ventricular endocardium during the administration of dipyridamole and electrophysiological changes indicative of ischaemia were recorded in areas of myocardium showing reversible perfusion defects as demonstrated by technetium 99m hexakis-2-methoxy-2-methylpropyl-isonitrile (Tc-99m-MIBI) and tomography.

3.4.3. PATIENTS AND METHODS

Summary

Following routine cardiac catheterisation, recordings were made of monophasic action potential from the left and/or right ventricular endocardium during intravenous infusion of dipyridamole. At a time when the drug was expected to exert maximal pharmacological effect (4 minutes following dipyridamole infusion), Tc-99m-MIBI was administered for subsequent single photon emission computed tomographic (SPET) imaging. The position of the monophasic action potential recording catheter in the ventricle was documented by biplane cinematography. Changes in the action potential duration were then related to the perfusion characteristics in the area of recording.

Study Patients

Thirty three patients undergoing routine cardiac catheterisation for investigation of chest pain were selected at random from the waiting list. Patients with unstable angina, atrial fibrillation, overt congestive heart failure and obstructive airways disease were excluded from the study. Antianginal drugs were continued up to 12 hours prior to the study. Substances containing methyl xanthines were withheld for 24 hours prior to the study. Written informed consent was obtained from each patient and the study protocol was approved by the ethical committee of the Middlesex Hospital, London.

Electrophysiological measurements

Purpose built bipolar pressure contact catheters with silver/silver chloride electrodes (Cordis (UK) Ltd; 7 French size) were used to record endocardial monophasic action potentials. Recordings were made from the right or left ventricle or both. Details of signal acquisition and processing have been described in section 3.3.3.

Myocardial scintigraphy

Technetium 99m hexakis-2-methoxy-2-methylpropyl-isonitrile (Tc-99m-MIBI) was used for myocardial perfusion scintigraphy with single photon emission tomography employing a two day, two dose protocol.
Details of image acquisition and reconstruction are described in section 3.3.3. Reconstructed tomographic slices were oriented in short axis, horizontal long, and vertical long axes for display and visual analysis.

Procedure

Routine left ventricular and coronary angiography was performed via the femoral route using the Judkin's technique and employing a non-ionic

angiographic dye (Omnipaque 350, Nycomed (UK) Ltd.). This was followed by positioning of monophasic action potential catheters in the left and/or right ventricular endocardium using femoral arterial and/or venous sheaths. A bipolar temporary atrial pacing electrode was introduced into the right atrium through a second femoral venous sheath. Atrial pacing was established at 20% above the patient's resting heart rate to maintain a constant heart rate during the procedure.

Arterial blood pressure was monitored through the side arm of the femoral arterial cannula. The electrocardiogram was continuously monitored using limb leads I and II and chest lead V5. Monophasic action potentials were recorded from a single site in the left and/or right ventricle after pacing had been established for 3 minutes (to allow for rate adaptation of the action potential duration). Dipyridamole was infused through a forearm vein at a rate of 0.142 mg /kg per min over 4 minutes. Recordings of monophasic action potentials, arterial pressure, and electrocardiogram were made every minute for a total of 15 minutes.

Four minutes after completion of dipyridamole infusion, 350 to 400 MBq of Tc-99m-MIBI was administered intravenously. If any patient developed severe angina or haemodynamic upset, the effect of dipyridamole was reversed using 125 to 250 mg of aminophylline i.v. given 2 minutes after Tc-99m-MIBI. Patients were returned to the ward and encouraged to eat a light fatty meal to facilitate hepatic clearance of the isotope. Myocardial perfusion imaging with tomography was carried out between one and two hours after injection of the tracer. Rest imaging was performed 24 hours later with the patient fasting for 4 hours and after a second dose of 350 to 400 MBq of Tc-99m-MIBI. Data acquisition was commenced between one and two hours

post injection after a light fatty meal as described above. A summary of the protocol employed in the study is shown in figure 3.4.1.

Data analysis

Coronary angiographic data was interpreted by visual inspection and degree of stenosis classed as mild, moderate or severe. Regional collateralisation was established on the basis of retrograde filling of distal segments of totally or subtotally occluded arteries. Regions supplied by arteries with greater than 50% stenosis (but non-occluded) and without angiographically obvious collateralisation were designated non-collateralised areas.

Myocardial perfusion images were analysed and interpreted by one observer blinded to the angiographic and monophasic action potential data. In case of equivocal results, a consensus opinion was accepted. Images were read as normal if they were without defect in the initial set or abnormal if there were reversible or fixed defects. Defect localisation was related to coronary distribution according to the standard anatomic relationship where anterior and septal abnormalities were considered to be in the territory of the left anterior descending artery, lateral or postero-lateral defects in the territory of the left circumflex artery and inferior abnormalities in the territory of the right coronary artery. Apical defects could relate to the territory of any major coronary artery. (Borges-Neto et al, 1988)

The duration of the monophasic action potentials was made at both 70% and 90% repolarisation. Methodology employed for measurement of action potential duration is described in section 3.3.3. Since both 70% and 90% values registered a similar pattern, values at 70% repolarisation have been used for analysis.

The position of the monophasic action potential catheter in the ventricle was documented at the time of cardiac catheterisation by biplane cinematography and subsequently related to the perfusion abnormalities on the perfusion scan. Based on the relation of the catheter tip to areas of scintigraphic perfusion defects and coronary anatomical data for the recording site, recordings were grouped into: Group 1 = recordings from areas with a normal perfusion pattern; Group 2 = recordings from areas with reversible perfusion pattern but subtended by stenosed arteries without angiographically apparent collateral vessels; Group 3 = recordings from areas with reversible pattern but subtended by totally or subtotally occluded arteries with collateral filling of the distal vessel.

Statistical analyses of comparisons between groups were made using analysis of variance with planned comparison of means (ANOVA). For graphical representation and to facilitate visual display, changes in action potential duration were expressed as ratios: for each single site recording, the control value of action potential duration was taken to represent 100% and any alteration related to the control value. Pooled data are expressed as means with the standard error of the mean.

3.4.4. RESULTS

Of 35 patients studied, heart rate control during dipyridamole infusion was lost in 4 patients and as the action potential duration is dependent on cycle length, these four patients were excluded from analysis. The results reported are on the 31 patients in whom steady state single site recordings of action potentials were obtained from the left and/or right ventricles. Patient details are given in table 3.4.1. Median age of the patients studied was 56 years with a range of 33 to 72 years. All except two patients had significant coronary artery disease defined as greater than 50% stenosis in any major

epicardial artery; 12 of these had single vessel disease, 12 patients had two vessel disease, and 5 had triple vessel disease. Previous aorto-coronary bypass grafting had been performed in three patients.

Sensitivity of myocardial perfusion scintigraphy with dipyridamole

All patients with significant coronary artery disease developed perfusion abnormalities on imaging. Among 51 coronary arteries with significant stenoses, 44 had related perfusion abnormalities on scintigraphy thus yielding a per vessel sensitivity of 86.3%. When fixed defects due to previous myocardial infarction were excluded, the sensitivity fell to 83.7%.

Monophasic action potential recordings

Figure 3.4.2 shows typical catheter placements during action potential recording. Fifty single site recordings were obtained from 31 patients (simultaneous recordings from right and left ventricles in 19 patients, the left ventricle alone in 4 patients, and right ventricle alone in 8 patients). Based on the perfusion characteristics of the recording site, 30 recordings were assessed to be from normally perfused areas (group 1). Of the 20 recordings from abnormally perfused areas, 10 were from non collateralised areas (group 2), and 10 from collateralised areas (group 3). Although the pacing rates used in each set of recordings varied, there was no significant difference in the paced heart rates between the three groups (mean ±SE values: group 1 = 87.8±1.9, group 2 = 84±3.0, and group 3 = 86±2.7; p=NS).

The standard deviation for percentage change from control values for the normal zone recordings was 1.5%. Shortening of the action potential duration by 3% (2 standard deviations) or greater occurred in 8 of the 10 recordings in group 2 and in all recordings in group 3. Maximal changes were apparent at 8 minutes from the start of dipyridamole infusion. An

example of the development and regression of action potential duration shortening indicative of ischaemia is shown in figure 3.4.3. Patient 19 had occlusion of native vessels and grafts to the territory of the left coronary circulation which was retrogradely collateralised by vessels from the right coronary artery and graft. Dipyridamole infusion produced marked angina and ischaemic changes in monophasic action potential recordings from the lateral wall of the left ventricle. Shortening of the action potential duration is maximal at 8 minutes. Regression of changes is apparent at 12 minutes, in this case accelerated by 125 mg of aminophylline at 10 minutes into the protocol.

Illustrative studies

Figures 3.4.4 and 3.4.5 show coronary angiographic appearances, tomographic myocardial perfusion images and typical examples of changes in monophasic action potential in studies representative for the three groups. Figures 3.4.4, A, 3.4.4, B, and 3.4.4, C are data on patient 11 in table 3.4.1. Coronary angiography shows a normal right coronary and left anterior descending artery producing a normal perfusion pattern in areas subtended by these arteries. The left main circumflex artery has a tight lesion without collateral supply. Perfusion images show reversible perfusion abnormalities in the lateral wall of the left ventricle. Monophasic action potential signals recorded from the normally perfused septum show no change in the duration of the signals whereas those from the abnormally perfused lateral wall of the left ventricle demonstrate shortening of the duration by 12 msecs. Figures 3.4.5,A, 3.4.5, B, and 3.4.5, C are from patient 22. The left anterior descending artery is occluded at its origin. The region is collateralised by a vessels fed by the right coronary artery. A large area of anterior and septal perfusion defect is produced with dipyridamole which partially reverse on the 'rest' images. The duration of the action potentials

recorded from the right ventricular septum demonstrate shortening by 32 msecs whereas those from a normally perfused left ventricular lateral wall remain unchanged.

Analysis of pooled data

The duration of the monophasic action potentials measured at 70% repolarisation altered from a mean (SEM) of 253.1 (4.9) msecs to 254.3 (5.3) msecs for group 1 (p=NS), 262.4 (5.5) msecs to 249.2 (5.2) for group 2 recordings ($p< 0.001$), and 244.4 (4.4) msecs to 220.4 (4.0) for group 3 recordings ($p< 0.0001$). Figure 3.4.6 depicts the changes in action potential normalised to the control values in relation to dipyridamole infusion for the three groups. Significant ischaemia is apparent in the group 2 recordings compared to the group 1 but the extent of ischaemia is substantially greater for group 3 recordings (changes in action potential duration from control to 8 minute values were significantly different between the three groups at 0.01 level).

Simultaneous normal and abnormal zone recordings

Simultaneous recordings were obtained from the right and left ventricles in 18 patients. In twelve of these patients, biventricular recordings were obtained wherein one of the recording sites was normally perfused and the other abnormally perfused. Mean (SEM) change in action potential duration in msecs between control values and 8 minute values were as follows: 258.0 (8.6) to 257.3 (9.6) for group 1 ($n = 12$), p = NS; 261.0 (6.4) to 247.0 (5.6) for group 2 ($n = 8$), $p < 0.003$; 247.0 (8.8) to 221.0 (9.6) for group 3 ($n = 4$), $p < 0.002$ (see figure 3.4.7). These changes were significantly different between the three groups ($p<0.01$).

Angina and ST segment changes with dipyridamole infusion

24 of the 31 patients developed angina during infusion of dipyridamole. This included the two patients with angiographically normal coronary arteries and normal perfusion images. ST segment depression was evident in only 8 patients, all with collateralised areas of viable myocardium (see table 3.4.1).

3.4.5. DISCUSSION

To date, there has been inconclusive evidence to settle the contentious question as to whether transient defects detected by perfusion scintigraphy using dipyridamole stress represent ischaemia or merely blood flow redistribution. This study using the monophasic action potential has shown shortening of the monophasic action potential duration characteristic of ischaemia in areas of myocardium with a reversible perfusion pattern whereas in areas showing a normal perfusion pattern, the action potential duration remained unchanged. These data support the view that some degree of ischaemia is almost invariably provoked by selective coronary vasodilation in the presence of significant stenoses. Ischaemia was most marked in areas subtended by occluded arteries with distal vessel collateralisation from adjacent vessels.

Coronary angiography provides anatomical information with little indication of the pathophysiological importance of coronary narrowings. Perfusion scintigraphy on the other hand, is often held as depicting the physiological correlate to anatomical lesions and therefore, more meaningful in the clinical context. This may hold true for perfusion deficits induced by exercise where there is considerable increase in oxygen demand. In perfusion scintigraphy using dipyridamole stimulus, the above may not be the case as the predominant mechanism of action is blood flow

increments in excess of demand. In areas of low tracer uptake, blood flow is therefore, not reduced in an absolute sense and such areas should not be considered necessarily ischaemic. Arguments against ischaemia have been advanced on the basis of a relative lack of electrocardiographic changes during dipyridamole infusion occurring in only 20 to 30% of patients. (Zhu et al, 1988) Provocation of angina with dipyridamole is an unreliable marker for ischaemia since the drug acts via the intermediary of adenosine. Adenosine is not only a vasodilator but also a messenger for the sensation of angina. (Lagerquist et al, 1990) Nevertheless, regional wall motion asynergy occurs in a proportion of patients given intravenous dipyridamole. (Sochor et al, 1984; Picano et al, 1985; Pennell et al, 1990) This would suggest that flow differences induced by the drug is of sufficient magnitude to provoke pathophysiological consequences namely, ischaemia leading to transient contractile dysfunction. The present study, using an independent marker, provides good evidence for ischaemia as a significant component of dipyridamole perfusion imaging.

At present, indices of localised ischaemia that can be practically deployed in clinical studies to assess ischaemia with dipyridamole are the surface electrocardiogram and demonstration of wall motion asynergy using any of the several existing imaging techniques. The surface electrocardiogram may be relatively insensitive to early myocardial ischaemia and not readily applicable to to the interpretation of regional myocardial ischaemia. (Taggart et al, 1989) Although wall motion abnormalities are known accompaniments of early ischaemia, they have been documented in only 50 to 60% of patients with significant coronary artery disease given a coronary vasodilator. (Pennel et al, 1990; Picano et al, 1989) This may be due to the relative insensitivity of the currently available imaging techniques to discern small changes resulting from early subendocardial myocardial

ischaemia. I therefore chose the monophasic action potential signal which records from an area of myocardium approximately 5 mm in diameter and is therefore suitable for exploring localised electrophysiological events.

Monophasic action potential signals recorded from the surface of the myocardium reflect an averaged intracellular action potential for the group of cells beneath the exploring electrode. (Olsson et al, 1985; Franz, 1991) The amplitude of the signal is attenuated in comparison with the intracellular action potential but the duration has been shown to be reliable for the entire repolarisation phase (Hoffman, 1959; Franz et al, 1986; Ino et al, 1988). Ischaemia shortens the action potential duration by mechanisms which are probably multifactorial. (Janse and Wit, 1989) Monophasic action potential recordings have been used to monitor ischaemia on both endocardial and epicardial surfaces in animal studies and in humans. (Donaldson et al, 1983; Donaldson et al, 1984; Franz et al, 1984; Taggart et al, 1986; John et al 1992 b) In endocardial studies, it is possible that the ability of these recordings to detect early changes of ischaemia which are typically localised to small areas, may be reduced by the volume conductor properties of intracavitary blood. The exploring electrode is in direct contact with the endocardium. The reference electrode which is situated 5 mm proximal to the catheter tip makes contact with the endocardium via the intracavitary blood. Solid angle theory would therefore predict that a large area of ischaemia would be relatively undiluted by more distant contact with the surrounding normal myocardium. (Holland and Arnsdorf, 1977) A small area of ischaemia on the other hand, would subtend only a small angle to the reference electrode which might be expected to influenced to a greater extent by the surrounding normal myocardium. In this study, any error that may have occurred would be an error of underestimation of the severity of ischaemia.

