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LA THÈSE A ÉTÉ MICROFILMÉE TELLE QUE NOUS L'AVONS REÇUE

The relationship of the availability of the slow inward current to the genesis of cardiac arrhythmias during ischemic conditions and reperfusion

by

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at

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THIS THESIS IS DEDICATED TO MY PARENTS, ROLAND AND EVELYN,

AND TO MY SISTERS PAT AND CAMILLE

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ABSTRACT

The role of the slow inward calcium current in cellular mechanisms of arrhythmia was examined in isolated canine Purkinje fiber-papillary muscle preparations during ischemic conditions and reperfusion. Microelectrode recordings were made simultaneously from Purkinje and muscle tissues. Ischemic conditions caused a decrease in membrane potential, depressed excitability and induced conduction block between Purkinje and muscle tissues. In the presence of calcium channel blocking agents, ischemic conditions resulted in greater depolarization of Purkinje tissue and increased incidence of inexcitability or conduction block. Return to nonischemic conditions (reperfusion) in the absence of drugs resulted in a complex sequence of responses in Purkinje tissue: oscillatory afterpotentials (OAP's), temporary depolarization, inexcitability, depolarization-induced automaticity (DIA), and parasystole. In the presence of calcium channel blocking agents OAP's, DIA, and parasystole were abolished. To determine changes in the availability of the slow inward current during ischemic conditions and reperfusion, DIA were elicited in isolated Purkinje fibers by external application of current. Ischemic conditions increased the cycle length of DIA, prevented sustained DIA, and increased the depolarization required to elicit DIA. Reperfusion was associated with a second period during which DIA were inhibited. However DIA returned later when membrane potential began to recover. Reperfusion also changed cycle length of DIA and initially decreased the threshold voltage of DIA. To determine whether cyclic changes in the availability of the slow inward current were responsible for the cyclic inhibition of DIA, isolated Purkinje fibers were exposed to ischemic conditions and reperfusion in the presence of BAY-K8644. BAY-K8644 enhances calcium influx through the slow channel. BAY-K8644 further inhibited DIA during ischemic conditions. Reperfusion in the presence of BAY-K8644 resulted in initial appearence of OAP's and return of DIA. BAY-K8644 prevented inhibition of DIA associated with early reperfusion. Therefore BAY-K8644 failed to promote DIA during ischemic conditions but not during reperfusion.

ABBREVIATIONS

ATP	Adenosine triphosphate
A-V	Atrio-ventricular
BCL	Basic cycle length
Ca^{2+}	Calcium
Ca^{2+}_i	Intracellular calcium
DAD	Delayed afterdepolarization
DIA	Depolarization-induced automaticity
ERP	Effective refractory period
Isi	Slow inward current
K	Potassium
KCL	Potassium chloride
$[K]_o$	Extracellular potassium concentration
LAD	Left anterior descending
M	Molar
mM	Millimolar
MDP	Maximum diastolic potential
mg	Milligram
ml	Millilitre
mm	Millimeter
msec	Milliseconds
mV	Millivolt
MΩ	Megohm

Na	Sodium
Na_i	intracellular sodium
OAP	Oscillatory afterpotential
TI	Transient inward current
uA	Microamps

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INTRODUCTION

"In the great majority of cases where sudden death is caused by cardiac failure, there is no doubt, an altered and impaired state of nutrition in the cardiac tissues, sometimes rendered palpable by degenerative changes recognisable with the microscope or pointed to by the presence of disease in the coronary arteries or conditions indicating a changed coronary supply." These words were written by John MacWilliam (1889) who was the first to recognize the relationship between myocardial ischemia and sudden cardiac death. MacWilliam also was the first to suggest that sudden cardiac death was often caused by ventricular fibrillation. In his paper published in 1889, MacWilliam gave a colorful description of ventricular fibrillation: "The cardiac pump is thrown out of gear, and the last of its vital energy is dissipated in a violent and prolonged turmoil of fruitless activity in the ventricular walls."

Sudden cardiac death may be defined as death occurring within 1 hour of the onset of symptoms. Sudden cardiac death is the leading cause of fatality in the industrially developed world. In patients monitored electrocardiographically at the time of sudden death, ventricular fibrillation has almost invariably been the mechanism of sudden death. Therefore in patients threatened with sudden death, there is an underlying electrical instability of the heart. The clinical expression of the underlying electrical instability is the occurrence of ventricular premature beats. Ventricular premature beats may lead to tachycardia and fibrillation. The mechanism underlying these abnormalities of

heart rhythm, or arrhythmias, remain unclear. In most victims of sudden death, autopsy studies have revealed severe occlusive atherosclerotic disease of the major epicardial coronary arteries. However sudden death may also occur in the absence of new morphologic lesions. This latter finding suggests a role of transient coronary spasm in sudden death. Recent advances in coronary bypasss surgery, percutaneous transluminal angioplasty, and thrombolytic procedures have provided alternatives for the effective reversal of coronary occlusion and for treatment of evolving infarction. The basis for such direct reperfusion treatment of myocardial ischemia remains controversial, particularly in relation to the acute phase of reflow. Some studies have shown significant improvement of myocardial function in patients following early revascularization, whereas others report no changes or even further deterioration of cardiac function. Arrhythmias also occur upon restoration of blood flow. The mechanism of reperfusion arrhythmias, although poorly understood, appear to be different from those occurring during coronary occlusion.

The results in this thesis deal primarily with the role of the slow inward calcium current in the genesis of arrhythmias due to ischemia and reperfusion. The following introduction section will review the literature discusssing the electrophysiological changes that take place during coronary artery occlusion and reperfusion.

A) ELECTROPHYSIOLOGICAL CHANGES DURING THE ACUTE PHASE OF CORONARY ARTERY OCCLUSION

Harris and Rojas (1943) were among the first to investigate the

electrophysiological changes that take place during the initial part of ischemia. In this classic study, occlusion of the left anterior descending (LAD) coronary artery in dogs, resulted in frequent occurrence of ventricular arrhythmias. When ventricular fibrillation occurred, it occurred during the first 10 minutes of occlusion. Between the time of occlusion and fibrillation there was an accelerating series of ventricular ectopic beats which was maximum in frequency between 6-10 minutes. Electrocardiographic recordings from normal, ischemic and partially ischemic border zone suggested that the origin of the ectopic beats was the ischemic border zone. Ectopic activity ceased or nearly ceased within 10-20 minutes of occlusion in hearts that did not fibrillate during the first few minutes of occlusion. Attempts to determine specific cellular electrophysiologic changes during acute coronary artery occlusion were not made until the 1970's. Knowledge of the cellular electrophysiological changes that take place during coronary artery occlusion is a prerequisite for understanding the mechanisms underlying arrhythmias.

1. Changes in resting membrane potential

A number of reviews have discussed changes in resting membrane potential during ischemia (Elharrar and Zipes, 1977; Janse and Kleber, 1981; Janse, 1982). The earliest electrophysiological change during ischemia is a decrease in resting membrane potential of cells. Kardesch et al. (1958) demonstrated depolarization in cells of rabbit and dog hearts subjected to global ischemia. Samson and Scher (1960) showed a similar depolarization in dog hearts subjected to occlusion of the LAD

coronary artery. In both of these early studies, intracellular recordings were made from the ischemic area in the in situ heart. Kardesch et al. (1958) also found that the resting membrane potential decreased by 15 mV within the first 6 min of ischemia, and that electrical activity ceased when the resting potential decreased to -54 mV. However, it was not until the late 1970's that extensive studies of changes in transmembrane potentials following occlusion were made. Kleber et al. (1978) recorded transmembrane potentials from the subepicardium of isolated perfused porcine hearts subjected to occlusion of the LAD coronary artery. Resting membrane potentials between -60 and -65 mV were recorded within 7-10 minutes after coronary occlusion. The decrease in resting membrane potential was reflected also by a T-Q depression in the local DC electrograms. Cells usually became unresponsive at resting potentials of about -65 mV. However, between 20-40 min of occlusion, an increase in resting potential along with a resulting decrease in T-Q depression was observed (Cinca et al., 1980). This improvement was only temporary, and ischemic cells lost their excitability and no longer exhibited action potentials after 40-60 min of occlusion.

Changes in resting membrane potential in response to ischemia are probably due to accumulation of potassium in the extracellular space. The following reviews discuss changes in K^+ during ischemia (Gettes et al., 1982; Kleber, 1984). Hill and Gettes (1980) studied changes in extracellular potassium (K) activity after LAD coronary artery occlusion in pigs. The changes in potassium occurred in 3 phases: a rapidly rising phase detectable as early as 15 seconds and lasting 5-15 minutes,

a plateau phase that lasted approximately 15 minutes, and a slowly rising phase that extended to 60 minutes of occlusion. Kleber (1983) correlated changes in resting membrane potential with extracellular K activity in guinea pig hearts subjected to global ischemia. The changes in resting membrane potential were found to approximate the K equilibrium potential.

2. Changes in transmembrane action potential

Kardesch et al. (1958) reported decreased amplitude and marked shortening of action potential duration within minutes of global ischemia in rabbit and canine hearts. Similar results have been reported in other studies conducted in canine (Samson and Scher, 1960) and porcine hearts (Downar et al., 1977a; Kleber et al., 1978). Intracellular recordings from the subepicardium of the ischemic zone revealed that ischemia leads to a gradual, heterogenous and progressive decrease in action potential duration, resting membrane potential, overall action potential amplitude and maximal upstroke velocity (Downar et al., 1977a; Russel and Oliver, 1977). These deterioriations occured over a period of several minutes and were most pronounced in the center of the ischemic zone. The decrease in action potential amplitude may be responsible for the S-T segment elevation in the local electrogram (Cinca et al., 1980). Slow conduction in ischemic tissue leads to delayed activation of ischemic cells and therefore a late intrinsic deflection of the extracellular signal.

Depression of the maximal upstroke velocity of phase 0 of the action potential by ischemia might be due to inactivation of the sodium

current in response to depolarization. Thus during ischemia, the action potential might be dependent on the slow inward current. However, Cardinal et al., (1981) have shown that lidocaine, which blocks the sodium channel, further depressed action potential amplitude and increased activation delay of ischemic cells. This finding suggests that depolarization of ischemic tissue may not completely inactivate the fast inward sodium current.

Electrical alterans may also occur during ischemia. During alterans the action potential morphologies alternate between large and small amplitude responses. This phenomenon has been observed to preceed the onset of ventricular fibrillation (Downar et al., 1977a). Janse and Kleber (1981) have also suggested that the electrical alterans seen during ischemia may reflect an activation of the slow inward current only during every second beat.

3. Changes in conduction through ischemic myocardium

Various reviews discussing changes in conduction during ischemia have been published (Elharrar and Zipes, 1977; Lazzara et al., 1978; Janse and Kleber, 1981). Depresssion of conduction is a prominent effect of ischemia. However, within 2-3 min of coronary occlusion in the porcine heart, there is an initial speeding of conduction in ischemic tissue (Holland and Brooks, 1976). The transient increase in conduction velocity is manifested by an earlier onset of the epicardial electrogram in the ischemic zone. Following the decrease in conduction time, there is a period of delayed activation in the ischemic zone (Holland and Brooks, 1976; Elharrar and Zipes, 1977). The prolongation of conduction

time between an electrode placed in the ischemic epicardial zone to that placed in the normal epicardial zone may be greater in one direction than another (Elharrar et al., 1977a). Conduction delays occur not only between ischemic and non-ischemic tissue, but also within the ischemic zone, ie. endocardial to epicardial delays (Penkoske et al., 1978; Murdock et al., 1980). Penkoske et al. (1978) recorded simultaneous bipolar electrograms at various depths from epi- to endocardium in ischemic and normal zones of cats undergoing LAD coronary artery occlusion. They reported an increase in conduction time through the ventricular wall of the ischemic zone before the onset of arrhythmias. After the arrhythmias had subsided, conduction time from endocardium through mid-myocardial layers of the ischemic zone returned to normal. However, conduction time to the epicardial area of the ischemic zone remained prolonged.

Attempts have been made to correlate conduction delay during occlusion with incidence of arrhythmias. Williams et al. (1974) found that the magnitude of the conduction delay within the ischemic zone (measured epicardially) increased exponentially with time following ligation to the time of occurrence of arrhythmias. Kaplinsky et al. (1979a) have suggested that the first half hour of occlusion can be subdivided into periods of immediate (2-10 minutes) and delayed (12-30 minutes) arrhythmias, which are correlated with conduction delays in specific areas. Conduction delay in subepicardial layers was strongly associated with immediate but not delayed arrhythmias. After 30 minutes of occlusion, an increase in the amplitude of epicardial potentials was

observed, conduction normalized, and ventricular ectopic activity disappeared (Scherlag et al., 1974). Thus conduction delay or delay in activation of the ischemic zone may play an important role in arrhythmias. Factors which influence conduction in ischemic tissue may therefore influence the incidence of occlusion arrhythmias. For example, Scherlag et al. (1974) showed that decreased heart rate (by vagal stimulation) improved epicardial activation during occlusion and also abolished ventricular arrhythmias. Similarly, Elharrar et al. (1977b) reported that drugs that depressed conduction in the ischemic myocardium were found to promote development of ventricular arrhythmias, whereas those that improved conduction were found to be protective.

Although the subepicardial layer of the ischemic zone appears to be the main source of delayed activation, it is not clear how this abnormal electrical activity spreads to the normal myocardium. Following delayed activation of the epicardial ischemic zone, the first site of reactivation to the rest of the heart was via the Purkinje system (Kaplinsky et al., 1979b). This suggests that the exit point for re-excitation of the heart following delayed activation, may be by the way of the subendocardial Purkinje system.

4. Changes in excitability and refractoriness

Changes in membrane potential during ischemia can have profound effects on excitability of ischemic tissue. The changes in transmembrane action potentials during ischemia indicate that changes in excitability and refractoriness occur in ischemic tissue. A number of reviews have been published which discuss changes in excitabilty and

refractoriness during ischemia. These include: Elharrar and Zipes, 1977; Lazzara et al., 1978; Janse, 1982; Lazzara and Scherlag, 1984; Janse et al., 1985a.

Various methods of stimulation used to measure changes in diastolic excitability have shown ischemic tissue gradually becomes inexcitable. In those areas that retained some degree of excitability, an increase in diastolic threshold occurred (Brooks et al., 1960; Tsuchida, 1965). However, an initial increase in excitability occurs during the first few minutes of occlusion. The time required for these changes in excitability to occur was much longer than the time to onset of early ischemia-induced arrhythmias. Elharrar et al. (1977a) measured changes in excitability following acute coronary occlusion in dogs. Changes in diastolic excitability were measured by systematically decreasing the duration of regular pacing stimuli until the ventricles failed to respond. In both ischemic epicardium and endocardium, there was an initial increase in excitability between 1-3 min of occlusion. The increase was followed by a rapid decrease in excitability. There was also a gradient of decreasing excitability from normal to ischemic tissue. The gradient of excitability also passed through a heterogenous ischemic border zone with areas of decreased and increased excitability. These changes in excitability may also influence the determination of refractory period durations (Elharrar et al., 1977a).

Most studies on changes in retractoriness of ischemic tissue have been performed with extracellular stimulating electrodes. Electrograms recorded at varying distances from the stimulating electrodes were used

to detect propagated responses. Downar et al. (1977a) observed the duration of the refractory period to lengthen in the ischemic zone in the first 2 minutes after coronary artery occlusion in porcine hearts. A similar finding has been reported in the canine heart (Russel and Oliver, 1978). Jansé and Kleber (1981) suggested that prolongation of refractory periods might be caused by a slight increase in action potential duration. Following the initial lengthening, the refractory period shortens (Downar et al., 1977a; Russel and Oliver, 1978). After 5-10 minutes of occlusion, post-repolarization refractoriness develops (Downar et al., 1977a). Post-repolarization refractoriness is said to occur when expiration of the refractory period occurs well after repolarization is complete (Lazzara et al., 1975). This phenomena was originally described in isolated His-Purkinje fibers excised several hours after coronary occlusion. Post-repolarization refractoriness may be caused by depolarization secondary to accumulation of potassium ions in the extracelllar space (Kodama et al., 1984). Post-repolarization refractoriness can be prolonged by increased heart rate (Lazzara et al., 1975, 1978). However, the influence of heart rate on post-repolarization refractoriness has only been measured in infarcted hearts or infarcted preparations removed from infarcted hearts. Some investigators have also questioned the interpretation of studies on changes in refractoriness during ischemia. Most studies which have reported changes in refractoriness and excitability have utilized a method in which the interval of a premature stimulus is progressively decreased until no response is observed. The minimum interval which results in a

propagated response is the effective refractory period. Lazzara et al. (1978) and Janse et al. (1985a) have pointed out that recovery of excitability in ischemic myocardium should not be expressed in terms of intervals between stimuli alone. They suggested that the quality of premature responses should also be taken into account. They found that responses elicited in the ischemic zone well after completion of repolarization have markedly reduced amplitudes and upstroke velocities.

5. Arrhythmias

The various changes in action potential characteristics observed during ischemia may underly mechanisms of ventricular arrhythmias. Heterogeneity of the electrophysiological characteristics of cells during ischemia may be the factor linking various transmembrane alterations to the occurrence of ventricular tachycardia and fibrillation. Fragmented electrograms recorded from the ischemic zone often bridge the diastolic interval between normally conducted and ectopic beats (Boineau and Cox, 1973; Waldo and Kaiser, 1973; Scherlag et al., 1974; El-Sherif et al., 1975). This suggested that circulating excitation might play a role in generation of arrhythmias during acute ischemia. Fractionation of activity suggests that ischemic tissue activates with delay and that excitation is irregular. In circus-movement reentry, the impulse encounters an area of unidirectional block, and propagates around the area via an alternate pathway. The impulse can then retrogradely activate the zone of unidirectional block and emerge as a reentrant wavefront at the site of origin. The exact pathway of excitation during ischemia could not be detected in early studies. Simultaneous recordings at

multiple sites on the ischemic and non-ischemic regions were needed to show that wavefronts leading to a local circus movement were responsible for premature activation of the whole heart. The work of Janse et al. (1980) incorporated ventricular mapping in porcine and canine hearts to demonstrate that reentry does indeed occur in epicardial regions during established ventricular tachycardia induced by coronary occlusion. These investigators used 60 DC-extracellular electrograms recorded simultaneously from epicardial and intramural sites on the left ventricle. Recordings were made during the first 15 minutes of coronary occlusion. The results of this important study may be summarized as follows: (1) The earliest activity during single premature beats (or initial beats of a series of ectopic activation) was found in the normal myocardium adjacent to the ischemic border zone. The same activation pattern was found for the initial beats leading to tachycardia and fibrillation. (2) There was no evidence for reentrant activity bridging the gap between the latest activity during the last basic beat and the earliest activity in the first ectopic beat. (3) Purkinje activity preceeded myocardial activity in ectopic activations and in the initial beats of tachycardia and fibrillation. (4) Epicardial activation patterns were similar during spontaneous ectopic beats and during stimulation of subendocardial sites where Purkinje activity was recorded. Therefore no evidence was found for reentry being responsible for single premature beats or for the initial ectopic beats of tachycardia. However, there was evidence of circus movement of wavefronts around an area of conduction block in the ischemic zone during maintained tachycardia.

Localization, revolution time and size of circus movements changed from beat to beat. Tachycardias were characterized by the presence of a single fairly large circus movement. When the ventricles fibrillated, conduction patterns in the ischemic zone were different, ie. reentrant patterns were multiple, seldom completed, and had small diameters (0.5 cm).

Janse et al. (1980) suggest that ectopic impulses which initiate ventricular tachycardia and fibrillation in early ischemia are not due to reentry. Janse et al. (1980) also show that Purkinje tissue may be the source of earliest ectopic impulse that initiates ventricular tachycardia. What mechanism could initiate ectopic activity in normal Purkinje tissue within 3 minutes of coronary occlusion? Katzung et al. (1975) and Kleber et al. (1978) have proposed that current flow between ischemic and normal tissue may be arrhythmogenic. Local current circuits are thought to be set up across the ischemic border because of potential differences between intracellular compartments of ischemic and non-ischemic tissue (Janse and Kleber, 1981). This current of injury flowing at the boundary between ischemic and normal tissue may contribute to the genesis of ectopic activity (Hoffman, 1966). The magnitude and direction of these currents are thought to change throughout the different phases of the cardiac cycle. In diastole, because of membrane potential differences between ischemic and non-ischemic cells, current flows from the intracellular compartments of ischemic cells, across the ischemic border, to the inside of normal cells. When both normal and ischemic tissue are activated, the

flow of current is in the opposite direction. That is, since activation of ischemic cells is delayed then the intracellular compartment of normal cells is positive with respect to the intracellular compartment of ischemic cells. Therefore current will flow intracellularly from normal cells to ischemic cells (Janse et al., 1980). Kleber et al. (1980) and Janse et al. (1980) have measured the magnitude of the injury current during different phases of the cardiac cycle. The currents flowing across the ischemic border were maximal when normal cells had repolarized and ischemic cells were still in their plateau phase. At that moment normal cells had recovered their excitability and the injury current therefore has a depolarizing effect on normal cells. This current might be strong enough to re-excite normal cells. However for injury current flow to occur, the apposition of fully repolarized and not yet repolarized cells requires a region of inexcitability (Janse and Kleber, 1981 -review). That is, when a region of inexcitability exists between normal and the ischemic tissue displaying delayed activation, electrotonic currents could be generated by the ischemic tissue. The region of inexcitability may be the ischemic border zone (Janse and Kleber, 1981). The electrotonic current could flow across the ischemic border and depolarize normal cells and thus be excitatory. Information on the presence of inexcitable zones in the ischemic border close to the sites of current sources is absent. Janse et al. (1980) have suggested that the injury current flowing between ischemic tissue and adjacent Purkinje fibers would be sufficient to prevent complete repolarization in Purkinje tissue. Purkinje tissue may therefore display automaticity,

depending on the membrane potential at which repolarization was prevented. Therefore Janse et al. (1980) have proposed that 2 different arrhythmogenic mechanisms occur during the first 10 minutes of ischemia. One mechanism is responsible for single or repetitive premature depolarizations. They proposed an automatic mechanism located at the normal side of the ischemic border. Automaticity is possibly induced by injury currents in normal Purkinje fibers. A second mechanism, proposed to underly maintained ventricular tachycardia was circus movement reentry. Ventricular tachycardia degenerated into fibrillation when activation wavefronts became fragmented into multiple independent wavelets of varying size and number (ie. micro-reentry). The role of injury currents in ischemia-induced arrhythmias has been discussed extensively by Janse and Kleber (1980).

Controversy still exists as to whether and to what extent activity of Purkinje tissue is affected by ischemia. The work of Scherlag (1974) and Janse et al. (1980) support the concept that Purkinje fibers are affected by ischemia. Janse et al. (1980) reported Purkinje activity always preceeded myocardial activity in the initial ectopic beats of tachycardia during ischemia. Recently Janse et el. (1985b) studied the involvement of the subendocardium in arrhythmogenesis during acute ischemia in isolted dog and rabbit hearts. The subendocardium was destroyed by filling the ventricular cavities with phenol. Ischemia still produced arrhythmias even when the subendocardial border zone, including the Purkinje system was destroyed. Reentry within the ischemic myocardium was also demonstrated. However tachycardia never

degenerated into fibrillation in hearts without a subendocardial border zone. Ectopic activity was seen in hearts without a subendocardial border zone and subjected to ischemia, even though ectopic activity could not be demonstrated in these hearts prior to ischemia. The ectopic activity also differed from that seen during ischemia in intact hearts (eg. fusion beats occurred). They postulated that triggered activity, abnormal automaticity or injury currents might be responsible.

Kaplinsky et al. (1979a) suggested that acute ischemia-induced arrhythmias occur in 2 phases: immediate (2-10 minutes after ligation) and delayed (12-30 minutes after ligation). The evidence from Janse et al. (1980) discussed above, suggests that the immediate phase of arrhythmias are reentrant in origin. Little is known about the second phase (delayed) of arrhythmias. Russel et al. (1984) recently studied differences in the electrophysiology of cardiac tissues during these 2 phases of arrhythmias in the dog. Greater mean epicardial delays and spatial heterogeneity of epicardial delays occurred during the first phase of arrhythmias than in the second phase. They have suggested that amelioration of epicardial delays and heterogeneity argues against reentry in the epicardium being the primary mechanism of arrhythmias during the second phase. However they did not rule out the possibility of reentry occurring at other sites. Thus it is possible that the 2 phases of arrhythmias occurring during ischemia may arise from different mechanisms.

B) ELECTROPHYSIOLOGICAL CHANGES DURING REPERFUSION AFTER ACUTE CORONARY ARTERY OCCLUSION

Tennant and Wiggers (1935) described the effects of coronary occlusion on the changes in regional myocardial contraction in dogs. They observed a progressive decrease in regional contractility of the ischemic area with the duration of ligation. They also re-established blood flow (reperfusion) by releasing the occlusion, and found that ventricular fibrillation often occured. Despite this early demonstration that arrhythmias may occur upon reperfusion, there was little immediate experimental attention directed towards studying the electrophysiologic changes that occur during reperfusion. Reperfusion can occur clinically as a consequence of relief of coronary spasm or during angioplastic or thrombolytic procedures. Reperfusion in response to therapeutic thrombolysis results in an increase in idioventricular rate and premature ventricular beats (Goldberg et al., 1983). The realization that reperfusion arrhythmias occur clinically as well as experimentally, and that reperfusion arrhythmias differ from ischemia-induced arrhythmias, has recently generated a marked increase in experimental investigation of reperfusion arrhythmias.

1. Changes in resting membrane potential

There have not been many studies on changes in resting membrane potential during reperfusion in intact hearts. Downar et al. (1977a) showed that heterogenous recovery of electrical activity in the ischemic zone upon reperfusion was associated with the appearence of arrhythmias. Their intracellular recordings from subepicardium show that

there may be an initial increase in membrane potential upon reperfusion after 6 min of LAD coronary occlusion. Penny and Sheridan (1983) investigated cellular electrophysiologic changes in epicardium during "low" flow or zero flow ischemia in isolated guinea pig hearts. They showed that after 20-30 minutes of ischemia, reperfusion resulted in recovery of action potential characteristics and that fibrillation sometimes occurred. From their microelectrode recordings it appears that there is an initial increase in membrane potential within one minute of reperfusion. However, when ventricular fibrillation occurred, between 1 and 2 minutes of reperfusion, the membrane potential decreased. Membrane potential increased after 9 minutes of reperfusion and the hearts no longer displayed arrhythmias. Although these two studies indicated that reperfusion may be associated with initial recovery of membrane potential in epicardial tissue, more detailed studies are needed to fully document changes in membrane potential and their relation to arrhythmias.

2. Effects on transmembrane action potentials

Penkoske et al. (1978) recorded simultaneous bipolar electrograms (epi-, endocardial, and intramural) from the ischemic and normal zones of cat hearts sublected to LAD coronary occlusion and reperfusion. In contrast to marked alterations in electrogram parameters induced by occlusion, reperfusion did not significantly alter the width, rise time, dV/dt or amplitude of electrograms recorded from all three areas of the ischemic zone, compared to values just before reperfusion. Both electrogram width and dV/dt returned to control levels in epicardial regions

within 30 seconds after reperfusion and before the onset of arrhythmia. However, intramural and endocardial regions exhibited continued depression of dV/dt. The significance of improvement of dV/dt was attributed to an improvement in localized conduction. At the time of termination of arrhythmia upon reperfusion, all electrogram parameters in all 3 regions returned to pre-occlusion values. Therefore heterogenous recovery of tissue from previous ischemia may be one mechanism of reperfusion arrhythmias. Kaplinsky et al. (1981) have shown a similar heterogenous improvement of electrograms recorded from the previously ischemic zone early in reperfusion in dog hearts. These investigators also have shown that two periods of ventricular arrhythmias exist during reperfusion: instantaneous (occurring within 1-2 minutes of reperfusion) and delayed (occurring 2-7 minutes of reperfusion). During the initial part of reperfusion, the increase in amplitude of local electrograms from the ischemic area was followed by premature ventricular beats. This arrhythmia degenerated into fibrillation seconds later. By 3 minutes of reperfusion, electrical activity was again synchronous in the previously ischemic zone.

Downar et al. (1977a) showed that reperfusion following 6 minutes of LAD coronary occluson in porcine hearts resulted in a heterogenous recovery of action potentials within the ischemic zone. Cells that were previously inexcitable during occlusion, exhibited near normal action potentials within seconds. However, the recovery of electrical activity following reperfusion didn't occur at the same rate in all cells within the previously ischemic zone. For example 2:1 responses occurred upon

reperfusion in a cell that was previously inexcitable. Another cell from the ischemic zone displayed large action potential alternating with small ones (Janse, 1982; Downar et al., 1977a). Penny and Sheridan (1983) also looked at changes in action potential characteristics in isolated guinea pig hearts and found that Vmax of action potentials improved just before the onset of fibrillation. Vmax usually increases as membrane potential increases. Vmax is also used as an index of conduction velocity. Penny and Sheidan (1983) suggested that improvement of Vmax may indicate that some activation of fast Na channels may be necessary for spread of arrhythmias upon reperfusion.

These results taken together indicate that reperfusion initially results in a heterogeneity of electrical activity in the previously ischemic zone. The heterogenous recovery of action potentials may be responsible for arrhythmias occurring during early reperfusion. However, "delayed" arrhythmias upon reperfusion occur at a time when membrane potential and Vmax have recovered within the previously ischemic zone.

3. Effects of reperfusion on conduction

Penkoske et al. (1978) measured conduction in cat hearts subjected to 35 min of coronary occlusion and then reperfusion. Reperfusion appeared to cause an improvement of conduction through epicardial regions of the previously ischemic zone (as assessed by increased dV/dt of local electrograms). However, myocardial and endocardial regions exhibited continued localized depression of conduction (decreased dV/dt). Also, these investigators found other discrepancies when they measured conduc-

tion time through the different regions. Within 1 minute of reperfusion conduction time showed no delay between endocardial and intramural regions. However there was conduction delay from intramural to epicardial layers. Conduction delay was seen just before the onset of arrhythmias due to reperfusion. They noted that although conduction time normalizes through mid-myocardial areas early after reperfusion, localized areas of depressed conduction velocity still existed. This heterogeneity of recovery after reperfusion may lead to arrhythmias. Murdock et al. (1980) also reported that after 10 minutes of occlusion, reperfusion resulted in improvement of conduction in the previously ischemic subepicardium and subendocardium of dogs. Improvement of conduction occurred within 5-10 seconds of reperfusion, and progressed towards control pre-occlusion values. They also used large bipolar composite electrodes to detect appearance of delayed or fractionated electrograms in the reperfused zone. They found that reperfusion resulted in progressive fractionation of electrograms and simultaneous arrhythmias. Delayed electrical activation also occurred in areas that did not show this during the late ischemic period. They considered this to represent re-emergence of activity from regions of slowly conducting tissue. Reperfusion also resulted in improvement of ischemia-induced conduction delay. Murdock et al. (1980) suggested that reperfusion re-established slow conduction through severely depressed areas and thereby caused reentrant pathways to form.

Localized areas of depressed conduction upon reperfusion may account for the heterogeneity of recovery upon reperfusion. The im-

provement in conduction through previously ischemic tissue may reflect re-establishment of slow conduction through severely depressed areas. Improvement of conduction during initial reperfusion always preceeded the onset of arrhythmias. Penny and Sheridan (1983) also showed an improvement of conduction time which preceeded the onset of arrhythmias during reperfusion. Other investigators (Levine et al., 1978) have shown that reperfusion accelerated conduction to supernormal values within 1-2 minutes of reperfusion.

4. Changes in excitability and refractoriness

Although reperfusion results in rapid restoration of amplitude of electrograms from an ischemic area, desynchronization of electrical depolarization takes several minutes to completely resolve (Penkoske et al., 1978; Kaplinsky et al., 1981). Reports on changes in refractoriness during reperfusion are conflicting. Naimi et al. (1977) reported that within seconds of reperfusion of ischemic myocardium in dogs, there was a shortening of the effective refractory period (ERP) within the ischemic zone (ie. further shortening from ischemia) compared to the non-ischemic zone. There was also an increased dispersion of ERP within the ischemic zone, which was greater in the transmural versus transepicardial direction. The shortening of ERP during early reperfusion resulted in maximal dispersion of refractoriness between ischemic and non-ischemic tissue, at a time when reperfusion arrhythmias were most often observed. These changes in ERP upon reperfusion, occurred after occlusion of 5 or 15 minutes duration. However the increased dispersion of ERP was greater after the 5 minute ligation. Shortening of ERP was

short lived, and ERP returned to pre-ligation values after 2 minutes of reperfusion. Penny and Sheridan (1983) also studied changes in ERP using microelectrode recordings from isolated guinea pig hearts undergoing low flow or zero flow ischemia. Action potential duration and ERP further shortened during reperfusion after 30 min ischemia. The shortening of duration and ERP occurred prior to the onset of reperfusion induced fibrillation. Abbreviation of ERP occurred during reperfusion following 30 minutes of ischemia, but not after shorter (5 min) or longer (60 min) periods of ischemia. In contrast some reports have indicated lengthening of ERP during reperfusion (Levites et al., 1975; Stewart et al., 1980). However in one of these studies (Levites et al., 1975), the ERP was measured 3-5 min after ligature release, which is well after the time at which shortening of ERP was reported in other studies.

Thus, it appears that there is an initial decrease of ERP upon reperfusion which preceeds the onset of ventricular arrhythmias. The dispersion of refractoriness between ischemic and non-ischemic zones adds to the general inhomogeneity of recovery between these two zones, and may be responsible for early arrhythmias upon reperfusion.

5. Arrhythmias

The mechanisms responsible for ventricular arrhythmias upon reperfusion remain unclear. Recent reviews have been published which discuss mechanisms responsible for reperfusion arrhythmias (Corr and Witowski, 1983; Manning and Hearse, 1984; Witowski and Corr, 1984). Studies by Kaplinsky et al. (1981) and Kabell et al. (1985) have indicated that

both reentry and automaticity may be responsible for reperfusion arrhythmias.

The heterogeneity of electrophysiological parameters early in reperfusion was associated with frequent periods of ectopic activity (Levites et al., 1975). At 1 minute of reperfusion, epicardial delay was present just before the onset of arrhythmias. Fractionation of electrical activity within the ischemic zone early in reperfusion was associated with return of arrhythmias (Murdock et al., 1980). In this latter study, increasing the pacing rate just before reperfusion enhanced the frequency and severity of reperfusion arrhythmias. These investigators assumed this to be an exacerbation of a reentrant mechanism. However, increased arrhythmic activity might also be caused by triggered activity. Kaplinsky et al. (1981) and Kabell et al. (1985) also reported increased fractionation of activity and delay of electrograms from reperfused zones. Janse et al. (1980) reported that circus movement reentry occured upon reperfusion. In their study, the initial beats leading to reentry were initiated outside the ischemic border and were not due to reentry. All of these authors have proposed that reentry is responsible for early reperfusion arrhythmias. Reentry was proposed because reperfusion resulted in non-homogenous electrophysiological characteristics which might provide the necessary conditions for reentry (ie. slow conduction; one-way conduction block, circuituous pathways). This hypothesis was further tested by Bolli et al. (1983). In their study, reperfusion arrhythmias were studied after 20 min ligations in dogs. Two sites on the LAD coronary artery were occluded.

However to increase tissue heterogeneity, one group of dogs was subjected to incomplete reperfusion. At the time of reperfusion a distal occlusion was maintained and only the proximal occlusion was released. Thus, three zones resulted: a normal zone; an ischemic zone; and a reperfused zone. This increased the tissue heterogeneity during reperfusion, compared to dogs which underwent complete reperfusion. The size of the ischemic and reperfused zones were determined by post-mortem dye perfusion. During incomplete reperfusion, there was an increased incidence of ventricular tachycardia and fibrillation, compared to dogs that underwent complete reperfusion. These investigators attributed the increase in arrhythmias to an enhanced generation of reentrant circuits.

Fujimoto et al., (1983) have suggested that different mechanisms may be responsible for arrhythmia induction and arrhythmia maintenance during reperfusion. They suggested that arrhythmia induction was initiated by rapid non-uniform recovery processes in the reperfused area. However maintenance of these arrhythmias and their degeneration into fibrillation may involve reentry utilizing the maximally depressed ischemic cells.

In contrast to the above studies, other investigators believe that changes in automaticity may play an important role in the genesis of reperfusion arrhythmias. Penkoske et al. (1978) studied reperfusion arrhythmias in cats subjected to 35 minutes of LAD coronary occlusion. They determined the intrinsic idioventricular rate by inducing complete A-V block with intense stimulation of the right cervical vagus nerve. Idioventicular escape rate was unchanged during occlusion arrhythmias,

but increased during reperfusion arrhythmias. The arrhythmias induced by reperfusion were prevented by rapid atrial pacing (ie. overdrive) in dogs without A-V block. Penkoske et al. (1978) therefore suggested that enhanced ventricular pacemaker activity may play a role in initiation of ventricular tachycardia upon reperfusion. Since epicardial conduction delay was present during the tachycardia, these workers proposed that slowed conduction was reponsible for maintenance of the tachycardia. Similar increases in ventricular automaticity have been reported in dogs upon reperfusion (Kaplinsky et al., 1981). However, in this latter study the onset of enhanced ventricular automaticity was seen late in reperfusion (2-7 minutes).

Sheridan et al. (1980) have attributed the increase in idioventricular rate after reperfusion in cat hearts to an increased rate of stimulation of alpha-receptors by catecholamines released during reperfusion. Ideker et al. (1981) showed that ectopic activity during reperfusion appeared in the epicardium at the border zone, and suggested the subendocardium as the source of ectopic activity. Kabell et al. (1985) have suggested that the earliest sites of activation during reperfusion arrhythmias were indicative of automatic foci in the subendocardium of the reperfused zone. Clinical demonstration of reperfusion arrhythmias have also shown a role for enhanced automaticity. Goldberg et al. (1983) have shown, in patients undergoing coronary recanilization by intracoronary thrombolysis, that transient arrhythmia developed upon restoration of antegrade coronary blood flow. Accelerated idioventricular rhythm was most often noted upon repertusion. No evidence for

increased ventricular automaticity has been found in 2 studies (Levites et al., 1975; Murdock et al., 1980). However, as pointed out by Corr and Witowski (1983), these studies used formaldehyde injection into the A-V node/His region to study ventricular automaticity. Corr and Witowski (1983) pointed out that formaldehyde may have either destroyed efferent sympathetic nerve fibres to the ventricles (i.e. adjacent to the A-V node) or that it destroyed potential pacemakers in the low A-V node or His-bundle. They reasoned that either action might abolish automaticity upon reperfusion.

Reperfusion arrhythmias may therefore involve different mechanisms of arrhythmias at different times during reperfusion. Kaplinsky et al. (1981) and Kabell et al. (1985) have provided evidence that reentry and automaticity are responsible for reperfusion arrhythmias. Reentry is thought to contribute to the malignant arrhythmias occurring immediately (within the first minute) following reperfusion. Automatic mechanisms predominate minutes later when non-sustained ventricular tachycardia occurs, but when fibrillation is less common.

C) CALCIUM AND ISCHEMIA AND REPERFUSION ARRHYTHMIAS

1. Calcium homeostasis during ischemia and reperfusion

Shen and Jennings (1972a,b) were the first to show that free intracellular calcium (Ca^{2+}) accumulates in the ischemic and reperfused myocardium of the dog. An increased uptake of Ca^{2+} was shown in dog hearts subjected to 40 minutes of LAD coronary occlusion followed by 20 minutes reperfusion. This protocol mimicked a transient ischemic episode since permanent damage to cells didn't occur. Cells that were irreversibly

injured after 60 minutes of occlusion and 20 minutes of reperfusion, did not show any increase in calcium uptake.

a) Calcium accumulation during ischemia

Nayler (1981) showed that intracellular accumulation of Ca^{2+} occurs during 60-90 minutes of global ischemia in isolated perfused rabbit hearts. Similar results have been shown by Bourdillon and Poole-Wilson (1981), who showed an accompanying increase in resting tension and decrease in developed tension. Bourdillon and Poole-Wilson (1981) attributed the effect of increased intracellular free Ca^{2+} to a redistribution of intracellular Ca stores. The increase in intracellular Ca^{2+} may in part be caused by leakage of extracellular myocardial Ca^{2+} (Hearse, 1977). However, there is strong evidence that it results from a decrease in Ca^{2+} sequestration at various subcellular sites and depletion of Ca chelating agents such a citrate and ATP (as a direct result of mitochondrial failure during ischemia) (Hearse, 1977). Nayler (1983) has hypothesized that a sequence of events can cause Ca^{2+} overloading during ischemia and reperfusion. As ATP stores decrease during ischemia, cytosolic sodium (Na^+) rises (because of inhibition of Na-K ATPase) and cytosolic Ca^{2+} increases (as a result of decreased activity of sarcolemmal and sarcoplasmic Ca^{2+}-ATPase). Added to this is the possibiity of enhanced entry of Ca^{2+} in exchange for accumulating intracellular Na^+. Catecholamines released during ischemia may activate slow Ca^{2+} channels which furhther contributes to raised cytosolic Ca^{2+}. Jennings and Reimer (1983) have suggested that ischemia may be divided into reversible and irreversible phases of damage. They have shown epi-

sodes of ischemia lasting 15 minuts were reversible in dog hearts. However irreversible injury started after 20 minutes of occlusion, and the first cells to be affected were cells within the subendocardial zone of ischemia including the left posterior papillary muscle. This result suggests that the subendocardium is affected after 20 minutes of occlusion in dog hearts.

The calcium channel blocking agent nifedipine prevents induction of myocardial contracture by ischemia (Henry et al., 1977; Nayler, 1980a,b). Similar results have been obtained with verapamil (Nayler 1980a,b). This suggests a direct role of the slow inward current in contributing to raised cytosolic Ca and subsequent increase in energy demands during ischemia. Perfusion with low extracellular concentration of Ca also prevents the heart from developing contracture during ischemia (Koomen et al., 1983). These results suggest that entry of Ca^{2+} into the cell via the slow inward current during ischemia may play a role in Ca^{2+} accumulation.

b) Calcium accumulation during reperfusion

Shen and Jennings (1972a,b) reported that 40 min of ischemia followed by 10 minutes of reperfusion led to a 10 fold increase in calcium uptake. The Ca uptake was linear during the first 10 minutes of reperfusion and was thought to be energy dependent. Also seen upon reperfusion were explosive cell swelling, mitochondrial and myofibrillar damage and the appearance of contraction bands and intra-myocardial Ca-phosphate deposits. Nayler (1981) hypothesized that cellular Ca^{2+} overload contributes to the onset of irreversible cell injury during

ischemia. Increased Ca-uptake upon reperfusion has been shown by other investigators (Bourdillon and Poole-Wilson, 1981; Nayler, 1981). The possible causes of massive Ca^{2+} overloading has been hypothesized by Nayler (1983) and was outlined in the previous section. Shen and Jennings (1972a,b) showed that the source of Ca contributing to overloading is the plasma reperfusing the tissue.

