AUTOMATICITY AND REFRACTORINESS IN MAMMALIAN VENTRICULAR MYOCARDIUM: AN ELECTROPHYSIOLOGICAL STUDY OF DRUG EFFECTS

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Augustus Oliver Grant
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MB.ChB., University of Edinburgh, 1971

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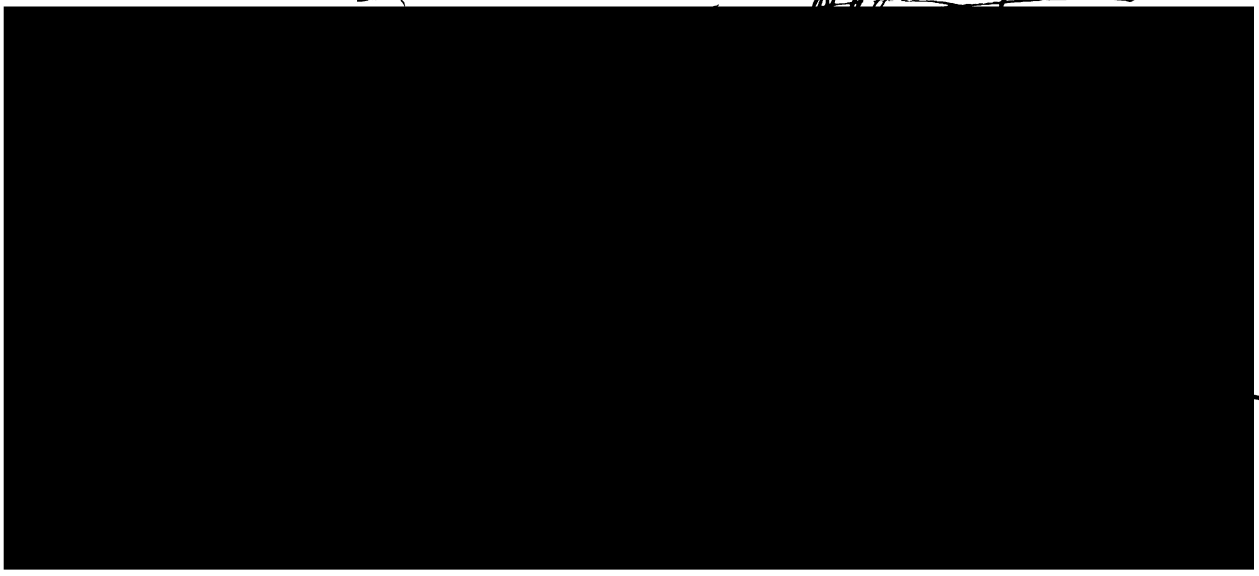

ABSTRACT

AUTOMATICITY AND REFRACTORINESS IN MAMMALIAN VENTRICULAR MYOCARDIUM: AN ELECTROPHYSIOLOGICAL STUDY OF DRUG EFFECTS

A.O. Grant

Automaticity.

Automaticity can be induced over a range of membrane potentials in normally quiescent ventricular myocardial fibres by depolarizing current. This study examined the effects of verapamil 1 - 5 mg/L, quinidine 2 - 4 mg/L, and droperidol 1.9 - 3.8 mg/L on automaticity in true ventricular myocardial fibres.

Papillary muscles from guinea pig were mounted in a sucrose gap chamber and transmembrane potential recorded by standard microelectrode techniques. Automaticity was induced with depolarizing currents of varying strength.

Verapamil reduced phase 4 slope (diastolic depolarization rate) at all membrane potentials. It also caused a selective reduction of the amplitude of action potentials arising from less negative diastolic potentials. The role of blockade of the slow inward current in these effects was examined by increasing the extracellular calcium, $[Ca^{2+}]_o$, or addition of epinephrine during exposure to verapamil. Increased $[Ca^{2+}]_o$ partially restored action potential overshoot, but phase 4 slope was further reduced. Epinephrine caused a partial or complete reversal of phase 4 slope depression, but did not restore action potential amplitude. All the effects of verapamil on ventricular automaticity therefore cannot be explained on the basis of calcium blockade.

Quinidine reduced phase 4 slope at all maximum diastolic potentials. There was a less marked reduction of the amplitude of action potentials arising from low maximum diastolic potentials.

Epinephrine caused a partial reversal of the reduction of phase 4 slope produced by quinidine.

Droperidol also reduced phase 4 slope at all maximum diastolic potentials. However, this drug was more depressant for action potentials arising from more negative potentials. There was no consistent effect on action potential overshoot. Epinephrine reversed the depression of phase 4 slope produced by droperidol.

During myocardial infarction, internally generated "currents of injury" may lead to repetitive activity in ventricular myocardial fibres, similar to that induced by current from an external source. Such repetitive activity may be responsible for ventricular extrasystoles, parasystole, or may trigger re-entrant arrhythmias. Antiarrhythmic drugs could abolish such arrhythmias by suppression of ectopic impulse formation in true ventricular myocardial fibres.

Refractoriness

The effects of droperidol .38 - 15.2 mg/L and quinidine 1 - 4 mg/L on the recovery kinetics of dV/dT max of transmembrane action potentials recorded from guinea pig papillary muscle *in vitro* were examined. Droperidol caused marked slowing of the recovery kinetics of dV/dT max. This effect was exaggerated in potassium depolarized preparations and partially reversed by increasing $[Ca^{2+}]_o$. Quinidine did not slow the recovery kinetics of dV/dT max.

Droperidol prolonged the effective refractory period (ERP) at all drug concentrations. However, the ERP:action potential duration (APD) ratio was only prolonged at 15.2 mg/L. Quinidine prolonged the ERP, but did not increase the ERP:APD ratio in the

concentrations studied. Slowing of the recovery kinetics appears to be important in prolonging the ERP:APD ratio only at high concentrations of droperidol.

Droperidol decreased dV/dT max in a rate dependent manner, the effect of the drug being more pronounced at high drive rates. This rate dependent decrease of dV/dT max was more marked in potassium depolarized preparations, and was partially reversed by elevation of $[Ca^{2+}]_o$. It is concluded that this effect may be dependent on slowing of the recovery kinetics of dV/dT max.

To: Stephanie, Mum, and Dad

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The technical assistance of Messers. C. Cotner and C. Lee in preparation of the figures is much appreciated. I am indebted to Ms. J. Carnes for typing this manuscript at very short notice.

ABBREVIATIONS

APD: Action potential duration.

ERP: Effective refractory period.

MDP: Maximum diastolic potential.

TABLE OF CONTENTS

1. INTRODUCTION

Cardiac arrhythmias are thought to arise as a result of disturbances in impulse initiation and/or impulse propagation. Automaticity and refractoriness are specific aspects of these two disturbances. The present investigation examined the effects of verapamil, quinidine and droperidol on automaticity and refractoriness in the ventricular myocardium of the guinea pig.

1.1. Automaticity

1.1a. Normal Impulse Formation in the Heart

Progress in our understanding of normal and abnormal impulse formation in the heart has been dictated by the availability of techniques. By the simple procedure of ligation along normal anatomical boundaries, Stannius showed that the impulse for initiating the heart beat arose in the sinus venosus of the amphibian heart. However, his experiment indicated (Stannius ligature 2) that other parts of the heart were capable of impulse formation (reviewed by Gaskell, 1900). The early morphologists had described ganglion cells in relation to the sinus venosus (Remak's ganglion) and the atrioventricular junction (Bidder's ganglion). The suggestion was made that activity arose in the ganglion cells. This was strongly challenged by Gaskell (1900). In a lucid review of the whole question, he pointed out that the longitudinal tube from which the heart develops is capable of spontaneous "rhythmicity". He suggested that during differentiation, the atrial and ventricular myocardium had become specialized to subserve a contractile function, whereas other fibres, retaining their primitive state, were

responsible for impulse initiation and conduction. In other words, the nodes and conducting system(s) which today we call "specialized" may in fact be the more primitive (less differentiated). He summarized his position in the following conjecture: "Is it not that, whereas all parts of the muscular tissue of the heart possess the power of rhythmical contraction to a greater or less degree, some parts possess that power to such an extent that their contractions are automatic?"

Using records of mechanical activity, Gaskell confirmed and extended the original observations in the amphibian heart. He showed that a hierarchy of inherent rhythmicity also extended to the heart of poikilotherms.

The next important advance was the work of Keith and Flack (1906-1907). On the basis of comparative embryology, they concluded that the remnants of the sinus venosus were to be found in relation to the union of the superior vena cava and the true auricle(atrium) of the mammalian heart. They therefore asked the question whether there was any morphological substrate for spontaneous impulse formation in this region of the heart in a spectrum of mammalian species. Tawara had previously described "peculiar muscular fibres" in the atrio-ventricular junction and the main (His) bundle. Keith and Flack found that indeed, in all hearts examined, there was cellular specialization in this region, including

(i) Small pale staining cells embedded in dense connective tissue.

(ii) A high density of innervation.

(iii) A large central artery. Parenthetically, James (1973) considers this close relationship of functional significance.

The constancy of their findings led them to conclude of the pale cells "...it is in them that the dominating rhythm of the heart normally begins". By a combination of epicardial mapping with extracellular electrodes and subsequent microscopy, Lewis et al., (1910-1911) showed conclusively that the normal cardiac impulse originated in the sinoauricular (sinoartrial) node.

Several excellent reviews have appeared on this subject (e.g. West 1972). My object in recalling the work done at the turn of the century (see also "Electrical current as an inducer of repetitive activity" below), was to ask the following: At the time when our current ideas of impulse formation in the heart were defined was there anything by way of experimental evidence or inference to suggest that only the "specialized tissues" were capable of impulse formation? To the contrary, the authorative summary of Gaskell at that time suggested otherwise.

1.1b. Cellular Physiology

Early investigation of the cellular basis of impulse formation involved the recording of electrical events of groups of cells with extracellular electrodes. In 1942, Bozler published observations on automaticity in the vertebrate heart using the above technique. He noted that at the pacemaker site, "each impulse is preceded by a period of gradually rising negativity", termed a prepotential. Such a prepotential could be induced in quiescent

preparations by epinephrine. Bozler distinguished repetitive activity dependent on prepotentials from that dependent on "afterpotential" mechanism(s).

The ultramicroelectrode introduced by Ling and Gerard made possible the recording of electrical activity of single cells. Draper and Weidmann (1951) applied the technique to cardiac tissue, (Purkinje fibres of dog and kid) and confirmed the presence of diastolic depolarization (phase 4) in spontaneously active fibres. Similar phase 4 depolarization was demonstrated in the sinus venosus of the frog (Hutter and Trautwein 1956). Using the floating micro-electrode technique, West (1955) recorded activity from fibres of the sinoatrial node of the rabbit. He showed that following a given action potential, the membrane potential underwent slow diastolic depolarization, passing smoothly into the upstroke of the subsequent action potential.

It was recognized by these investigators that slow depolarization during diastole may be the result of:

(i) A gradual increase in sodium permeability.

(ii) A gradual decline in potassium permeability.

(iii) Reduction of the activity of an electrogenic sodium pump.

(iv) A combination of any of the above.

The relative membrane resistance was shown to increase progressively during diastole (Weidmann 1951; Dudel and Trautwein 1958; Vassalle 1966). This suggests that mechanism (i), if present could only play a minor role. Further work at this time centered around the effects of changes in extracellular ion concentration. The results are summarized in table 1.

TABLE I

	Sinus Node	Purkinje Fibre
Increased $[K^+]_o$	+ and -: Trautwein 1963*	-: Vassalle 1965
	0: Toda and West 1967	
Decreased $[K^+]_o$	+: Trautwein 1963	+: Vassalle 1965
Increased $[Ca^{2+}]_o$	+ and -: Seifen et al., 1964**	+: Temte and Davis 1967
		0: Weidmann 1955b
Decreased $[Ca^{2+}]_o$	-: Seifen et al., 1964	0: Temte and Davis 1967
Decreased $[Na]_o$	-: Trautwein 1963***	-: Draper and Weidmann 1951
	Cranefield 1975	

+ ≡ Increased; 0 ≡ no effect; - ≡ Decreased.

* Increase followed by decrease.

** Increase up to about 7 mM, then decrease.

*** 20% of normal Na or less.

A detailed analysis of the ionic mechanism(s) of cardiac automaticity as had been done for the nerve action potential (Hodgkin and Huxley 1952b) had to await a suitable cardiac preparation for voltage clamping. Such a preparation, the shortened Purkinje fibre, was described by Deck et al., (1964). While clearly unsuitable for analysis of rapid current transients, the slow currents underlying diastolic depolarization could be examined.

In their initial studies (Deck and Trautwein 1964), they identified a positive (outward) current termed the "diastolic current" at the termination of depolarizing clamp steps. The current had a large time constant and reversed sign around the potassium equilibrium potential. They suggested that this current was triggered by repolarization. The current was decreased to 1/4 - 1/3 of its initial value when choline was substituted for sodium. The current was nevertheless identified as a potassium current, the "cholinergic" effect of choline being suggested as the reason for the marked decrease in choline solution although the methods clearly stated that atropine was added to the choline solution. Deck and Trautwein (1964) proposed that decrease of this "potassium" current during diastole could account for diastolic depolarization.

These initial observations were extended by Vassalle (1966). He voltage clamped spontaneously beating Purkinje fibres at the maximum diastolic potential. The current record showed a time dependent inward current. Such a current would cause depolarization of the unclamped membrane. The time dependence of the current disappeared around the potassium equilibrium potential.

Noble and Tsien (1968, 1969) performed a detailed voltage clamp analysis of the outward potassium currents in cardiac Purkinje fibres of sheep. They identified the "pacemaker current" as a component, IK_2. IK_2 is described as the product of an activation variable s, a rectifier function, and a driving force $(E_m - E_k)$. s varies from 0 - 1 in the range of pacemaker potential (-90 to -70 mV). Their conclusions as to the genesis of the pacemaker prepotential may be summarized as follows:

(i) During the action potential, s increases from 0 to full activation ($s \sim 1$), the long duration of the action potential and the shorter time constant at less negative potentials ensuring full activation of this slow current.

(ii) At the time of repolarization to the maximum diastolic potential $s = 1$, but the steady state value of s for this potential is close to 0, i.e., $s_\infty \sim 0$. s therefore declines towards its steady state value.

(iii) With a declining outward current, in the face of a steady background inward current, diastolic depolarization ensues.

(iv) The decreasing membrane potential causes s to approach s_∞. The potential at which $s = s_\infty$ should be a stable point.

(v) However, IK_2 has a negative slope in its current voltage relationship and this causes a terminal acceleration of diastolic depolarization to the sodium threshold. The nature of the background

(v) (cont.)

inward current was not studied.

Several valid criticisms can be levied at Noble and Tsiens' work (e.g. see Johnson and Liebermann 1971). However, subsequent work (Peper and Trautwein 1969; Hauswirth et al., 1972) has confirmed in principle their conclusions and no work has appeared to seriously contradict it.

A possible contributory role of electrogenic ion transport in the genesis of the pacemaker potential is far from settled. There is evidence for electrogenic active transport in cardiac muscle (e.g. Carpentier and Vassalle 1971; Haas 1972; Noma and Irisawa 1974; Isenberg and Trautwein 1974). However, a precise role in initiating or modulating normal pacemaker activity is not defined.

1.1c. Pharmacology

Drugs may modify the frequency of pacemaker discharge by:

(i) Alterations of the maximum diastolic potential.

(ii) Alterations of the slope of diastolic depolarization.

(iii) Alterations in the threshold potential.

(iv) Alteractions in action potential duration.

(v) Combinations of the above.

Autonomic Mediators

Initial studies of autonomic effects involved stimulation of the extrinsic nerves to the sinus venosus or transmural stimulation in the region of the sinus node (e.g. Hutter and Trautwein 1956; Vincenzi and West 1963). In some species, a vagosympathetic trunk is present rather than separate parasympathetic and sympathetic

entities. Also, with transmural stimulation, both sympathetic and parasympathetic nerve endings are stimulated. The use of pharmacologic blocking agents permitted meaningful analysis (see review by West 1972).

"Parasympathetic stimulation" slowed pacemaker activity in the sinus venosus by increasing the maximum diastolic potential and reducing phase 4 slope (Hutter and Trautwein 1956). The increase in membrane potential shifted the maximum diastolic potential towards the potassium equilibrium potential (Dudel and Trautwein 1958). It was therefore concluded that acetylcholine suppressed automaticity by increasing potassium conductance. In contrast, an early review indicated that acetylcholine is "almost wholly without effect on Purkinje fibres" (Hoffman and Cranefield 1960). This question has been re-examined recently, and the indications are that acetylcholine does decrease automaticity in Purkinje fibres (Bailey et al., 1972: Tse et al., 1975).

Sympathetic stimulation increases pacemaker frequency by accelerating diastolic depolarization (Hutter and Trautwein 1956; West et al., 1956; Hoffman and Cranefield 1960). The effect on the threshold potential has been variously reported as unchanged, decreased, or increased (Hoffman and Cranefield 1960; Kassebaum 1964).

The mechanism of action of epinephrine on automaticity has been examined by the voltage clamp technique in Purkinje fibres (Hauswirth et al., 1968; Tsien 1974a, b). Epinephrine accelerates the pacemaker potential by displacing the kinetic parameters of IK_2 along the voltage axis. The size of the background current is unchanged. The effect appears

to be mediated by an increase in intracellular c-AMP. However, the adrenergic receptor is located on the outer membrane surface (Reuter 1974).

Digitalis

The influence of digitalis on cardiac automaticity has been reviewed in detail by West (1972). Since that review, a number of important papers have been published on the subject. While confirming that toxic concentrations of digitalis increase repetitive activity, the mechanism may be different than hitherto conceived. For $[K^+]_o$ greater than 2.7 mM, digitalis does not change (Rosen et al., 1973) or may decrease normal pacemaker slope (Ferrier et al., 1973). However, digitalis induces diastolic oscillations, designated as transient depolarization (Ferrier et al., 1973; Hashimoto and Moe 1973; Ferrier and Moe 1973) or low amplitude potentials (Rosen et al., 1973). That digitalis can induce diastole scintillations was first reported by Reiter (1961) on the basis of mechanical studies. The membrane oscillations seem to arise by an afterpotential mechanism and are calcium dependent (Ferrier and Moe 1973; Rosen et al., 1974). Ventricular myocardial fibres also show these oscillations on exposure to toxic concentrations of digitalis (Katzung, unpublished). Digitalis induced arrhythmias may in part arise from these oscillations.

The effects of ouabain on the outward potassium currents have been examined by the voltage clamp technique (Aronson et al., 1973, Isenberg and Trautwein 1974). Unfortunately, both studies were carried out with $[K^+]_o$ 2.7 mM. Both studies indicate that ouabain decreases the time dependent potassium currents. Such a decrease would enhance automaticity.

Antiarrhythmic Drugs

I have summarized the effects of representative antiarrhythmic drugs in Table II. While their effects on action potential characteristics may differ, most antiarrhythmic drugs suppress spontaneous automaticity or that induced uner a variety of conditions. In general the similarity of effects on Purkinje and sinoatrial node fibres is striking, even though the mechanisms underlying automaticity in the two tissues are probably different (Wit et al., 1974). The effects of lidocaine appear to be an exception. Sinus node automaticity is only decreased by toxic concentrations of lidocaine in vitro (Mandel and Bigger 1971; Parameswaran and Goldberg 1973).

There is little information as to the effects of these drugs on the ionic mechanism(s) underlying automaticity. Arnsdorf and Bigger (1972), examined the effect of lidocaine on the current-voltage relationship of Purkinje fibre in Na free solution. Their results indicated that lidocaine increases potassium permeability. However, it is difficult to extrapolate from such measurements of total membrane characteristics to the specific current(s) underlying automaticity. A recent abstract, (Weld and Bigger 1974) reported the effects of lidocaine on IK_2 under voltage clamp conditions. Lidocaine decreased the magnitude and slowed the kinetics of IK_2. The slowing of the kinetics of IK_2 would be consistent with the observed decrease in automaticity. On the other hand, procainamide does not alter IK_2, but may decrease the background inward current (Weld and Bigger 1972).

There is a dearth of information on drug effects on auto-

TABLE II

Drug	Sinus Node	Purkinje Fibre	
		Effect	Initiating factor
Lidocaine	0: Mandel and Bigger 1971 Paramiswaran et al. 1973	+	(a) Spontaneous: Bigger and Mandel 1970 (b) Stretch: Davis and Temte 1969 (c) Epinephrine: Davis and Temte 1969 Bigger and Mandel 1970
Quinidine	+: West and Amory 1960	+	(a) Spontaneous: Hoffman 1958; Weidmann 1955b (b) Epinephrine: Hoffman 1958 (c) Low $[Ca^{2+}]_o$: Hoffman 1958
Diphenylhydontoin	+: Strauss et al. 1968	+	(a) Spontaneous: Bigger et al. 1968 (b) Isoproterenol: Bigger et al. 1968 (c) Ouabain: Bigger et al. 1968
Propranolol		+	(a) Epinephrine: Davis and Temte 1968
Procainamide		+	(a) Spontaneous: Hoffman 1958; Weidmann 1955b (b) Epinephrine: Hoffman 1958 (c) Low $[Ca^{2+}]_o$: Hoffman 1958
Verapamil	+: Tritthart 1971	+	(a) Spontaneous, $[K^+]$ 2 mM: Rosen et al.1974 (b) Na-free Ca-rich solution: Cranefield et al. 1974 (c) Electrical: Cranefield et al. 1974.

+ ≡ Slowing of automaticity

0 ≡ No effect

maticity in atrial and ventricular myocardial fibres. This may relate to the fact that these tissues show automaticity only under special conditions (e.g. Müller 1965; Antoni and Oberdisse 1965; Kaufmann and Theophile 1967; Antoni 1971). Many of these inducing factors share one thing in common:- they cause a decrease in resting potential. A technique of modulating membrane potential in a "normal *in vitro* ionic environment" would therefore be of some interest.

1.1d. Electrical Current as an Inducer of Repetitive Activity

One of the earliest reports of the effect of constant current stimulation on the vertebrate heart was the work of Foster and Dew-Smith (1876). Mechanical activity was recorded from the lower 2/3 of the ventricle of the frog and toad *in vitro*. This preparation contained no "ganglia" (their term for AV nodal tissue) and was not spontaneously active. With weak current pulses, their observations were: no effect at all or a beat at the making and/or termination of the pulse. With currents of greater intensity, repetitive activity "indistinguishable from normal beats" was observed. The period of the repetitive beats decreased with increasing current intensity. The effects could be observed independent of the size of the tissue, the present of atropine or "urari" (curare). However, Foster and Dew-Smith failed to elicit repetitive activity in the *in situ* heart. They concluded that "...although the lower moiety of the ventricle does not under ordinary circumstances exhibit a spontaneous pulsation, its behavior under constant current warrants the conclusion that the power of rhythmic pulsations though latent is not absent, and only needs favourable circumstances for its complete manifestion."

Further observations of the effect of constant current were made by Segers (1940). Strips of tortoise heart were mounted in a 2 compartment chamber and mechanical activity observed. The preparation was normally quiescent even with adrenaline, $BaCl_2$ or $CaCl_2$ added to one compartment. However, electrical stimulation elicited repetitive contraction. "Triggered activity" may have been responsible for the repetitive activity.

At the cellular level, Trautwein and Kassebaum (1961) examined the effect of current on automaticity in rabbit sinus node and Purkinje fibres. Automaticity was enhanced by depolarizing current and depressed by hyperpolarizing current. Similar induction or modulation of automaticity by hyperpolarizing or depolarizing current has been further described in Purkinje fibres (Imanishi 1971, Aronson and Cranefield 1974), amphibian atrial myocardium (Brown and Noble 1969); sinus node fibres (Lenfant et al., 1972) and chick heart (Sperelakis 1972). In the study of Trautwein and Kassebaum quoted earlier, they failed to induce automaticity in ventricular trabeculae of the sheep with constant current. Automaticity induced in the mammalian ventricular myocardium by hyperpolarizing current was originally demonstrated by Antoni and Tagtmeyer (1965). Recently, it was shown that automaticity could be more reproducibly and regularly induced in this tissue by depolarizing current clamps (Katzung 1974a, b; Surawicz and Imanishi 1974; Imanishi and Surawicz 1974).

By varying the current intensity, automaticity can be induced over a range of membrane potentials in the above tissue. The automatic action potentials which arise from maximum diastolic potentials close to the resting membrane potential have slow diastolic depolarization rates, but rapid phase 0 depolarization. Conversely, activity arising

from membrane potentials of -55 mV or less negative have steep phase 4 slope which pass imperceptibly into phase 0. The transition between the two types of activity is gradual. They are probably equivalent to the slow and fast responses (reviewed by Wit et al., 1974).

The ionic mechanisms underlying fast and slow response activity may differ. In Purkinje fibres, the time dependent outward current responsible for diastolic depolarization in the range of potential -90 to -70 mV is IK_2 (Noble and Tsien 1968). At membrane potentials positive to -70 mV, IK_2 is fully activated and would not show time dependent change. Hauswirth et al., (1969) have shown that another component, IX_1, is responsible for diastolic depolarization at low membrane potentials. The current(s) responsible for the genesis of the spike may also differ. At the membrane potentials at which the slow response is seen, the rapid inward sodium current is largely inactivated (Weidmann 1955a). The spike of the slow response is probably dependent on current through the "slow channel" (Cranefield et al., 1972, 1974; Trautwein 1973; Reuter, 1973). Further, subthreshold depolarization at potentials at which sodium inactivation is almost complete may further depolarize the fibre to the threshold for the slow inward current (Cranefield 1975). I shall defer any speculation as to the nature of the corresponding current(s) in ventricular myocardial fibres till the discussion.

1.2. REFRACTORINESS

1.2a. Cellular Physiology

The effective refractory period is defined as that period following a given stimulus during which a propagated response cannot be elicited by a second stimulus (Hoffman and Cranefield 1960). Following a given depolarization, the ability of excitable membranes to undergo a subsequent depolarization is dependent on at least 2 variables: (a) voltage (b) time (Hodgkin and Huxley 1952b; Hoffman et al., 1957). With regard to the first, the cell membrane must repolarize to a potential approximately 2/3 the resting value before a second "fast response" can be elicited. At such a potential, the fractional availability of the "sodium system" is restored to a level at which a sufficiently large sodium current can be activated and lead to regenerative depolarization. However, with repolarization to a given potential, time is required for the removal of inactivation such that the membrane will assume the steady state availability for that potential. The membrane must therefore repolarize to a higher potential than the steady state value before a propagated response can be elicited from the incompletely repolarized membrane.

In nerve, the removal of sodium inactivation is extremely rapid (Hodgkin and Huxley 1952b). Although the time constants are voltage dependent, their small value makes time a variable of less importance in this tissue. It had long been assumed that the system of equivalent inactivation-reactivation cycles with relatively short time constants also obtained in mammalian cardiac tissue (Weidmann 1955a; Dudel 1972). However, in the atrial trabecula of the frog,

Haas et al., (1971) demonstrated that the kinetics of the two processes are different. Removal of inactivation was a much slower process. This slow removal of inactivation probably accounts for the long ERP in frog atria which may outlast complete repolarization by several hundred msec. Strauss and Bigger (1972) have also demonstrated slow reactivation in the sinoatrial perinodal fibres of the rabbit.

Recently, Gettes and Reuter (1974) have shown that in mammalian Purkinje and ventricular myocardial fibres, the kinetics of removal of inactivation are different from those of inactivation. Reactivation time constants as long as 200 msec were obtained at low resting membrane potentials. The kinetics of reactivation at low resting potential would be a pertinent determinant of the ERP. In the work of Gettes and Reuter, membrane potential was varied by changing the extracellular potassium concentration. Unfortunately, they did not discuss the possibility that change in $[K^+]_o$ per se might alter the reactivation kinetics. In the squid giant axon, Adelman and Palti (1969) have shown that very large increases in $[K^+]_o$ slow the kinetics of sodium reactivation independent of changes membrane potential.

The recovery kinetics of the sodium carrying system is but one facet of the complex problem of refractoriness. There is also the question of the variation of threshold stimulating current during the cardiac cycle (reviewed by Hoffman and Cranefield 1960). As a bare minimum, one has to consider:

(i) The membrane potential at the time an extra stimulus is introduced.

(ii) The threshold potential - determined by the "sodium system".

(iii) The mean membrane resistance between the two potentials in (i) and (ii).

During the repolarization phase of the action potential, the membrane resistance is determined largely by the potassium permeability. In the terminal part of phase 2, and phase 3, potassium permeability increases and finally decreases to the resting value soon after complete repolarization. Variations in threshold stimulating current probably at least reflect in part these changes in potassium permeability. Recently Spear and Moore (1974) have demonstrated the dependence of the recovery of threshold on variation of $[K^+]_o$. Their study also showed that a phase of 'supernormal' excitability is observed only in certain parts of the ventricular conduction system and in 2.7 mM $[K^+]_o$.

1.2b. Pharmacology

Antiarrhythmic drugs may prolong the effective refractory period by:

(i) An increase in action potential duration.

(ii) Shift of the sodium channel activation membrane-potential relationship ("h") curve to more negative potentials.

(iii) Slowing the recovery kinetics of the sodium channel availability.

(iv) Altering the membrane resistance during phase 3 of the action potential.

The degree and probable mechanisms by which antiarrhythmic drugs, in clinically relevant concentrations, alter the ERP remain controversial. Some of the difficulties of interpretation may relate to the following:

(i) The different species of test animals used.

(ii) The concentration of ions, particularly potassium in the salt solutions used.

(iii) The concentration of the drugs used. Quinidine shall be considered in some detail.

In the light of our present knowledge no definitive statement can be made about which species are most similar to man. This must await the accumulation of a sufficiently large volume of data on the electrophysiological characteristics of human cardiac tissue in vitro and in vivo. I think that discrediting data wholly on the basis of the species in which the experiments were performed is unwarranted at this time (c.f. Bassett and Wit 1973).

It is now clear that the effects of antiarrhythmic drugs are very dependent on the concentration of extracellular electrolytes. In general, antiarrhythmic drugs are more depressant the higher the extracellular potassium concentration (Watanabe et al., 1963; Katzung and Jensen 1970; Jensen and Katzung 1970; Singh and Vaughan Williams, 1971). In some cardiac tissues, e.g. the N region of the A-V node (Watanabe and Dreifus 1967) or for some drugs, e.g. procainamide in Purkinje fibres (Rosen et al., 1973), the converse may be true. The normal range of serum potassium is 3.5 - 5 mM (Castleman and McNeely 1974). Therefore studies that have been done with 2.7 or 3 mM $[K^+]$ should be considered as special but not

dismissed. (Patients with low serum potassium do have arrhythmias!)

There are still major uncertainties as to what are the appropriate drug concentrations to use *in vitro*. The frequency quoted therapeutic concentration range of quinidine sulphate is 4 - 9 mg/L (Sokolow and Edgar 1950). Further, a recent study using a more exact assay technique indicated a range of 2.3 - 5 mg/L (Kessler *et al.*, 1974). Quinidine is 80% bound to serum albumin (Conn and Luchi 1961). It is probably only the free drug that is active. This still would not indicate what the appropriate concentrations would be in a given species *in vitro*. Hondeghem (1973) has suggested the use of "functionally corresponding dosages". In other words, one should use concentrations which have similar effects e.g. termination of an arrhythmia induced under similar *in vitro* test conditions, in the studying and comparing the effects of drugs. It is clear that drug concentrations which render otherwise "normal" tissues inexcitable, should be considered toxic. Cross perfusion experiments (Rosen *et al.*, 1972) may provide a more definitive answer in the near future. The available data clearly indicate that the upper limit of the therapeutic *in vivo* concentration range is toxic *in vitro*.

Vaughan Williams and Szekeres (1961) have shown that quinidine sulfate 2.5 - 6 mg/L ($[K_o^+]$ 5.6 mM) prolongs the ERP 10 - 60% in rabbit atria. Hoffman (1958) reported that quinidine 3 - 6 mg/L ($[K_o^+]$ 2.7 mM) prolonged the ERP "out of proportion to any increase in action potential duration" in canine Purkinje and ventricular myocardial fibres. In a review article of 1971, Bassett and Hoffman suggested that quinidine and the other "group I

antiarrhythmic drugs" prolong the ERP relative to the action potential duration by shifting the "membrane responsiveness" curve to more negative potentials (see figure 1). For a regenerative depolarization, the fractional availability of the sodium carrying system must assume some value, A. From figure 1, it is evident that in the absence of drug, this hypothetical membrane must repolarize to a potential of about -62 mV as compared to -75 mV in the presence of drug. If the drug causes little change in the rate of repolarization, the prolongation of the ERP is explained. The hypothesis is based on the data of Weidmann (1955b). He showed that in the Purkinje fibres of calf and sheep quinidine sulfate, 10 mg/L ($[K_o^+]$ 2.7 mM) shifted the membrane responsiveness curve to more negative potentials. The preparations were inexcitable in 1 - 2 hours. It must be concluded that such shifts represent toxic effects. Chen et al. (Circ Res., in press 1975) have shown that quinidine 6 - 16 mg/L ($[K^+]_o$ 5.4 mM) does not shift the normalized dV/dT membrane potential relationship curve on the voltage axis. In considering the group II antiarrhythmic drug, Bassett and Hoffman failed to discuss the shift which these drugs produced in the membrane responsiveness curve in relation to the ERP. Bigger and Mandel (1970) have shown that concentrations of lidocaine of up to 12.5 mg/L ($[K^+]_o$ 3 mM) prolong the ERP:APD ratio and do not change, or move the membrane responsiveness curve to less negative potentials. It is evident that changes in the membrane responsiveness curve would not account for the effects of lidocaine on the ERP. Further, the technique of measuring the dV/dT -membrane potential relationship by introducing extrasystoles during phase 3 of repolarization does not distinguish between voltage and

time dependent processes. The data with diphenylhydantoin is essentially similar to that for lidocaine (Bassett and Hoffman 1971).

