SYMPATHETIC MODULATION OF VENTRICULAR AUTOMATICITY

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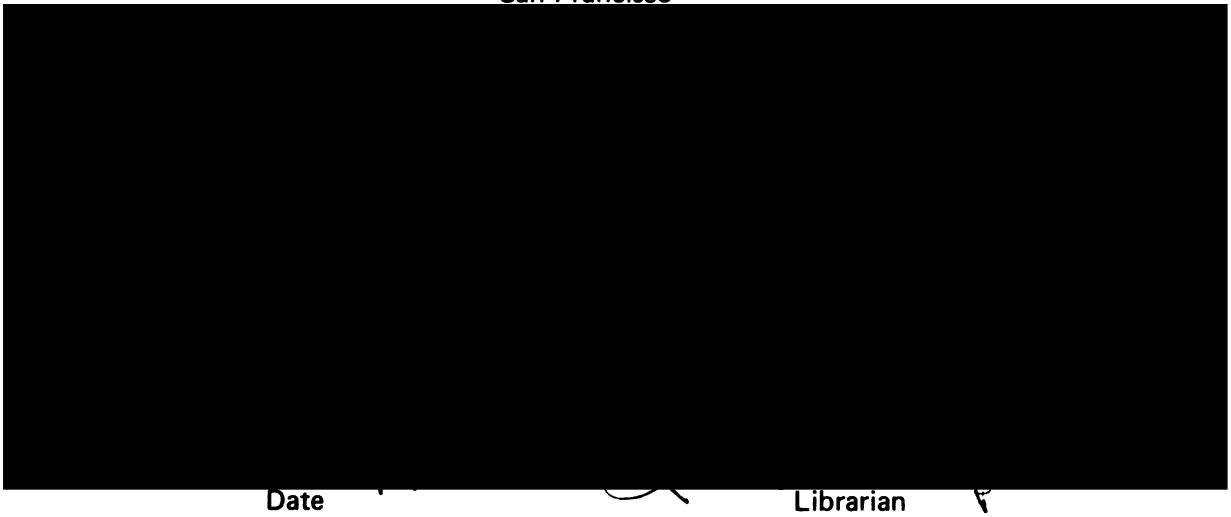

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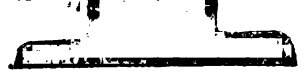
Thesis Abstract

Sympathetic Modulation of Ventricular Automaticity

Right ventricular papillary muscles from guinea pigs were mounted in a single sucrose gap chamber, and intracellular membrane potentials were recorded using conventional microelectrode techniques. The application of small depolarizing currents across the gap, elicits automaticity in ventricular myocardial cells over the range of maximum diastolic potentials of -65 to -30 mV. Previous work (Katzung, B. G. Circ. Res. 37:118-127, 1975) has suggested that two inward currents underlie automaticity in this preparation: a fast inward Na^+ current over the more negative range of -65 to -50 mV, and a slow inward (Ca^{2+}/Na^+) current over the less negative range of -50 to -30 mV. The first objective of this project was to determine if the effects of catecholamines upon depolarization-induced automaticity (DIA), are mediated exclusively via beta-adrenoceptor activation, or by both alpha- and beta-adrenoceptor activation. The second objective was to determine whether or not endogenous catecholamines are involved in the maintenance of this form of ventricular automaticity.

A comparison of the effects of three adrenergic agents, phenylephrine, norepinephrine, and isoproterenol, upon various parameters of DIA was made. These experiments suggested that stimulation of either alpha- or beta-adrenoceptors can produce an increased pacemaker rate over the more negative range of potentials. This increased rate appeared to be due to a decrease in the action potential duration in the case of phenylephrine and isoproterenol. DIA over the less negative range of potentials appeared to be enhanced by isoproterenol, due primarily to an increase in the rate of diastolic depolarization. Phenylephrine characteristically suppressed DIA over the less negative range of potentials, due primarily to a decrease in action potential amplitude. The other important characteristic of isoproterenol was that more current was consistently required to depolarize the cell in the presence of the drug. The results with norepinephrine appeared to reflect a mixture of alpha and beta effects. It is tentatively concluded from these experiments that stimulation of alpha-adrenoceptors can modulate ventricular automaticity in ways distinctly different from beta-adrenoceptor stimulation.