Nifedipine has been shown to permit complete mechanical recovery of hearts subjected to ischemia and reperfusion (Henry et al., 1977). Nayler (1980a,b) showed that nifedipine, verapamil and β-blocking drugs improved recovery of active tension during reperfusion. These drugs also attenuated the rise in resting tension and decreased the massive uptake of Ca^{2+} by mitochondria. However, these drugs had to be given during ischemia to elicit a protective effect. Therefore it appears that Ca-influx through slow Ca channels contributes to massive overloading of Ca^{2+} upon reperfusion. However Nayler (1980b) has pointed out that Ca^{2+} accumulation does occur upon reperfusion in the presence of calcium channel blocking agents. Therefore their effectiveness cannot be attributed to an effect of simply counteracting Ca^{2+} influx through slow channels. Nayler (1980b) suggests that, because calcium channel blocking agents slow Ca^{2+} influx early in ischemia, they may slow ATP utilization. Sufficient ATP may be spared to maintain intracellular Ca^{2+} homeostasis, and thereby prevent mitochondrial Ca^{2+} overload. Grinwald (1982) has shown that reperfusion enhanced uptake of Ca^{2+} is strongly dependent on the concentration of extracellular Na. This suggests that Na-Ca exchange is active during reperfusion. Grinwald

(1982) suggested that enhanced Na-Ca exchange during reperfusion may be due to increased cytosolic Na.

In summary, during ischemia and reperfusion, cells become overloaded with Ca^{2+}. The possible specific routes of Ca^{2+} entry include the slow calcium channel and Na-Ca exchange. Protection against cellular Ca-overload can be achieved with calcium channel blocking agents, β-blocking agents and perfusion with low extracellular concentrations of Ca. Cellular Ca-overload may lead to cell death by activating energy consuming enzymes, and by disruption of the mitochondria. It is therefore possible that enhanced entrance of Ca or Ca-overload may be responsible for arrhythmias during ischemia and reperfusion.

2. The slow inward Ca current

A number of reviews have been published which discuss the properties of the slow Ca channel, the slow inward current, and their modulation by various agents. Some of the more recent reviews are: Kohlhardt, 1981; McDonald, 1982; Keung and Aronson, 1983; Reuter, 1983; Tsien, 1983; Sperelakis, 1984; Gilmour and Zipes, 1985; Trautwein and Cavalié, 1985. The importance of the slow inward current in excitation-contraction coupling has been reviewed by Fabiato (1983).

The configuration of the potentials recorded from Purkinje fibers is characterized by "4 phases". The action potential is characterized by a negative maximum diastolic potential (MDP) (-80 to -90 mV) and a rapidly rising upstroke (phase 0). The upstroke has an overshoot (+ 30 mV), followed by an early phase of repolarization (phase 1), and a prolonged plateau phase of slow repolarization (phase 2). Phase 3 corre-

sponds to the final repolarization phase of the action potential. Following phase 3, Purkinje fibers often show a spontaneous diastolic depolarization (phase 4). The action potential of ventricular muscle is similar to that of Purkinje fibers, but phase 1 and 2 are less prominent and phase 4 does not show spontaneous diastolic depolarization. When the membrane of a Purkinje fiber or ventricular muscle is depolarized to threshold (-60 to -70 mV) the membrane becomes highly permeable to sodium (gNa) (Beeler and Reuter, 1970; Dudel and Rudel, 1979; New and Trautwein, 1972). During the upstroke and phase 1 of the normal action potential, the membrane is in a range of potentials (-40 mV and more positive) at which another inward current, called the slow inward current (Isi) becomes activated. Initial evidence for a Ca-dependent Isi came from voltage clamp experiments performed in Purkinje fibers (Reuter, 1967). Isi is activated during the plateau phase of cardiac action potentials and also is the primary inward current during spontaneous activity of the sinus node. Isi in sinus nodal cells is also reduced when there is a decrease in $[Na]_o$. Therefore Isi in sinus nodal cells may not be a pure calcium current. When INa is inactivated by depolarization to potentials positive to -50 mV, cardiac fibers are able to generate action potentials. The action potentials that occur when the fast inward sodium current is inactivated have been called "slow responses" (Rougier et al., 1969). A number of agents have been shown to affect Isi. The calcium channel blocking agents, verapamil and nifedipine have been shown to selectively inhibit the slow inward current (Kohlhardt et al., 1972; Kohlhardt and Fleckenstein, 1977). β-

adrenergic agonists have been shown to increase Isi (Reuter, 1974; Reuter and Scholtz, 1977). Recently, a new agent, BAY-K8644 which is a dihydropyridine like nifedipine, has been shown to increase conductance of Ca through the slow calcium channel (Thomas et al., 1985). This agent has therefore been termed a "calcium agonist" by Schramm et al., (1983). However this term does not fully explain the specific action of BAY-K8644 to increase Ca-conductance through the slow Ca^{2+} channel.

3. Effects of calcium channel blocking agents on arrhythmias induced by ischemia and reperfusion

Ca accumulates during ischemia and reperfusion, and calcium channel blocking agents may reduce this accumulation. The route of entry of Ca for this accumulation may therefore be the slow inward current. The slow inward current may be involved in various mechanisms of arrhythmia. The effects of decreasing Ca influx on arrhythmias due to ischemia and reperfusion have been studied. The electrophysiological parameters that have been measured during ischemia and reperfusion with calcium channel blocking agents are usually changes in conduction and suppression of arrhythmias.

a) Calcium channel blocking agents and ischemia-induced arrhythmias

Kaumann and Aramedia (1968) were the first to demonstrate that verapamil provides nearly complete protection against fibrillation in dogs subjected to coronary artery ligation. This early report on the antifibrillatory and antiarrhythmic actions of verapamil has been confirmed by others in dogs (Elharrar et al., 1977b; Fondacaro et al.,

1978; Brooks et al., 1980) and in pigs (Bergey et al., 1984). Similar protection has been demonstrated with diltiazem (Clusin et al., 1982). Studies with nifedipine (and other dihydropyridines) are more controversial. Antiarrhythmic action of nifedipine during occlusion has been shown in the rat (Fagbemi and Parrat, 1981; Thandroyen, 1982), but not in the pig (Bergey et al., 1984). Parrat (1982) has reviewed some of the possible mechanisms that may be involved in the antiarrhythmic action of calcium channel blocking agents during ischemia. Possible mechanism include: (1) blockade of slow calcium channels in ischemic myocardium, (2) improvement of conduction within the ischemic region, (3) increase in blood flow within the ischemic region and (4) preservation of cell integrity within the ischemic region. Reentrant excitation is thought to be responsible for ischemia-induced arrhythmias. Slow conduction may be present during ischemia. Potassium (Hill and Gettes, 1980) and catecholamines (Riemersma, 1982- review) are released from ischemic tissue. Extracellular accumulation of both favour development of slow response action potentials. Slow response action potentials, if present during ischemia, would be inhibited by calcium channel blocking drugs. However, there is no direct evidence that slow response Ca-mediated action potentials are responsible for arrhythmias during ischemia. Electrograms recorded from ischemic tissue are of low voltage, prolonged duration and exhibit slow conduction. Ischemic tissue may be the source of slow conduction. Slowed conduction is thought to result in electrical inhomogeneity and is believed to play an important role in generating reentry. One may expect that calcium

channel blocking agents would further increase conduction delay within the ischemic zone. However, verapamil (Elharrar et al., 1977; Fondacaro et al., 1978; Nakaya et al., 1980; Peter et al., 1983) and diltiazem (Nakaya et al., 1980) have been shown to improve conduction during ischemia. Nifedipine (Peter et al., 1983) does not improve conduction during ischemia. Improvement in conduction is observed within the first few minutes of ischemia, at a time when early ventricular arrhythmias occur. Similar changes are not seen with other classes of antiarrhythmic drugs. Drugs such as lidocaine increase ischemia-induced conduction delay (Nakaya et al., 1980). The reason for the improvement of conduction in the presence of calcium channel blocking agents remain unknown. Nakaya et al. (1980) ruled out the possibility that the coronary vasodilator action of these agents may play a role in reducing conduction delay. Parrat (1982) suggested that these agents may improve conduction by reducing myocardial oxygen demands. Myocardial oxygen demands would be reduced as a consequence of bradycardia, reduction of afterload, or depression of myocardial contractile function.

Clusin et al. (1983) have suggested another mechanism for the antiarrhythmic action of calcium channel blocking drugs during ischemia. They have proposed that ischemia-induced fibrillation is caused by cellular Ca overload which activates a depolarizing injury current. Diltiazem and reduced extracellular ionized Ca (by infusion with sodium citrate) were found to have a similar antiarrhythmic effect (Clusin et al., 1982). Clusin et al. (1984) have also shown that the antifibrillatory effect of diltiazem correlated with inhibition of "injury

currents" and increase in membrane potential. In summary, the electrophysiologic mechanisms whereby calcium channel blocking agents inhibit ischemia-induced arrhythmias is incompletely understood.

b) Calcium channel blocking agents and reperfusion-induced arrhythmias

There is considerable controversy as to the effectiveness of calcium channel blocking agents in suppressing reperfusion arrhythmias and fibrillation. Verapamil has been shown to protect against reperfusion induced arrhythmias in the dog heart (Ribiero et al., 1981; Brooks et al., 1980; Sugiyama et al., 1980). Other studies in canine hearts failed to show any protective effects of verapamil (Naito et al., 1981; Pelleg et al., 1985). Diltiazem did not affect reperfusion arrhythmias in dog hearts (Sheehan and Epstein, 1982), whereas protection was evident in rat hearts (Weishaar and Bing, 1980). Nifedipine has been shown to be antiarrhythmic in rat hearts (Parrat and Coker, 1983). The mechanisms underlying the effectiveness of calcium channel blocking agents in suppression of reperfusion arrhythmias are not known. Reperfusion arrhythmias may be associated with enhanced ventricular automaticity and rapid idioventricular rate (Penkoske et al., 1978). Protection afforded by calcium channel blocking agents may be through inhibition of this mechanism of arrhythmia. Corr and Witowski (1983) have suggested that the increase in idioventricular rate during reperfusion may be related to increased Ca^{2+} uptake with subsequent induction of oscillatory afterpotentials similar to those induced by digitalis (Ferrier, 1977- review). Carbonin et al. (1981) showed that verapamil

inhibited both reperfusion and digitalis-induced arrhythmias in rat hearts. They suggested that the protective action of verapamil in both situations might be due to abolition of oscillatory afterpotentials. Corr and Witowski (1983) have also suggested that alpha-receptor mediated adrenergic influences may increase the slow inward current during reperfusion, and that the protection afforded by calcium channel blocking agents is through their alpha-receptor blocking properties.

D) THE ROLE OF CALCIUM AND THE SLOW INWARD CURRENT IN MECHANISMS OF ARRHYTHMIA

Basic mechanisms of arrhythmia can be classified as follows: abnormalities of impulse initiation; abnormalities of impulse conduction; or a combination of both. This section will deal with the role of the slow inward current and increased intracellular calcium in the generation of these mechanisms in ventricular tissues.

1. Arrhythmias caused by abnormal impulse initiation

a) Normal automatic mechanism

Automaticity is a normal property of cells of the His-Purkinje system of the ventricles. The basis for automaticity is a slow decrease in membrane potential during diastole. This is referred to as phase 4 depolarization or slow diastolic depolarization. In the normal heart, the automaticity of cells of the sinus node is rapid enough to overdrive potentially automatic cells elsewhere in the heart. However any intervention which decreases intercellular coupling might be expected to increase automaticity of latent pacemakers. Uncoupling can be caused by an increase in intracellular calcium (Dahl and Isenberg, 1980). For

example, digitalis inhibits the Na-K ATPase and causes an increase in Na_i with resultant increase in Ca_i via Na-Ca exchange, and ultimately cellular uncoupling (Weingart, 1977).

Normal automaticity of Purkinje tissue can be suppressed by pacing (Dangman and Hoffman, 1983). This phenomena is called overdrive suppression. A recent review of mechanisms of overdrive suppression has been published (Vassalle, 1985). Musso and Vassalle (1982) have also suggested that overdrive may also lead to an accumulation of calcium. Accumulation of calcium initially causes an increase in the slope of diastolic depolarization but also shifts the threshold to less negative potentials. The latter effect contributes to temporary suppression of automaticity in Purkinje tissue.

b) Abnormal automatic mechanisms

Ventricular muscle cells don't normally show spontaneous diastolic depolarization. However, when the resting potential of these cells is decreased to less than -60 mV, spontaneous diastolic depolarization may occur and may initiate regenerative depolarizations (Katzung, 1974; Imanishi and Surawicz, 1976). The abnormal automaticity that results has been called depolarization-induced automaticity (DIA). Reviews on DIA have been published by Elharrar and Zipes (1980) and Surawicz (1980). DIA has also been observed in Purkinje tissue (Hauswirth et al., 1969; Imanishi, 1971). Because of the low level of membrane potential at which this automaticity occurs, the action potentials are slow responses (ie. action potentials with upstrokes dependent on the slow inward current) (Cranefield, 1975). DIA has been shown to occur in

Purkinje fibers surviving in canine infarcts (Friedman et al., 1973; Lazzara et al., 1973), and in diseased human ventricular myocardium (Gilmour et al., 1983). Unlike normal automaticity, DIA can't be suppressd by rapid pacing (Ferrier and Rosenthal, 1980; Dangman and Hoffman, 1983). DIA is accelerated by catecholamines (Katzung, 1974; Imanishi and Surawicz, 1976). Catecholamines are believed to exert this effect by increasing the slow inward current responsible for DIA. Elevation of extracellular Ca concentration increases the slope of phase 4, the overshoot, dV/dt, and increases the range of membrane potentials at which DIA occurs (Katzung, 1975). DIA can be inhibited by verapamil (Elharrar and Zipes, 1980). Therefore DIA is inhibited by maneuvers which inhibit Isi and is promoted by agents which increase Isi.

Triggered activity, another mechanism of abnormal impulse initiation, is caused by afterdepolarizations. Afterdepolarizations are subthreshold depolarizations that are coupled to preceeding action potentials and which may occur before or after repolarization is complete. Afterdepolarizations that occur before complete repolarization are referred to as early afterdepolarizations (Cranefield, 1977). Early afterdepolarizations may reach the threshold potential of the slow inward current, and thereby result in 1 or more repetitive action potentials at a low membrane potential. These action potentials are presumably slow responses (Cranefield, 1975; Cranefield, 1977). There are problems associated with characterization of early afterdepolarizations as triggered activity. The spontaneous action potential that follows the normal action potential arising from a high membrane poten-

tial is triggered. However, if a series of spontaneous action potentials arise before the cell repolarizes, then the phenomenon is presumably the same as DIA. Early afterdepolarizations are dependent on the slow inward current for generation of regenerative responses. Early afterdepolarizations have been shown to be caused by a number of experimental interventions (Cranefield, 1975). These include reduction of $[K]_o$ (Gadsby and Cranefield, 1977), catecholamines (Cranefield, 1975), and drugs such as the β-blocker sotalol (Strauss et al., 1970), N-acetylprocainamide (Dangman and Hoffman, 1981), and quinidine (Roden and Hoffman, 1985).

Afterdepolarizations may occur after repolarization of the action potential is complete. These afterdepolarizations are referred to as a delayed afterdepolarization (DAD) (Cranefield, 1977) or oscillatory afterpotential (Bozler, 1943; Ferrier, 1977). These afterdepolarizations may be subthreshold or may attain threshold and initiate premature responses. Therefore, the resulting action potentials are triggered. These afterdepolarizations have been found to occur in Purkinje fibers intoxicated with digitalis (Ferrier et al., 1973) and are called oscillatory afterpotentials (OAP's). They are also found in ventricular muscle fibers intoxicated with digitalis (Ferrier, 1976). The mechanism responsible for afterdepolarization in the presence of digitalis is thought to be through inhibition of the Na-K ATPase. This results in an increase in intracellular Na, which in turn leads to increase in intracellular Ca via sodium-calcium exchange. The increase in intracellular Ca is thought to activate an oscillatory current underlying OAP's (ie.,

the transient inward current-TI) via an oscillatory release of Ca from the sarcoplasmic reticulum (Kass et al., 1978a,b). OAP'S can be suppressed by agents which block the slow inward current, such as manganese (Ferrier and Moe, 1973) and verapamil (Rosen and Danilo, 1980). Therefore the slow inward current modulates or enhances the amplitude of OAP's by enhancing Ca influx. Kass et al. (1978a) have shown that the amplitude of the TI varies directly with the extracellular calcium concentration, and can be blocked by manganese. Vassalle and Mugelli (1981) have suggested that the TI current may be a normal component of cardiac electrical activity, and may be enhanced as intracellular calcium levels are increased. On the other hand, Wit and Rosen (1983) have suggested that triggered activity may terminate spontaneously due to Na or Ca accumulation in the cell in response to rapid heart rate. This may be the same mechanism as that of overdrive suppression in normal Purkinje pacemakers (Musso and Vassalle, 1982).

Triggered activity arising from afterdepolarizations may also be induced by other agents or procedures which enhance calcium influx via increase in Isi such as catecholamines (Nathan and Beeler, 1975; Wit and Cranefield, 1976; 1977). Afterdepolarizations also occur in hypertrophied ventricular myocardium (Aronson, 1981) and diseased human ventricular myocardium (Gilmour et al., 1983). Triggered activity is accelerated rather than depressed by overdrive pacing. The amplitude of the afterdepolarizations increase with increasing driving rate. In contrast, normal automaticity in Purkinje tissue can be suppressed with increasing driving rate.

Thus OAP's (DAD's) are enhanced by interventions which increase intracellular calcium. These afterdepolarizations are enhanced by agents which increase the slow inward current and can be blocked by agents which block this current.

2. Arrhythmias caused by abnormalities of impulse propagation: reentry

The basic requirement for reentry is conduction block (transient or unidirectional). The impulse travels around an area of block and approaches the point of block from the opposite direction. If the returning impulse succeeds in traversing the area of block retrogradely, it may re-excite the rest of the heart. The magnitude of the Na inward current and the rapidity with which this current reaches it's maximum intensity (the upstroke velocity of phase 0) is one determinant of the speed of propagation. Moderate depolarization of myocardial cells will result in decreased availability of the Na channels and therefore slowing of conduction. The action potential in this situation is referred to as a depressed fast response. Depressed fast responses may contribute to the genesis of reentrant arrhythmias. However, if the membrane potential is decreased to a level at which Na-channels are completely inactivated, Ca-dependent slow responses may also contribute to re-entrant arrhythmias because of their slow conduction velocities (Cranefield, 1975). Wit et al. (1972a,b) demonstrated that re-entrant excitation can occur in short bundles of canine Purkinje fibers superfused with high concentrations of K^+ and epinephrine. Slow conduction in this model depended on abolition of the fast sodium upstroke and initiation of slow response action potentials. Therefore, Cranefield et

al. (1972) suggested that re-entrant excitation may depend on the appearance of areas in which the normal action potential is replaced by the slow response (Cranefield, 1975).

Recently Antzelevitch et al. (1980) described a kind of reentry, called reflection, which occurs in unbranched bundles of Purkinje fibers. In their model of reflected reentry, slow conduction doesn't occur along the bundle. Instead there is delayed activation of part of the bundle, resulting from electrotonic activation of a region distal to an inexcitable segment. The inexcitable segment of the bundle does not generate action potentials. Therefore impulses will be blocked at this segment. However, action potentials proximal to the site of block will generate axial current flow through the inexcitable cable. The electrotonic manifestation of the impulse can depolarize excitable fibers distal to the area of inexcitability. The action potential initiated distal to the inexcitable segment can cause retrograde axial current flow through the inexcitable segment and thereby reexcite the proximal segment. Thus impulse transmission occurs over the same pathway in both directions. Antzelevitch and Moe (1981) have shown that when slow response action potentials are present in the inexcitable segment, reflection does not occur. Therefore, in contrast to Cranefield's proposal, slow action potentials may not be necessary for reflected reentry.

3. Arrhythmias generated by a combination of automaticity and impulse conduction: parasystole

Parasystole is classically thought to be caused by ectopic discharges from a pacemaker which is protected by entrance block from discharges of the sinus node. Therefore discharges from the sinus node cannot invade (i.e. entrance block) and excite the automatic focus. The ectopic discharges may occur from a low resting membrane potential (and therefore is presumably DIA) or from a high membrane potential. Entrance block is a common characteristic of depolarized areas of tissue which are automatic and which are connected to normally polarized tissue (Ferrier and Rosenthal, 1980). Jalife and Moe (1976, 1979) have also shown that entrance block may occur in regions of normal automaticity if they are surrounded by depolarized or inexcitable tissue. Although parasystolic foci exhibit entrance block, they may exhibit exit conduction. Thus arrhythmias are generated by extrasystoles that propagate out of the focus.

The discharge pattern of a parasystolic focus may be electronically modulated by activity outside the focus (Jalife and Moe, 1976, 1979; Ferrier and Rosenthal, 1980). That is, subthreshold depolarizations arriving early in diastole of the pacemaker will delay subsequent discharge of the pacemaker, whereas impulses arriving late in diastole accelerate the discharge of the pacemaker.

Parasystolic activity may occur at low membrane potential (Ferrier and Rosenthal, 1980) and is therefore dependent on Isi for pacemaker activity in this situation. Gilmour et al. (1983) have shown that auto-

maticity in diseased ventricular myocardium which shows varying degrees of modulation may be suppressed by verapamil.

Reviews which discuss mechanisms of cardiac arrhythmias in detail have been published by Hoffman and Rosen, 1981; Wit and Rosen, 1983; Gilmour and Zipes, 1985.

4. A model of ischemia and reperfusion arrhythmias

Technical difficulties prevent study of cellular electrophysiological changes in hearts subjected to occlusion and reperfusion. Therefore, various investigators have studied cellular electrophysiological changes in isolated tissues exposed to ischemic conditions. One model was recently developed by Ferrier et al. (1985a). In this model, cellular electrophysiological changes occurring in Purkinje and muscle tissues were studied using standard microelectrode techniques. Preparations were exposed to ischemic conditions, which included hypoxia, acidosis, 20 mM lactate and zero substrate (Table 1, and Ferrier et al., 1985a). It is known that ventricular myocardium deprived of its oxygen supply quickly undergoes metabolic and electrophysiologic changes. Reduction in oxygen availability will result in cessation of ATP production. The ischemic conditions employed in the model by Ferrier et al. (1985a) were specifically used to inhibit energy metabolism in Purkinje and muscle tissue. Hypoxia was chosen to inhibit aerobic metabolism. Substrate exclusion was chosen to mimic substrate extraction in poorly perfused tissue. Both of these conditions aid in inhibiting aerobic metabolism in muscle tissues. However, Purkinje tissues contain large stores of glycogen which can be metabolized anaerobically (Opie, 1969).

Therefore low pH and lactate were included to inhibit anaerobic metabolism (Rovetto et al., 1975). All of these conditions have been shown to occur in the ischemic myocardium (Elharrar and Zipes, 1977 - review). This model is incomplete since it doesn't contain elevated K^+ (Hill and Gettes, 1980), lysophospholipids (Sobel et al., 1978), and catecholamines (Riemersma, 1982 - review), all of which accumulate in the ischemic myocardium. However, Ferrier et al. (1985a,b) have shown that superfusion of canine Purkinje fibers attached to papillary muscle with the "ischemic" solution results in several distinct electrophysiological changes associated with arrhythmogenic mechanisms. Exposure to ischemic conditions for 40 min resulted in decrease in membrane potential, abbreviation of action potentials, and bi-directional block between muscle and Purkinje tissue. Ischemic conditions also decreased developed tension in Purkinje tissue (Ferrier et al., 1985b). Return to non-ischemic conditions (reperfusion) resulted in a sequence of responses in Purkinje tissue: initial hyperpolarization, progressive depolarization, inexcitability, and final repolarization. The depolarizing phase was associated with appearance of oscillatory afterpotentials (OAP). Final repolarization was associated with depolarization induced automaticity (DIA), during which Purkinje tissue functioned as a parasystolic focus. The mechanical response in Purkinje tissue upon reperfusion was as follows: initial return of developed tension, followed by a decline of developed tension, and then final recovery of developed tension. The electrophysiological responses during ischemic conditions suggest an alteration in the slow inward current, ie. shortened action potentials.

The mechanical response during ischemic conditions also suggest alterations in the slow inward current - or calcium handling. The appearance of OAP's on reperfusion suggest that calcium overload occurs upon reperfusion. Reperfusion-induced inexcitability followed by automaticity at low membrane potential suggests a sequence of "inactivation" and "reactivation" of the slow inward current. Reperfusion also was associated with a cyclic return, disappearance, and return of contractility of Purkinje tissue.

E) Objective

The objective of the investigations described in this thesis was to determine the contribution of the slow inward current to the genesis of mechanisms of arrhythmia in the model of ischemia and reperfusion proposed by Ferrier et al. (1985a). The specific objectives were:

a) To evaluate protection afforded by calcium channel blocking agents during ischemic conditions and reperfusion. This would determine which mechanisms of arrhythmia were related to the slow inward current.

b) To determine the changes in the availability of slow response action potentials during ischemic conditions and reperfusion. The changes in availability of slow response action potentials during ischemic conditions and reperfusion can be related to mechanisms of arrhythmia and cyclic changes in the availability of the slow inward current.

c) To determine whether the inactivation of slow response action potentials can be reversed by a "calcium agonist", BAY-K8644, which

increases Ca-conductance through the slow channel. This would more clearly determine whether cyclic changes in the availability of the slow inward current are responsible for the cyclic dissapearance and reapperance of DIA.

METHODS

A. TISSUE PREPARATION

Adult male mongrel dogs (15-20 Kg) were anesthetized with sodium pentobarbital administered intravenously (30 mg/kg). Previous unpublished studies from our lab have indicated that the response of preparations from female dogs to ischemic conditions and reperfusion are inconsistent during the year. The changes in MDP and spontaneous activity were attenuated in preparations removed from female dogs. The heart was rapidly removed through a parasternal incision in the chest. The heart was fibrillated to facilitate removal of preparations. Preparations consisting of false tendons (specialized conducting Purkinje tissue) attached to papillary muscle were dissected from both ventricles. Preparations were kept in a beaker containing modified Tyrode's solution at room temperature. The Tyrode's solution in the beaker was bubbled continuously with a gas mixture of 95% oxygen and 5% carbon dioxide (Union Carbide, Canada Limited).

For study, a preparation was mounted with the endocardial surface facing upwards in a wax tissue bath (Figure 1). The preparation was fastened in the bath with stainless steel pins. The preparation was slightly raised above the bottom of the wax bath to allow perfusion of the underside of the tissue. In other studies, isolated free running Purkinje fiber preparations dissected from papillary muscles, were mounted in a wax tissue bath. One end of the Purkinje fiber was fastened with a stainless steel pin (Figure 1).

Figure 1

Diagrammatic representation of experimental preparations. A: Schematic representation of a Purkinje fiber-papillary muscle preparation. Microelectrode recordings were made from Purkinje and muscle tissue. Stimulation was applied to the endocardial surface of muscle or to the Purkinje fiber. Developed tension was also monitored from Purkinje tissue (not illustrated). B: Schematic representation of an isolated Purkinje fiber preparation. Current was applied to the surface of the preparation immediately adjacent to the test segment from which electrical and mechanical records were made.

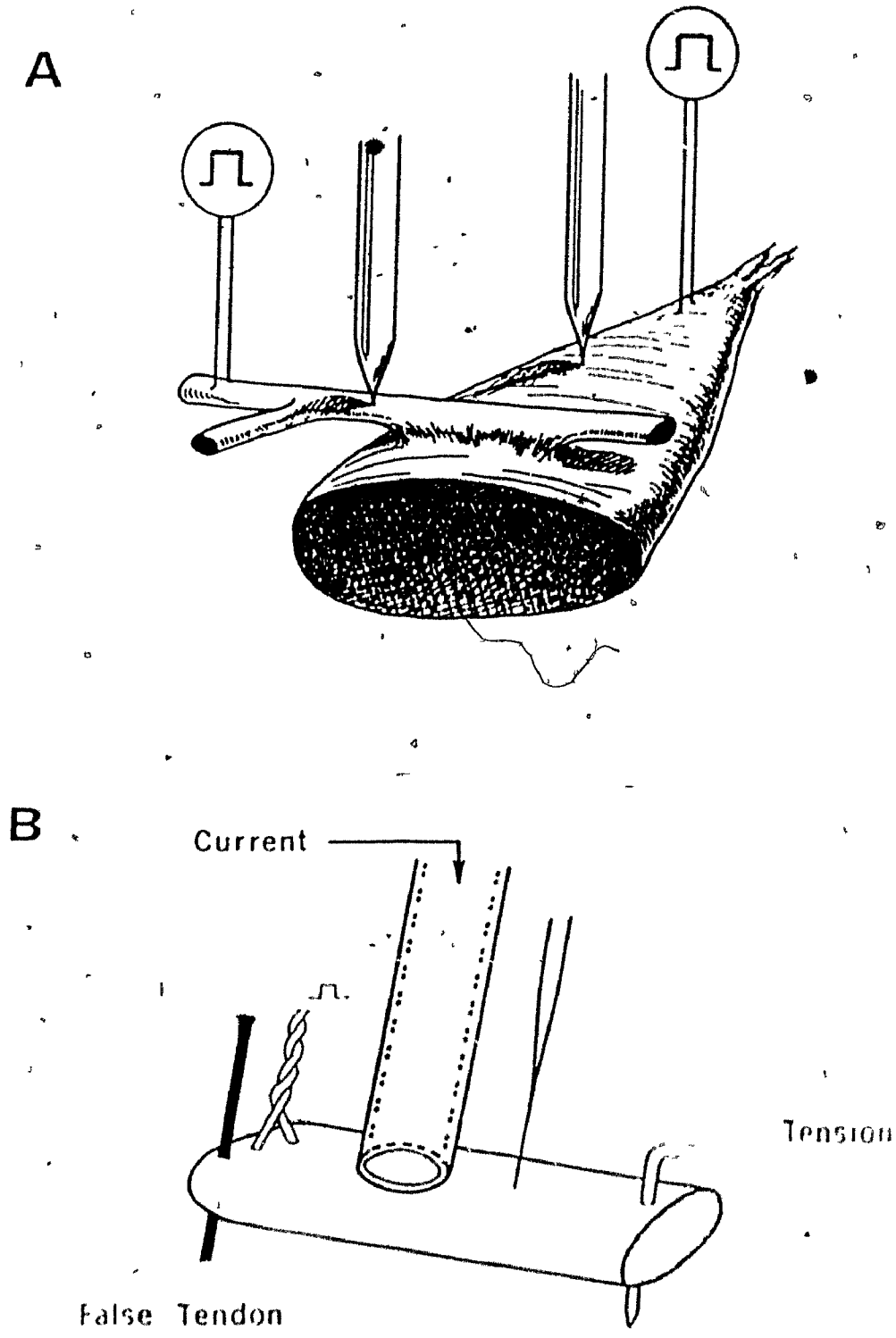

Figure 1

B. STIMULATION PROTOCOL

1. Purkinje fiber-papillary muscle preparations

Preparations were stimulated with bipolar silver electrodes placed on the Purkinje fiber or apex of the papillary muscle (Figure 1). Stimuli were rectangular pulses, 3 msec in duration and approximately 1.5 times the threshold voltage. The threshold voltage was the minimum voltage which elicited contractions in the preparation. Contraction of the preparation was observed with a microscope placed above the tissue bath. The pattern of stimulation consisted of trains of 15 pulses separated by 3 sec pauses (Pulsar 4i and 6i, Frederick Haer and Co.). The basic cycle length (interval between stimuli) during the train was set using a Pulsar 4i. After a train of 15 pulses from the Pulsar 4i, a "done" pulse from the pulsar 4i triggered the external start of the Pulsar 6i (Figure 2a). The Pulsar 6i was set to generate a pause (3 sec) between trains of stimulation. At the end of the pause in stimulation, a "done" pulse from the Pulsar 6i triggered the external start of the Pulsar 4i (Figure 2a). The basic cycle length (BCL) was usually 500 msec but was varied in some experiments.

2. Isolated Purkinje fiber preparations

The stimulation protocol used in these experiments was the same as that used for the Purkinje fiber-papillary muscle preparations. However, during the pause in stimulation, 3-sec long current pulses of varying amplitude were delivered through a glass pipette that contained a chlorided silver wire and was filled with Tyrode's solution. Current pulses were obtained from a digital stimulator (Pulsar 6i) operating in

Figure 2a

Schematic representation of electrophysiologic apparatus used for stimulation and recording from Purkinje fiber-papillary muscle preparations.

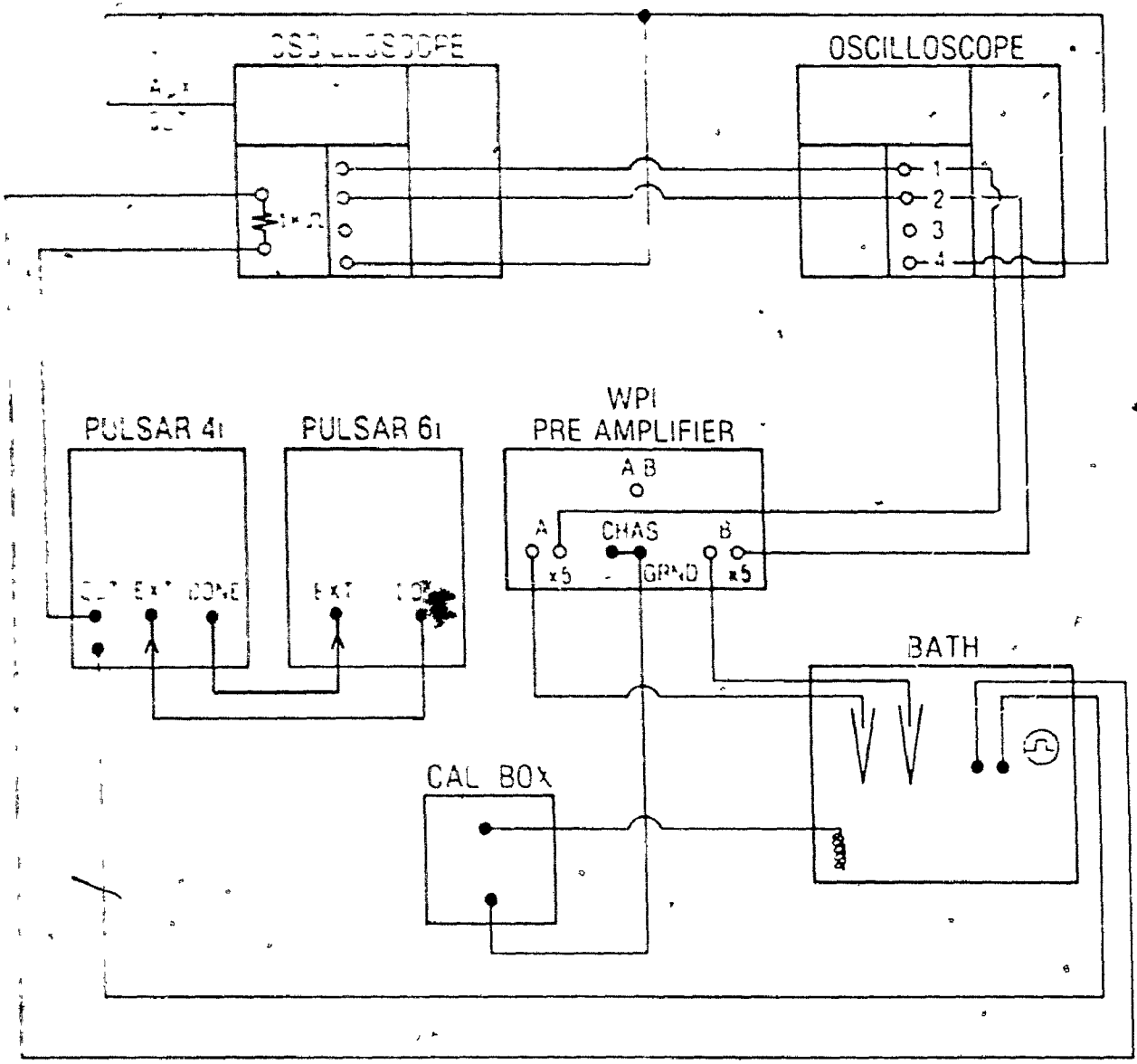
OSCILLOSCOPE
OSCILLOSCOPE
1
2
3
4
PULSAR 4i
PULSAR 6i
WPI
PRE AMPLIFIER
A B
A
CHAS
B
x5
GRND
x5
BATH
CAL BOX

Figure 2d

the constant voltage mode (Figure 2b). The current pulses were initiated 20 msec after the last stimulus of the train. The current pulses were turned on during the refractory period so that no extra beats could be elicited by direct stimulation. The current pulses elicited during the pause in stimulation were used to depolarize the membrane to potentials less than -65 mV, at which Purkinje fibers exhibit automaticity. In some experiments continuous current pulses were applied during the train of stimulation so as to hyperpolarize the membrane.

C. ELECTRODES

1. Stimulating electrode

Preparations were stimulated using a bipolar silver electrode which was placed on the surface of the preparation. The bipolar silver electrode was connected to "stimulator 1". "Stimulator 1" was used to set the basic cycle length, and the voltage for stimulation.

2. Ground electrode

The ground electrode was fabricated using a silver wire placed in an electrolytic solution containing 0.1 N HCL. Chloride was electroplated on the silver wire using a 1.5 V dry cell. The electrode was then gently rinsed with deionized water to wash off any excess HCL. This electrode was then placed in the wax tissue bath. The electrode was secured in place with the use of plastocene outside the tissue bath. This electrode was used as a reference for intracellular membrane potential measurements i.e. the electrode served as a reference ground for the tissue bath (Figure 2).

Figure 2b

Schematic representation of electrophysiologic apparatus used for extracellular current application and recording from isolated Purkinje fibers.

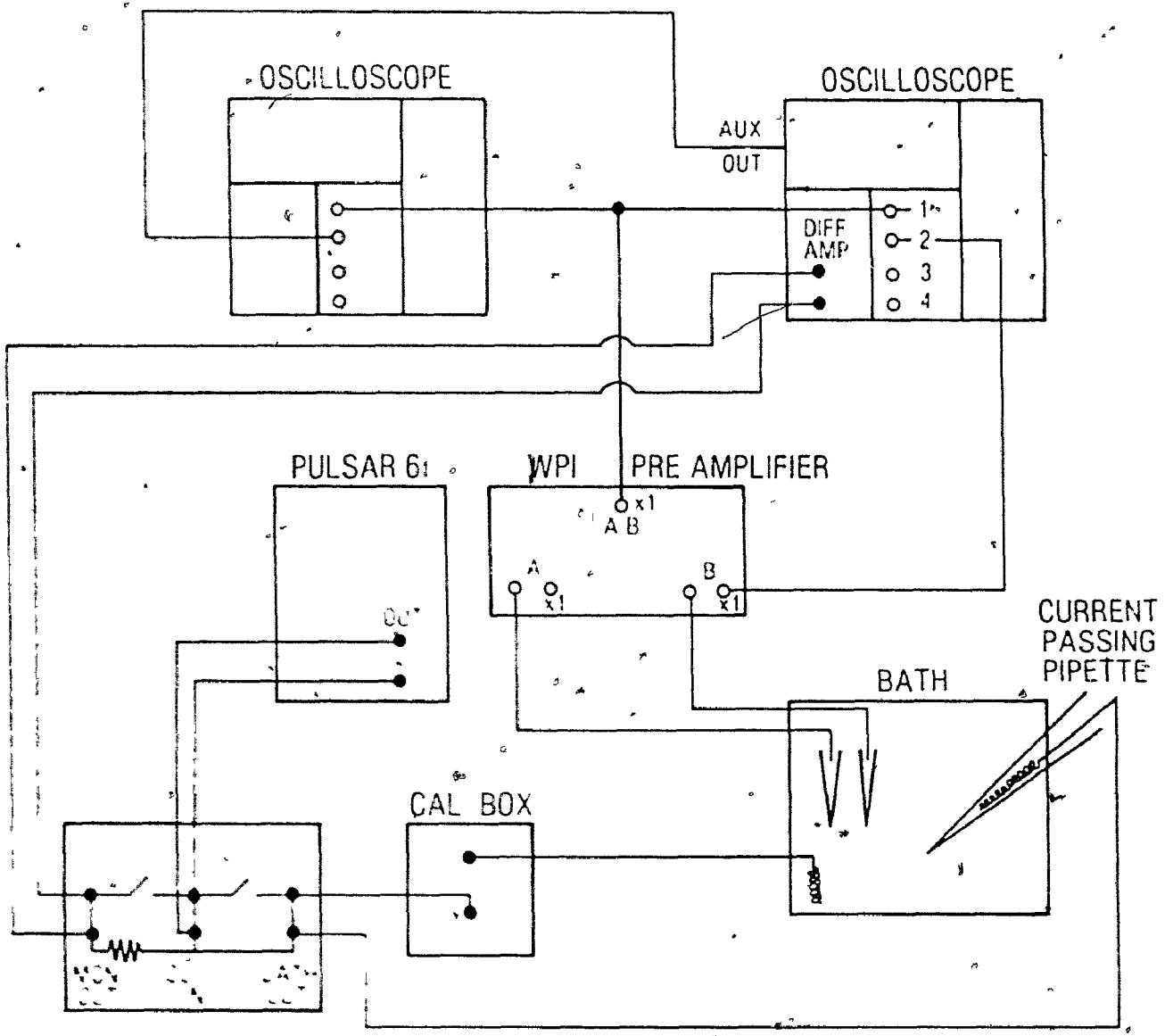
OSCILLOSCOPE
OSCILLOSCOPE
AUX
OUT
DIFF
AMP
1
2
3
4
PULSAR 6
WPI
PRE AMPLIFIER
x1
A B
A
B
x1
x1
CURRENT
PASSING
PIPETTE
BATH
CAL BOX

Figure 2b

3. Current passing electrode pipette

Current was delivered through a soft glass Pasteur pipette (Maple Leaf Brand Pasteur Pipette) that contained a chlorided silver wire and was filled with Tyrode's solution. The tip of the pipette was made smooth by fire polishing. The other end of the pipette was attached to a thin plastic tubing. The end of the plastic tubing was further attached to a 10 ml plastic syringe. Gentle suction could be applied through the pipette by pulling the plunger on the plastic syringe. Suction was applied through the pipette so that a firm seal could be established between the pipette and the Purkinje fiber.