The literature on the effects of antiarrhythmic drugs on reactivation kinetics is rather sparse. Johnson and Robertson (1958) showed that in the presence of quinidine sulfate 5 mg/L, an extrasystole introduced in the period soon after complete repolarization had a markedly decreased maximal upstroke velocity compared to the basic drive beat. In atrial muscle, this effect was reversed by acetylcholine. They suggested that quinidine caused "a dissociation between repolarization and restoration of the sodium carrying system". The tissues, atria and ventricles of the rabbit, subsequently became inexcitable. Using a similar technique of extrasystoles at variable coupling intervals, Strauss et al., (1968) could demonstrate no effect of diphenylhydantoin on reactivation kinetics in Bachmann bundle fibres. The voltage clamp technique was used by Driot and Garnier (1972) to examine the effects of quinidine sulfate .78 - 78 mg/L (10^{-6} - 10^{-4} M) on reactivation kinetics. They showed that quinidine sulfate at a concentration of 39 mg/L more than doubled the time constant of reactivation. A similar study by Kern et al., (1971) showed that the neuroleptic droperidol markedly slowed Na reactivation. Both of the above studies were carried out in frog atrial trabecula. The quantitative (and possibly qualitative) differences of reactivation kinetics in frog atrium and mammalian ventricular myocardium have already been pointed out. In the ventricular myocardium of guinea pig quinidine in concentrations of 2 - 16 mg/L does not slow the recovery kinetics of dV/dT of phase 0 (Gettes et al., 1973). On the other hand lidocaine in therapeutic concentrations slowed the kinetics

of reactivation in ventricular myocardial and Purkinje fibres (Gettes et al., 1973; Weld and Bigger 1973). It therefore appears that while most antiarrhythmic drugs prolong the ERP:APD, the underlying mechanisms may differ.

An area of cardiac electropharmacology closely related to the question of refractoriness is the frequency dependence of the effects of antiarrhythmic drugs. Johnson and McKinnon (1957) showed that whereas quinidine caused little change in dV/dT of phase zero in guinea pig ventricular myocardial fibres at low drive rates, dV/dT progressively decreased as the rate was increased. West and Amory (1960) reported similar effects in rabbit atrium. Heistracher (1971) showed that dV/dT max of the first response of previously quiescent quinidine treated fibres was similar to control. With successive beats dV/dT decreased to a new steady state. The number of beats rather than the duration of the period of stimulation determined how soon a steady state was achieved. Similar rate dependence has been reported for other drugs by Katzung and Jensen (1970), Jensen and Katzung (1970), Tritthart et al., (1971), Arita and Surawicz (1972). Voltage dependence of dV/dT max probably does not explain the decrease of dV/dT max as the rate is increased. The decreases in membrane potential observed at high rates are small and transient (Carpentier and Vassalle 1971). Time dependent recovery processes may be important in this case.

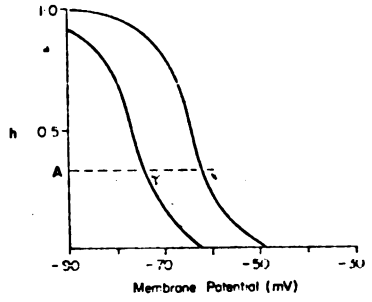


Figure 1. Hypothetical curve of the relationship between membrane potential and availability of the sodium carrying system. A measure of availability e.g. normalized dV/dT max is plotted on the ordinate; the membrane potential on the abcissa. The broken line A designates the level to which the membrane must repolarize before a second action potential can be elicited. Line X is the control; line Y obtained after a drug which displaces the curve to more negative potentials.

2. THE RESEARCH PROPOSAL

2.1. Statement of the Problems

2.1a. Ventricular Automaticity

As I have indicated in the introduction, there is relatively little information of the effects of antiarrhythmic drugs on automaticity in ventricular myocardial fibres. Although impulse initiation does not normally occur in these fibres, there is no certainty that impulse initiation does not occur in them in the disease states in which arrhythmias arise. One cannot make unqualified extrapolations from data obtained in one fibre type to another. The response of various fibre types to noxious stimuli such as ischemia are different (Wit and Friedmann 1975), so may the effects of antiarrhythmic drugs. An analysis of the effects of antiarrhythmic drugs on ventricular automaticity may help to give a more complete understanding of the mode of action of these drugs. It might also help to explain why certain drugs are more effective in a given arrhythmia than others. Electrically induced automaticity provides a model for studying automaticity in normally quiescent ventricular myocardial fibres.

The slow response which is observed at low maximum diastolic potentials is also of interest in another regard. The action potentials have reduced amplitude, low upstroke velocity and would be conducted slowly. Their very slow conduction implies that the path length for re-entry of such responses is realistic in terms of area of disease and overall geometry of the cardiac ventricle (Wit *et al.*, 1972a,b). In most studies to date, the slow response has been

elicited in an altered ionic environment, e.g. high potassium and sympathomimetic amines (Pappano 1970, Wit et al., 1972a,b); or sodium free, calcium rich solutions (Aronson and Cranefield 1973). The effects of drugs may be different in these altered milieu. The use of current to modulate membrane potential permits the study of the slow response during automaticity occurring in normal Tyrode solution.

2.1b. Refractoriness

The shortcomings of considering only the voltage dependence of the sodium carrying system as it affects refractoriness have been outlined. A slowing of the recovery kinetics of the sodium carrying system could account for some of the contradictory findings in the literature. A slower recovery process would mean that more time has to elapse during the repolarization phase before reactivation, adequate for a second response, has occurred. Relatively small changes would be important since recovery time constants are large at the relevant membrane potentials.

Pertaining to the frequency dependence of dV/dT of phase 0, a drug may prolong the time for reactivation far beyond complete repolarization. Increases in rate might eventually cause excitation to occur during the period of partial recovery. This would lead to a decrease of dV/dT max until a new steady state value of decreased magnitude was achieved. Indeed such a change in dV/dT max is not unlike that observed when previously quiescent quinidine treated fibres are driven.

A demonstration of slowing of the recovery kinetics of dV/dT max by antiarrhythmic drugs would therefore provide an additional or alternative explanation for some of the effects.

2.2. The Specific Questions

2.2a. Ventricular Automaticity

(i) "What are the effects of quinidine, droperidol and verapamil on automaticity in ventricular myocardial fibres?"

I elected to study quinidine as the prototype of antiarrhythmic drugs. Its clinical efficacy is well established. There is a body of data on its electrophysiological effects *in vitro*, including its effects on automaticity in other cardiac fibres.

Droperidol is a neuroleptanalgetic that has been shown to have antiarrhythmic activity in laboratory and human experiment arrhythmias (Yelnosky *et al.*, 1964; Long *et al.*, 1967). Long (1967) had suggested that droperidol was antiarrhythmic because of its hypotensive action. However, it has been shown to be efficacious in arrhythmias in which changes in blood pressure were unimportant (Betolo *et al.*, 1972). Although some of its electrophysiological effects *in vitro* have been described, its effects on cardiac automaticity have not been examined.

Verapamil was originally introduced as an anti-anginal agent. It has recently been shown to be an effective antiarrhythmic agent (reviewed by Krikler, 1974). Verapamil suppresses automaticity in Purkinje fibres (Rosen *et al.*, 1974), sinus and antrioventricular nodal fibres (Wit and Cranefield 1974; Tritthart *et al.*, 1971). Verapamil and its methoxy derivative D-600 block the slow inward current in feline ventricular muscle (Kohlhardt *et al.*, 1972). Singh and Vaughan Williams (1972) have postulated that the anti-

arrhythmic effects of verapamil are dependent on blockade of the slow inward current. Therefore, should any effects on ventricular automaticity be observed, a corollary would be:

"Do interventions which increase the slow inward current reverse the effects of verapamil?"

2.2b. Refractoriness

(i) "Can slowing of the recovery kinetics of dV/dT max of phase zero of the action potential be excluded as a possible mechanism of prolongation of the ERP by droperidol and quinidine?"

The case for studying these two drugs is in part similar to that stated above. The available literature is based largely on work done in frog atria. There are no data on the kinetic effects of droperidol in mammalian ventricular myocardial fibres.

(ii) "Are the effects of droperidol on dV/dT max of phase zero of the action potential rate dependent?"

There is no literature available on this question. The final question is a corollary to the second.

(iii) "If the effects of droperidol on dV/dT max of phase zero of the action potential is rate dependent, can changes of the recovery kinetics of dV/dT max be excluded as a basis for such an effects?"

3. METHODS

3.1. Ventricular Automaticity

Guinea pigs, 250 - 350 G, were stunned by a blow to the head and exsanguinated. Papillary muscles approximately 0.7 - 1.0 mm in diameter were excised from the right ventricle and mounted in a three compartment chamber (Reuter and Scholz, 1968), Figure 2. The anterior and posterior compartments were perfused with a modified oxygenated Tyrode solution (for composition of solutions, see below). The middle compartment was perfused with deionized sucrose solution. Tyrode solution was saturated with 95% oxygen, 5% CO_2; sucrose solution with 100% oxygen. The temperature was monitored with a telethermometer (Yellow Springs Inst. Co.) and maintained at 36°C.

Transmembrane potential was measured differentially between two conventional glass micro-electrodes (10 - 35 M ohms resistances), one electrode impaling a fibre, the other on the surface of the bundle. The microelectrodes were coupled via Ag/AgCl wires to matched voltage followers (VF in figure 2), having high input impedence (10^{13} ohms) and input capacity neutralization (Winston Electronics Co.). The potential difference was amplified by the vertical amplifier (3A3, differential input) of an oscilloscope (Tektronix 565). The transmembrane potential was differentiated electronically, and the filtered time derivative displayed on a separate beam of the oscilloscope. Schematics of the circuits of the differentiator and active filter appear in the appendix. The differentiator was linear in the range 1 - 1100 V/sec.

An operational amplifier (Philbrick ML 100, C in figure 2)

provided constant current pulses between large Ag/AgCl electrodes located in the anterior and posterior chambers. The electrode in the anterior chamber was connected to a current-to-voltage transducer (T in figure 2). The I-V transducer gave a voltage output proportional to the total current across the sucrose gap. This voltage was inverted, (inverter I in figure 2) and compared with command pulses from a Grass stimulator (S 44) through summing resistors at the inverting input of the clamp amplifier (C in figure 2). The output of the clamp amplifier was led to the distal chamber of the bath via an Ag/AgCl wire. The total current was also displayed on the oscilloscope. The oscilloscope traces were photographed with a kymographic camera (Grass Instruments Co., C4).

The preparation was initially driven at a rate of 1 Hz with a stimulus 1 1/2 times threshold and duration 2.5 msec. A period of 1 hour was allowed for equilibration while a stable impalement was established. An impalement was considered stable if 10 minutes after the initial penetration there was no further change in membrane potential and the repolarizing and resting phases of the membrane potential were smooth. The drive rate was reduced to 0.05 - 0.75 Hz. After a minimum period of 4 minutes, control action potentials were recorded. Constant current pulses of long duration (1.2 to 1.5 sec) and of variable strength were then applied to induce automaticity. Increments in current were chosen to achieve 5 - 10 mV changes in MDP. The survey was terminated when the responses at low MDPs were heavily damped. This survey was repeated once. The basis drive rate of 1 Hz (2.5 msec pulses) was then resumed. The preparation was then exposed to the drugs: Verapamil 1 - 5 mg/L or droperidol 1.9 - 3.8 mg/L

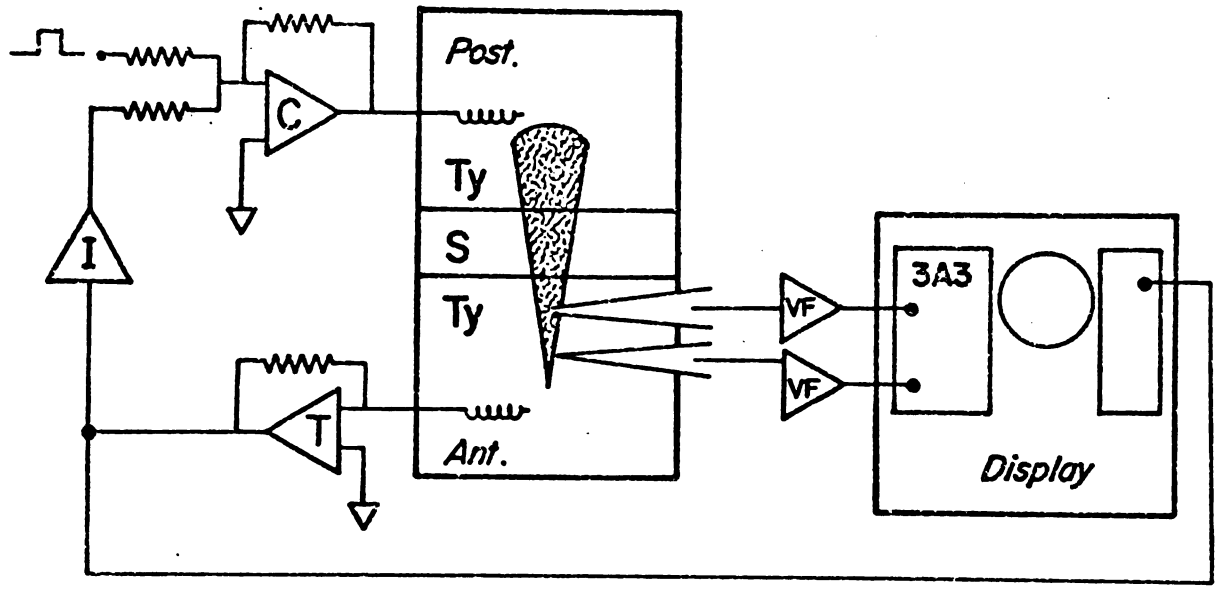


Figure 2. The experimental setup. VF = matched voltage followers; display, Tektronix 565 oscilloscope with 3A3 differential amplifier; T = current-to-voltage transducer; I = inverter; C = clamp amplifier. The posterior (post) and anterior (ant.) compartments of the chamber were perfused with Tyrode solution (Ty), the middle compartment with isotonic sucrose solution (S).

for 1/2 hour or quinidine 2 - 4 mg/L for 1 hour. Automaticity was surveyed as described above every 15 minutes during exposure to verapamil and droperidol, and every 30 minutes during quinidine. A washout of 1 - 2 hours, increase in $[Ca^{2+}]_o$ to 7.2 mM, or addition of epinephrine 10^{-5} M was then performed. Only experiments in which the impalement was maintained in a single fibre are reported.

The measurements of phase 4 slope and overshoot are described in the initial section of the results. Mean phase 4 slopes and overshoot before and during exposure to drug were compared by an unpaired t test (2 sided).

3.2. Refractoriness

Two different techniques were employed in this section. Since the difference between them related to the technique of stimulation, I shall describe them under the terms "Extracellular and Intracellular Stimulation".

3.2a. Extracellular Stimulation

Right ventricular papillary muscles from guinea pig hearts were fixed to the waxed base of a 10 ml perfusion chamber with stainless steel pins. The chamber was perfused with oxygenated Tyrode solution at a rate of 10 ml/min. A dual inlet allowed rapid changes of solution. An insulating water jacket maintained the temperature at 36°C.

Transmembrane potential was measured with a glass micro-electrode (10 - 35 M ohms resistance). The micro-electrode was coupled via an Ag/AgCl wire to a voltage follower having high input impedence and input capacity neutralization. The perfusion chamber

was connected to ground via a Tyrode-agar bridge. The output of the voltage follower was amplified by the vertical amplifier (3A3, used in the single ended configuration) of a Tektronix oscilloscope (565). The action potential was differentiated electronically, and the filtered time derivative displayed on a separate beam of the oscilloscope. The oscilloscope traces were photographed with a kymographic camera.

Driving (S1) and test pulses (S2), 2.5 msec duration and 1 1/2 times threshold were provided by a digital stimulator with a programmable delay. The stimuli were isolated from ground by a stimulus isolation unit (Grass Instruments Co., SIU5), and delivered to the preparation by a Teflon coated bipolar silver electrode. In the initial experiments, amplitude and duration of the drive and test stimuli were not independently variable. In later experiments, the output of two stimulators were summed by two stimulus isolation units, and S1 and S2 were then independently variable. The stimulating and recording electrodes were at least 2 mm apart.

3.2b. "Intracellular" Stimulation

This method utilized the sucrose gap chamber as described for the study of ventricular automaticity. It differed only in the source of stimulating current. The constant voltage output of the stimulator was modified to a constant current source by addition of a 1 Mohm series resistor. This system replaced the clamp amplifier as the current source between the large electrodes located in the anterior and posterior chambers.

3.2c. Experimental Protocol

The preparation was initially driven at 1 Hz during the

equilibration period of 1 hour. A stable impalement was established in the interim. The drive rate was then reduced to 0.2 Hz. Control action potentials were recorded a minimum of 4 minutes after reduction of stimulation rate. Recovery kinetics of dV/dT max of phase 0 were then determined using the technique of repeated extrasystoles (Gettes and Reuter 1974). Test stimuli (S2), were introduced at decreasing diastolic intervals following complete repolarization of every 2nd - 3rd basic beat. This was conveniently done by the programmable delay of the stimulator which could provide formats with decrements of the S1 - S2 interval of 100, 10 and 1 msec. The largest decrement was used for long S1 - S2 intervals, the shorter decrements for short S1 - S2 intervals. The stimulus strength was varied to keep the latency approximately constant. Following the survey of kinetics, action potentials were obtained at 1, 1.3, 2 and 4 Hz in some experiments.

The effective refractory period was determined in some of the experiments done with the sucrose gap technique only. With the drive rate of 1 Hz, a test stimulus (1 1/2 times threshold and 2.5 msec in duration), was introduced every 5th basic beat during the absolute refractory period. The S1 - S2 interval was then increased in 1 msec steps until an extrasystole was elicited. The ERP was taken as the shortest S1 - S2 interval for which 3 successive responses were observed to the test pulses of that coupling interval.

The preparation was then exposed to drug containing Tyrode solution, droperidol for 30 - 45 minutes, quinidine for 1 hour, and the protocol repeated.

In four droperidol and two quinidine experiments, the extracellular potassium concentration was increased to 10 mM after the initial

control observations were obtained. When the resting membrane potential had decreased to a stable level, the protocol was repeated. The perfusate was again changed to 5 mM K Tyrode solution. When the resting membrane potential had returned to control levels, the preparation was exposed to drug containing Tyrode solution. The entire protocol was repeated in 30 - 60 minutes.

In 3 droperidol experiments, the effects of increased calcium concentration (8 mM) on kinetics were also investigated.

The relationship of maximum upstroke velocity (dV/dT max) to resting membrane potential - the "h curve" in Hodgkin-Huxley terms - was studied by depolarization in 20 mM K Tyrode solution. Records were obtained from a fibre stimulated at 0.2 Hz as it gradually depolarized in the high K solution. The preparation was then washed with 5 mM K Tyrode solution. When the control resting potential was regained, the preparation was exposed to droperidol containing solution for 30 - 45 minutes. Potassium depolarization was then repeated with 20 mM K Tyrode solution containing droperidol.

APD, ERP and dV/dT max at various steady drive rates during control drug exposure were compared by a paired t Test (2 sided).

3.3. Solutions and Drugs

Initially, a modified Tyrode solution of the following composition (mM) was used: NaCl 137, $NaHCO_3$ 11.9, NaH_2PO_4.4, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1.1 and glucose 5.5. When saturated with carbogen (95% O_2, 5% CO_2), the pH of this solution was 7.1 - 7.2. In the latter part of the study, I therefore changed the composition of the solution to the following (mM): NaCl 128, $NaHCO_3$ 22, NaH_2PO_4 .45, KCl 5,

$CaCl_2$ 1.8, $MgCl_2$ 1.1 and glucose 5.5. When gassed this solution had a pH of 7.40 - 7.45. Similar results were obtained with either solution. The deionized sucrose solution had the following composition (mM): sucrose 300, and glucose 5.5. $CaCl_2$ 10^{-4} M (New and Trautwein 1972; Kleber 1973) was added to the deionized sucrose solution. The sucrose solution was gassed with 100% O_2. When the calcium or potassium concentration of the Tyrode solution was increased, I made no corrections for the changes in osmolality since the maximum change was 9%. All reagents used were of analytical grade.

The following drugs were employed: verapamil hydrochloride (generously provided by Knoll Pharmaceutical Co., Orange, NJ); quinidine gluconate injection (Eli Lilly and Co., Indianapolis, Ind.); droperidol injection and powder form (generously provided by McNeil Laboratories Inc., Fort Washington, Pa); epinephrine hydrochloride with sodium sulfite as preservative (Vitamix Pharmaceutical Inc., Philadelphia, Pa). Verapamil was made up in a stock solution of 5 mg/ml and aliquots added to the Tyrode solution. Aliquots of quinidine were obtained from a 1:10 dilution of the form supplied. Droperidol injection was added directly to the Tyrode solution in the form supplied. Alternatively, droperidol powder was dissolved in 0.01 M citric acid to give a stock solution of 3 mg/ml and aliquots added to the Tyrode solution. The drug levels quoted refer to the salt. For convenient reference, a table of concentrations of the drugs used and their equivalent molar concentrations is provided below.

Drug	Concentration, mg/L	Concentration M
Verapamil HCl	1 - 5	2×10^{-6} - 10^{-5}
Quinidine gluconate	1 - 4	1.9×10^{-6} - 7.6×10^{-6}
Droperidol	0.38 - 15.2	10^{-6} - 4.10^{-5}
Epinephrine HCl	2.2	10^{-5}

3.4. Limitations of the Techniques

3.4a. Extracellular Stimulation

Very large stimulating currents were sometimes required to maintain the latency constant. As the source of the current was localized, this could lead to damage to the fibres below the stimulating electrode. Further, in attempting to study recovery kinetics with this technique in 10 mM K Tyrode solution, time constants much longer than those previously reported were obtained. I therefore abandoned this technique in favour of the sucrose gap method.

3.4b. Intracellular Stimulation

(i) Ventricular Automaticity

The sucrose gap presents certain advantages over an intracellular microelectrode as a current passing technique (Johnson and Lieberman 1971; Trautwein 1973). Current is injected across a disk equivalent to the cross-sectional area of the preparation. This assures a fairly uniform current density in the anterior (test) compartment (Morad and Goldman 1973; Reuter 1973). Further, the problem of loss of impalement of the current passing electrode is

eliminated. However, the technique also has disadvantages. A fraction of the total current across the sucrose gap is through an extracellular shunt. With time, the longitudinal intracellular impedence increases (New and Trautwein 1972; Kleber 1973). Therefore a smaller fraction of the total current is transmembrane current. The addition of calcium to the sucrose solution retards the increase in longitudinal impedance. Because of this problem, I have compared overshoots and diastolic depolarization rates at equivalent diastolic membrane potentials rather than that elicited by equal currents.

(ii) Refractoriness

The accepted definition of the ERP in cardiac tissues refers to propagated responses (Hoffman and Cranefield 1960). With a short muscle segment in the anterior compartment, no propagation should occur and a "membrane action potential" is measured. The accepted definition of the ERP is therefore not applicable. The measurement that was described in the experimental protocol was therefore a "minimum inter-response time". My impression is that in reality, propagation occurred in at least some of the preparations used. I shall give evidence for propagation later.

There were two problems (sometimes interrelated) associated with the choise of stimulus strength.

(i) When the preparation was driven at a steady rate, dV/dT max varied with the stimulus strength. In some preparations, dV/dT max decreased as stimulus strength increased; in other preparations, the converse was observed. Changes of as much as 40% were observed. The changes were sometimes quite abrupt, and were often accompanied by changes in latency. The cause of this

variation in dV/dT max with stimulus strength is not clear. One possibility is that it may be partly the result of sodium inactivation occurring during the stimulus. An increase in stimulus strength would increase the degree of depolarization occurring during the stimulus. However, this possibility of stimulus induced sodium inactivation is rather unlikely. The time constant of sodium inactivation increases with decreasing membrane potential (Weidmann, 1955a). The degree of inactivation during a brief 2.5 msec pulse should be small. Excitation along geometrically different pathways may be important. In all experiments, a range of stimulus strength could be found over which there was no variation of dV/dT max. A stimulus within this range was used during the survey of kinetics.

(ii) Frequently, as extrasystoles were introduced progressively earlier after repolarization of the basic drive beat, changes in latency were observed. As such changes in latency may by themselves lead to changes in upstroke velocity, this would have a confounding effect on this technique of studying recovery kinetics. My own and the observation of others (e.g. Gettes and Reuter 1974) are that if the latency is maintained approximately constant, dV/dT max of the test extrasystole is a continuous function of the diastolic interval. Abrupt changes in dV/dT max of the extrasystole occurring with changes in latency would lead to discontinuities in such a plot. Therefore, stimulus strength was changed as required in order to maintain a fairly constant latency.

4. RESULTS

4.1. Automaticity

4.1a. Characteristics of Electrically Induced Automaticity in Ventricular Myocardial Fibres

A typical survey of automaticity in drug free solution is shown in figure 3. Panel A shows the action potential elicited by a brief (2.5 msec) stimulus. The maximum upstroke velocity of phase 0 was 290 V/sec. No phase 1 is evident, and the plateau is in the positive range of membrane potential. During diastole, the resting membrane potential remained stable. Such features are characteristic of the action potential of a ventricular myocardial fibre (Hoffman and Cranefield 1960). In panel B, a prolonged current pulse of 15 μA elicited a single action potential, followed by a relatively flat phase 4 period. With an increase in current strength to 16 μA, panel C, the first (elicited) action potential repolarized to -83 mV. However, this was followed by diastolic depolarization terminating in another regenerative action potential. The potential at which the trace abruptly disappeared was taken as the apparent threshold potential (Weidmann 1955b). Division of the difference between the maximum diastolic and apparent threshold potentials by the interval between them gave a mean diastolic depolarization rate. This procedure is summarized in figure 4. At less negative potentials, e.g. F in figure 3, an apparent threshold was not evident and the mean slope of depolarization between the maximum diastolic potential (MDP) and a point 10 mV positive to this was calculated. The amplitude of the automatic action potential (2nd AP in panels C-F of figure 3), was measured from the maximum diastolic potential to the

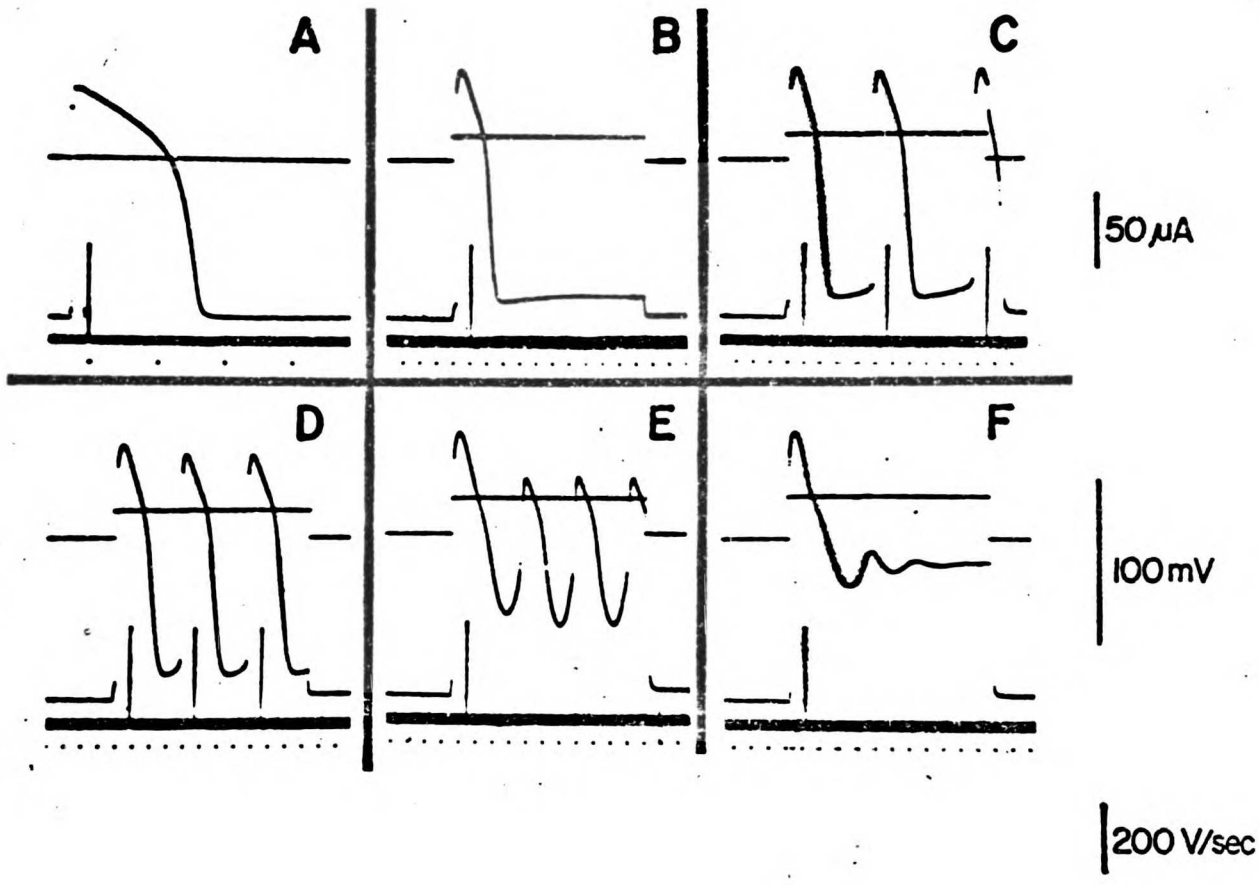


Figure 3. A survey of automaticity under control conditions. In each panel, the beginning and end of the upper trace is the zero reference for membrane potential while the trace itself displays the total current. The second trace is the transmembrane potential. The third trace is the first time derivative of the transmembrane potential. On the lowest trace are time markers at 100 msec. intervals. Panel A is the action potential elicited by a brief (2.5 msec) stimulus, panels B-F, activity during prolonged (1.2 sec) constant current pulses.

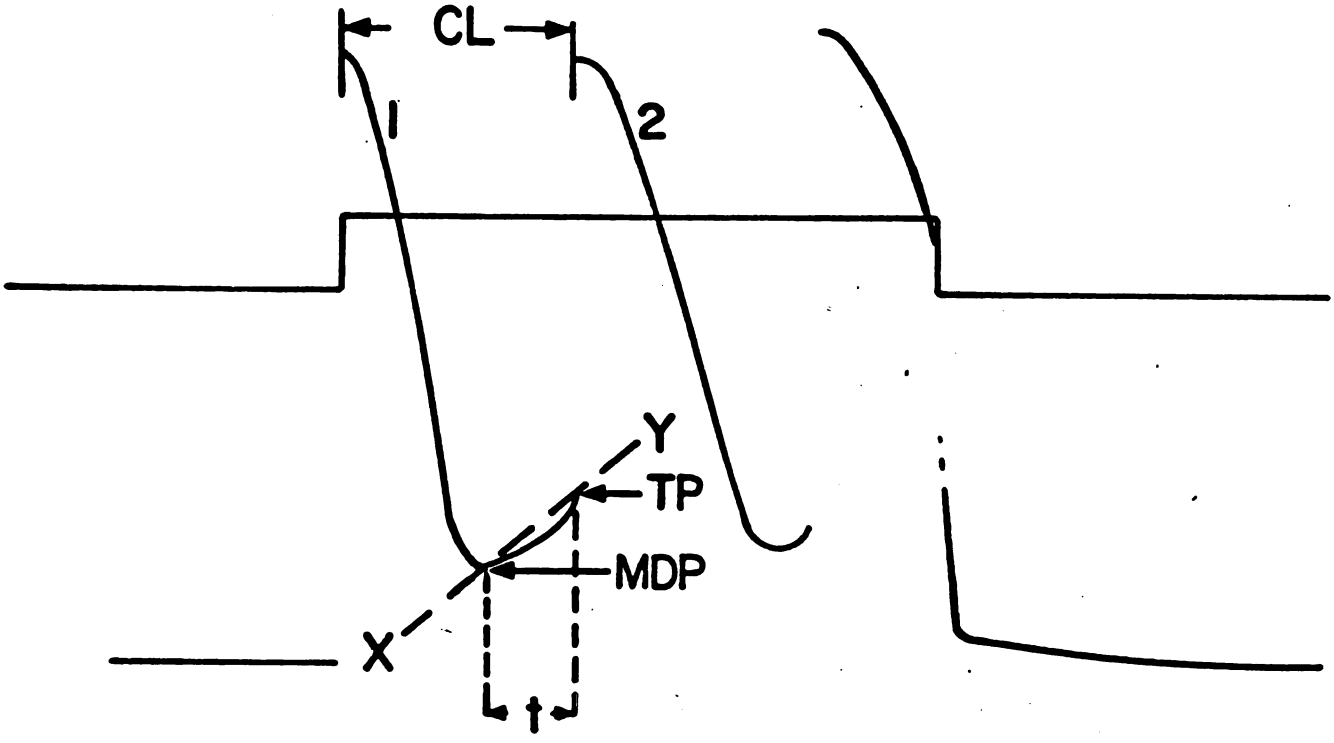


Figure 4. Facsimile of activity during prolonged pulses to show measurement procedure. The time intervals between the peaks of action potentials 1 and 2 is the measured cycle length, CL. MDP indicates the maximum diastolic potential; TP, the threshold potential. The slope of the line XY was obtained by division of (MDP - TP) by t. This gave a mean diastolic depolarization rate.

peak of the action potential; the overshoot from the zero level of membrane potential to the peak of the action potential. In general, phase 4 slopes were greatest for maximum diastolic potentials in the range -55 to 45 mV; the cycle intervals were shortest in this potential range. Panels C and D show automatic activity arising from high MDPs, with APs having fast upstroke velocities. On the other hand, panel E shows activity arising from a low MDP with APs having upstroke velocities of less than 20 V/sec. These are analogous to the fast and slow response respectively. At the extreme of the automaticity range (-28 mV in panel F), positively damped oscillations were observed.