The action potential duration shortening that has been observed in the present study is likely to have been a direct result of ischaemia rather than changes related to alterations in catecholamine and potassium levels. Neither of these parameters vary during the course of the protocol employed (unpublished observations). Dipyridamole would be expected to produce increased myocardial levels of adenosine but the latter substance has no significant effect on the ventricular action potential duration. (O'Nunain et al, 1991) Furthermore, in the patients where simultaneous recordings were obtained from abnormally and normally perfused areas, the action potential duration remained stable throughout the entire 15 minutes of recording in the normally perfused areas.

The majority of patients in this study were on beta receptor blocker or calcium channel blocker medications. Neither of these agents are conventionally recognised as influencing the ventricular action potential duration. (Vaughan Williams, 1984) Antianginal medications are known to reduce the ischaemic response to dipyridamole without limiting the the hyperaemic flow pattern induced by the drug. (Picano, 1989) However, there were insufficient numbers of patients off all medications to meaningfully compare and assess the influence of these drugs on the extent of ischaemia. Nevertheless, it unlikely that either group of medications would have encouraged the development of ischaemia which is the key finding in this study.

The importance of this study is that we have provided strong evidence for the presence of some degree of ischaemia as an integral part of vasodilator myocardial perfusion imaging. Particularly interesting was the striking difference between the action potential recordings from ischaemic areas showing collateralisation on angiography compared to areas without

collaterals. Collateral circulation in man develops in response to stimuli arising from prolonged periods of blood flow limitation and possibly depend on genetically mediated angiogenic factors. (Gould, 1989; Kurachi et al, 1985) These vessels are generally considered protective to compromised areas of myocardium. In the context of local coronary vasodilators however, the reverse would seem to be the case. Such deleterious effects of epicardial collateralisation have been suggested in previous reports. (Demer et al, 1986; Chambers et al, 1988) The likely mechanism would be a local steal phenomenon by virtue of a relative stenosis at the junction of the feeding vessel with the collateral origin. (Gould, 1989)

The finding of a high incidence of ischaemia in this study is somewhat contrary to previous studies examining the question of ischaemia with coronary vasodilation in coronary artery disease. (Josephson et al, 1982; Leppo et al, 1982; Okada et al, 1987) Previous studies have focussed on electrocardiographic and wall motion abnormalities as indicators of ischaemia. Whereas profound wall motion changes are known to occur within few beats of major coronary artery occlusion in experimental studies, the situation with regard to local subendocardial ischaemia induced by a steal phenomenon maybe different and may not produce discernible wall motion abnormalities. An endocardial to epicardial blood flow steal phenomenon induced by dipyridamole has been previously suggested, (Picano, 1989; Meerdink et al, 1989) and is the probable mechanism in the genesis of the ischaemia for the non-collateralised areas in this study. It is nevertheless unlikely that such early ischaemia contributes significantly to the differential uptake of radiotracers in vasodilator perfusion imaging. The relatively large areas of perfusion abnormalities that is commonly seen with vasodilation would be more in keeping with a redistribution phenomenon as the primary mechanism. Ischaemia appears to be provoked in the course

of such blood flow redistribution and to greater extent when myocardial viability is dependent on collateralisation.

3.4.6. CONCLUSION

In this study, I set out to investigate whether dipyridamole induced reversible perfusion defects on myocardial perfusion scintigraphy was associated with the presence of myocardial ischaemia. I would interpret the results as indicating that some degree of ischaemia is almost always provoked in the course of the coronary flow redistribution induced by dipyridamole in the presence of significant coronary stenoses. Such ischaemia is of greater intensity in areas of viable myocardium with collateral supply. These observations have important implications in the physiological interpretation of a diagnostic test which is rapidly gaining popularity.

Table 1: Details of patients in the study.

Patient Number	Age Sex	Drugs	Coronary artery anatomy LAD	LCX	RCA	Paced rate (bpm)	Angina/ ST change	Perfusion scan	MAP catheter position	Group
1	54, M	Atenolol Diltiaz	Moderate NC	Moderate NC	Severe NC	80	AP+ ST-	Inferior, lateral and antero-apical RPD; septum normal	Lateral wall of LV RV mid septum	LV - 2 RV- 1
2	44, M	Atenolol	Normal	Moderate NC	Occluded C	80	AP+ ST -	Inferior FD lateral RPD	Lateral wall of LV RV mid septum	LV- 2 RV- 1
3	58, M	Propran Nifedip Nitrate	Moderate NC	Moderate NC	Occluded C	75	AP+ ST+	Inferior RPD	RV mid septum	RV- 1
4	50, M	Atenolol Diltiaz	Severe NC	Normal	Occluded C	80	AP+ ST-	Inferior and antero-septal RPD	Infero-posterior wall of LV	LV- 3
5	56, M	Atenolol Nifedip	Normal	Normal	Normal	80	AP+ ST-	Normal	RV mid septum	RV- 1
6	62, M	Atenolol	Normal	Moderate NC	Occluded C	80	AP- ST-	Inferior FD; lateral RPD	Postero-lateral wall of LV; RV mid septum	LV- 2 RV- 1
7	48, M	Atenolol	Moderate NC	Normal	Occluded C		AP+ ST-	Inferior and antero-apical RPD	Lateral wall of LV	LV- 1
8	35, M	Atenolol Nitrate	Severe NC	Mild	Normal	80	AP- ST-	Anterior and septal RPD	Lateral wall of LV RV mid septum	LV- 1 RV- 2
9	68, M	Metop Nifedip	Severe NC	Normal	Severe NC	80	AP- ST-	Anterior, septal and inferior RPD	Lateral wall of LV	LV- 1
10	59, M	Atenolol	Severe NC	Moderate NC	Mild	90	AP+ ST-	Anterior and septal RPD	Lateral wall of LV RV mid septum	LV- 1 RV- 2
11	49, M	Nil	Normal	Severe NC	Normal	100	AP+ ST-	Inferolateral RPD	Lateral wall of LV RV mid septum	LV- 2 RV- 1

12	55, M	Atenolol Nifedip	Mild	Occluded NC	Non Dominant	80	AP+ ST-	Inferior wall partial RPD	Lateral wall of LV RV mid septum	LV- 1 RV- 1
13	57, M	Nifedip	Mild	Occluded C	Normal	90	AP+ ST+	Inferior and lateral RPD	Lateral wall of LV RV mid septum	LV- 3 RV- 1
14	50, M	Atenolol	Severe NC	Mild	Occluded C	90	AP+ ST+	Antero-septal and inferior RPD	RV infero - posterior septum	RV- 3
15	54, M	Atenolol Nifedip	Normal	Normal	Normal	90	AP+ ST-	Normal	Lateral wall of LV RV apex	LV- 1 RV- 1
16	56, M	Atenolol Nifedip	Severe NC	Normal	Occluded C	80	AP+ ST-	Anteroseptal RPD Inferior FD	Lateral wall of LV RV mid septum	LV- 1 RV- 2
17	50, M	Nil	Mild	Moderate NC	Severe NC	100	AP+ ST-	Inferior and lateral RPD	Lateral wall of LV RV mid septum	LV- 2 RV- 1
18	58, M	Metop	Poor graft run off- NC	Normal	Normal	80	AP- ST-	Antero-septal partial RPD	RV apical septum	RV- 2
19	68, M	Metop	Occluded C	Occluded C	Patent graft	90	AP+ ST+	Anterior FD Lateral wall RPD	Lateral wall of LV	LV- 3
20	57, M	Metop Diltiaz	Moderate NC	Normal	Normal	90	AP+ ST-	Antero-apical RPD; septum normal	RV mid septum	RV- 1
21	33, M	Atenolol	Moderate NC	Normal	Normal	80	AP+ ST-	Mild antero-apical RPD	LV septum RV mid septum	LV- 1 RV- 1
22	59, M	Atenolol Nifedip	Occluded C	Normal	Normal	100	AP+ ST-	Anterior and septal RPD; inferior partial RPD	Lateral wall of LV RV mid septum	LV- 1 RV- 3
23	54, M	Atenolol	Occluded C	Normal	Moderate NC	80	AP+ ST-	Anterior, septal and inferior RPD	Lateral wall of LV RV anterior wall	LV- 1 RV- 1
24	54, M	Nil	Occluded C	Occluded C	Normal	90	AP+ ST+	Antero-apical FD Lateral wall RPD Septum normal	Lateral wall of LV RV mid septum	LV- 3 RV- 1
25	46, M	Diltiaz	Normal	Occluded C	Non Dominant	80	AP+ ST+	Lateral wall RPD Partial RPD in inferoapical region	Lateral wall of LV RV posterior septum	LV- 3 RV- 3

26	60, M	Atenolol Diltiaz	Mild	Normal	Severe NC	100	AP+ ST-	Inferior and apical RPD	Lateral wall of LV RV apex	LV- 1 RV- 1
27	48, M		Moderate NC	Moderate NC	Occluded NC	110	AP- ST-	Anteroseptal FD; inferior RPD	Lateral wall of LV RV apex	LV- 1 RV- 1
28	72, M	Nifedip	Occluded C	Moderate NC	Occluded C	90	AP+ ST+	Anterseptal RPD Inferior FD	RV mid septum	RV- 3
29	66, M	Atenolol Nifedip	Patent graft	Patent graft	Occluded C	90	AP- ST-	Inferior wall partial RPD	RV mid septum	RV- 1
30	59, M	Atenolol Nifedip	Normal	Normal	Severe NC	70	AP- ST-	Inferior wall and inferior septal RPD	RV postero-inferior septum	RV- 2
31	64, M	Atenolol Nifedip	Severe C	Moderate NC	Occluded C	70	AP+ ST+	Inferior wall FD Antero-septal RPD	High lateral wall of LV; RV mid septum	LV- 1 RV- 3

KEY TO ABBREVIATIONS FOR TABLE 3.4.1: **LAD**, left anterior descending; **LCX**, left circumflex; **RCA**, right coronary artery; **MAP**, monophasic action potential; **NC**, non collateralised; **C**, collateralised; **AP**, angina pectoris; **ST**, ST segment of electrocardiogram; **LV**, left ventricle; **RV**, right ventricle; **FD**, fixed defect; **RPD**, reversible perfusion defect; **Dilitiaz**, dilitiazem; **Propran**, propranolol; **Metop**, metoprolol; **Nifed**, nifedipine.

GROUPS: **Group 1** = action potential recordings from endocardial areas with a normal perfusion pattern; **Group 2** = recordings from endocardial areas with perfusion deficits due to coronary artery disease *without* collateral circulation; **Group 3** = recordings from endocardial surfaces with perfusion deficits due to coronary artery disease *with* collateral circulation.

No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 (Minutes)
1	244	236	240	248	244	244	248	244	240	240	244	248	240	240	244	244
2	284	284	280	276	280	280	280	284	284	284	288	284	284	280	280	280
3	252	248	248	248	252	252	252	252	252	252	248	252	252	248	252	248
4	264	264	264	264	268	264	264	264	264	256	260	264	260	260	252	252
5	236	236	236	236	240	230	232	232	232	240	240	236	244	240	240	236
6	268	268	268	268	268	268	264	268	268	268	264	268	272	268	268	268
7	284	284	284	288	292	296	296	296				288	288	292	292	292
8	264	264	264	264	268	268	268	268	268	268	264	268	264	268	268	268
9	324	324	320	320	328	324	328	328	328	324	328	332	332	328	332	332
10	272	272	272	272	272	276	272	272	272	272	276	276	276	276	280	272
11	272	272	276	276	276	276	276	276	276	276	276	272	272	272	276	272
12	212	208	212	208												
13	212	212	208	208	208	208	208	208	212	212	212	216	216	216		212
14	280	284	280	280	280	280	284	280	284	280	280		280	284	280	280
15	264	264	264	264	260	264	264	264	284	268	268	272	268	264	264	268
16	254	260	260	256	250	256	256	256	256	256	256	252	248	248	248	248
17	240	240	240	232	240	240	240	232	232	236	236	240	236	236		232
18	224	228	224	224	228	224	224	224	224	224	224	224	228	224	224	228
19	252	248	248	248	248	248	244	244	244							
20	264	264	260	264	264	268	264	268	268	268	268	268	268	268	272	268
21	288	288	288	288	288		288	292	292	288						
22	252	252	252	252		252			252							
23	236	236	236	232	232	232	236	236	232	232	288	228	228	232	232	232
24	264	264	264	264	264		264	264	264	264	264	264	264	264	264	264
25	216	216	216	212	212	212	216	216	220	216	220	220	224	220	216	220
26	236	236	240	240	232	232	232	232	232	232	232	232	232	236	236	236
27	272	268	272	272	272	268	222	272	272	272	268	268	272		272	268
28	228	232	232	232	228	232	232	228	232	232	232	232	232	228	232	228
29	200	200	200	200	204	200	204	196	200	200	200	200	200	200	196	200
30	236	236	236	236	232	236	236	236	236	236	232	236	236	236	236	236

Table 3.4.2, A: Monophasic action potential duration at 70% repolarisation in msecs for the 15 minutes of the study protocol for Group 1 recordings (normally perfused areas).

No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 (Minutes)
1	252	252	248	244	244	240	240	240	240	244	244	244	248	248	248	248
2	284	284	284	272	272	256	248	248	252	260	264	264	268	264	268	268
3	288	284	284	276	280	276	276	276	272	276	280	280	284	280	276	280
4	240	240	240	236	236	232	224	224	224	224	228	228	224	232	236	240
5	244	244	244	240	244	240	240	240	240	240	240	244	244	244	244	244
6	264	264	260	260	264	264	260	264	260	260	264	264	264	264	264	264
7	244	244	240	232	228	232	228	232	228	224	228	228	236	240	244	244
8	260	260	260	260	256	256	252	252	248	244	248	252	252	252	256	256
9	268	268	268	268	264	256	256	260	256	260	260	264	268	268	264	268
10	280	280	276	276	272	268	272	268	272	272	272	272	272	276	276	276

Table 3.4.2, B: Monophasic action potential duration at 70% repolarisation in msecs for the 15 minutes of the study protocol for Group 2 recordings (Abnormally perfused but non-collateralised areas).