4. Microelectrodes

Microelectrodes were fabricated using glass capillaries which contained an inner filament (WPI Kwik-fil Glass Capillaries, W-P Instruments INC., New Haven, Conn., U.S.A). The length of the pipettes were 4 inches. The outside diameter of the pipette was 1.0 mm $\pm$ 0.1 mm whereas the inside diameter was 0.58 mm. Micropipettes were pulled to a small tip diameter using a micropipette puller (Ml Micropipette Puller, Industrial Science Associates INC., Flushing, N.Y., U.S.A). Micropipettes were filled with 2.7 M KCL, and had a resistance between 15-30 MΩ. Microelectrode resistance was determined 2 ways. First, the resistance was determined using an ohmmeter (Dynamatic vacuum tube voltmeter, B and K, Chicago, Illinois, U.S.A.). Microelectrodes were then placed in microelectrode holders (MEH-1S, W-P Instruments) which were filled with 2.7 M KCL. The holder with the microelectrode was attached to a miniature probe (Series 700 Probes, W-P Instruments). The miniature probe

incorporates the first stage of the preamplifier. Two microelectrodes were used in each experiment. The two miniature probes were connected to separate inputs of a dual channel microelectrode amplifier (Model 750 Dual Microprobe, W-P Instruments). The output from the microelectrode amplifier was displayed on a Tektronix 5110 Oscilloscope (Tektronix Inc., Beaverton, Oregon, U.S.A). The second way in which the resistance was checked was performed after the microelectrodes were attached to the micro-probe amplifiers. After the electrodes were placed in the bath, determination of microelectrode resistance was done by depressing the electrode test button on the front panel of the amplifier. When this switch is closed, a calibrated current is injected through the electrode attached to the probe input. When this is done, an offset voltage equal to 1 millivolt/megOhm is generated at the X5 output. Thus the resistance of the microelectrode can be determined from the deflection of the voltage tracing for that microelectrode on the oscilloscope.

D. RECORDING

1. Microelectrode Recordings

A 100 mV calibrated current pulse was delivered between the ground electrode and the microelectrode from a calibration box. The pulse was used to calibrate 100 mV deflectons for both oscilloscopes. The transmembrane potential of Purkinje cells and muscle cells were recorded simultaneously with microelectrodes. In experiments involving application of electric current, transmembrane potentials of Purkinje cells in isolated Purkinje fibers were recorded differentially using 2 glass microelectrodes and the following technique. The impaling electrode was

positioned immediately outside the cell to be impaled. Then, while passing short current pulses, the indifferent electrode (second microelectrode) was positioned to eliminate the effect of current passage. The recording electrode was then impaled within a cell. This method eliminated spurious voltage changes caused by electrode position or the resistance of the ground electrode. This procedure was repeated for each impalement in those experiments where the impalement was lost or the recording site changed. Microelectrode recordings were recorded differentially from the A-B output on the Dual Channel Microelectrode Amplifier (W-P Instruments) and further amplified on a Tektronix 5A14N four channel amplifier (Tektronic Inc.). These recordings were displayed on a Tektronix 5110 oscilloscope. Microelectrode recordings were also displayed on another Tektronix 5110 oscilloscope which was attached to a recording camera (Model C4, Grass Instruments, Quincy, Mass., U.S.A.). The lens of the camera and oscilloscope screen were enclosed in a cardboard light screen. The camera used 35 mm film (Kodak RAR Film 2495, Eastman Kodak Company, Rochester, N.Y., U.S.A.) which was developed in a Versamat Film Processor (Kodak, U.S.A.). The versamat film processor developed, fixed and dried the 35 mm film. Transmembrane potentials were also recorded on an instrumentation Magnetic Tape Recorder (Vetter Model D, A.R. Vetter Co., Rebersburg, Pennsylvania, U.S.S.) using magnetic tape (Maxell UD 35-90 Magnetic tape, Hitachi Maxell, Tokyo, Japan or TDK LX 35-90, TDK, Japan).

2. Developed Tension Recordings

A 100 mg weight was placed at the end of the force transducer to calibrate the system for measuring changes in developed tension. In some experiments force was measured isometrically from the free end of the Purkinje fiber with a Grass FT.03 force transducer (Quincy, Mass., U.S.A.) attached to a Grass low level DC Amplifier. Resting tension was applied slowly to the Purkinje fiber. One hundred to 300 mg of resting tension was usually used. The magnitude of the contractions produced in the Purkinje fiber depended on the size of the fiber and the amount of resting tension used. If further increases in resting tension produced a decrease in the magnitude of the contractions, then the resting tension used was that which produced the greatest magnitude of contractions. Force was also measured in isolated Purkinje fibers. One end of the Purkinje fiber was attached to a pin which was attached to the force transducer, whereas the other end was attached by a pin to the wax tissue bath. The force displacement transducer was maintained on an X,Y,Z micromanipulator (Prior, England). The output signal from the transducer circuit, corresponding to the force developed, was amplified with a Grass low level DC amplifier. The output signal from this amplifier was monitored using one of the channels of each oscilloscope. The force displacement recordings were also displayed on the oscilloscope and photographed with a Grass camera.

3. Current

In experiments in which extracellular current was applied to induce DIA in isolated Purkinje fibers, the current was also monitered. The

current pulses were obtained from a digital stimulator operating in the constant voltage mode and delivered through a current limiting resistor. The return electrode consisted of a ground chloride silver coil immersed in the bath (i.e. ground electrode). The current was monitored by measurement of voltage developed across a smaller resistor (1KΩ) in series with the extracellular electrode. The voltage was measured with a Tektronix 5A21N differential amplifier in the monitor oscilloscope. A record of the output from the differential amplifier was also displayed on the oscilloscope used for photographic recording.

E. SOLUTIONS

All preparations were superfused continuously with modified Tyrode's solution. Solutions were placed in glass perfusion bottles above the Faraday cage. The solutions then passed through a very thin plastic tubing placed in a water bath below the tissue bath. The temperature of the water bath was maintained by a YSI Model 73A Instrument Temperature Controller (Yellow Springs Instrument Co.). The temperature of the superfusate entering the tissue bath was monitored by a YSI series 400 thermister temperature probe (Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A.). The thermister temperature probe was attached to the instrument temperature controller. The temperature of the superfusate in the tissue bath was maintained at 37°C. Therefore, the solutions were heated to 37°C as they passed through the coil in the water bath. The flow rate of the solution entering the tissue bath was controlled by attaching a CAIR clamp to the tubing from the perfusion bottles. By adjusting the thumbwheel on the CAIR clamp, the

flow of solution through the tubing and therefore the flow rate of solution entering the tissue bath could be regulated. Control and test solutions were delivered to the tissue chamber via separate conduits at a flow rate of 10-15 ml per minute. The tissue bath held a volume of 10-15 ml. Thus complete transition from one solution to another in the tissue bath took a few minutes for full exchange. Therefore transition from one solution to another was rapid although not instantaneous.

1. Normal Tyrode's Solution

Preparations were equilibrated for at least one hour with a modified Tyrode's solution (Table 1). Stock solutions were made with deionized water and all compounds except KCl, $MgSO_4 \cdot 7H_2O$, and $CaCl_2 \cdot 2H_2O$. Separate solutions containing KCl, $MgSO_4 \cdot 7H_2O$, and $CaCl_2 \cdot 2H_2O$ were made up in deionized water. These separate solutions were added to the stock solution on the day of use. Normal Tyrode's solution was bubbled with a gas mixture of 95% oxygen and 5% carbon dioxide. The pH of the solution at 37°C was 7.2-7.4.

2. "Ischemic" Tyrode's solution

After equilibration with Tyrode's solution, preparations were then superfused for 40 minutes with Tyrode's solution which had been further modified to mimic several of the conditions occurring during ischemia (Elharrar and Zipes, 1977-review) (Table 1). The "Ischemic" Tyrode's solution was made in the same sequence as the Normal Tyrode's solution i.e. a separate set of solutions were added to the stock solution on the day of use. Lactate was also added to the "Ischemic" Tyrode's solution on the day of use. The "Ischemic" Tyrode's solution was bubbled with

Table 1: Composition of Tyrode's Solutions

	Normal Tyrode's Solution (mM)	"Ischemic" Tyrode's Solution (mM)
NaCl	129.0	123.0
KCl	4.0	4.0
$NaH_2PO_4 \cdot H_2O$	0.9	0.9
$CaCl_2 \cdot 2H_2O$	2.5	2.5
$MgSO_4 \cdot 7H_2O$	0.5	0.5
$NaHCO_3$	20.0	6.0
Dextrose	5.5	---
Sodium Lactate	---	20.0
Gas	95% O_2/5% CO_2	90% N_2/10% CO_2
pH	7.2-7.4	6.8
PO_2	---	48 mm Hg

90% Nitrogen/10% Carbon dioxide gas mixture and had a pH of 6.8 at 37°C. This "Ischemic" solution mimicked severe hypoxia (PO_2 = 48mm Hg), acidosis, lactate accumulation and substrate deprivation (Ferrier et al., 1985a).

In all experiments, preparations were exposed to the "Ischemic" solution for 40 minutes and superfusion with control Tyrode's was then reinstated for an additional hour to simulate reperfusion. This protocol was then repeated in the presence of drugs (see below). We have observed that a second cycle of exposure to ischemic conditions and reperfusion results in identical changes in MDP and mechanisms of arrhythmia as that observed during the first cycle. We have shown previously that elevation of extracellular potassium concentration (10 mM) during ischemic conditions does not substantially change the response of these tissues upon reperfusion (Ferrier et al., 1985b).

F. DRUGS

1. Verapamil

Verapamil was generously supplied by G.D. Searle and Company of Canada Limited (Rexdale, Ontario, Canada). Verapamil was initially dissolved in deionized water to yield a stock solution with a concentration of 10^{-3} M. One ml of this stock solution was added to 1 liter of Tyrode's solution to yield a final concentration of 10^{-6} M. Verapamil was added to the "Ischemic" Tyrode's and Normal Tyrode's solution for reperfusion.

2. Nifedipine

Nifedipine was generously supplied by Miles Laboratories, Rexdale, Ontario, Canada. Nifedipine was initially dissolved in a small amount of ethanol. Deionized water was added to yield a stock concentration of $3X10^{-4}$ M. One ml of this stock solution was added to 1 litre of Tyrode's solution to yield a final concentration of $3X10^{-7}$ M. The final ethanol concentration superfusing the tissue never exceeded 0.001% in the superfusing medium. Nifedipine was added to the "Ischemic" and Normal Tyrode's solution for reperfusion. Since nifedipine demonstrates photolytic degradation, preparation of all solutions of nifedipine were performed in a darkened enviorment. Also, all glassware and tubing were covered with aluminum foil.

3. BAY-K8644

BAY-K8644 was supplied by Miles Pharmaceuticals, Division of Miles Laboratories, Inc., West Haven, Connecticut, U.S.A., and was kindly donated by Dr. P.E. Dresel. BAY-K8644 was dissolved in Polyethylene Glycol (Sigma Chemical Company, St. Louis, Missouri, U.S.A.) and deionized water to yield a stock concentration of $3X10^{-3}$ M. A glass vial containing the stock solution was placed in a sonicator and in a Vortex mixer for about 30 seconds to aid in dissolving the compound. Dilution into 1 liter of Tyrode's solution yielded a final concentration of $1X10^{-6}$ M BAY-K8644. Since BAY-K8644 demonstrates photolytic degradation, the preparation of all solutions was performed in a darkened environment and all glassware and tubing were covered with aluminum foil.

G. ANALYSIS OF RECORDS

Only those experments where continuous impalement was maintained or when the impalement was lost and a cell with identical electrophysiological characteristics was obtained, were analyzed. Also, only those preparations which displayed normal action potential characteristics were used for subsequent studies. A preparation was considered to display normal action potential characteristics when the MDP of Purkinje tissue was between -90 and -93 mV, and when there was no automaticity. Action potentials were considered normal for muscle tissues when the MDP was between -80 and -90 mV.

Photographic records on 35mm clear base film were magnified using a 35 mm film enlarger (Dagmar Super Model A, Audio-Visual Research, Waseca, Minessota, U.S.A.) and projected onto a digitizer pad (DT-11 Hipad Digitizer, Bausch and Lomb, Texas, U.S.A.). Analysis of action potential and contractile characteristics (see below) was performed on an Apple IIe dual disc drive computer with an Apple IIe CCS Board Interface (RS-232) for the Hipad Digitizer. The programs for the analysis of the data was written by Dr. A. Lukas and are listed at the end of this thesis.

A) Parameters Utilized

1- Basic Cycle Length (BCL)= The interval between stimuli during a train of stimulation. The BCL was usually 500 msec, but was varied in some experiments.

2- Pause= The time between trains of stimulation. The pause was usually 3 sec, but was varied in some experiments.

3- Maximum Diastolic Potential (MDP)= The most negative potential attained during diastole following the last driven action potential in a train. The MDP was measured in millivolts (mV).

4- Current Magnitude= The amount of current delivered through the extracellular pipette and which depolarized or hyperpolarized the membrane potential. The current magnitude was measured in microamps (uA).

5- Developed Tension= The amplitude of the last contraction elicited in Purkinje tissue by the last stimuli of a train. Developed tension was measured in milligrams (mg).

6- Cycle Length of Depolarization-Induced Automaticity= The interval between the peaks of the first two automatic action potentials elicited by a depolarizing current pulse. The cycle length of DIA was measured in milliseconds (msec).

7- MDP of DIA= The membrane potential of the first automatic action potential elicited by a depolarizing current pulse. The MDP of DIA was measured in millivolts (mV).

H. STATISTICS

Data were analyzed using a paired T-test or analysis of variance followed by the Student-Neuman-Keuls (SNK) multiple range test. Differences between means were considered significant for $P<0.05$.

RESULTS

A) EFFECTS OF CALCIUM CHANNEL BLOCKING AGENTS ON THE ELECTROPHYSIOLOGICAL RESPONSE OF CANINE VENTRICULAR TISSUES TO ISCHEMIC CONDITIONS AND REPERFUSION

1. Ischemic conditions

a. Changes in MDP

Figure 3 (left side of figure) shows changes in MDP of Purkinje tissue exposed to ischemic conditions for 40 minutes in the absence of drug or presence of either 1×10^{-6} M verapamil or 3×10^{-7} M nifedipine. Ischemic conditions resulted in an initial rapid decrease of membrane potential, followed by a period during which the membrane potential remained relatively stable (approximately 25 minutes). However, there was a greater variability in MDP changes between 35-40 minutes. In the presence of verapamil or nifedipine ischemic conditions resulted in a markedly greater depolarization of Purkinje tissue within the first 15 minutes. The difference was statistically significant. However, in the presence of nifedipine, the membrane potential returned to control levels by 20 minutes and was not significantly different for the remainder of ischemic conditions. In contrast, the membrane potential remained at a more depolarized level for the remainder of the ischemic period in the presence of verapamil. However, the membrane potential of Purkinje tissue at the end of exposure to ischemic conditions in the presence of verapamil was not significantly different from that of the control or nifedipine treated preparations. These results suggest that calcium channel blocking drugs promote significantly greater depolarization of Purkinje tissue in response to ischemic conditions.

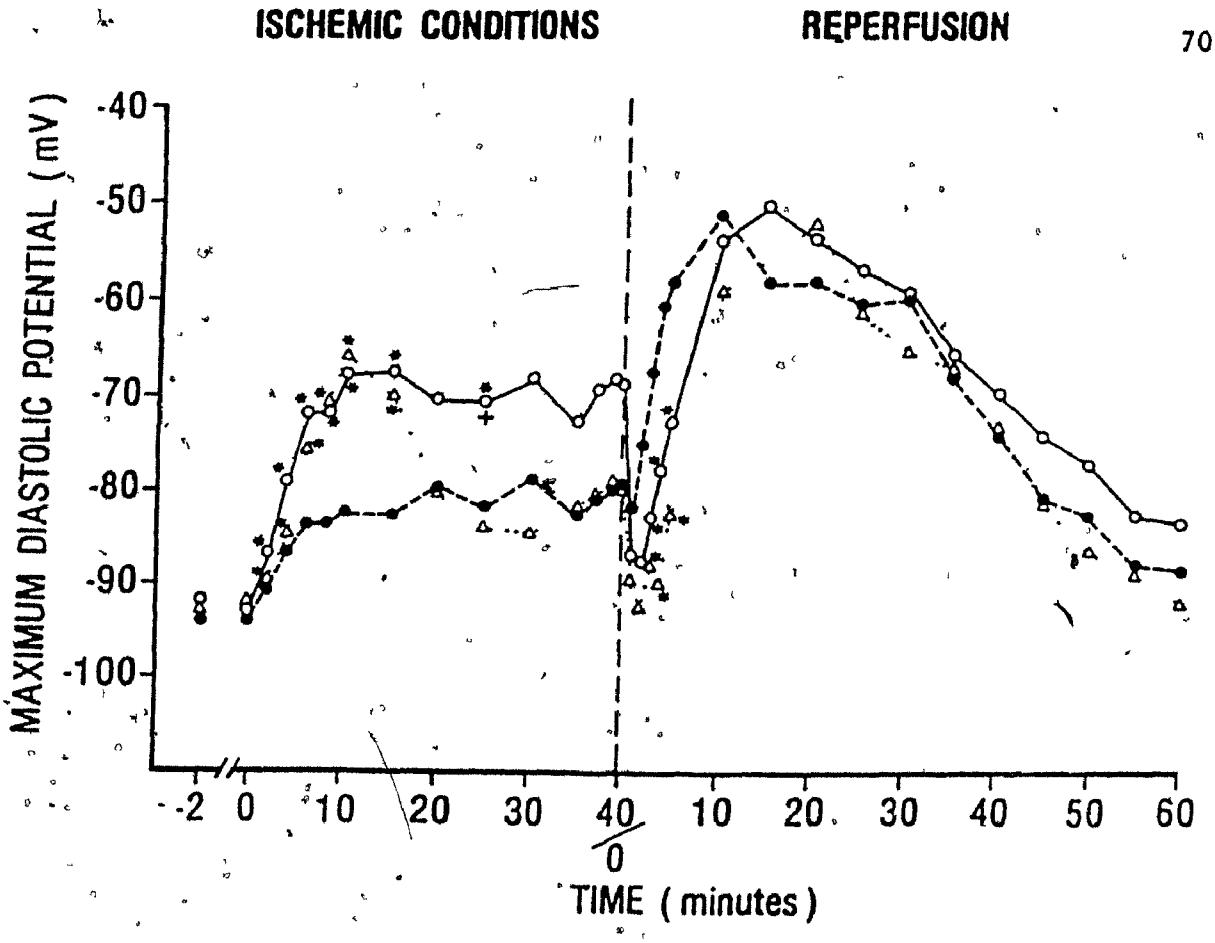

Figure 3

Changes in maximum diastolic potential in Purkinje tissue in the absence and presence of verapamil or nifedipine in response to ischemic conditions followed by non-ischemic conditions. The ordinate indicates the maximum diastolic potential in mV as a function of duration of exposure to ischemic conditions (to left of vertical line) and duration of reperfusion after return to control Tyrode's solution (to right of vertical line). Data points represent means. Data were analyzed for significance using ANOVA with SNK. ●- - -● Control (n= 9-16), △· · ·△ Nifedipine (n= 5-9), o——o verapamil (n= 5-9). * Significantly different from control ($P<.05$), + significantly different from nifedipine treated ($P<.05$).

Figure 4 (left side of figure) shows changes in MDP of muscle tissues during exposure to ischemic conditions for 40 minutes in the absence of drug and presence of either verapamil or nifedipine. Ischemic conditions resulted in a decrease of MDP of muscle tissues which appeared within 10 minutes and was maintained for the remainder of the ischemic period. Ischemic conditions in the presence of nifedipine also resulted in a decrease of MDP within 10 minutes which was not significantly different from control. However after 35 minutes the MDP of muscle tissues began to decrease more than under control ischemic conditions or ischemic conditions in the presence of verapamil. In contrast, verapamil resulted in a statistically significant attenuation of depolarization in muscle tissue during the first 10 minutes of exposure to ischemic conditions. By the end of the ischemic period, the MDP of control and verapamil treated tissues were not significantly different. Thus, unlike Purkinje tissue, initial depolarization of muscle tissue in response to ischemic conditions was not strongly promoted by calcium channel blockers.

b. Conduction defects

The electrophysiologic consequences of verapamil or nifedipine-enhanced depolarization in response to ischemic conditions are illustrated by Figure 5. Panels A-D were recorded during exposure to ischemic conditions in the absence of drug. Ischemic conditions resulted in moderate depolarization of Purkinje tissue (top trace in each panel). During a second cycle of "ischemia" and reperfusion the same preparation was exposed to nifedipine. Panels E-H were recorded during the second

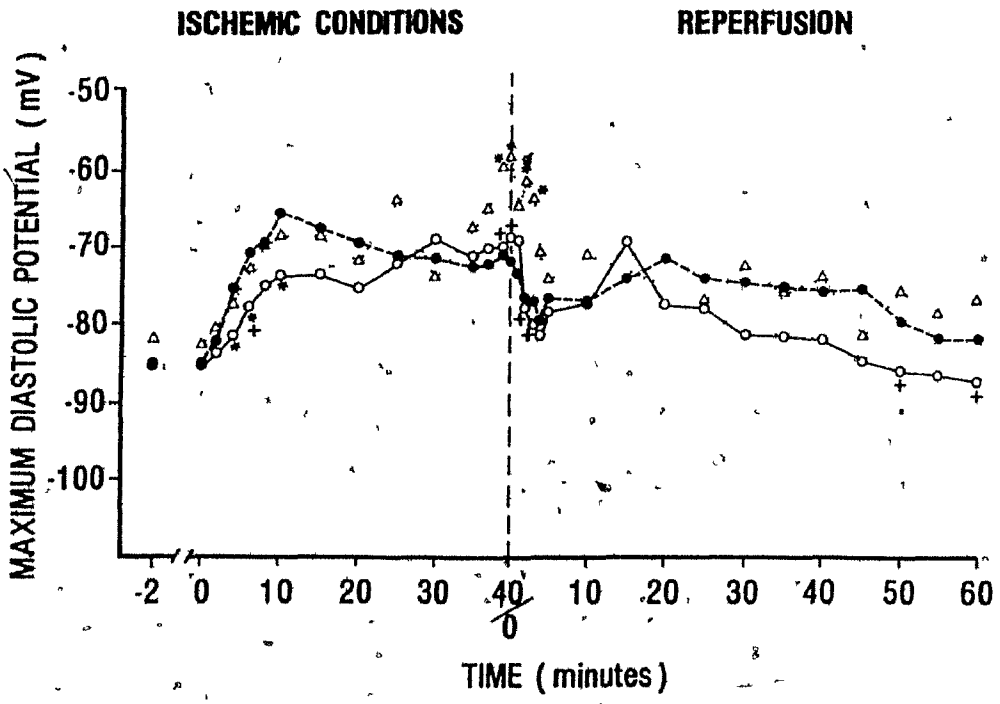

Figure 4

Changes in maximum diastolic potential in muscle tissue in the absence and presence of verapamil or nifedipine in response to ischemic conditions followed by non-ischemic conditions. Figure legend is the same as Figure 3.

exposure to ischemic conditions. Ischemic conditions in the presence of nifedipine resulted in a much greater depolarization of Purkinje tissue. In this experiment the stimulating electrode was placed on the papillary muscle. Depolarization of Purkinje tissue eventually resulted in complete failure of propagation of active action potentials to the recording site in Purkinje tissue (panel G). Only passive electrotonic depolarizations were observed. However the membrane potential gradually recovered and some active action potentials were observed during the train of stimulation (responses 1,3, and 5)(panel H). Similar effects were observed during ischemic conditions in the presence of verapamil. However in the presence of verapamil the recovery of membrane potential was not as great as in the presence of nifedipine, although active action potentials returned.

Table 2 summarizes the incidence of conduction defects occurring in Purkinje tissue in preparations from either right or left ventricle. Most of the endocardial surface of these preparations have subendocardial Purkinje fibers overlying muscle. Because preparations were usually stimulated on the endocardial surface of the papillary muscle, the conduction defect that occurred in Purkinje tissue may represent conduction block between muscle and Purkinje tissues, or depressed excitability in one or both tissues. Conduction defects occurred in only 3 of 20 preparations exposed to ischemic conditions alone. Nifedipine caused a greater incidence of conduction defects in Purkinje tissue exposed to ischemic conditions (4 of 7 preparations). Verapamil resulted in a similar increase in the incidence of conduction defects (5 of 9 prepara-

Figure 5

Effects of ischemic conditions on the electrophysiological characteristics of canine Purkinje and muscle tissues in the absence and presence of nifedipine. In each panel, the top trace is a microelectrode recording from Purkinje tissue and the second trace is a recording from muscle tissue. The last trace at the bottom of each column is a record of stimulation pattern. Panels A-D show changes in electrical activity during exposure to ischemic conditions. Panels E-H show changes in electrical activity in the same preparation during exposure to ischemic conditions in the presence of nifedipine.

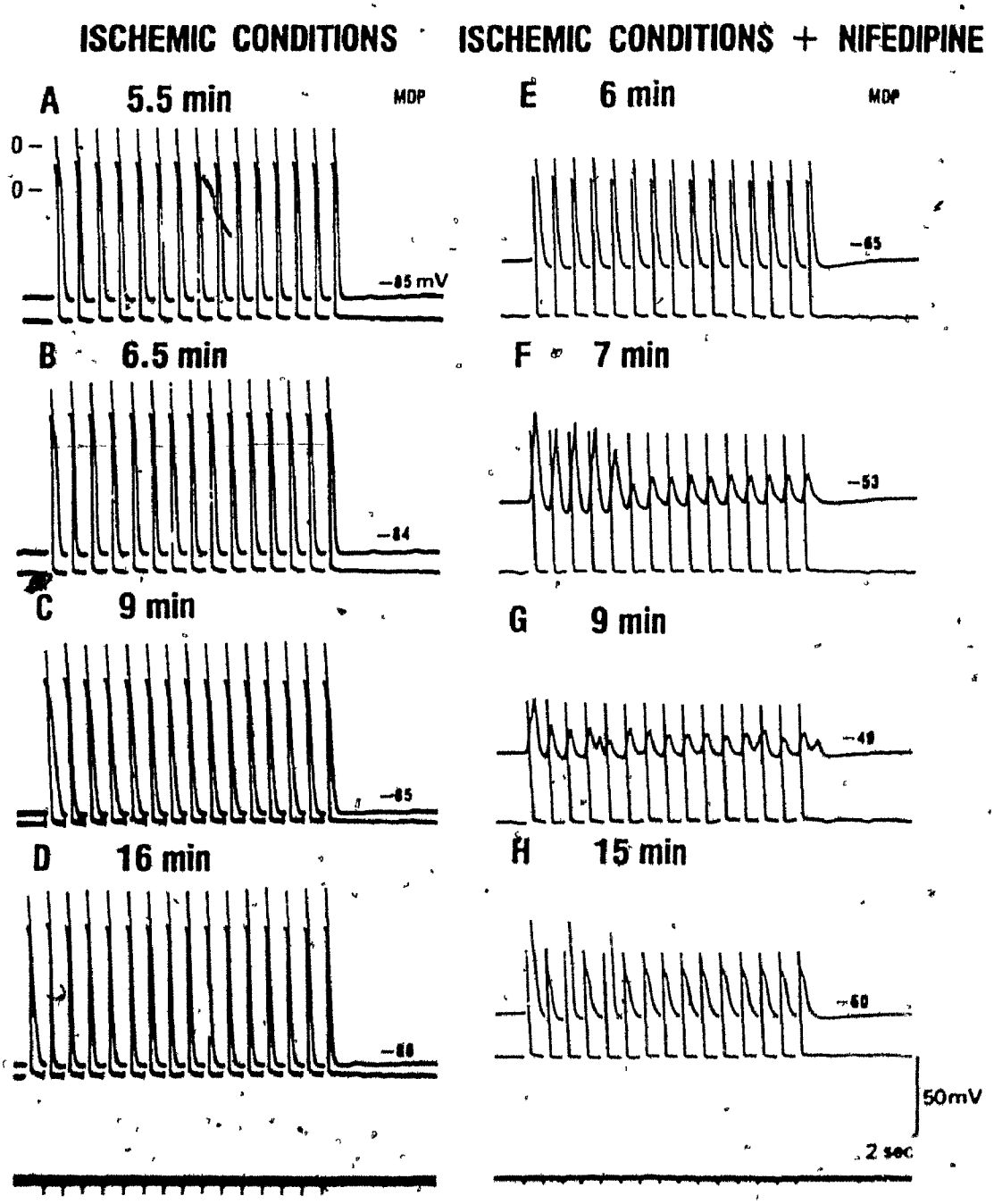
ISCHEMIC CONDITIONS
ISCHEMIC CONDITIONS + NIFEDIPINE
A 5.5 min
MDP
0 –
0 –
–85 mV
B 6.5 min
–84
C 9 min
–85
D 16 min
–88
E 6 min
MDP
–85
F 7 min
–53
G 9 min
–49
H 15 min
–60
50mV
2 sec

Table 2: Incidence of Conduction Defects During Ischemic Conditions

	Purkinje Tissues	Muscle Tissues
Control	3/20	9/20
Nifedipine	4/7	4/7
Nifedipine (30 min preequil)	4/4	4/4
Verapamil	5/9	2/7

tions). Thus both calcium channel blocking agents depressed conduction and/or excitability in Purkinje tissues during ischemic conditions.

A previous report has suggested greater protection of the ischemic heart with verapamil when the heart is pretreated with the agent (Bersohn and Shine, 1983). These investigators demonstrated greater preservation of phosphocreatine and ATP levels in verapamil pretreated hearts subjected to low flow ischemia and reperfusion compared to hearts that were treated with verapamil after ischemia had begun. They also showed enhanced recovery of mechanical function upon reperfusion in hearts pretreated with verapamil, compared to control hearts or hearts treated with verapamil during ischemia. Therefore the possibility that pretreatment with nifedipine might afford protection of tissues from electrical disturbances when exposed to ischemic conditions followed by reperfusion was also investigated in this study. Four preparations were pre-equilibrated for 30 minutes with nifedipine before exposing them to ischemic conditions plus nifedipine. Table 2 shows that pre-equilibration with nifedipine resulted in conduction block during ischemic conditions in all preparations tested. Although pre-equilibration with nifedipine exacerbated conduction block during ischemic conditions, pretreatment did not cause significantly greater depolarization of Purkinje tissue (not illustrated).

Ischemic conditions result in bidirectional depression of conduction between Purkinje and muscle tissues (Ferrier et al., 1985a). In the present study exposure to ischemic conditions resulted in 4 of 20 preparations displaying conduction block in muscle tissues (Table 2)

when stimulation was delivered to Purkinje tissue (free running false tendon). In the presence of nifedipine, ischemic conditions caused conduction block in 4 of 7 preparations. However, these preparations were stimulated at a site on the papillary muscle. Therefore these observations may represent either conduction block or failure of excitation in muscle or Purkinje tissue. All preparations in this group that displayed conduction block/inexcitability in muscle during ischemic conditions with nifedipine, also displayed conduction block in Purkinje tissue. All preparations pre-equilibrated with nifedipine displayed conduction block in muscle when stimulation was applied to Purkinje tissue. In contrast, exposure to ischemic conditions in the presence of verapamil resulted in only 2 of 7 preparations displaying conduction block in muscle when stimulation was delivered to Purkinje tissue. This ratio is less than that of control. These results indicate that ischemic conditions in the presence of nifedipine results in a greater incidence of conduction block/inexcitability in muscle tissues compared to verapamil. Also, pre-equilibration with nifedipine appears to enhance conduction block in both Purkinje and muscle tissues during ischemic conditions.

c. Changes in developed tension

Figure 6a shows the effects of verapamil on the changes in developed tension of Purkinje fibers during ischemic conditions. In the absence of drug developed tension declined to zero after 10 minutes of exposure to ischemic conditions. In the presence of verapamil and ischemic conditions the developed tension was significantly reduced com-

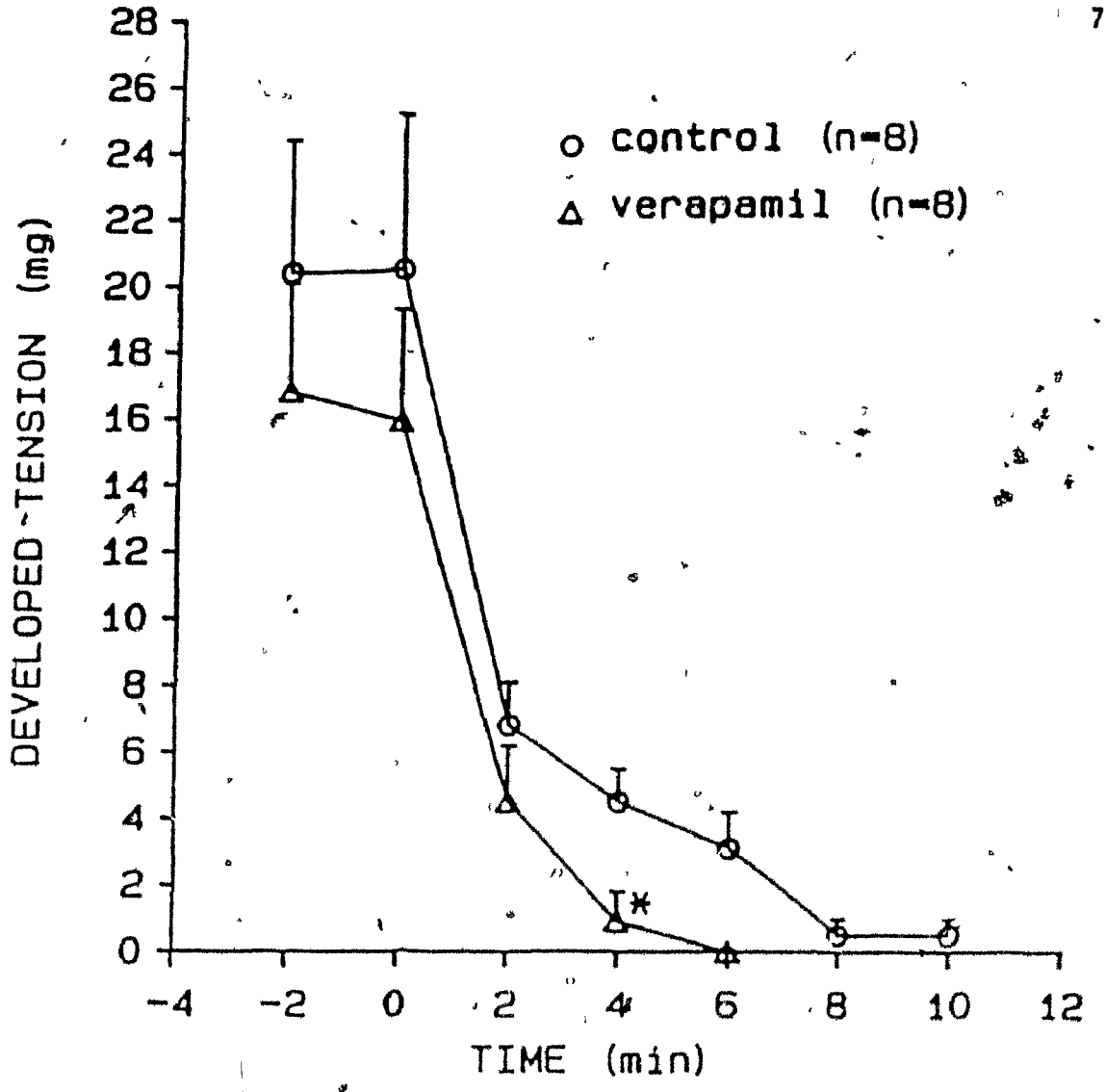

Figure 6a

Effects of ischemic conditions on changes in developed tension in Purkinje tissue in the absence and presence of verapamil. The ordinate indicates the developed tension in mg as a function of the duration of exposure to ischemic conditions. Ischemic conditions began at 0 minutes. Data points represent mean±standard errors. Data were analyzed for significance using a paired t-test.

* significantly different from control, $P<.05$

pared to the control at 4 minutes. Developed tension in Purkinje tissue declined to zero with 6 minutes of exposure to ischemic conditions in the presence of verapamil. The effects of nifedipine on developed tension during ischemic conditions are shown in Figure 6b. In the presence of nifedipine, developed tension was significantly less than control at 4 and 8 minutes. The time required for developed tension to reach zero during control ischemic conditions was greater than 10 minutes. The time required for developed tension to reach zero in the presence of nifedipine and ischemic conditions was 8 minutes. Figure 6c shows changes in developed tension during ischemic conditions plotted as a percentage of the pre-ischemic value. The controls for nifedipine treated and verapamil treated preparations were pooled. The rate of decrease in developed tension during ischemic conditions in the presence of verapamil or nifedipine was similar i.e. the lines are parallel and the slopes are similar. However the rate of decrease in developed tension was much greater than control ischemic conditions (after 2 minutes). These results suggest that ischemic conditions in the presence of calcium channel blocking agents cause a significantly greater decrease in developed tension in Purkinje tissue compared to ischemic conditions alone. Also, the time to reach zero developed tension in Purkinje tissue during ischemic conditions was much shorter in the presence of calcium channel blocking drugs.

2. Reperfusion

a. Changes in MDP

Reperfusion with control Tyrode's solution following 40 minutes of

Figure 6b

Effects of ischemic conditions on changes in developed tension in Purkinje tissue in the absence and presence of nifedipine. Figure legend same as Figure 6a.

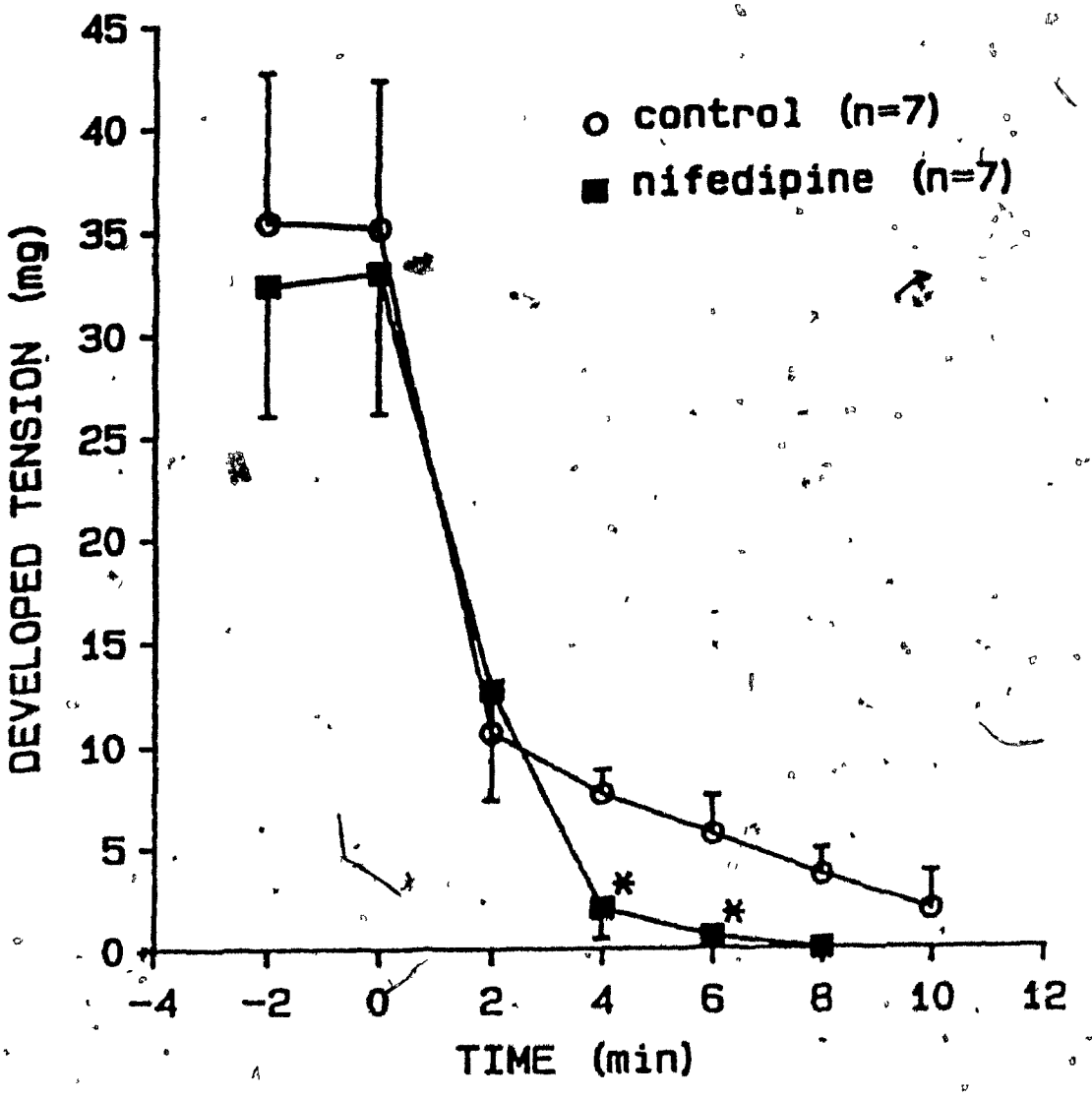
45
40
35
30
25
20
15
10
5
0
DEVELOPED TENSION (mg)
control (n=7)
nifedipine (n=7)
*
*
-4
-2
0
2
4
6
8
10
12
TIME (min)

Figure 6b

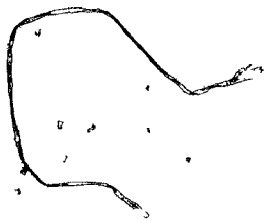

Figure 6c

Change in developed tension as a percentage of the pre-ischemic value. The ordinate indicates the developed tension as a percentage of the pre-ischemic value. The respective controls for nifedipine and verapamil treated preparations were pooled. Data points represent mean percentage of the pre-ischemic value of developed tension.