4.1b. Variability of Electrically Induced Automaticity

Automaticity was observed in approximately 95% of preparations examined. The mean of the most negative MDP at which repetitive activity was observed was -73.2 ± 1.3 mV (mean ± S.E., N = 38), with a range of -89 to -57 mV. The upper limit of the most positive MDP for automaticity was -24.7 ± 1.5 mV, with a range of -43 to -8 mV. The latter values are somewhat arbitrary as the survey was usually terminated at the potential at which the repetitive activity was heavily damped.

Some of the variability between preparations is highlighted in figure 5. The normal action potentials from the 3 muscles depicted (panels A, D, and G) have similar characteristics. Panel B shows the activity in muscle 1 at the MDP at which repetitive activity was first observed. There was a difference of 28 mV between the resting and maximum diastolic potentials. The diastolic depolarization following

the elicited beat was linear, with terminal acceleration to threshold. There was a smooth transition between phase 4 and phase 0 of the automatic beat. This suggests that the impaled cell might be the pacemaker or that the preparation was responding in a homogeneous manner. Similar transition between diastolic depolarization and phase 0 of the automatic beat was observed at the less negative diastolic potentials e.g. panel C. In muscle 2, there was a mere 6 mV between the resting and maximum diastolic potentials at which repetitive activity was first observed (panel E). The flat diastolic potential following repolarization of the first (elicited) beat was abruptly interrupted by a second action potential. The simplest interpretation of this record is that the impaled cell was being activated by a propagation impulse. This implies spatial inhomogeneity in the preparation. At less negative MDPs, e.g. panel F, diastolic depolarization was evident. There was a difference of 20 mV between the resting potential and the MDP at which repetitive activity was first observed in muscle 3 (panel H). Again, the diastolic potential was initially stable and activity was probably propagated. The upstroke velocity of the automatic beat was appreciably greater than that of the elicited beat, although the former was arising from a much lower membrane potential. This was not an infrequent observation. On some occasions when the above was evident, I obtained a series of action potentials with 2.5 msec pulses of varying strength. In the majority of cases, action potentials elicited with a threshold stimulus had a high upstroke velocities similar to that seen in the automatic beat. When the stimulus strength was further increased, a decrease in maximum upstroke velocity was observed. In muscle 3, when the amplitude of

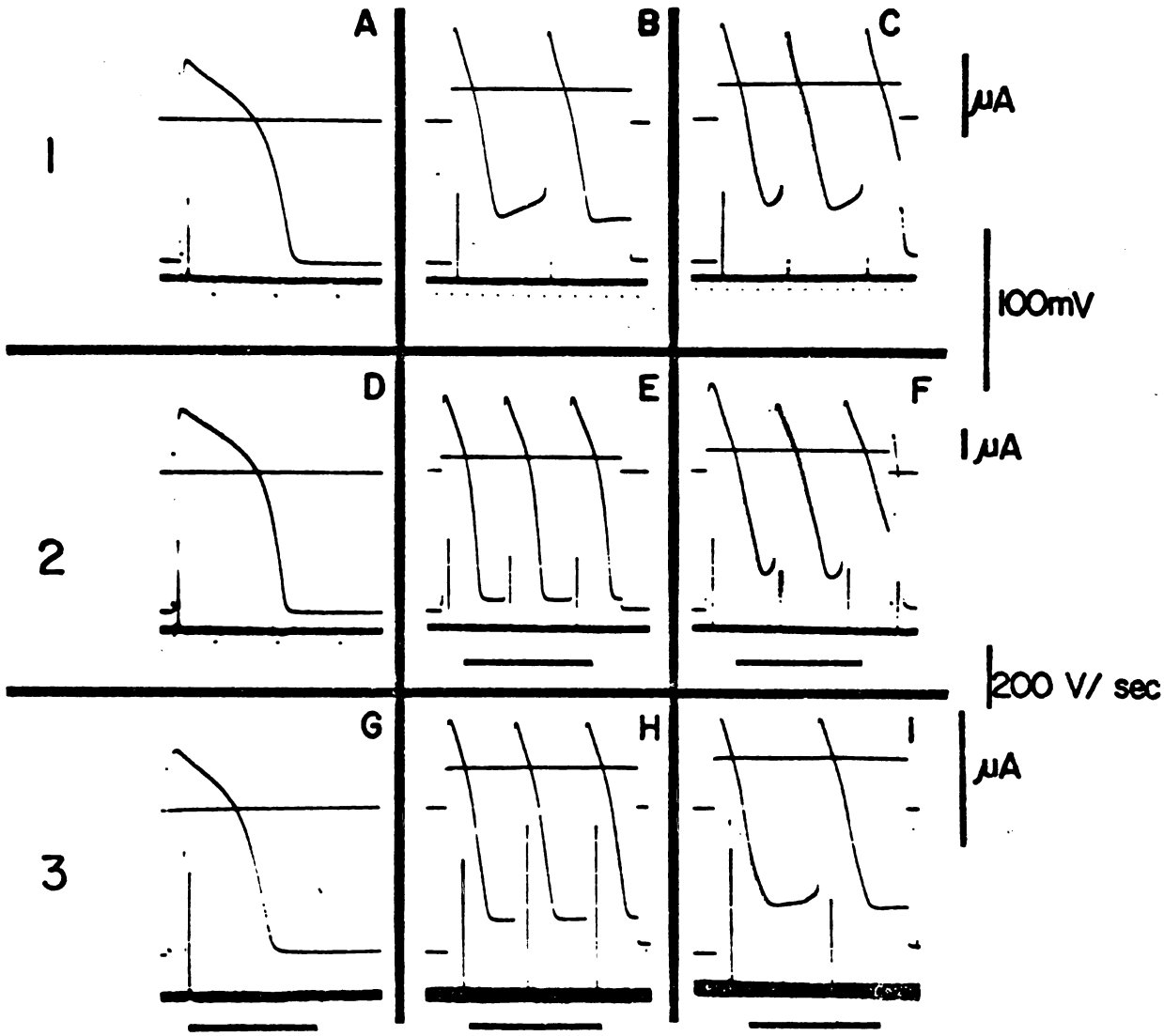


Figure 5. Variability of electrically induced automaticity between preparations. The traces are as in figure 3. Rows 1, 2 and 3 show activity during brief and long current pulses in 3 papillary muscles under control conditions. Panels A, D, and G are the normal action potentials. Panels B, C, E, F, H and I, activity during prolonged current pulses. The current calibration for each row is 50 μA. The time calibration below panels E, F, H and I is 800 msec, that below panel G 200 msec.

the current pulse was increased, diastolic depolarization was evident at lower MDPs (panel I).

In 3 control experiments, automaticity was surveyed after 1/2 to 1 hour during superfusion with drug free Tyrode solution. Only small changes in phase 4 slope were observed (see figure 12).

4.1c. Further Evidence of Lack of Spatial Homogeneity

Panels A, B and C of figure 6 are also from muscle 3 of the previous section. Diastolic depolarization was terminally interrupted by a convex electrotonic potential (see panel 6C). The impaled cell although showing diastolic depolarization was clearly triggered by another cell. The former was therefore a latent pacemaker.

Evidence of interaction between cells was not limited to the interval of diastolic depolarization. The activity depicted in panels D-F of figure 6 is from another muscle. The repolarization phase of the automatic action potential in panel E was interrupted by an "extrasystole". Although an initial electrotonic interaction is not evident (panel F), I believe this also indicates propagated activity.

The question of lack of spatial uniformity is best assessed by multiple simultaneous impalements in every preparation used (New and Trautwein 1972; Tarr and Trank 1974). This was not attempted in the present study.

4.1d. The Effects of Verapamil

The effects of verapamil 1 - 5 mg/L were examined in 12 experiments. Figure 7 shows the result of a typical experiment with

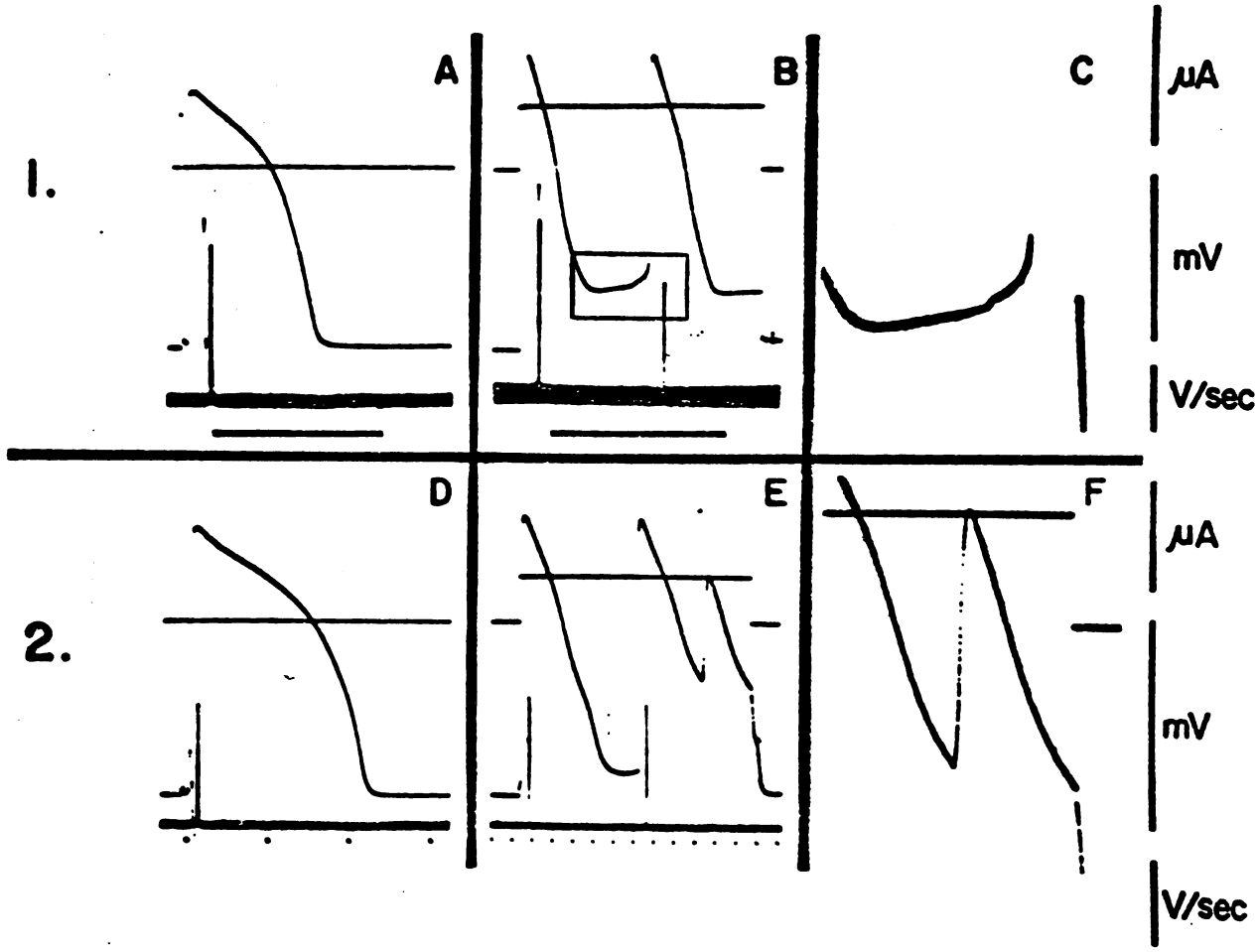


Figure 6. Further evidence of inhomogeneity during prolonged current pulses. The traces are as in figure 3. Rows 1 and 2 show activity during brief and prolonged current pulses from 2 papillary muscles. Panels A and D are the normal action potentials; panels B and E activity during prolonged current pulses. An enlargement of the insets in panel B and E are depicted in panels C and F respectively. In panel C, the arrow indicates the potential at which electrotonic interaction with a neighbouring cell is evident. The current calibrations are 50 μA for rows 1 and 2; the voltage calibration 75 mV for row 1 and 100 mV for row 2; dV/dT calibration is 100 V/sec for row 1 and 200 V/sec for row 2. The time calibration is 200 msec for panel A; 800 msec for panel B.

verapamil 2.5 mg/L. Except for a slight prolongation of duration, there was little change in the normal action potential (panel A) after 30 minutes of exposure to verapamil (panel D). In the case of the regenerative responses arising from high (more negative) MDPs, panels B and E, verapamil reduced phase 4 slope and decreased the action potential amplitude. The reduction of upstroke velocity on exposure to verapamil (compare dV/dT max of the 2nd beat in panels B and E), is at least in part accounted for by the lower threshold potential during exposure to the drug. In the case of repetitive activity arising from low (less negative) MDPs, panels C and F, there was also a reduction of phase 4 slope. However, the reduction of overshoot was more pronounced compared to that for activity arising from more negative MDPs. There was a small decrease in threshold potential. However, this variable could only be measured consistently for the action potential with fast upstroke velocities i.e., those arising from high MDPs. An increase in the action potential duration at high and low MDPs contributed to the increased cycle length evident in panels E and F. Mean effects observed in a group of preparations are shown in table III.

The effects of verapamil were of relatively rapid onset, being clearly detectable in 15 minutes. From observations made at 45 minutes and 1 hour in 2 preparations, it was evident that a steady state was not reached at the end of the usual 30 minute period.

At a drug concentration of 1 mg/L, simple reduction of phase 4 slope and overshoot at low MDPs was observed. At the higher drug levels of 2.5 and 5 mg/L, automatic beats were abolished in some preparations (e.g. see figure 10). Complete elimination of the drug

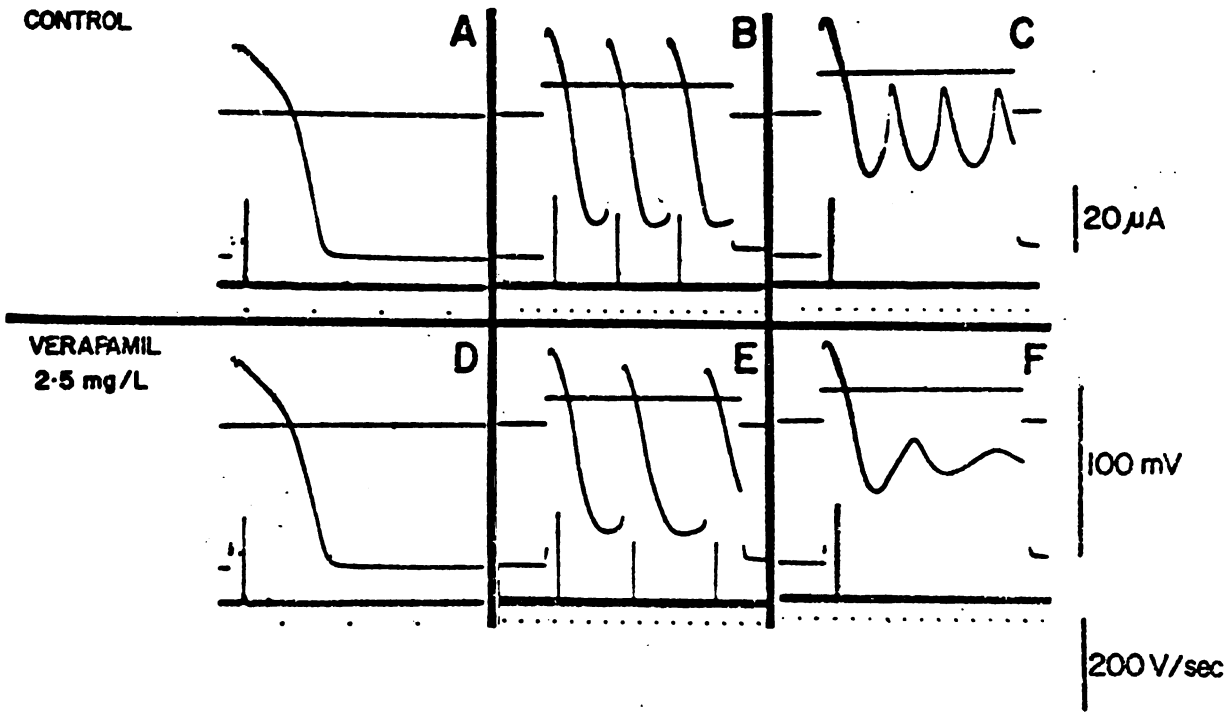


Figure 7. The effects of verapamil 2.5 mg/L on ventricular automaticity. The traces are as in Figure 3. Panels A and D are the action potentials for short (2.5 msec) pulses, panels B, C, E, and F show automaticity during long (1.2 msec) constant current pulses. Panels A-C are control; panels D-F 30 minutes after exposure to verapamil. In this and subsequent figures of oscilloscope traces, panels during the prolonged pulse have been matched for equivalent MDPs (e.g. MDP in panel B $\triangleq$ MDP in panel E).

TABLE III

Effects of verapamil 2.5 mg/L on overshoot and phase 4 slope during electrically induced automaticity. The data is from all 7 muscles examined at this drug concentration. However, data from a given muscle was included in a decade of MDP only if automaticity persisted in that decade during drug exposure. Consequently, some muscles which showed the greatest response to the drug, namely, loss of activity, have not been included. This accounts for the small "n" in some decades of MDP.

	-79 to -70	-69 to -60	-59 to -50	-49 to -40	-39 to -30	-29 to -20	-19 to -10
			Slope mV/sec				
Control	68.2±11.0	88.0±14.5	229.2±15	193.2±16	149.8±6.1	119.1±5.8	119.3±7
n	21	19	3	5	12	17	5
Verapamil 2.5 mg/L	31.8± 7.9	103.8±42.9	194.5± 7.9	133.3±18	78.2±5.8*	+	+
n	6	3	8	6	8		
			Overshoot, mV				
Control	40 ± 1.1	43.7± 1.2	29.3± 2.7	21.2±.4	16.2±1.6	5.3±3.4	5.8±3.8
	22	19	3	5	12	17	5
Verapamil 2.5 mg/L	32.3± 3.7	29.3± 6.7	15.8± 1.9*	4.2± 3.5*	-14.5±2.1*		
	6	3	8	6	8		

*P < .05 + Activity present in these decades during control, but not during drug exposure.

effect was not achieved even after prolonged washing with drug free solution (also shown in figure 10).

4.1e. Reversal of the Effects of Verapamil

Singh and Vaughan Williams (1972) postulated that the basic action of verapamil was to block the slow inward (calcium) current. Therefore, I tested two interventions which are known to increase the slow inward current. In 5 experiments, the extracellular calcium concentration was increased from 1.8 to 7.2 mM during exposure to verapamil; in 3 experiments, epinephrine 10^{-5} M was employed. Figure 8 shows an experiment in which both interventions were performed sequentially on the same fibre. Again, verapamil had little effect on the normal action potential, panels A and D. In the more negative range of MDPs, (panels B and E), the slope and overshoot of the automatic action potentials were both moderately decreased. However in the less negative range of MDPs, (panels C and F), verapamil caused a marked reduction in the number, phase 4 slope and amplitude of the automatic action potentials. $[Ca^{2+}]_o$ was then increased to 7.2 mM, panels G to I. The normal action potential plateau duration and upstroke velocity were decreased, and the action potential amplitude increased. At the more negative MDPs (-72 mV in H, -74 mV in B and E), automaticity was abolished. For the action potential arising from low MDPs, panel I, the phase 4 slope, already diminished by verapamil was further decreased by the high $[Ca^{2+}]_o$. However, the drug induced decrease in overshoot was partially reversed. These effects contrasted with those of epinephrine, panels J to L. The amplitude and plateau duration of the normal action potential were

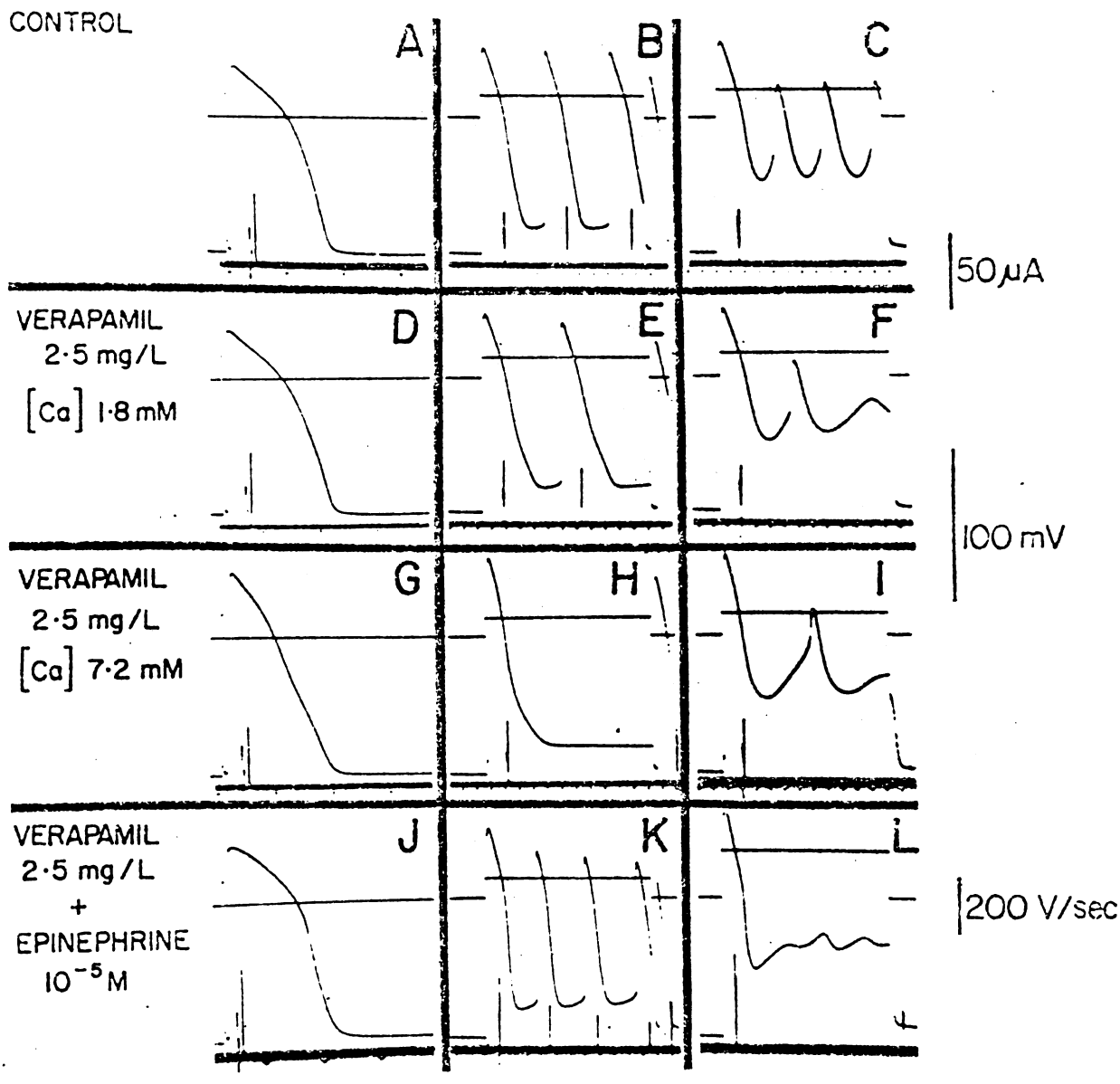


Figure 8. Effect of increased $[Ca]_o$ and epinephrine on automaticity during suppression by verapamil 2.5 mg/L. The traces are as in Figure 3. Panels A-C are control responses; panels D-F were obtained during superfusion with verapamil 2.5 mg/L, $[Ca]_o$ 1.8 mM. Panels G-I show the effects of increasing $[Ca]_o$ to 7.2 mM in the presence of verapamil. Between I and J, the $[Ca]_o$ was returned to 1.8 mM. Panels J-L show the effects of epinephrine 10^{-5} M in the presence of verapamil.

increased. Automaticity was clearly increased at the higher MDP. In the less negative potential range, only damped oscillations of fairly high frequency were observed, panel L. The effects on phase 4 slope in this experiment for the entire potential range are summarized in figure 9. The biphasic relationship between MDP and phase 4 depolarization rate under control conditions is clearly shown. In this experiment, the effects of verapamil were marked only in the less negative range of membrane potentials. Elevation of the extracellular calcium in the presence of verapamil further reduced the phase 4 slopes, although as noted in figure 8, it increased action potential amplitude at those potentials at which automaticity persisted. Epinephrine on the other hand restored phase 4 slope towards normal in the mid-range of MDPs, but did not restore regenerative spike activity in the less negative potential range. In all experiments, epinephrine shifted the range of potentials at which automaticity was observed to more negative potentials. Both epinephrine and increased $[Ca^{2+}]_o$ decreased the duration of the action potential during the prolonged current clamps. Because marked increases of $[Ca^{2+}]$ have been reported to slow automaticity, (Seifen et al., 1964), I also increased $[Ca^{2+}]_o$ in a stepwise manner, i.e., 1.8, 3.6, 5.4, and 7.2 mM, during exposure to verapamil in a single experiment. At no calcium concentration did I observe any restorative effect on phase 4 slope.

Figure 10 further illustrates the effect of epinephrine and also that of prolonged washing. During control, the automatic action potential in the high range of MDP in panel B had an MDP of -63 mV and a maximum upstroke velocity of 250 V/sec. Exposure to verapamil 2.5 mg/L for 30 minutes abolished repetitive activity in both ranges

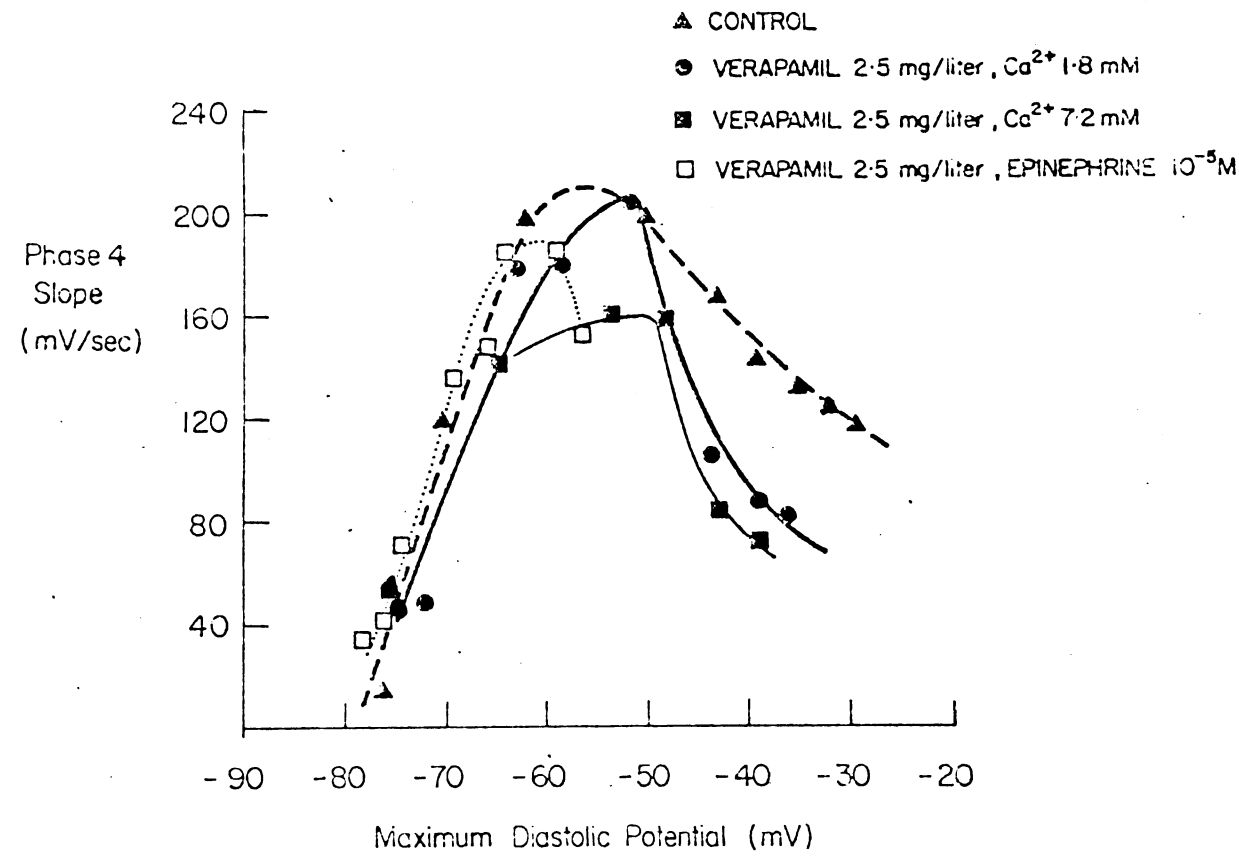


Figure 9. Effect of verapamil 2.5 mg/L, increased $[Ca]_o$ and epinephrine on phase 4 slope. The curves show mean diastolic depolarization rates in a single muscle during control, verapamil in $[Ca]_o$ 1.8 mM, verapamil in $[Ca]_o$ 7.2 mM, and verapamil with epinephrine 10^{-5} M.

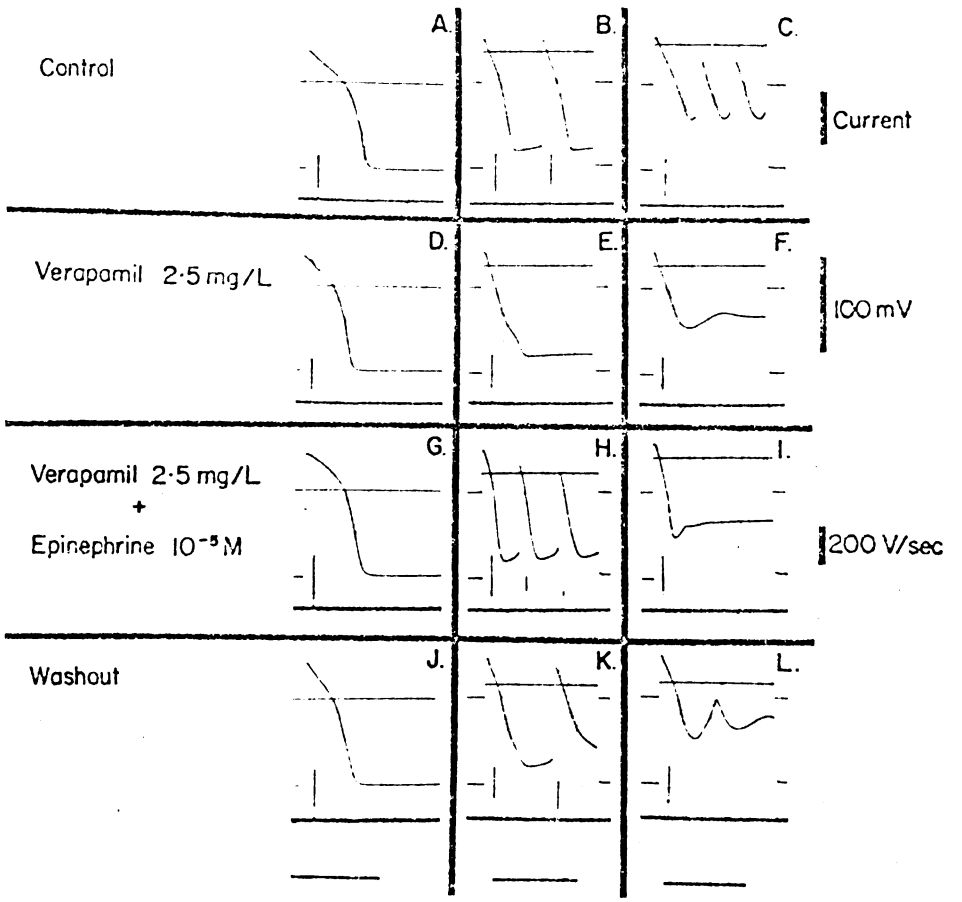


Figure 10. Effects of epinephrine and washing on ventricular automaticity during suppression by verapamil 2.5 mg/L. The traces are as in Figure 3. Panels A-C are during control; panels D-F during exposure to verapamil; panels G-I during exposure to verapamil and epinephrine 10^{-5} M; panels J-L at the end of a 1 hour superfusion with drug free solution. The current calibration is 25 uA for panels B, C, E, H and I; 50 uA for panels K and L. The time calibration at the bottom of the left column is 200 msec; those below the middle and right columns, 800 msec.

of MDP (panels E and F). The addition of epinephrine 10^{-5} M in the continued presence of verapamil restored activity in the higher range of MDPs only (panels H and I). Superfusion with drug free solution was accompanied by a partial reversal of the effects of verapamil (panels K and L).