No.	0	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15 (Minutes)
1	232	232	232	224	220	220	216	216	216	220	228	228	232	232	232	236
2	236	236	236	232	220	220	216	216	212	220	216	224	228	228	228	232
3	252	252	252	252	252	236	224	224	220	220	228	232	248	248	252	248
4	256	256	256	256	248	248	244	232	224	228	244	240	240	240	248	248
5	260	260	256	244	244	240	236	228	228	236	244	252	252	260	260	260
6	228	228	228	228	224	216	216	200	200	208	216	208	216	224	224	224
7	224	224	224	220	220	216	212	208	208	212	216	220	224	224	224	224
8	244	244	248	244	236	224	228	224	220	224	228	236	244	244	248	244
9	248	248	244	244	240	232	232	232	232	236	236	232	236	236	236	236
10	264	264	268	260	260	252	248	248	244	244	252	256	256	260	268	268

Table 3.4.2, C: Monophasic action potential duration at 70% repolarisation in msecs for Group 3 (Abnormally perfused but collateralised areas)

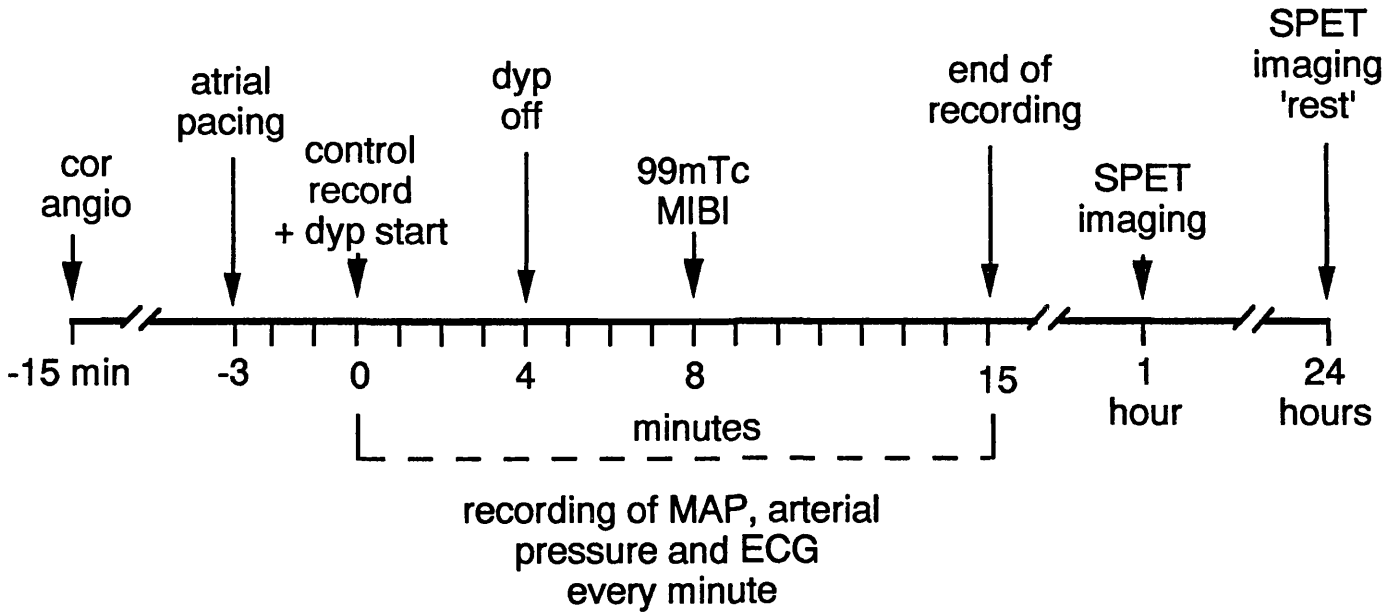

Figure 3.4.1: Protocol for the study. ***cor angio,*** *coronary angiography;* ***dyp,*** *dipyridamole;* ***MAP,*** *monophasic action potentials.*

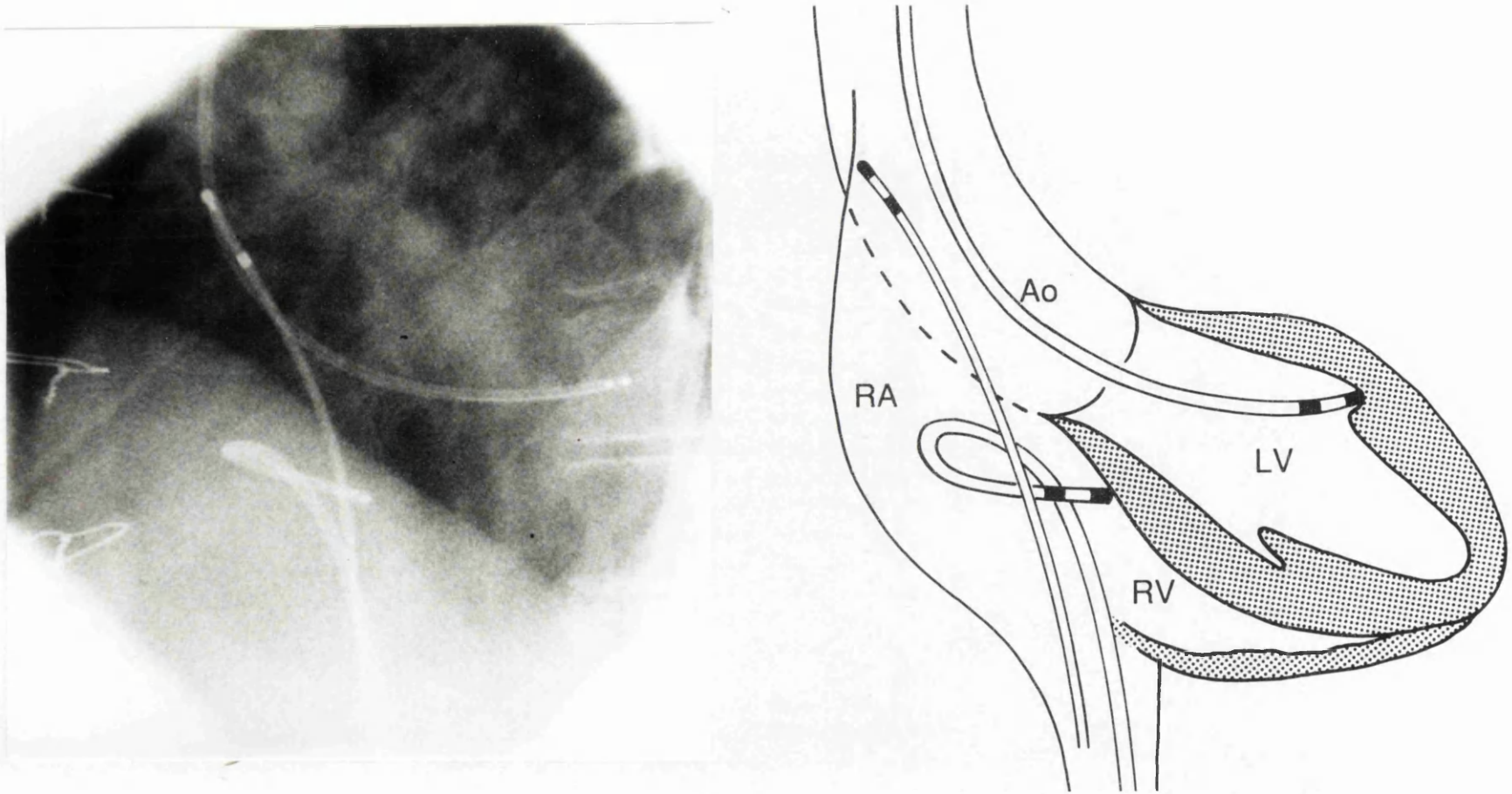

Figure 3.4.2: Typical catheter placements shown in the left anterior oblique view. In this study, MAP recording catheters were placed in the right and left ventricles. The right ventricular catheter has been manipulated on to the interventricular septum and in the left ventricle, the catheter has been left in contact with the endocardium of the potero-lateral wall. A temporary pacing catheter is shown in the right atrium.

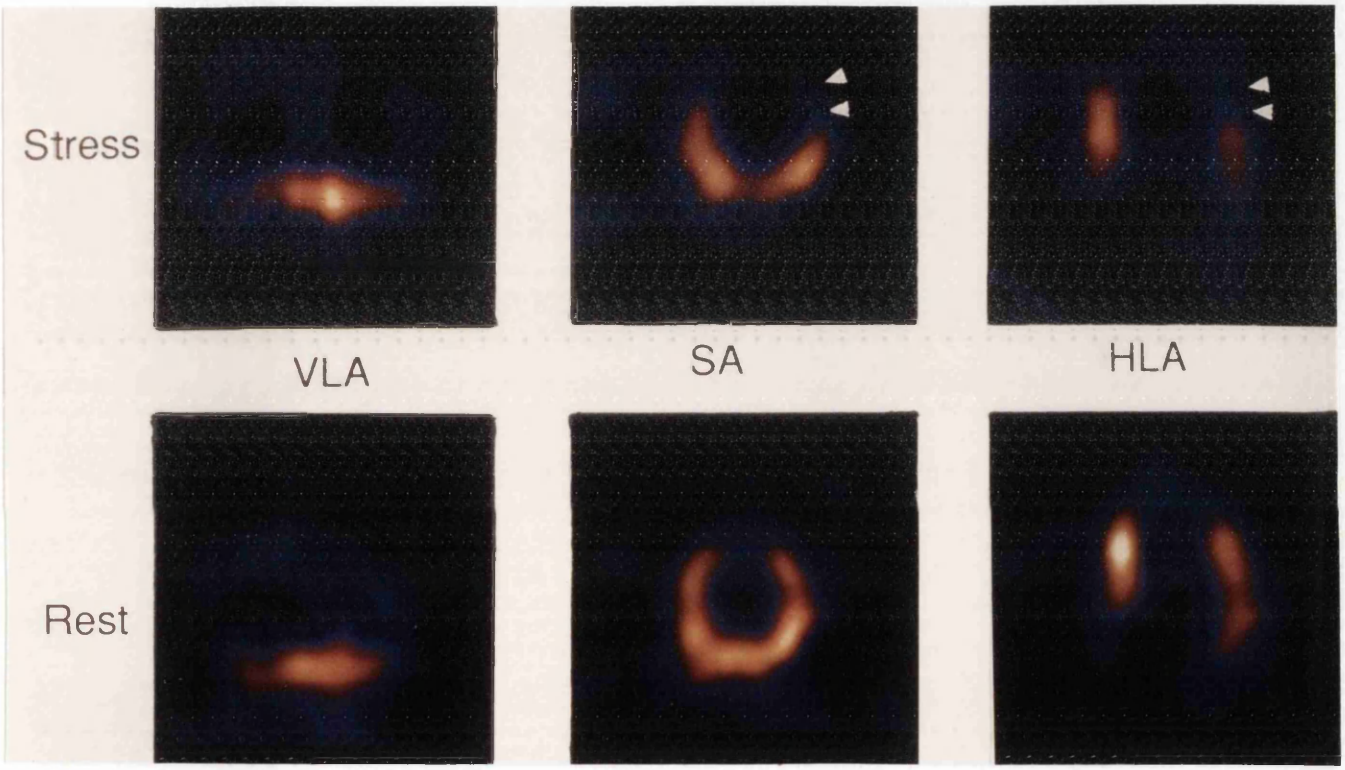

Figure 3.4.3, A: *SPET images of patient 19 who had occlusion of the native vessels and grafts to the territory of the left coronary circulation. This territory was retrogradely collateralised from the right coronary artery and graft. A fixed defect is seen in the anterior wall of the left ventricle. The lateral left ventricular wall develops marked hypoperfusion with dipyridamole which reperfuses at 'rest'.*

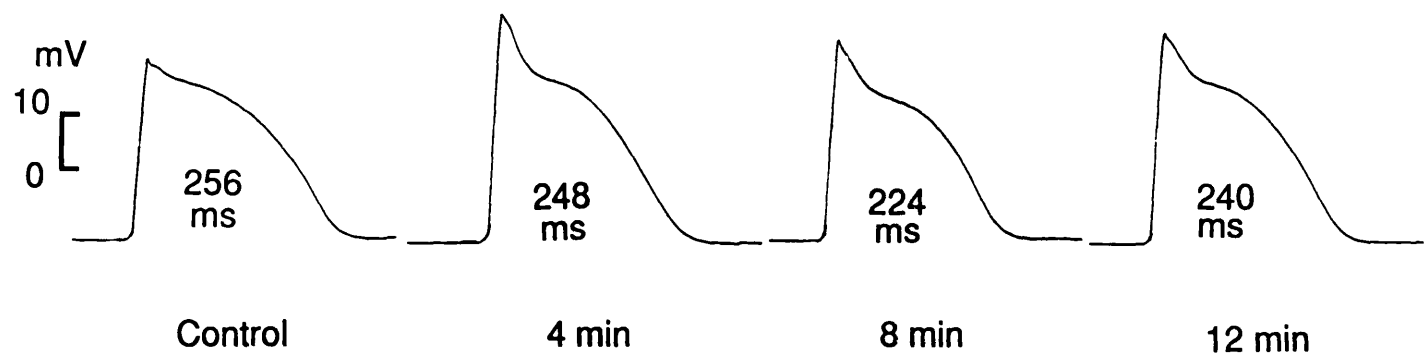

Figure 3.4.3, B: Serial steady state MAP signals were recorded from the lateral left ventricular wall. Dipyridamole infusion produced marked shortening of the action potential duration which becomes apparent at 4 minutes from the start of dipyridamole infusion. Peak changes occur at 8 minutes into the protocol when the action potential duration shortens by 32 msecs compared to the control value. Regression of these changes is seen at 12 minutes, in this case enhanced by the administration of 125 mg of aminophylline given at 10 minutes into the protocol. MAP duration in msecs measured at 70% repolarisation is shown within each action potential signal. ***MAP,*** *Monophasic action potential.*

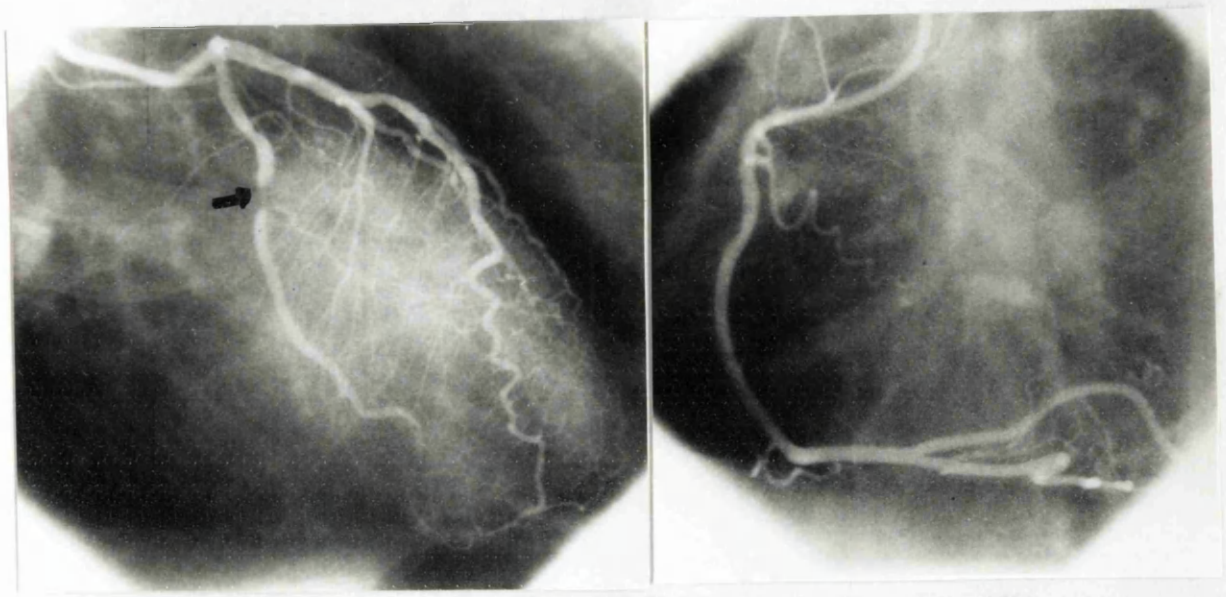

Figure 3.4.4, A: Coronary angiogram of patient 11. The left coronary artery in the right anterior oblique view (left panel) shows a tight stenosis (arrow) in the proximal course of the left circumflex artery. There is no obvious collateral supply from the left anterior descending or right coronary artery (right panel) to the area subtended by the stenosed vessel.