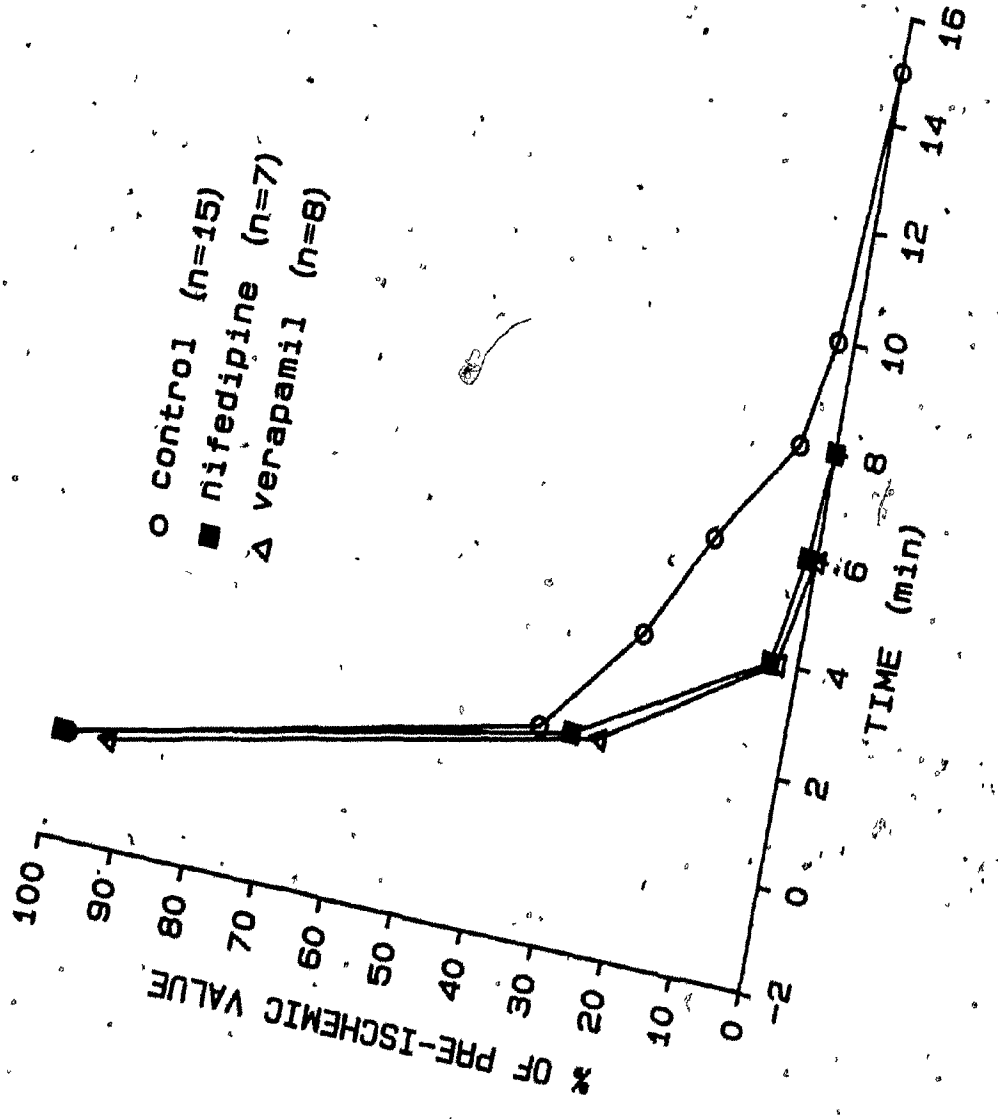
o control (n=15)
■ nifedipine (n=7)
Δ verapamil (n=8)
% OF PRE-ISCHEMIC VALUE
100
90
80
70
60
50
40
30
20
10
0
-2
0
2
4
6
8
10
12
14
16
TIME (min)

Figure 6c

exposure to ischemic conditions caused marked changes in MDP in Purkinje tissue (Figure 3, right panel). Several distinct phases of arrhythmogenic activity were closely related to these cyclic changes in MDP. Reperfusion resulted in initial repolarization (which in some cases exceeded the pre-ischemic MDP). The magnitude of the control repolarization is obscured in Figure 3 because only mean responses are shown. The magnitude of the mean response was attenuated because of the transient nature of the repolarization/hyperpolarization and because of slight variations in the precise time at which peak membrane potentials were achieved. The incidence of initial repolarization or hyperpolarization is summarized in Table 3. Repolarization was followed by rapid depolarization to membrane potentials between -45 and -55 mV. Purkinje tissue remained at this depolarized level between 10 and 25 minutes of reperfusion (Figure 3). Gradual recovery of membrane potential began after approximately 30 minutes of reperfusion. Recovery was complete after 60 min of reperfusion. Reperfusion in the presence of nifedipine or verapamil did not alter any of the changes in MDP that occurred in Purkinje tissue after the initial 5 minutes of reperfusion. However the time required for depolarization to low membrane potential was significantly greater in the presence of nifedipine, with or without pre-equilibration (Table 3). Thus the MDP of Purkinje tissue in verapamil treated and nifedipine treated preparations were significantly greater than the control preparations between 3-5 minutes (Figure 3 and Table 3). Depolarization, during reperfusion culminated in a period of

TABLE 3

Incidence and Time Course of Reperfusion Effects On Purkinje Tissue Exposed To Ischemic Conditions

Treatment		Hyperpolarization	OAP'S	Reperfusion-induced inexcit.	DIA	Modulated Parasystole	§ Recovery
Control	Incidence	7/19	8/19	17/21	17/21	9/21	21/21
	Time (min ± S.E.)	1.4 ± .2*	1 ± .7	6.9 ± .7	23.0 ± 2.6		44.0 ± 2.0
Nifedipine	Incidence	5/7	0/7	6/7	0/7	0/7	7/7
	Time (min ± S.E.)	2.4 ± .5*		10.8 ± 1.5+			41.4 ± 3.6
Nifedipine (30 min. Preequil.)	Incidence	3/4	0/4	3/4	0/4	0/4	3/4
	Time (min ± S.E.)	3.4 ± .9*		14.1 ± 2.1 ∓			44.3 ± 3.0
Verapamil	Incidence	5/9	0/9	9/10	0/10	0/10	9/10
	Time (min ± S.E.)	2.1 ± .4*		7.9 ± .7			42.5 ± 4.9

Time: time elapsed since start of reperfusion; *time at peak; OAP: oscillatory afterpotential; DIA: depolarization-induced automaticity.
+ significantly greater than control ($P<0.05$)
∓ significantly greater than verapamil treated ($P<0.05$)
§ membrane potentials ≥ -80 mV and cessation of automaticity

conduction block and inexcitability in Purkinje tissue. The time elapsed from the start of reperfusion to the onset of inexcitability was measured. The time to reach inexcitability was 6.9 ± .7 minutes for the controls, 10.8 ± 1.5 min for nifedipine, 14.1 ± 2.1 min for pre-equilibration with nifedipine, and 7.9 ± .7 min for verapamil treated preparations. There was no significant difference in the time to inexcitability in the presence of verapamil as compared to the control. However, preparations took significantly longer to become inexcitable in the presence of nifedipine.

The changes in MDP occurring in muscle tissues upon reperfusion are shown in Figure 4 (right panel). Reperfusion resulted in an initial phase of repolarization lasting for 10 minutes in muscle tissues followed by a phase of moderate depolarization (between 15 and 45 minutes), and finally recovery by the end of 60 minutes. In the presence of nifedipine the MDP was significantly lower than in control or verapamil treated preparations during the first 5 minutes. In the presence of verapamil the membrane potential after 5 minutes was significantly greater than nifedipine treated but not control preparations. The MDP of verapamil treated preparations also was significantly greater than nifedipine treated preparations (but not control preparations) within the last 10 minutes of reperfusion.

b. Changes in developed tension during reperfusion

The effects of nifedipine on changes in developed tension during reperfusion are shown in Figure 7a. In the absence of drug developed tension in Purkinje tissue increased from 0 to 24.0 mg within 3 minutes

Figure 7a

Effects of reperfusion on changes in developed tension in Purkinje tissue in the absence and presence of nifedipine. The ordinate indicates the developed tension in mg as a function of the duration of exposure to reperfusion. Data points represent mean±standard errors. Data were analyzed for significance using a paired T-test.

* significantly different from control, $P < .05$

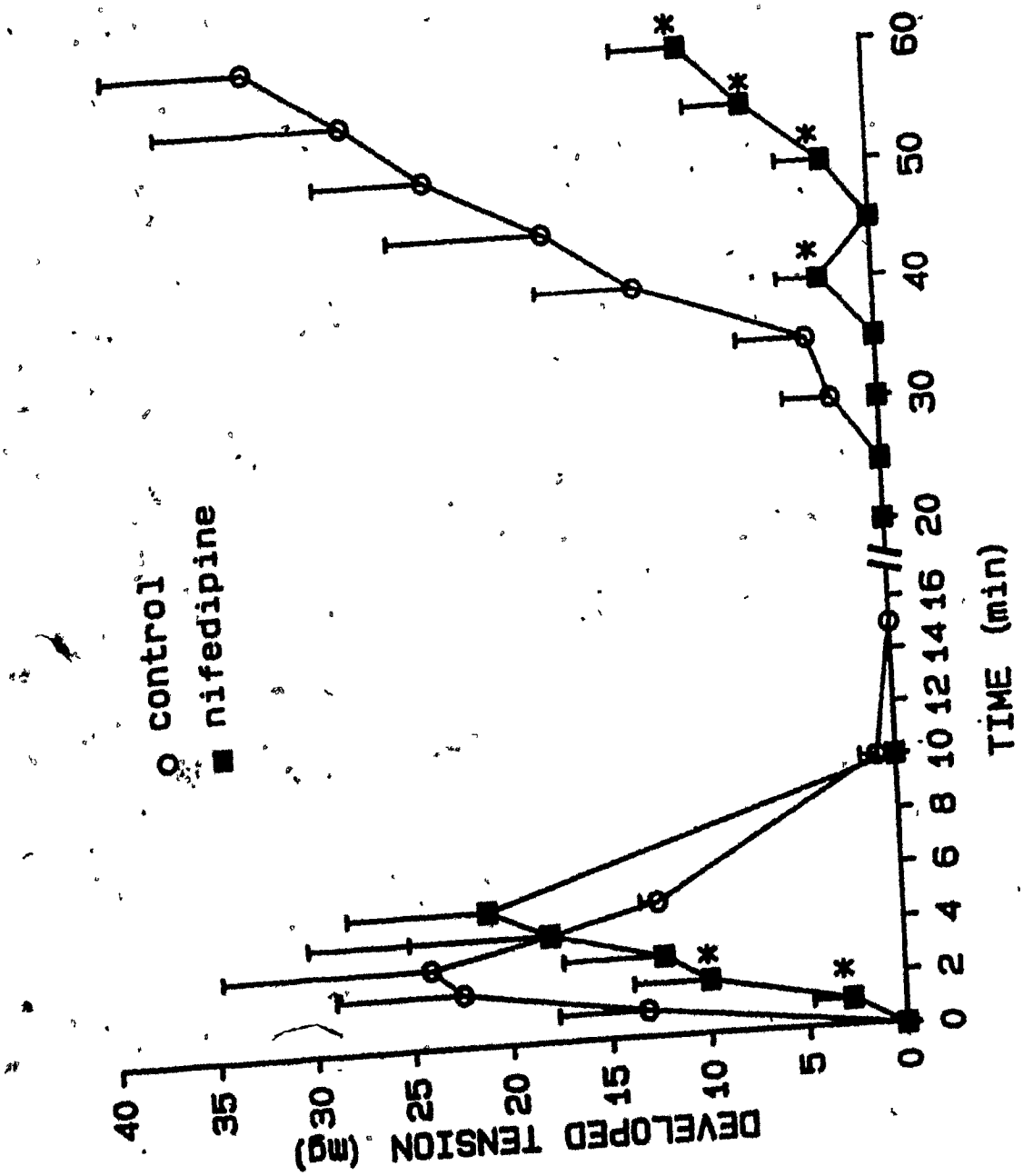
DEVELOPED TENSION (mg)
40
35
30
25
20
15
10
5
0
TIME (min)
0
2
4
6
8
10
12
14
16
20
30
40
50
60
control
nifedipine
*

Figure 7a

of reperfusion. This was followed by a rapid decline in developed tension to 0 mg by 10 minutes of reperfusion. Developed tension returned by 30 minutes and increased progressively thereafter during 60 minutes of reperfusion. Reperfusion in the presence of nifedipine significantly reduced the developed tension at 1 and 2 minutes of reperfusion. Developed tension also decreased to 0 by 10 minutes of reperfusion in the presence of nifedipine. Contractility returned in the presence of nifedipine but was significantly less than control between 50-60 minutes of reperfusion.

The changes in developed tension in the absence and presence of verapamil are shown in Figure 7b. In the absence of verapamil, contractility returned in Purkinje tissue within 1 minute of reperfusion. The developed tension declined to 0 after 5 minutes of reperfusion. Contractility then returned between 15 and 30 minutes of reperfusion. After 30 minutes of reperfusion, contractility progressively increased to the end of reperfusion. In the presence of verapamil (Figure 7b), contractility returned upon initial reperfusion and was not significantly less than the control. Contractility in the presence of verapamil then decreased to 0 after 10 minutes of reperfusion. Contractility began to return after 40 minutes of reperfusion but was significantly less than the control by the end of 60 minutes of reperfusion.

Figure 7c shows the combined data for developed tension of all controls (pooled data) compared to verapamil and nifedipine treated preparations. The values are represented as a percentage of the

Figure 7b

Effects of reperfusion on changes in developed tension in Purkinje tissue in the absence and presence of verapamil. Figure legend is the same as Figure 7a.

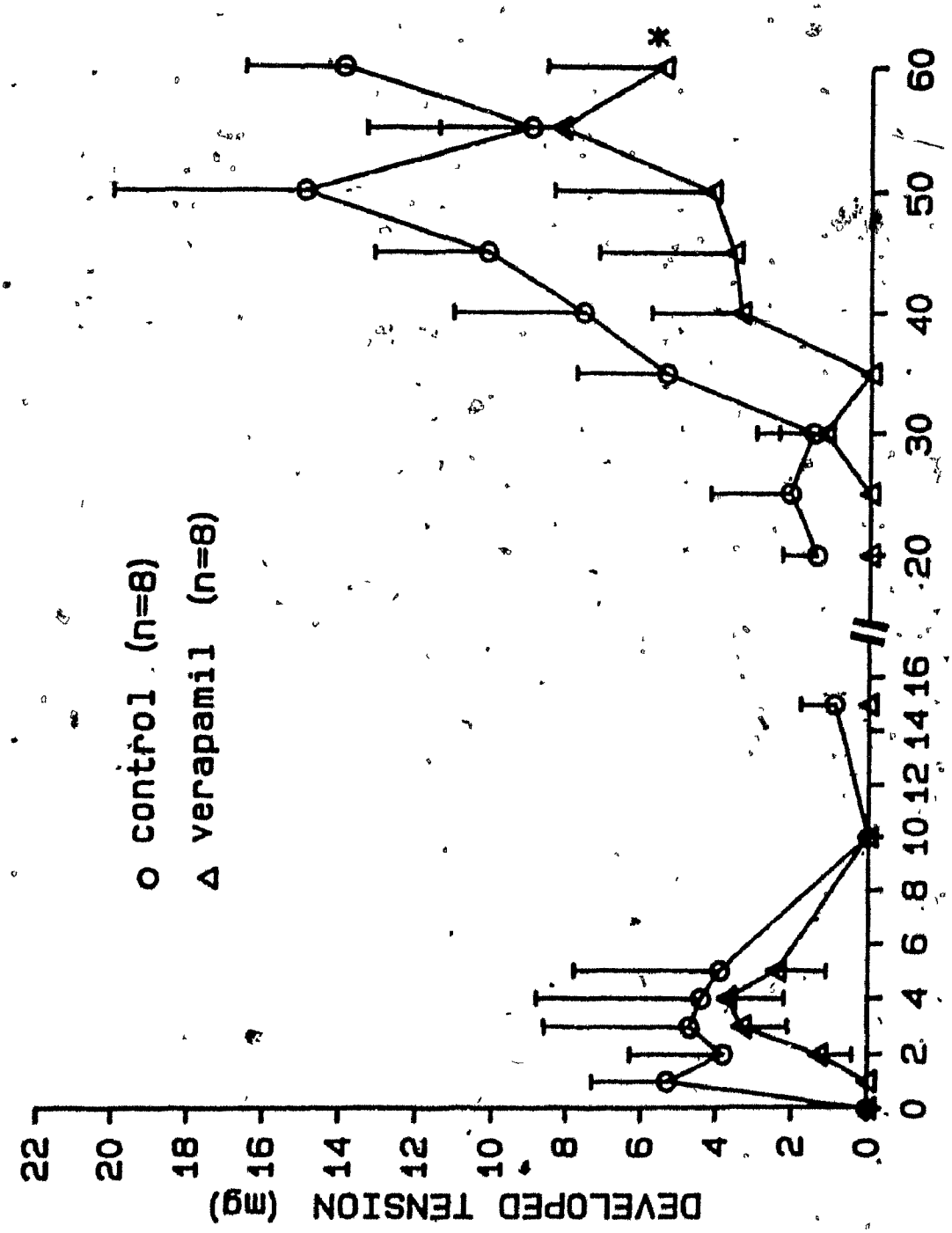

Figure 7b

Figure 7c

Change in developed tension during reperfusion as a percentage of the pre-ischemic value. The ordinate indicates the developed tension as a percentage of the pre-ischemic value. The respective controls for nifedipine and verapamil treated preparations were pooled. Data points represent mean percentage of pre-ischemic value.

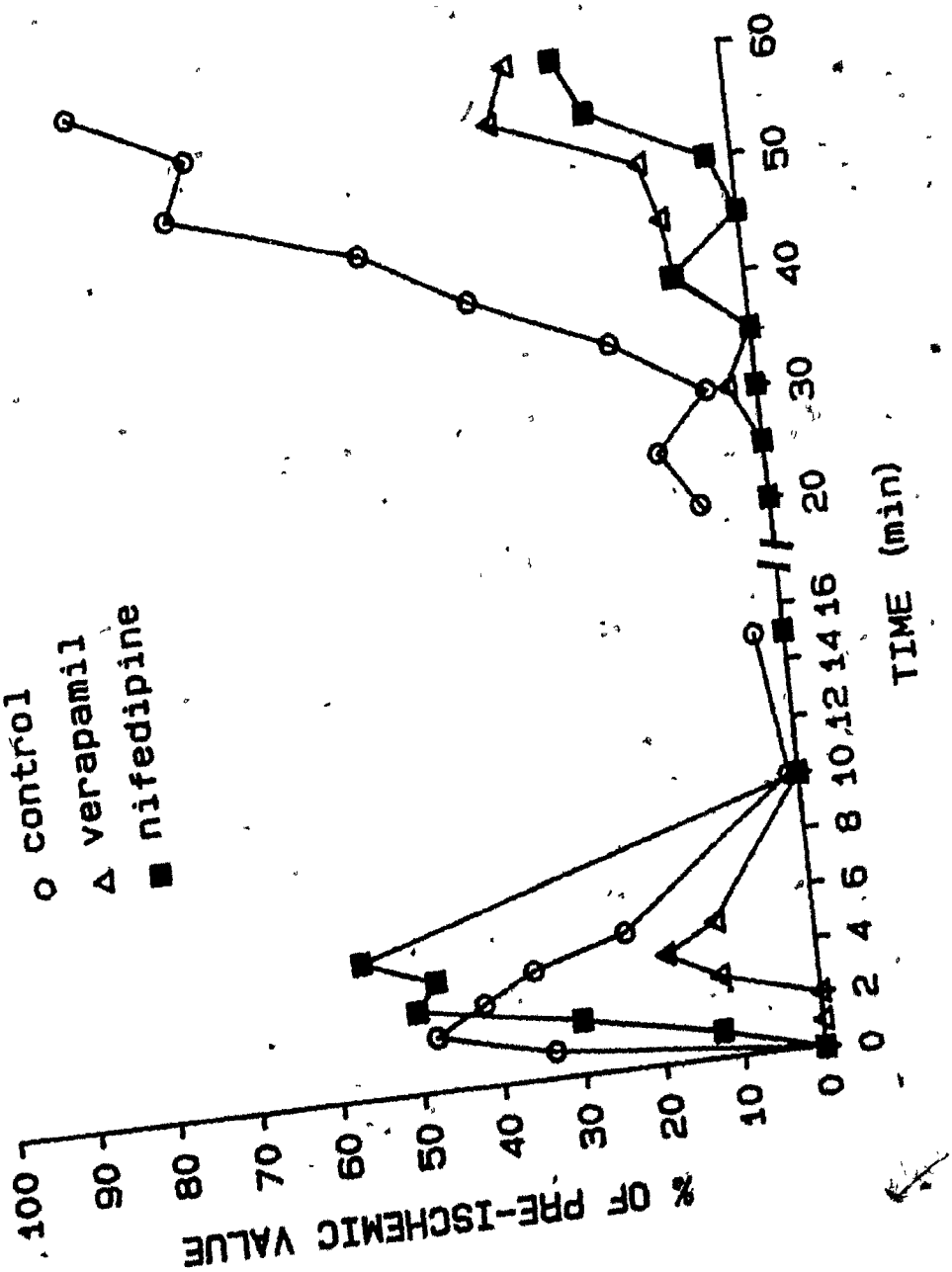
o control
Δ verapamil
■ nifedipine
% OF PRE-ISCHEMIC VALUE
100
90
80
70
60
50
40
30
20
10
0
0 2 4 6 8 10 12 14 16
TIME (min)
20
30
40
50
60

Figure 7c

pre-ischemic value. The pattern is similar to that seen in the graphs displaying the raw data (Figure 7a and Figure 7b). There was a return of contractility within the first 5 minutes followed by a decrease to zero developed tension within 10 minutes of reperfusion. Contractility reappeared after 20 minutes of reperfusion. Final return of contractility in the presence of verapamil or nifedipine began between 30 and 40 minutes of reperfusion. However return of contractility was slower than control and less complete in the presence of calcium channel blocking agents after 50 minutes of reperfusion.

These results show that reperfusion results in an initial return of contractility in Purkinje tissue (within 5 minutes). The return of contractility in Purkinje was not significantly decreased by the calcium channel blocking agents after 1 minute of reperfusion. After 5 minutes of reperfusion, contractilty decreased to zero both in the absence or presence of these agents. Contractility appeared again after 20 minutes of reperfusion. Final return of contractilty occurred much later in the presence of the calcium channel blocking agents (40 min reperfusion) and contractility remained significantly lower than control at 60 minutes of reperfusion.

c. Occurrence of oscillatory afterpotentials

Oscillatory afterpotentials (OAP's) appeared in Purkinje tissue but not in muscle during the initial part of reperfusion when the Purkinje tissue began to depolarize. Figure 8 shows OAP's recorded in a representative control experiment. OAP can be seen following the last driven action potential recorded from Purkinje tissue in each panel (panels A

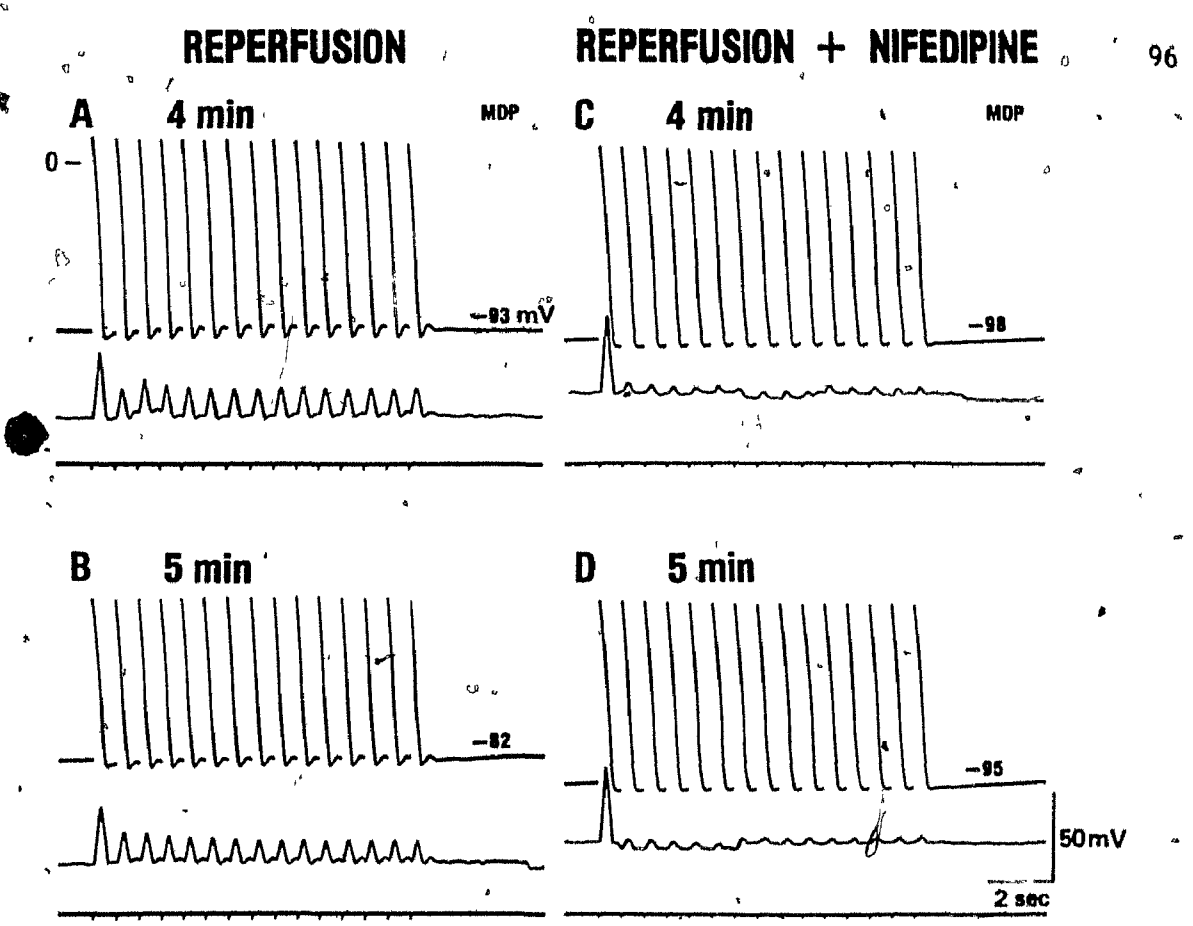

Figure 8

Representative experiment demonstrating oscillatory afterpotentials (OAP's) in Purkinje tissue during reperfusion. The top trace in each panel shows a record of transmembrane electrical activity from Purkinje tissue, the middle trace is a recording of contractile activity of the same tissue, and the bottom trace indicates the stimulus pattern. Panels A and B show the occurrence of OAP's at 4 and 5 minutes respectively during reperfusion in the absence of nifedipine. Also note the occurrence of aftercontractions following driven contractions in Panels A and B. Panels C and D show electrophysiological and contractile activity in the same preparation during reperfusion in the presence of nifedipine. Nifedipine abolished OAP's and aftercontractions, and reduced developed tension during reperfusion.

and B, top trace). A record of contractile activity is also shown in each panel. Small aftercontractions appeared in the contractile record from Purkinje tissue when OAP were present. The same preparation was exposed to nifedipine during a second cycle of ischemic conditions and reperfusion. Panels C-D were recorded during the second reperfusion. Purkinje tissue did not exhibit either OAP's or aftercontractions. Panels A and D allow comparison of responses at similar membrane potentials whereas panels A and C or B and D permit comparison at similar times. Table 3 summarizes the incidence of OAP's occurring in Purkinje tissue upon reperfusion. In control experiments OAP's occurred in 8 of 19 preparations during reperfusion. In the presence of verapamil, nifedipine, or pre-equlilibration with nifedipine OAP's did not occur. Thus, the calcium channel blocking agents abolished OAP's upon reperfusion.

d. Reperfusion-induced inexcitability

After the initial repolarization phase seen upon reperfusion, Purkinje tissue depolarized to membrane potentials less than -70 mV. During this phase preparations could no longer be driven by stimuli applied to Purkinje tissue. When stimulation was applied to the endocardial surface of papillary muscles, active responses were still elicited in muscle. However, these impulses failed to propagate to the recording sites in false tendon. Figure 9, panel A, shows an example recorded in a representative control experiment. The Purkinje tissue depolarized to -52 mV and became inexcitable. Action potentials were recorded from a site in muscle tissue. However, the recording from

Figure 9

Effects of nifedipine on the sequence of electrical activity occurring in Purkinje tissue in association with reperfusion-induced depolarization. Panels A-C show reperfusion in the absence of nifedipine. Panels D-F show reperfusion with nifedipine in the same preparation. In each panel the top trace (with reference to MDP during the pause) is a microelectrode recording from Purkinje tissue, the middle trace is a recording from muscle tissue, and the bottom trace shows the stimulus pattern. Voltage and time calibrations are shown in panel D.

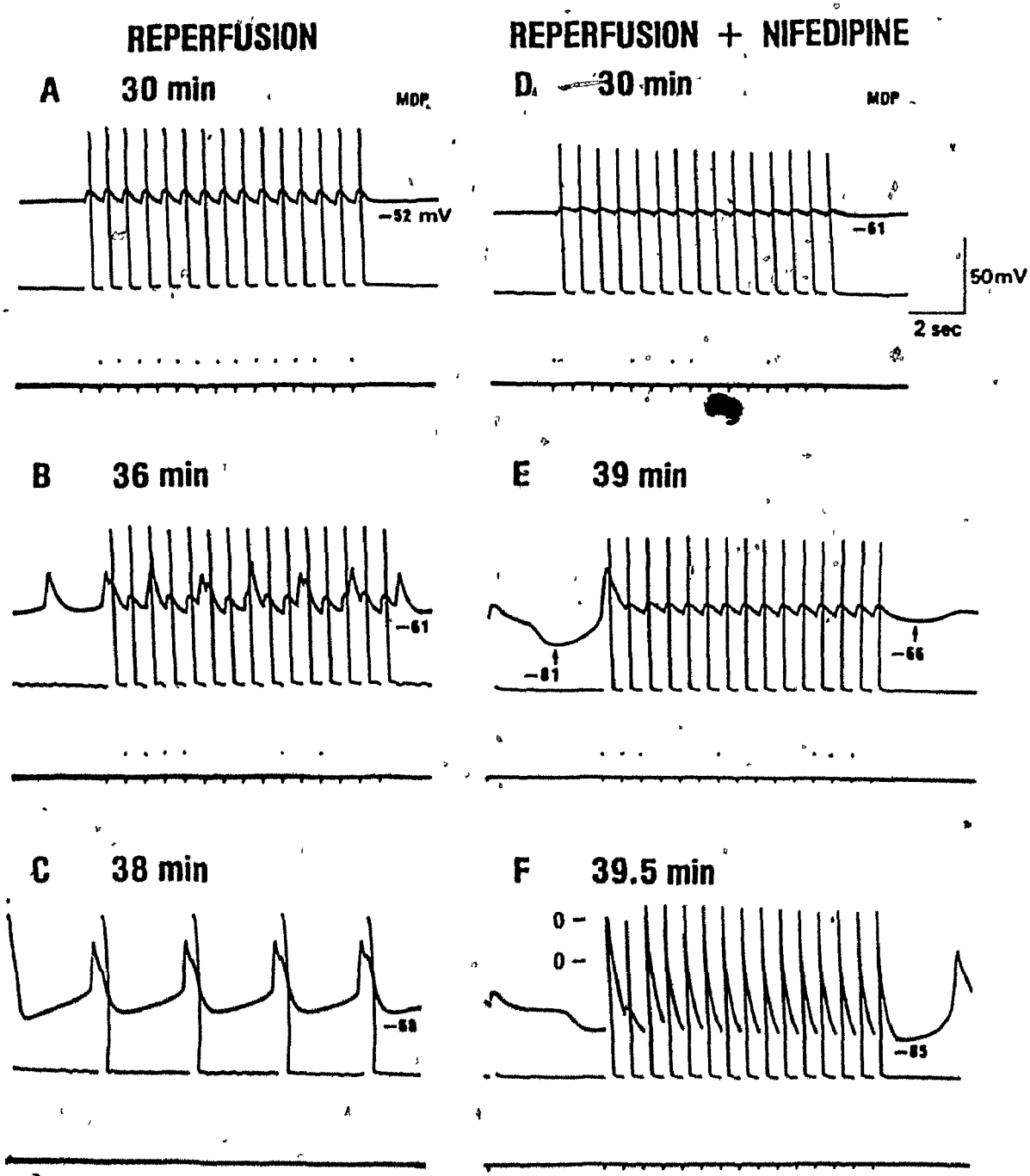
REPERFUSION
REPERFUSION + NIFEDIPINE
A 30 min
MDP
−52 mV
D 30 min
MDP
−61
50mV
2 sec
B 36 min
−61
E 39 min
−61
−66
C 38 min
−68
F 39.5 min
0 −
0 −
−85

Figure 9

Purkinje tissue showed no active action potentials. Only small electrotonic (passive) deflections were recorded. When the same preparation was subjected to reperfusion in the presence of nifedipine (Figure 9, panel D) the preparation again depolarized and became inexcitable. In the absence of calcium channel blocking agents, reperfusion-induced inexcitability occurred in 17 of 21 preparations upon reperfusion (Table 3). Reperfusion-induced inexcitability occurred in 6 of 7 preparations exposed to nifedipine, 3 of 4 pre-equilibrated and exposed to nifedipine, and 9 of 10 exposed to verapamil. Thus, although the time to reach inexcitability was greater in the presence of nifedipine, neither drug altered the incidence of depolarization or inexcitability.

e. Depolarization-induced automaticity

The beginning of recovery of Purkinje tissue is signalled by the appearence of automaticity at low membrane potentials (MDP<-65 mV). This type of pacemaker activity, which occurs at membrane potentials characterized by "slow response" or calcium mediated action potentials, has been described in Purkinje tissue (Hauswirth et al., 1969; Imanishi 1971) and muscle (Katzung and Morgenstern, 1977). In the latter study it has been referred to as depolarization-induced automaticity (DIA). Figure 9 shows an example of this type of automaticity occurring upon reperfusion. During the pause in stimulation (panel B, extreme left), automatic beats occurred in Purkinje tissue but did not propagate to muscle. During the train of stimulation approximately one third of impulses in muscle induced low amplitude potentials with slow upstrokes in Purkinje tissue. When stimulation was turned off 2 minutes later, the

membrane potential had increased from -61 to -68 mV and Purkinje tissue still displayed automaticity (panel C). The lower trace, recorded from a site in muscle, demonstrates that the spontaneous activity originating in Purkinje tissue propagated to muscle at this time. Following full recovery, the same preparation was exposed to ischemic conditions and reperfusion in the presence of nifedipine. Panels D-F were recorded during the second reperfusion. Automaticity did not occur while Purkinje tissue remained severely depolarized. As membrane potential recovered, Purkinje tissue frequently exhibited two levels of resting potential as described by Gadsby and Cranefield (1977). Panel E was recorded while the preparation was exhibiting different maximum transmembrane potentials in alternate pauses. When membrane potential remained low, automaticity was absent (far right) (this contrasts sharply with panel B, left). Only when membrane potential increased sufficiently to allow reactivation of sodium channels did automaticity appear (Panel E, far left). Also there were no driven responses in Purkinje tissue during the train of stimulation when the membrane potential remained low enough to inactivate sodium channels. Purkinje tissue abruptly repolarized to -85 mV 30 seconds later and driven action potentials were immediately elicited in Purkinje tissue. Table 3 summarizes the incidence and time of appearance of low membrane potential automaticity (depolarization-induced automaticity- DIA) in Purkinje tissue upon reperfusion. DIA occurred in 17 of 21 preparations upon reperfusion. However, none of the preparations displayed DIA upon reperfusion in the presence of verapamil, nifedipine, or with pre-equilibration with

nifedipine. Thus, calcium channel blocking agents abolished reperfusion-induced DIA.

f. Parasystole

Tissues exhibiting automaticity at low membrane potentials and which are connected to more polarized areas may function as parasystolic foci upon reperfusion (Ferrier and Rosenthal, 1980; Ferrier et al., 1985a). These investigators have shown that areas of tissue exhibiting automaticity upon reperfusion may display varying degrees of entrance and exit block (Rosenthal and Ferrier, 1983; Ferrier et al., 1985a). When entrance block is present, the spontaneous cycle length of DIA may be modulated by activity initiated outside the automatic focus. Figure 10 shows modulated parasystolic activity occurring in Purkinje tissue upon reperfusion. Panel A shows an example of parasystole with entrance block and exit conduction. Automaticity occurring in Purkinje tissue resulted in spontaneous impulses that propagated to the recording site in muscle. During the sequence illustrated in panel A, two stimuli were delivered to muscle. Both stimuli elicited action potentials that failed to propagate to the recording site in Purkinje tissue. However the first driven muscle action potential imposed an electronic depolarization late in the automatic cycle of Purkinje tissue and caused a marked acceleration of the next spontaneous firing of the Purkinje fiber (compared to last two spontaneous beats in panel A). The accelerated beat failed to propagate to the recording site in muscle. The next driven muscle action potential occurred early in the spontaneous cycle of Purkinje tissue. The action potential failed to propagate to the

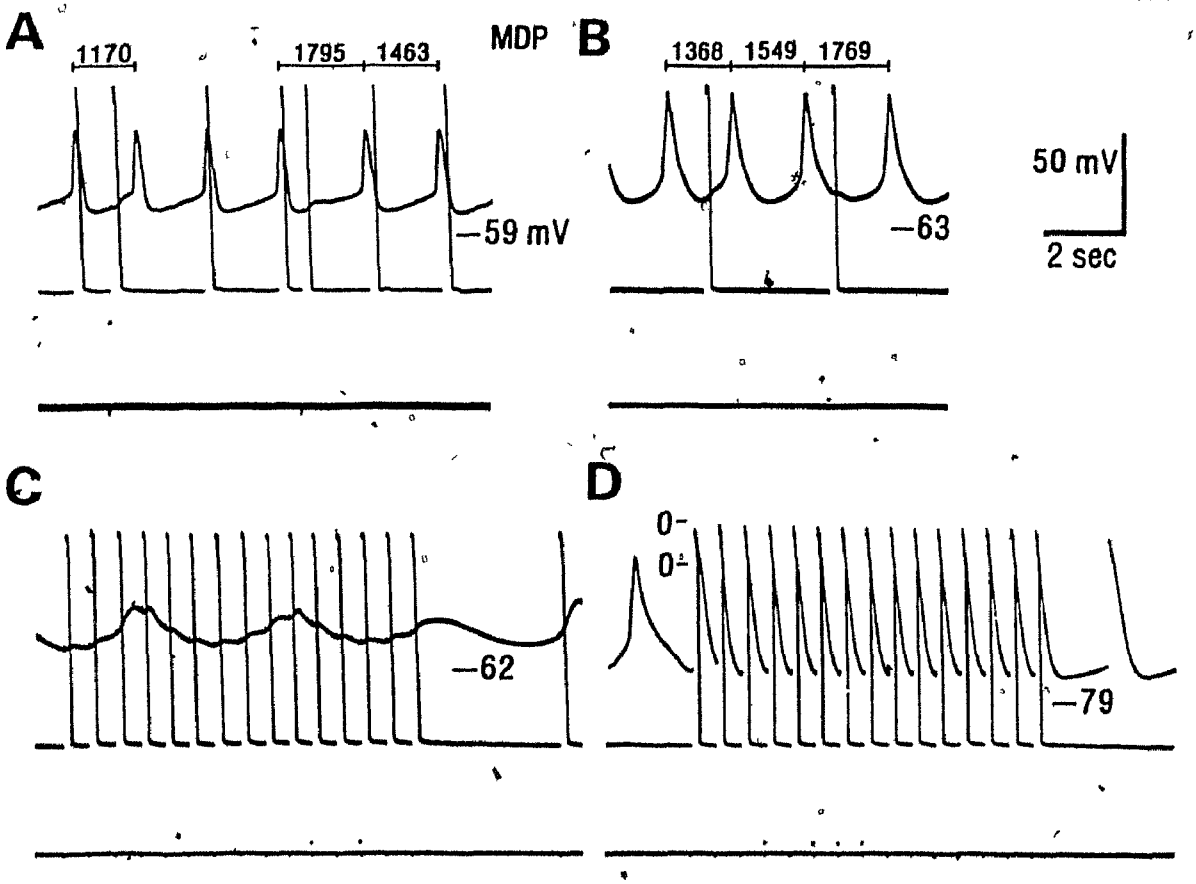

Figure 10

Panel A: Modulated parasystolic activity with entrance block and exit conduction induced by reperfusion. Panel B: Modulated parasystolic activity with entrance block. Panels C and D: Reperfusion with verapamil in the same preparation as in panel B. Traces are arranged as described for Figure 9. Voltage and time calibrations are shown in panel B.

recording site in Purkinje tissue. However, it imposed an electrotonic depolarization early in the spontaneous pacemaker cycle of Purkinje tissue and caused a marked delay in the generation of the next spontaneous impulse in Purkinje tissue. The spontaneous action potential propagated to the recording site in muscle. Thus, Purkinje tissue exhibited entrance block to test beats plus modulation of cycle length of automaticity. Panel B shows a similar example of entrance block with modulation recorded from another preparation. However this preparation exhibited complete exit block. Following full recovery the preparations were subjected to ischemic conditions and reperfusion in the presence of drug. Panels C and D were recorded from the same preparation as in panel B with a second reperfusion in the presence of verapamil. Low membrane potential automaticity was inhibited in the presence of verapamil. Fifteeen seconds later (panel D), the Purkinje tissue abruptly repolarized to a higher membrane potential, and active action potentials were elicited in Purkinje tissue with each stimulus of a train (i.e. no entrance block). Only at this higher membrane potential was automaticity observed in Purkinje tissue. Thus entrance block disappeared abruptly with the return of active action potentials. Table 3 summarizes the incidence and time of appearance of modulated parasystolic activity occurring in Purkinje tissue upon reperfusion. Parasystole was said to occur when Purkinje tissue displayed low membrane potential automaticity (MDP<-65mV), entrance block, and modulation. Parasystolic activity occurred in 9 of 21 preparations upon reperfusion. Neither entrance block nor modulation of automaticity could be

demonstrated in any of the preparations exposed to calcium channel blocking drugs. Thus the calcium channel blocking agents prevented modulated parasystolic activity.

g. Recovery of electrical activity upon reperfusion

We have found that not all preparations attain the same membrane potential at the end of 60 minutes of reperfusion, compared to the preischemic value. However, preparations do not display automaticity at membrane potentils greater than -80 mV during reperfusion. Therefore recovery was defined as having occurred when the membrane potential of Purkinje tissue exceeded -80mV and when automaticity ceased. All preparations in the control group recovered within 60 minutes of reperfusion (Table 3). Similarly all preparations recovered in the presence of nifedipine. In the presence of pre-equilibration with nifedipine 3 of 4 preparations recovered, and in the presence of verapamil 9 of 10 recovered. The time required for recovery is also summarized in Table 3. Recovery occurred at 44.0±2.0 minutes during the control reperfusion. In the presence of nifedipine or pre-equilibration with nifedipine, recovery occurred at 41.4±3.6 minutes and 44.3±3.0 minutes. Recovery in the presence of verapamil took 42.5±4.9 minutes. These results show that calcium channel blocking agents do not alter the recovery of electrical activity of Purkinje tissue following reperfusion.

3. Summary

Ischemic conditions caused a decrease in membrane potential, depressed excitability, and induced conduction block between muscle and Purkinje tissue. The presence of verapamil or nifedipine resulted in a

significantly greater depolarization of Purkinje tissue and increased incidence of inexcitability or conduction block in Purkinje and muscle tissues during ischemic conditions. Ischemic conditions also resulted in a decrease in developed tension in Purkinje tissue. In the presence of verapamil or nifedipine, developed tension in Purkinje tissue decreased much faster.

Return to non-ischemic conditions in the absence of drugs resulted in a complex sequence of responses in Purkinje tissue: prompt hyperpolarization, progressive depolarization to inexcitability, and final repolarization to control membrane potentials. Final repolarization included a phase of automaticity at low membrane potentials, during which Purkinje tissues functioned as a parasystolic foci. In the presence of verapamil or nifedipine reperfusion-induced depolarization of Purkinje tissue was unchanged. However occurrence of oscillatory afterpotentials, low membrane potential automaticity, and parasystole was abolished. Return to non-ischemic conditions in the absence of drugs also resulted in a partial return in developed tension within the first 5 minutes of reperfusion. After 5 minutes of reperfusion, contractility decreased to zero. Return of contractile activity was observed after 20 minutes of reperfusion. In the presence of calcium channel blocking agents, a similar return and decline in contractility was observed upon initial reperfusion. Final return of contractile activity in the presence of calcium channel blocking agents occurred much later in reperfusion and was significantly attenuated in comparison to control at the end of 60 minutes of reperfusion.