4.1f. The Effects of Quinidine

The effects of quinidine 2 - 4 mg/L were examined in 12 experiments. Figure 11 illustrates an experiment with quinidine at both concentrations. Following exposure to quinidine 2 mg/L, the duration of the normal action potential was increased, but there was little change in dV/dT max of phase 0 (compare panels A and D). For automatic responses arising from high MDP, e.g., those shown in panels B and E, there was a small reduction in phase 4 slope and overshoot. At less negative MDP, panel F, phase 4 slope was also decreased, but there was little change in overshoot. Panels G - I show the effect of increasing the quinidine concentration to 4 mg/L. There was no further increase in the duration of the normal action potential (panel G), but there was some decrease in dV/dT max of phase 0. In the higher range of MDPs, the phase 4 slope of the automatic beat was further decreased, but the overshoot did not change. At the low MDPs, phase 4 slope and overshoot were further reduced. The cycle interval between the elicited and first automatic action potential was increased in both potential ranges by quinidine 2 and 4 mg/L. Panels J - L were obtained after 2 hours superfusion with drug free solution. The normal action potential duration returned towards control, and dV/dT max of phase 0 was somewhat greater than

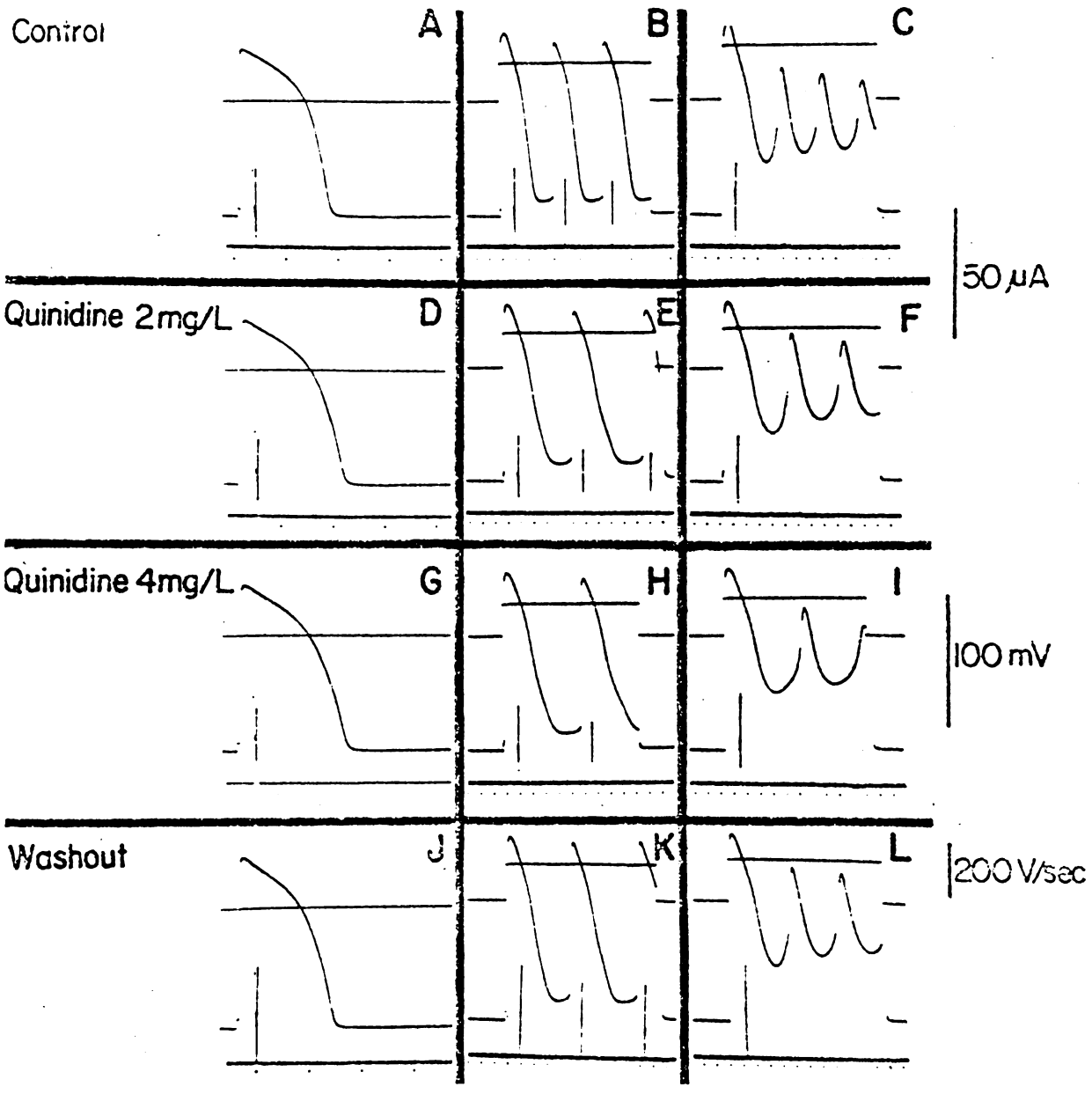


Figure 11. Effects of quinidine 2-4 mg/L on ventricular automaticity. The traces are as in Figure 3. Panels A-C are control records. Panels D-F were obtained during exposure to quinidine 2 mg/L, panels G-I during exposure to quinidine 4 mg/L. Panels J-L were obtained at the end of a 2 hour period of drug free superfusion.

control. For action potential in both ranges of MDP, phase 4 slope and overshoot returned towards control.

Figure 12 shows mean phase 4 slopes as a function of MDP for a typical papillary muscle during control, a 1/2 hour drug free superfusion, and quinidine 4 mg/L for 1/2 and 1 hour. During the drug free period, phase 4 slope was little changed. However, during exposure to quinidine, there was a reduction of phase 4 slope at all potentials.

The mean effect of 4 mg/L of quinidine on a group of muscles are shown in Table IV. The effect on threshold was variable. In some experiments, a decrease in threshold to less negative potentials was observed, in others, there was no change. It was my consistent observation that prolongation of the action potential duration contributed as much as the decrease in phase 4 slope to an increase in cycle length.

The bradycardia and A - V block produced by a large dose of quinidine are reversed by epinephrine (Finnegan and Trounce, 1954). I therefore examined the effect of epinephrine 10^{-5} M on ventricular automaticity during depression by quinidine 4 mg/L in 3 experiments. The results of one such experiment are depicted in figure 13. Quinidine prolonged the normal action potential duration and decreased dV/dT max of phase 0. The drug markedly decreased phase 4 slope at high MDPs (panel E). There was no change in overshoot. At low MDPs, both phase 4 slope and overshoot were decreased (panel F). The effects of epinephrine 10^{-5} M are shown in panels G - I. For the normal action potential, panel G, the classical effects of epinephrine

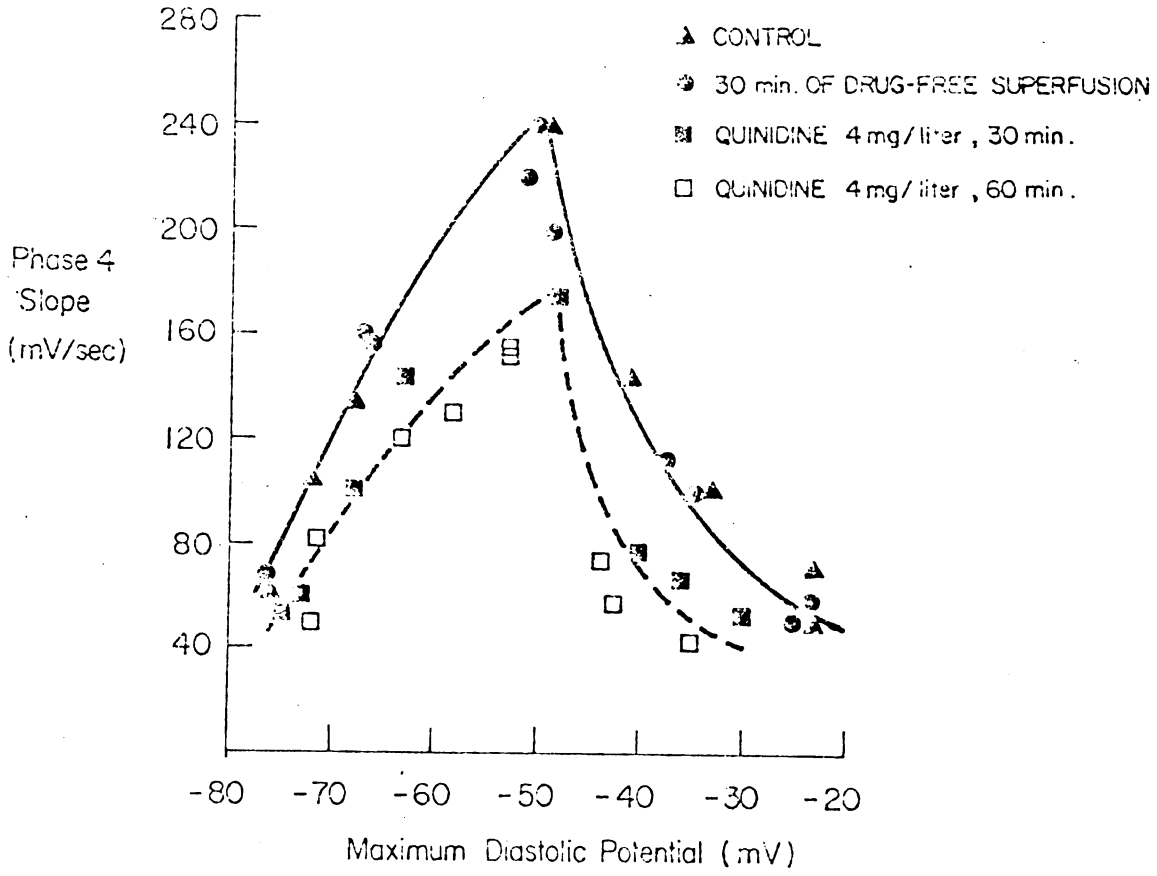


Figure 12. Phase 4 slope plotted as a function of maximum diastolic potential during the control period, 30 minutes of drug free superfusion, and quinidine 4 mg/L for 1/2 hour and 1 hour. The data is from a different muscle from that in Figure 11.

TABLE IV

The effects of quinidine 4 mg/L on phase 4 slope and overshoot. The principle of selection of data for this table are outlined in the legend of Table III. The mean resting membrane potential in this group of muscles was 2 mV higher thatn that in Table II.

	-89 to -80	-79 to -70	-69 to -60	-59 to -50	-49 to -40	-39 to -30	-29 to -20	-19 to -10
				Slope, mV/sec				
Control	63.4± 3.4	65.4± 6	160.8±14.7	214 ±10.7	158.3± 6.7	142.6± 2.6	123.1± 6.2	133.8± 3.5
n	4	47	38	13	22	17	8	3
Quinidine 4 mg/L	29.8± 4*	50.7± 5	112.1± 8.4*	127.4± 7*	85.7± 5.6*	86.6± 4.1*	120.8±10.1	90.2± 8.8
n	3	45	44	20	29	16	5	3
				Overshoot, mV				
Control	38.3± .24	44.6± .8	40 ± 1.3	34 ± 1.3	23.4± 2.3	15.7± 2.7	19.9± 6.2	37.7± 1.3
n	4	47	38	13	22	17	8	3
Quinidine 4 mg/L	24.7± .66*	38.9±1.5*	31.9± 1.7*	29.5± 1.5	13.6± 3.4	5.4± 4	16.8± 4.8	12 ± 4.1*
n	3	45	44	20	29	14	5	3

* $p < .05$.

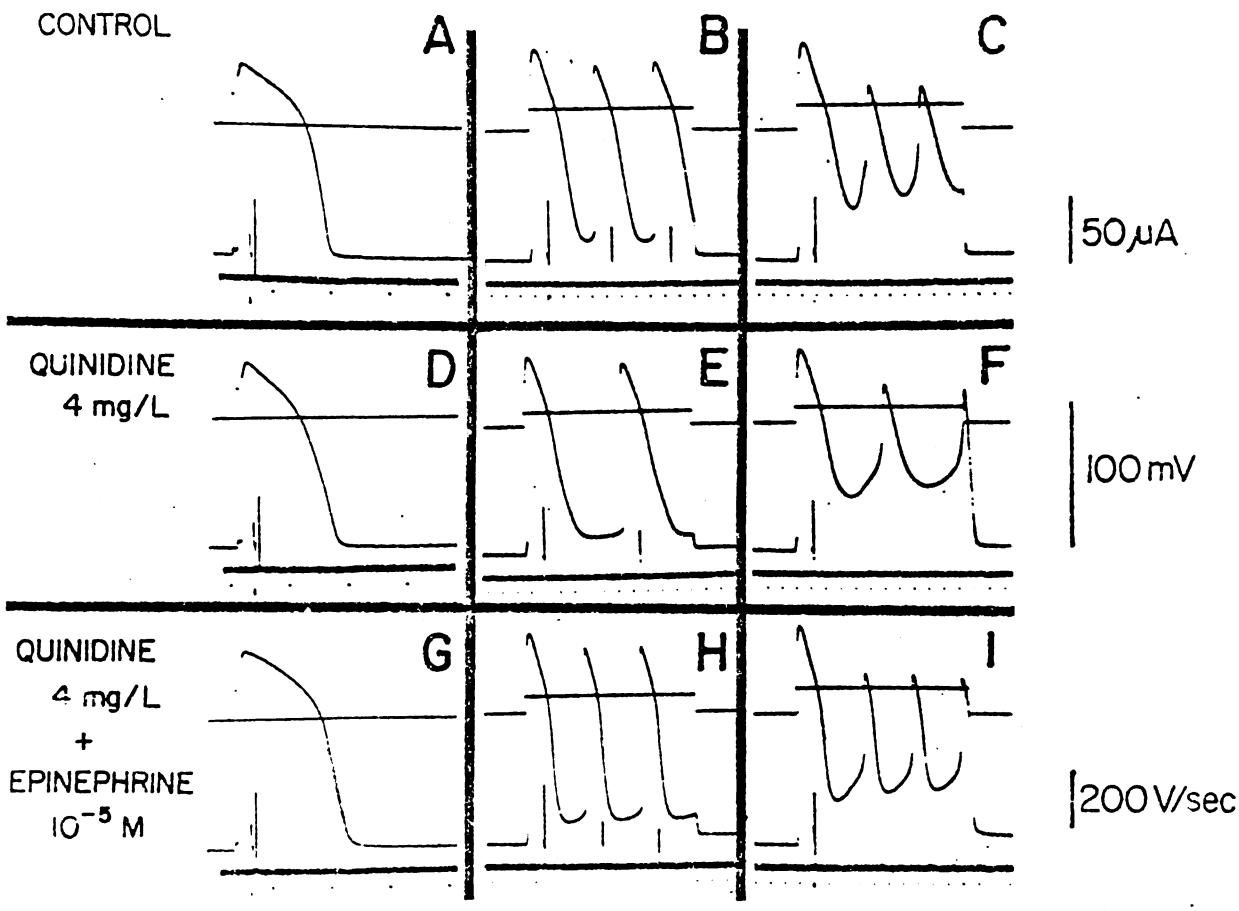


Figure 13. Effect of epinephrine 10^{-5} M on ventricular automaticity during suppression with quinidine 4 mg/L. The traces are as in Figure 3. Panels A-C are control responses; panels D-F were obtained at the end of a 1 hour exposure period to quinidine 4 mg/L. Panels G-I show the effects of epinephrine in the continued presence of quinidine.

were evident, viz, an increase in action potential amplitude and a prolongation of the plateau duration (Reuter 1974). Phase 4 slope was increased, but overshoot remained unchanged in the high range of MDP (panel H). At low MDPs (e.g. panel I), there was a small increase in phase 4 slope, but little change in amplitude. Following exposure to epinephrine, the least negative MDP at which automaticity was observed was shifted from -34 to -46 mV. Similarly, the maximum on the phase 4 slope - MDP plot was shifted from -60 to -64 mV. In one experiment, the shift were less marked, and an increase in overshoot was observed at low MDPs. A part of the decrease in cycle length which epinephrine produced was the result of a shortening of the action potential duration during the prolonged current pulse.

4.1g. Effects of Droperidol

The effects of droperidol 1.9 - 3.8 mg/L were examined in 11 experiments. An experiment with droperidol 3.8 mg/L is illustrated in figure 14. Following exposure to droperidol for 30 minutes, the duration of the normal action potential was increased (panel D). In the more negative range of MDP, the cycle length was markedly increased (panel E). Both a reduction of phase 4 slope and a prolongation of action potential duration contributed to the increase in cycle length. The duration of the action potential to the zero mV potential level was little changed. However, the terminal phase of repolarization was delayed. This delay in repolarization was even more marked in some preparations where action potentials of as much as 800 msec duration were observed. The amplitude of the automatic action potentials at the high MDP was increased. This was not a usual observation. At

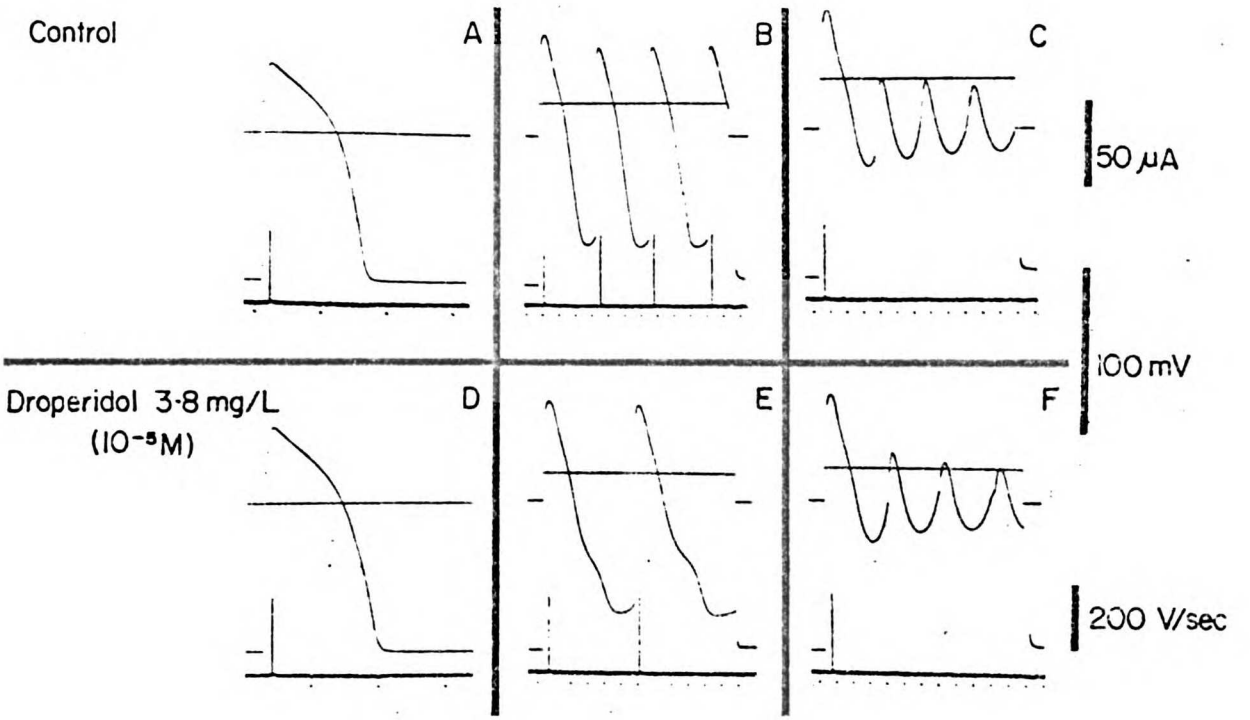


Figure 14. The effects of droperidol 3.8 mg/L on ventricular automaticity. The traces are as in Figure 3. Panels A - C are control; panels D - F were obtained at the end of a 30 minute period of superfusion with droperidol.

the low MDP depicted in panels C and F, an increase in cycle length with a reduction in phase 4 slope was also observed. The overshoot was little changed. There was an appreciable change in the size of current needed to obtain a similar degree of depolarization in the less negative range of membrane potential.

In general, droperidol was more depressant to action potentials arising in the more negative range of membrane potentials. This is illustrated in Figure 15. Again, the duration of the normal action potential was prolonged on exposure to droperidol 3.8 mg/L for 15 minutes. During the control period, automaticity was easily demonstrated in both ranges of membrane potential (panels B - E). However, on exposure to droperidol, it was not possible to induce automaticity in the more negative range of membrane potentials. A current of 9.2 μA elicited a single action potential followed by a flat diastolic period. An increase in current strength of less than 0.25 μA caused a 33 mV change in MDP. Automaticity persisted in the less negative range of MDP, with decreased phase 4 slope and little change in amplitude (compare panels E and H).

The presence of a "forbidden zone" in the more negative region of membrane potential suggests that the mean membrane current-voltage relationship had a flat (or negative) slope in this region of potential. In one experiment, I measured the relative membrane resistance in this range of membrane potential with 1 μA pulses, 50 msec in duration and of frequency 5 Hz. At the resting membrane potential of -86.5 mV, the 1 μA pulses produced a membrane displacement of less than .5 mV. However, at the depolarized level of -79 mV during the long constant current pulse, the membrane displacement was

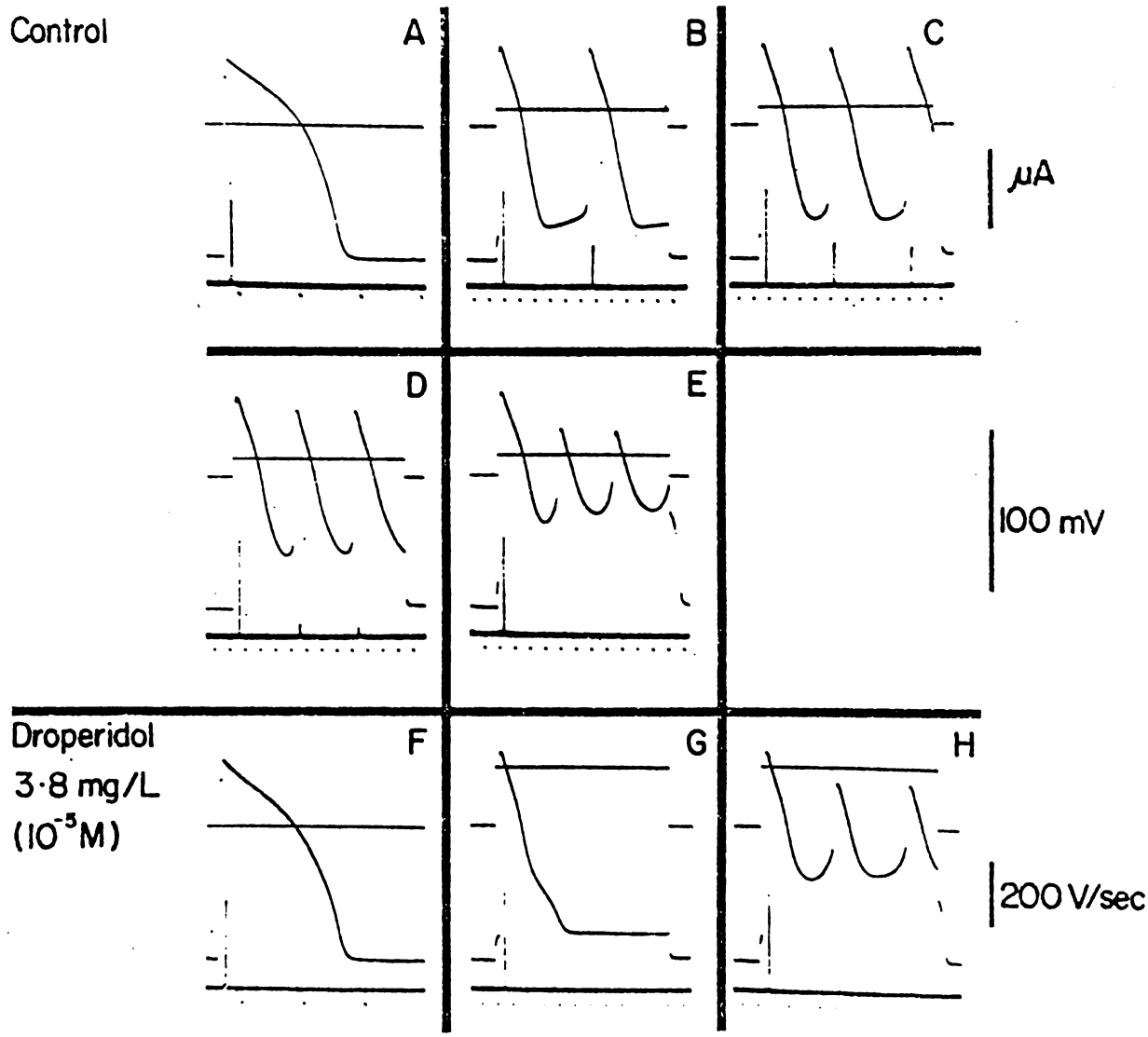


Figure 15. Effects of droperidol 3.8 mg/L on ventricular automaticity. Panels A - E are control. Panels F - H were obtained after exposure to droperidol 3.8 mg/L for 15 minutes. The current calibration is 50 uA for panels B - E, 12.5 uA for panels G and H.

1 mV. This indicates anomalous (inward) rectification in this potential range. When the amplitude of the long current pulse was increased to a level at which automaticity was observed, the 1 μA pulses led to stimulated action potentials. There was no change in relative membrane resistance at potentials (-86 mV and -78 mV) similar to control, during exposure to droperidol for 15 and 30 minutes. However, if the longitudinal intracellular resistance increased appreciably over this period of time, a smaller fraction of the applied current would be transmembrane current. This would counteract any true increase in membrane resistance.

The effects of droperidol 3.8 mg/L are summarized in Table V. A depression of automaticity was also observed in the three experiments performed with droperidol 1.9 mg/L.

The effects of droperidol were evident after 15 minutes of exposure to the drug. No reversal of the effect of droperidol was observed on washing with drug free solution for 1 hour.

I attempted to reverse the depressant effects of droperidol with epinephrine 10^{-5} M in three experiments. The results of one of these experiments is depicted in figure 16. The action potential duration was prolonged during exposure to droperidol 3.8 mg/L (compare panels A and D). Droperidol increased the cycle length of automatic action potentials at high MDP (panel E). Phase 4 slope and overshoot were both decreased during drug exposure. At the low MDP, cycle length was increased and phase 4 slope decreased (panel F). Overshoot was little changed. The addition of epinephrine 10^{-5} M in the presence of droperidol increased the maximum upstroke velocity, amplitude and duration of the normal action potential (panel G). At the high MDPs,

TABLE V

The effect of droperidol 3.8 mg/L on phase 4 slope and overshoot. Principle of data selection are outlined in the legend to Table III

MDP	-79 to -70	-69 to -60	-59 to -50	-49 to -40	-39 to -30	-29 to -20	-19 to -10
			Slope mV/sec				
Control	50.6± 3.7	132.7±10.6	252.7± 8.7	214.1±12.9	172.2± 8	151.4± 6.5	155.4± 6.5
n	50	25	11	18	26	22	7
Droperidol 3.8 mg/L	34 ± 2.5*	60.6± 5.3*	127.8±10.2*	122.8± 9.1*	108.3± 8*	115.2± 4.8	118.1± 3.6*
n	32	22	2	19	49	24	5
			Overshoot				
	mV						
Control	48.5± .5	45.2± .9*	33.9± 1.4	29.8± 2.3	26.7± 1.7	24.3± 2	26.4± 1.2
n	50	25	11	18	26	22	7
Droperidol 3.8 mg/L	47.4± .8	50.7± 1.4	29 ± 6	26.3± 2.4	19.7± 1.6*	22.5± 2.3	21.9± 1.2
n	32	22	2	19	49	24	5

* $p < .05$.

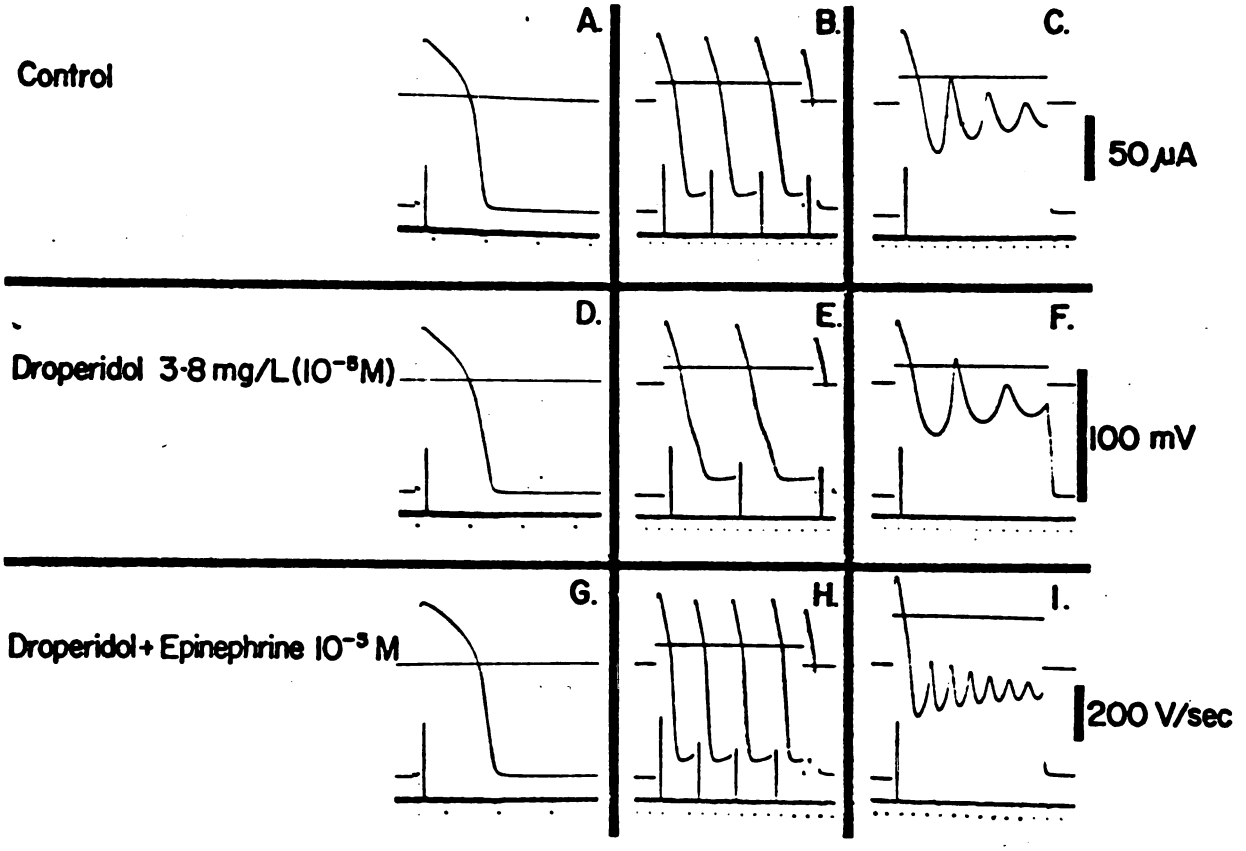


Figure 16. The effects of epinephrine 10^{-5} M on ventricular automaticity during suppression by droperidol 3.8 mg/L. The traces are as in figure 3. Panels A - C are control; panels D - F during exposure to droperidol 3.8 mg/L; panels G - I during exposure to epinephrine in the presence of droperidol.