Several different approaches were used to evaluate the influence of endogenous catecholamines upon DIA. Tyramine elicited a positive chronotropic effect. *In vitro* reserpine abolished DIA over the less negative range, without affecting DIA over the more negative range. In low doses, l-propranolol abolished DIA over the less negative range, whereas d-propranolol at identical concentrations was without effect. Intravenous pretreatment of several animals with 6-hydroxydopamine produced a 95% reduction in right ventricular catecholamine levels. Papillary muscles from these animals displayed automaticity only over the more negative range and showed no response to tyramine. Loss of DIA by all of these interventions could be restored by norepinephrine or isoproterenol. These experiments suggest therefore, that endogenous catecholamines play a role in the maintenance of ventricular automaticity induced by depolarizing currents.



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It would be interesting to know whether the autonomic nerves within the myocardium are stimulated by all currents sufficient to stimulate the muscle within which they lie and, for that matter, whether the action potential of the heart muscle itself ever serves to excite them, in the way that of skeletal muscle may excite motor nerves.

John R. Blinks and Jan Koch-Weser
(Pharmacol. Rev. 15: 559, 1963.)

1.0 INTRODUCTION

1.1 Neurogenic and Myogenic Theories Regarding Initiation of the Heartbeat

Concepts regarding the origin of the heartbeat have been the subject of controversy since the early eighteenth century. Two mutually exclusive theories, "neurogenic" and "myogenic" theories on the origin of the heartbeat, have dominated the controversy. The myogenic theory was first put forth by Haller in 1754, who became impressed with the inherent rhythmical activity of embryonic heart tissue. Early anatomical studies, however, had discovered the existence of nerve fibers in the heart, which gave strong impetus for a neurogenic theory regarding origin of the heartbeat. Despite the fact that the neurogenic theory predominated in the eighteenth and nineteenth centuries, it was not without opposition. In addition to philosophical opposition, there began to accumulate evidence which proved that the heart could beat independently of the central nervous system. Studies showed that despite complete ablation of the central nervous system or removal of the heart from the body, it continued its rhythmic activity.

The discovery of ganglion cells within the heart, near the site of impulse initiation, however, led to the development of a modified neurogenic theory. This modified theory proposed that ganglia serve as a reflex center which initiate and regulate the heartbeat, in a manner similar to the known rhythmical discharges of nerve cells of the respiratory center. This modified theory was expressed by James Paget, in the Croonian Lecture delivered in 1857 before the

Royal Society of London. The theory apparently gained wide acceptance, with such supporters as Stannius and Schmiedeberg. By 1884, Ransom wrote:

> "The progress of inquiry into the function of the different tissues of the animal body has been marked by a general tendency to exalt the importance of nerve and correspondingly to deprecate the powers of muscle.
>
> Thus, while contractility has always been patent as a property of muscle, it has remained for modern times to establish the doctrine...that muscle is irritable to stimuli other than those of nerve. And, at the present time, of the three main characteristics of living protoplasm - Contractility, Irritability, and Automatism, while the second is granted as common to both, Automatism is generally held to be confined to nerve as is Contractility to muscle."

Gaskell performed a series of experiments on isolated frog hearts (1882) and concluded that the rhythm of the heart is caused by discrete motor impulses passing to the muscular tissue from certain motor ganglia. This conclusion was based upon the observation that sections of various portions of the frog heart continued to beat while other sections remained quiescent and ganglia cells were always found in the former sections but not in the latter sections. Also, the application of a clamp to the auriculo-ventricular groove, which separated the ventricle from the sinus and auricles, failed to produce an independent ventricular rhythm.

Gaskell became quite influenced by earlier work on the effects of electrical current on the heart by Michael Foster, founder of the Journal of Physiology. Foster (1869-70) first showed that the lower half of the frog's ventricle when separated from the rest of the heart would never beat spontaneously. However, if the tissue is submitted to the influence of "interrupted current", spontaneity ensued. Foster and Dew-Smith (1875) also showed that during the

application of constant current (10 sec. duration) to snail hearts, a rhythmic pulsation occurred. Since previous anatomical studies had shown the complete absence of any nervous structures in the snail heart, Foster and Dew-Smith concluded that the rhythmic activity produced was of a "purely protoplasmic nature". Similar results and conclusions were found in tortoise ventricles (1876).