B) EFFECTS OF ISCHEMIC CONDITIONS ON CALCIUM-DEPENDENT ACTION POTENTIALS

Sperelakis and Schneider (1976) proposed that calcium channels in ischemic cells may not be available for activation because of decreased ATP levels, decreased pH or accumulation of some metabolite. Ischemic conditions in the present model caused depression of contractions in Purkinje tissue. Reperfusion caused Purkinje tissue to depolarize to a low membrane potential and was associated with inexcitability and depression of contractions in this tissue. Final repolarization of Purkinje tissue was associated with a phase of automaticity at low membrane potentials. Contractions returned in Purkinje tissue during recovery of membrane potential. These observations suggest that ischemic conditions and reperfusion may have effects on inactivation and recovery of the slow inward calcium current in Purkinje tissue. If this is so, ischemic conditions and reperfusion may affect the induction of calcium-dependent action potentials. The effects of ischemic conditions and reperfusion on calcium-dependent action potentials in Purkinje tissue were investigated using a current clamp method to depolarize Purkinje tissue. Automaticity observed in depolarized canine Purkinje fibers (MDP<-65mV) is dependent on the slow inward calcium current for its activation (Imanishi, 1971) and will be referred to as depolarization-induced automaticity (DIA).

1. Ischemic Conditions

a. Induction of DIA

The effects of ischemic conditions on DIA are shown in Figure 11. During control conditions (panel A), depolarization of Purkinje tissue to a MDP of -39 mV elicited DIA with a cycle length of 463 msec. Panels B and C show electrical activity in Purkinje tissue during ischemic conditions. Traces in panels B and C were recorded from Purkinje tissue depolarized to similar membrane potentials as in panel A. The cycle length of DIA increased to 609 msec after exposure to ischemic conditions for 15 minutes (panel B). Also DIA was not sustained for the duration of the current clamp. The current clamp elicited 2 automatic beats followed by a sub-threshold oscillation. DIA was not elicited when the membrane potential was depolarized to -41 mV in panel C. In this preparation there was total inhibition of calcium-dependent action potentials after 25 minutes of exposure to ischemic conditions. Inhibition was said to occur when automaticity could not be elicited at membrane potentials less than -65 mV. The effects of ischemic conditions on the electrophysiological changes in DIA were investigated in 10 Purkinje fibers. All 10 preparations showed an increase in cycle length of DIA. Nine of 10 preparations showed inhibition of sustained DIA, similar to that shown in Figure 11, panel B. Four of 10 preparations showed a cessation of DIA (similar to Figure 11, panel C) at all membrane potentials tested. Thus, ischemic conditions strongly inhibited DIA.

Figure 11

A representative experiment demonstrating the effects of ischemic conditions on depolarization-induced automaticity (DIA) in a Purkinje fiber. Traces from top to bottom in each panel are: Purkinje electrical activity, record of current application, and stimulus pattern. Panel A shows induction of DIA in response to a depolarizing current pulse. Panels B and C show the effects of ischemic conditions on DIA. At 15 minutes of exposure to ischemic conditions (Panel B), DIA was not sustained during the application of depolarizing current. The cycle length of the initial two automatic beats was also increased. DIA was inhibited 10 minutes later (Panel C).

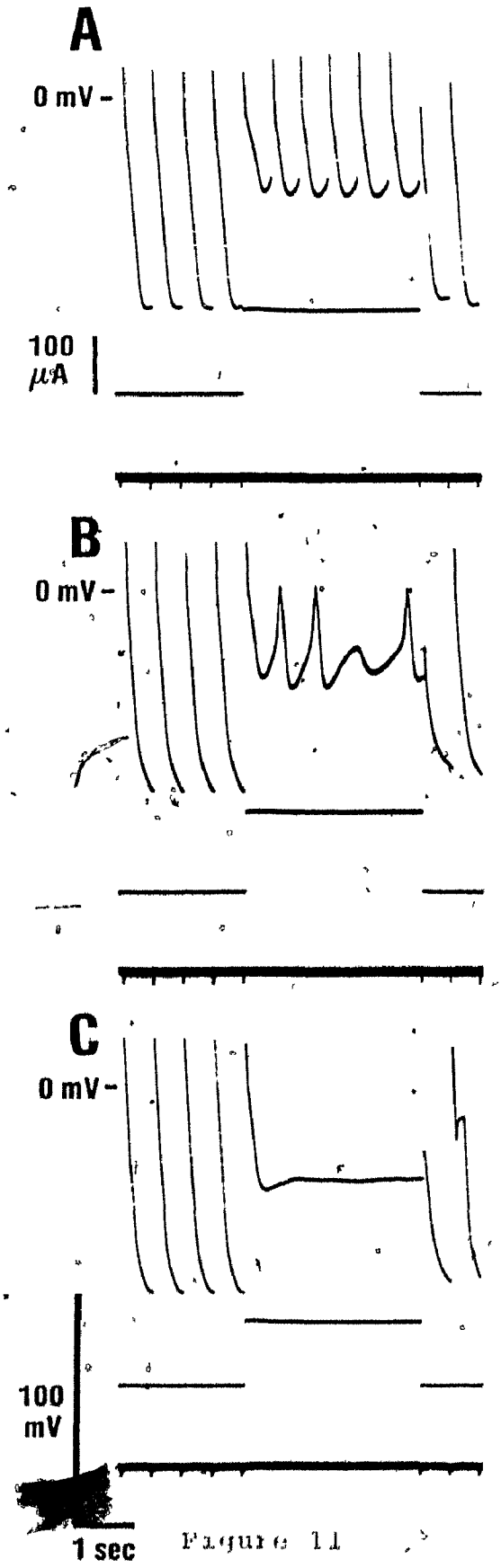
A
MDP = −39 mV
CL = 463 msec
0 mV
100 μA
B
−36 mV
609 msec
0 mV
C
−41 mV
0 mV
100 mV
1 sec

Figure 11

b. Cycle length of DIA

The changes in cycle length of DIA in Purkinje tissue during ischemic conditions was also determined. This was done 2 ways. First, the changes in cycle length of DIA at one membrane potential were determined. Figure 12 shows changes in cycle length of DIA at a MDP of -50 ±2 mV in a preparation that displayed automaticity at this membrane potential for the duration of ischemic conditions. Within the first 5 minutes of ischemic conditions, there was an abbreviation of the cycle length of DIA. After 5 minutes of exposure to ischemic conditions, the cycle length progressively increased with continued exposure to ischemic conditions. At the end of the period of ischemic conditions the cycle length of DIA was greater than the pre-ischemic value.

Changes in the cycle length of DIA over a range of membrane potentials were also determined during ischemic conditions. Figure 13 shows changes in cycle length of DIA as a function of membrane potential between -60 mV and -10 mV. Within 20 minutes of initiating ischemic conditions, the cycle length of DIA had increased with respect to control at all MDP's between -45 and -55 mV. Similar measurements after 30 minutes of exposure to ischemic conditions demonstrated a further increase in cycle length of DIA. All 10 preparations showed an increase in cycle length of DIA. These results indicate that ischemic conditions cause a similar progressive increase in the cycle length of DIA at all membrane potentials.

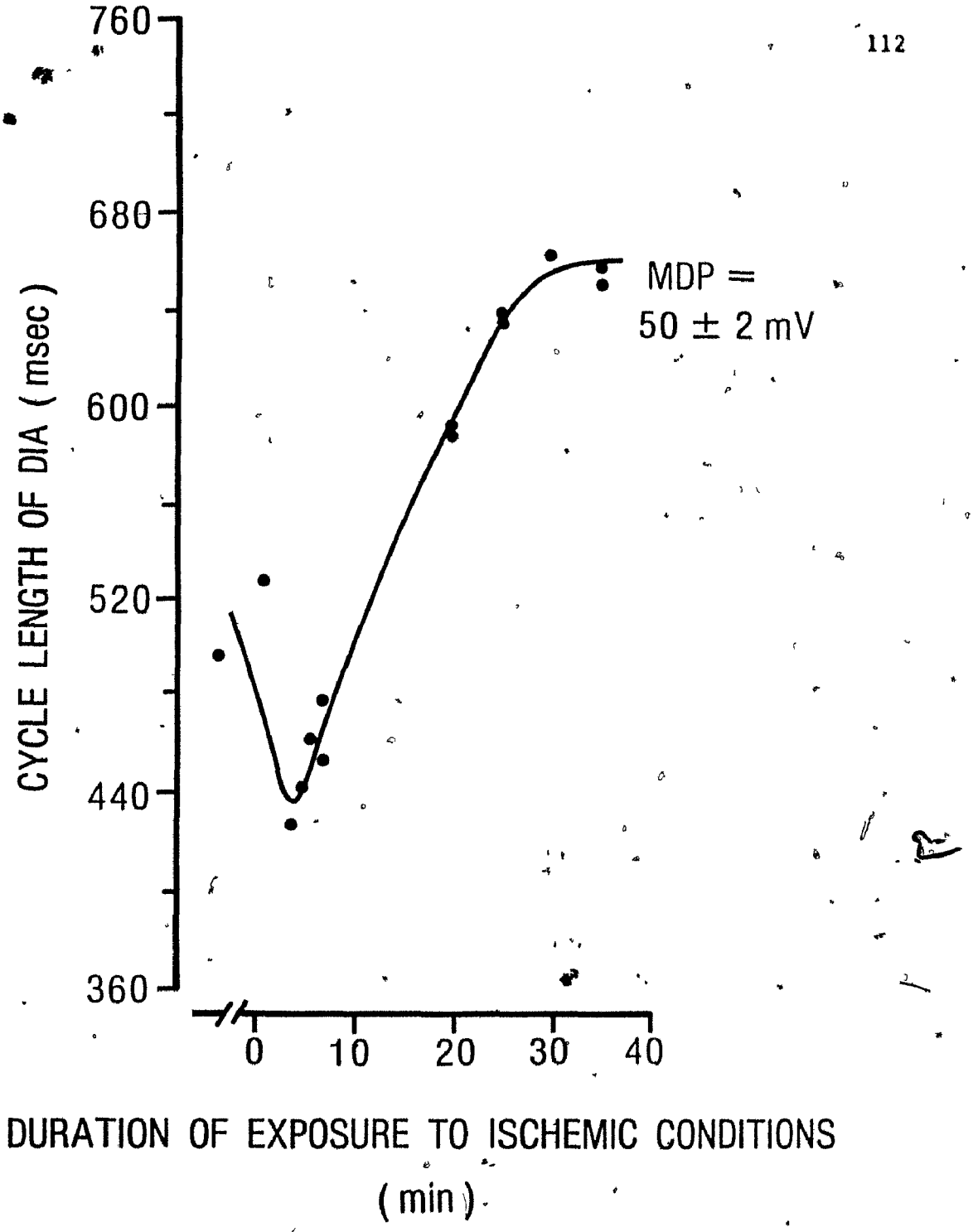

Figure 12

Effect of ischemic conditions on changes in cycle length of DIA at a membrane potential of -50 mV in a Purkinje fiber.

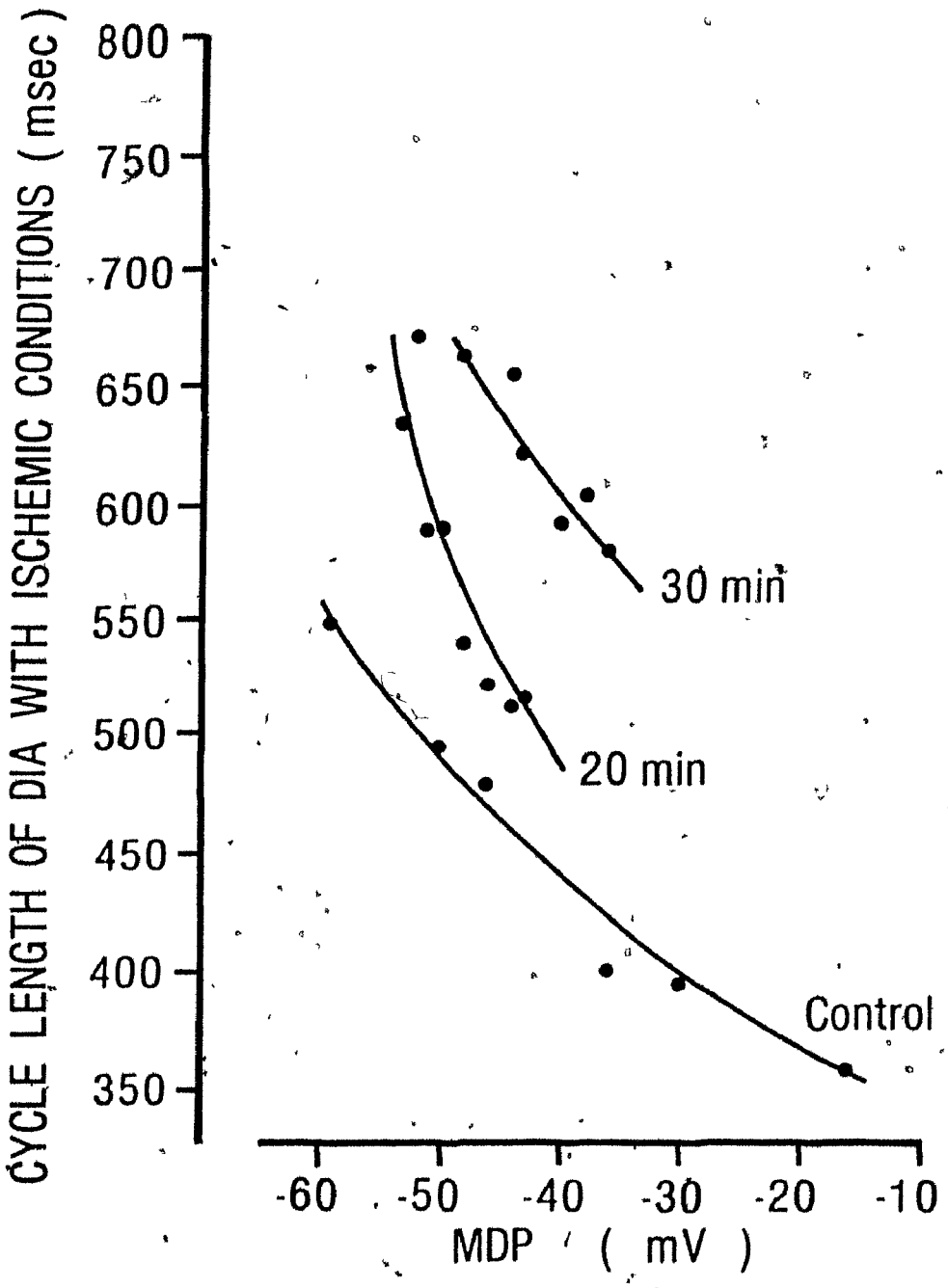

Figure 13

The effects of membrane potential on cycle length of DIA during exposure to ischemic conditions. Control indicates the cycle length of DIA obtained prior to exposure to ischemic conditions. The cycle length of DIA increased over the range of membrane potentials tested at 20 and 30 minutes of exposure to ischemic conditions.

c. Threshold voltage for induction of DIA

Ischemic conditions may inactivate only a certain fraction of calcium channels and thereby shift the membrane potential at which calcium-dependent action potentials can be elicited to a more depolarized level (i.e. less negative). Thus, ischemic conditions may inhibit calcium-dependent action potentials at membrane potentials very close to the threshold for activation of the calcium current, but not at more negative membrane potentials. Figure 14 shows results from an experiment in which changes in the threshold voltage for DIA were measured during exposure to ischemic conditions. DIA could be elicited at MDP's ranging between -65 and -15 mV during the pre-ischemic period. Between 0 and 10 minutes of exposure to ischemic conditions DIA was obtained between MDP's of -46 and -15mV. However DIA could not be elicited by depolarizing the tissue to MDP's between -57 and -46mV. At 20, 25, and 30 minutes of exposure to ischemic conditions, DIA could not be elicited at MDP's more negative than -36mV. After 35 to 40 minutes of exposure to ischemic conditions DIA was only elicited by the current clamp between MDP's of -36 and -33mV. DIA could not be elicited between -33 and -15mV. These results suggest that ischemic conditions may shift the threshold voltage for DIA to less negative membrane potentials and also decrease the range of membrane potentials over which DIA can be elicited.

d. Action potential configuration

The action potential of Purkinje tissue is characterized by a distinct plateau. The slow inward current is believed to contribute to

Figure 14.

The effects of ischemic conditions on threshold voltage of DIA in a Purkinje fiber.

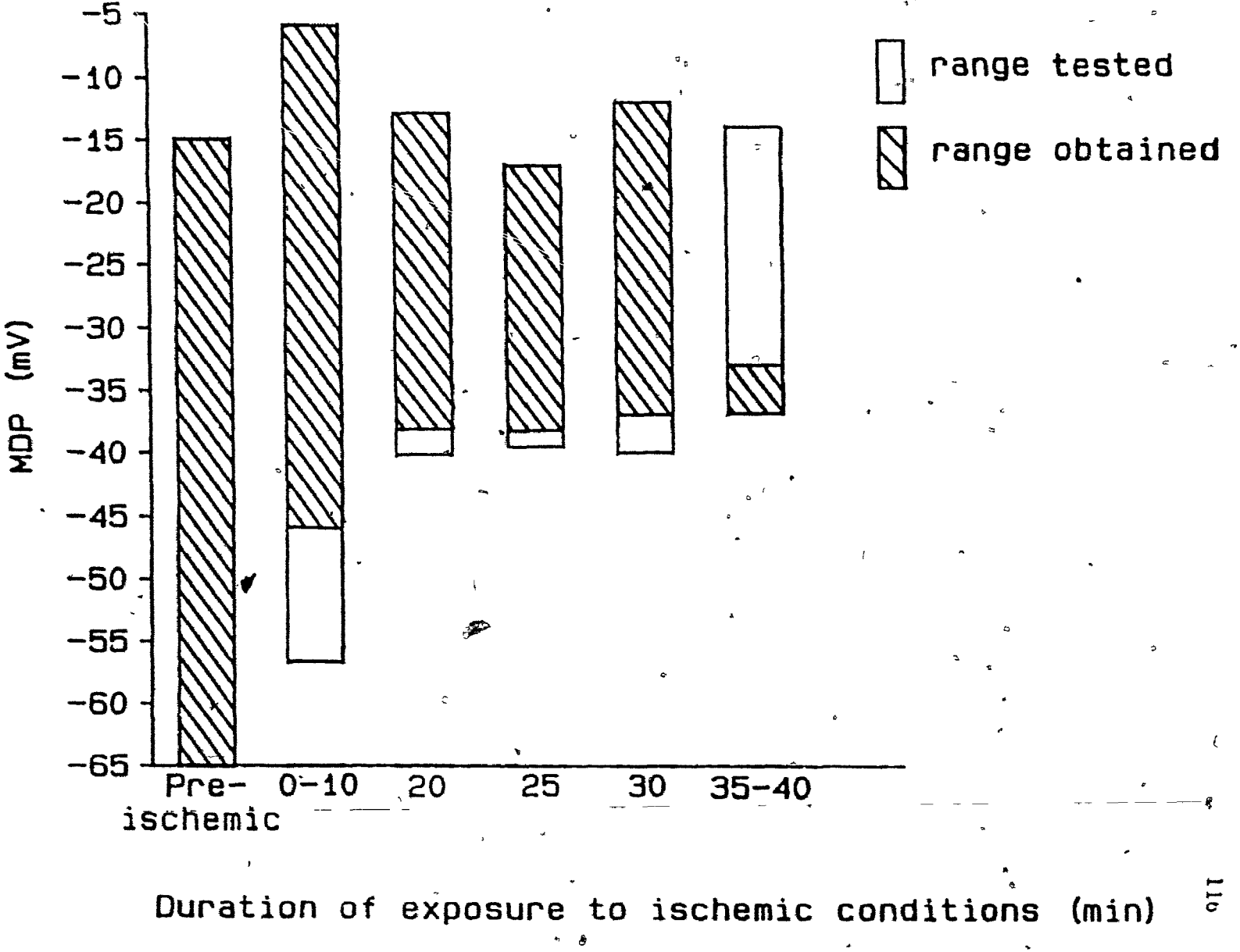
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
-60
-65
MDP (mV)
range tested
range obtained
Pre-ischemic
0-10
20
25
30
35-40
Duration of exposure to ischemic conditions (min)

Figure 14

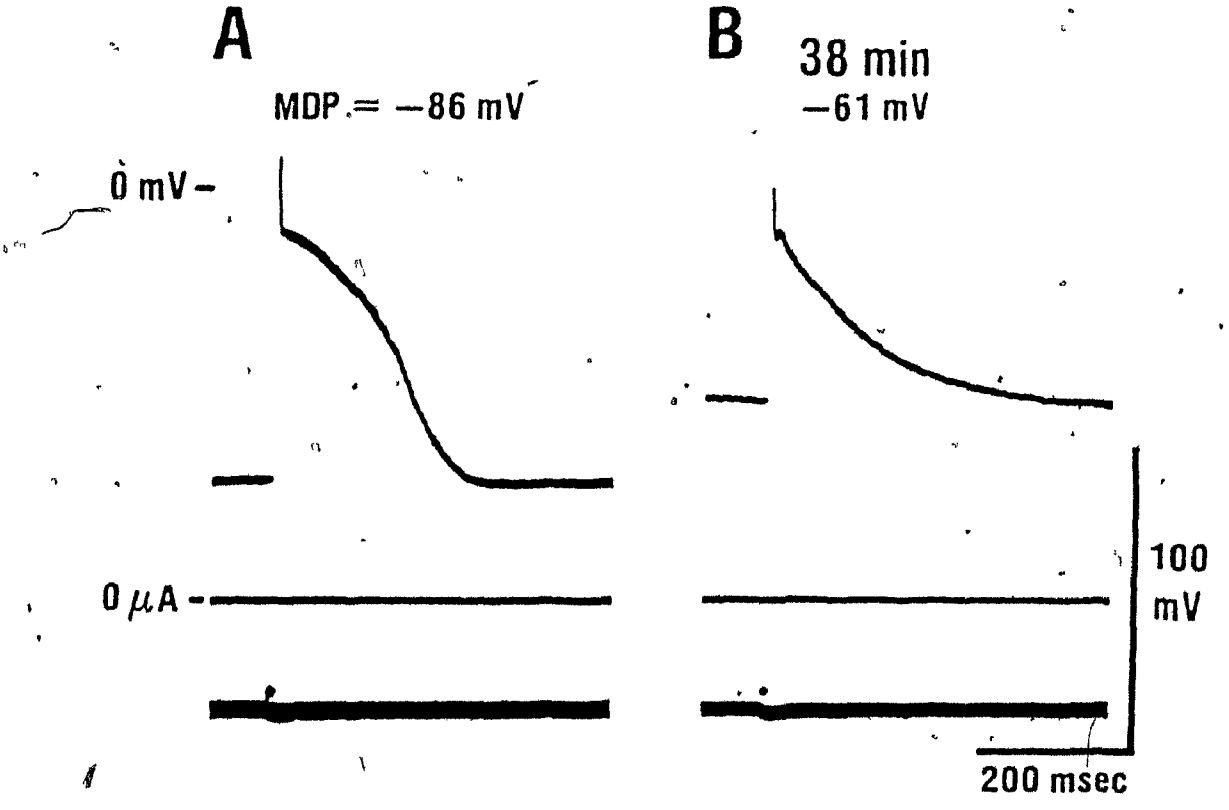

Figure 15

Change in action potential configuration in Purkinje tissue with exposure to ischemic conditions. Panel A shows a control action potential configuration. Panel B was recorded after 38 minutes of exposure to ischemic conditions.

the plateau phase of the action potential. Inhibition of the slow inward current results in abbreviation of the action potential plateau (Kass and Tsien, 1975). Therefore the effects of ischemic conditions on the action potential plateau were also measured. Figure 15 shows the action potential configuration of a Purkinje cell before exposure to ischemic conditions. A distinct plateau can be observed. After 38 minutes of exposure to ischemic conditions, the Purkinje cell had depolarized, and the plateau was essentially abolished. A similar abbreviation of action potential plateau was seen in all 10 preparations at the end of exposure to ischemic conditions.

2. EFFECTS OF REPERFUSION ON CALCIUM -DEPENDENT ACTION POTENTIALS

a. Induction of DIA

One might predict that, if ischemic conditions result in inhibition of calcium mediated action potentials then return to non-ischemic conditions should result in restoration of calcium mediated action potentials. Figure 16 illustrates the effects of reperfusion on DIA (same preparation as Figure 11). In panel A, at 1 minute of reperfusion, DIA returned but the activity was not sustained. One minute later, depolarization to a similar membrane potential did not elicit any automaticity in Purkinje tissue. The tissue was depolarized in the absence of externally applied electric current at this time, and a couple of minutes later became inexcitable. DIA could not be elicited at any membrane potential between 2 and 20 minutes of reperfusion. After 20 minutes of reperfusion Purkinje tissue remained depolarized but DIA could be elicited again when the MDP was decreased to -37 mV by applied current.

Figure 16

Electrophysiological changes in DIA during reperfusion after ischemic conditions in the same preparation as in Figure 11. Traces from top to bottom in each panel are: Purkinje electrical activity, record of current application, and stimulus pattern. MDP= maximum diastolic potential of the first action potential elicited by a current pulse. CL= cycle length of the first two automatic beats elicited by a current pulse.

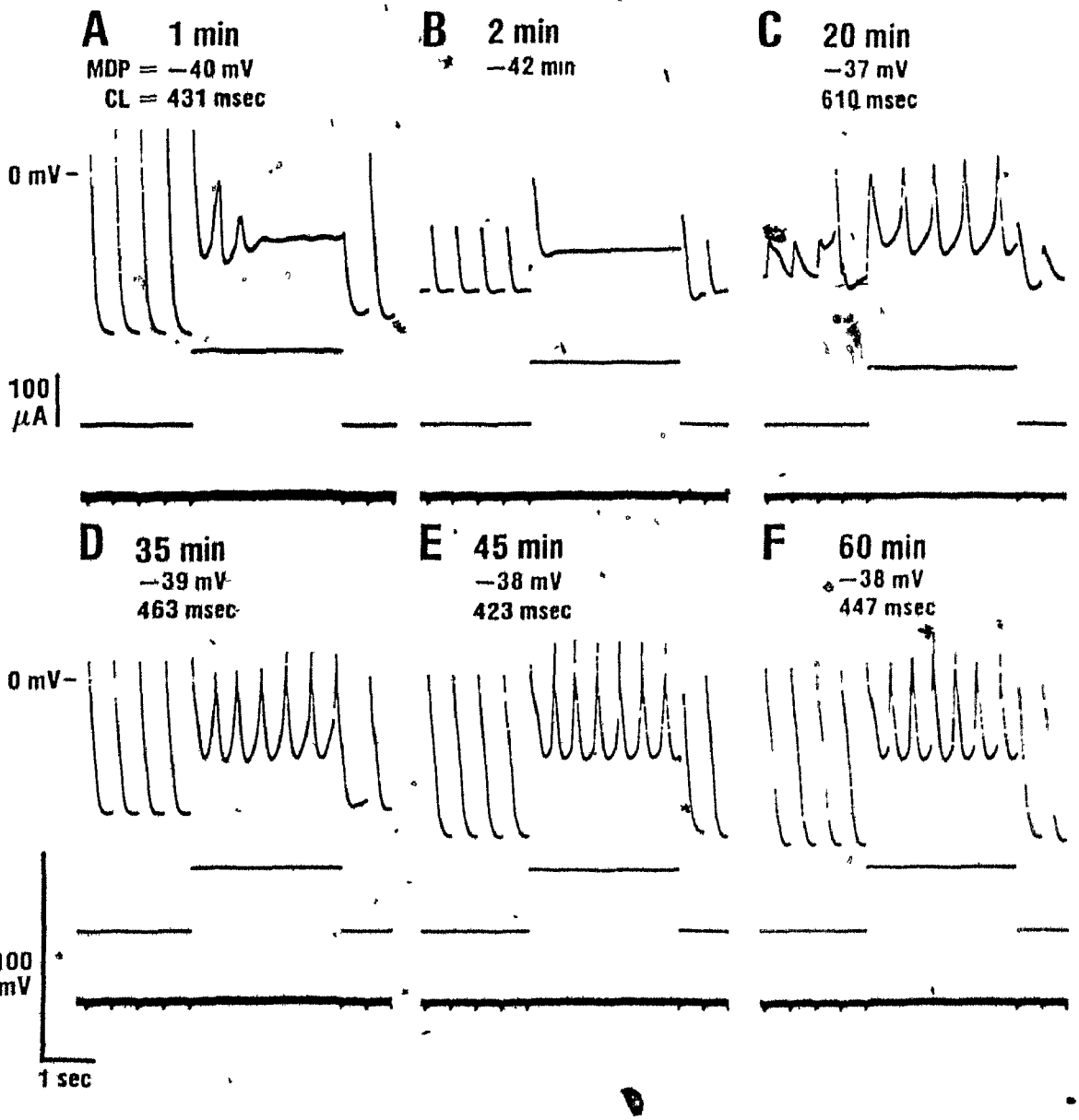
A 1 min
MDP = −40 mV
CL = 431 msec
B 2 min
−42 min
C 20 min
−37 mV
610 msec
0 mV
100 μA
D 35 min
−39 mV
463 msec
E 45 min
−38 mV
423 msec
F 60 min
−38 mV
447 msec
0 mV
100 mV
1 sec

Figure 16

Therefore depolarization in response to reperfusion was not the cause of inactivation of DIA. Automaticity had a longer cycle length, than observed at a similar membrane potential after 1 minute of reperfusion. With further recovery, Purkinje tissue regained excitability, and active responses propagated to the recording site. As spontaneous MDP gradually increased, the cycle length of DIA elicited by depolarization to -37 to -39 mV decreased (panels D-F).

Inhibition of DIA occurred frequently early in reperfusion. Figure 17 shows the number of preparations in which DIA were inhibited at all membrane potentials during reperfusion. Four preparations displayed inhibition of DIA at the end of the "ischemic" period. After 2 minutes of reperfusion, the number of preparations displaying inhibition of DIA increased to 7. Inhibition of DIA gradually decreased with time. After 20 minutes of reperfusion, DIA remained inhibited in only 2 of 10 preparations. Subsequently, all preparations exhibited DIA with depolarizing current pulses. These results suggest that reperfusion may cause an initial inhibition of DIA above that seen during ischemic conditions.

b. Cycle length of DIA

In addition to causing a second inhibition of DIA, reperfusion also altered the cycle length of DIA. The changes in cycle length of DIA at a MDP of -50mV±2mV during reperfusion are shown in Figure 18. The pre-ischemic value for the cycle length of DIA at a MDP of -50 ± 2mV is also indicated. DIA at this membrane potential was not abolished during reperfusion in the example illustrated. The cycle length of DIA decreased during the initial 20 minutes of reperfusion to values similar to that

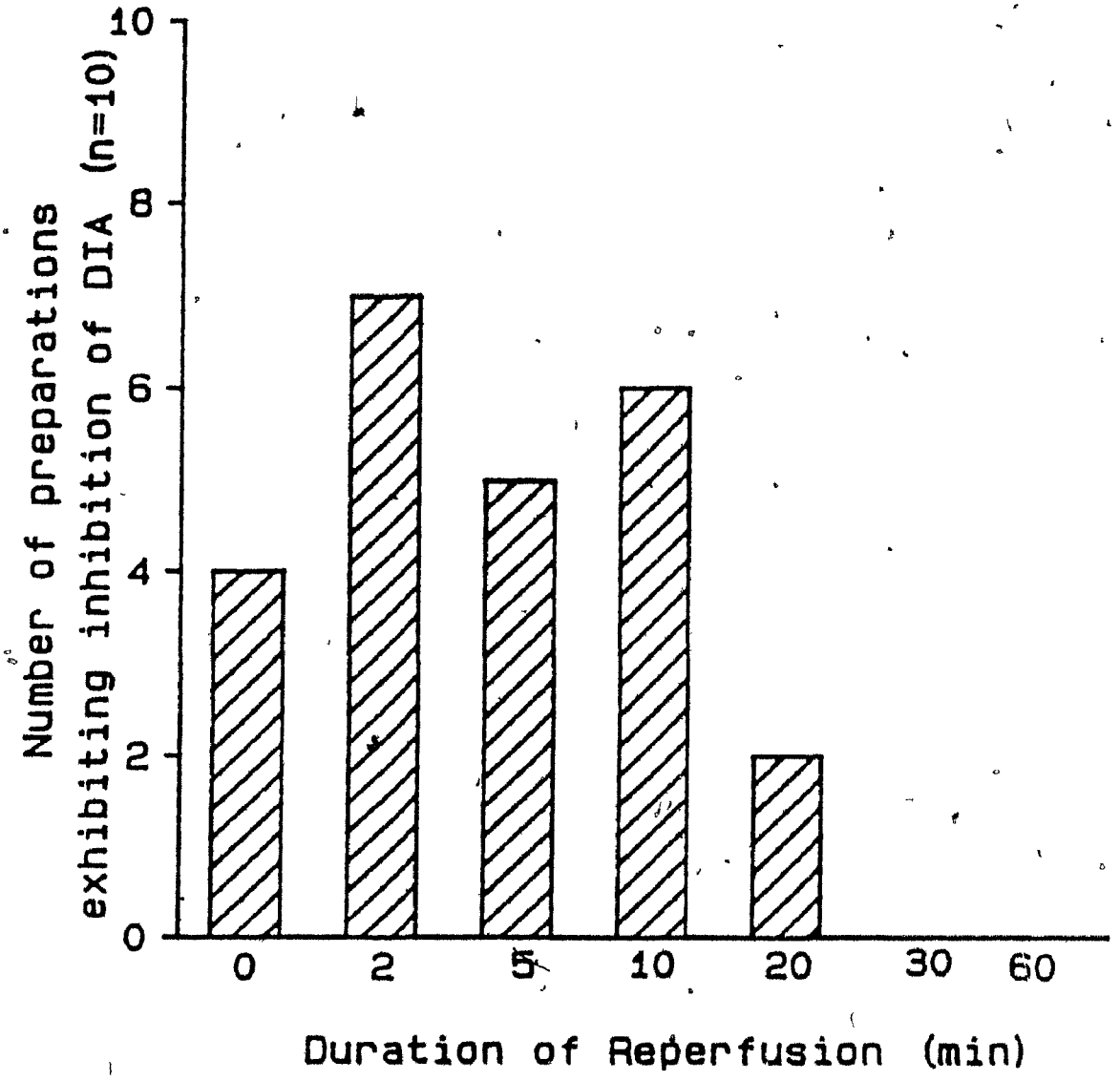

Figure 17

The number of preparations in which DIA were inhibited at all membrane potentials during reperfusion.

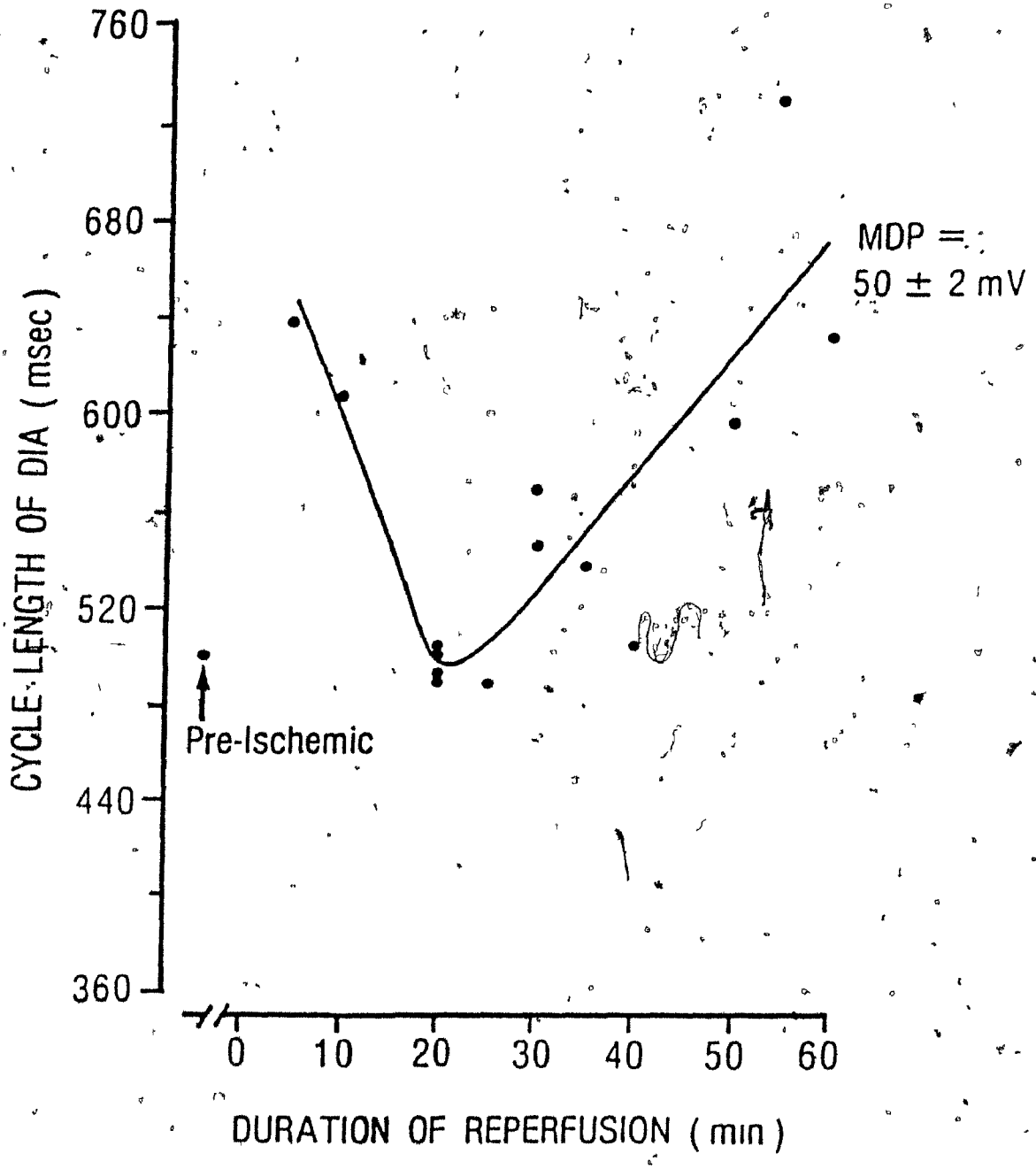

Figure 18

Effect of reperfusion on changes in cycle length of DIA at a membrane potential of -50 mV in a Purkinje fiber.

obtained during the pre-ischemic control. The cycle length of DIA then increased gradually for the remainder of reperfusion.

The changes in cycle lengths of DIA over a range of membrane potentials were also investigated. Figure 19 shows changes in cycle lengths of DIA over a range of membrane potentials during reperfusion. At 20 minutes of reperfusion, the cycle lengths of DIA at all membrane potentials tested were similar to those obtained before exposure to ischemic conditions. However as the duration of reperfusion increased, there was a progressive increase in cycle length of DIA at all membrane potentials tested.

The effects of reperfusion on changes in cycle length of DIA are summarized in Table 4 for all 10 preparations studied. The cycle length of DIA during reperfusion was compared to the cycle length of DIA obtained during the pre-ischemic period. If the cycle length of DIA obtained during reperfusion was different from the pre-ischemic value by greater than 10%, then the appropriate increase or decrease was designated. Initially, upon reperfusion (at 2 minutes), the three preparations displaying DIA, had cycle lengths greater than the respective controls. After 20 minutes of reperfusion, 4 of 8 preparations displaying DIA also showed an increase in cycle length of automaticity. However 3 of the remaining 4 preparations had cycle lengths of DIA similar to their respective pre-ischemic values. At 30 and 60 minutes of reperfusion, 7 of 10 preparations showed an increase in cycle length of DIA. These results suggest that initially upon reperfusion (within the first 20 minutes), there may be abbreviation of the cycle length of DIA to

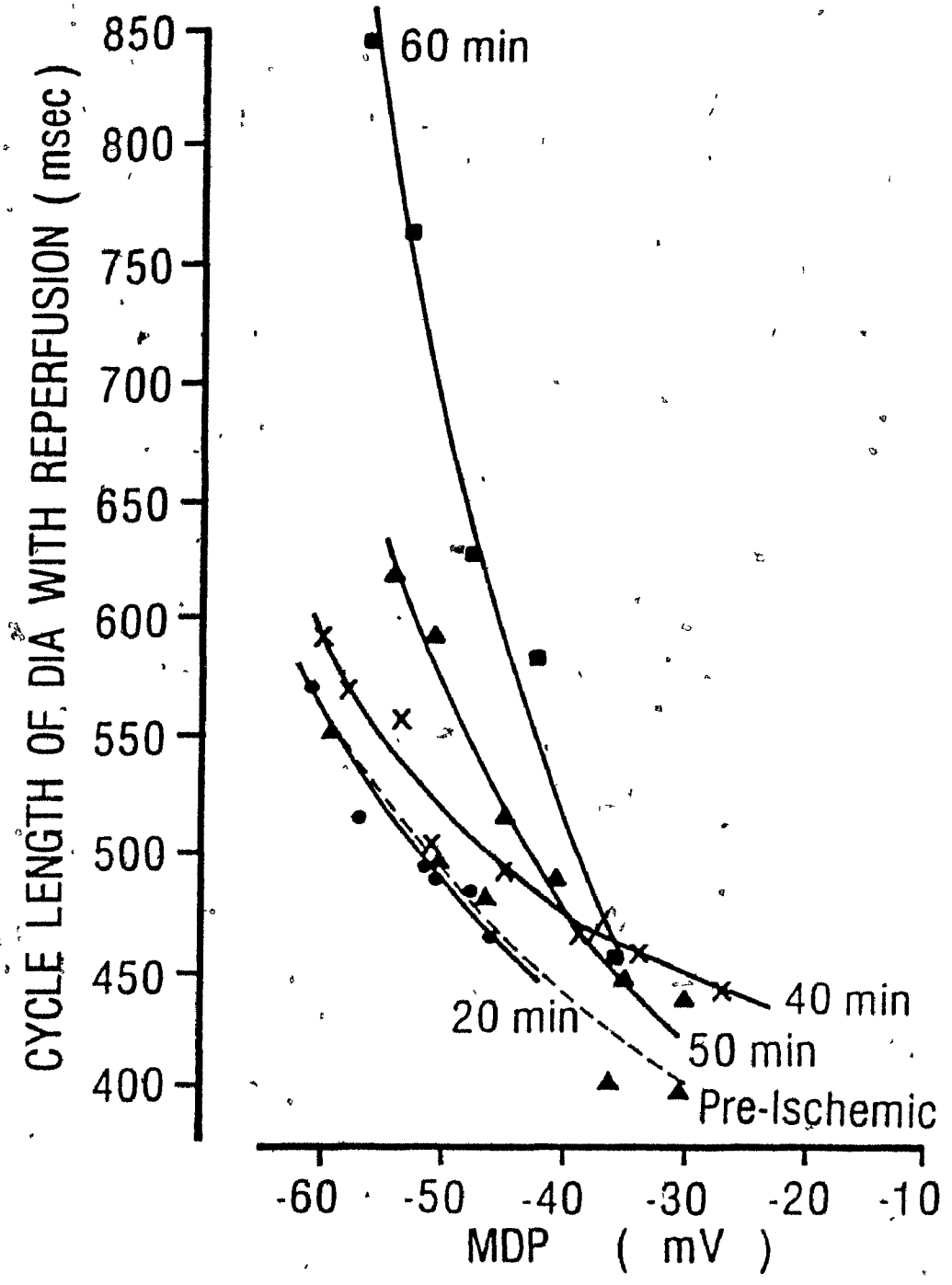

Figure 19

The effects of membrane potential on cycle length of DIA during exposure to reperfusion.