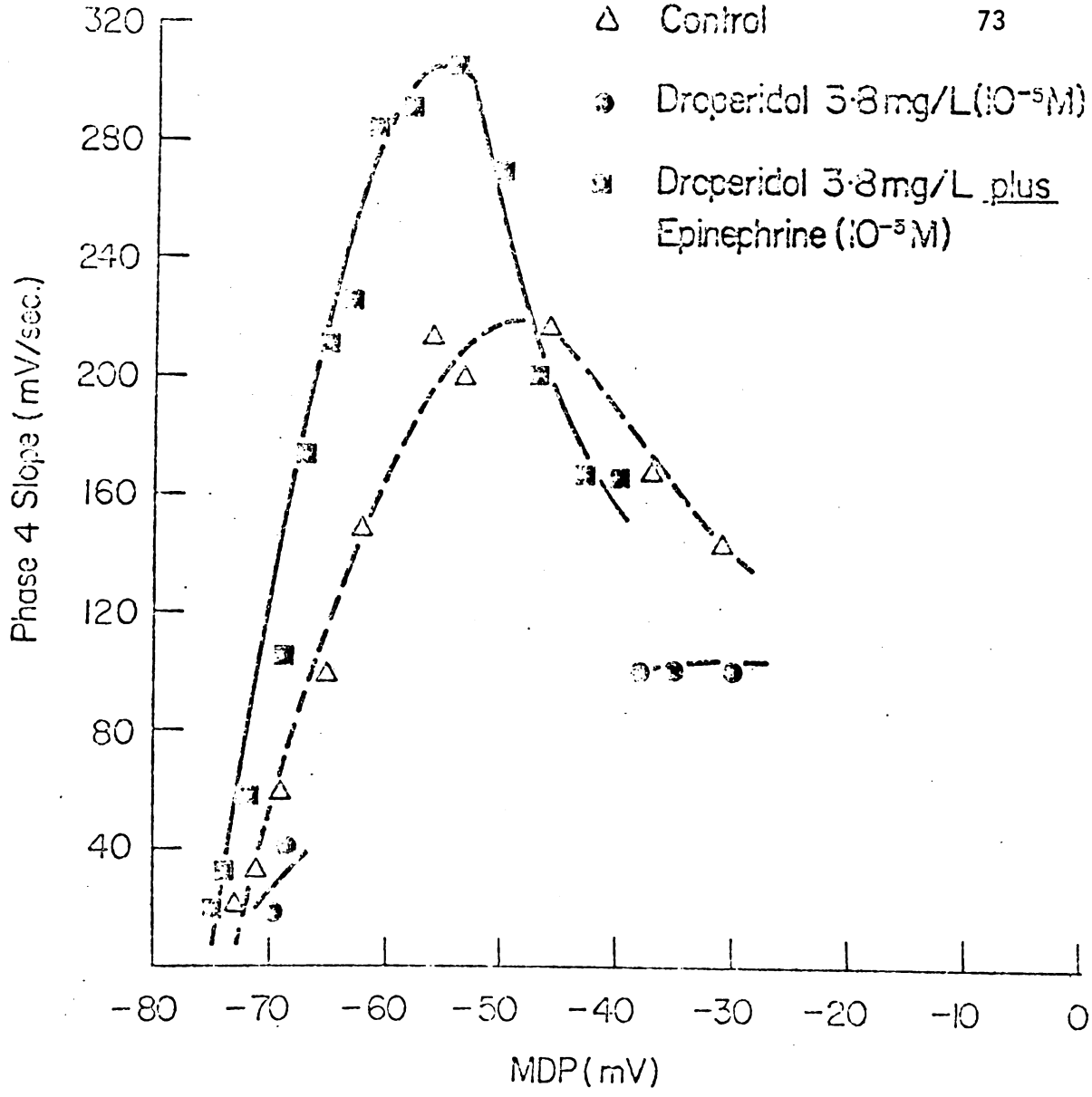


Figure 17. Summary of the effect of epinephrine 10^{-5} M on diastolic depolarization rate during suppression by droperidol 3.8 mg/L. The data is from the same muscle depicted in Figure 16.

e.g., panel H, the cycle length was now decreased below the control value. This resulted from a decrease in action potential duration, and an increase in phase 4 slope. Action potential amplitude remained unchanged. In the less negative range of MDP, damped oscillations of high frequency were observed (panel I). Considerably more current was required to achieve equivalent depolarizations in the less negative range of MDP. The effects on phase 4 slope for the entire range of MDP in this experiment is shown in figure 17. On exposure to droperidol, there was a zone between -67 and -37 mV in which it was not possible to define automaticity. At the extremes of MDP in which automaticity persisted, there was a decrease of phase 4 slope. Addition of epinephrine increased phase 4 slope to values greater than control at most MDPs. This effect was most marked at intermediate MDPs. The least negative MDP at which automaticity was observed in the presence of droperidol was -30 mV; on addition of epinephrine this was shifted to -40 mV. At the most negative MDP, there was a 5 mV shift to a higher (more negative) MDP.

Summary of Automaticity Results

(i) Verapamil decreased diastolic depolarization rate and suppressed action potential amplitude during electrically induced automaticity. These effects were more marked in action potentials arising from less negative MDPs.

(ii) Elevation of $[Ca^{2+}]_o$ in the presence of verapamil further decreased diastolic depolarization rate, but restored action potential amplitude towards normal.

Summary of Automaticity Results (Cont.)

(iii) Epinephrine reversed the depressant effects of verapamil on diastolic depolarization rate, but had no restorative effects on action potential amplitude.

(iv) Quinidine decreased diastolic depolarization rate at most MDPs and had a variable effect on action potential amplitude.

(v) The depressant effects of quinidine on diastolic depolarization rate were reversed by epinephrine.

(vi) Droperidol also decreased diastolic depolarization rate, and had a variable effect on action potential amplitude.

(vii) Epinephrine reversed the depressant effects of droperidol on diastolic depolarization rate. At low MDPs, action potential amplitude was actually decreased in some preparations.

4.2. REFRACTORINESS

4.2a. Effects of Droperidol on the Recovery Kinetics of dV/dT max of Phase Zero of the Action Potential

The effects of droperidol 10^{-6} - 4.10^{-5} M on the recovery kinetics of dV/dT max of phase 0 were examined in 16 experiments. The principle of the two pulse techniques of determing the recovery kinetics of dV/dT max is illustrated by the control records in figure 18. A test stimulus of varying coupling (diastolic) intervals elicited an extra response following complete repolarization of the basic drive beat (panels B - F). The diastolic interval is defined as that period beginning with repolarization of the basic drive beat to a point 4 mV positive to the resting value and ending with the onset of phase zero of the test response. As the test response was elicited from the same membrane potential as the basic drive beat, a plot of dV/dT max of the test response vs. diastolic interval describes the time dependent (voltage independent) recovery of dV/dT max. In the experiment illustrated in figure 18, there was little change in the upstroke velocity of the test response (panels B - F) even at the shortest diastolic interval (167.5 V/sec - panel A and - 157.5 V/sec - panel F, respectively for the basic beat and test response). This suggests that under control conditions, the recovery of dV/dT max from inactivation is almost complete on full repolarization when the resting potential is high (-87 mV in this fibre). This contrasts with the effects observed after the same fibre was exposed to droperidol 10^{-5} M for 45 minutes, figure 19. The upstroke velocity of the basic beat decreased from 167.5 to 137.5 V/sec and the action potential duration

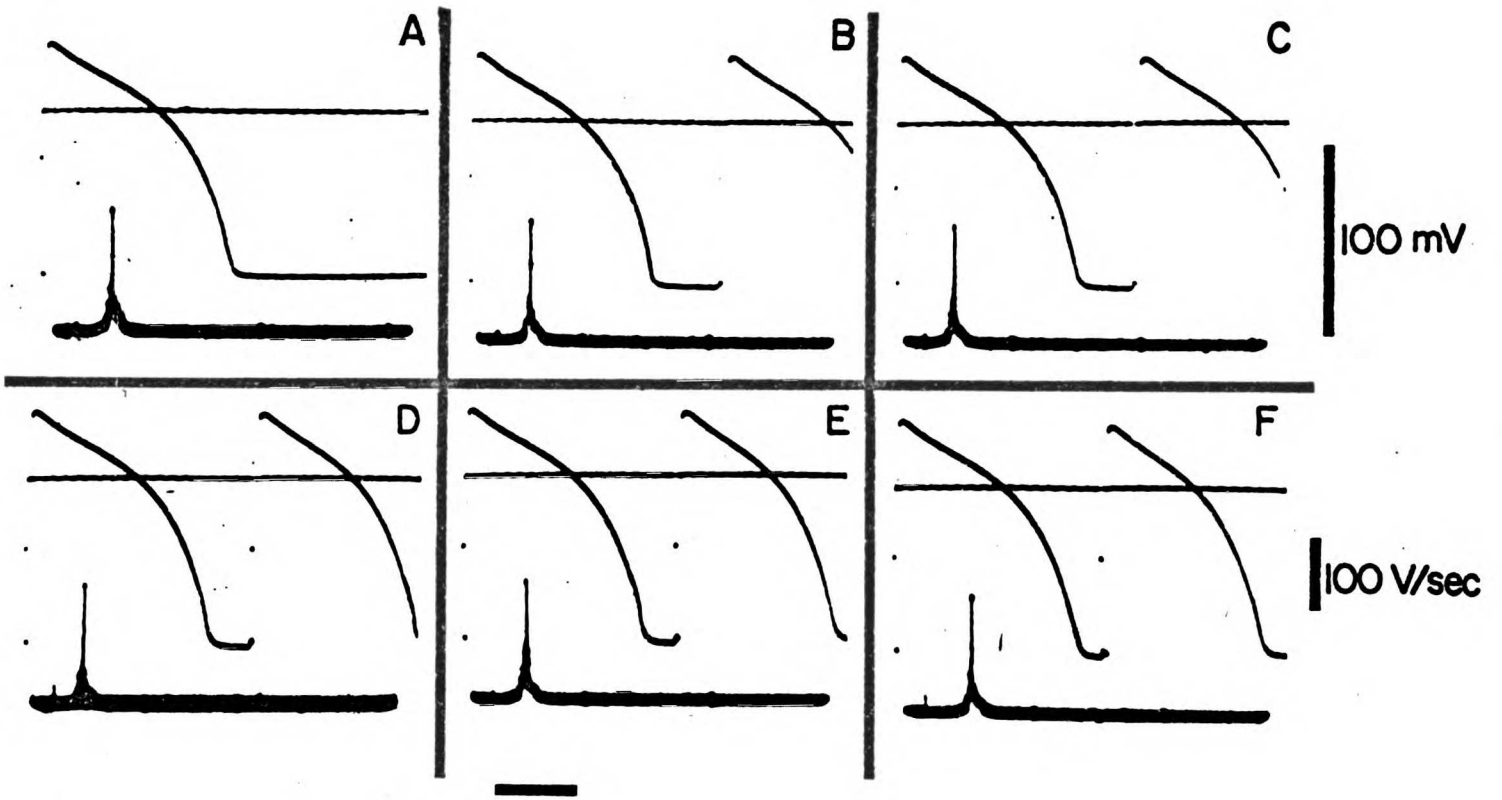


Figure 18. Recovery kinetics of dV/dT max during control. In figures 18, 19 and 23 the upper trace is the zero level of transmembrane potential. The middle trace is the transmembrane action potential. The third trace is the first time derivative of the action potential. Panel A shows the basic drive beat. Panels B - F show the basic drive beat, the test response and dV/dT max of the TEST RESPONSE. The time calibration below panel E is 100 msec.

was prolonged. As test responses were elicited at shorter and shorter diastolic intervals, their maximal upstroke velocity decreased progressively (100 V/sec for the test response in panel F).

The maximum upstroke velocity of the test responses during control and exposure to droperidol in this muscle have been expressed as a percentage of the corresponding basic drive beats and plotted against diastolic interval in figure 20. At a diastolic interval of 100 msec, dV/dT max of the test response was 95% that of the basic beat during drug free superfusion. On the other hand during exposure to droperidol, dV/dT max was still less than 95% that of the basic beat at a diastolic interval of 600 msec. The deviations from 100%, delta in figure 20, have been plotted on a logarithmic scale against diastolic interval in figure 21. The straight lines through the control points and those after drug treatment were drawn by eye. The points at the shorter diastolic intervals (less than 100 msec) during drug exposure appear as though they may lie along a different line of steeper slope. I elected to use a single exponential as there was not a sufficient number of points at very short diastolic intervals to clearly define a second line. In the majority of preparations, the fit to a single exponential was better than that in figure 21. The recovery process is conveniently described by the time constant and the y intercept. The lines have been extrapolated to the ordinate, and the times taken for delta to decrease to 1/e of the intercepts interpolated. These times are the recovery time constants of dV/dT max. Droperidol caused a 10 fold increase in the recovery time constant.

The results from all 16 experiments are summarized in Table VI. Because time constants of less than 20 msec could not

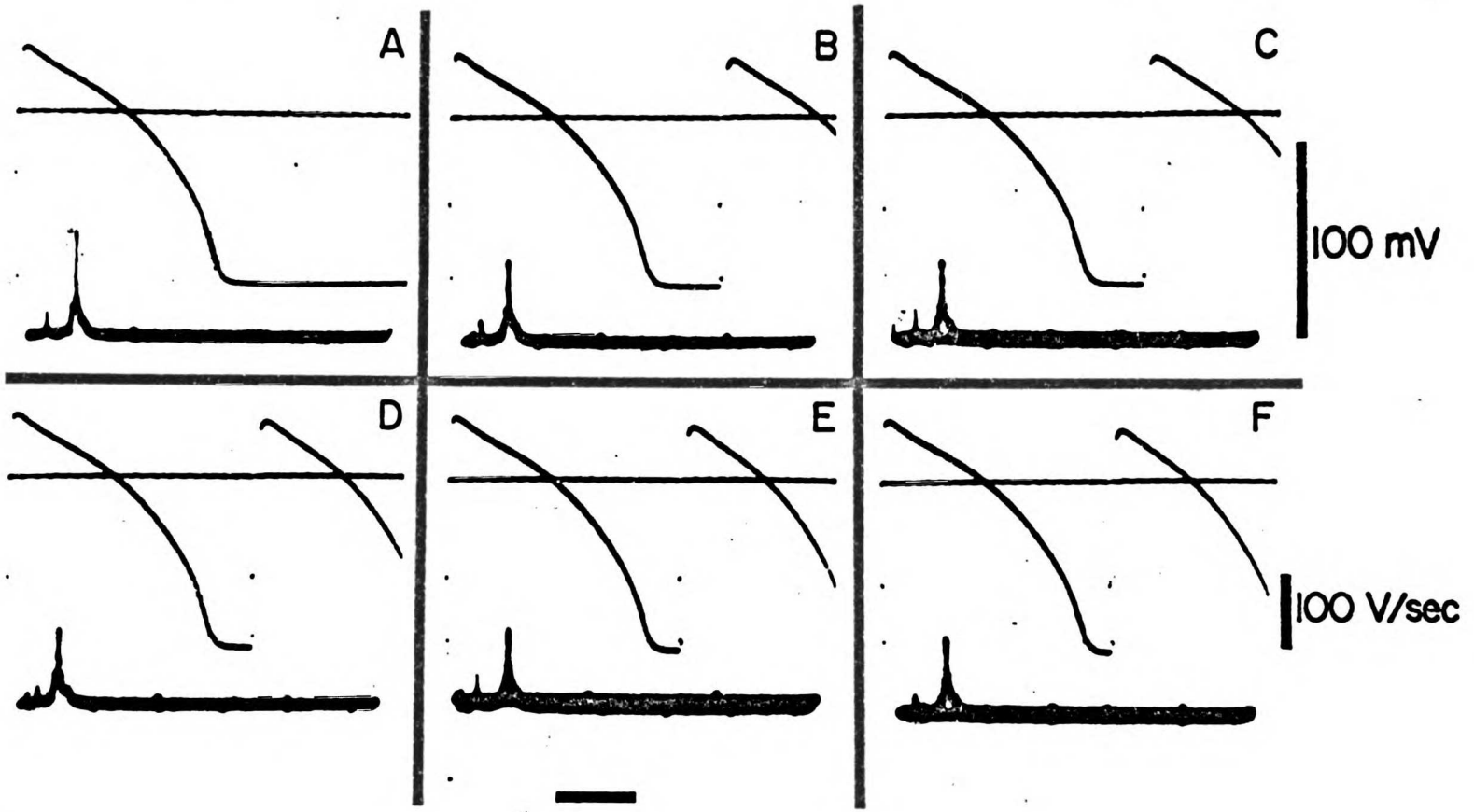


Figure 19. Recovery kinetics of dV/dT max during exposure to droperidol 10^{-5} M. For explanation of traces see Figure 18. The time calibration below panel F is 100 msec.

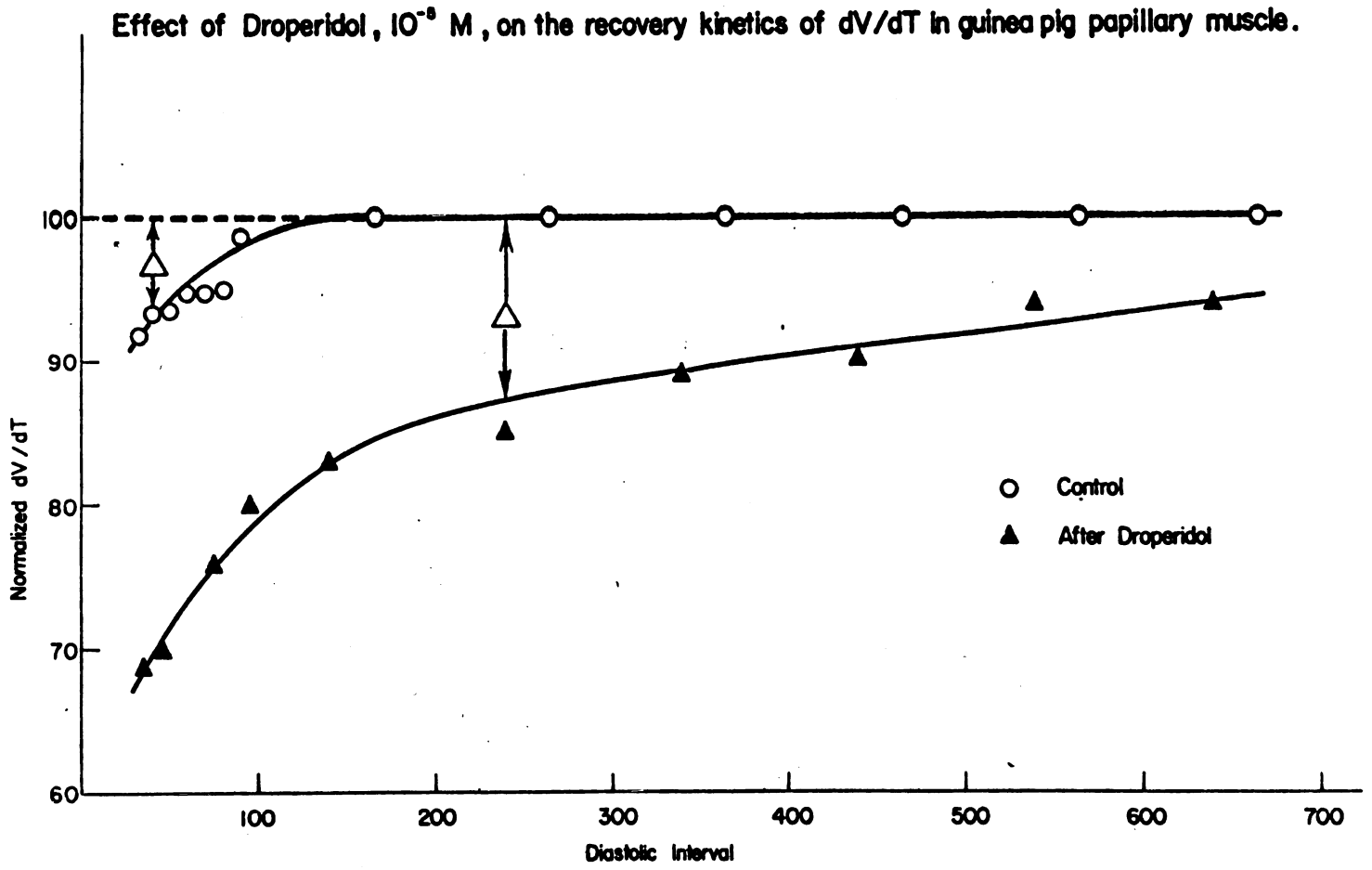


Figure 20. dV/dT max of the test response has been expressed as a % of the basic drive beat during control and exposure to droperidol and plotted against diastolic interval. Δ represent the deviation of dV/dT max of the test response from that of the basic drive beat.

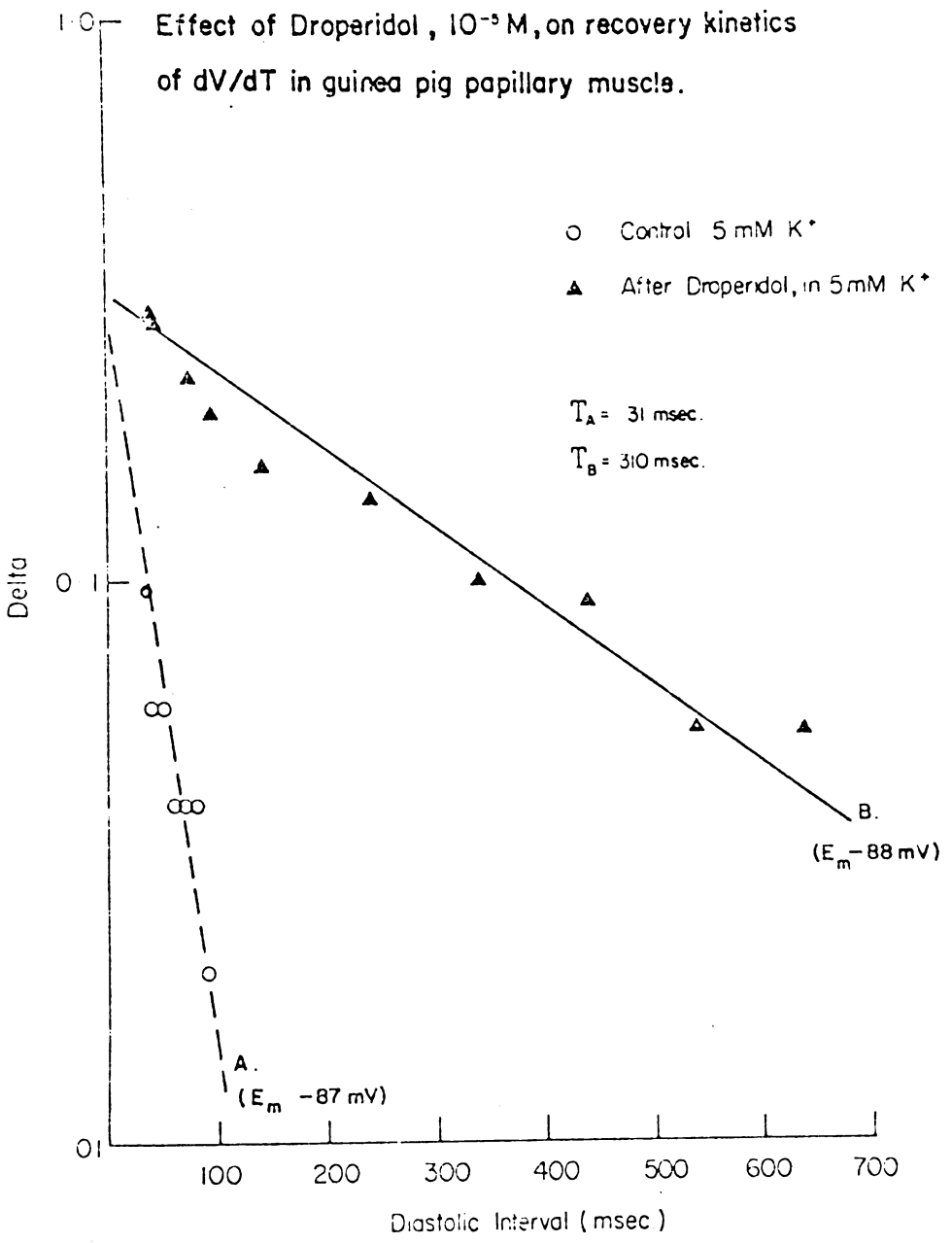


Figure 21. The deviations of dV/dT max of the test response from 100% have been plotted on a logarithmic scale against the diastolic interval. The data is from the same muscle in Figure 20.

be reliably quantitated, values falling below this level are listed in the table as "< 20 msec". In fact in most of the control determinations, dV/dT max of the test response was already equal to that of the basic drive beat for the shortest diastolic intervals. For a mean resting membrane potential of -88.6 mV in these muscles, the expected control time constant is less than 20 msec (Gettes and Reuter 1974). At 10^{-6} M, droperidol did not change the apparent recovery time constant of dV/dT max. At concentrations from 5.10^{-6} - 4.10^{-5} M, droperidol caused a tenfold or greater increase in the recovery time constants. There was marked variability between muscles. For example, at 10^{-5} M, the range of time constants after exposure to droperidol was 155 - 354 msec. In four experiments, the drug concentration was increased during the continuous impalement of single fibres. In three of these experiments, there was no slowing of the recovery kinetics of dV/dT max at the low drug concentrations (10^{-6} M in Exp. 1 and 3, 5.10^{-6} M in Exp. 6), but slowing was evident at the higher drug concentrations. In the 4th experiment (Exp. 5), the time constant remained unchanged as the drug concentration was increased from 5.10^{-6} M to 2.10^{-5} M. However, the intercept increased from .08 to .4. This means that the hypothetical zero delay test response had a smaller normalized upstroke velocity in the higher drug concentration.

The effects of droperidol on the recovery kinetics were only partially reversed after prolonged superfusion with drug free solution. An example of the effects of droperidol 2.10^{-5} M, and prolonged washout after exposure to droperidol is shown in figure 22.

In 4 experiments, recovery kinetics were determined in both

TABLE VI

Effect of droperidol on the recovery kinetics of dV/dT max. The intercept is derived from the ratio of two upstroke velocities and is dimensionless (i.e. has no units).

Drug Conc.	Exp. No	Control			Droperidol		
		dV/dT V/sec	Time Constant Msec	Intercept	dV/dT V/sec	Time Constant Msec	Intercept
10^{-6}	1	119.5	20	.135	112	20	.135
	2	195	< 20		207.5	< 20	
	3	270	37	. 2	280	37	. 2
	4	190	< 20		190	< 20	
5.10^{-6}	1	119.5	20		116	510	. 2
	5	269	< 20		260	440	. 08
	6	215	< 20		195	< 20	
10^{-5}	7	252	24.5	.125	225	330	.265
	8	241	< 20		235	320	. 18
	9	167.5	31.5	. 3	137.5	310	. 32
	6	215	< 20		195	354	.265
	10	272.5	22	. 13	245	210	. 39
	11	455	< 20		473	155	.385
2.10^{-5}	12	310	< 20		280	305	. 57
	13	253	< 20		225	362	.235
	14	385	< 20		355	310	.285
	3	270	37	. 2	272.5	327	. 5
	5	269	< 20		245	440	.4
4.10^{-5}	15	180	*		145	295	. 93
	16	310	< 20		230	325	. 6

*Control kinetics not done, experiment performed at the end of a h curve experiment.

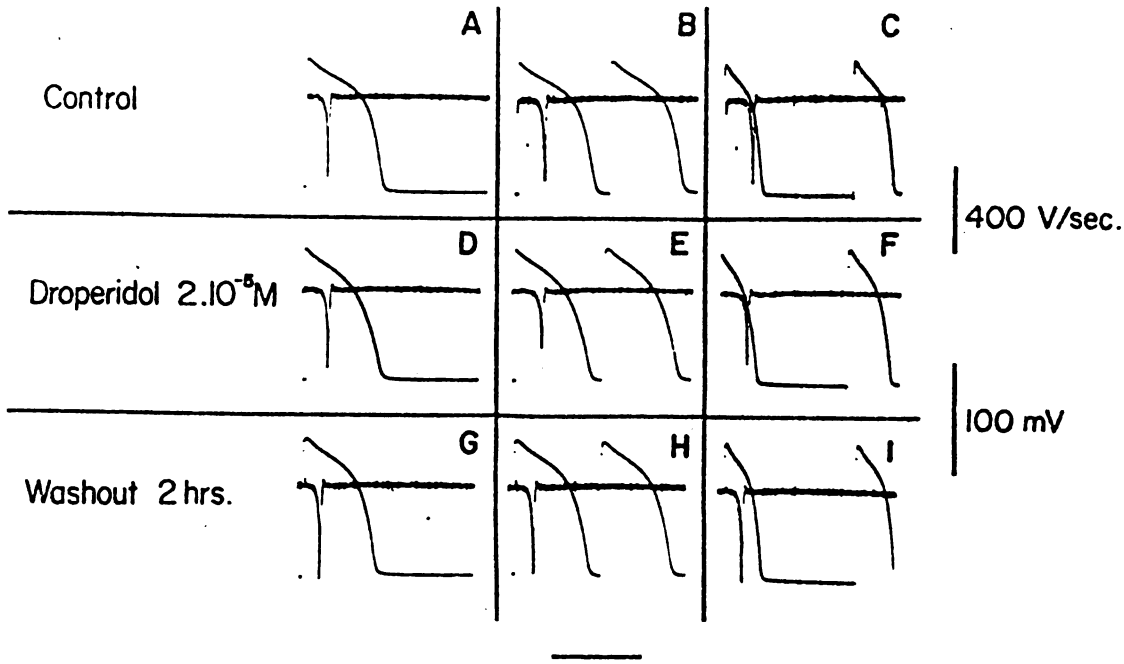


Figure 22. Effects of Droperidol 2.10^{-5} M on the recovery kinetics of dV/dT max. The upper trace in each panel is first time derivative of the transmembrane potential; the lower trace is the transmembrane potential. Panels A, D, and G show the basic drive beats and their time derivative. Panels B, C, E, F, H and I show the basic drive beat, the test response and the upstroke velocity of the test response. Note that during the control period there is no decrease of dV/dT max of the test response at the shorter diastolic interval, panel B. However, on exposure to droperidol dV/dT max of the test responses are appreciably less than that of the basic beat at both diastolic intervals (panels D - F). This effect is partially reversed by washing with drug free solution for 2 hours (panels G - I).

5 mM and 10 mM K^+ Tyrode solution during control and exposure to droperidol. Two experiments were done at each of the concentrations 10^{-6} and 10^{-5} M. Although 10^{-6} M droperidol did not slow the recovery kinetics in 5 mM K^+ Tyrode solution, I included that concentration in this section of the protocol to see if partial depolarization increased the sensitivity of the preparation to the depressant effects of the drug.