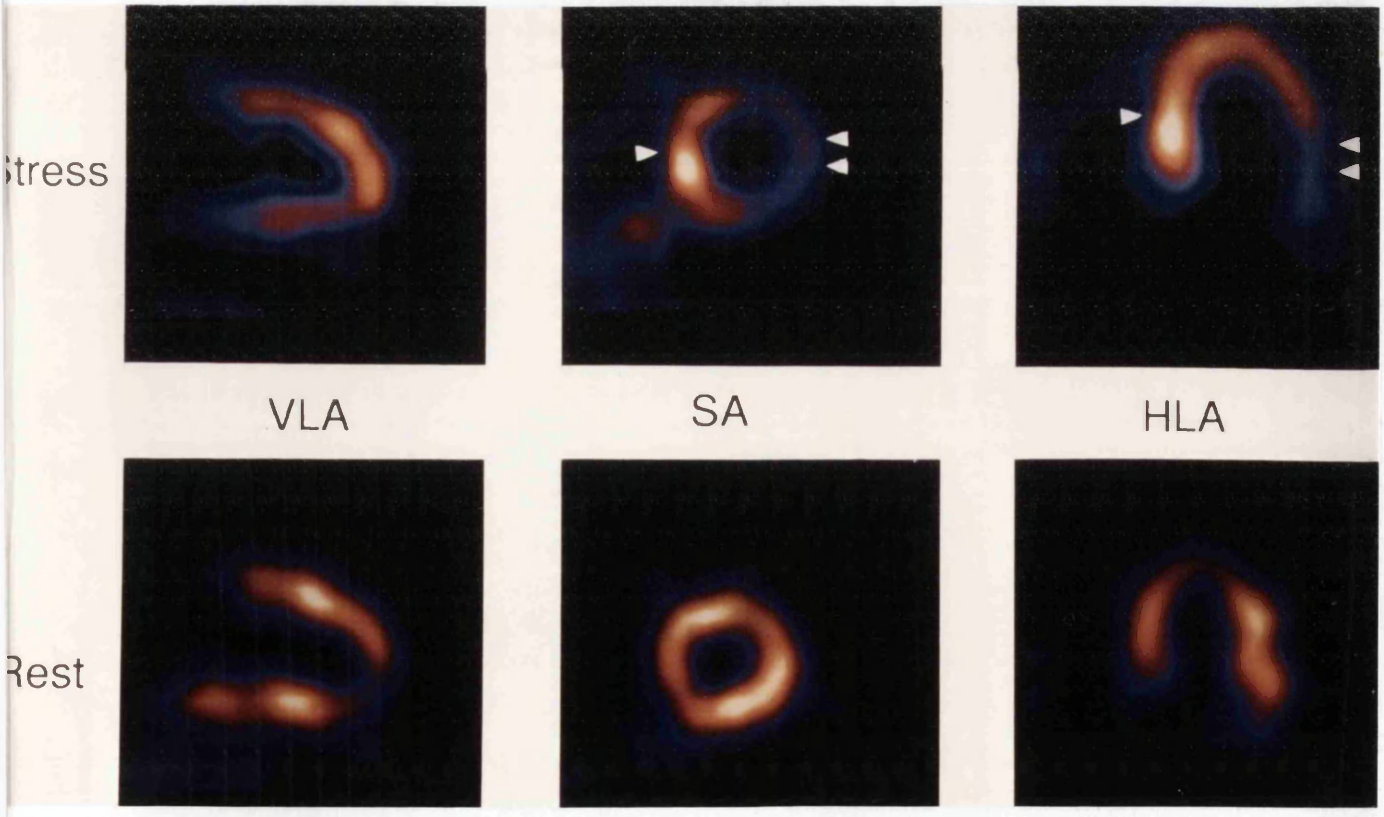

Figure 3.4.4, B: *Tc-99m-MIBI SPET images show marked reversible perfusion abnormality in the area of supply of the left circumflex artery*

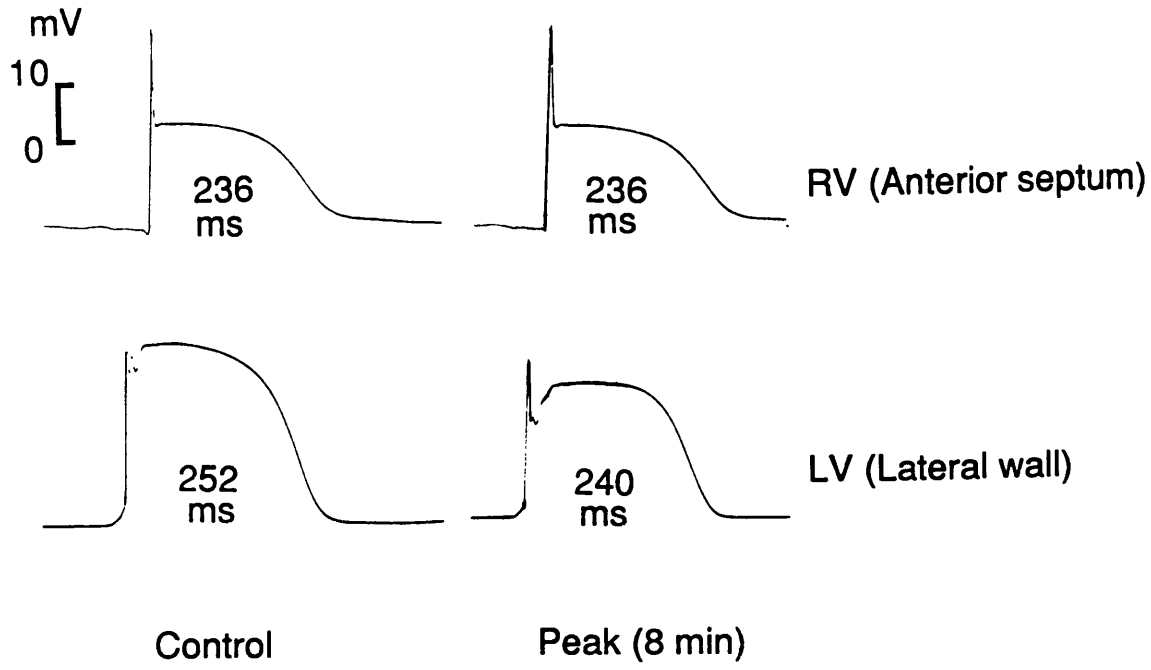

Figure 3.4.4, C: *MAP signals were recorded simultaneously from the right ventricular mid septum and left ventricular lateral wall in this patient (approximate recording sites represented on the scintigram in figure 3.4.4, B are indicated - single arrow for right ventricular septum and double arrows for left ventricular lateral wall). Steady state MAP duration measured at 70% repolarisation (shown within signals) at 8 minutes into the protocol remain unchanged from control value for the recording from the normally perfused septum whereas that for the non collateralised but abnormally perfused left ventricular lateral wall shortens by 12 msecs.* ***VLA,*** *vertical long axis;* ***SA,*** *short axis;* ***HLA,*** *horizontal long axis;* ***MAP,*** *monophasic action potential.*

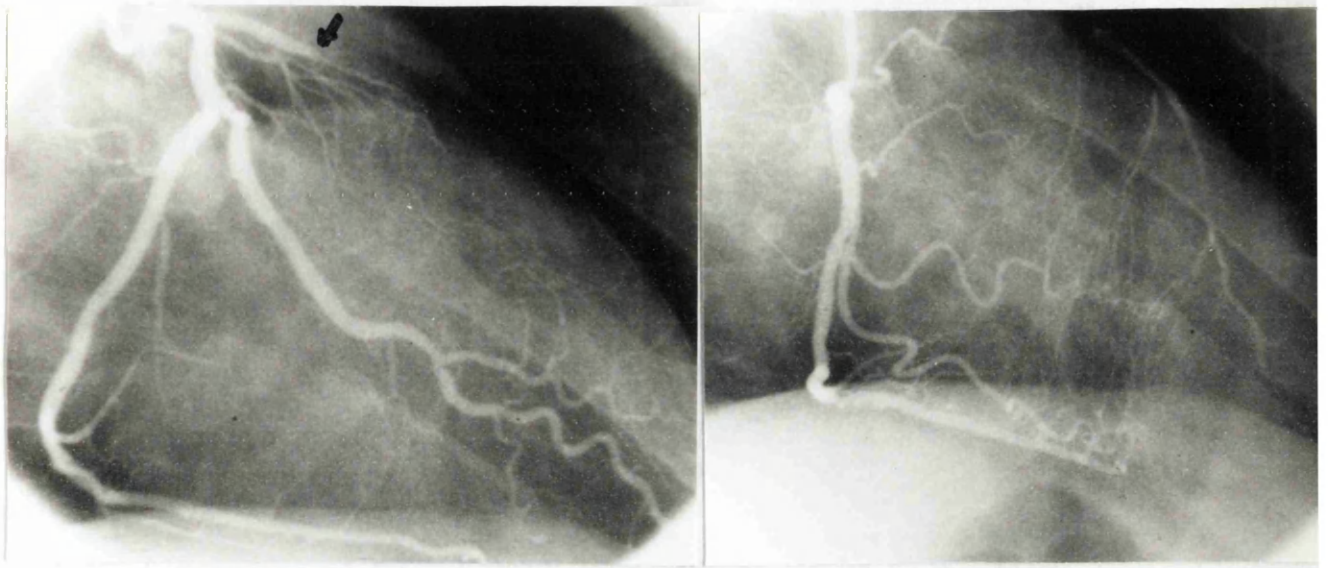

Figure 3.4.5, A: Right anterior oblique views of coronary angiograms in patient 22. The left anterior descending artery (left panel) is occluded at its origin (arrow). The distal vessel is filled retrogradely by extensive collaterals from the normal right coronary artery (right panel).

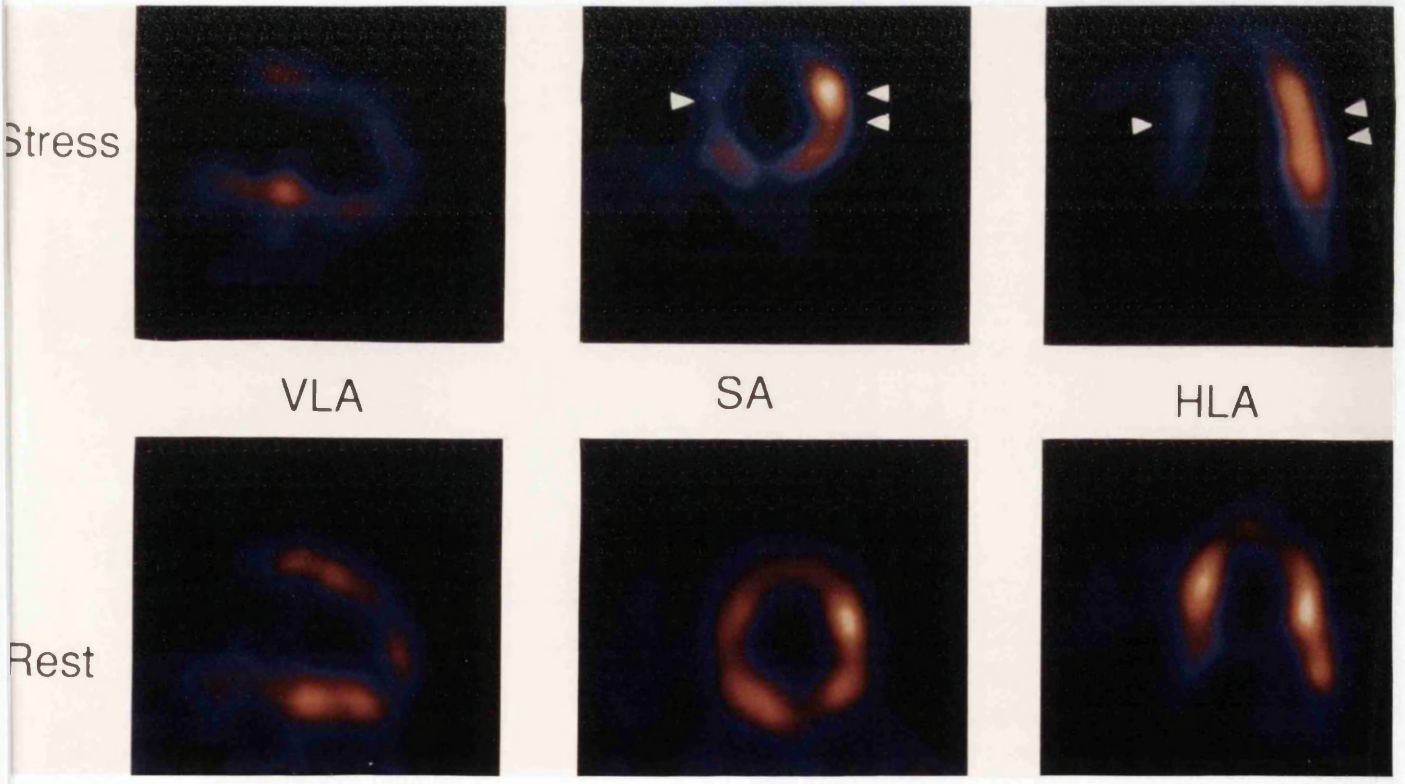

Figure 3.4.5, B: Tc-99m-MIBI SPET images demonstrate anterior, apical and septal hypoperfusion with dipyridamole stress with partial reperfusion of these areas during 'rest'.

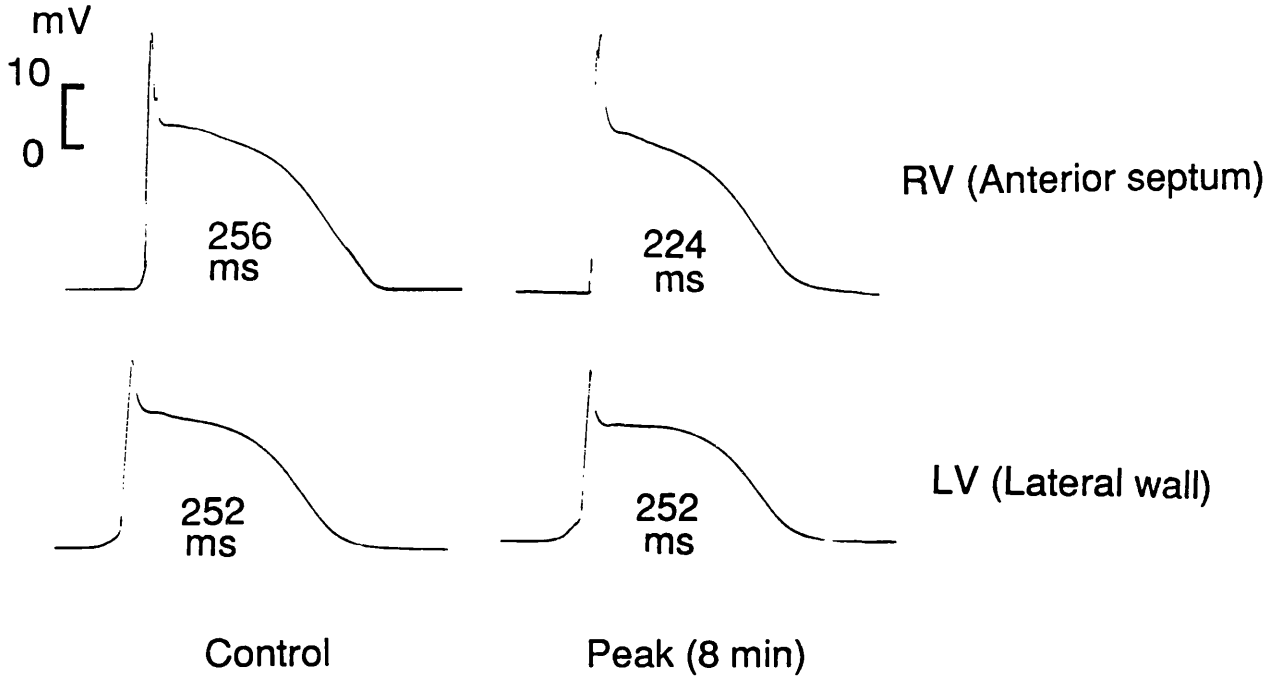

Figure 3.4.5, C: MAPs were recorded from the right ventricular septum and the left ventricular lateral wall in this patient (approximate recording sites indicated by arrows on the scintigram in figure 3.4.6, B - single arrow for right ventricular septum and double arrows for left ventricular lateral wall) . Steady state MAP duration shortens by 32 msecs between the control and 8 minute recordings for the right ventricular septum (abnormally perfused area with collateralisation) whereas recordings from the normally perfused left ventricular lateral wall show no alteration in the duration from control value (MAP duration at 70% repolarisation is shown within signals). VLA, vertical long axis; SA, short axis; HLA, horizontal long axis; MAP, monophasic action potential.