Table 4: Effect of Reperfusion on Changes in Cycle Length of DIA

Time (min)	CL Decreased	CL Same	CL Increased	DIA Abolished
2			3	7
5	1	1	3	5
10	1	1	2	6
20	1	3	4	2
30	2	1	7	0
60	1	2	7	0

N=10

CL=Cycle length of DIA

Comparison made with the pre-ischemic control cycle length of DIA.

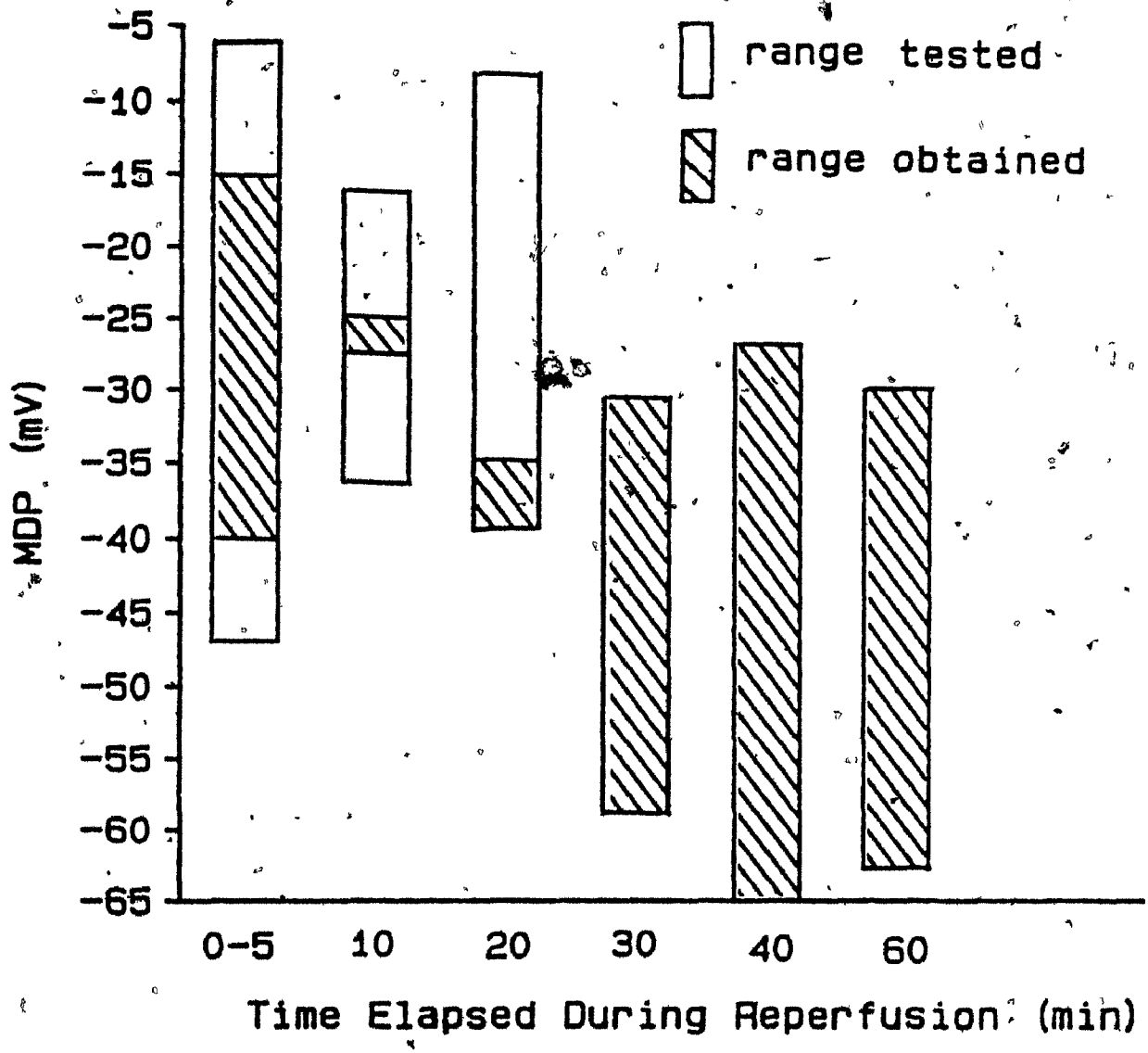

Figure 20

The effect of reperfusion on threshold voltage for activation of DIA.

values similar to pre-ischemic controls. However after 20 minutes of reperfusion, the cycle length of DIA increased above pre-ischemic values.

c. Threshold voltage for induction of DIA

Because, reperfusion caused inhibition of DIA, the effects of reperfusion on the range of membrane potentials at which DIA could be elicited were also investigated. Figure 20 shows an example of the range of membrane potentials at which DIA could be elicited upon reperfusion (same experiment as Figure 14). Within 5 minutes of reperfusion, the range of MDP's at which DIA could be elicited was increased compared to the range obtained at the end of ischemic conditions (compare Figure 14 at 35-40 min to Figure 20 at 0-5 min). This was seen in 7 of 10 preparations. However at 10 minutes of reperfusion, DIA could only be elicited at -26mV. This inhibition remained at 20 minutes of reperfusion at which time DIA could only be elicited between -37 and -35mV. However at 30, 40, and 60 minutes of reperfusion, DIA could be elicited over a wide range of membrane potentials. Similar results were seen in all 10 preparations. These results indicate that reperfusion initially resulted in narrowing of the range of MDP at which DIA could be elicited. However final recovery of Purkinje tissue during reperfusion was associated with return of DIA over a wide range of membrane potentials.

d. Action potential configuration

Figure 21 shows changes in action potential configuration in a preparation during reperfusion. The effects of ischemic conditions in this preparation are shown in Figure 15 panel B and were discussed in an

Figure 21

The effect of reperfusion on changes in action potential configuration in a Purkinje fiber. Panels A-D were recorded from the same preparation as in Figure 16. In panel A, at 5 minutes of reperfusion, a hyperpolarizing current pulse was applied to increase the membrane potential to the same as that of the control (Figure 16, panel A). In panel B a similar hyperpolarizing current pulse was applied to increase the membrane potential to that of the control. In panels C and D, the membrane potential of Purkinje tissue had recovered to control values.

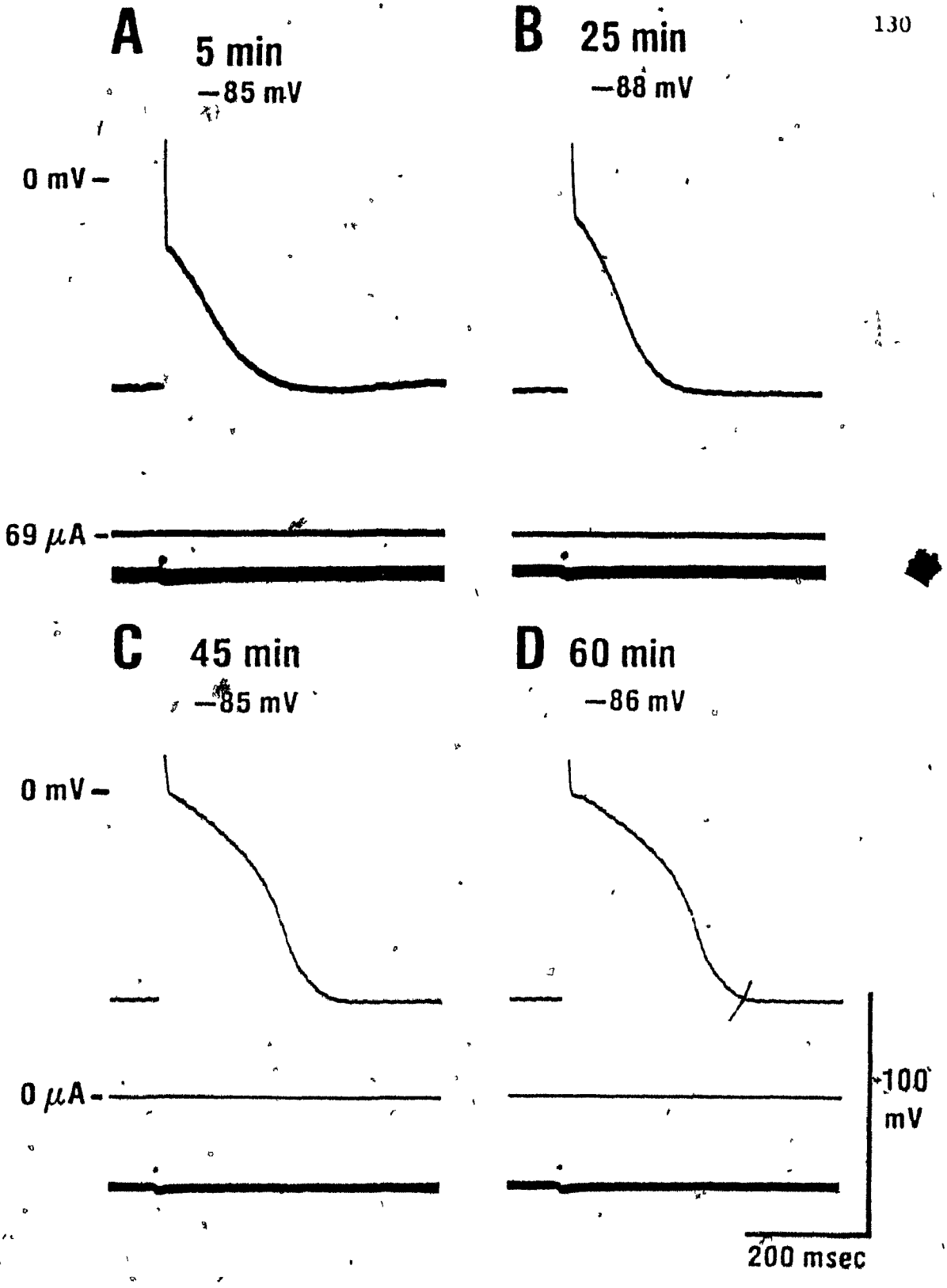
A
5 min
−85 mV
B
25 min
−88 mV
0 mV
69 μA
C
45 min
−85 mV
D
60 min
−86 mV
0 mV
0 μA
100 mV
200 msec

Figure 21

earlier section. In panel A, at 5 minutes of reperfusion, the preparation became inexcitable at a low membrane potential. At this time DIA could not be elicited at any membrane potential. To compare the action potential configuration at this time to the control, the tissue was hyperpolarized by extracellular application of current to a membrane potential similar to the control. Within 5 minutes of reperfusion there was a marked abbreviation of the action potential plateau plus a shift of the plateau to more negative membrane potentials (compare Figure 21 panel A to Figure 15 panel A). However a large fast sodium upstroke could still be elicited at this time when the cell was hyperpolarized. At 25 minutes of reperfusion, the tissue was still depolarized. At this time DIA could be elicited, but only at very low membrane potentials. Hyperpolarization to a membrane potential similar to control (Figure 15 panel A) allowed a stimulated action potential to be elicited. However the plateau remained abbreviated at this time. At 45 minutes of reperfusion, the membrane potential returned to control levels. Therefore a hyperpolarizing pulse was not needed to return the membrane potential back to control levels for comparisons. At this time DIA could be readily elicited at a wide range of membrane potentials. Panel D shows the configuration of the action potential at this time. The configuration of the plateau had returned close to that of the control action potential. The plateau of the action potential remained at 60 minutes of reperfusion (panel D). These observations demonstrate that early in reperfusion there is an abbreviation of the action potential plateau, at a time when DIA cannot be elicited. These results provide additional

evidence that the calcium channel is inactivated during reperfusion. Furthermore, although the calcium channel was inactivated, the fast sodium response was still available during reperfusion, as long as the membrane potential returned close to control values. It should be noted that hyperpolarizing current pulses were used throughout the stimulated action potentials shown in Figure 21 (Panels A and B). Therefore, some of the reduction in the plateau may be attributed to enhanced repolarization due to the hyperpolarizing current pulse.

3) SUMMARY

Ischemic conditions increase the cycle length of DIA, prevent sustained DIA [redacted] may result in complete cessation of DIA. The increase in cycle length of DIA during ischemic conditions occurred over a wide range of membrane potentials. Ischemic conditions lowered the MDP's at which DIA could be elicited (i.e. shifted the threshold voltage to less negative values). Inhibition of DIA during ischemic conditions was associated with abbreviation of the action potential plateau.

Reperfusion initially resulted in return of DIA in 30% of preparations, but only at very depolarized membrane potentials. Reperfusion was also associated with a secondary inhibition of DIA in 50% of preparations. However DIA returned later in reperfusion when membrane potential began to recover in Purkinje tissue. Reperfusion also caused changes in cycle length of DIA. The cycle length of DIA during initial reperfusion (within the first 20 minutes) shortened to values similar to those achieved during the pre-ischemic period. After 20 minutes of reperfusion, the cycle length of DIA progressively increased above the

pre-ischemic values. Reperfusion initially decreased the membrane potential range at which DIA could be elicited. However as the duration of reperfusion increased, DIA could again be elicited over a wide range of membrane potentials. Inhibition of DIA during reperfusion was associated with an abbreviation of the action potential plateau. As DIA returned later in reperfusion, there was an increase in the action potential plateau.

C) EFFECTS OF BAY-K8644 ON CALCIUM-DEPENDENT ACTION POTENTIALS DURING ISCHEMIC CONDITIONS AND REPERFUSION

The availability of calcium-mediated action potentials was decreased during ischemic conditions and reperfusion. BAY-K8644 is a dihydropyridine (a nifedipine analogue), which has been found to have a positive inotropic effect and vasoconstricter action (Schramm et al., 1983). This drug has been shown to prolong the mean open time of the calcium channel (Kokubun and Reuter, 1984) and to increase the magnitude of the calcium current, although some Ca-antagonists properties have been reported (Hess et al., 1984). Therefore BAY-K8644 might be expected to prevent inactivation of the calcium channel in a dynamic system such as ischemia and reperfusion. The effects of BAY-K8644, "a calcium agonist", on changes in DIA during ischemic conditions and reperfusion were investigated in 6 preparations exposed to ischemic conditions and reperfusion. Following recovery from a control reperfusion a second exposure to ischemic conditions and reperfusion in the presence of BAY-K8644 was performed.

1. Effects of Ischemic Conditions in the absence and presence of BAY-K8644 on DIA

a. Cycle length of DIA

Because BAY-K8644 increases Ca-conductance, it might be expected to decrease the cycle length of DIA. The effects of ischemic conditions in the absence and presence of BAY-K8644 on changes in cycle length of DIA at one membrane potential were determined. Figure 22 shows changes in cycle length of DIA (MDP = -50 ± 2mV) during ischemic conditions. Control ischemic conditions initially caused a decrease in cycle length (within 5 minutes) followed by a progressive increase in cycle length of DIA. In the presence of BAY-K8644 ischemic conditions caused a greater decrease in cycle length of DIA within 5 minutes of exposure to ischemic conditions. However after 10 minutes of exposure to ischemic conditions with BAY-K8644, the cycle length of DIA progressively increased to levels greater than observed with ischemic conditions alone. There was complete inhibition of DIA by 25 minutes of exposure to ischemic conditions and BAY-K8644. Table 5 summarizes the data from all 6 preparations. All comparisons of DIA were made to the respective pre-ischemic control values for DIA. DIA was abolished in 1 preparation early during exposure to ischemic conditions, whereas 1 preparation showed a decrease in cycle length of DIA, and 4 showed an increase in cycle length of DIA. Later during the control ischemic conditions (35-40 minutes), 2/3 of preparations showed an increase in cycle length of DIA and 1/3 of preparations showed an inhibition of DIA. Early during ischemic conditions in the presence of BAY-K8644, half of the preparations showed a

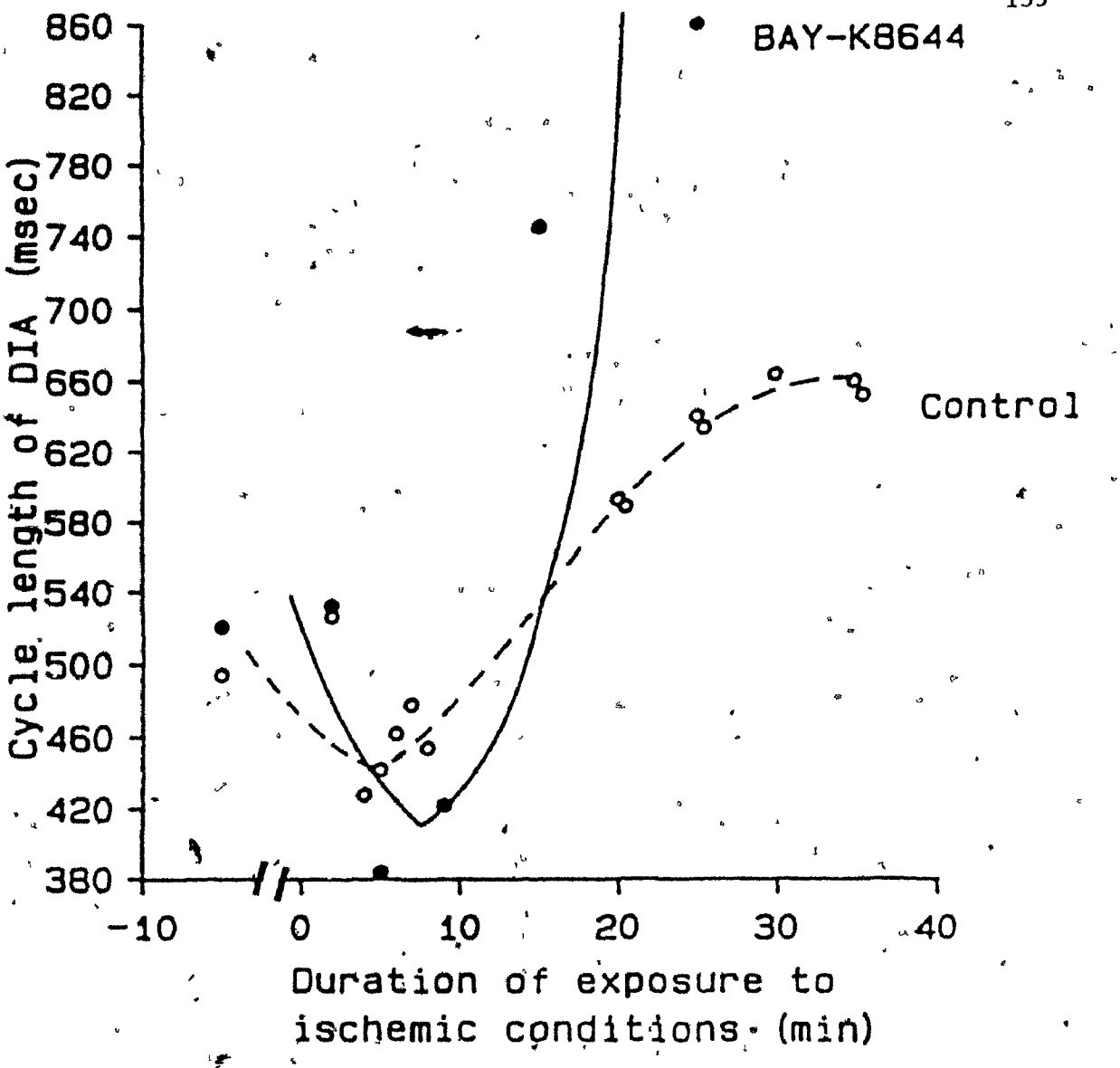

Figure 22.

Change in cycle length of DIA at -50 mV during ischemic conditions in the absence and presence of BAY-K8644 in a Purkinje fiber. DIA was inhibited after 35 minutes of exposure to ischemic conditions. In the presence of BAY-K8644 and ischemic conditions, DIA was inhibited after 25 minutes.

Table 5: Effects of Ischemic Conditions on DIA in the Absence and Presence of BAY-k8644

Time (min)	Intervention	CL Decreased	CL Increased	DIA Inhibited
15	I	1	4	1
	I + Bay-k8644	3	3	-
20	I	-	4	2
	I + Bay-k8644	2	4	-
30	I	-	4	2
	I + Bay-k8644	-	3	3
35-40	I	-	4	2
	I + Bay-k8644	-	2	4

N=6

I=Ischemic conditions

Comparison made with the respective pre-ischemic control value.

decrease in cycle length of DIA, and half showed an increase in cycle length of DIA. Later in ischemic conditions in the presence of BAY-K8644 (30 minutes) half of the preparations showed an increase in cycle length of DIA and half displayed cessation of DIA. By the end of ischemic conditions in the presence of BAY-K8644, 1/3 of preparations showed an increase in cycle length of DIA, whereas 2/3 of preparations displayed cessation of DIA.. These results suggest that initially during ischemic conditions, BAY-K8644 tends to prevent inhibition of DIA. However later during exposure to ischemic conditions, BAY-K8644 was unable to prevent the increase in cycle length or abolition of DIA.

b. Threshold voltage of DIA

If BAY-K8644 is able to stimulate calcium-dependent action potentials, then it might be expected to decrease the degree of depolarization needed to elicit DIA during ischemic conditions. Figure 23 shows the effects of BAY-K8644 on the range of membrane potentials at which DIA were elicited during ischemic conditions. In the absence of BAY-k8644, ischemic conditions caused a progressive decrease in the threshold voltage for initiation of DIA (see previous section also). After the tissue had recovered from the first cycle of ischemic conditions and reperfusion, a second cycle was conducted. The second pre-ischemic control was performed in the absence of BAY-K8644. As shown in Figure 23, DIA could be elicited only at more depolarized potentials during the second pre-ischemic control. Between 0-10 minutes of exposure to ischemic conditions in the presence of BAY-K8644, DIA could be elicited at higher membrane potentials than the corresponding time in the

Figure 23

Change in threshold voltage for activation of DIA in the absence and presence of BAY-K8644. The open bars indicate the range of membrane potentials tested for DIA. The shaded bars indicate the range of membrane potentials in which DIA was obtained.

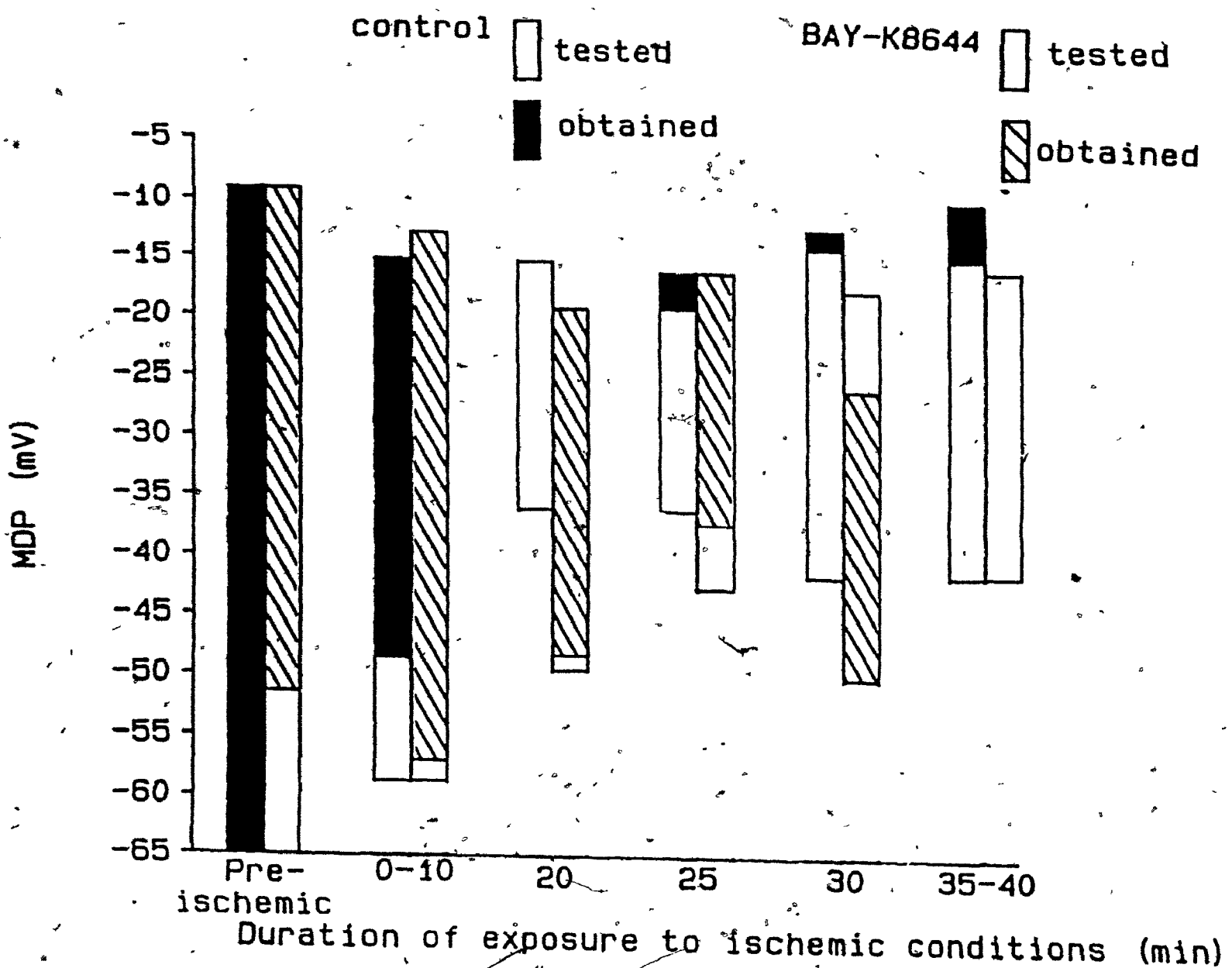
control
tested
obtained
BAY-K8644
tested
obtained
-5
-10
-15
-20
-25
-30
-35
-40
-45
-50
-55
-60
-65
MDP (mV)
Pre-
ischemic
0-10
20
25
30
35-40
Duration of exposure to ischemic conditions (min)

Figure 23

presence of ischemic conditions alone. This effect of BAY-K8644 remained at 20, 25, and 30 minutes of exposure to ischemic conditions and BAY-K8644. Therefore in the presence of ischemic conditions and BAY-K8644 there was an increase in the range of membrane potentials over which DIA could be elicited compared to ischemic conditions alone. However in the presence of BAY-K8644, ischemic conditions also decreased threshold voltage for initiation of DIA. At 35-40 minutes of exposure to ischemic conditions and BAY-k8644, DIA was abolished. However in the presence of ischemic conditions alone, DIA could still be elicited, although only at potentials much less than in the pre-ischemic control. Table 7 summarizes the effects of BAY-k8644 during ischemic conditions on the change in threshold voltage for DIA at comparable times evaluated during exposure to ischemic conditions alone. Between 0-10 minutes of exposure to ischemic conditions and BAY-k8644, only 2 preparations were available for comparison, since the other 4 preparations were not tested in the same membrane potential range as the control ischemic conditions. In these 2 preparations, DIA occurred at a higher membrane potential when compared to ischemic conditions alone. At 20 minutes of exposure to ischemic conditions and BAY-k8644, 4 preparations were available for comparison. One of the 4 preparations showed inhibition of DIA. The other 3 preparations displayed DIA at a higher membrane potential in the presence of ischemic conditions and BAY-k8644 compared to without BAY-k8644. At 25 and 30 minutes, 3 preparations displayed DIA at a higher membrane potentials. Between 35 and 40 minutes, 2 preparations displayed DIA at a higher membrane potential, whereas inhibi-

Table 6: Effect of BAY-k8644 on Threshold Voltage for Activation of DIA During Ischemic Conditions

Time (min)	*Higher Membrane Potential	Inhibition of DIA
0-10	2	-
20	3	1
25	3	2
30	3	3
35-40	2	4

N=6

*Compared to control ischemic conditions in the absence of BAY-k8644.

Figure 24

Occurrence of OAP's in a Purkinje fiber during reperfusion in the presence of BAY-K8644. The top trace in each panel shows a record of transmembrane electrical activity from Purkinje tissue, the middle trace is a recording of contractile activity of the same tissue, and the bottom trace indicates the stimulus pattern. Panels A and B show the occurrence of OAP's at 2 and 3 minutes respectively during reperfusion in the presence of BAY-K8644. Also note the occurrence of after-contractions following driven contractions in Panels A and B. Panel C shows the electrophysiological and contractile activity at 4 minutes of reperfusion in the presence of BAY-K8644. The tissue depolarized and became automatic at a low membrane potential. Reperfusion in the absence of BAY-K8644 is shown in Figure 16.

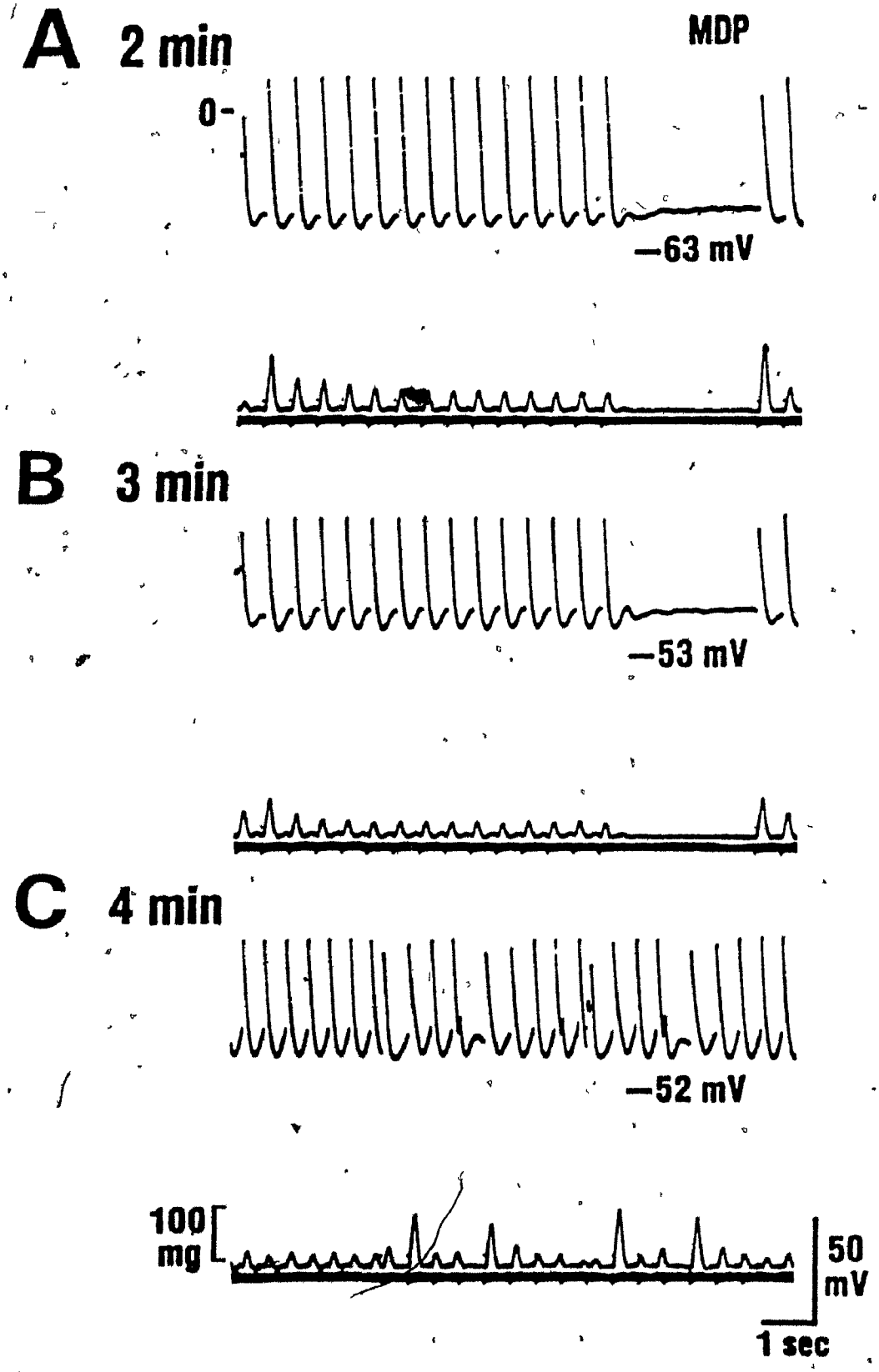

Figure 24

tion occurred in 4 preparations. Therefore these results suggests that even though BAY-K8644 does not prevent inhibition of DIA during ischemic conditions, it still exerts an effect on DIA by increasing the threshold voltage for DIA.

2. EFFECTS OF BAY-K8644 ON DIA DURING REPERFUSION

a. Electrophysiological changes in DIA in the absence and presence of BAY-K8644

Figure 24 shows the effects of reperfusion in the presence of BAY-K8644 in a representative experiment. During the control reperfusion, DIA was inhibited in this preparation (Figure 16). In the presence of Bay-k8644, DIA was not inhibited by reperfusion (panel C). OAP also appeared initially upon reperfusion at a time when DIA could be elicited (Figure 24, panels A and B). The occurrence of OAP suggests that BAY-K8644 may contribute to a calcium overload upon reperfusion. In the presence of BAY-K8644, 5 of 6 preparations displayed OAP upon reperfusion.

Figure 25 shows the number of preparations in which DIA was inhibited by reperfusion in the absence and presence of BAY-K8644. At the end of the control period of ischemic conditions, 2 preparations out of 6 showed inhibition of DIA, whereas DIA was inhibited in 4 of 6 preparations exposed to BAY-K8644 DIA. In both groups, only 1 of 6 preparations showed inhibition within the first 5 minutes of reperfusion. However, after 10 minutes of reperfusion, DIA was inhibited in 4 control preparations but only 1 preparation in the BAY-K8644 treated group. At 20 minutes of reperfusion 1 control preparation displayed inhibition of

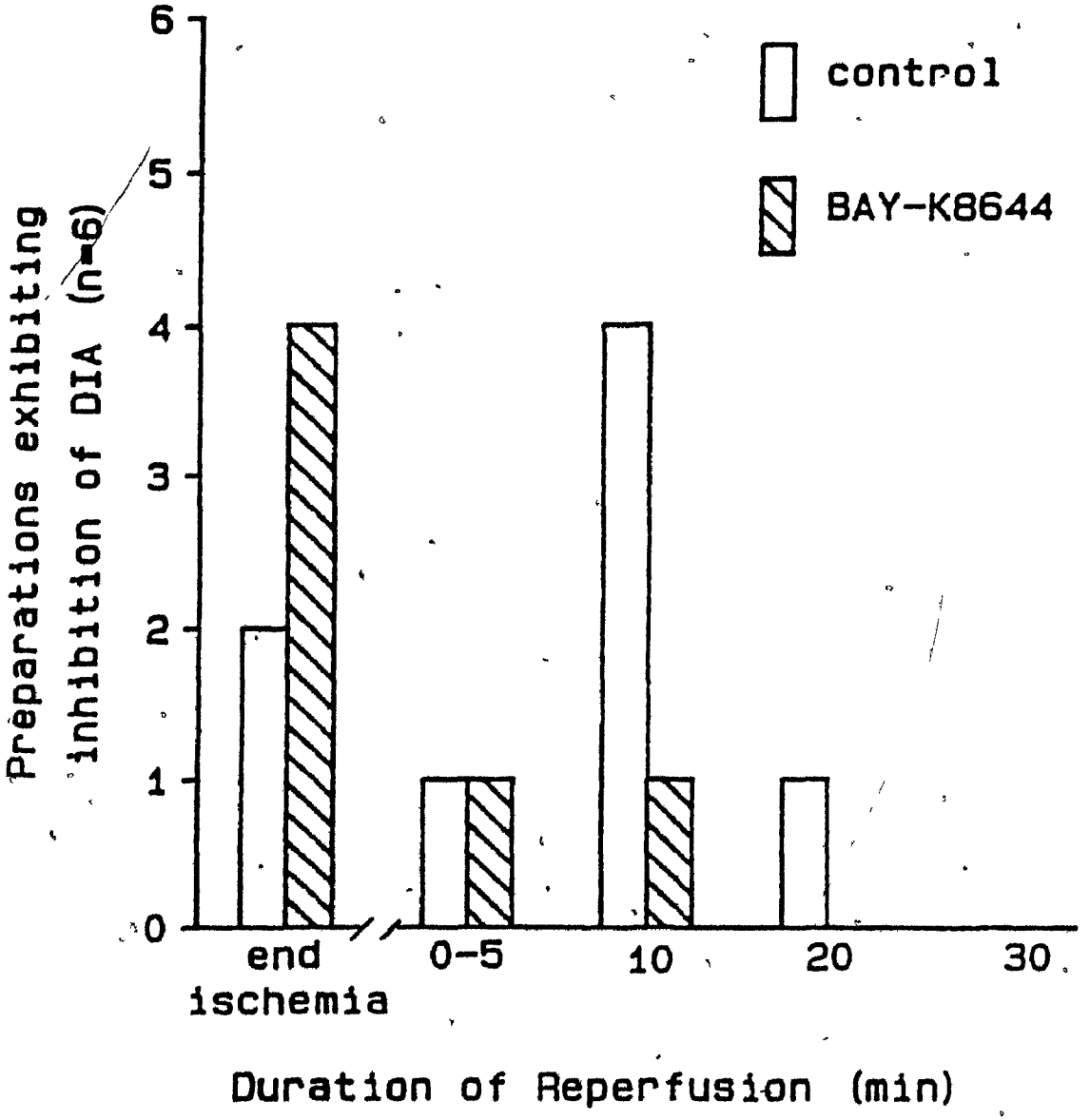

Figure 25

Inhibition of DIA during reperfusion in the absence and presence of BAY-K8644. The ordinate indicates the number of preparations in which DIA could not be elicited as a function of duration of exposure to reperfusion.

Figure 26

Effects of reperfusion in the absence and presence of BAY-K8644 on changes in cycle length of DIA at a MDP of -50 mV.