Recovery kinetics during control in 5 mM and 10 mM K^+ Tyrode solution are illustrated in Figure 23. In 5 mM $[K^+]_o$, the basic drive beat had a resting membrane potential of -88 mV and a maximum upstroke velocity of 241 V/sec. At the shortest diastolic interval, panel B, the upstroke velocity of the test response was the same as that of the basic drive beat. Again, this indicates that recovery of dV/dT max was coincident with or earlier than, full repolarization. On increasing $[K^+]_o$ to 10 mM, the resting membrane potential decreased to -74 mV and the upstroke velocity to 190 V/sec (panel E). Test responses elicited at two short diastolic intervals (panels F and G), clearly had decreased upstroke velocities. At the longest diastolic interval, panel H, the upstroke velocity of the test response was the same as that of the basic drive beat. This indicates, that depolarization of the membrane causes marked slowing of the recovery kinetics of dV/dT max in absence of drug. Recovery kinetics were repeated in the same fibre during exposure to droperidol 10^{-5} M. The results are illustrated in Figure 24. In 5 mM $[K^+]_o$ the upstroke velocity of the basic beat decreased from 241 V/sec to 235 V/sec and the resting membrane potential was -90 mV. The action potential duration was increased (compare panel A in Figure 23 and 24). The

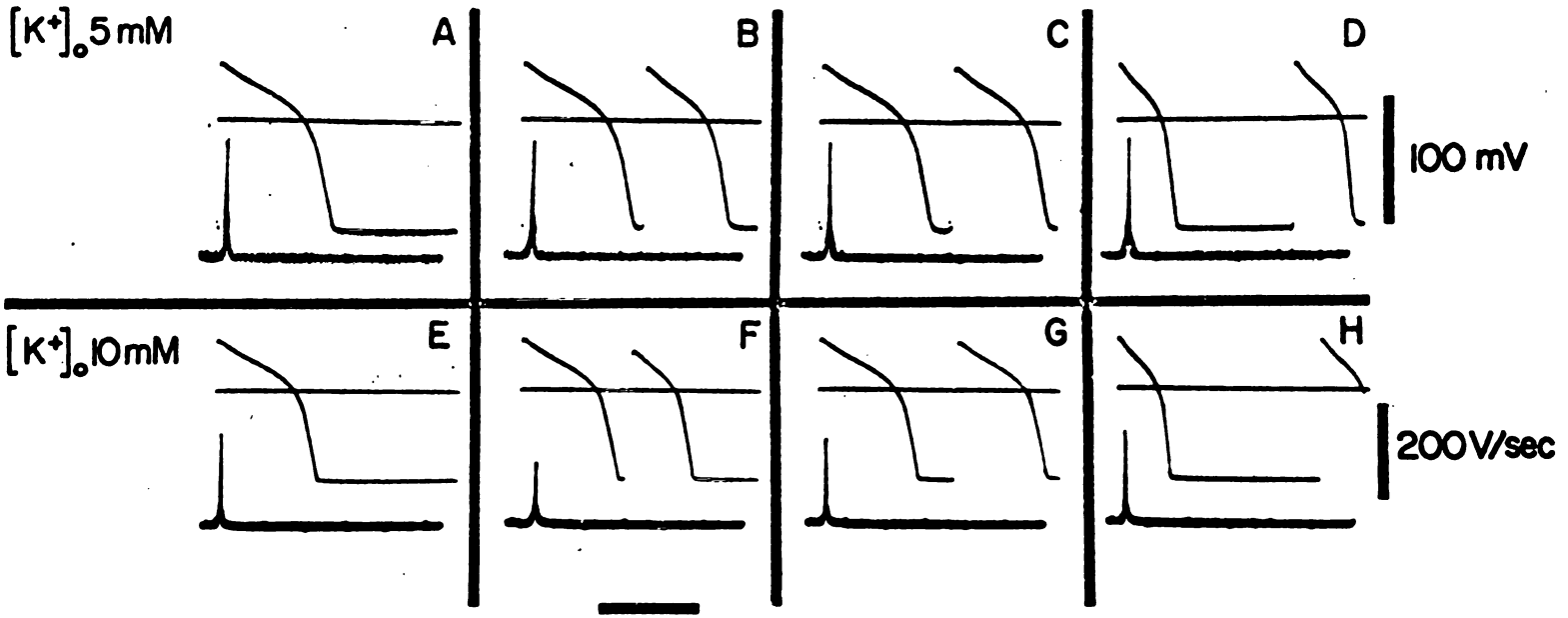


Figure 23. Recovery kinetics of dV/dT max in 5 and 10 mM K Tyrode solution (drug free). The traces are explained in figure 18. The time calibration below panel F is 200 msec for panels A - C and E - G, 400 msec for panels D and H. Traces have been retouched.

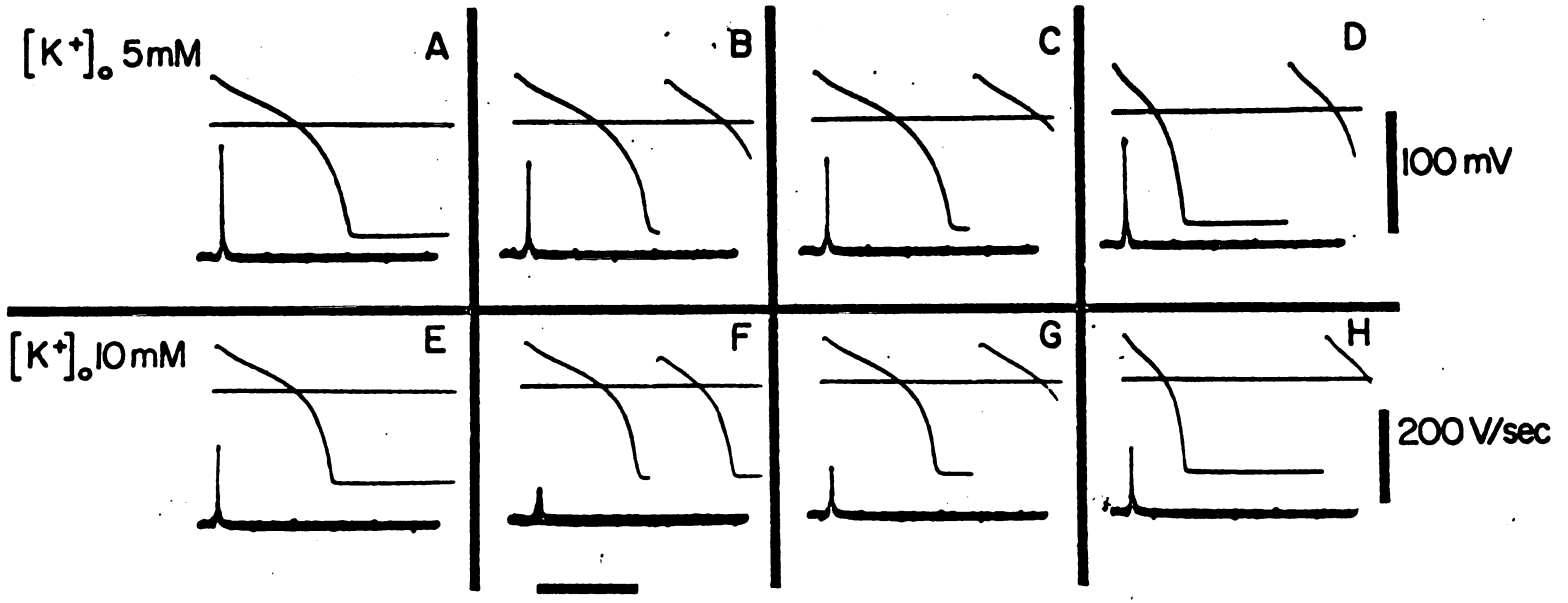


Figure 24. Recovery kinetics of dV/dT max in 5 and 10 mM K^+ Tyrode solution during exposure to droperidol (10^{-5} M). The traces are explained in figure 18. The time calibration below panel F is 200 msec for panels A - C and E - G, and 400 msec for panels D and H. Traces have been retouched.

upstroke velocity of the test responses was clearly decreased at the shorter diastolic intervals (panels B and C). At the longest diastolic interval, almost complete recovery of the test response dV/dT max had occurred. These contrast with the effects observed in 10 mM $[K^+]_o$ in the continued presence of droperidol. The resting membrane potential was decreased to -75 mV and the upstroke velocity to 165 V/sec by the higher $[K^+]_o$. The upstroke velocity of a test response at a diastolic interval similar to that in panel B was depressed to a greater degree (panel F). Even at the longest diastolic interval shown, panel H, the upstoke velocity of the test response was still appreciably smaller than that of the basic drive beat.

The results of all 4 experiments are summarized in Table VII. The time constants of recovery and intercepts were both increased when $[K^+]$ was increased from 5 to 10 mM. Although droperidol 10^{-6} M did not slow the recovery kinetics in 5 mM [K] Tyrode solution, slowing was evident in both preparations partially depolarized in 10 mM [K].

Recently Gettes and Reuter (1974) have reported that a four-fold increase in extracellular calcium concentration partially reversed the slowing of recovery kinetics observed in K^+ depolarized preparations. I was interested in ascertaining whether this partial reversal produced by calcium was specific for kinetics slowed by increased $[K^+]_o$ or whether the slowing of kinetics produced by other agents could also be reversed by calcium. I therefore increased $[Ca]_o$ to 8 mM during exposure to droperidol 10^{-5} M in 3 experiments. Increased $[Ca^{2+}]_o$ caused a small increase in the resting membrane potential (mean -90 to -92) and a decrease in upstroke velocity

TABLE VII

Effect of droperidol on the recovery kinetics of dV/dT max in 5 and 10 mM K^+ Tyrode solution.

Expt. No.	Control						Droperidol					
	5 mM K^+			10 mM K^+			5 mM K^+			10 mM K^+		
	dV/dTmax V/sec	Time Constant Msec	Inter-cept	dV/dTmax V/sec	Time Constant Msec	Inter-cept	dV/dTmax V/sec	Time Constant Msec	Inter-cept	dV/dTmax V/sec	Time Constant Msec	Inter-cept
	Control						Droperidol 10^{-6} M					
1	119.5	20	.135	98	90	.33	112	20	.135	100	125	.45
2	195	< 20		141	40	.33	207.5	< 20		126	285	.35
	Control						Droperidol 10^{-5} M					
8	241	< 20		190	75	.24	235	320	. 18	165	460	.44
9	167.5	< 20		103	77.5	.83	137.5	310	. 32	50	420	.8

TABLE VIII

Effect of increased $[Ca^{2+}]_o$ on the recovery kinetics of dV/dT max during slowing by droperidol 10^{-5} M. In the column to the far right is shown the percentage change in intercept and time constant observed on increasing $[Ca^{2+}]_o$ from 1.8 to 8 mM.

	Control			Droperidol 10^{-5} M $[Ca^{2+}]_o$ 1.8 mM			Droperidol 10^{-5} M $[Ca^{2+}]_o$ 8.0 mM			%Change	
Expt. No.	dV/dT V/sec	Time Constant Msec	Inter-cept	dV/dT V/sec	Time Constant Msec	Inter-cept	dV/dT V/sec	Time Constant Msec	Inter-cept	Time Constant	Inter-cept
6	215	< 20		195	354	.26	173	258	.175	27	33
9	167.5	< 20		137.5	320*	.41*	120	220	. 37	31	10
11	455	< 20		473	155	.38	320	115	. 29	26	24

*Time constant and intercept determined 1/2 hour after that listed in Table VI.

(mean 269 to 205 V/sec). In all 3 experiments, increased $[Ca^{2+}]_o$ decreased the recovery time constant and the intercept. The results are summarized in Table VIII.

The decrease in dV/dT max of the test response observed during control, exposure to droperidol or high K^+ Tyrode solution could result from a declining outward current activated during the preceding basic beat (Gettes and Reuter 1974). This hypothesis was tested in the experiment shown in figure 25. At varying intervals during diastole (17.5 - 135 msec measured from 4 mV of repolarization) a constant current pulse of 3.5 μA and 20 msec duration was introduced. At both the short and the long diastolic intervals respectively, (panels B and C respectively) the membrane displacement produced was 2.5 mV. This suggests that there was no appreciable change in membrane resistance. Test responses elicited at corresponding diastolic intervals had maximal upstroke velocities equal to that of the basic beat. The fibre was then depolarized in 10 mM K^+ Tyrode solution and the resting membrane potential decreased to -60 mV. Test pulses of the same strength and duration caused membrane displacements of 1.5 mV at both short and long diastolic intervals (panels H and I). This indicates that the relative membrane resistance had decreased on exposure to 10 mM K^+ Tyrode solution, and that there was no appreciable change in membrane resistance during the diastolic interval (10 - 95 msec). Action potentials elicited at corresponding diastolic intervals both had reduced upstroke velocities, indicating slowing of the recovery kinetics. Slowing of the kinetics of dV/dT max can therefore be observed without changes in diastolic resistance. Hauswirth (1968) showed that droperidol 10^{-5} and 5.10^{-5} M did not change diastolic

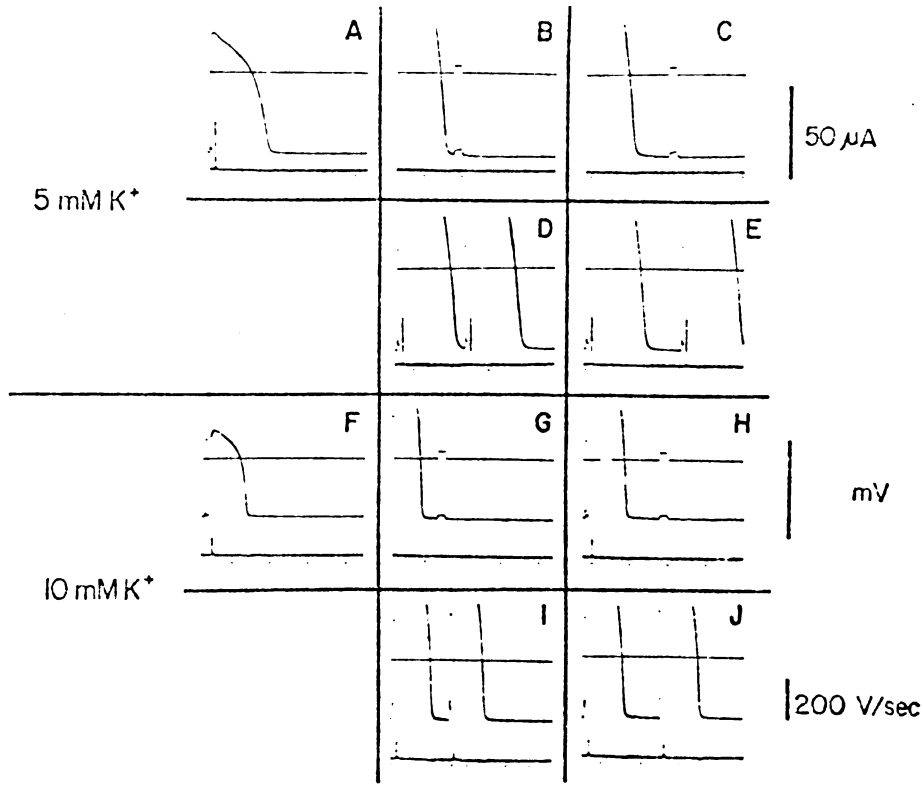


Figure 25. Relative membrane resistance during diastole in 5 mM and 10 mM K^+ Tyrode solution. The upper trace is the 0 membrane potential reference, and also displays the total current. The second trace is the transmembrane potential. The third trace is the first time derivative of the transmembrane potential. Time markers at 100 msec intervals are dislayed in the lowest trace. Panels A and F show the action potentials elicited by a brief stimulus. Panels B and C, and G and I show the terminal phase of the basic drive beat and 20 msec pulses of 3.5 uA introduced during diastole. Panels D and E, and I and J show the basic drive beat and test responses introduced at corresponding diastolic intervals. The voltage calibration is 100 mV for panels A and F, 50 mV for the remaining panels.

membrane resistance in Purkinje fibres.

Summary of the effects of droperidol on the recovery kinetics of dV/dT max:

(i) Droperidol 5.10^{-6} - 4.10^{-5} M slowed the recovery kinetics of dV/dT max markedly.

(ii) The slowing of the recovery kinetics was greater in partially depolarized preparations. A concentration of 10^{-6} M slowed the recovery kinetics only in potassium depolarized preparations.

(iii) Increasing the extracellular calcium from 1.8 to 8 mM partially reversed the slowing of the recovery kinetics produced by droperidol.

4.2b. Effects of Quinidine on the Recovery Kinetics of dV/dT max of Phase Zero of the Action Potential

I examined the effects of quinidine 1.9 - 7.6 x 10^{-6} M on the recovery kinetics of dV/dT max in 5 experiments. The results of four of these experiments are summarized in Table IX. By displaying the terminal phase of repolarization of the basic beat on an expanded time scale, I was better able to judge when complete repolarization had occurred in these experiments. This allowed the estimation of time constants of less than 20 msec in some of these preparations. Quinidine had little effect on the recovery time constans of dV/dT max. There were some changes in intercept. These concentrations of quinidine did not cause appreciable changes in upstroke velocity (see Table IX), however, increases in action potential duration of

TABLE IX

Effect of Quinidine on the Recovery Kinetics of dV/dT max

Drug Conc. M	Exp. No	$[K^+]_o$	Control			Drug		
			dV/dT V/sec	Time Constant Msec	Intercept	dV/dT V/sec	Time Constant Msec	Intercept
1.9×10^{-6}	1	5	372.5	13	.28	347.5	12.5	.41
1.9×10^{-6}	2	5	327.5	14	.36	320	22	.45
3.8×10^{-6}	3	5	167.5	22.5	.18	145	22	.28
3.8×10^{-6}	4	5	242.5	< 20		242.5	29.5	.145
1.9×10^{-6}	1	10	275	52.5	.49	197.5	42.8	.44

11-23% were observed. In the 5th experiment, quinidine 7.6 10^{-6} M was studied. The recovery time constant was less than 20 msec during control. On exposure to quinidine for 1 hour, dV/dT max of the test response was about 5% less than that of the basic drive beat and did not change appreciably with diastolic interval. No recovery time constant could be obtained.

In 1 experiment recovery kinetics were examined in 5 and 10 mM K^+ Tyrode solution. The time constants in 5 mM and 10 mM K^+ are listed in Table IX (Exp. No 1.). In 10 mM K Tyrode solution, the initial time constant of 52 msec decreased to 42.5 msec. on exposure to quinidine 1.9 10^{-6} M.

Summary of the effects of quinidine on the recovery kinetics of dV/dT max:

(i) Quinidine 1.9 - 7.6 10^{-6} M had little effect on the recovery kinetics of dV/dT max.

4.2c. Droperidol and the dV/dT max Membrane Potential Relationship

The effects of droperidol 10^{-6} - 4 10^{-5} on the dV/dT max - membrane potential relationship were examined in 5 experiments. Membrane potential was varied by increasing the $[K^+]_o$ from 5 to 20 mM. In the data presented below, dV/dT max at each membrane potential has been expressed as a fraction of that in 5 mM K^+ Tyrode solution and plotted against membrane potential. This normalizing procedure circumvents the decrease in dV/dT max produced by the drug and allowed comparison between drugs concentrations of displacements of the h curve along the voltage axis. The results of one experiment (No. 3, Table X) are depicted in figure 26. The sigmoid shape of the curves both

during control and exposure to droperidol is similar to that obtained by the "voltage clamp" technique (e.g. Weidmann 1955a) or by potassium depolarization (Gettes and Reuter 1974; Hogan and Spitzer 1975). Droperidol caused a dose dependent shift of the h curve along the voltage axis to more negative potentials. The results of all 5 experiments, including absolute values of dV/dT max, are summarized in Table X. At 10^{-6} M, voltage shifts to more negative potentials were observed in 2 of 3 experiments. Higher concentrations of droperidol consistently produced similar shifts in the h curve.

The dV/dT max - membrane potential relationship is frequently determined by introducing extrasystoles during phase 3, the period of incomplete repolarization of the action potential. This relationship is called a membrane responsiveness curve (Bigger and Mandel 1970). As I have pointed out above, such a technique does not distinguish between voltage and time dependent effects. Since droperidol has the dual effect of slowing the recovery kinetics of dV/dT max and displacing the h curve along the voltage axis, it might be predicted that it would shift the membrane responsiveness curve to a greater extent than the h curve. This hypothesis was tested in the single experiment illustrated in figure 27. During control, dV/dT max decreased at much more negative potentials along the membrane responsiveness curve than along the h curve. This suggests that other factors in addition to membrane voltage, e.g. time dependent recovery, contributed to the decrease in membrane responsiveness curve. Droperidol 10^{-5} M shifted the h curve 7.1 mV along the voltage axis (E_m 50 = 73.5 mV). In contrast, the membrane responsiveness curve was shifted along the voltage axis by 10 mV (E_m 50 = 86.5 mV).

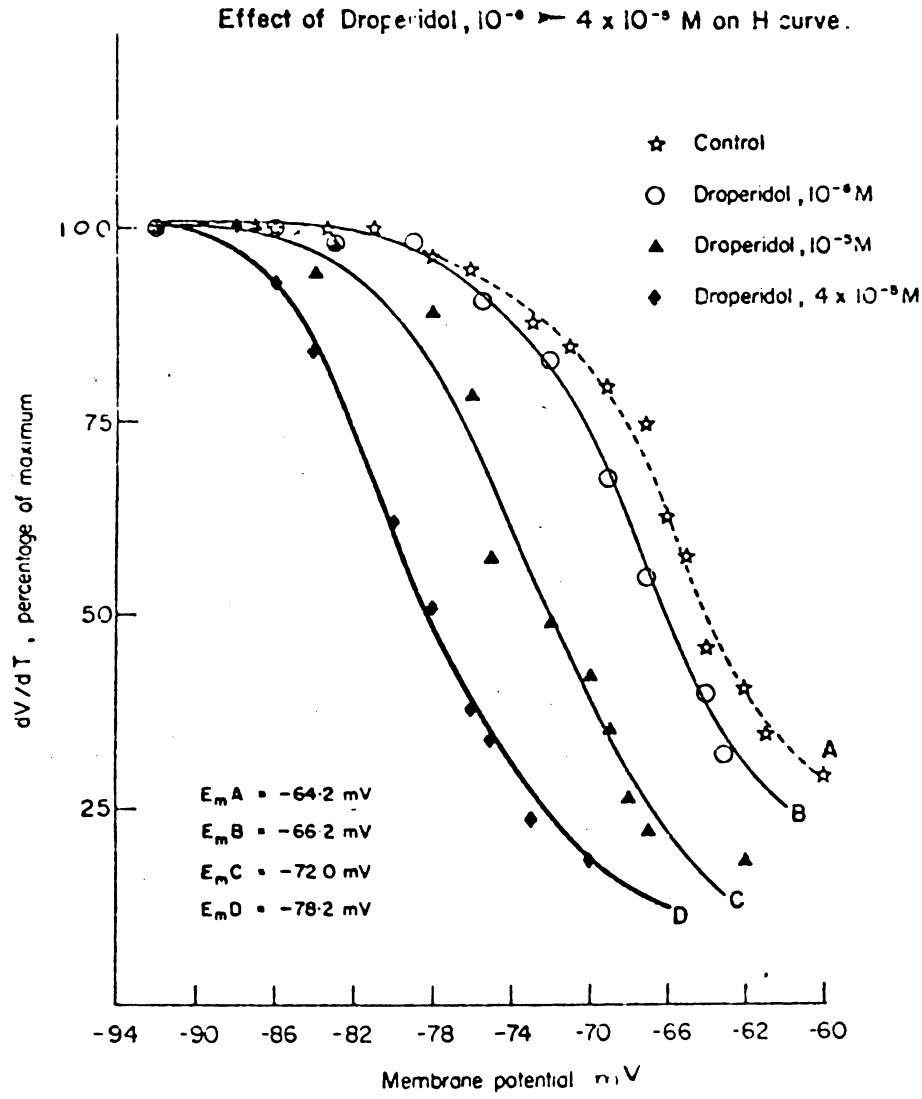


Figure 26. Membrane potential during control and drug exposure were varied by rapid depolarization in 20 mM potassium. Action potentials were obtained at decreasing membrane potentials as the preparation depolarized. dV/dT max of phase 0 at each potential was normalized by expressing it as a % of dV/dT max obtained in 5 mM potassium. The normalized dV/dT max have been plotted on the ordinate; membrane potential on the abcissa. The parameter E_m(A-D) describes the potential at which dV/dT max decreased by 50%. The drive rate was 0.2/sec.

TABLE X

The effects of droperidol on dV/dT of phase 0 of the action potential as a function of membrane potential. Em is the resting membrane potential in 5 mM [K] Tyrode solution. The parameter Em50 described the membrane potential at which dV/dT max decrease by 50%

Experiment No	Drug Conc. M	Control			During Drug		Shifts mV
		dV/dT max V/sic	Em,mV	Em50, mV	dV/dT max V/56	Em50	
1	10^{-5}	180	-82.5	-63.5	167.5	-67.8	-4.3
	2.10^{-5} M				157.5	-70.0	-6.5
	4.10^{-5} M				147.5	-72.8	-9.3
2	5.10^{-6}	215	-94	-66.4	196	-71	-4.6
	10^{-5}				196	-73.5	-7.1
3	10^{-6}	300	-92	64.2	305	-66.2	-2.0
	10^{-5}				285	-72	-7.8
	4.10^{-5}				265	-78.2	-14.0
4	10^{-6}	187.5	-86	59.5	190	60.8	-3.4
5	10^{-6}	157.5	-86	63.0	145	63.5	+.5

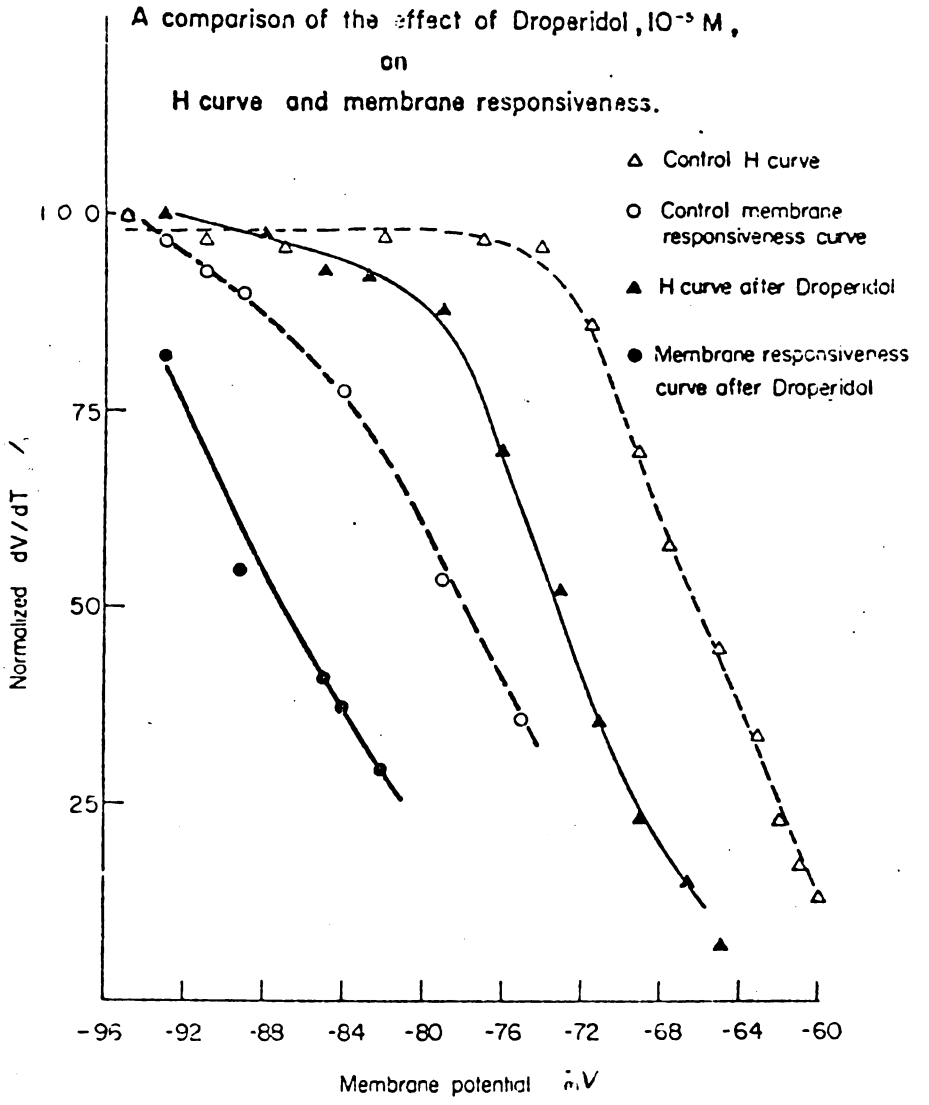


Figure 27. h curve was determined by rapid depolarization in 20 mM K^+; action potentials were obtained after every 4-8 mV of membrane depolarization. Membrane responsiveness was determined by introducing extrasystole during phase 3 of the action potential. Data from Experiment No. 2, Table X.

Summary of the effects of droperidol on the dV/dT membrane potential relationship:

(i) Droperidol 10^{-6} to 4.10^{-5} M shifted the steady state dV/dT max membrane - potential curve (h curve) along the voltage axis to more negative potentials.

4.2d. Effects of Droperidol and Quinidine on the Effective Refractory Period

In a limited number of experiments, I tried to ascertain whether the concentrations of drugs employed in the kinetics study also prolonged the ERP. The results are listed in Table XI. The small number of experiments precluded a rigorous statistical analysis at each drug concentration. Droperidol prolonged theERP in all experiments. Because the action potential duration was also increased in most of the preparations, the ERP:APD ratio was prolonged only at the high drug concentration of 4.10^{-5} M. In both experiments at this concentration, the ERP outlasted the APD by over 100 msec during exposure to droperidol. While the recovery time constants in these two experiments (295 and 325 msec) were similar to those obtained at lower drug concentrations, the intercepts .93 and .6, were the highest observed in the study.

The results of 2 experiments with quinidine 1.9 and 7.6 x 10^{-6} M are also listed in Table XI. Both APD and ERP were prolonged and there was little change in ERP:APD ratio.

TABLE XI

The effect of droperidol and quinidine on the effective refractory period. The action potential duration was measured from the onset of phase 0 to 4 mV of full repolarization. The drive rate was 1/sec; the stimulus strength 1 1/2 times threshold. The mean time constant and intercept are shown in the 2 columns to the right. N = no. of preparations.

Conditions (n)	APD msec	ERP msec	ERP/ APD	Time Constant msec	Inter-cept
Control (3)	186.3± 2.8	181.8± 2.9	.97	20	.135
Droperidol 10^{-6}M	205.4±11.2+	197.3±15.3	.97	20	.135
Control (5)	199.8±15	192.8±16	.96	24.5	. 21
Droperidol 10^{-5}M	234.5±22+	225 ±23+	.96	280	. 35
Control (2)	199.3±18	196.8±18	.98	37	. 2
Droperidol $2x10^{-5}$M	210.6±29	218 ±36	.98	237	. 5
Control (2)	201.7±15.2	197.7±17.7	.98	< 20	
Droperidol $4x10^{-5}$M	197.5±27.5	357 ±60	1.8	310	. 76
Control (1)*	177.5	172.5	.97		
Quinidine $1.9x10^{-6}$M	200	192.2	.96		
Control (1)*	193	180	.92		
Quinidine $7.6x10^{-6}$M	220	204.4	.93		

*ERP only done in these 2 experiments.

+P < .05.

4.2e. Effect of Droperidol on dV/dT max as a Function of Steady Drive Rate

From the long recovery time constants of dV/dT max which droperidol produced, it would be predicted that the increasing drive frequency would decrease dV/dT max in the presence of drug. The results of a series of experiments examining this phenomenon are listed in Table XII. At the shortest interstimulus interval of 250 msec, dV/dT max was about 15% less than that at an interstimulus interval of 5,000 msec during drug free superfusion. In the presence of droperidol, a reduction of as much as 45% was observed at the shortest diastolic intervals. The decrease was significant ($p < .05$, 2 sided paired t test) at the higher drug concentration in spite of the small number of muscles studied.

I have previously shown that two interventions modified the recovery kinetics of dV/dT max during slowing by droperidol, viz:

(i) Depolarization in 10 mM K^+ Tyrode solution potentiated the slowing of recovery kinetics.

(ii) Elevation of the extracellular $[Ca^{2+}]_o$ to 8 mM partially reversed the slowing of the recovery kinetics.

It was therefore of interest to examine the effects of these two interventions during the rate dependent decrease of dV/dT max produced by droperidol.

Figure 28 illustrates the results of an experiment during changes in $[K^+]$. In 5 mM K^+ Tyrode solution, droperidol 10^{-6} M had no effect on dV/dT max even at the shortest interstimulus interval. On the other hand, dV/dT max was decreased to a greater extent in

TABLE XII

Effect of droperidol on dV/dT of phase 0 as a function of steady drive rate. (N) = the number of preparations studied. To facilitate comparison between muscles, dV/dT during control and drug exposure at interstimulus intervals (ISI) of 1000 msec or less have been expressed as a fraction of that at 5000 msec.

Drug Conc. M (N)	ISI 5000 msec		ISI 1000 msec		ISI 750 msec		ISI 500 msec		ISI 250 msec	
	Control	Drug	Control	Drug	Control	Drug	Control	Drug	Control	Drug
10^{-6} (3)	1[=195V/s]	1[=198V/s]	.97±0	.97±0	.96±0	.98±0	.92±.03	.92±0	.85±.02	.77±.01
5.10^{-6}(2)	1[=194V/s]	1[=184V/s]	.98±00	.93±04	.97±0	.90±.05	.96±0	.85±.8	.89±.8	.58±.25
10^{-5} (3)	1[=303V/s]	1[=292V/s]	.96±00	.94±.03	.95±.03	.90±.02	.93±.02	.81±.05	.89±.00	.66±0*
2.10^{-5}(5)	1[=297V/s]	1[=276V/s]	.98±.00	.90±.03	.97±.00	.85±.03*	.96±.00	.68±.09*	.85±.03	.54±.05*
3.10^{-5}(1)	1[=435V/s]	1[=370V/s]	.97	.99	.97	.85	.96	.74		

*P < .05 (paired t test, 2 sided).