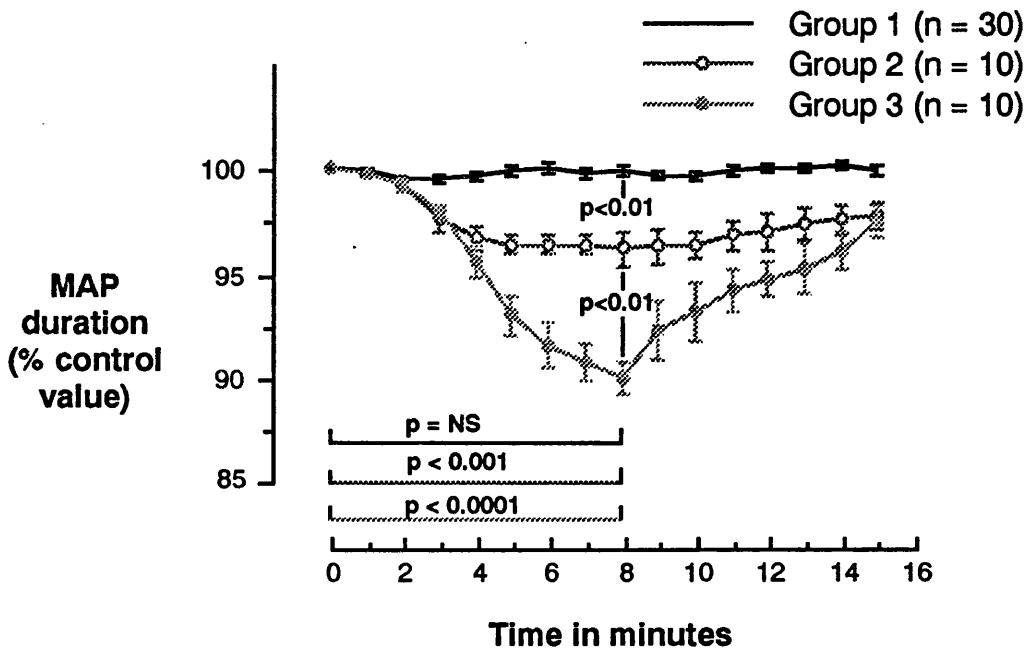

Figure 3.4.6: Mean changes in MAP duration for the three groups is shown as a percentage of the control value. During dipyridamole infusion, the group 1 (normal zone) recordings show no change. Group 2 (abnormal zone without collateralisation) recordings shorten significantly when compared to group 1. The changes are maximal for group 3 (collateralised abnormal zone) with MAP shortening significantly greater than for groups 1 and 2. Peak changes are apparent at 8 minutes after dipyridamole administration is commenced. MAP, monophasic action potential.

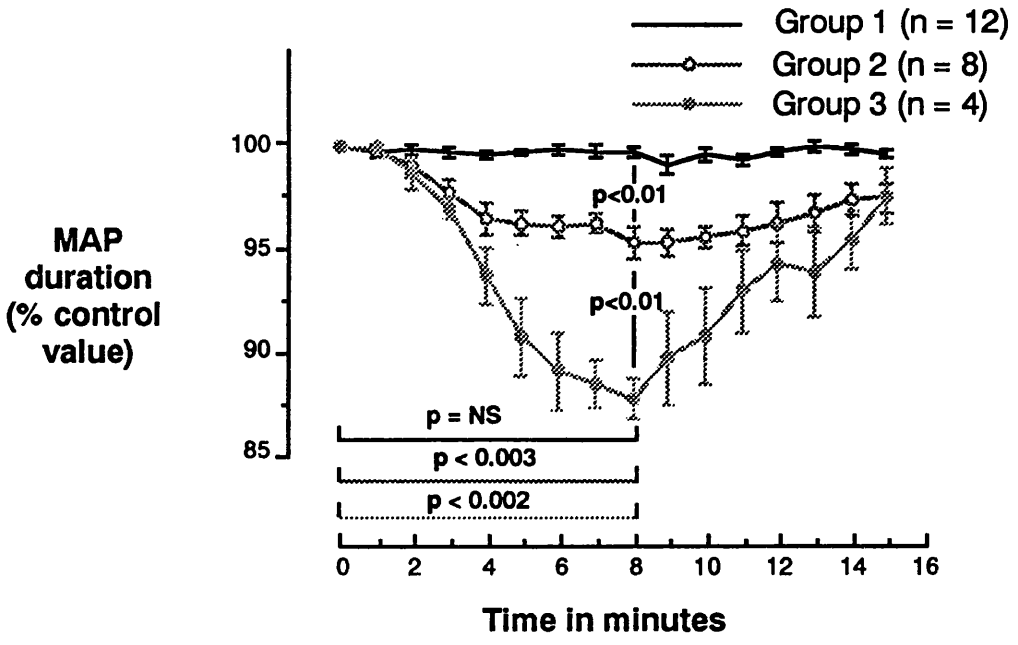

Figure 3.4.7: Pooled data for 12 patients in whom simultaneous biventricular recordings were obtained wherein one of the recording sites was normally perfused and the other abnormally perfused. The MAP duration expressed as percentage of the control value in the normal zone recordings (group 1) remain unchanged throughout the entire 15 minutes of recording. Group 2 recordings show a significant shortening compared to group 1. The extent of MAP shortening is greatest in the group 3 recordings. MAP, monophasic action potential.

CHAPTER 3.5.

DIRECT EFFECT OF DOBUTAMINE ON ACTION POTENTIAL DURATION IN ISCHAEMIC COMPARED TO NORMAL AREAS IN THE HUMAN VENTRICLE

3.5.1. ABSTRACT

Background: The relative benefits of beta blockers over other conventional antiarrhythmic drugs indirectly infer the importance of beta agonism as an arrhythmogenic mechanism in myocardial ischemia. However, studies of effects of beta receptor stimulation on myocardial repolarization in the ischaemic human myocardium has not been previously undertaken.

Objectives: The study was designed to examine the differential effect of beta adrenergic receptor stimulation on ventricular action potential duration and hence dispersion of repolarization in potentially ischaemic versus non-ischaemic human ventricular myocardium.

Methods: Steady state recordings of monophasic action potentials were obtained simultaneously from the right and left ventricular endocardium in 14 patients (28 recording sites) during infusion of dobutamine at incremental doses (dose range 5 to 15 μg/kg/min; low dose = 5μg/kg/min, high dose = 10 to 15 μg/kg/min) during atrial pacing. Perfusion at the action potential recording site was assessed by incorporating myocardial perfusion scintigraphy with injection of technetium 99m hexakis-2-methoxy-2methylpropyl-isonitrile during the monophasic action potential recording procedure at peak doses of dobutamine. Action potential duration during dobutamine was compared to that during atrial pacing to identical pacing rates in the absence of dobutamine.

Results: In 21 recordings from the normally perfused zones, dobutamine infusion produced a variable effect on action potential duration over and above that produced by atrial pacing to identical heart rates either lengthening or shortening the action potential duration. The mean (±sem) value for the additional effect of dobutamine with low doses was 0.9 ± 2.5 ms and with high doses was -4 ± 2.6 ms (p=NS). In 7 recordings from ischaemic zones, the low doses of dobutamine had a similar effect altering the action potential duration by -3.4 ± 6.5 ms (p=NS compared to normal zone values). However, the higher dose of dobutamine invariably shortened the action potential duration producing a mean change of -22.9 ±2.9 ms. ($p< 0.05$ compared to low dose in ischaemic areas and $p<0.01$ compared to the normal zone recordings).

Conclusions: These results suggest a different effect of beta adrenergic stimulation in potentially ischaemic compared to non-ischaemic human ventricular myocardium. Abnormal dispersion of repolarization thus created may well be important in beta receptor mediated arrhythmogenesis during myocardial ischaemia. Beta receptor blockade would be expected to reduce such dispersion of repolarisation between adjacent areas of the myocardium and reduce arrhythmogenic potential in the ischaemic setting.

3.5.2. INTRODUCTION

The consistent ability of beta adrenergic blockers to prevent sudden cardiac death and reduce overall mortality in ischaemic heart disease imply beta agonism as an important arrhythmogenic mechanism in the context of myocardial ischaemia. (Yusuf et al, 1985) However, studies of the effect of

beta stimulation on arrhythmogenesis have been largely limited to isolated tissue and animal studies. (Schwartz and Priori, 1990) The importance of evaluating arrhythmogenic and antiarrhythmic mechanisms in the normal and ischaemic human heart, as complimentary to laboratory studies, has recently been emphasized. (Task Force of the Working Group on Arrhythmias for the ESC, 1991) One important arrhythmogenic mechanism is increased dispersion of repolarisation. (Han and Moe, 1964; Kuo et al, 1983; Surawicz, 1989) I have therefore studied the effect of beta receptor stimulation on action potential duration in the ischaemic and non-ischaemic human myocardium in order to assess any differential response. I used dobutamine, a widely used therapeutic and diagnostic beta-1 receptor agonist and recorded monophasic action potentials simultaneously from the right and left ventricular endocardium in patients with coronary artery disease. In order to disassociate the rate related effects from the direct membrane effects, recordings during steady state atrial pacing at a rate just above that induced by dobutamine were compared to those obtained during atrial pacing alone to identical heart rates in the absence of dobutamine. The results show a significant difference in the electrophysiological response to dobutamine infusion in ischaemic areas compared to non-ischaemic areas. Such differences maybe relevant to beta receptor mediated arrhythmogenic mechanisms.

3.5.3. PATIENTS AND METHODS

Summary

Recordings of the monophasic action potentials were obtained simultaneously from single sites on the right ventricular septum and left ventricular postero-lateral wall during steady state atrial pacing at heart rates expected to be achieved during dobutamine infusion. Dobutamine was infused in incremental doses and steady state recordings of action potentials

obtained at paced rates just above that induced by dobutamine. At peak dose of dobutamine, technetium 99m hexakis-2-methoxy-2-methylpropyl-isonitrile (Tc-99m-MIBI) was administered for subsequent scintigraphy and identification of ischemic areas.

Patients

Fourteen patients (6 female and 8 male patients with an age range of 40 to 73 years (median 53)) undergoing routine cardiac catheterisation for investigation of chest pain were selected at random for the study. All except three patients had significant coronary artery disease; single vessel disease in 8 patients and two vessel disease in 3 patients. Patients with unstable angina, significant left main stem stenosis, atrial fibrillation, significant conduction abnormalities and inadequately controlled hypertension were not included in the study. None of the patients were receiving antiarrhythmic drugs conventionally considered to influence ventricular repolarization. Beta blockers were discontinued 48 hours prior to the study. Patients also received 10 mg Diazepam prior to the study. Written informed consent was obtained from each patient and the study protocol was approved by the hospital ethical committee.

Electrophysiological measurements

Monophasic action potentials were recorded using the pressure contact technique with purpose built catheters (Cordis (UK) Ltd; size 7 French) on which were mounted silver/silver chloride electrodes. Details of catheter design, and signal acquisition have been discussed in section 3.3.3.

Myocardial perfusion scintigraphy

Technetium 99m hexakis-2-methoxy-2-methylpropyl-isonitrile (Tc-99m-MIBI) was used for myocardial perfusion scintigraphy with single photon

emission computerized tomography (SPET) employing a two day, two dose protocol. Images were acquired with a single detector rotating gamma camera/computer system (IGE 400 AC - Starcam) equipped with a low-energy, high-resolution collimator. Sixty four 30 second images were acquired over a 360° circular arc of rotation. Details of acquisition and reconstruction parameters have been described in section 3.3.3. Reconstructed tomographic slices were reoriented in the short axis, horizontal long and vertical long axes for display and visual analysis.

Procedure

Patients were fasted for 4 to 6 hours. Routine left ventricular and coronary angiography was performed via the femoral route using the Judkin's technique and employing a non-ionic angiographic dye (Omnipaque 350, Nycomed (UK) Ltd.). Limb leads 1 and 2 and precordial lead V5 were used for continuous electrocardiographic monitoring.

Catheter placements:

A monophasic action potential recording catheter was introduced via short femoral sheaths into each of the right and left ventricle. Stable recording positions were obtained in the postero-lateral wall of the left ventricle and anterior aspect of the right ventricular side of the interventricular septum. Gentle apposition of the tip of the endocardial surface resulted in monophasic action potential signals of 20 to 40 mV in amplitude. A 6F temporary pacing electrode (Cordis UK) was placed in the right atrial appendage or right atrial-superior vena caval junction for atrial pacing.

Control MAP recordings during pacing alone:

Baseline recordings of the MAP were obtained at paced heart rates commencing at the nearest multiple of 10 beats above the patient's resting

heart to a maximum of 120 ppm or that permitted without the development of significant angina or atrio-ventricular block. Pacing rates were incremented by 10 beats at a time and each pacing rate maintained for 2 minutes to obtain steady state action potential recordings at each heart rate.

Dobutamine infusion:

Atrial pacing was discontinued and infusion of dobutamine commenced at a rate of 5μg/kg/min and incremented by 5 μg/kg/min at 5 minute intervals to a maximum dose of 20μg/kg/min or to the point of angina. After 3 minutes of each incremental dose of dobutamine, atrial pacing was established for 2 minutes at a rate to the nearest multiple of 10 beats above that induced by dobutamine and monophasic action potentials recorded.

Myocardial perfusion scintigraphy:

At peak dobutamine dose, 350 to 400 MBq of Tc-99^{m}-MIBI was administered intravenously. The patient was returned to the ward and encouraged to eat a light fatty meal to facilitate hepatic clearance of the isotope. Myocardial perfusion imaging with single photon emission computerized tomography (SPET) was carried out between one and two hours after injection of the tracer. Rest imaging was performed 24 hours later with the patient fasting for 4 hours and after a second dose of 350 to 400 MBq of Tc-99^{m}-MIBI.

Analysis of data

Significant coronary artery disease was defined as greater that 50% stenosis in a major epicardial vessel. Coronary angiographic data was interpreted by visual inspection and degree of stenosis classed as mild (representing <50% stenosis), moderate (50 to 70% stenosis) or severe (>70% stenosis).

The duration of the monophasic action potentials was measured at both 70% and 90% repolarization. Measurements at 90% repolarization was made by drawing a tangent to the fastest phase of the repolarization curve. (Taggart et al, 1992) Methodology employed for measurements of the monophaisc action potential duration is described in section 3.3.3. Both 70% and 90% values registered similar changes. Values at 70% repolarization have been used for analysis.

Myocardial perfusion SPET images were interpreted by one observer who was blinded to the angiographic and monophasic action potential data. In case of equivocal results, a consensus opinion was accepted. Scintigrams were read as normal if they were without defect in the initial set or abnormal if there were reversible or fixed defects. Myocardial segments with diminished Tc-99m-MIBI uptake without reversal of defect on the rest study were considered 'fixed defects'. Segments with partial reversal as evidenced by incomplete filling of the initial perfusion defect, were considered partially ischaemic and those segments with complete reversal were considered ischaemic. Criteria for relating perfusion defects on the SPET images to coronary anatomical lesions were as described in section 3.3.3.

The position of the monophasic action potential catheter in the ventricle was documented at the time of cardiac catheterisation by biplane cinematography and subsequently related to the perfusion abnormalities on the perfusion scan. Recordings from sites in the left ventricle or right ventricular side of the interventricular septum that corresponded to areas on the scintigram showing reversible perfusion defects were considered ischaemic zone recordings and those from areas with normal perfusion pattern as normal zone recordings.

Although the original study protocol intended a maximum dose of 20μg/kg/min, the maximum dose had to be curtailed to 15 μg/kg/min due to patient discomfort. The doses of dobutamine used in this study therefore ranged (in increments of 5 μg/kg/min) from 5 to 15 μg/kg/min. For the purposes of differentiating low dose effects from high dose effects, low dose was defined as 5 μg/kg/min. Doses of 10 μg/kg/min and above were considered high dose.

Values are reported as mean values ± SEM. Statistical analyses of comparisons between groups were made using analysis of variance and paired comparison of means (ANOVA). A p value of <0.05 was considered statistically significant.

3.5.4. RESULTS

Simultaneous recordings of monophasic action potentials from the right and left ventricle were obtained in 14 patients (table 3.5.1). Based on the perfusion scintigraphic appearances for the recording sites, 21 of these recordings were from non-ischaemic areas and 7 were from ischaemic areas. Recordings of action potentials during dobutamine were matched to control recordings with identical atrial paced rates obtained prior to the dobutamine infusion. Incremental pacing showed the expected shortening of action potential duration as heart rate increased. Dobutamine produced an additional effect on the action potential duration. Figure 3.5.1 shows the changes in the action potential duration over and above those induced by pacing. Responses for non-ischaemic and ischaemic zones were as follows:

Non-ischaemic zone

In recordings from non-ischaemic territory, dobutamine produced either a lengthening or a shortening of the action potential duration over and above

the effect of pacing (lengthening in 9, shortening in 7 and no change in 5). The mean change in the action potential duration was 0.9 ± 2.5 ms. A similar response was observed with the higher dose dobutamine infusion in response to which 6 recordings showed lengthening, 12 showed a shortening and 3 showed no change, the mean change being -4 ± 2.6 ms (p = NS compared to low dose).