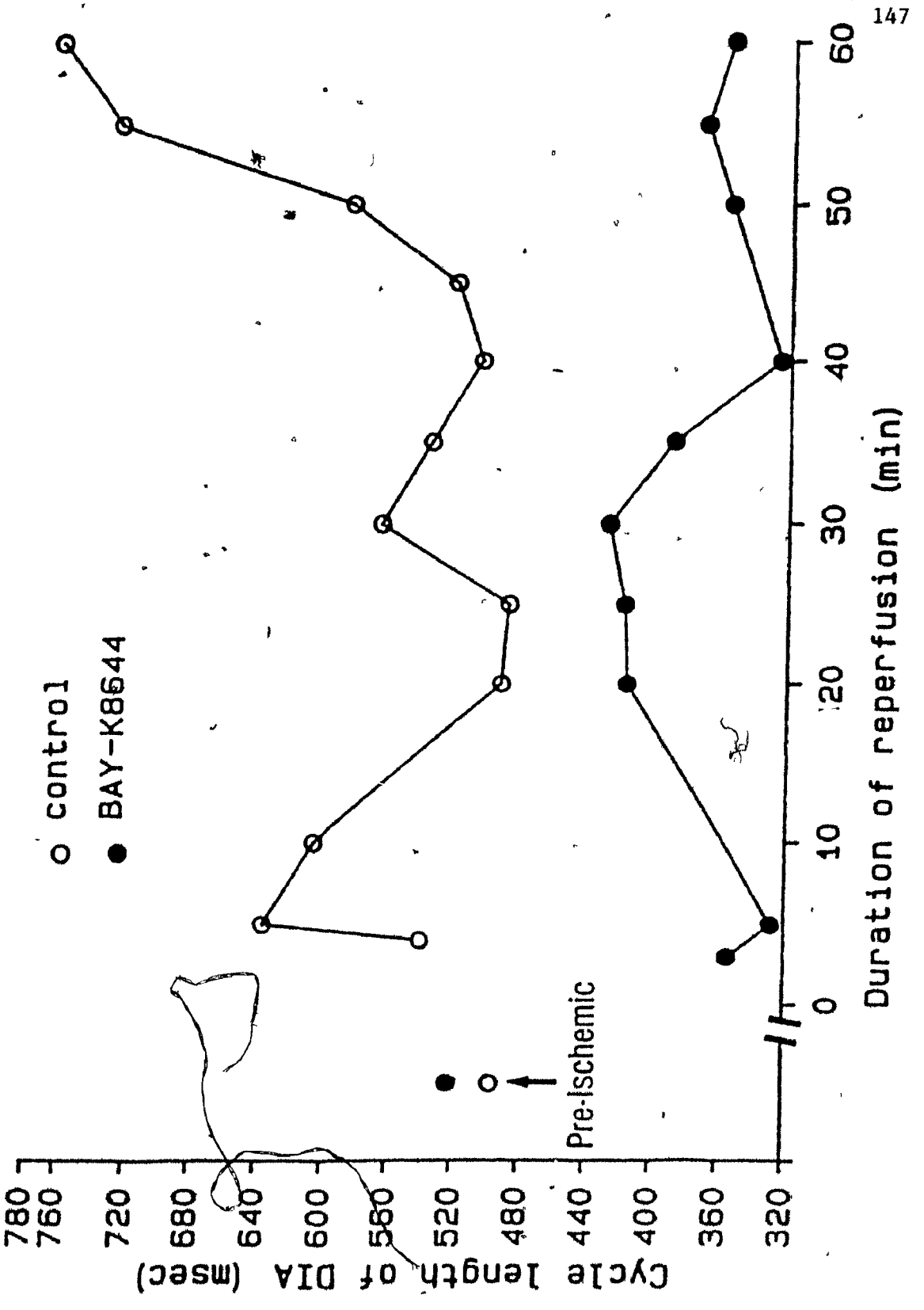
Cycle length of DIA (msec)
780
760
720
680
640
600
580
520
480
440
400
360
320
Pre-Ischemic
Duration of reperfusion (min)
0
10
20
30
40
50
60
control
BAY-K8644

Figure 26

DIA, whereas none displayed inhibition in the BAY-K8644 treated group. No preparations in either group showed inhibition of DIA at 30 minutes of reperfusion. These results suggest that BAY-K8644 may prevent inhibition of DIA upon reperfusion.

b. Effects of BAY-K8644 on changes in cycle length of DIA

Reperfusion tends to cause an increase in cycle length of DIA as well as inhibition of DIA. Since BAY-K8644 prevents inhibition of DIA upon reperfusion, then BAY-K8644 may decrease the cycle length of DIA during reperfusion. Figure 26 shows changes in cycle length of DIA at a MDP of -50 ± 2mV during reperfusion in the absence and presence of BAY-K8644. Also indicated in Figure 26 are the respective pre-ischemic values for cycle length of DIA at this MDP. In the absence of BAY-K8644, there was an initial increase in cycle length of DIA within the first 10 minutes of reperfusion. This was followed by a decrease in cycle length of DIA to values similar to pre-ischemic levels (by 20 minutes of reperfusion) and finally a progressive increase in cycle length of DIA (from 40 minutes to 60 minutes of reperfusion). In the presence of BAY-K864., there was an initial shortening of cycle length of DIA compared to the pre-ischemic level (within the first 5 minutes). This was followed by an increase in cycle length of DIA (between 5 and 25 minutes). However, the values remained below those of the pre-ischemic period throughout and the control reperfusion period. Between 25 and 60 minutes of reperfusion in the presence of BAY-K8644, the cycle length of DIA progressively decreased. Table 7 summarizes the effects of BAY-K644 on changes in cycle length of DIA in response to reperfu-

Table 7: Effect of BAY-k8644 on the Cycle Length of DIA During Reperfusion

Duration of Reperfusion (min)	Intervention	CL Decreased	CL Same	CL Increased	DIA Abolished
0-5	R	1	0	4	1
	R + BAY-k8644	3	1	1	1
10	R	0	0	2	4
	R + BAY-k8644	4	0	1	1
20	R	0	1	4	1
	R + BAY-k8644	5	1	0	0
30	R	1	1	4	0
	R + BAY-k8644	5	0	0	0
60	R	2	0	4	0
	R + BAY-k8644	6	0	0	0

N=6

R=Reperfusion

CL=Cycle length of DIA

Comparisons were made with the respective pre-ischemic control cycle length.

sion. Designations of increase or decrease are with respect to changes of 10% from the pre-ischemic control cycle length. Between 0 and 5 minutes of reperfusion, most control preparations showed an increase in cycle length of DIA, whereas those that were reperfused in the presence of BAY-K8644 showed a decrease in cycle length of DIA. This trend continued for the duration of reperfusion. At 60 minutes of reperfusion, 4 of 6 preparations reperfused in the absence of BAY-K8644 showed an increase in cycle length of DIA, whereas the other 2 preparations showed a decrease in cycle length of DIA. However reperfusion in the presence of BAY-K8644 results in all 6 preparations showing a decrease in cycle length of DIA at 60 minutes of reperfusion. These results therefore suggest that reperfusion in the presence of BAY-K8644 results in return of DIA at cycle lengths lower than that of the pre-ischemic control. Therefore it appears that BAY-K8644 promotes calcium mediated action potentials upon reperfusion and therefore acts as a calcium agonist.

c.. Threshold voltage of DIA

BAY-K8644 appears to promote calcium dependent action potentials upon reperfusion. The effects of BAY-K8644 on calcium mediated action potentials during reperfusion may be through an increase in range of membrane potentials over which DIA can be elicited. Therefore the effects of BAY-K8644 on changes in threshold voltage for DIA were determined during reperfusion. Table 8 summarizes the effects of reperfusion with BAY-K8644 on shifts in threshold voltage for DIA (compared to the range of membrane potentials from the corresponding control at the same time). Within 5 minutes of reperfusion with BAY-K8644, DIA was elicited

Table 8: Effects of BAY-K8644 on Threshold Voltage of DIA During Reperfusion

Duration of Reperfusion (min)	*Same	*Higher Membrane Potential	DIA Abolished
0-5	3	2	1
10	1	3	1
20	3	3	0
30	6	0	0
60	4	1	0

*Comparisons were made to reperfusion in the absence of BAY-k8644. Higher membrane potential indicates an increase of greater than 10% of the control reperfusion.

at the same threshold voltage as control in in 3 preparations. Two preparations displayed DIA at higher membrane potentials than the control threshold. At 20 minutes of reperfusion with BAY-K8644, 3 preparations displayed DIA at a higher membrane potential. The other 3 preparations displayed DIA at the same membrane potentials. At 30 minutes and 60 minutes of reperfusion with Bay-k8644, 6 and 4 preparations respectively displayed DIA at the same membrane potentials. These results suggests that initially upon reperfusion with BAY-K8644 (within first 20 minutes), DIA could be elicited at a higher membrane potential compared to control. However as reperfusion progressed (after 30 minutes of reperfusion), there were no differences in the threshold voltage at which DIA could be elicited in control and the BAY-K8644 exposed preparations.

3. SUMMARY

BAY-K8644 decreased the cycle length of DIA initially during ischemic conditions. However BAY-K8644 did not prevent inhibition of DIA during ischemic conditions, and eventually enhanced this inhibition. Even though BAY-K8644 promoted inhibition of DIA during ischemic conditions, it increased the threshold voltage of DIA. Reperfusion in the presence of BAY-K8644 resulted in initial appearence of OAP's and return of DIA. BAY-K8644 also prevented inhibition of DIA associated with early reperfusion. BAY-k8644 decreased the cycle length of DIA during reperfusion compared to control. BAY-K8644 increased the threshold voltage of DIA early in reperfusion. However, there was no difference between the threshold voltage of DIA in preparations reperfused in

the absence or presence of BAY-K8644 (after 30 minutes of reperfusion). These results together suggest that BAY-K8644 fails to act as a "calcium agonist"during ischemic conditions. However BAY-K8644 appears to act as a "calcium agonist" during reperfusion because it promotes induction of DIA.

DISCUSSION

A) THE EFFECTS OF CALCIUM CHANNEL BLOCKING AGENTS DURING ISCHEMIC CONDITIONS AND REPERFUSION

1. Ischemic conditions

Calcium channel blocking drugs were found to depress contractility early during ischemic conditions. These agents also enhanced depolarization of Purkinje tissue during ischemic conditions. Also, these agents failed to prevent and frequently increased conduction defects occurring during ischemic conditions.

Ischemic conditions abolished contractile activity in Purkinje tissues. The cause of contractile dysfunction during ischemia is not understood. The metabolic hypothesis proposes that depletion of high-energy phosphates causes reduction of tension development. However, most studies have failed to show that high-energy phosphates fall rapidly enough to explain the immediate decrease in developed tension during ischemia. An alternative hypothesis proposes that development of intracellular acidosis might explain the rapid decline in contractile function (Tennant and Wiggers, 1935). Acidosis may account for decreased contractility of Purkinje tissue, because the pH of ischemic conditions is 6.8. However Weiss *et al.* (1984) have shown that decline in contractile function observed in the isolated arterially perfused rabbit interventricular septum subjected to ischemia or hypoxia without glucose is not related to acidosis. Weiss *et al.* (1984) have suggested that decline in contractility during ischemia and metabolic inhibition is due to metabolic factors. Since ischemic conditions in the present

model inhibits aerobic and anaerobic metabolism in Purkinje tissue, the early decrease in developed tension in Purkinje tissue may be due to metabolic factors. Such metabolic factors may include the total cellular content of high-energy phosphates. Kubler and Katz (1977) proposed that the high-energy phosphate pool necessary for contraction may become more rapidly depleted than is reflected by the total cellular content of high-energy phosphates. Alternatively, they have suggested that intracellar phosphate accumulation resulting from breakdown of high energy phosphates could lead to reduced availability of Ca^{2+} to the myofilaments. The intracellular phosphate accumulation was proposed to trap the calcium and therefore decrease its availability to the myofilaments. Another mechanism whereby ischemia may lead to decreased contractility is by inhibition of the slow inward current. The slow inward current can be blocked by anoxia, acidosis or hypoxia (Sperelakis and Schneider, 1976 - review). Since the slow inward current is responsible for entrance of Ca^{2+} which is necessary for excitation-contraction coupling (Fabiato, 1983 - review), then decline in contractility may be through inhibition of this current during ischemic conditions. The ischemic conditions employed in the present model were hypoxia and acidosis. Therefore these conditions may have inhibited the slow inward current and caused a decrease in contraction of Purkinje tissue.

The decline in contractility of Purkinje tissue during ischemic conditions was much faster in the presence of the calcium channel blocking drugs. A negative inotropic effect of verapamil and nifedipine would be expected to occur, since these agents block the slow inward

current (Kohlhardt et al., 1972; Kohlhardt and Fleckenstein, 1977). However these agents appear to have a greater effect on contractility during ischemic conditions, compared to ischemic conditions alone. Greater inhibition of calcium channels in the presence of verapamil or nifedipine would lead to a greater reduction of the slow inward current and a faster decline in contractility. Smith et al. (1976) have also shown that verapamil selectively depressed contractility in the ischemic myocardium of dogs. Acidosis has also been shown to sensitize cat papillary muscles to the negative inotropic effects of both verapamil and nifedipine (Brisco and Smith, 1982). Therefore the enhanced negative inotropic effect of verapamil and nifedipine on Purkinje tissue during ischemic conditions may be due to an enhanced sensitivity of tissues to these agents due to acidosis. Partial inhibition of slow channels by acidosis or hypoxia may be the reason for the increase sensitivity to calcium channel blockers during ischemic conditions.

Ischemic conditions also caused a gradual loss of MDP in Purkinje tissues within 10 minutes. Since K is known to accumulate extracellularly during ischemia (Hill and Gettes, 1980), the initial depolarization of Purkinje tissue may be due to K accumulation. However it is not known if the ischemic conditions in the present model cause potassium efflux, even though intracellular acidosis has been suggested to cause K efflux (Skinner and Kunze, 1976). Lactate may also cause depolarization of Purkinje tissue (Wissner, 1974), although hyperpolarization has also been reported (Coraboeuf et al., 1976). Ischemic conditions may also decrease Na-K ATPase activity which would result in K accumulation and

therefore depolarization. Brown et al. (1978) have shown that decreasing pH from 0.2-0.6 pH units is capable of producing depolarization of sheep Purkinje fibers. Increasing $[K]_o$ antagonized the effects of acidosis on Na-K ATPase inhibition. Therefore K accumulaton, lactate and acidosis may all contribute to a decrease in MDP of Purkinje tissue.

The decrease in MDP of Purkinje tissue was enhanced by verapamil or nifedipine. Under normal conditions these agents have not been reported to cause large decreases in MDP. However free sarcoplasmic calcium controls steady state potassium conductance (Isenberg, 1977). A decrease in intracellular Ca^{2+} could lead to a decrease in potassium conductance and therefore depolarization. The calcium channel blocking agents D600 (Kass and Tsien, 1975) and verapamil (Posner et al., 1975) have been shown to decrease potassium conductance. Therefore, some of the depolarization seen during ischemic conditions with the calcium channel blockers may be through a decrease in potassium conductance. Since ischemic conditions may inhibit calcium channels (Sperelakis and Schneider, 1976), potassium conductance would decrease and membrane potential would also decrease. Greater inhibition of calcium channels in the presence of verapamil or nifedipine could lead to a greater reduction of potassium conductance and greater depolarization of Purkinje tissue.

It is not clear why the membrane potential returned to control ischemic levels after 15 minutes in the presence of nifedipine but not verapamil. This difference might be explained by the hypothesis of Sperelakis and Schneider (1976) and its relation to the mechanism of

calcium channel blockade by calcium channel blocking drugs. If calcium channels inactivate or partially inactivate with ischemic conditions, binding of one calcium channel blocker may be favoured over the other. Verapamil demonstrates use dependent and voltage dependent block (Kanaya et al., 1983) ie. block of calcium channel is enhanced by increasing frequency of stimulation and by more positive holding potentials. This led these investigators to postulate that verapamil preferentially binds to the inactivated state of calcium channels. This would mean that verapamil may preferentially bind to calcium channels during ischemic conditions since these channels would be more in the inactivated state. This could account for the persistent depolarization in the presence of verapamil during ischemic conditions. On the other hand nifedipine doesn't demonstrate voltage or frequency dependence (Hirth et al., 1983) and preferentially binds to the resting state of the calcium channel. Therefore, if the calcium channel is beginning to inactivate after 20 minutes of exposure to ischemic conditions, this would favour unbinding of nifedipine. Therefore nifedipine's effect on membrane potential would be attenuated. However Sanguinetti and Kass (1984) have found that dihydropyridines bind to inactivated channels 1000 times stronger than to resting channels, and have shown that these agents demonstrate use-dependent block.

The effects of acidosis on membrane potential can also be influenced by calcium channel blocking agents. Recently Lauer et al. (1984) have reported the effects of low pH (6.0) on membrane depolarization in isolated canine Purkinje fibers. They found that low pH resulted in de-

polarization ("sensitive cells") of Purkinje tissue by 45 mV within 20 minutes, whereas some tissues only depolarized about 10 mV ("resistant cells"). These investigators found that exposure of "resistant cells" to verapamil and low pH resulted in a large depolarization (about 45 mV). They attributed the large depolarization of "sensitive cells" to a decrease in potassium permeablity by low pH. Therefore the ability of verapamil to enhance depolarization in resistent cells during acidosis was due to its ability to indirectly reduce potassium permeability. A similar mechanism may be operative in the present model, although the pH here is 6.8.

The calcium channel blocking agents also failed to prevent and frequently increased conduction defects occurring during ischemic conditions. Ferrier et al. (1985a) have previously reported that conduction block occurring between Purkinje and muscle tissue during ischemic conditions is bi-directional. The present results show that ischemic conditions caused conduction block and/or inexcitability in Purkinje tissue in a small number (3/20) of preparations. Conduction block however was greater in muscle tissues (9/20 preparations) during ischemic conditions. This is in agreement with the results of Gilmour et al. (1984). These investigators found that a combination of hyperkalemia, hypoxia and acidosis prevented antegrade impulse propagation from Purkinje to muscle tissues but did not inhibit retrograde propagation from muscle to Purkinje tissue. They reasoned that this was due to the fact that current is "funneled" more readily from the large muscle mass into the narrow junction of Purkinje tissue than in the opposite direction. A

similar mechanism may explain the discrepancy of occurrence of conduction block within the two tissues in the present model. The reason why conduction block and or inexcitability occurs in Purkinje tissue during ischemic conditions is not known. Hypoxia and acidosis are known to increase internal resistance of myocardial and Purkinje fibers (Brown et al., 1978; Wojtczak, 1978; DeMello, 1980; Ikeda and Hiraoka, 1982). This increase in resistance would decrease cell-to-cell coupling and may account for the conduction block/inexcitability that occurred in Purkinje tissue. Conduction block/inexcitability occurring in Purkinje tissue during ischemic conditions was exacerbated by the calcium channel blocking agents. This finding is in contrast to previous in vivo studies in ischemic myocardium, where calcium channel blocking agents have been shown to improve conduction (Elharrar et al., 1977b; Nakaya et al., 1980). However, there have been no investigations reported on the influence of calcium channel blockers on Purkinje tissue during ischemic conditions. One possible reason why calcium channel blocking agents may increase conduction block/inexcitability in Purkinje tissue, is that they enhance depolarization during ischemic conditions. Since the depolarization of Purkinje tissue is great enough to inactivate the Na current, only the slow inward current would be available for activation. However because these agents block the slow inward current, conduction block/inexcitability would occur in Purkinje tissue.

Ischemic conditions also increased conduction block in muscle tissues. Conduction block to muscle tissue occurred when Purkinje tissue was stimulated. The reason for increased conduction block in

muscle tissues may be due to uncoupling of the Purkinje-myocardial junction by hypoxia and acidosis (Gilmour et al., 1984). Conduction block/inexcitability in muscle tissues was exacerbated in the presence of nifedipine. The reason for this enhancement of conduction block by nifedipine is unknown. In contrast, ischemic conditions in the presence of verapamil resulted in a decreased incidence of conduction block in muscle tissue. An improvement of conduction has been reported in the presence of verapamil, during conditions of hypoxia, hyperkalemia, and acidosis (Kimura et al., 1982). However in this study by Kimura et al. (1982), verapamil improved conduction in epicardial but not endocardial cells. The reasons for these discrepancies are not known.

Ischemic conditions in the present model did not contain elevated extracellular potassium. Ischemic conditions with 10 mM potassium have been used previously in the present model of ischemia (Ferrier et al., 1985a,b). Elevation of extracellular potassium during ischemic conditions was not necessary to elicit a strong reperfusion response (Ferrier et al., 1985a). The electrophysiological response of Purkinje tissue upon reperfusion in both cases was almost identical. However, ischemic conditions with elevated potassium caused a significantly greater depolarization of Purkinje and muscle tissues, compared to ischemic condition without elevated potassium. Downar et al. (1977b) concluded that elevated potassium was not essential to elicit the electrophysiological changes occurring during coronary occlusion in porcine hearts. However, elevated potassium during ischemic conditions may modify the action of calcium channel blocking drugs. Kimura et al. (1982) however

showed that verapamil does not alter the electrophysiological changes occurring in canine ventricular muscle during superfusion with a combination of hypoxia, acidosis and hyperkalemia.

2. Reperfusion

The calcium channel blocking drugs suppressed two mechanisms of arrhythmia upon reperfusion: oscillatory afterpotentials (OAP's) and depolarization induced automaticity (DIA). However, these agents did not alter changes in MDP elicited by reperfusion. The calcium channel blocking drugs also did not alter changes in contractility early in reperfusion.

Contractility returned in Purkinje tissue within the first 5 minutes of reperfusion. After 5 minutes of reperfusion, contractility decreased to zero. Return of contractility in Purkinje fibers was observed at 20 minutes of reperfusion. In the presence of calcium channel blocking agents, a similar return and decline in contractility was observed upon initial reperfusion. Final return in contractility in the presence of these agents was observed much later in reperfusion and was significantly less than the control at the end of 60 minutes of reperfusion. The failure of calcium channel blocking drugs to reduce the return of contractility observed early in reperfusion suggests that this effect is not mediated through the slow inward current. Another possible source for the inotropic effect of early reperfusion may be an increase in intracellular Ca^{2+} which would be available to the myofilaments. The source for increased intracellular Ca^{2+} may be through Ca^{2+} accumulated via a Na^{+}-Ca^{2+} exchange mechanism. Enhanced Na^{+}-Ca^{2+}

exchange may occur as a consequence of inhibition of Na-K ATPase during ischemia, as postulated by Nayler (1981). Grinwald (1982) also suggested that Na^{+}-Ca^{2+} exchange occurs upon reperfusion. Yee et al. (1985 - abstr.) recently has shown evidence for increased intracellular Ca^{2+} upon reperfusion using the present model. Yee et al. (1985 - abstr.) showed a significant increase in resting tension after 10 minutes of reperfusion. The reason for the decrease in contractility to zero after 5 minutes of reperfusion is not known. Since at this time the Purkinje tissue becomes inexcitable at a low membrane potential, the slow inward current may become inactivated at a low membrane potential. Therefore, the slow inward current which serves as the "trigger" for excitation-contraction coupling (Fabiato, 1983 - review) is inactive at a low membrane potential and contractions would be abolished. Another possible explanation for decrease in contractility may be due to severe conduction block in the specialized conducting system. A similar decline in contractility along with depolarization to inexcitability, was observed in the presence of calcium channel blocking agents.

Final return of contractility was observed after 20-30 minutes of reperfusion, which coincides with the time when Purkinje tissue regained it excitability. Therefore conduction block into Purkinje tissue may not be present anymore. Also at this time the slow inward current would be "reactivated", and could serve as a "trigger" for excitation-contraction coupling. In the presence of the calcium channel blocking agents, the return of contractility in Purkinje tissue occurred much later in reperfusion. This may be due to the fact that calcium channels were in-

hibited by these agents when Purkinje tissue was depolarized at a low membrane potential. However, active action potentials returned in Purkinje tissue at high membrane potentials in the presence of the calcium channel blocking agents. This allowed more calcium channels to become available for activation upon depolarization, although some of these channels would be blocked by the calcium channel blocking agents. Therefore the slow inward current would be reduced, but not abolished by these agents. The reduction of the slow inward current may have led to a decrease in contractility at the end of reperfusion in the presence of the calcium channel blocking agents.

Reperfusion initially resulted in repolarization and in some cases a hyperpolarization in Purkinje tissues. Repolarization was followed by a phase of depolarization to a low level of membrane potential. Following this, there was a phase of recovery of MDP of Purkinje tissue to control pre-ischemc values. The initial repolarization (hyperpolarization) may be caused by stimulation of the sarcolemmal Na-K ATPase upon reperfusion. An increase in intracellular sodium stimulates sodium extrusion via Na-K ATPase. Because the extrusion of sodium is electrogenic (Vassalle, 1985 - review), it brings about a hyperpolarization of the membrane potential. This hypothesis requires an increase in intracellular sodium early in repolarization. Benditt et al. (1982) have shown that low pO_2 inhibits rapid-drive-induced hyperpolarization of Purkinje fibers. Reduced pO_2 presumably inhibited some Na-K ATPase activity. Therefore the low pO_2 present during ischemic conditions may have inhibited the Na-K ATPase. This inhibition might result in

increased Na_i. Yee et al. (1985 - abstr.) reported an increase in intracellular Na activity in Purkinje fibers after 40 min of exposure to ischemic conditions identical to ours. Thus stimulaton of the Na-K ATPase via accumulation of intracellular Na may be the mechanism for the early repolarization/hyperpolarization upon reperfusion. Yee et al. (1985 - abstr.) found that the Na_i rapidly decreases to below pre-ischemic control levels within 10 minutes of reperfusion. This is further evidence for stimulation of the Na-K ATPase upon reperfusion.

Following the phase of repolarization, Purkinje tissue depolarized to a low membrane potential and became inexcitable. The reason for this depolarization is not known. We have previously reported that ischemic conditions and reperfusion in the presence of low Ca^{2+} prevented the severe depolarization upon reperfusion (Mohabir et al., 1983 - abstr.). Therefore enhanced entry of calcium may be responsible for the severe depolarization of Purkinje tissue upon reperfusion. Further evidence for increase in intracellular Ca^{2+} occurring during the depolarization phase of reperfusion, is shown by the increase in resting tension of Purkinje fibers after 10 minutes of reperfusion (Yee et al., 1985 - abstr.). Failure of calcium channel blocking agents to prevent depolarization upon reperfusion, suggests that entry of Ca^{2+} through the slow channel doesn't mediate this depolarization. After the phase of depolarization, membrane potential recovered in Purkinje tissue. The reason for recovery of the membrane potential also is unknown. Since it appears that increase in $[Ca^{2+}]_i$ is responsible for depolarization, extrusion or recompartmentalization of increased intracellular Ca^{2+} may be responsible for recovery of membrane potential.

The changes in MDP in Purkinje upon reperfuson were associated with different mechanisms of arrhythmia. OAP's have been shown to occur in Purkinje fibers in this model of ischemic conditions and reperfusion in a previous study (Ferrier et al., 1985a). OAP's also can be induced by digitalis intoxication (Ferrier et al., 1973). The mechanism whereby digitalis intoxication leads to the appearence of OAP's is thought to involve an intracellular calcium overload secondary to inhibition of the sarcolemmal Na-K ATPase. Intracellular Ca^{2+} overload is believed to cause an oscillatory release of Ca^{2+} from the sarcoplasmic reticulum which activates the current underlying OAP's (TI) (Kass et al., 1978a,b). It is not known whether the current causing OAP's upon reperfusion is the same as that underlying digitalis induced OAP's. Ferrier and Moe (1973) have shown that manganese can eliminate acetyl-strophanthidin-induced OAP's. Rosen and Danilo (1980) have shown that verapamil can eliminate ouabain-induced OAP's. The present study shows that verapamil and nifedipine can also eliminate reperfusion-induced OAP's. The TI current is largely carried by sodium and is not directly dependent on the slow inward current. However, it is enhanced by a wide variety of procedures which increase intracellular Ca^{2+} levels. Calcium channel blocking agents, by decreasing entrance of Ca^{2+} may prevent the occurrence of OAP's. Whether the intracellular Ca^{2+} overload responsible for OAP's upon reperfusion may be partly mediated by the slow inward current cannot be determined by the present results.

After the phase of OAP's, Purkinje tissues depolarized to a low level of membrane potential and did not display any action potentials.

This phase of inexcitability leads to severe conduction block in the specialized conducting system. Depolarization of Purkinje tissue to a low membrane potential should result in the appearance of low membrane potential automaticity (Hauswirth et al., 1969). However, low membrane potential automaticity only occurred after the phase of inexcitability. Therefore it appears that the calcium channel may become "inactivated", resulting in a phase of inexcitability, and then become "reactivated", resulting in low membrane potential automaticity. Recently Lee et al. (1985) have shown that inactivation of calcium channels in Purkinje fibers is both voltage dependent and $[Ca]_i$ dependent. If intracellular $[Ca^{2+}]$ is initially high upon reperfusion, this might cause inactivation of the calcium channel. Calcium channel blocking agents did not prevent the phase of inexcitability at low membrane potential.

The cycle length of DIA can be modulated when entrance block is present (Ferrier and Rosenthal, 1980; Ferrier et al., 1985a). Entrance block was shown to be an intrinsic property of DIA occurring at a site connected to normally polarized tissue (Ferrier and Rosenthal, 1980). Therefore, DIA may function as a parasystolic focus with modulation. The present results indicate that calcium channel blocking agents can selectively abolish modulated parasystole. Residual automaticity occurred only at higher membrane potential and did not exhibit entrance block or modulation.

3. Relevance to previous studies

The present results with calcium channel blocking agents during ischemic conditions and reperfusion may be related to some extent to

studies with these agents during coronary artery occlusion and reperfusion. However caution should be used in extrapolating results obtained during exposure to ischemic conditions with results obtained in the whole heart. There are many factors involved in arrhythmias seen in the whole heart during coronary occlusion and reperfusion which are not present in the in vitro model of ischemic conditions and reperfusion. Such factors include catecholamines, and a border zone which separates ischemic tissue from normal tissue.

Verapamil has been shown to improve conduction during coronary artery ligation (Elharrar et al., 1977b; Fondacaro et al., 1978; Nakaya et al., 1980; Peter et al., 1983). However, most of these studies measured conduction delay occurring within the epicardium and not endocardium. Also most of these studies utilized short periods (5-10 minutes) of occlusion. Only one of the studies (Peter et al., 1983) used longer periods of occlusion (15 and 30 minutes) and measured endocardial delay. In this latter study (Peter et al., 1983), verapamil only improved conduction within the ischemic endocardium in the antegrade direction at 15 and 30 minutes of occlusion. Conduction in the retrograde direction only improved at 30 minutes of occlusion in the presence of verapamil. In contrast, nifedipine only improved conduction in ischemic endocardium in the antegrade direction at 15 minutes of occlusion. The present results showed that conduction block can occur in endocardial tissues exposed to ischemic conditions. Bi-directional depression of conduction was found to occur between Purkinje and muscle tissues. Depression of conduction was exacerbated by nifedipine but not

verapamil. Extrapolation of these findings to those in the whole heart become difficult. The difference in the present findings and those from the whole heart may be due to use of different durations of "ischemia" or differences in the response of epicardial versus endocardial tissues to the action of calcium channel blocking agents. A difference in the response of canine epicardial tissue compared to endocardial tissue to combined hypoxia, hyperkalemia and acidosis in the presence of verapamil has been reported (Kimura et al., 1982). Kimura et al. (1982) showed that verapamil improved action potential amplitude and V_{max} and reduced conduction delay in epicardial cells but not endocardial cells. Therefore differences in tissue response may explain differences in the present results and those from in vivo studies with coronary artery ligation.

Parratt (1982) suggested that calcium channel blockers may protect ischemic myocardium by partly increasing blood flow and/or from reduced myocardial oxygen demands and that this might be another mechanism by which these agents may improve conduction within the ischemic myocardium of intact hearts. These effects may occur as a consequence of bradycardia or the effects of reduced after-load or depressed contractile function (Smith et al., 1976). Therefore Parratt (1982) suggested that calcium channel blocking agents improve the balance between oxygen supply and demand which may lead to an improvement of ischemia-induced conduction delay. Factors such as blood flow or after-load reduction do not contribute to elecrophysiological changes in an in vitro superfusion model. Therefore the beneficial effect of calcium channel blocking agents may not be seen in an in vitro model of ischemic conditions.

The present model of ischemic conditions and reperfusion shows 2 mechanisms of abnormal automaticity (OAP, DIA) which were abolished by the calcium channel blocking agents. Penkoske et al. (1978) showed that reperfusion after 35 minutes of LAD coronary occlusion, produces an increase in idioventricular rate. The increase in idioventricular rate may be mediated by OAP and/or DIA. Therefore calcium channel blocking agents may be antiarrhythmic during reperfusion by decreasing idioventricular rate caused by OAP or DIA. In fact, Corr and Witowski (1983) have suggested that increase in idioventricular rate during reperfusion may be related to increased Ca^{2+} uptake resulting in OAP. Carbonin et al. (1981) have also shown that verapamil inhibited both reperfusion and digitalis-induced arrhythmias in rat hearts. They have also suggested that the protective action of verapamil in both situations may be due to inhibition of OAP. Whether the antiarrhythmic and antifibrillatory actions of verapamil seen in the whole heart upon reperfusion (Brooks et al., 1980; Sugiyama et al., 1980; Ribiero et al., 1981) are due to inhibition of OAP and/or DIA cannot be determined by the present results.

Extrapolation of the present findings to the clinical situation should also be done with caution. There have been few well-controlled clinical trials on the antiarrhythmic effects of calcium channel blocking gents during ischemia and reperfusion. Furthermore it is not known whether slow responses can be related to ventricular tachycardia in man (Gilmour and Zipes, 1985). However Sclarovsky et al. (1983) have shown that verapamil abolished accelerated idioventricular rhythm in 4 of 6

patients with acute myocardial infarction. Also Sung et al. (1983) have shown that verapamil suppressed ventricular tachycardia in 3 patients. In this latter study, the mechanism underlying the ventricular tachycardia was attributed to triggered activity arising from delayed afterdepolarizations.

Another clinical phenomenon which might be relevant to the present study relates to the occurrence of parasystole. Parasystole with modulation recently has been shown to occur in patients (Castellanos et al., 1984). The present study and those of Ferrier (Ferrier and Rosenthal, 1980; Ferrier et al., 1985a) have shown that parasystole only occurs at low membrane potential in Purkinje tissue. The present results show that parasystole can be abolished by calcium channel blocking agents. Therefore these agents may be useful in the future as a pharmacologic therapy for arrhythmias due to parasystole.

B. The Effects of Ischemic Conditions and Reperfusion on Calcium-Dependent Action Potentials

1. Ischemic Conditions

The effects of ischemic conditions on Ca-dependent action potentials were: increase in cycle length of DIA, non-sustained DIA, total cessation of DIA.

Sperelakis (1984 - review) has shown that hypoxia (Belardinelli et al., 1979), metabolic inhibitors (Schneider and Sperelakis, 1974) and acidosis (Vogel and Sperelakis, 1977) inhibit Ca^{2+}-dependent action potentials. Therefore, it was proposed that Ca^{2+} channels may inacti-

vate during ischemia (Sperelakis and Schneider, 1976 - review). However these investigators studied Ca^{2+}-dependent action potentials by depolarizing muscle or Purkinje tissue to a low membrane potential with high extracellular K. Ca^{2+}-dependent action potentials were then elicited by addition of isopreterenol. Therefore the effects of various interventions such as hypoxia etc. were only studied at one membrane potential (ie. there is no "control" of membrane potential with addition of high K). The present results show that ischemic conditions can inhibit Ca^{2+}-dependent action potentials (DIA) in Purkinje tissue. However the present study utilized electrical currents to depolarize Purkinje tissue, and therefore a range of membrane potentials could be studied. Ca^{2+}-dependent action potentials were not always totally inhibited. Inhibition was sometimes seen at some membrane potentials, but when the tissue was depolarized further, Ca^{2+}-dependent action potentials were again elicited. This finding may indicate a change in the threshold voltage for Ca^{2+}-dependent action potentials, and/or a change in the pacemaker current for DIA. The present results show that ischemic conditions do indeed reduce the MDP at which DIA could be elicited. Therefore ischemic conditions reduce the threshold voltage of Ca^{2+}-dependent action potentials.

At low membrane potentials, the pacemaker activity generated in Purkinje fibers is due to changes in the same outward current, I_{x1} (Hauswirth et al., 1969), which is responsible for phase 1 repolarization in normal fibers. The I_{x1} current deactivates slowly at low membrane potentials and constitutes a decaying outward current component

during diastole. DIA is the result of the deactivation of this time-dependent outward current and the concomitant activation of the slow inward current (Katzung and Morgenstern, 1977). Since ischemic conditions always produced an increase in cycle length of DIA, then the pacemaker current for DIA may be reduced during ischemic conditions. Elharrar and Zipes (1980) have shown that elevation of extracellular K (8 and 12 mM) increases the cycle length of DIA in Purkinje fibers. Furthermore, these investigators have suggested that since extracellular K increases during ischemia (Hill and Gettes, 1980), that DIA would be suppressed during ischemia. It is not known whether ischemic conditions in the present model lead to accumulation of extracellular K. However, increase in extracellular K may be the mechanism whereby ischemic conditions cause an increase in cycle length of DIA. Recently Jaeger and Gibbons (1985) have suggested that the slow inward current may produce many of the effects attributed to the I_{x1} current. Therefore the effects of ischemic conditions on cycle length of DIA may not be due to the inhibition of a K pacemaker current, but due to inhibition of the slow inward current.

The inhibition of conductance to an ion may be reflected by an increase in membrane resistance. Hypoxia without glucose is known to increase longitudinal resistance in ventricular muscle (Wojtczak, 1978). Acidosis also increases intracellular resistance of Purkinje fibers (DeMello, 1980). Hypoxia and acidosis are thought to increase longitudinal resistance through a rise in intracellular Ca^{2+}. Yee et al. (1985 - abstr.) reported that ischemic conditions caused an increase

in resting tension in Purkinje tissue which was reversible at the end of exposure to ischemic conditions. This latter finding may indicate an increase in $[Ca^{2+}]_i$ during ischemic conditions. Therefore ischemic conditions may increase relative membrane resistance through a rise in $[Ca^{2+}]_i$.

The implications of blockade of slow Ca^{2+} channels during ischemia have been outlined by Sperelakis and Schneider (1976). They have proposed that inhibition of calcium channels may serve as a protective mechanism for control of Ca^{2+} influx. It has been postulated that a membrane protein must be phosphorylated in order for the slow channel to become available for voltage activation (Sperelakis and Schneider, 1976). The basis for this hypothesis has come from the work of Tsien et al. (1972) on the effects of cAMP on plateau currents in Purkinje fibers. Interventions which decrease ATP such as acidosis, hypoxia and metabolic inhibition would make less channels become available for voltage activation. Therefore the myocardial cell would become inactivated and suppress its own Ca^{2+} influx during adverse conditions. Sperelakis (1984) referred to this as the "protection hypothesis". Sperelakis (1984) proposed that inhibition of calcium channels during ischemia is protective because the cells would contract weakly and therefore conserve ATP stores. Another implication of the present finding is that slow conducton due to calcium-dependent action potentials may not play a role in ischemia-induced arrhythmias, since slow channels would not be available for activation.

2. Reperfusion

The changes in availability of Ca^{2+}-dependent action potentials during reperfusion have not been studied before. The present study shows that reperfusion initially causes further inactivation of DIA. Later in reperfusion, DIA returned, but the cycle length was longer than the pre-ischemic control cycle lengths.

The reason for further inhibition of DIA upon reperfusion is unknown. Since reperfusion results in return to "normal" conditions, then the reason for inhibition of Ca-dependent acton potentials could not be the same as that for ischemic conditions. Initially upon reperfusion, there is evidence for an intracellular Ca-overload, as shown by the appearance of OAP's and by the increase in resting tension of Purkinje fibers (Yee et al., 1985 - abstr.). Lee et al. (1985) have shown that inactivation of Ca^{2+}-channels in Purkinje fibers is both voltage dependent and $[Ca^{2+}]_i$ dependent. Therefore if increase in intracellular Ca^{2+} is high initially upon reperfusion, then this may account for inactivation of Ca^{2+} channels. However if this was the reason for inactivation of DIA upon reperfusion, then why does DIA return later in reperfusion? As Yee et al. (1985 - abstr.) reported, there was no significant decrease in resting tension at the end of reperfusion compared to after 10 minutes of reperfusion. Therefore the $[Ca^{2+}]_i$ may still be high at the end of reperfusion. However recovery of electrical and mechanical activity in Purkinje tissue suggests that free $[Ca^{2+}]_i$ may recover to normal by the end of reperfusion. Another mechanism may therefore be responsible for inhibition of Ca^{2+}-dependent action potentials initially upon reperfusion.

The cycle length of DIA returned to pre-ischemic control cycle lengths at 20 minutes of reperfusion in those preparations where there was no cessaton of DIA upon reperfusion. This may mean that reperfusion may initially restore activitiy of the pacemaker current, I_{xl}, responsible for DIA. However, after 20 minutes of reperfusion, the cycle length of DIA increased over the pre-ischemic control values. Therefore, the pacemaker current, I_{xl}, may be reduced later in reperfusion. These changes in cycle length of DIA could not be related to changes in threshold voltage for DIA. As reperfusion progressed (after 20 minutes), DIA could be elicited over a wide range of membrane potentials.

The implications of changes in availability of Ca^{2+}-dependent action potentials during reperfusion are that they may account for different mechanisms of arrhythmia. DAD's seen initially upon reperfusion occur at a time when Ca^{2+}-dependent action potentials occur. This may mean that the slow inward current may be responsible for some of the intracellular Ca^{2+} overload upon reperfusion. Conduction block/inexcitability upon reperfusion occur at a time when there was further inhibition of calcium-dependent action potentials. This may be the reason why low membrane potential automaticity is not seen immediately upon reperfusion. Return of Ca^{2+}-dependent action potentials occur at a time when DIA/parasystole are seen. Therefore a "reactivation" of the calcium channel may be responsible for the occurrence of low membrane potential automaticity.

One factor that may contributed in DIA during ischemia conditions and reperfusion may be catecholamine released by nerve stimulation site

the current clamp. Catecholamine depletion may contribute to the secondary inhibition of DIA during reperfusion. However, if this were the case, then DIA should not return at the end of reperfusion.

C. Effects of BAY-K8644 on Ca-Dependent Action Potentials During Ischemic Conditions and Reperfusion

BAY-K8644 is a dihydropyridine which has a positive inotopic effect and vasoconstrictor action (Schramm et al., 1983). Thus Schramm et al. (1983) have called BAY-K8644 a "Ca-agonist" because it appears to activate Ca^{2+}-channels and therefore promote Ca^{2+} influx. Kokobun and Reuter (1984) have shown that BAY-K8644 prolongs the mean open time of the calcium channel, and therefore increases the Ca^{2+}-current. Wahler and Sperelakis (1984) have shown that BAY-K8644 induces slow action potentials in guinea pig papillary muscle depolarized with high potassium. Since Sperelakis (1984) assumes that agents which induce slow action potentials after depolarization with high potassium will increase the number of available slow channels for activation, BAY-K8644 should increase the number of available slow channels for activation. Therefore BAY-K8644 may be expected to open the calcium channel under conditions where it may become inactivated (eg. ischemic conditions).

1. Ischemic Conditions

Initially during ischemic conditions, BAY-K8644 caused a decrease in cycle length of DIA, which was greater than the control ischemic conditions. Therefore BAY-K8644 appears to act as a "Ca-agonist" initially during ischemic conditions. However as the duration of exposure to ischemic conditions progressed, half of the preparations showed an in-

crease in cycle length of DIA, and the other half displayed cessation of DIA. Therefore the drug failed to act as a "Ca-agonist" later during ischemic conditions. By the end of exposure to ischemic conditions, more preparations displayed cessation of DIA compared to the control ischemic conditions. Therefore BAY-K8644 failed to prevent the inactivation of the calcium channel during ischemic conditions. However BAY-K8644 did appear to have some effect on the calcium channel during ischemic conditions, since it increased the threshold voltage for DIA, compared to ischemic conditions alone. The reason why BAY-K8644 failed to act as a "Ca-agonist" during ischemic conditions is not known. BAY-K8644 also enhanced inhibition of DIA during ischemic conditions. Ischemic conditions may either alter the activity of the drug through ionization, or may alter the binding site of the drug. Recently Nayler et al. (1985) have reported that 60 minutes of ischemia in rat hearts reduces the Bmax of dihydropyridine binding sites. Since BAY-K8644 is a didydropyridine, the binding sites for this agent may be reduced by ischemic conditions. Alternatively the effects of ischemic conditions on the calcium channel may not be reversed by BAY-K8644. If the effects of ischemic conditions on inactivation of the calcium channel is dependent on the lowering of the metabolic dependence (i.e. lowering ATP levels) of the channel, then BAY-K8644 might fail to increase the availability of the calcium channel since it doesn't work by a metabolic action.

2. Reperfusion

Reperfusion in the presence of BAY-K8644 resulted initially in the appearance of OAP's. Also, reperfusion in the presence of BAY-K8644 prevented further inhibition of DIA. During reperfusion in the presence of BAY-K8644, the cycle length of DIA was decreased below that of pre-ischemic control.

BAY-K8644 may enhance Ca-overload upon reperfusion, since the appearence of OAP's upon reperfusion is promoted by this compound. Nilius (1984a,b) reported that high concentrations (greater than 10^{-6} M) of BAY-K8644 cause OAP's in guinea-pig papillary muscles exposed to high K. However, this was the only report to show that this compound may produce OAP's. Even though the concentration of BAY-K8644 used in the present study was 1×10^{-6} M, we have found no evidence for OAP's in canine ventricular tissues exposed to BAY-K8644 alone. However, the concentration of BAY-K8644 used in the present study promoted OAP's upon reperfusion. OAP's were elicited at a time during reperfusion when DIA could be elicited in Purkinje tissue. Therefore reperfusion may induce a Ca^{2+}-overload (as discussed previously) which is exacerbated by BAY-K8644. BAY-K8644 may enhance Ca^{2+}-overload upon reperfusion by activating Ca^{2+}-channels. Nayler et al. (1985) have also shown that BAY-K8644 enhances Ca^{2+} uptake upon reperfusion, after 60 minutes of ischemia in rat hearts.

BAY-K8644 also prevented further inhibition of DIA upon reperfusion. Therefore this compound appears to act as a "Ca-agonist" upon reperfusion. The prevention of inhibition of DIA during reperfusion, but

not during ischemic conditions, suggests that restoration of "normal" conditions (ie. pH, O_2, glucose) allows BAY-K8644 to act as a "Ca-agonist". This is further evidence that ischemic conditions may either alter the structure of BAY-K8644 or alter the binding site for this compound.