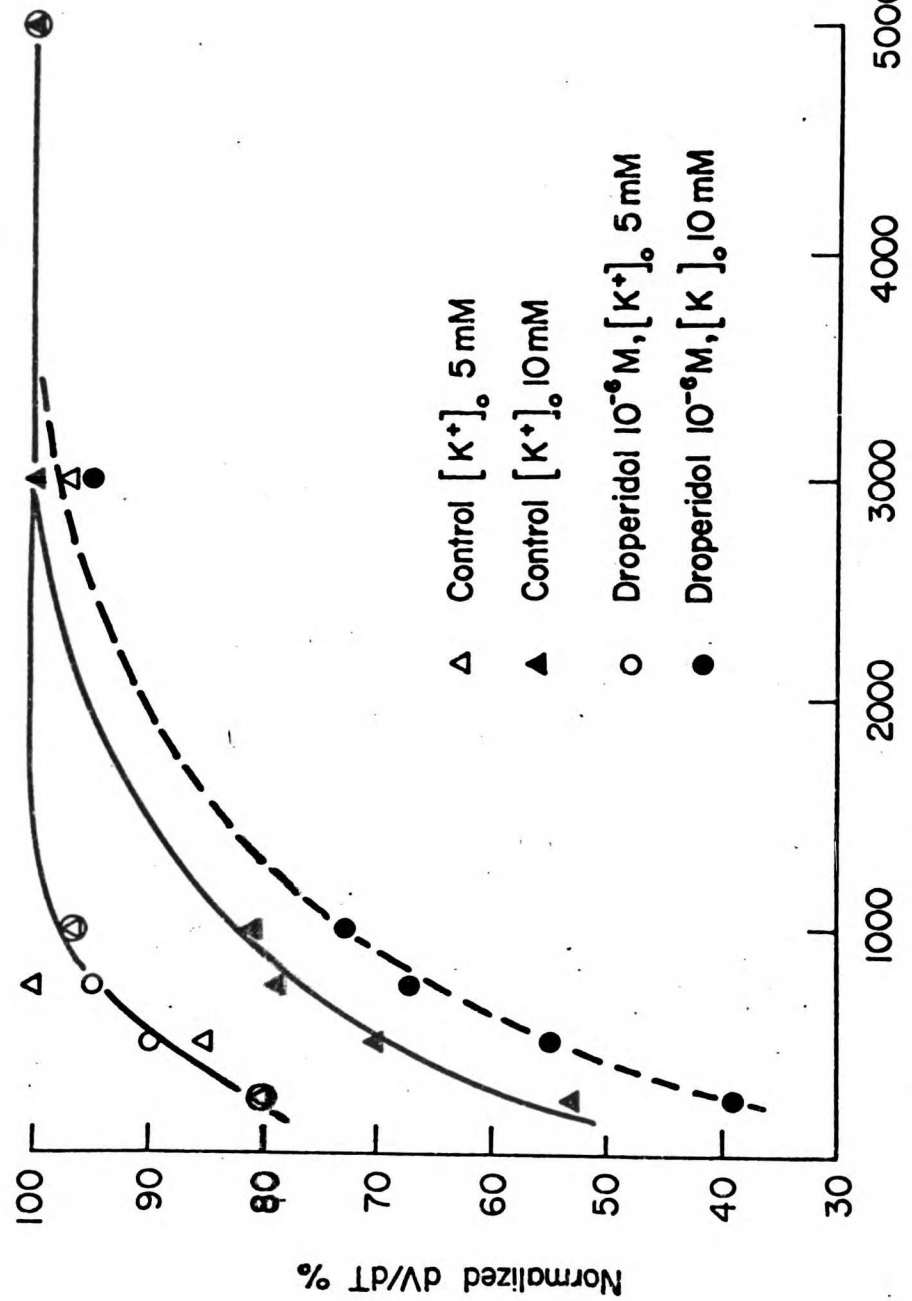


Figure 28. Effect of droperidol 10^{-6} M on dV/dT max as a function of steady drive rates in 5 and 10 mM K^+. dV/dT max during control and drug exposure have been normalized by expressing it as a % of dV/dT max at the longest interstimulus interval (5,000 msec).

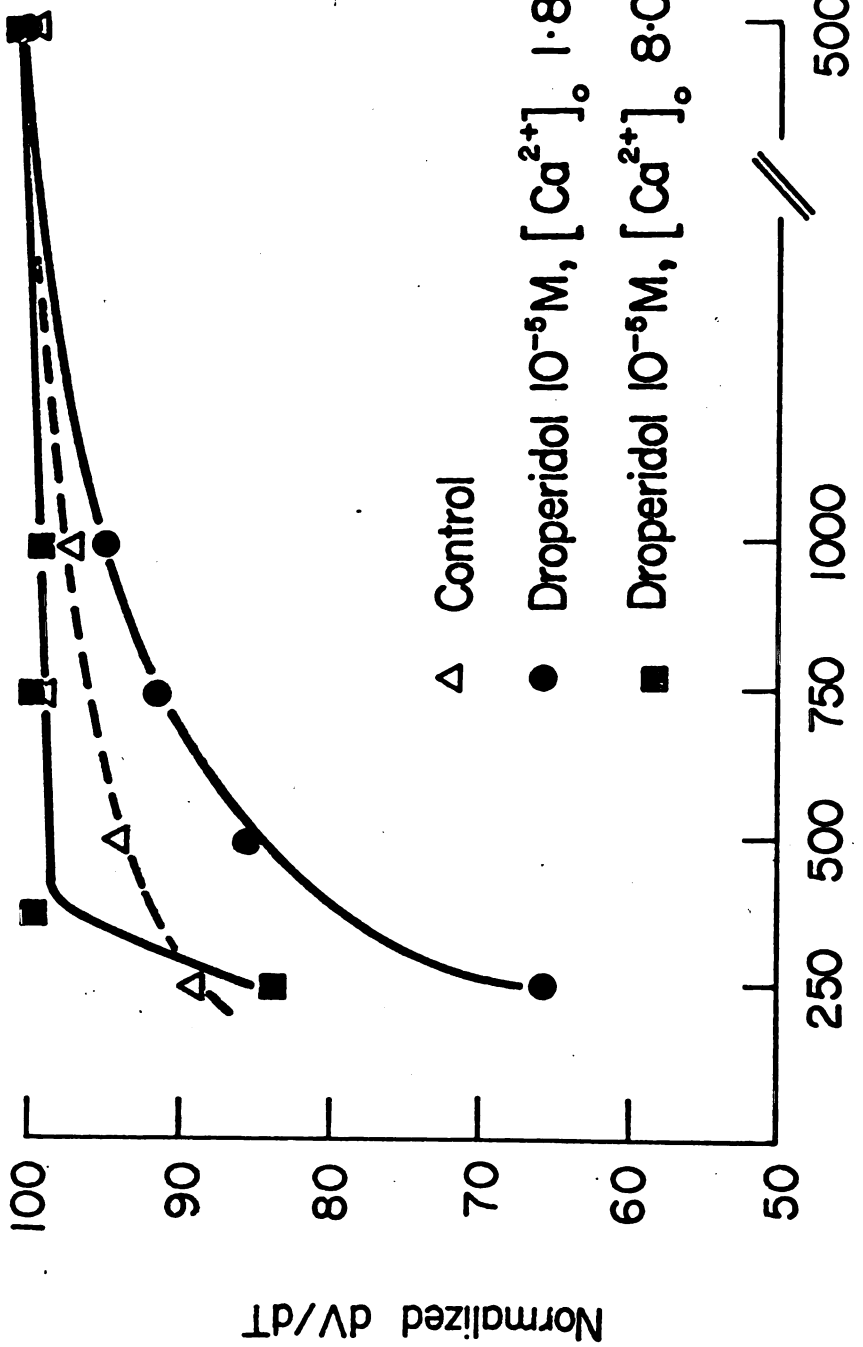


Figure 29. Effects of droperidol 10^{-5} M on dV/dT max as a function of steady drive rates in 1.8 and 8 mM Ca Tyrode.

the presence of droperidol 10^{-6} M and a $[K^+]$ of 10 mM. Similar results were obtained in 1 other experiment at this concentration. It will be recalled that whereas droperidol 10^{-6} M had no effect on the recovery kinetics of dV/dT max in 5 mM K^+ Tyrode solution, it did produce slowing in 10 mM K^+ Tyrode solution.

The single experiment in which $[Ca^{2+}]_o$ was increased to 8 mM is illustrated in figure 29. At interstimulus intervals of 1,000 msec or less, droperidol 10^{-5} M decreased dV/dT max. However, when the $[Ca^{2+}]_o$ was increased to 8 mM in the presence of droperidol, dV/dT decreased only at the shortest interstimulus interval of 250 msec. At the longest interstimulus interval of 5,000 msec, dV/dT max was 455 V/sec during control and 473 V/sec during exposure to droperidol. When $[Ca^{2+}]_o$ was increased to 8 mM in the presence of droperidol, dV/dT max decreased to 320 V/sec.

Summary

(i) Droperidol decreased dV/dT max of phase zero when the interstimulus interval was decreased to 750 msec or less.

(ii) The frequency dependent decrease of dV/dT max was exaggerated in potassium depolarized preparations (2 experiments) and partially reversed by increasing $[Ca^{2+}]_o$ (1 experiment).

5. DISCUSSION

5.1. Automaticity

5.1a. The Experimental Approach

Since the validity of any study is dependent on the methodology used, this shall be discussed first. Major aspects of the methods used in studying automaticity were (a) identity of the cells studied (b) electrotonic conduction of diastolic activity from adjacent or distant cells (c) nature of the depolarizing current(s) during phase 4.

Were the cells studied true ventricular myocardial fibres? Hecht (1965) has suggested that the demonstration of spontaneous rhythmicity in ventricular myocardial preparations is "suspicious of the presence of Purkinje tissue in the sample". Although the automaticity which was examined in the present study is clearly not spontaneous, Hecht's point is still relevant. Papillary muscles do contain Purkinje fibres. However, in the majority of mammals examined to date, Purkinje fibre are either sparse or absent from the terminal 1 - 2 mm of the papillary muscle (Draper and Maya-Tu 1959; Hondeghem and Stroobandt 1974). Further, the fibres that were impaled in the present work could be identified as true ventricular myocardial fibres by 2 absolute criteria:

(i) A stable membrane potential during diastole.

(ii) A plateau in the positive range of membrane potential (Hoffman and Cranefield 1960).

Every fibre reported on in this study satisfied these criteria. In addition, the great majority of fibres had a maximum upstroke velocity of less than 500 V/sec. However, occasionally fibres showed maximum

upstroke velocities of as much as 600 V/sec. This rate of rise was still in the linear range of the differentiator and in at least 1 experiment, the high rate of rise was verified by direct measurement of phase 0 on an expanded time scale. At no time was it necessary to terminate an impalement because it did not satisfy the absolute criteria. For example, spontaneous diastolic depolarization was never observed in an impaled fibre. A selection of an appropriate cell was therefore not necessary during the experimental protocol.

Automatic depolarization recorded from a cell identified as being a true ventricular fibre may result from activity in a neighbouring Purkinje or ventricular fibre. This consideration is related to the broader question of spatial uniformity in the preparations used. Spatial uniformity can be documented only by recording from multiple cells simultaneously in every preparation studied (Johnson and Lieberman 1971; Tarr and Trank 1974). New and Trautwein (1972), Reuter (1973), and Morad and Trautwein (1968) have shown by means of such double impalement experiments that in suitably selected papillary muscles, current spread and voltage control during voltage clamps (appropriate to predicted cable properties) is homogenous during all parts of the cardiac cycle except the phase of rapid sodium current. Therefore, I selected only those preparations which were likely to have spatial uniformity, namely:

(i) Preparations that were free of branching.

(ii) Limitation of the segment in the test compartment to about 1-1.5 mm.

In spite of this, occasional records (e.g. Figure 5D to I and Figure 6) showed evidence of non-uniformity. It must be pointed out that of

the muscles reported in the present study, those illustrated in Figure 5D to I and Figure 6 showed the greatest evidence suggestive of non-uniformity.

There is enough data available to make a "reasoned guess" regarding the effects of spatial non-uniformity. Consider a non-uniform preparation in which the impaled cell is quiescent, while a cell at some distance develops a regenerative action potential. Further, the following two assumptions are made:

(i) The action potential in the distal cell may be approximated by square wave.

(ii) The preparation in the test compartment can be approximated by a simple cable.

Then in such a model, the theoretical analysis and experimental observations indicate that the voltage change in the impaled cell is convex upwards (Hodgkin and Rushton 1946). The first assumption is not critical. With regard to the second, the tip of the papillary muscle in the test compartment is clearly not a simple one dimensional cable. Nevertheless, in this study, the voltage displacement recorded on application of a subthreshold pulse of constant current had a rising phase which was <u>always</u> convex upwards. It is reasonable to conclude that during phase 4, activity in a distant cell would be recorded as a voltage displacement convex upwards. In the present experiments, diastolic depolarization was either rectilinear or concave upwards. In a few experiments, e.g. Figure 6C, phase 4 was interrupted by propagated activity in the form of a convex upward voltage displacement. The electrotonic activity formed the foot of the ensuing action potential. As spatial uniformity decreases, such electrotonic

interactions should occur earlier during phase 4 and produce larger voltage displacements. In the worst case, membrane voltage during phase 4 should be entirely convex upwards. This was never observed in the present study. Nevertheless, a minor contribution of spatial non-uniformity to the diastolic depolarization observed in the present experiments cannot be totally excluded at this time.

Automatic depolarization in response to applied current, is not specific for the ventricular myocardium of guinea pig. Similar activity has been induced in ventricular myocardial fibres of rabbit, dog, and the cat (Katzung unpublished observation; Grant unpublished observation). However, others have suggested that automaticity cannot be induced in sheep myocardium by depolarizing current applied by means of a microelectrode (Trautwein and Kassebaum 1961).

In the previous reports of Katzung (1974a, b) and in this study, automaticity was induced by injecting current across a sucrose gap. The available evidence indicates that the induction of automaticity in ventricular myocardial fibres is not specific for this technique. I have already alluded to the simple experiments of Foster and Dew-Smith (1876) in which repetitive activity was induced by applying the output of electromotive cells across a strip of frog ventricular myocardium. Imanishi and Surawicz (abstracts 1974) induced automaticity in guinea pig ventricular myocardium by applying current across two electrically isolated chambers. There are some qualitative differences between the repetitive activity observed in this laboratory and that reported by Imanishi and Surawizc. However, these differences appear to be predictable on the basis of the different modes of current

application and do not suggest any major difference in interpretation of results.

It is concluded that the methodology is a valid experimental approach to the study of automaticity in true ventricular myocardial fibres.

5.1b. Possible Mechanisms of Electrically Induced Automaticity and the Action of Antiarrhythmic Drugs

The ionic mechanisms underlying automaticity have been defined in some detail in Purkinje and atrial fibres. In these tissues, phase 4 depolarization is dependent on:

(i) Deactivation of a slow outward potassium current.

(ii) A steady background inward current (Deck and Trautwein 1964; Vassalle 1966; Noble and Tsien 1968; Brown and Noble 1969; Lenfant et al., 1972).

Diastolic depolarization reduces the membrane potential to the threshold of an inward current component and a regenerative action potential ensues. At low resting potentials, a threshold may not exist and activity is essentially oscillatory, being dependent on (i) and (ii) above (Hauswirth et al., 1969; Cranefield 1975).

A similar analysis of the mechanisms underlying automaticity in ventricular fibres has yet to be performed. However, it is useful to examine the extent to which current components analogous to those mentioned above, have been identified in ventricular myocardial fibres in general and specifically those of the guinea pig.

Outward Currents: Outward current may be species specific. Ochi (1970) described a slow outward current in the ventricular myocardium of the

guinea pig. The current was activated at potentials positive to -10 mV and had a reversal potential between -60 to -80 mV (data from a fibre with a resting potential of -84 mV). A reversal potential positive to the resting potential would explain why this current would not lead to automaticity under ordinary circumstances. The results have been negative or equivocal in the sheep, calf, and rabbit ventricle (Giebisch and Weidmann 1971; Harrington and Johnson 1973; McGuigan 1974). On the other hand, an outward K^+ current has been identified in dog myocardium (Beeler and Reuter 1970). In the discussion of his paper, McGuigan (1974) points out that outward K^+ current can lead to automaticity and in the cat ventricular myocardium in which he could identify an outward K^+ current, automaticity was observed. The absence of a detectable outward K^+ current in sheep may be relevant to the failure of Trautwein and Kassebaum (1961) to induce automaticity in this tissue.

Background Inward Current: During depolarization induced automaticity the current from the external source (clamp amplifier) provides at least a part of the background current. Katzung (Circ. Res in press) has examined the effect of changes in $[Ca^{2+}]_o$ and $[Na^+]_o$ on phase 4 slope and action potential amplitude during ventricular automaticity. Decrease of $[Ca^{2+}]_o$ 1.8 to 0.45 mM decreased phase 4 slope. On the other hand, elevation of $[Ca^{2+}]_o$ from 1.8 to 5.4 mM increased phase 4 slope in 1/2 of the muscles studied and decreased it in the remainder. Reduction of $[Na^+]_o$ by 75% reduced phase 4 slope at all MDPs. One interpretation is that both Na^+ and Ca^{2+} contribute to the background current. Alternatively, Na^+ and Ca^{2+} may modulate the conductance of channel(s) responsible for the background current.

Spike Electrogenesis: Inward currents which could be responsible for regenerative spikes have been well identified in ventricular myocardial fibres (for review, see Reuter 1973; Trautwein 1973). At more negative MDPs, the spike could be generated by the transient inward Na current responsible for phase 0 of the normal action potential. At less negative MDPs, inactivation of the sodium current is almost complete and the slow inward Ca current is probably responsible for spike electrogenesis.

In guinea pig ventricle, the amplitude of the fast spike may also be Ca dependent (Coraboeuf and Otsuka 1956; Stanley and Reiter 1965). The results of Katzung (cited above) also suggest dependence of the amplitude of fast spikes on $[Ca^{2+}]_o$. The experiments of Ochi (1970), showed that the slow inward current is activated at about -40 mV in guinea pig ventricle. The spike could therefore only be totally dependent on the slow inward current in this range of MDPs where slowly rising APs are observed. Katzung's results also indicate some Na dependence of action potentials arising from low MDPs. Possible explanations are:

(i) Wide ion selectivity of the slow channel (Ochi 1970; Reuter 1973).

(ii) A current component through another channel (e.g. Mclean et al., 1974).

There is no information on the possible role of active transport in electrically induced automaticity. Experiments with digitalis glycosides and other transport inhibitors would be of great interest.

The possibility of the decline in K conductance being totally voltage dependent also exists. To exclude this possibility, the voltage and time dependence of the changes in permeability will have to be separated by voltage clamp analysis.

Effects of Antiarrhythmic Drugs

Verapamil: The major effect of verapamil was a reduction of the amplitude and overshoot of APs arising from low MDPs. As indicated in Table III, automatic activity was observed in the decades of MDP -29 to -20 mV and -19 to -10 mV during control in some preparations. However, automaticity was abolished in these decades during exposure to verapamil 2.5 mg/L in all preparations. There was a reduction of phase 4 slope particularly in action potentials arising from low MDPs. In some muscles, activity was abolished at all MDPs by verapamil. It is important to note that this includes action potentials arising from high MDPs and having fast upstroke velocities (e.g. figure 10E). The overall effect of verapamil were more marked than Table III would suggest as the latter includes data only from those muscles in which automaticity persisted during exposure to verapamil.

In voltage clamped feline ventricular myocardium, the major effect of verapamil was a reduction of the slow inward calcium current (Kohlhardt et al., 1972). My own observation of a marked suppression of the amplitude of action potentials arising from low MDPs, support the hypothesis that such action potentials are dependent of the slow inward current.

I attempted to reverse the effect of verapamil by two interventions which increase the slow inward current, namely increased

$[Ca^{2+}]_o$ and epinephrine (Reuter 1973). For the action potential elicited by a brief stimulus, increased $[Ca^{2+}]_o$ in the presence of verapamil increased action potential amplitude, but decreased the plateau duration. Increased $[Ca^{2+}]_o$ produces similar effects in the absence of verapamil (Hoffman and Cranefield 1960; Stanley and Reiter 1965). In the experiments of Stanley and Reiter, the decrease in plateau duration in high $[Ca^{2+}]_o$ was associated with an increase in contractility. An intervention which increased the slow inward current only would be expected to increase the plateau duration (Reuter 1973; Gettes and Reuter 1974). This suggests that increased $[Ca^{2+}]_o$ had effects other than increasing the slow inward current e.g. an increase in K^+ conductance Isenberg (1975). During depolarization induced automaticity, increased $[Ca^{2+}]_o$ in the presence of verapamil restored action potential amplitude towards control, but further decreased phase 4 slope. If increased $[Ca^{2+}]_o$ is assumed to increase action potential amplitude by increasing the slow inward current, the reduction of action potential amplitude which verapamil produced might be dependent on blockade of the slow inward current. The further reduction of phase 4 slope might be accounted for by:

(i) A continuing effect of verapamil in spite of increased $[Ca^{2+}]_o$.

(ii) Increased $[Ca^{2+}]_o$ to 7.2 mM, independent of the presence of verapamil may reduce diastolic depolarization rate.

(iii) The effect of verapamil in diastolic depolarization rate may not be dependent on slow inward current blockade.

As a steady state was not reached at a time when $[Ca^{2+}]_o$ was increased in the presence of verapamil, the first possibility is a real one. The second possibility may also be contributory in some muscles as Katzung (cited above) observed a decrease in phase 4 slope in 1/2 the muscles studied when $[Ca^{2+}]_o$ was increased from 1.8 - 5.4 mM. The results in other excitable tissues provide evidence for and against the third possibility. This shall be discussed in more detail below. The important point to note, however, is that increased $[Ca^{2+}]_o$ did not increase phase 4 slope towards control in the continued presence of verapamil.

The addition of epinephrine during exposure to verapamil was associated with an increase in both the amplitude and plateau duration of the action potential elicited by a brief stimulus. This suggests that the concentration of epinephrine used, 10^{-5} M, was enough to increase the slow inward current (Reuter 1974). During depolarization induced automaticity, epinephrine reversed the effect of verapamil on phase 4 slope, but did not restore action potential amplitude. Epinephrine and increased $[Ca^{2+}]_o$ therefore having differing effects on action potential amplitude during ventricular automaticity in the presence of verapamil. The mechanism by which epinephrine and increased $[Ca^{2+}]_o$ increase the slow inward current are different. The magnitude of the slow inward current is given by the product of an activation variable, an inactivation variable, a limiting conductance $\bar{g}_{ca}$ and a driving force $\{E - E_{ca}\}$; E being the membrane potential, E_{ca} the reversal potential of calcium (Bassingthwaighte and Reuter 1972). Epinephrine increases the limiting conductance

variable $\bar{g}_{ca}$ while increased $[Ca^{2+}]_o$ increases the driving force $\{E - E_{ca}\}$. However, the differences in the effects of these two interventions may relate to other actions. Epinephrine consistently shifted the range of potentials at which automaticity was observed to more negative MDPs e.g. figures 8L and 10I. As a result of this shift, automaticity was not observed in the range of MDPs in which the slow inward current should be activated. Recently, Freeman and Turner (1974) have reported that concentrations of epinephrine and isoproterenol which increase phase 4 slope also decrease overshoot in sinoatrial fibres of rabbits.

Singh and Vaughan Williams (1972) have reported that the effects of verapamil 10^{-7} - 10^{-5} M on the action potential characteristics of atrial and ventricular fibres are different from those of most antiarrhythmic drugs. They speculated that the antiarrhythmic effects of verapamil were dependent on blockade of a calcium dependent "aberrant spike". Slowly conducted regenerative responses arising from low membrane potentials have been described in Purkinje fibres (Carmeliet and Vereecke 1969; Verdonck and Carmeliet 1971; Cranefield et al., 1972; Wit et al., 1972a, b; Aronson and Cranefield, 1973, 1974), ventricular muscle (Reuter and Scholz 1968; Mascher 1971) and atrial muscle (Pappano 1970; Thyrum 1974). These slow responses are though to depend on a Ca current through the "slow channel". Normal activity in the sinus and A - V nodes is also believed to be slow channel dependent (Lenfant et al., 1968, 1972; Lu and Brook 1969; Zipes and Mendez 1973).

Verapamil and its methoxy derivative, D-600, block (i.e. reduce the amplitude) of the slow response in Purkinje fibres

(Aronson and Cranefield 1973; Cranefield et al., 1974), atrial muscle (Thyrum 1974) and normal activity in the sinus and A - V nodes (Wit and Cranefield 1974). My own results indicate that similar responses induced by depolarizing current in ventricular myocardial fibres are also suppressed by verapamil. Similar results in ventricular myocardial fibres have recently appeared in abstract form (Imanishi and Surawicz 1975). Together, they support the hypothesis that slow response activity is "slow channel" dependent, i.e. Ca dependent, in all parts of the heart.

In common with most clinically effective antiarrhythmic drugs, verapamil decreases the slope of phase 4 in Purkinje fibres (Rosen et al., 1974) and sinus and A - V nodal fibres (Wit and Cranefield 1974; Tritthart et al., 1971). The present study shows that verapamil reduces phase 4 slope in ventricular fibres during electrically induced automaticity. However, it also suggests that this effect of verapamil is not dependent on blockade of the slow inward current. Firstly, reduction of phase 4 slope is also observed at potentials negative to that required for activation of the slow inward current. Secondly, blockade of the slow inward current by verapamil is completely reversed by elevation of $[Ca^{2+}]_o$ (Kohlhardt et al., 1972). Elevation of $[Ca^{2+}]_o$ further reduced phase 4 slope in my experiments. Similar reduction has been observed both in vitro (Purkinje fibres of dog - Rosen et al., 1974) and in vivo (SA node - Zipes and Fischer 1974). On the other hand, partial reversal by increased $[Ca^{2+}]_o$ has been reported in Purkinje fibres (in Na free Ca rich solutions Cranefield et al., 1974) and for pacemaker activity in guinea pig taenia coli (Riemer et al., 1974).

Quinidine

The major effect of quinidine was a reduction of phase 4 slope at most MDPs. Some reduction of action potential amplitude at more negative MDPs was also observed. Compared to verapamil, quinidine has been shown to produce a general rather than a specific reduction of the various ionic currents under voltage clamp conditions in frog atria (Driot and Garnier 1972; Ducouret et al., 1972). In these studies quinidine exerted a prominent effect on the kinetics of both the inward and outward currents. The decrease in phase 4 slope which I observed could be the result of a slowing of the deactivation kinetics of an outward potassium current. The reduction of the amplitude of the fast response may be a sodium dependent effect. Quinidine decreases the upstroke velocity of the normal action potential. This reduction of dV/dT max is accompanied by little change in overshoot. On the other hand, the decrease in overshoot of automatic action potentials arising from high MDPs was about 25%. However, the lower MDPs from which automatic action potentials arise may account for this difference.

While the effects of therapeutic concentrations of quinidine on Purkinje and sinus node fibres have been well described (Hoffman 1958; West and Amory 1960; West 1972), this is the first detailed description of similar effects in ventricular myocardial fibres. Ventricular fibres show repetitive activity on exposure to aconitine. Typical diastolic depolarization is sometimes observed under these circumstances (Heistracher and Pillat 1962). These authors showed that quinidine transiently decreased discharge frequency in aconitine poisoned fibres. Quinidine also suppresses the

repetitive activity induced by depolarizing current in frog atrium (Ducouret et al., 1972). It appears that while the specific ionic currents underlying automaticity in the various types of cardiac fibres may differ, they have similar susceptibilities to anti-arrhythmic drugs.

Epinephrine reversed the depressant effects of quinidine at most MDPs. The most prominent effect was on diastolic depolarization rate. This restorative action of epinephrine may be dependent on an effect of potassium conductance as suggested by Tsien and discussed above. Epinephrine reversal of the effect of quinidine on phase 4 slope is consistent with previous work. Matsumura and Takaori (1959) showed that epinephrine restored activity in quinidine arrested rabbit atria. Similarly Gottsegen and Ostor (1963) and Finnegan and Trounce (1954) have reported that sympathomimetic amines reverse the cardiotoxic effects of quinidine in vivo.

Droperidol

This drug reduced diastolic depolarization in all ranges of MDPs. At most MDPs, there was little change in action potential amplitude. The suppression of diastolic depolarization was most marked at the more negative MDPs. In some preparations (e.g. figure 15), automaticity was abolished in the more negative range. In one experiment in which automaticity was abolished at the more negative MDPs, a brief (2.5 msec) pulse superimposed on the long constant current pulse elicited a regenerative response. This suggests that the abolition of action potentials at the more negative MDP is the results of an effect on the prepotential generating mechanism rather than the spike generating mechanism.

In most droperidol experiments, less current was required to achieve a given MDP during drug exposure. This suggested an alteration in overall membrane current voltage relationship. I explored this relationship in a semiquantitative fashion by measuring the relative membrane resistance. (Reference is also made to this technique in the section on refractoriness). In a space clamped preparations, membrane resistance at two points in time or at two different potentials can be compared by the ratio of the amplitude of the voltage displacement, ΔV, which small constant current pulses produce. The square of the ratio of the voltage displacement is proportional to the ratio of the resistances (Hodgkin and Huxley 1947). When 1 μA 50 msec constant current pulses were superimposed upon the longer current clamp pulses during the survey of automaticity, the voltage displacement was always larger at the reduced MDPs following the 1st (elicited) AP than during the resting state of more negative membrane potentials. This suggests significant anomalous (inward) rectification. However, the ΔV during the resting state and the long pulse was unchanged by exposure to droperidol over the limited range of potentials studied. The negative result may in part reflect the low sensitivity of the technique.

There are no data of the effects of droperidol on the specific ionic conductance in ventricular myocardial fibres. In fact a recent paper was suggested that droperidol 10^{-5} M M has no significant effect on the action potential canine ventricular myocardium (Wojtczak and Beresewicz 1974). The absence of any consistent effect on AP amplitude during ventricular automaticity in my experiment suggest that in the concentrations used (5.10^{-6} - 10^{-5} M)

droperidol did not markedly suppress the spike generating mechanism(s). On the other hand, the marked effect of the drug on diastolic depolarization rate suggests an effect of potassium conductance.

Wojtczak and Beresewicz (cited above) have reported that droperidol suppressed automaticity induced by stretch in Purkinje fibres. The suppression of automaticity in both Purkinje and myocardial fibres confirms a suggestion of Bertolo et al., (1972) that at least some of the cardiac effects of droperidol may be direct.

As with verapamil and quinidine, the effect of droperidol on diastolic depolarization rate are reversed by epinephrine.

Significance

Hoffman and Cranefield (1960) have state that ventricular arrhythmias during myocardial infarction do not arise in ventricular myocardial fibres, but in the terminal Purkinje network. It has been stated (e.g. Hoffman 1966) that certain arrhythmias during myocardial infarction can arise as a result of a "current of injury" between relatively normal and damaged cells. The ST segment changes observed in the electrocardiogram during myocardial ischaemia are thought to arise from the injury current (Goldman 1967). Using the D - C magnetocardiogram, Cohen and Kaufman (1975) have confirmed the association between myocardial ischaemia, current of injury and ST segment changes in the E.K.G. Such currents of injury would enhance pacemaker activity in Purkinje fibres. Similarly, Sano and Sawanobori (1972) have shown that the demarcation current which results from marked temporal dispersion can lead to repetitive activity in Purkinje fibres.

The recent demonstration of depolarization induced auto-

maticity in ventricular myocardial fibres (Katzung 1974a, b; Surawicz and Imanishi 1974; Imanishi and Surawicz 1974) suggest that these fibres can also be a site of ectopic activity. Injury currents from damaged fibres could lead to depolarization and repetitive activity in true ventricular myocardial fibres in the normal zone. Such automaticity could provide a focus of parasystole, lead to conduction disturbances, or trigger re-entrant excitation. Solberg et al., (1972; see also figure 6 of Wit and Friedman 1975) have shown that repetitive activity with phase 4 depolarization occur in ventricular myocardial fibres of dog following acute coronary ligation. Katzung et al., (submitted for publication) have shown that internally generated current of injury can lead to repetitive activity in guinea pig papillary muscles.

Drugs could abolish such repetitive activity by:

(i) Increasing the rate of the normal pacemaker to a rate greater than that of the ectopic pacemaker, i.e. overdrive suppression; or

(ii) Suppression of the ectopic focus to a greater extent than the sinus node such that the sinus node maintains dominance.

Each mechanism demands a different sensitivity to drug effect between sinus and the ectopic automatic focus. With regard to differential effects at the two sites, autonomic innervation is crucial. It is clear that the sinus and A - V nodes have a more abundant innervation than the ventricular myocardium (Anderson and del Castello 1972). Therefore, drugs possessing direct and autonomic effects can lead to a differential effect on the nodes and working myocardium.

My results indicate that verapamil, quinidine and droperidol can suppress depolarization induced automaticity in ventricular myocardial fibres. The critical question is whether the concentrations at which these effects were observed is less than that required to abolish sinus node automaticity. I cannot give a direct answer to this question. The concentration of quinidine gluconate that I used, 2 - 4 mg/L provides a lower concentration of free base compared to that which suppresses sinus node automaticity (6 mg/L of quinidine sulphate, West and Amory 1960), and is similar to the therapeutic concentration of free base. In fact, therapeutic concentrations of quinidine may actually lead to an acceleration of sinus rate by an antivagal action (Goldberg 1974). The concentrations of verapamil employed (1 - 5 mg/L) were much higher than that which suppresses automaticity in the sinus node of rabbit (0.1 - 1 mg/L). 1 mg/L of verapamil almost totally abolished sinus node activity in vitro (Wit and Cranefield 1974). On the other hand, only bradycardia is observed when verapamil 5 mg/L is injected into the sinus node artery of dog in vivo (Zipes and Fischer 1974). The in vivo therapeutic concentration range of verapamil is not clearly defined. The pharmaco kinetic of verapamil are unusual in that the drug disappears from the circulation within minutes of administration, but its therapeutic effect may last for several hours (Krikler 1974). There are no data on the direct effects of droperidol on sinus node rate.