Ischaemic zone

In recordings during the low dose dobutamine infusion, action potential lengthening was observed in 3 sites and shortening in 4, the mean change registered being -3.4 ± 6.5 ms (p = NS compared to normal zone values). The high dose dobutamine resulted in shortening of action potential duration in all 7 recordings. The changes observed with the higher dose of dobutamine which averaged -22.9 ±2.9 ms were significantly different from those during low dose in the ischaemic region ($p<0.05$) and the high dose in the normal zone ($p<0.01$).

Figure 3.5.2 shows monophasic action potential signals recorded in patient 1. The patient had a tight stenosis in the proximal left circumflex artery. The right ventricular recording site was a non-ischaemic zone and the left ventricular site was a zone which showed reversible perfusion defects in response to dobutamine on the Tc-99m-MIBI perfusion images. Increasing pacing rate in the absence of dobutamine from 70 to 100 bpm produced the expected shortening in both right (figure 3.5.2, A) and left ventricular (figure 3.5.2, B) recordings. At the right ventricular site, addition of the dobutamine infusion did not significantly influence the action potential duration for either the low dose (5 μg/kg/min; paced rate 70 bpm) or for the high dose (15 μg/kg/min; paced rate 100 bpm). In the left ventricular site, dobutamine infusion at the low dose (before the onset of ischaemia) resulted

in a small increase in action potential duration. However, at higher doses (in the presence of ischaemia) a substantial shortening of action potential duration in excess of that produced by identical pacing rate is seen.

Patient response to pacing and dobutamine

During the pacing alone section of the protocol, none of the patients developed angina or ST segment changes on the monitored surface ECG leads. During the dobutamine infusion, 10 patients developed chest pain which was associated with ST segment changes in 5 patients (ST depression in 4 and elevation in 1). In three patients, troublesome palpitation occurred necessitating cessation of dobutamine infusion (see table 3.5.1).

3.5.5. DISCUSSION

Main findings of the present study

A number of studies have examined the effects of beta stimulation on the various phases of the cardiac action potential. The majority of these studies have confined observations to isolated tissue and animal studies, (Autenrieth et al, 1975 b; Mataba et al, 1979; Kass and Wiegers, 1982; Taggart et al, 1988) In recent years, it has become clear that experimental findings in the *in vitro* setting cannot be directly extrapolated to the human heart where a number of neural and autonomic variables interact.

The effects of beta adrenergic stimulation on the human cardiac action potential have not been fully evaluated. More importantly, the differential effect between ischaemic and normal human ventricular myocardium has not been previously studied. Accordingly, I studied the effect of dobutamine, a widely used beta-1 receptor agonist, on human ventricular action potential duration. Recordings from normal and potentially ischaemic areas of myocardium were compared. Presence or absence of myocardial ischaemia at the recording site was identified by perfusion scintigraphy using Tc-99m-MIBI injected at the time of the monophasic action potential recording procedure. The main findings were that with higher doses of dobutamine, recordings from the ischaemic area registered consistent shortening of action potential duration in excess of that induced by pacing to an identical heart rate in the absence of dobutamine. This finding contrasted with recordings from the normal myocardium in which a

variable response to dobutamine was observed, either lengthening or shortening of action potential duration.

Relation to previous studies and mechanisms

The variable response of action potential duration in the normal zone (and during low dose dobutamine infusion in the ischaemic zone before the onset of ischaemia) is consistent with the diversity of response to beta stimulation in the literature. Studies examining the influence of beta receptor stimulation on the ventricular action potential duration have demonstrated lengthening, (Mitchell et al, 1986; Callewaert et al, 1984) shortening (Autenrieth et al, 1975 b; Giotti et al, 1973) or a biphasic effect. (Kass and Wiegers, 1982) These differences have been attributed to varying drug concentrations, relative alpha and beta components and differences in receptor density. (Janse and Wit, 1989) Opthof et al have recently demonstrated wide variations in individual hearts in dogs in the response of refractoriness (and by inference, action potential duration) to sympathetic stimulation. (Opthof et al, 1991) The explanation for this variable response is not clear. Although dobutamine is predominantly a beta 1 receptor agonist, it is known to have alpha receptor effects. Alpha receptor stimulation is known to prolong action potential duration by its action predominantly on calcium currents at the plateau level and to a lesser extent effects on potassium conductance and the transient outward current. (Fedida et al, 1989) Beta 1 receptor stimulation on the other hand, is generally recognized as shortening the action potential duration by accelerating the time dependant currents. However, numerous additional membrane effects of beta stimulation have been suggested in recent years and the net effect on the action potential duration will depend on which of, and to what extent the various currents are altered. (Gadsby, 1990) Hence, a variable

effect in the non-ischaemic myocardium as observed in the present study is therefore, not surprising.

The pronounced and consistent shortening of action potential duration in the potentially ischaemic myocardium in response to the higher doses of dobutamine is probably in the main due to induction of ischaemia and enhancement of action potential duration shortening which occurs as a direct effect of ischaemia. (Franz et al, 1984; Taggart et al, 1986; John et al, 1991) This would be expected in view of the known effect of dobutamine to increase myocardial oxygen demand. Furthermore, the presence of ischaemia at the time of the recordings and at the site was documented by the Tc-99m-MIBI perfusion scintigraphy. However, alternative or additional mechanisms may well be important in the consistent shortening of action potential duration during high dose dobutamine infusion in the ischaemic zone. They may include a direct effect of beta agonists on membrane currents and interactive effects of beta stimulation on rate adaptive mechanisms of action potential duration. (Lindemann and Watanabe, 1990; Taggart et al, 1990a)

Dispersion of action potential duration and relevance to arrhythmogenesis

The invariable shortening of action potential duration in an ischaemic area compared to the variable effect in the normal area would by inference, alter dispersion of action potential duration in the ischaemic and non-ischaemic sites. Dispersion of repolarization and consequent inhomogeneity of recovery of excitability is important in the sustenance of re-entry arrhythmias. In the normal myocardium the time course of refractoriness roughly parallels the time course of repolarization. (Lee et al, 1992) In the

ischaemic myocardium however, refractoriness outlasts repolarization (post repolarization refractoriness). (Downar et al, 1977; Janse and Wit, 1989) It is not possible therefore to equate our observations on action potential duration precisely with dispersion of refractoriness between the ischaemic and non-ischaemic regions although, in the initial phases of ischaemia, changes in the two parameters go hand in hand for the most part. (Downar et al, 1977) It is also not possible to be exact as to the degree of dispersion required to initiate re-entrant arrhythmia in view of the wide disparity in substrates, nature and size of re-entry circuits. Experimental results have indicated values ranging from about 80 ms to 10 ms. (Surawicz, 1989; Allessie et al, 1977; Gough et al, 1985) An alteration of a few milliseconds maybe all that is necessary for initiation of re-entrant tachyarrhythmias given the appropriate wavelength. The degree of alteration in dispersion that we have observed in this study could therefore be of sufficient magnitude to influence re-entrant mechanisms.

Implications

Beta blockers have emerged the only antiarrhythmic drug that can effectively reduce sudden cardiac death in coronary artery disease. (Norwegian Multicentre Study Group, 1981; Rydén et al, 1983; ß-blocker Heart Attack Trial Research Group, 1983; Cardiac Arrhythmia Suppression (CAST) Investigators, 1989) However, the mechanism by which beta blockers exert their antiarrhythmic effect in ischaemia has been variously described and remain unclear. Catecholamines have been shown to promote or induce arrhythmias in normal cardiac tissue by enhanced automaticity, triggered activity or by re-entry mechanisms. (Janse and Wit, 1989; Priori et al, 1988; Zipes, 1991) The present study through demonstrating differential effects of beta stimulation on the action potential duration in the normal and potentially ischaemic human heart would

suggest a salutary role of beta blockade by reducing dispersion of repolarization in adjacent areas of myocardium.

Limitations of the present study

An important limitation of the study protocol is the inability to exclude the possibility of a degree of ischaemia due to the pacing itself. However, during the pacing alone section of the protocol, no patient experienced angina or showed ST segment changes on the monitored ECG leads unlike with dobutamine. Hence, any ischaemia induced by the pacing alone is likely to have been minor. Another possible limitation is that the results in the present study may have been influenced by background sympathetic activity of the individual patients. Some of the patients had been receiving long term beta blockers including Atenolol which was discontinued 48 hours prior to the study. It is possible that there may still have been residual effects at the time of the study. In addition, beta blockers may affect receptor levels for some time after discontinuation. Individual variability may have resulted from differences in beta receptor sensitivity following discontinuing medications. However, there was no significant difference in the resting heart rate between patients who had been receiving ß blockers and who had not. It is therefore unlikely that variations in background sympathetic activity would have has a significant influence on the overall effect. Finally, tolerance to maximum dobutamine dosage differed between patients as a result of the variable severity of their coronary artery disease. This meant that observations had to be made over a range of heart rates dictated by individual tolerances. Although it is known that the effect of interventions on the action potential duration maybe influenced by basic cycle length, such influence would be minor over the relatively narrow range of cycle lengths employed in the present study.

3.5.6. CONCLUSIONS

This study examines the direct effects on the action potential duration of dobutamine, a commonly used beta 1 receptor agonist. In normal areas of myocardium, a variable effect has been demonstrated consistent with experimental studies. In areas susceptible to ischaemia, ischemia inducing doses of dobutamine consistently shortened the action potential duration. Such regional effects of beta stimulation can theoretically alter dispersion of action potential in adjacent areas of normal and ischaemic myocardium. Beta blocking agents by reducing such dispersion would be expected to minimize the propensity for re-entrant arrhythmias involving the ischaemic myocardium.

Table 3.5.1: Details of patients in the study.

Pt No.	Age/Sex	Drugs	Left ventricular wall motion	Coronary anatomy			Dobutamine dose (μg/kg/min).		Paced rates corresponding to low and high doses of dobutamine (ppm).		Patient response to peak dose of dobutamine	Perfusion scan in area of recording
				LAD	LCX	RCA	Lowest	Highest	Lowest	Highest		
1	48, M	Nil	Mild inferior	Normal HK	Severe	Normal	5	15	70	100	Chest pain ST shift	RV- N LV-I
2	60, M	Atenolol	Anterior HK	Occluded distally	Normal	Normal	5	15	70	100	Chest pain	RV- N LV- N
3	50, F	Atenolol Diltiazem	Normal	Normal	Normal	Normal	5	15	70	80	Chest pain	RV- N LV- N
4	52, F	Atenolol	Normal	Normal	Normal	Severe	5	15	80	100	Chest pain	RV- N LV- N
5	41, M	Atenolol	Infero-posterior HK	Mild	Severe	Normal	5	15	70	120	Palpitation	RV- N LV- I
6	73, M	Metoprolol	Normal	Moderate	Mild	Normal	5	15	80	100	Chest pain	RV- N LV- N
7	54, F	Nil	Normal	Normal	Severe	Normal	5	10	90	110	Chest pain ST shift	RV- N LV- I
8	50, M	Atenolol	Normal	Moderate	Severe	Normal	5	10	80	100	Chest pain ST shift	RV- N LV- I
9	57, M	Atenolol Nifedipine	Anterior HK	Severe	Normal	Normal	5	10	100	110	Chest pain	RV- I LV- N
10	54, M	Diltiazem	Inferior HK	Moderate	Normal	Severe	5	15	70	90	ST shift	RV- N LV- N
11	47, F	Nil	Normal	Normal	Normal	Normal	5	15	100	120	Palpitation	RV- N LV- N
12	54, M	Nil	Inferior HK	Normal	Severe	Occluded	5	10	100	110	Chest pain	RV- N LV- I
13	60, F	Nifedipine	Normal	Severe	Mild	Normal	5	10	80	110	Chest pain ST shift	RV- I LV- N
14	40, F	Nil	Normal	Normal	Normal	Normal	5	10	90	120	Palpitation	RV- N LV- N

Key to abbreviations: **LAD**, Left anterior descending artery; **LCX**, Left circumflex artery; **RCA**, Right coronary artery; **RV**, Right ventricle; **LV**, Left ventricle; **HK**, Hypokinesia; **N**, Normal; **I**, Ischaemic.

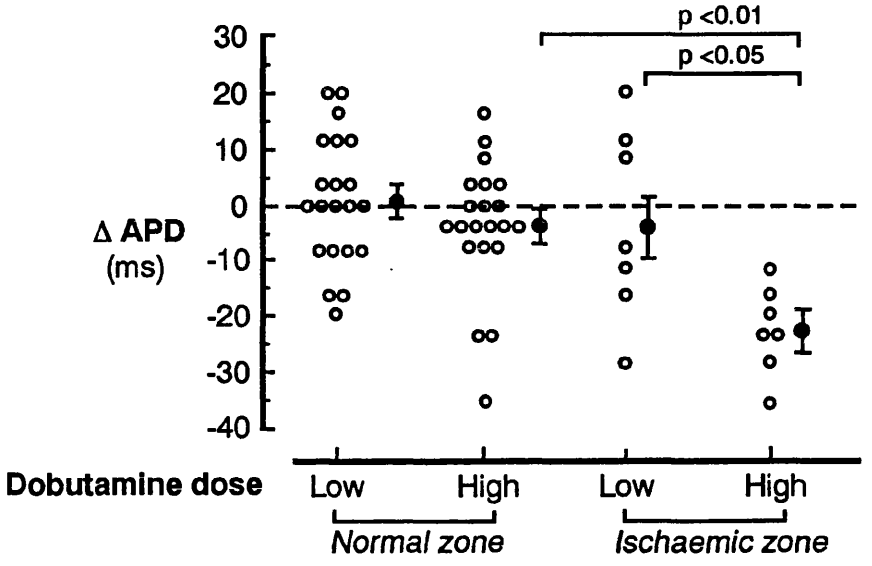

Figure 3.5.1: *Change in steady state monophasic action potential duration during dobutamine infusion. Each point represents the additional effect of dobutamine over and above that of the effect of pacing. In the normal zone recordings, dobutamine produces lengthening in some and shortening in others. A similar pattern was observed for the low dose dobutamine infusions in the ischaemic zones before the onset of ischaemia. The higher doses of dobutamine in the ischaemic zones invariably produced shortening of APD in all the recordings.* ***APD,*** *Action potential duration.*

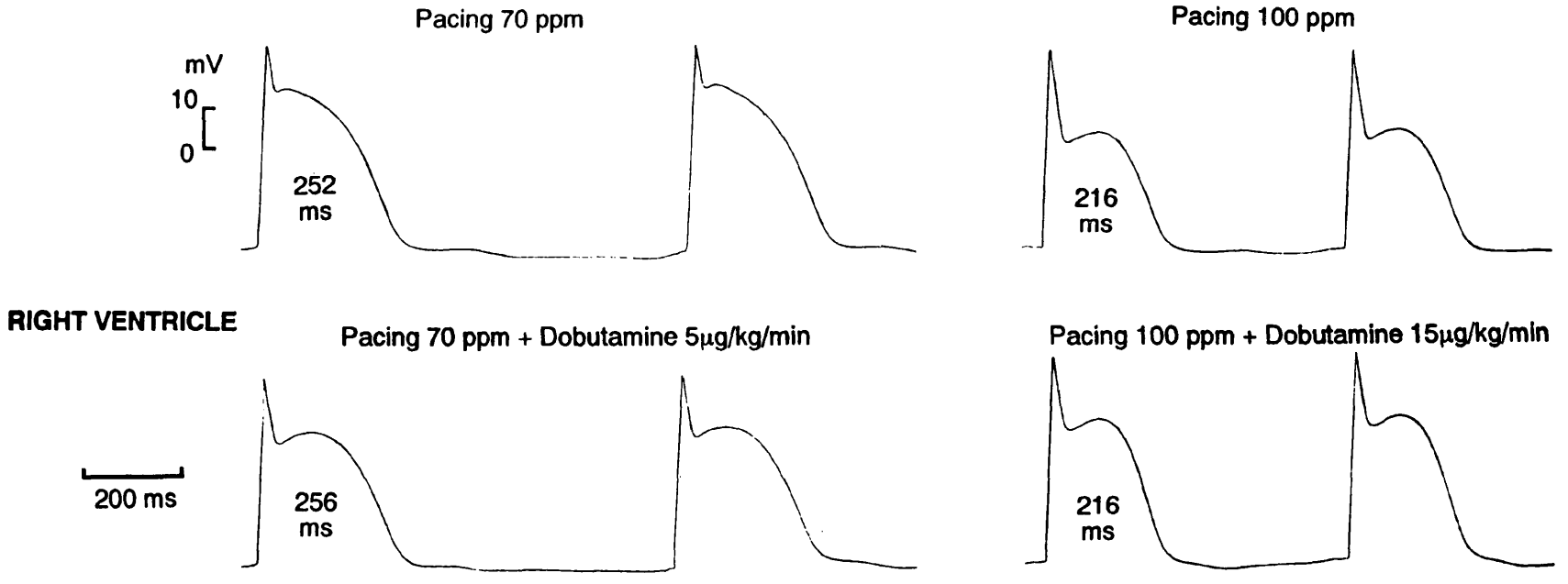

Figure 3.5.2, A: Monophasic action potentials (MAPs) recorded from the right ventricluar septum in patient 1. The recording site on the Tc-99m-MIBI SPET images showed a normal perfusion pattern. Increment in paced rate from 70 to 100 ppm in the absence of dobutamine produces the expected rate related shortening of action potential duration (top panel). Dobutamine infusion does not significantly influence the MAP duration for either the low dose (5μg/kg/min; paced rate 70 ppm) or for the high dose (15 μg/kg/min; paced rate 100 ppm) (bottom panel). MAP duration measured at 70% repolarization in milliseconds is shown within the signals.