Return of DIA during reperfusion in the presence of BAY-K8644 was associated with a decrease in cycle length of DIA below pre-ischemic control values (obtained in the absence of BAY-K8644). The decrease in cycle length of DIA during reperfusion in the presence of BAY-K8644 may be due to an increase in pacemaker current, I_{x1}, and/or an increased availability of slow Ca^{2+} channels. If BAY-K8644 increases the availability of slow Ca^{2+} channels then BAY-K8644 would be expected to increase the threshold voltage for DIA. An increase in threshold voltage for DIA was obtained up to 30 minutes of reperfusion, as compared to reperfusion in the absence of BAY-K8644. However, after 30 minutes of reperfusion, BAY-K8644 did not increase the threshold voltage for DIA over the control reperfusion. This may indicate that after 30 minutes of reperfusion in the presence of BAY-K8644 the maximum number of Ca^{2+} channels was activated by BAY-K8644. Any additional reduction of cycle length of DIA after 30 minutes of reperfusion in the presence of BAY-K8644, may be due to an increase in the pacemaker current, I_{x1}.

Preparations reperfused in the absence of BAY-K8644 showed an inhibition of DIA. Reperfusion in the presence of BAY-K8644 prevented further inhibition of DIA. Therefore BAY-K8644 may prevent inhibition of DIA during reperfusion.

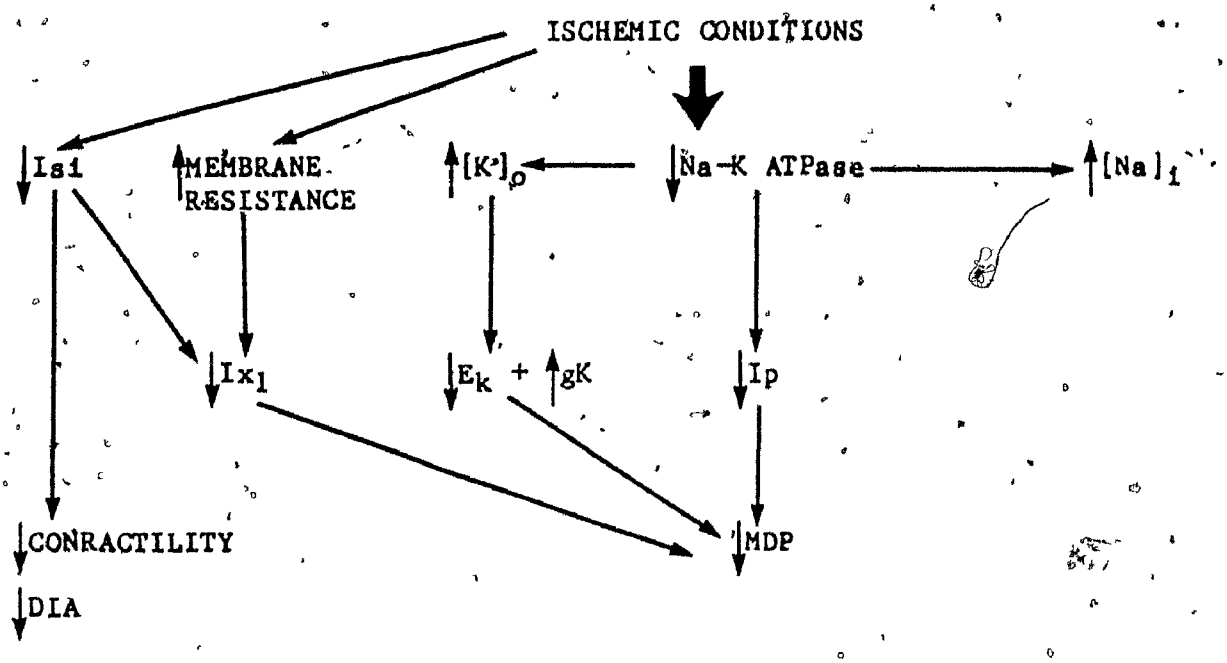

Figure 27

Possible sequence of changes occurring during ischemic conditions.

D. PROPOSED MODEL OF ELECTROPHYSIOLOGICAL CHANGES DURING ISCHEMIC CONDITIONS AND REPERFUSION

The results obtained from the present work may contribute to an explanation of the electrophysiological changes that take place in Purkinje tissue during exposure to ischemic conditions and reperfusion. Figures 27 and 28 show a possible sequence of changes that take place during ischemic conditions and reperfusion respectively.

Ischemic conditions may cause some inhibition of the electrogenic Na-K ATPase pump (Figure 27). Inhibition of the Na-K ATPase would lead to a decrease in the "pump" current (Ip) and therefore depolarization (↓MDP). Inhibition of the Na-K ATPase would also lead to an increase in K concentration outside the cell, and increase in Na inside the cell. The increase in $[K]_o$ would lead to an increase in K conductance (↑gK) and to a decrease in the equilibrium potential for potassium (↓Ek). These two factors would also contribute to a depolarization of Purkinje tissue. Ischemic conditions also decrease the slow inward current (↓Isi). The decrease in Isi will lead to a decrease of potassium conductance, and therefore depolarization. Greater inhibition of Isi in the presence of calcium channel blockers would lead to a further reduction of potassium conductance and therefore a greater depolarization of Purkinje tissue. Decrease in Isi also contributes to inhibition of DIA. Decrease in Isi may also lead to decrease of contractility in Purkinje tissue. Ischemic conditions may also cause an increase in membrane resistance of Purkinje. The increase in membrane resistance could also reflect a decrease in conductance to Ca (and therefore decreased Isi) and/or

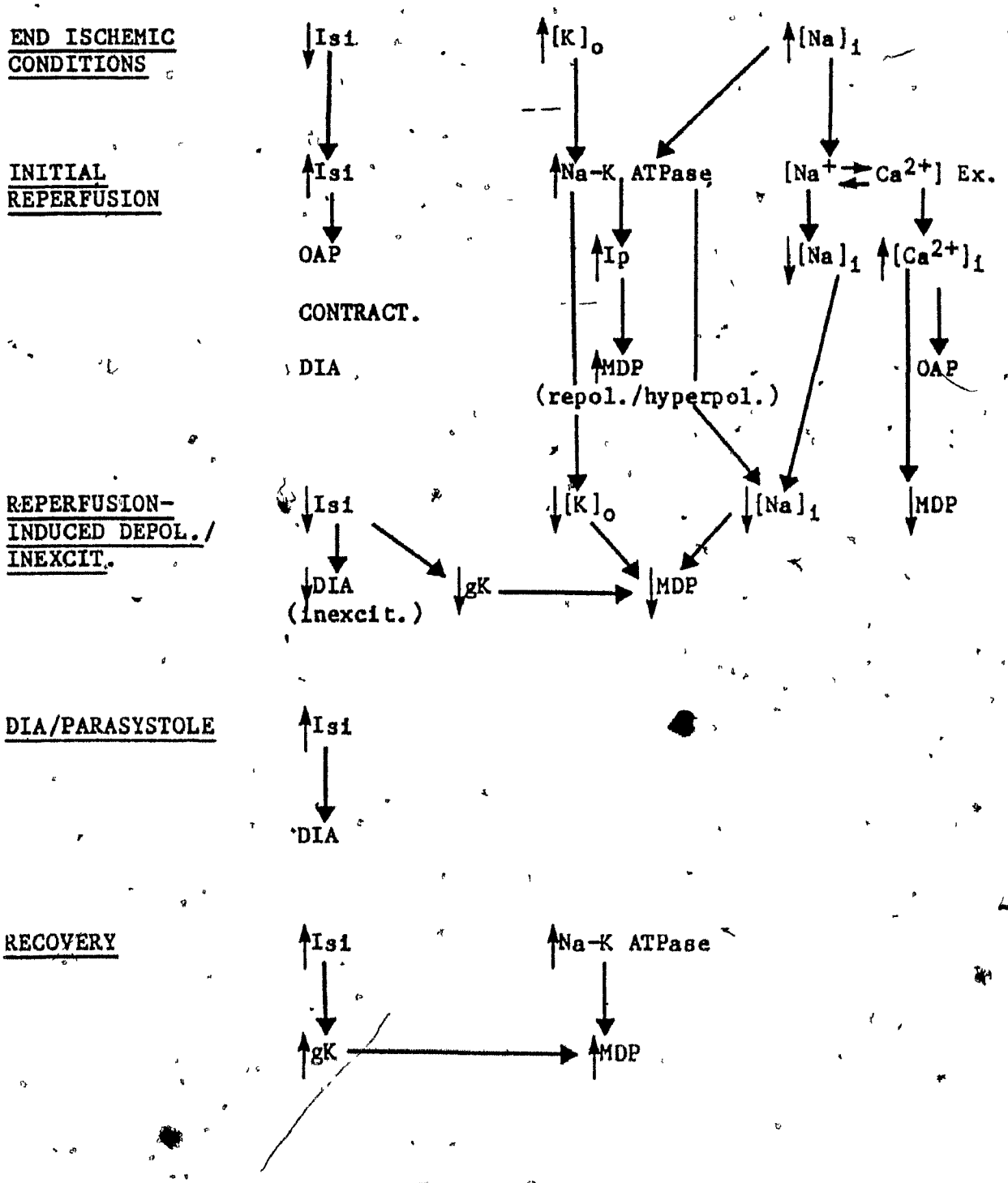

Figure 28

Possible sequence of changes occurring during reperfusion.

potassium conductance. Therefore an increase in membrane resistance may also contribute to depolarization as well as inhibition of DIA. Also, increases in $[K]_o$ may also contribute to inhibition of DIA.

The changes that occur in Purkinje tissue during reperfusion are much more complicated than those occurring during ischemic conditions. Figure 28 shows a diagram outlining possible changes occurring in Purkinje tissue upon reperfusion. At the end of exposure to ischemic conditions there is a decrease in Isi. At the same time $[K]_o$ is increased and $[Na]_i$ is increased, due to inhibition of the Na-K ATPase. Upon initial reperfusion, the Na-K ATPase becomes "reactivated". The resulting increase in Na-K ATPase activity results in extrusion of $[Na]_i$ in return for $[K]_o$. This results in the initial repolarization/hyperpolarization seen in Purkinje tissue. Also, the increase in $[Na]_i$ at the start of reperfusion stimulates the Na-Ca exchange mechanism, resulting in increase $[Ca^{2+}]_i$. The electrical manifestation of increased $[Ca^{2+}]_i$ is the appearance of OAP's. It is not known why the Na-Ca exchange may be stimulated only upon reperfusion. There is no evidence from the present work that the Na-Ca exchange mechanism occurs to promote Ca influx during ischemic conditions. Also initially upon reperfusion, there may be a reactivation of the Ca-channel and therefore the slow inward current. The Ca^{2+} entering through the slow inward current may contribute to a Ca-overload, and therefore the appearance of OAP's. The contribution of the slow inward current to OAP's upon reperfusion was determined by the fact that calcium channel blockers abolished OAP's. Also, BAY-K8644 which increases the slow inward current, facilitated the

appearance of OAP's. The "reactivation" of the Ca-channel also coincides with a time when contractility returned in Purkinje tissue.

After the initial repolarization/hyperpolarization phase with the appearance of OAP's, Purkinje tissue depolarized to a low membrane potential and became inexcitable. The reason for this depolarization is not known. Overstimulation of the Na-K ATPase could result in a large decrease in $[K]_o$ in the narrow intercellular clefts. This would decrease potassium conductance and therefore decrease MDP despite an increase in potassium equilibrium. At the same time, the Na-Ca exchange mechanism would result in increase $[Ca]_i$. If this exchange were electrogenic and exchanges 3 Na^+ for 1 Ca^{2+} (Horackova, 1984- review), then this would favour depolarization upon reperfusion. Also at this phase of depolarization and inexcitability, the Isi becomes inactivated. Further inactivation of Isi may be due to increased $[Ca^{2+}]_i$ (Lee et al., 1985). Inhibition of Isi may result in inhibition of potassium conductance and therefore depolarization. The further inhibition of Isi may be reason why Purkinje tissue did not display any activity initially upon depolarization.

Following the phase of inexcitability at low membrane potential, Purkinje tissue displayed automaticity at low membrane potential. The reason for the "reactivation" of the slow inward current is not known. "Reactivation" of the slow inward current may be due to decrease in intracellular Ca^{2+}, since this was presumably the reason for inactivation of the slow inward current. Following the phase of DIA/parasystole, Purkinje tissue slowly recovered MDP to pre-ischemic levels by the end

of reperfusion. Coinciding with this phase, is the reappearance of contractions in Purkinje tissue. The reason for recovery of MDP of Purkinje tissue may be due to a return of all ions influencing the MDP back to normal levels. Presumably at this time, the Na-K ATPase is functioning normally and interstitial K levels have returned to normal. Also at the same time, "reactivation" of Isi will result in increase in K conductance and therefore a repolarizing influence on Purkinje tissue. Also, recovery should result in return of $[Ca]_i$ levels back to normal. Whether return of $[Ca]_i$ to normal levels is due to extrusion of excess Ca^{2+} or due to sequestraton of Ca^{2+} intracellularly is not known.

It should be noted that the role of the slow inward current in studies outlined in ths thesis, were assessed using indirect indices of calcium current activation and inhibition. However changes in action potential configuration and DIA in the absence and presence of various agents and conditions point to a direct involvement of the slow inward current.

E. Future Work

Although the present work was aimed at elucidating the role of the slow inward current in ischemia and reperfusion arrhythmias, there are many questions left unanswered. Preliminary work from our laboratory with @ and § blockers suggests that these agents don't afford any protection against arrhythmias due to ischemic conditions and reperfusion. Therefore catecholamines are not released during ischemic conditions in the present model. Since catecholamines are released during ischemia (Riemersma, 1982 - review), the effect of catecholamines on the availa-

bility of Ca-dependent action during ischemic conditions should be investigated. Preliminary work from our laboratory has shown that catecholamines failed to prevent the decline in contractility in Purkinje tissue during ischemic conditions (A. Lukas - personal communication). However, the effects of catecholamines on electrically induced DIA during ischemic conditions have not been studied. The effects of catecholamines on the availability of Ca-dependent action potentials during reperfusion should also be investigated. Preliminary investigations have shown that reperfusion with noradrenaline prevented the inexcitable phase at low membrane potential in Purkinje tissue (A. Lukas - personal communication). This suggests that reperfusion with catecholamines may prevent further inhibition of Ca-dependent action potentials. The ideal way to investigate changes in the slow inward current during ischemic conditions and reperfusion is with voltage clamp. Through voltage clamp one would be able to study the alteration in the kinetics of the slow inward current under conditions of ischemia and reperfusion, and how catecholamines, etc. may influence the kinetics of the slow inward current.

Another important Ca-dependent mechanism that should be investigated is the role of the Na-Ca exchange mechanism during ischemic conditions and reperfusion. The proposed scheme of electrophysiological changes (Figures 30 and 31) suggested that Na-Ca exchange should occur during ischemic conditions, although there was no evidence that Na-Ca exchange occurs. Therefore ischemic conditions may inhibit Na-Ca exchange. The effects of ischemic conditions on Na-Ca exchange should

therefore be investigated. Since reperfusion appears to cause OAP's via a Na-Ca exchange mechanism, then the effects of inhibition of Na-Ca exchange should be investigated. Recently, a new Na-Ca exchange inhibitor, called dichlorobenzamil, has been synthesized (Bush et al., 1985-abstract). Therefore the effects of this agent on reperfusion induced OAP's can be investigated. Another interesting pharmacological agent is BAY-K8644 which promotes appearence of OAP's upon reperfusion. The characteristics of OAP's caused by BAY-K8644 such as cycle length dependency and membrane potential dependency should be investigated. It would also be interesting to use voltage clamp techniques to see if this agent also activates the TI current similarly to digitalis.

Finally, although the work in this thesis concentrated on the slow inward current, the involvement of the fast inward sodium current in arrhythmias due to ischemic conditions and reperfusion should also be investigated. The work in this thesis was not meant to imply that Ca is responsible for all mechanisms of arrhythmia seen during ischemic conditions and reperfusion. In fact, the present model of ischemic conditions and reperfusion demonstrates the difficulty of determining many factors which might cause specific electrophysiological changes during ischemic conditions and reperfusion. This is because the addition of any pharmacological agent or the reduction or addition of any ion will probably exert several effects instead of effects on a specific phase during ischemic conditions or reperfusion.

Computer program for analysis of electrophysiological and mechanical records

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10  REM --- DIGIT.BAS -- DEC 1/83 --TONY LUKAS --
20  REM --- THIS PROGRAM INPUTS DATA FROM THE DIGITIZER, ANALYSES
30  REM --- THE DATA AND STORES IT ON A DISKETTE.
40  REM ---
50  HOME :D$ =  CHR$ (4): PRINT D$;"PR#3": PRINT
60  HOME : VTAB 4:A$ = "ANALYSIS PROGRAM FOR": GOSUB 7000
70  VTAB 6:A$ = "HIPAD DIGITIZER": GOSUB 7000
80  DIM X(300,6)
85  PRINT D$;"BLOADATAB"
90 XNOW = 0:YNOW = 0:OLD = 1:TFS = 256:DIGITIZE = 775:XMSB = 769
100 XLSB = 770:YMSB = 772:YLSB = 773: TEXT : CALL  - 963
110  REM --- PROCEDURE SELECTION CODING
120  HOME : VTAB 2:A$ = "MODE SELECTION SUBROUTINE": GOSUB 7000
130  VTAB 5:A$ = "NEW DATA ANALYSIS OR DISPLAY": GOSUB 7000
140  VTAB 7:A$ = "EXISTING FILE. (TYPE N OR D)": GOSUB 7000
150  INPUT V1$: IF V1$ = "D" THEN  GOSUB 3000: GOSUB 4000: GOTO 8000
160  VTAB 10:A$ = "TENSION TRACINGS ? (TYPE Y OR N)": GOSUB 7000
170  INPUT Z$
180  IF Z$ = "Y" THEN : VTAB 13:A$ = "NUMBER OF mgs PER DIVISION": GOSUB
     7000
190  IF Z$ = "Y" THEN  INPUT SEN
200  IF V1$ = "N" THEN : IF Z$ = "Y" THEN  GOSUB 1000: GOSUB 2000
210  IF Z$ = "Y" THEN  GOTO 8000
220  IF V1$ = "N" THEN : IF Z$ = "N" THEN  GOSUB 1500: GOSUB 2000: GOTO 8
     000
400  GOTO 8000
1000  REM --- DIGITIZER DATA INPUT SUBROUTINE WITH TENSION
1010  HOME : VTAB 2:A$ = "DATA INPUT SUB WITH TENSION": GOSUB 7000
1020  VTAB 5:A$ = "RESET DIGITIZER IN BOTTOM LEFT CORNER": GOSUB 7000
1030  CALL DIGITIZE
1040  VTAB 8:A$ = "ENTER POSITION OF STIMULUS TRACE": GOSUB 7000
1050  VTAB 10:A$ = "THEN TOPS AND BOTTOMS OF CHANNEL": GOSUB 7000
1060  VTAB 12:A$ = "A AND B  100 mV CAL SIGNALS. NEXT.": GOSUB 7000
1065  VTAB 14:A$ = "ENTER A 1000 msec TIME CAL.": GOSUB 7000
1070  FOR J = 1 TO 5: CALL DIGITIZE
1080 CX = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1090 CY = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1100 C(J) = CY
1110  NEXT J
1120 AC = 100 / (C(2) - C(4)):BC = 100 / (C(3) - C(5))
1130 PA = C(4) - C(1):PB = C(5) - C(1)
1132  FOR L = 1 TO 2: CALL DIGITIZE
1133 S(L) = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1134 CF = 1000 / (S(2) - S(1))
1135  NEXT L
1140  HOME : VTAB 2:A$ = "ENTER STIMULUS LINE, THEN MDP OF": GOSUB 7000
1150  VTAB 4:A$ = "CHANNEL A AND B, AND LASTLY THE": GOSUB 7000
1160  VTAB 6:A$ = "TOP AND BOTTOM OF THE CONTRACTION": GOSUB 7000
1165  IF BX > 0 THEN  GOTO 1320
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1170  FOR I = 1 TO 10000
1180  FOR K = 1 TO 5
1190  CALL DIGITIZE
1200 XNOW = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1210 YNOW = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1220  IF YNOW ( 250 THEN : IF XNOW ( 830 THEN  RETURN
1225  IF YNOW ( 250 THEN : IF XNOW ) 1725 THEN  GOSUB 6000: GOTO 1140
1227  IF YNOW ( 250 THEN : IF XNOW ) 830 THEN  GOSUB 5000: GOTO 1140
1230 BX = 0:T(K) = YNOW
1240  NEXT K: VTAB 10: HTAB 15: PRINT "DATA SET # ";I
1250 X(I,1) = (100 - ((T(2) - T(1) - PA) * AC))
1260  VTAB 12: HTAB 5: PRINT "CHANNEL A (mV) : ";: PRINT X(I,1)
1270 X(I,2) = (100 - ((T(3) - T(1) - PB) * BC))
1280  VTAB 14: HTAB 5: PRINT "CHANNEL B (mV) : ";: PRINT X(I,2)
1290 TCF = SEN / (((C(2) - C(4)) + (C(3) - C(5))) / 8)
1300 X(I,3) = (T(4) - T(5)) * TCF
1310  VTAB 16: HTAB 5: PRINT "TENSION (mg) : ";: PRINT X(I,3)
1320  NEXT I
1330  RETURN
1500  REM --- DIGITIZER DATA INPUT SUBROUTINE WITHOUT TENSION
1510  HOME : VTAB 2:A$ = "DATA INPUT SUB WITHOUT TENSION": GOSUB 7000
1520  VTAB 5:A$ = "RESET DIGITIZER IN BOTTOM LEFT CORNER": GOSUB 7000
1530  CALL DIGITIZE
1540  VTAB 8:A$ = "ENTER POSITION OF STIMULUS TRACE": GOSUB 7000
1550  VTAB 10:A$ = "THEN TOPS AND BOTTOMS OF CHANNEL": GOSUB 7000
1560  VTAB 12:A$ = "A AND B  100 mV CAL SIGNALS. NEXT,": GOSUB 7000
1565  VTAB 14:A$ = "ENTER A 1,000 msec TIME CAL": GOSUB 7000
1570  FOR J = 1 TO 5: CALL DIGITIZE
1580 CX = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1590 CY = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1600 C(J) = CY
1610  NEXT J
1620 AC = 100 / (C(2) - C(4)):BC = 100 / (C(3) - C(5))
1630 PA = C(4) - C(1):PB = C(5) - C(1)
1632  FOR L = 1 TO 2: CALL DIGITIZE
1633 S(L) = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1634 CF = 1000 / (S(2) - S(1))
1635  NEXT L
1640  HOME : VTAB 2:A$ = "ENTER STIMULUS LINE, THEN MDP OF": GOSUB 7000
1650  VTAB 4:A$ = "CHANNEL A THEN CHANNEL B.": GOSUB 7000
1655  IF BX ) 0 THEN  GOTO 1780
1660  FOR I = 1 TO 10000
1670  FOR K = 1 TO 3
1680  CALL DIGITIZE
1690 XNOW = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1700 YNOW = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1710  IF YNOW ( 250 THEN : IF XNOW ( 830 THEN  RETURN
1715  IF YNOW ( 240 THEN : IF XNOW ) 830 THEN  GOSUB 5000: GOTO 1640
1720 BX = 0:T(K) = YNOW
1730  NEXT K: VTAB 10: HTAB 15: PRINT "DATA SET # ";I
1740 X(I,1) = (100 - ((T(2) - T(1) - PA) * AC))
1750  VTAB 12: HTAB 5: PRINT "CHANNEL A (mV) : ";: PRINT X(I,1)
1760 X(I,2) = (100 - ((T(3) - T(1) - PB) * BC))
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```
1770  VTAB 14: HTAB 5: PRINT "CHANNEL B (mV) : ";: PRINT X(I,2)
1780  NEXT I
1790  RETURN
2000  REM --- STORE DATA FILE ON DISKETTE SUBROUTINE
2010  HOME : VTAB 2:A$ = "DATA STORAGE SUBROUTINE": GOSUB 7000
2020  VTAB 5: INPUT "FILENAME     ";W$
2030  VTAB 8: INPUT "DRIVE NO     ";DR$
2040  VTAB 11:A$ = "DATE OF EXPERIMENT ?": GOSUB 7000: INPUT M1$
2050  VTAB 14:A$ = "ANIMAL TYPE, SEX AND WT ?": GOSUB 7000: INPUT M2$
2060  VTAB 17:A$ = "PREPARATION ?": GOSUB 7000: INPUT M3$
2070  PRINT D$;"OPEN";W$",D";DR$
2080  PRINT D$;"WRITE";W$
2090  PRINT M1$: PRINT M2$: PRINT M3$: PRINT I - 1
2100  FOR J = 1 TO I - 1: PRINT X(J,1): PRINT X(J,2)
2110  PRINT X(J,3): PRINT X(J,4): PRINT X(J,5): PRINT X(J,6)
2120  NEXT J
2130  PRINT D$;"CLOSE";W$
2140  RETURN
3000  REM --- READ DISKETTE FILE SUBROUTINE
3010  HOME : VTAB 2:A$ = "READ FILE SUBROUTINE": GOSUB 7000
3020  VTAB 5: INPUT "FILENAME    ";W$: VTAB 8: INPUT "DRIVE NO    ";DR$
3030  PRINT D$;"OPEN";W$",D";DR$: PRINT D$;"READ";W$
3040  INPUT M1$: INPUT M2$: INPUT M3$: INPUT I
3050  FOR J = 1 TO I: INPUT X(J,1): INPUT X(J,2)
3060  INPUT X(J,3): INPUT X(J,4): INPUT X(J,5): INPUT X(J,6)
3070  NEXT J
3080  PRINT D$;"CLOSE";W$
3090  RETURN
4000  REM --- DATA DISPLAY SUBROUTINE
4010  HOME : VTAB 2:A$ = "DATA DISPLAY SUBROUTINE": GOSUB 7000
4020  VTAB 5: PRINT "     ";M1$;: PRINT "     ";M2$;
4030  PRINT "     ";M3$
4040  FOR J = 1 TO I: VTAB 8: HTAB 15: PRINT "DATA SET # ";J
4050  VTAB 10: HTAB 5: PRINT "CHANNEL A (mV) : ";
4055  PRINT X(J,1);: PRINT "          "
4060  VTAB 12: HTAB 5: PRINT "CHANNEL B (mV) : ";
4065  PRINT X(J,2);: PRINT "          "
4070  VTAB 14: HTAB 5: PRINT "TENSION (mg) : ";
4075  PRINT X(J,3);: PRINT "          "
4080  VTAB 16: HTAB 5: PRINT "OAP AMPLITUDE (mV) : ";
4085  PRINT X(J,4);: PRINT "          "
4090  VTAB 18: HTAB 5: PRINT "COUPLING INTERVAL (msec) : ";
4100  PRINT X(J,5);: PRINT "          "
4110  VTAB 20: HTAB 5: PRINT "PRECEEDING BCL (msec) : ";
4120  PRINT X(J,6);: PRINT "          "
4130  GET U$
4140  NEXT J
4150  RETURN
5000  REM --- OAP CALCULATIONS SUBROUTINE
5010  HOME : VTAB 2:A$ = "OAP CALCULATIONS SUBROUTINE": GOSUB 7000
5020  VTAB 5:A$ = "ENTER THE BCL PRECEEDING THE END": GOSUB 7000
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5030  VTAB 7:A$ = "OF THE TRAIN, THEN THE STIMULUS": GOSUB 7000
5040  VTAB 9:A$ = "ARTEFACT AND CENTER OF OAP, LASTLY": GOSUB 7000
5050  VTAB 11:A$ = "THE MDP AND PEAK OF THE OAP": GOSUB 7000
5060  FOR Q = 1 TO 4: CALL DIGITIZE
5070 BX = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
5090 B(Q) = BX
5100  NEXT Q
5110  FOR R = 1 TO 2: CALL DIGITIZE
5120 OAB = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
5130 O(R) = OAB
5140  NEXT R
5150 X(I,4) = (O(2) - O(1)) * AC: VTAB 15: HTAB 5
5160  PRINT "OAP AMPLITUDE (mV) = ";: PRINT X(I,4)
5180 X(I,5) = (B(4) - B(3)) * CF: VTAB 17: HTAB 5
5190  PRINT "COUPLING INTERVAL (msec) = ";: PRINT X(I,5)
5200 X(I,6) = (B(2) - B(1)) * CF: VTAB 19: HTAB 5
5210  PRINT "PRECEEDING BCL (msec) = ";: PRINT X(I,6)
5220  VTAB 22: HTAB 5: INVERSE
5230  PRINT "PRESS CURSOR BUTTON TO CONTINUE": NORMAL
5240  CALL DIGITIZE
5250  RETURN
6000  REM --- CHANGE TENSION SENSITIVITY SUBROUTINE
6010  HOME : VTAB 2:A$ = "CHANGE TENSION SENS. SUBROUTINE": GOSUB 7000
6020  VTAB 5:A$ = "ENTER NEW TENSION SENSITIVITY": GOSUB 7000
6030  VTAB 7:A$ = "NUMBER OF mgs PER DIVISION ? ": GOSUB 7000
6040  VTAB 9: INPUT SEN:BX = 1
6050  RETURN
7000  REM --- CENTER STRING A$ SUBROUTINE
7010 B =  INT (20 - ( LEN (A$) / 2)): IF B < 0 THEN B = 1
7020  HTAB B: PRINT A$: RETURN
8000  PRINT  CHR$ (17)
8010  END
```

Computer program for analysis of cycle length of DIA

```
10  REM --- AUTODIG.BAS --- MARCH 12/84 --- TONY LUKAS ---
20  REM --- THIS PROGRAM INPUTS DATA FROM THE DIGITIZER, ANALYSES
30  REM --- THE DATA AND STORES IT ON A DISKETTE.
40  REM ---
50  HOME :D$ =  CHR$ (4): PRINT D$;"PR#3": PRINT
60  HOME : VTAB 4:A$ = "ANALYSIS PROGRAM FOR": GOSUB 7000
70  VTAB 6:A$ = "HIPAD DIGITIZER": GOSUB 7000
80  DIM X(300,7)
85  PRINT D$;"BLOADATAB"
90 XNOW = 0:YNOW = 0:OLD = 1:TFS = 256:DIGITIZE = 775:XMSB = 769
100 XLSB = 770:YMSB = 772:YLSB = 773: TEXT : CALL  - 963
110  REM --- PROCEDURE SELECTION CODING
120  HOME : VTAB 2:A$ = "MODE SELECTION SUBROUTINE": GOSUB 7000
130  VTAB 5:A$ = "NEW DATA ANALYSIS OR DISPLAY": GOSUB 7000
140  VTAB 7:A$ = "EXISTING FILE. (TYPE N OR D)": GOSUB 7000
150  INPUT V1$: IF V1$ = "D" THEN  GOSUB 3000: GOSUB 4000: GOTO 8000
160  VTAB 10:A$ = "TENSION TRACINGS ? (TYPE Y OR N)": GOSUB 7000
170  INPUT Z$
180  IF Z$ = "Y" THEN : VTAB 13:A$ = "NUMBER OF mgs PER DIVISION": GOSUB
     7000
190  IF Z$ = "Y" THEN  INPUT SEN
200  IF V1$ = "N" THEN : IF Z$ = "Y" THEN  GOSUB 1000: GOSUB 2000
210  IF Z$ = "Y" THEN  GOTO 8000
220  IF V1$ = "N" THEN : IF Z$ = "N" THEN  GOSUB 1500: GOSUB 2000: GOTO 8
     000
400  GOTO 8000
1000  REM --- DIGITIZER DATA INPUT SUBROUTINE WITH TENSION
1010  HOME : VTAB 2:A$ = "DATA INPUT SUB WITH TENSION": GOSUB 7000
1020  VTAB 5:A$ = "RESET DIGITIZER IN BOTTOM LEFT CORNER": GOSUB 7000
1030  CALL DIGITIZE
1040  VTAB 8:A$ = "ENTER POSITION OF STIMULUS TRACE": GOSUB 7000
1050  VTAB 10:A$ = "THEN TOPS AND BOTTOMS OF CHANNEL": GOSUB 7000
1060  VTAB 12:A$ = "A AND B  100 mV CAL SIGNALS. NEXT,": GOSUB 7000
1065  VTAB 14:A$ = "ENTER A 1000 msec TIME CAL.": GOSUB 7000
1070  FOR J = 1 TO 5: CALL DIGITIZE
1080 CX = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1090 CY = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1100 C(J) = CY
1110  NEXT J
1120 AC = 100 / (C(2) - C(4)):BC = 100 / (C(3) - C(5))
1130 PA = C(4) - C(1):PB = C(5) - C(1)
1132  FOR L = 1 TO 2: CALL DIGITIZE
1133 S(L) = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1134 CF = 1000 / (S(2) - S(1))
1135  NEXT L
1140  HOME : VTAB 2:A$ = "ENTER STIMULUS LINE, THEN MDP OF": GOSUB 7000
1150  VTAB 4:A$ = "CHANNEL A AND B, AND LASTLY THE": GOSUB 7000
1160  VTAB 6:A$ = "TOP AND BOTTOM OF THE CONTRACTION": GOSUB 7000
1165  IF BX > 0 THEN  GOTO 1320
1170  FOR I = 1 TO 10000
1180  FOR K = 1 TO 5
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1190  CALL DIGITIZE
1200 XNOW = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1210 YNOW = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1220  IF YNOW ( 250 THEN : IF XNOW ( 830 THEN  RETURN
1225  IF YNOW ( 250 THEN : IF XNOW ) 1725 THEN  GOSUB 6000:.GOTO 1140
1227  IF YNOW ( 250 THEN : IF XNOW ) 830 THEN  GOSUB 5000: GOTO 1140
1230 BX = 0:T(K) = YNOW
1240  NEXT K: VTAB 10: HTAB 15: PRINT "DATA SET # ";I
1250 X(I,1) = (100 - ((T(2) - T(1) - PA) * AC))
1260  VTAB 12: HTAB 5: PRINT "CHANNEL A (mV) : ";: PRINT X(I,1)
1270 X(I,2) = (100 - ((T(3) - T(1) - PB) * BC))
1280  VTAB 14: HTAB 5: PRINT "CHANNEL B (mV) : ";: PRINT X(I,2)
1290 TCF = SEN / (((C(2) - C(4)) + (C(3) - C(5))) / 8)
1300 X(I,3) = (T(4) - T(5)) * TCF
1310  VTAB 16: HTAB 5: PRINT "TENSION (mg) : ";: PRINT X(I,3)
1320  NEXT I
1330  RETURN
1500  REM --- DIGITIZER DATA INPUT SUBROUTINE WITHOUT TENSION
1510  HOME : VTAB 2:A$ = "DATA INPUT SUB WITHOUT TENSION": GOSUB 7000
1520  VTAB 5:A$ = "RESET DIGITIZER IN BOTTOM LEFT CORNER": GOSUB 7000
1530  CALL DIGITIZE
1540  VTAB 8:A$ = "ENTER POSITION OF STIMULUS TRACE": GOSUB 7000
1550  VTAB 10:A$ = "THEN TOPS AND BOTTOMS OF CHANNEL": GOSUB 7000
1560  VTAB 12:A$ = "A AND B  100 mV CAL SIGNALS. NEXT,": GOSUB 7000
1565  VTAB 14:A$ = "ENTER A 1000 msec TIME CAL": GOSUB 7000
1570  FOR J = 1 TO 5: CALL DIGITIZE
1580 CX = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1590 CY = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1600 C(J) = CY
1610  NEXT J
1620 AC = 100 / (C(2) - C(4)):BC = 100 / (C(3) - C(5))
1630 PA = C(4) - C(1):PB = C(5) - C(1)
1632  FOR L = 1 TO 2: CALL DIGITIZE
1633 S(L) = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1634 CF = 1000 / (S(2) - S(1))
1635  NEXT L
1640  HOME : VTAB 2:A$ = "ENTER STIMULUS LINE, THEN MDP OF": GOSUB 7000
1650  VTAB 4:A$ = "CHANNEL A THEN CHANNEL B.": GOSUB 7000
1655  IF BX ) 0 THEN  GOTO 1780
1660  FOR I = 1 TO 10000
1670  FOR K = 1 TO 3
1680  CALL DIGITIZE
1690 XNOW = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
1700 YNOW = ( PEEK (YMSB) * TFS) +  PEEK (YLSB)
1710  IF YNOW ( 250 THEN : IF XNOW ( 830 THEN  RETURN
1715  IF YNOW ( 240 THEN : IF XNOW ) 830 THEN  GOSUB 5000: GOTO 1640
1720 BX = 0:T(K) = YNOW
1730  NEXT K: VTAB 10: HTAB 15: PRINT "DATA SET # ";I
1740 X(I,1) = (100 - ((T(2) - T(1) - PA) * AC))
1750  VTAB 12: HTAB 5: PRINT "CHANNEL A (mV) : ";: PRINT X(I,1)
1760 X(I,2) = (100 - ((T(3) - T(1) - PB) * BC))
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1770  VTAB 14: HTAB 5: PRINT "CHANNEL B (mV) : ";: PRINT X(I,2)
1780  NEXT I
1790  RETURN
2000  REM --- STORE DATA FILE ON DISKETTE SUBROUTINE
2010  HOME : VTAB 2:A$ = "DATA STORAGE SUBROUTINE": GOSUB 7000
2020  VTAB 5: INPUT "FILENAME     ";W$
2030  VTAB 8: INPUT "DRIVE NO     ";DR$
2040  VTAB 11:A$ = "DATE OF EXPERIMENT ?": GOSUB 7000: INPUT M1$
2050  VTAB 14:A$ = "ANIMAL TYPE, SEX AND WT ?": GOSUB 7000: INPUT M2$
2060  VTAB 17:A$ = "PREPARATION ?": GOSUB 7000: INPUT M3$
2070  PRINT D$;"OPEN";W$",D";DR$
2080  PRINT D$;"WRITE";W$
2090  PRINT M1$: PRINT M2$: PRINT M3$: PRINT I - 1
2100  FOR J = 1 TO I - 1: PRINT X(J,1): PRINT X(J,2)
2110  PRINT X(J,3): PRINT X(J,4): PRINT X(J,5): PRINT X(J,6)
2115  PRINT X(J,7)
2120  NEXT J
2130  PRINT D$;"CLOSE";W$
2140  RETURN
3000  REM --- READ DISKETTE FILE SUBROUTINE
3010  HOME : VTAB 2:A$ = "READ FILE SUBROUTINE": GOSUB 7000
3020  VTAB 5: INPUT "FILENAME    ";W$: VTAB 8: INPUT "DRIVE NO    ";DR$
3030  PRINT D$;"OPEN";W$",D";DR$: PRINT D$;"READ";W$
3040  INPUT M1$: INPUT M2$: INPUT M3$: INPUT I
3050  FOR J = 1 TO I: INPUT X(J,1): INPUT X(J,2)
3060  INPUT X(J,3): INPUT X(J,4): INPUT X(J,5): INPUT X(J,6)
3065  INPUT X(J,7)
3070  NEXT J
3080  PRINT D$;"CLOSE";W$
3090  RETURN
4000  REM --- DATA DISPLAY SUBROUTINE
4010  HOME : VTAB 2:A$ = "DATA DISPLAY SUBROUTINE": GOSUB 7000
4020  VTAB 5: PRINT "    ";M1$;: PRINT "    ";M2$;
4030  PRINT "    ";M3$
4040  FOR J = 1 TO I: VTAB 8: HTAB 15: PRINT "DATA SET # ";J
4050  VTAB 10: HTAB 5: PRINT "CHANNEL A (mV) : ";
4055  PRINT X(J,1);: PRINT "          "
4060  VTAB 12: HTAB 5: PRINT "CHANNEL B (mV) : ";
4065  PRINT X(J,2);: PRINT "          "
4070  VTAB 14: HTAB 5: PRINT "TENSION (mg) : ";
4075  PRINT X(J,3);: PRINT "          "
4080  VTAB 16: HTAB 5: PRINT "BCL (msec) : ";
4085  PRINT X(J,4);: PRINT "          "
4090  VTAB 18: HTAB 5: PRINT "INTERPOLATED INTERVAL (msec) : ";
4100  PRINT X(J,5);: PRINT "          "
4110  VTAB 20: HTAB 5: PRINT "MODULATED CL (msec) : ";
4120  PRINT X(J,6);: PRINT "          "
4130  VTAB 22: HTAB 5: PRINT "SPONTANEOUS CL (msec) : ";
4140  PRINT X(J,7);: PRINT "          "
4150  GET U$
4160  NEXT J
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4170  RETURN
5000  REM --- PARASYSTOLE CALCULATIONS SUBROUTINE
5010  HOME : VTAB 2:A$ = "PARASYSTOLE CALCULATIONS SUB": GOSUB 7000
5020  VTAB 5:A$ = "ENTER THE BCL, THEN THE FIRST AND LAST": GOSUB 7000
5030  VTAB 7:A$ = "BEAT OF A MODULATED CYCLE, AND LASTLY": GOSUB 7000
5040  VTAB 9:A$ = "THE FIRST AND LAST BEAT OF A": GOSUB 7000
5050  VTAB 11:A$ = "SPONTANEOUS CYCLE": GOSUB 7000
5060  FOR Q = 1 TO 6: CALL DIGITIZE
5070 BX = ( PEEK (XMSB) * TFS) +  PEEK (XLSB)
5080 B(Q) = BX
5090  NEXT Q
5100 X(I,4) = (B(2) - B(1)) * CF: VTAB 14: HTAB 5
5120  PRINT "BASIC CYCLE LENGTH (msec) = ";: PRINT X(I,4)
5130 X(I,5) = (B(2) - B(3)) * CF: VTAB 16: HTAB 5
5140  PRINT "INTERPOLATED INTERVAL (msec) = ";: PRINT X(I,5)
5150 X(I,6) = (B(4) - B(3)) * CF: VTAB 18: HTAB 5
5160  PRINT "MODULATED CYCLE LENGTH (msec) = ";: PRINT X(I,6)
5180 X(I,7) = (B(6) - B(5)) * CF: VTAB 20: HTAB 5
5190  PRINT "SPONTANEOUS CYCLE LENGTH (msec) = ";: PRINT X(I,7)
5200  VTAB 22: HTAB 5: INVERSE
5210  PRINT "PRESS CURSOR BUTTON TO CONTINUE": NORMAL : CALL DIGITIZE
5220  RETURN
6000  REM --- CHANGE TENSION SENSITIVITY SUBROUTINE
6010  HOME : VTAB 2:A$ = "CHANGE TENSION SENS. SUBROUTINE": GOSUB 7000
6020  VTAB 5:A$ = "ENTER NEW TENSION SENSITIVITY": GOSUB 7000
6030  VTAB 7:A$ = "NUMBER OF mgs PER DIVISION ? ": GOSUB 7000
6040  VTAB 9: INPUT SEN:BX = 1
6050  RETURN
7000  REM ---- CENTER STRING A$ SUBROUTINE
7010 B =  INT (20 - ( LEN (A$) / 2)): IF B < 0 THEN B = 1
7020  HTAB B: PRINT A$: RETURN
8000  PRINT  CHR$ (17)
8010  END

]
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