Two other points may be of clinical relevance. Krikler (1974), in a recent clinical review, suggested the use of calcium gluconate or catecholamine in the treatment of verapamil induced

bradycardia or asystole. My results and those of Zipes and Fischer (1974) cast doubt on the effectiveness of calcium. However, the results lend weight to the possible effectiveness of sympathomimetic amines. The second point relates to possible drug interactions. Droperidol is frequency used in conjunction with fentanyl for neuro-lepanalgesia in poor risk surgical patients. The incidence of cardiac disease and therapy with antiarrhythmic drugs such as quinidine may be high in these patients. The demonstration of suppression of automaticity by droperidol in this study suggest the need for further study of potentially dangerous drugs interaction which may occur in such patients.

5.2. REFRACTORINESS

5.2a. The Methodology

The major objectives in this section of the research project were twofold:-

(i) Firstly, to determine the effects of droperidol and quinidine on the time dependent recovery of the rising phase of the action potential.

(ii) Secondly, to relate any such changes to alterations in the effective refractory period and rate dependent of dV/dT max.

Hodgkin and Katz (1949) showed that in the squid giant axon, dV/dT max is a good index of the increase in sodium conductance during the rising phase of the action potential. The early experiments of Draper and Weidmann (1951) confirmed the dependence of dV/dT max and overshoot of the cardiac Purkinje fibre action potential on $[Na^+]_o$. Initial studies of ventricular myocardial fibres of guinea pig suggested that this tissue might be an exception. Coraboeuf and Otsuka (1956) showed that the overshoot of the action potential recorded from guinea pig papillary muscle did not depend on $[Na^+]_o$. With $[Na^+]_o$ of 0 mM, the overshoot decreased only when a decline of resting membrane potential was observed. Subsequent studies of Deleze (1959) confirmed the lack of dependence of AP overshoot on $[Na^+]_o$ in this tissue. However, this study clearly showed that dV/dT max of phase 0 was a function of $[Na^+]_o$. Therefore, in guinea pig ventricular myocardium, AP overshoot does not depend on $[Na^+]_o$, but dV/dT max does and is probably a good index of sodium conductance.

I used a twin pulse technique to study the time dependent recovery of dV/dT max. The technique was initially introduced by Hodgkin and Huxley (1952a). They showed that the time constant of Na reactivation obtained by the twin pulse clamp technique was similar to that obtained by voltage clamping with conditioning steps. The technique has subsequently been applied to both clamped and unclamped cardiac tissues by a number of investigators e.g. Haas et al., 1971; Reuter et al., 1972; Strauss and Bigger 1972. In non-voltage clamped preparations, the technique is fairly straightforward when the intervals between the basic drive action potentials and test responses are long. However, at short diastolic intervals (< 10 - 20 msec) certain difficulties arise. Firstly, it is difficult to judge precisely the time required for complete repolarization. In order that time should be the only factor determining changes in dV/dT max of the test response, the latter must be elicited from the completely repolarized membrane. I therefore elicited test responses only at diastolic intervals at which repolarization was clearly complete. The diastolic interval was measured from 4 mV of repolarization to the resting potential of the basic beat to the onset of phase 0 of the test response. The points at the outset and termination of the diastolic interval could be measured down to a minimum of 2.5 msec. At short diastolic intervals, the complication of changes in latency also arose in some experiments. I have previously discussed the adjustments which were made to minimize same.

In two sets of experiments, I used increases in $[K^+]_o$ to decrease membrane potential. Recovery kinetics of dV/dT max were determined in 5 and 10 mM K^+ and the voltage dependence of dV/dT max

was studied by depolarization in 20 mM K^+. Voltage clamp experiments in the squid giant axon show that an increase in $[K^+]_o$ from 5 to 10 mM increase the time constant of inactivation by approximately 23% in this tissue independent of any effect on membrane potential (estimate from figure 6 of Adelman and Palti, 1969). In the squid giant axon the time constant of inactivation and reactivation are equal (Hodgkin and Huxley 1952b). Recovery time constants obtained in high $[K^+]_o$ may therefore be larger than that obtained by varying membrane potential by other techniques e.g. voltage clamping. Weidmann (1955a) showed that, in Purkinje fibres, increased $[K^+]_o$ does not alter the voltage dependence of dV/dT max. However, the possibility remains that high $[K^+]_o$ per se may modify the effect of some drugs on the voltage dependence of dV/dT max of phase 0. I shall discuss this point further in relationship to the effects of the specific drugs.

A graphic technique was used to determine the recovery time constant of dV/dT max. In some experiments, 2-3 of the experimental points obtained at short diastolic intervals appeared to lie along a second, steeper, line on the semilogarithmic plot. This was within the very time interval at which there was uncertainties about the experimental technique. Because of the small number of points in that region a monoexponential analysis was used. In an exponential process, "goodness of fit" can frequently be improved by increasing the number of exponential terms (Riggs 1963). Since the objective was to make a semiquantitative comparison of control and drug treated preparations, no inferences were made concerning the number of molecular processes involved in the reactivation of dV/dT max. This would have required a vigorous justification of the

number of exponential terms selected.

5.2b. Droperidol, Quinidine and the Recovery Kinetics of dV/dT max

Droperidol.

The results show that droperidol 5.10^{-6} - 4.10^{-5} M decreased dV/dT max of test responses elicited during diastole. Because of the difficulty of maintaining an impalement in a single fibre, I was unable to increase drug concentration in several steps in a single fibre. Such an approach is necessary to clearly demonstrate dose dependency of the drug effect. However, a 10 - 20 fold increase in drug concentration from the "no effect concentration" of 10^{-6} M, caused marked slowing of the recovery kinetics. If the concentration changes are viewed on a logarithmic scale, this suggests that the dose response curve is rather steep. The effect of the drug was only slowly reversed by prolonged washing. A similar slow reversal was reported by Hauswirth (1968), and contrasts with the short duration of the cardiovascular effects of droperidol *in vivo* (Yelnosky *et al.*, 1964).

There are a number of possible causes of the decrease in dV/dT max of the test responses produced by droperidol:

(i) The decrease in dV/dT max of the test responses could be the result of a drug effect on active transport.

(ii) The drug induced changes in dV/dT max of the test responses could be the result of an effect on K^+ permeability.

(iii) A third possibility is, that droperidol slows the process of reactivation of the sodium carrying system.

The sodium which enters the cell during phase 0 of the action potential has to be extruded subsequently by active transport. If droperidol slows the subsequent transport of sodium out of the cells, this could lead to an increase in intracellular $[Na^+]$. An increase in intracellular $[Na^+]$ would decrease the driving force for the sodium current and decrease dV/dT max. A progressive restoration of the normal concentration gradient during diastole would account for the increase in dV/dT max of the test response as the diastolic interval increases. This possibility is very unlikely. Firstly only very small amounts of sodium enter the cell during an action potential (Hodgkin and Keynes 1955). The amount could lead to a significant increase in intracellular $[Na^+]$ only if there is subsarcolemmal compartmentalization of intracellular sodium. There is no evidence for such compartmentalization of intracellular sodium. Secondly, a drug induced blockade of active transport is usually associated with marked abbreviation of action potential duration (Hoffman and Cranefield 1960). Concentrations of droperidol which decrease dV/dT max of test responses, may actually prolong the APD.

dV/dT max of phase 0 of the action potential is dependent on the net inward movement of ions across the cell membrane. If

the inward sodium current at each diastolic test interval were fixed, but outward K^+ current was declining as diastolic interval increases, this could lead to the initial low value and progressive increase of dV/dT max of the test responses observed. Outward K^+ currents have been observed in the ventricular myocardium of at least some species (e.g. guinea pig, discussed in detail above). Such outward currents are activated during the plateau of the action potential and decline during the subsequent diastolic period. However, I do not think that the decrease of dV/dT max of test responses in these experiments is the result of changes in K^+ permeability. Outward currents that have been identified to date in ventricular myocardial fibres are small. It is unlikely that they could account for the observed decreases of dV/dT max of test responses which were as great as 70%. Further, one would expect the decline of a large outward potassium current to be associated with a progressive rise in membrane resistance. Hauswirth (1968) has shown that droperidol does not cause a progressive increase in membrane resistance during diastole in sheep Purkinje fibres.

Following repolarization, time must elpase at a given potential for the "sodium channels" to be converted from an inactivated or refractory state, to a resting state capable of Na^+ transfer (Hodgkin and Huxley 1952b). A slowing of this recovery process by droperidol would account for the decline of dV/dT max of the test

responses as the diastolic interval shortens. There is no evidence to exclude this possibility. The available evidence suggests that the process of sodium reactivation involves definite molecular event(s), and so could be a substrate for drug action (Armstrong 1974). Using a different approach, Kern et al., 1971 showed that droperidol caused a progressive decline in the magnitude of sodium current during test pulses in voltage clamped atrial trabecula of frog. My conclusion is that the decreases in dV/dT max which I observed are the result of a slowing of sodium reactivation.

The results indicate that this slowing of the recovery kinetics is much more marked in partially depressed preparations. The threshold concentration for slowing of recovery kinetics was lowered about 5 fold for fibres depolarized in 10 mM K^+. This increase in sensitivity may represent an effect of $[K^+]_o$ per se or an effect of the lowered membrane potential. The present experiments do not permit a distinction between these alternatives. The increased sensitivity of partially depressed preparations is consistent with the observations of Hondeghem et al., (1974) that antiarrhythmic drugs exert a more marked effect on partially depressed preparations.

Increased $[Ca^{2+}]_o$ partially reversed the slowing of recovery kinetics produced by droperidol. As droperidol is an uncharged molecule (Yelnosky et al., 1964), it is probably not acting by direct competition with Ca^{2+}. Gettes and Reuter (1974), have previously reported that increased $[Ca^{2+}]_o$ partially reversed the slowing of recovery kinetics observed in K^+ depolarized ventricular fibres. This suggests that the partial reversal of slowing of recovery kinetics

may not be dependent on the cause of prior slowing.

Quinidine

Quinidine 1.9 - 7.6 x 10^{-6} M did not slow the recovery kinetics of dV/dT max. The highest concentration studied was greater than the threshold concentration of droperidol (5 x 10^{-6} M) which slowed recovery the kinetics of dV/dT max. Chen et al. (in press Circ. Res., 1975), have also obtained negative results with quinidine concentrations up to 3 x 10^{-5} M using the same technique and preparation which was employed in this study. If the drug does in fact slow the recovery kinetics of dV/dT max, the method is not sufficiently sensitive to detect the change. Further, recovery kinetics were assessed only at the drive rate of 0.2 Hz. Quinidine may exert a frequency dependent effect on recovery kinetics of dV/dT max. The effect of quinidine on dV/dT max at steady drive rates is frequency dependent (Johnson and McKinnon 1957). Studies of the effect of quinidine on recovery kinetics of dV/dT max at various drive rates would be of interest. The negative results in guinea pig papillary muscle are in contrast to the observations of Driot and Garnier (1972), who showed that quinidine 39 mg/L (5 x 10^{-5} M) slowed the recovery kinetics of dV/dT max in from atrial trabeculae. The tissue used, the technique - voltage clamping, or the higher drug concentration may account for the observed differences.

5.2c. Droperidol and the dV/dT max - Membrane Potential Relationship

Droperidol 5 x 10^{-6} - 4.10^{-5} M consistently shifted the dV/dT max - membrane potential relationship to more negative potentials. At least a part of this effect may be the result of the technique used

to vary membrane potential, that of K^+ depolarization. While obviating some of the problems of voltage clamping, it precludes the separation of potential dependent effects from effects of increased $[K^+]_o$ *per se*.

During exposure to 20 mM K^+ Tyrode solution, the resting potential of the fibre declines as the $[K^+]_o$ in the immediate extracellular spaces rises. If droperidol increased K^+ permeability, the $[K]_o$ in the immediate extracellular space would have to increase to a greater extent to cause the same degree of depolarization compared to control, i.e. at a given membrane potential, $[K^+]_o$ would be higher during drug exposure. Therefore, if increased $[K^+]_o$ by itself also increased the depressant effect of droperidol on phase 0 of the action potential, dV/dT max would be less at each potential compared to control. This would be evident as a shift of the dV/dT max - membrane potential relationship curve to more negative potentials. However, this could not be equated to a true shift in the voltage dependent parameter of sodium conductance. There are two reasons why I believe the above explanation is not correct:-

(i) In the section on recovery kinetics of dV/dT max, fibres were exposed to 10 mM K^+ Tyrode solution before and during exposure to droperidol. The difference in the final level of membrane potential was within 1.5 mV during control and exposure to droperidol. If droperidol increased K^+ permeability, one would anticipate a consistently higher membrane potential on exposure to droperidol in 10 mM K^+ Tyrode solution. The membrane potential was not consistently higher in 10 mM K Tyrode solution during exposure to droperidol in these experiments. I cannot make a definite comment

for 20 mM K^+ Tyrode solution. Inexcitability was observed before a stable resting potential was attained. I routinely started a washout of the 20 mM K^+ solution when the inexcitability was observed.

(ii) Kern et al., (1971) have reported that droperidol 5×10^{-5} M did not change K^+ efflux in frog atrial muscle. The shift in the h curve which was observed probably represents a definite shift in the voltage parameter controlling sodium conductance. In the study of Kern cited above, the h curve was determined by measuring dV/dT max of action potential elicited at the end of conditioning voltage clamps. A shift of the h curve of about 5 mV to more negative potentials was observed with droperidol 5×10^{-5} M.

Shifts along the voltage axis of a parameter controlling an ionic conductance may be the result of an effect on membrane surface charge (Gilbert 1971). Divalent cations e.g. Ca^{2+} are thought to displace the sodium h curve along the voltage axis by such an effect (Vogel 1974). However, droperidol is an uncharge species. It is therefore likely that droperidol produced its effect by a different mechanisms from the cations.

5.2d. Prolongation of the Effective Refractory Period by Droperidol and Quinidine.

During control and exposure to droperidol at concentrations of 2×10^{-5} M or less, the ERP: APD ratio was about .98. The overview presented in the introduction would suggest a smaller normal ratio. The much larger ratio which I observed may be the results of 2 factors:

(i) Action potential duration was measured to 4 mV of complete repolarization. The time to complete repolarization was about 10 - 15 msec longer.

(ii) A test stimulus of 1 1/2 times threshold and 2.5 msec duration was used. Hauswirth (1968) used a stimulus strength of twice threshold and 0.5 msec duration. He observed an ERP:APD ratio of 0.96. On the other hand, some investigators using pulses of 5 msec duration and 3 - 4 times threshold report ERP:APD ratios of about 0.75 (e.g. Bigger and Mandel 1970).

Droperidol 10^{-6} - 4 x 10^{-5} M prolonged the effective refractory period. The ERP:APD was prolonged (80% compared to control) by droperidol at the higher concentration of 4 x 10^{-5} M. A drug which slows the recovery kinetics of dV/dT max, and/or shifts the h curve to more negative potentials should prolong ERP: APD ratio. (Antoni 1971). Droperidol in a concentration of 5 10^{-6} M or greater had the dual effect of slowing the recovery kinetics of dV/dT max and shifting the h curve to more negative potentials. In explaining the discrepancy between my earlier and experimental results, a number of possibilities should be considered:

(i) The changes in the recovery kinetics of dV/dT max could have been an artifact of the methods used. This is most unlikely. I have already presented arguments justifying the validity of the methods used. Secondly, the effect of droperidol on recovery

kinetics of dV/dT max and h curve are in agreement with the work of another investigator using a different technique and preparation.

(ii) The stimulus strength which was used may have been a limiting factor in the determination of the ERP. Although the stimulus was 1 1/2 times diastolic threshold, both during control and exposure to droperidol, the relationship may not be the same at the lowered membrane potential at which test extrasystoles arose. If the drug increases membrane resistance at relatively low membrane potentials a constant current stimulus of 1 1/2 times diastolic threshold would produce a greater degree of depolarization compared to control. My negative results at lower drug concentrations suggest the need for further investigation of the determinants of refractoriness. Studies that would be of interest include:

(i) Determination of the ERP over a range of stimulus strength.

(ii) The effect of drugs on the recovery of threshold during phase 3 of the action potential.

A number of papers have reported the effects of droperidol on the effective refractory period. Bertolo et al., (1972) examined the effects of droperidol 6.5×10^{-7} - 1.3×10^{-6} M on the ERP in cat papillary muscle. From records of mechanical activity, they concluded that droperidol prolonged the ERP. Wojtczak and Beresewicz (1974) reported that droperidol 10^{-5} M does not prolong the ERP in the ventricular myocardium of dog. In comparing these reports and the present study, there is the obvious difference of species. Also, the potassium concentration used may be relevant: (a) Bertolo et al.

used $[K^+]_o$ of 5.6 mM and noted a prolongation of the ERP at a drug concentration of 6.5×10^{-7} M (b) A $[K^+]_o$ of 5 mM was used in the present study and ERP prolongation was noted at 10^{-5} M (c) Wojtczak and Beresewicz used $[K^+]_o$ of 4.4 mM and did not observe a prolongation of the ERP at a drug concentration of 10^{-5} M. This suggests that the effects of droperidol may depend on $[K^+]_o$.

Hauswirth (1968) reported that droperidol 5×10^{-5} M ($[K^+]_o$ 5.4 mM), increased the ERP:APD ratio 600% compared to control in sheep Purkinje fibres. This drug concentration subsequently rendered the tissue inexcitable. Droperidol 10^{-6} - 10^{-5} M increased the ERP:APD ratio only 5% compared to control in canine Purkinje fibres (Wojtczak and Beresewicz 1974).

In atrial trabecula of frog, Kern et al., (1971) reported that droperidol 5×10^{-5} M caused a transient shortening, followed by a marked prolongation of the ERP. The ERP:APD ratio was increased 560% compared to control. The tissue subsequently became inexcitable at this drug concentration.

My conclusion from this study and from the previous reports are that:

(i) Droperidol causes prolongation of the ERP: APD ratio greater than 5% compared to control only at high and probably toxic concentrations in "normal" in vitro preparations.

(ii) Droperidol slows the recovery kinetics of dV/dT max, but this effect need not be associated with a prolongation of the ERP:APD ratio.

Quinidine

Quinidine 1.9×10^{-6} and 7.6×10^{-6} M prolonged the ERP in 2 experiments. There was little effect on the ERP:APD ratio. These concentrations of quinidine did not slow the recovery kinetics of dV/dT max. In the limited number of experiments done, prolongation of the action potential duration is a sufficient explanation for the prolongation of the ERP. The concentrations used were non-toxic as the preparations retained excitability in the concentrations used.

Hoffman (1958) reported that quinidine sulfate 3 - 4 mg/L ($3.8 - 5.1 \times 10^{-6}$ M) prolonged the ERP:APD ratio in papillary muscle and Purkinje fibres of dog. Using the maximum following frequency as an index of the effective refractory period, Szekeres and Vaughan Williams (1962) reported that quinidine 6×10^{-6} M caused a 50% prolongation of the ERP:APD ratio.

While the maximum following frequency and the effective refractory period may have a similar ionic basis, the two should probably not be equated. The effective refractory period measured by test extrasystoles is determined at the same basic drive rate during control and drug exposure.

5.2e. The Frequency Dependence of dV/dT max: Effect of Droperidol

Droperidol decreased dV/dT max as a function of steady drive rates. The effect was most marked at concentrations of droperidol which slowed the recovery kinetics of dV/dT max. The results also suggest that the two are causally related for the following reasons:

(i) In two experiments conducted on single fibres, droperidol 10^{-6} M did not slow the recovery kinetics of dV/dT max and had little effect on dV/dT max at various drive rates when $[K^+]_o$ was 5 mM. However, when $[K^+]_o$ was increased to 10 mM, both slowing of the recovery kinetics and suppression of dV/dT max at high rates were observed.

(ii) Increased $[Ca^{2+}]_o$ partially reversed both the slowing of the recovery kinetics of dV/dT max and the frequency dependence of dV/dT max.

As the interstimulus interval is shortened, the diastolic interval decreases to a greater extent than the action potential duration. As a result, there is a shorter diastolic period and therefore less time for reactivation of the sodium carrying system. A drug like droperidol which slows Na reactivation should therefore cause a progressively greater decrease of dV/dT max as interstimulus interval is decreased. It would be predicted from this effect that droperidol would decrease the maximum follow frequency of ventricular muscle. Such a mechanism could protect the myocardium against high frequency stimulation.

With regard to the frequency dependence of dV/dT max, the effect of droperidol is similar to that of lidocaine, propranolol and prenylamine (Tritthart et al., 1971). There three drugs also slow the recovery kinetics of dV/dT max (Chen et al. cited above; Tarr et al., 1973; Haas et al., 1975). However, slowing of the recovery kinetics of dV/dT max is probably not the sine qua non of the frequency dependence of dV/dT max. Quinidine also causes a rate dependent decrease of dV/dT max at concentrations which apparently do not slow

the recovery kinetics of dV/dT max (Johnson and McKinnon 1957).

Significance

The effects of drugs on refractoriness relate to their effectiveness in re-entrant arrhythmias. Antiarrhythmic drugs may abolish re-entrant arrhythmias by prolonging the ERP:APD ratio (Antoni 1971; Bassett and Hoffman 1971). Droperidol caused no change in the ERP:APD ratio at concentrations of less than 2×10^{-5} M. A concentration of 1.3×10^{-6} M is antiarrhythmic *in vitro* for experimental arrhythmias in guinea pig heart (Bertolo *et al.*, 1972). This suggests that prolongation of the ERP:APD ratio may not be an important mechanism action of droperidol. One must add the proviso that this study was conducted principally on "normal" tissues. It would be erronous to attempt to completely explain the effects of a drug in the adverse conditions under which arrhythmias arise from studies on normal tissues. Hondeghem *et al.*, (1974) have shown that antiarrhythmic drugs are more depressant to already compromised cardiac tissue. My results showing greater slowing of recovery kinetics of dV/dT max in potassium depolarized preparations. At part of the selective depression of hypoxic tissue which Hondeghem *et al.* observed may the result of more marked slowing of the recovery kinetics of dV/dT max.

Recently, it has been suggested that antiarrhythmic drugs may be more usefully classified based on their "kinetic effects" e.g. effects on the recovery kinetics of dV/dT max (Gettes and Reuter 1974). There is sufficient experimental data to permit a classification of drugs into those with and without marked "kinetic

effects" (Tritthart et al., 1971). However, one has to ask the very pragmatic question "does the presence or absence of a 'kinetic effect' relate to specific antiarrhythmic action(s)?" Slowing of the recovery kinetics of dV/dT max may be related to a prolongation of the APD:ERP ratio. However, the present study suggest that the two need not necessarily be related. Re-entrant arrhythmias are frequently triggered by premature extrasystoles. Droperidol decreased the upstroke velocity of the earliest extrasystole that could be elicited during phase 3 of the action potential. Droperidol could therefore prevent the onset of re-entrant arrhythmias by a more depressant effect on early extrasystoles. Lidocaine on the otherhand, increases the upstroke velocity of the earliest propagated extrasystole and may prevent re-entry by improving the safety of conduction of premature impulses (Bassett and Hoffman 1971). Both lidocaine and droperidol slow the recovery kinetics of dV/dT max. Slowing of the recovery kinetics does not distinguish between these two different mechanisms of action. For these reasons, it is probably premature to adopt this new classification of antiarrhythmic drugs.

SUMMARY

Automaticity.

Automaticity can be induced over a range of membrane potentials in normally quiescent ventricular myocardial fibres by depolarizing current. This study examined the effects of verapamil 1 - 5 mg/L, quinidine 2 - 4 mg/L, and droperidol 1.9 - 3.8 mg/L on automaticity in true ventricular myocardial fibres.

Papillary muscles from guinea pig were mounted in a sucrose gap chamber and transmembrane potential recorded by standard microelectrode techniques. Automaticity was induced with depolarizing currents of varying strength.

Verapamil reduced phase 4 slope (diastolic depolarization rate) at all membrane potentials. It also caused a selective reduction of the amplitude of action potentials arising from less negative diastolic potentials. The role of blockade of the slow inward current in these effects was examined by increasing the extracellular calcium, $[Ca^{2+}]_o$, or addition of epinephrine during exposure to verapamil. Increased $[Ca^{2+}]_o$ partially restored action potential overshoot, but phase 4 slope was further reduced. Epinephrine caused a partial or complete reversal of phase 4 slope depression, but did not restore action potential amplitude. All the effects of verapamil on ventricular automaticity therefore cannot be explained on the basis of calcium blockade.

Quinidine reduced phase 4 slope at all maximum diastolic potentials. There was a less marked reduction of the amplitude of action potentials arising from low maximum diastolic potentials.

Epinephrine caused a partial reversal of the reduction of phase 4 slope produced by quinidine.

Droperidol also reduced phase 4 slope at all maximum diastolic potentials. However, this drug was more depressant for action potentials arising from more negative potentials. There was no consistent effect on action potential overshoot. Epinephrine reversed the depression of phase 4 slope produced by droperidol.

During myocardial infarction, internally generated "currents of injury" may lead to repetitive activity in ventricular myocardial fibres, similar to that induced by current from an external source. Such repetitive activity may be responsible for ventricular extrasystoles, parasystole, or may trigger re-entrant arrhythmias. Antiarrhythmic drugs could abolish such arrhythmias by suppression of ectopic impulse formation in true ventricular myocardial fibres.

Refractoriness

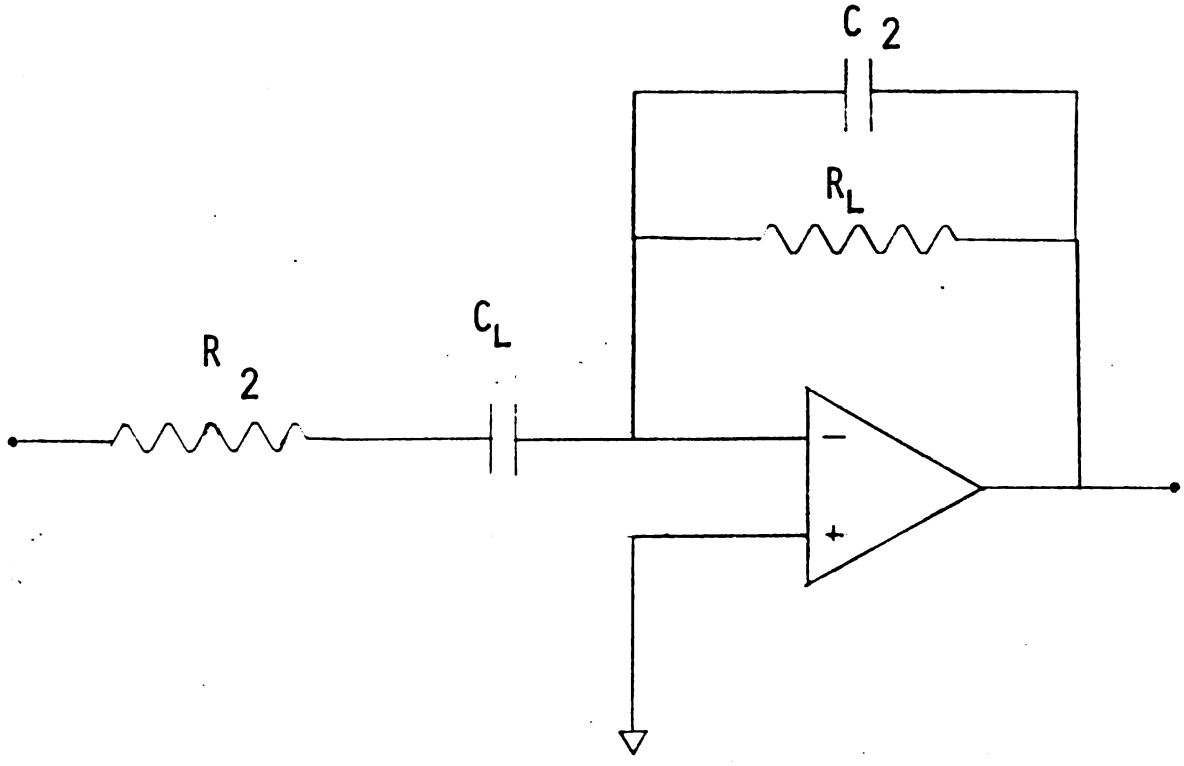
The effects of droperidol .38 - 15.2 mg/L and quinidine 1 - 4 mg/L on the recovery kinetics of dV/dT max of transmembrane action potentials recorded from guinea pig papillary muscle in vitro were examined. Droperidol caused marked slowing of the recovery kinetics of dV/dT max. This effect was exaggerated in potassium depolarized preparations and partially reversed by increasing $[Ca^{2+}]_o$. Quinidine did not slow the recovery kinetics of dV/dT max.

Droperidol prolonged the effective refractory period (ERP) at all drug concentrations. However, the ERP:action potential duration (APD) ratio was only prolonged at 15.2 mg/L. Quinidine prolonged the ERP, but did not increase the ERP:APD ratio in the

concentrations studied. Slowing of the recovery kinetics appears to be important in prolonging the ERP:APD ratio only at high concentrations of droperidol.

Droperidol decreased dV/dT max in a rate dependent manner, the effect of the drug being more pronounced at high drive rates. This rate dependent decrease of dV/dT max was more marked in potassium depolarized preparations, and was partially reversed by elevation of $[Ca^{2+}]_o$. It is concluded that this effect may be dependent on slowing of the recovery kinetics of dV/dT max.

I. Differentiator



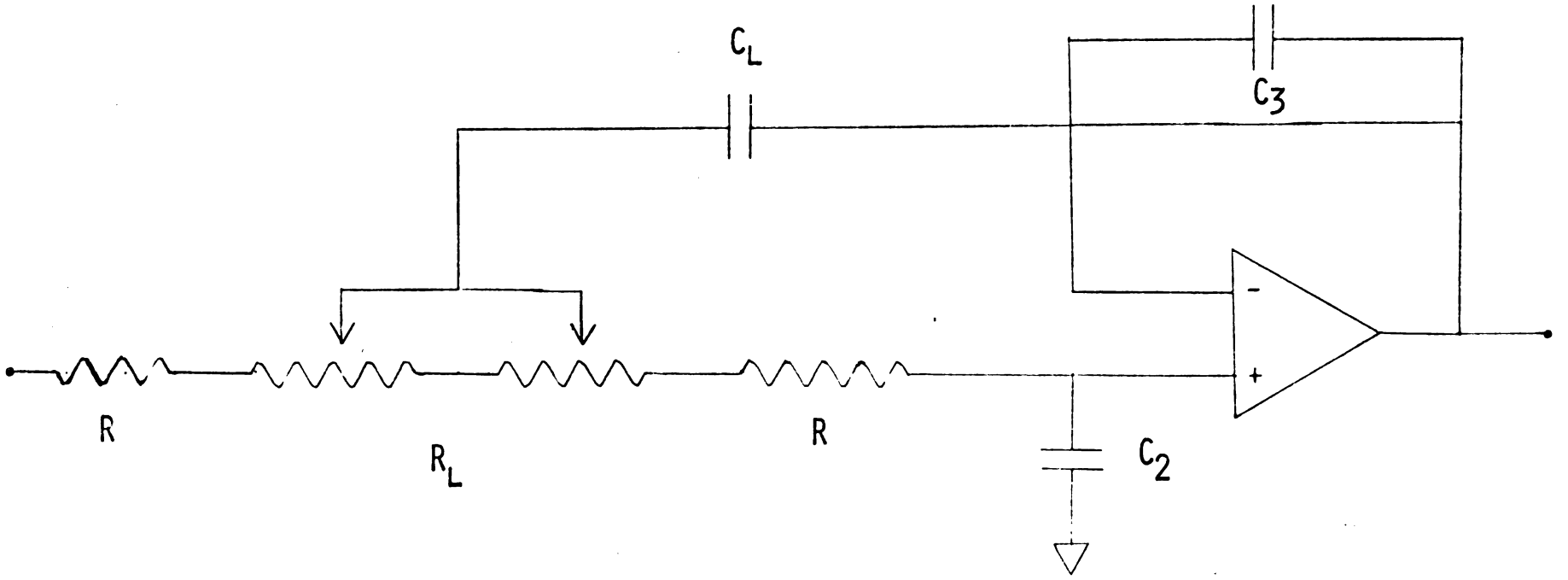
R_L = 100 K OHM

R_2 = 100 OHM

C_L = .1uF

C_2 = 100pF

2. Active filter



R = 10 K OHM

R_L = 250 K LOG DUAL POTENTIOMETER

C_L = 1000 PF

C_2 = 560 PF

C_3 = 5 PF

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