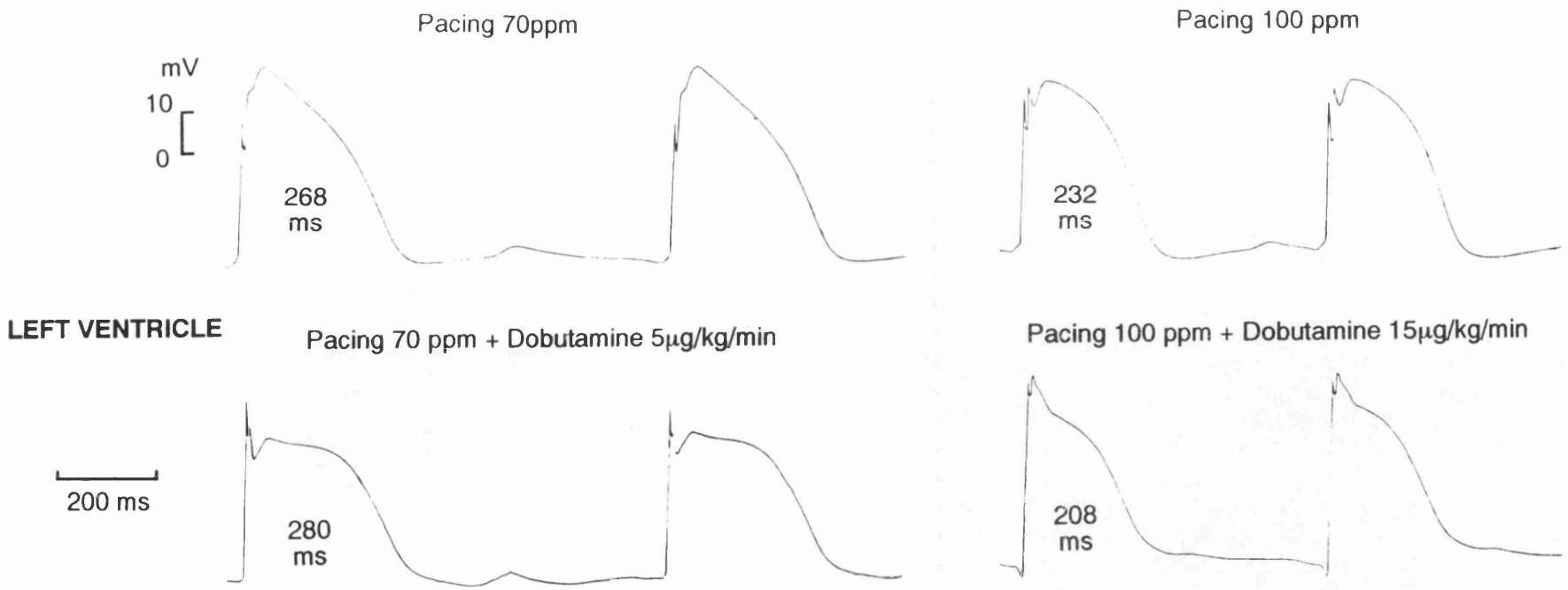

Figure 3.5.2, B: Monophasic action potentials (MAPs) recorded from the left ventricular postero-lateral wall simultaneous with the right ventricular recordings shown in figure 3.5.2, A. The recording site showed reversible perfusion defect on the SPET image (the patient had a tight proximal left circumflex artery stenosis). Atrial pacing from 70 to 100 ppm in the absence of dobutamine produces the expected rate related shortening of action potential duration (top panel). The addition of dobutamine at a low dose results in a small increase in MAP duration. At the higher doses however, a substantial shortening of the MAP duration in excess of that produced by identical pacing rates is seen (bottom panel). MAP duration measured at 70% repolarization in milliseconds is shown within the signals.

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Chapter 3.6.

SUMMARY AND GENERAL DISCUSSION

In the work presented in this thesis, I have used recordings of the monophasic action potential (MAP) from the endocardial surface of the heart to detect early electrophysiological changes of myocardial ischaemia. In addition, potential arrhythmogenic mechanisms of sympathetic stimulation in relation to ischaemia were addressed. In both animal and human studies (chapter 3.2 - paper 1 and chapter 3.3 - paper 2), endocardial recordings of the MAP consistently registered shortening in response to myocardial ischaemia. Although, such endocardial recordings have been employed in previous studies, a systematic comparison against other established parameters of ischaemia (wall motion changes in the case of animal studies or Tc-99m-MIBI perfusion defects in human studies) have not been previously undertaken. Having established the reliability of endocardial MAPs, I have applied the technique to dipyridamole scintigraphy and demonstrated strong evidence for the presence of myocardial ischaemia during the course of dipyridamole coronary vasodilation (chapter 3.4 - paper 3). Collateralised areas of myocardium were most susceptible to the ischaemic effects of dipyridamole.

The use of the MAP as a measure of ischaemia is attractive in combination with cardiac catheterisation techniques. The main utility of the technique lies in research protocols where therapeutic interventions during ischaemia can be monitored. The model for such studies in the cardiac catheter laboratory is the patient undergoing percutaneous coronary angioplasty. One drawback of using a localised recording such as the MAP is the need to position catheters in the appropriate location. A thorough knowledge of cardiac anatomy in relation to radiographic projections is therefore essential.

Further, the dependence of the MAP duration on heart rate implies that measurements should ideally be made at steady state heart rates after rate adaptation is complete (usually 90 to 120 seconds). I have defined the sensitivity and specificity for values of MAP duration changes per 100 ms change in cycle length that are representative of ischaemia as opposed to a rate related alteration. In protocols that employ an incremental pacing stress to provoke ischaemia, these values should prove useful.

A possible disadvantage of the duration of the MAP as a measure of ischaemia may be encountered during acute ischaemic studies in animal experiments. In studies where severe regional ischaemia is induced by total occlusion of the subtending artery, marked delay in the upstroke of the MAP signal may develop, the upstroke configuration then resembling that of a 'slow response' action potential. Measurement of the duration can, under these circumstances, prove difficult. However, such changes are usually seen only when ischaemia is extended over several minutes. For early myocardial ischaemia, changes in the MAP duration are reliable and sensitive.

The finding of differential response of endocardial and epicardial MAPs to early ischaemia in the animal experiments is of importance to arrhythmogenesis in the acute phases of ischaemia (chapter 3.2 - paper 1). In the normal heart, myocardial recovery from excitability (refractoriness) has a linear correlation with repolarisation time course. For orderly contraction of the myocardium, there has to be a normal dispersion of time course of repolarisation and hence refractoriness. However, when this normally existing order of dispersion is disrupted, refractoriness becomes distributed in a patchy manner and creates a substrate for re-entry which could then be triggered by a critically timed ectopic beat. The demonstration of a transient

divergence of endocardial and epicardial MAP duration during coronary occlusion would suggest that, in addition to alterations of repolarisation between circumjacent areas of myocardium, a transmural heterogeneity is created in the early phases of ischaemia. The abnormality documented in the study is however, too early in time to account for the Phase 1a arrhythmia (which typically occur after 2 minutes of ischaemia) as described by Janse and Wit (1989). Nevertheless, progressive heterogeneity within the surrounding ventricular wall (beyond the region of the recording electrodes in the study) may well create sufficient dispersion of refractoriness, and form a substrate for arrhythmias to coincide in time with the Phase 1a arrhythmias.

The combination of MAP recording and Tc99m-MIBI imaging was employed to define the effects on the action potential duration of beta adrenergic stimulation (chapter 3.5 - paper 4). The variable effects of beta stimulation on the action potential duration that was documented in the non-ischaemic myocardium is entirely consistent with the known variable effects of ß adrenergic stimulation on transmembrane ionic fluxes. In ischaemic areas, my results would suggest that these effects are overwhelmed by the onset of ischaemia. An alternate possibility to be considered to explain the uniform shortening of action potential duration in ischaemic myocardium, is a modifying effect of ischaemia on ß mediated actions. The end result is the creation of a significant alteration of the normal existing dispersion of action potential duration. Once again, the potential for arrhythmias is created on the basis of the mechanism discussed above. Such studies of arrhythmogenic mechanisms in the human heart and in the context of ischaemia are extremely important and difficult to accomplish.

The results of the Cardiac Arrhythmia Suppression Trial brings into focus the fact that antiarrhythmic drugs evaluated in isolated tissue preparations behave differently in the diseased human heart. Drug actions classified and defined on the basis of electrophysiological effects in isolated tissues cannot be reliably extrapolated to the human heart where a complex milieu of ischaemia, infarction, 'border zone' pathophysiology, and autonomic influences interact. Electrophysiological evaluation of these drugs, and their arrhythmogenic and antiarrhythmic mechanisms should therefore be undertaken in the human heart affected by the consequences of coronary artery disease. The MAP technique in conjunction with Tc-MIBI imaging offers a practical combination of methodologies for such investigations as exemplified by the series of studies that I have presented in this thesis.

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APPENDIX

European Heart Journal (1992) **13**, 310–315

The interrelation between the monophasic action potential duration, cycle length and ischaemia in the human left ventricle

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KEY WORDS: Monophasic action potential duration, ischaemia, cycle length, left ventricular endocardium, atrial pacing.

Steady state monophasic action potentials were recorded from a single site in the left ventricular endocardium during incremental atrial pacing to the point of angina in 25 patients. Ischaemic areas of the left ventricle were documented using a perfusion marker (99^mTc-MIBI) simultaneously with the action potential recording procedure. Recordings were obtained from an ischaemic area in 13 patients and from a non-ischaemic area in 12. A linear correlation between action potential duration and cycle length changes was demonstrated for both ischaemic and non-ischaemic zone recordings between cycle length changes of 750 and 428 ms. Ischaemia induced a shortening of the action potential duration significantly greater than that produced by cycle length changes ($P<0.0001$). Mean action potential duration shortening corrected for 100 ms change in cycle length for ischaemic zone recordings was 31.4 ± 4.2 (SD) compared to 23.3 ± 3.1 ms for non-ischaemic zone recordings. A range of values of action potential duration shortening in unit time was analysed for sensitivity and specificity for the detection of ischaemia. A value of 26.5 ms per 100 ms change in cycle length provided the optimum compromise with 88% sensitivity and specificity. Our data provide a means of employing the monophasic action potential duration to quantify early localized ischaemia in the presence of an alteration in cycle length.

Introduction

Regional inhomogeneity is a characteristic of myocardial ischaemia, the electrophysiological effects of which are well known to facilitate arrhythmia by re-entry mechanisms[1–3]. Small localized areas of ischaemia are sufficient to initiate arrhythmia by re-entry in diseased hearts. The ability to monitor such ischaemic changes may therefore be useful in developing therapeutic strategy. Currently available methods for the detection and quantification of early myocardial ischaemia in man have limitations. As early ischaemic changes tend to be localized, they maybe absent or delayed on the surface electrocardiogram[4,5]. Myocardial perfusion scintigraphy does not allow measurements to be made during sequential interventions within the confines of a single study. It would therefore be desirable to have a method that can monitor early ischaemia which maybe induced and reversed in a relatively short space of time, providing a model for testing therapeutic interventions.

The monophasic action potential recorded from the surface of the myocardium although of lower amplitude and upstroke velocity, represents a reliable measure of intracellular action potentials[6,7] and has been used in human studies for over a decade[8]. The action potential duration shortens in response to ischaemia. However, the use of the monophasic action potential duration as a measure of ischaemia is complicated by its dependence on cycle length insofar as shortened cycle length also shortens the action potential duration. In vivo studies designed to investigate ischaemia in man will mostly involve an increase in heart rate by use of incremental pacing protocols or the use of drugs which themselves have chronotropic effects. We have therefore explored the relationship between the monophasic action potential duration and cycle length in the left ventricular endocardium during incremental atrial pacing. Recordings were obtained from ischaemic or non-ischaemic areas to establish the influence of ischaemia on the action potential duration–cycle length relationship.

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Methods

Recordings of monophasic action potentials were made from the left ventricular endocardium using pressure contact electrodes during incremental atrial pacing. Areas of myocardial ischaemia were identified using radionuclide perfusion tomography. The position of the action potential recording catheter in the left ventricle was related to the perfusion characteristics and recordings classed as ischaemic or non-ischaemic zone recordings.

PATIENTS

We studied 25 patients (15 male; median age 59 years, range 39 to 73) randomly selected from those undergoing routine coronary angiography for investigation of chest pain. None of the patients were receiving anti-arrhythmic drugs conventionally considered to influence ventricular repolarization. Beta blockers (none with class III effect) were continued upto 6 h before the study. Patients also received 10 mg diazepam prior to the study. Approval

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for the study was obtained from the hospital ethical committee.

ELECTROPHYSIOLOGICAL MEASUREMENTS

Monophasic action potentials were recorded with bipolar pressure contact silver/silver chloride purpose built catheter electrodes (Cordis (UK) Ltd; size 7 French). Gentle apposition of the tip of the exploring electrode to the endocardium resulted in a monophasic action potential signal of usually 20–40 mV amplitude. Signal processing and data acquisition have been described elsewhere[4].

MYOCARDIAL PERFUSION SCINTIGRAPHY

99^{m}Technetium hexakis-2-methoxy-2-methylpropyl-isonitrile (99^{m}Tc-MIBI) was used for myocardial perfusion scintigraphy, with single photon emission tomography (SPET) employing a two-day, two-dose protocol. Images were acquired with a single detector rotating gamma camera/computer system (IGE 400AC-Starcam) equipped with a low-energy, high-resolution collimator. Sixty-four 30-s images were acquired over a 360° circular arc of rotation. Reconstructed tomographic slices were reoriented in the short axis, horizontal long and vertical long axes for display and visual analysis.

PROCEDURE

Following routine coronary angiography performed via the femoral route, a monophasic action potential recording catheter was introduced into the left ventricle via a short femoral arterial sheath. Recordings were taken from a single endocardial site in any individual patient. Atrial pacing was achieved using a bipolar 6 French temporary pacing lead (Cordis) which was positioned in the right atrial appendage or at the junction of the right atrium and superior vena cava. Pacing was commenced at 10 beats above the patient's resting heart rate and incremented by 10 beats until the patient developed angina, lost 1:1 atrioventricular conduction or achieved a heart rate of 150 b . min^{-1}. Each pacing train was maintained for 2 min to obtain steady state action potential recordings at each heart rate.

At peak paced heart rate, 370 MBq of 99^{m}Tc-MIBI was administered intravenously. To ensure adequate myocardial distribution of the tracer during pacing-induced ischaemia, pacing was continued for 2 min after the tracer injection. Myocardial perfusion imaging with SPET was carried out between one and two hours after injection of the tracer. Rest imaging was performed 24 h later after a second dose of 99^{m}Tc-MIBI.

The position of the monophasic action potential catheter in the ventricle was documented at the time of cardiac catheterization by biplane cinematography and subsequently related to the perfusion abnormalities on the perfusion scan. Recordings from sites in the left ventricle that corresponded to areas on the scintigram showing reversible perfusion defects were considered ischaemic zone recordings and those from areas with normal perfusion pattern as normal zone recordings.

DATA ANALYSIS

Myocardial perfusion SPET images were interpreted qualitatively by an observer blinded to the angiographic and monophasic action potential data. Defect interpretation was based on criteria previously described[9]. The duration of the monophasic action potential was measured at 90% repolarization. Steady state values of action potential duration obtained at the end of each 2 min pacing train have been employed for data analysis.

Statistical analysis of comparisons were made using the unpaired Student's t-test. Correlation between the action potential duration and cycle length was determined by regression analysis.

Results

Significant coronary artery disease defined as 70% or greater stenosis in any major epicardial artery was present in 22 of the 25 patients (nine with single vessel disease, 11 with two vessel disease and two with triple vessel disease). On the basis of the relation of the recording catheter tip to the perfusion characteristics for the area of the left ventricle, recordings were assessed to be from the ischaemic areas in 13 patients and non-ischaemic areas in 12 patients.

RELATION BETWEEN STEADY STATE ACTION POTENTIAL DURATION AND CYCLE LENGTH CHANGE

Table 1 shows the steady state action potential duration for various cycle lengths for individual patients. Each patient shows a linear relationship between steady state action potential duration and cycle length changes. The slope of the regression line for each patient is shown. A significant difference between the ischaemic and non-ischaemic group is demonstrated ($P < 0{\cdot}0001$).

Monophasic action potential recordings from the ischaemic zone showed a greater shortening of action potential duration per 100 ms change in cycle length than recordings from the non-ischaemic zone. Mean ($\pm$SD) values for the ischaemic group was 31·4 (4·2) ms per 100 ms change in cycle length compared to 23·2 (3·1) ms per 100 ms change in cycle length for the non-ischaemic group ($P < 0{\cdot}0001$).

REPRESENTATIVE PATIENTS

An example of monophasic action potential recordings from the normal zone is shown in Fig. 1 together with the perfusion scintigram. The scintigram shows a normal perfusion pattern during stress. The action potential duration shortens with cycle length decreases, in a manner consistent with rate effect. In Fig. 2, monophasic action potential recordings are shown together with the corresponding perfusion scintigram. The scintigram shows an area of reversible perfusion defect at the site of the action potential recording. During incremental atrial pacing, as cycle length shortens, action potential shortening is seen which is greater than that for the non-ischaemic zone in Fig. 1, owing to the additive effects of ischaemia and rate change.

Table 1 Steady state APD at various cycle lengths and linear regression data for relationship between APD and cycle lengths for individual patients

Patient No.	Cycle length in ms									r^2 value	Slope
	857	750	667	600	545	500	462	428	400		
Non-ischaemic zone recordings											
1	—	292	276	256	240	228	224	—	—	0·98	0·25
2	—	296	280	260	248	—	—	—	—	0·99	0·23
3	—	328	312	288	—	—	—	—	—	0·97	0·26
4	—	—	300	284	268	252	244	240	—	0·98	0·26
5	—	280	272	256	—	—	—	—	—	0·94	0·16
6	—	292	272	260	244	236	—	—	—	0·99	0·22
7	328	308	288	268	256	244	240	—	—	0·99	0·23
8	—	288	272	260	252	236	228	—	—	0·98	0·20
9	—	296	284	260	—	248	—	—	—	0·96	0·20
10	—	—	320	304	292	—	—	—	—	0·99	0·23
11	—	292	280	264	244	224	216	—	—	0·97	0·28
12	—	—	—	284	268	256	248	240	—	0·99	0·25
											Mean (±SD) 0·23 (0·03)*
Ischaemic zone recordings											
13	—	—	—	268	252	236	—	—	—	0·99	0·32
14	—	—	264	236	224	208	—	—	—	0·98	0·32
15	—	—	284	272	256	240	232	220	—	0·99	0·27
16	—	308	284	256	236	228	—	—	—	0·99	0·33
17	312	292	260	—	—	—	—	—	—	0·96	0·27
18	—	288	252	240	—	—	—	—	—	0·95	0·32
19	—	—	256	248	220	—	—	—	—	0·87	0·29
20	—	—	—	—	—	244	224	212	204	0·98	0·40
21	—	—	280	264	232	224	—	—	—	0·96	0·36
22	—	—	280	—	248	220	—	—	—	0·95	0·34
23	—	—	280	256	252	—	—	—	—	0·89	0·23
24	—	268	244	228	212	180	—	—	—	0·96	0·33
25	—	340	308	292	272	264	—	—	—	0·99	0·30
											Mean (±SD) 0·31 (0·04)*

*$P < 0·0001$
APD = action potential duration.

SENSITIVITY AND SPECIFICITY OF VALUES OF ACTION POTENTIAL DURATION

In order to test the applicability of this data to assessing the presence or absence of ischaemia in any individual patient, we have calculated the sensitivity and specificity for a range of action potential duration changes per unit time (Fig. 3). For example, for an action potential duration shortening of 20 ms/100 ms shortening of cycle length, the sensitivity is 100% but the specificity low at 17%. On the other hand, for a value of 40 ms/100 ms change in cycle length, although the specificity is 100%, the sensitivity is low at 8%. The optimum value of action potential duration shortening that represent ischaemia lies between 26 and 28 ms shortening per 100 ms decrement of the cycle length.

Discussion

We have documented the effect of ischaemia on the monophasic action potential duration in the presence of an altered cycle length for the human left ventricle. Our results show that a linear relationship between steady state monophasic action potential duration and cycle length changes is maintained for both ischaemic and non-ischaemic areas. Ischaemia, however, induces an abbreviation of the action potential duration over and above that produced by cycle length decrements. We have also analysed the data in terms of sensitivity and specificity for ischaemia during the standard incremental pacing protocol used in this study. On this basis, a range of action potential duration shortening between 26 and 28 ms/100 ms provides optimum sensitivity coupled with optimum specificity for the detection of ischaemia.

In the light of this and previous studies, it is likely that the monophasic action potential will be increasingly utilized as a measure of localized myocardial ischaemia. Our work is therefore important as it defines values of action potential duration shortening as representative of ischaemia as opposed to cycle length related changes. Although several studies have documented the monophasic action potential duration as a sensitive index of early and localized ischaemia, most have used recordings from the epicardial surface[10–12]. Monophasic action potential recordings from the endocardial surface have been less widely used

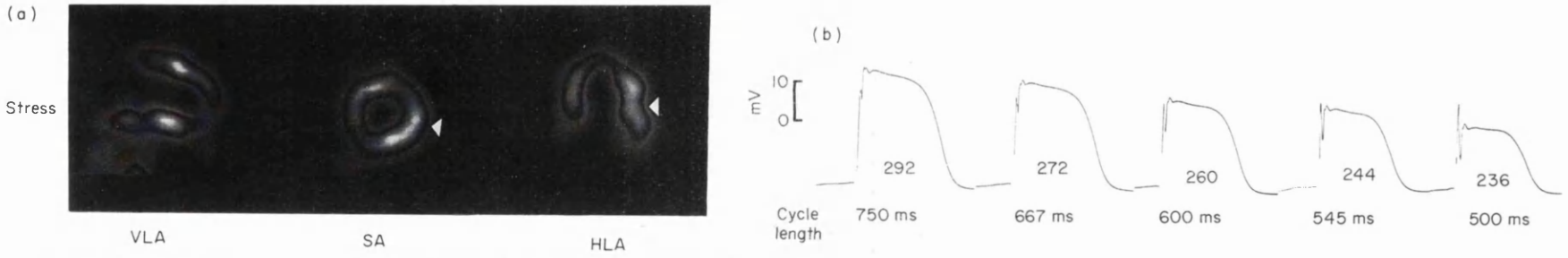

Figure 1 Example of a monophasic action potential recording from an area of myocardium with a normal perfusion pattern. (a) Shows SPET images of myocardial perfusion demonstrating a normal pattern of perfusion at a peak pacing rate of 120 b . min^{-1}. The approximate location of the catheter tip electrode is shown by an arrow. (b) Monophasic action potentials during incremental pacing from 80 to 120 b . min^{-1} (cycle length 750 to 500 ms) showing progressive shortening of the action potential duration due to rate effect alone. The duration of the action potential measured at 90% repolarization in ms is shown within each action potential signal.

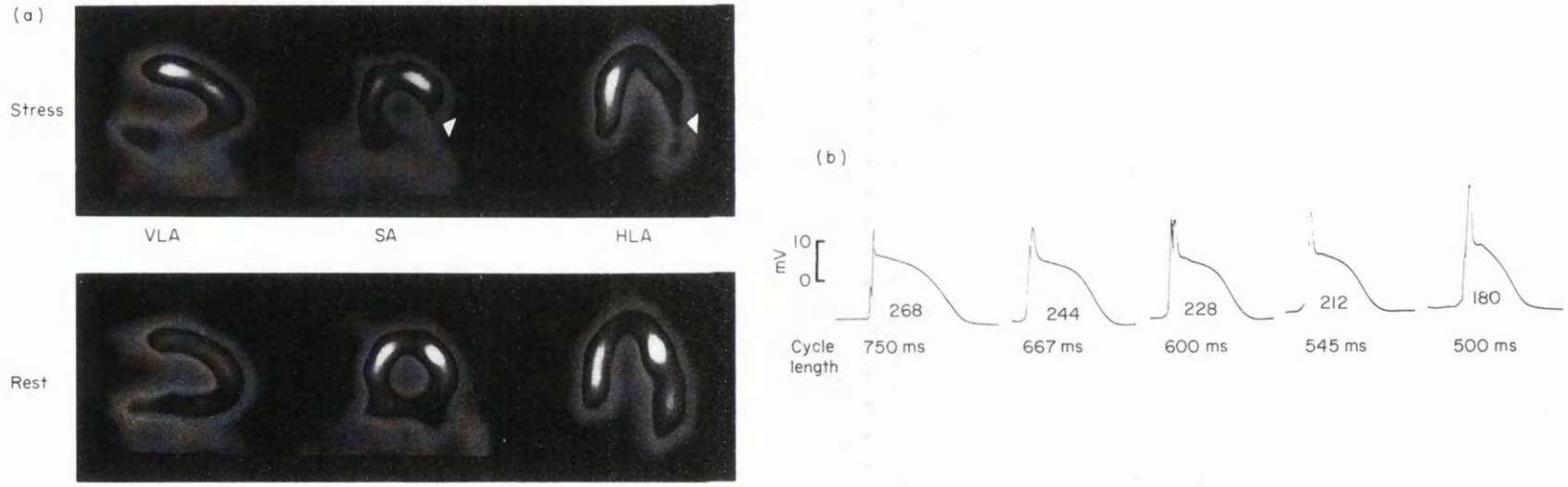

Figure 2 Example of a monophasic action potential recording for an area of myocardium showing a reversible perfusion defect. (a) SPET images of myocardial perfusion demonstrates an infero-lateral perfusion defect at a peak pacing rate of 120 b . min^{-1}. The rest images show reversal of the perfusion abnormality. The approximate location of the catheter tip electrode is indicated by an arrow. (b) Monophasic action potentials during incremental pacing from 80 to 120 b . min^{-1} (cycle length 750 to 500 ms) show progressive shortening. The extent of shortening in this recording is greater than that for the non-ischaemic zone (Fig. 1) due to the additive effect of rate and ischaemia.

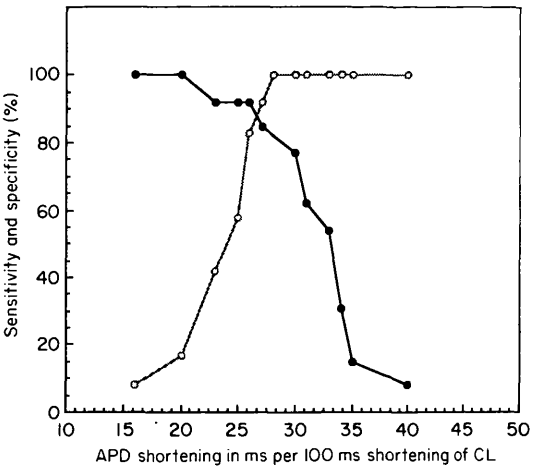

Figure 3 Sensitivity and specificity for the detection of ischaemia for a range of action potential duration shortening between 16 and 40 ms/100 ms shortening of cycle length. Optimum compromise occurs where the line for sensitivity (solid circles) and the line for specificity (open circles) cross at 26·5 ms. APD = action potential duration measured at 90% repolarization; CL = cycle length.

despite its potential application in conjunction with cardiac catheterization procedures. A theoretical uncertainty arises with regard to the volume conductor properties of intracavitary blood. The reference electrode of the monophasic action potential catheter makes contact with the endocardium via the blood and would therefore pick up electrophysiological events beyond the point of contact of the exploring electrode. This may dilute the local effects of ischaemia on the monophasic action potential duration thereby lowering sensitivity of the signal for early changes. Nevertheless, as the area of ischaemia extends, the influence of the volume conductor effect would be expected to rapidly diminish[12]. Another potential disadvantage of endocardial recordings of the monophasic action potential as a measure of ischaemia is its localized recording field. The electrode tip must be positioned precisely in an ischaemic area to register early changes. The development of steerable catheters should largely overcome the problem of accurate location of the catheter tip in desired areas of the ventricular endocardium.

Whereas previous studies have demonstrated the relationship between cycle length changes and action potential duration in isolated preparations[13–15] and for the human right ventricle[16,17], we have focused on the left ventricle, in view of its greater importance in relation to ischaemic heart disease. Our values for shortening of the action potential duration per 100 ms change in cycle length from the non-ischaemic areas of the left ventricle accord with those obtained by Franz *et al.* in the right ventricle[16]. The finding of a linear relation between action potential duration and cycle length changes in the ischaemic group, as shown in this study, is somewhat surprising. It might be expected that at the start of incremental pacing, the relationship between cycle length and action potential would be similar to recordings from the non-ischaemic territory and as ischaemia develops, this relationship would change producing a 'kink' in the slope relating the cycle length to action potential duration. A possible explanation for the finding to the contrary in our study maybe that ischaemia was present from an early stage. It is characteristic of monophasic action potential signals that they are particularly sensitive to early ischaemic changes. They would therefore have registered an ischaemic response before any other evidence (electrocardiographic changes or symptoms) of ischaemia became apparent. Most of the patients in our study had severe coronary artery disease and it is possible that the angiographic procedure itself had triggered early myocardial ischaemia.

Although ideally observations should have been made on a non-ischaemic site and an ischaemic site in the same patients, in this study we limited our observations to one pacing run and a single recording site in order to reduce the total duration of the study protocol. Ischaemia has several effects on the action potential signal, namely, loss of diastolic potential, reduction in amplitude, slowing of the upstroke and shortening of the duration[5,10,18]. We have confined our measurement to the action potential duration. The cellular mechanism by which shortening of the action potential duration occurs is unclear but involves the combined effect of a number of components of the ischaemic environment of the myocyte. For detailed discussion, see[18].

Conclusion

This study has demonstrated an electrophysiological means of quantifying early localized ischaemia in the presence of an alteration in cycle length. Our data employing an incremental pacing protocol indicate that ischaemia induces an abbreviation of the steady state monophasic action potential duration additive upon that produced by cycle length shortening. We have established values of action potential duration shortening in unit time that are representative of an ischaemic effect as opposed to a rate effect and appraised these in terms of sensitivity and specificity. Our data provides a methodological basis for future studies designed to assess the efficacy of therapeutic interventions on the early phases of ischaemia which maybe silent clinically and absent electrocardiographically.

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