Impressed with the results produced by electric current in Foster's experiments, Gaskell (1884) performed a series of experiments in tortoise hearts. Small strips of apex from tortoise ventricles were dissected and allowed to recover during a period in which single induction shocks were applied to one end of the muscle every ten seconds and the elicited contractions were observed. Weak interrupted current was applied through the entire strip periodically (current which was not strong enough to elicit contractions) and the effect of this interrupted current on the contractions elicited by single shocks observed. After such a period of recovery, the excitability of the muscle increased and eventually spontaneous contractions appeared. Gaskell stated, "Under the guidance of the interrupted current and the single induction shocks the rhythmical power inherent in the muscle strip has been developed and made manifest, the muscle has been taught to beat." Since spontaneous activity occurred in these small preparations which were devoid of any ganglia, Gaskell concluded that the power of rhythmical activity was inherent to the muscle and that all parts of the heart have this inherent property of automaticity, to a greater or lesser extent.

Of additional interest are the effects of the interrupted current on the contractions elicited by single shocks during the recovery period.

The height of the elicited contractions were diminished during the passage of interrupted current and upon cessation of the interrupted current, the induced contractions increased in force and attained a maximum greater than the original contractions. Gaskell remarked on the similarity of the effect of the interrupted current on contractions during the passage of current to the effect of stimulation of the vagus on atrial contractility. In the presence of "atropin", the effect of vagal stimulation was shown to be blocked and interestingly the contractile strength during the passage of interrupted current was found to increase in the presence of atropin.

Gaskell's studies were interpreted at the time as strong physiological evidence in support of a myogenic origin of the heartbeat. Although later work did prove the existence of true neurogenic pacemaker mechanisms dependent upon the discharge of ganglion cells in limulus hearts (Carlson, 1904-05) and in hearts of most crustaceans and some insects (Prosser, 1950), no evidence of a neurogenic pacemaker dependent upon the rhythmic discharge of ganglion cells has been shown in vertebrate hearts. Indeed, studies on embryonic chick hearts showed that spontaneous activity occurs prior to innervation (Pickering, 1893; Patton, 1949) and cultured heart cells believed devoid of neuronal elements were found capable of rhythmic activity by Burrows (1912). This has been interpreted as strong evidence refuting any possibility of a neurogenic pacemaker mechanism in vertebrate hearts (Brooks and Lu, 1972).

The purpose of this brief historical review on the long controversy which has surrounded the origin of the heartbeat has been to describe how our present ideas regarding the origin of the heartbeat

were formulated and upon what evidence the presently accepted myogenic origin of the heartbeat and cardiac automaticity rests. It is clear that the argument was settled early in the twentieth century, at about the same time that the active chemical substance of the adrenal gland, adrenaline, had been first isolated (Takamine, 1901), and Elliott (1904-05) had just shown that application of adrenaline to a variety of tissues, mimicked the effects of stimulation of the sympathetic nerves. It was not, however, until some 20 years later that the classic experiments of Otto Loewi (1921, 1926) proved the existence of chemical transmission. Thus, the refutation of a neurogenic mechanism underlying the heartbeat was in reality a refutation of a neurogenic mechanism based upon electrical transmission of the rhythmic discharge from a ganglionic cell to the cardiac musculature. What is also apparent is that investigations of the effects of electric current on cardiac tissue are historically linked to the first investigations on the origin of the heartbeat.

1.2 Early Studies on Pacemaker Activity and the Effects of Catecholamines

Localization of the site of the primary pacemaker of the heart, the sinoatrial node, was first made by the use of sensitive mechanical transducers. Gaskell (1882) thus localized the pacemaker of the amphibian heart in the sinus venosus and Wybauw (1910) and Lewis *et al* (1910) almost simultaneously determined the site of initial activity in dog hearts as the sinoatrial node. Although such techniques yielded information upon the site of initial activity, they provided little information on the processes at the pacemaker site which were responsible for its recurrent discharge. The nature of

these processes had been intriguing for a number of years and had earlier led Engelmann (1895, 1897) to postulate the existence of an "inner stimulus" which increases during diastole until a threshold is reached, resulting in discharge.

Soon after Bernstein's membrane theory (1868) was formulated and galvanometric recording devices became available, the search for the "inner stimulus" ensued. According to the membrane theory such an event would require the polarization of the conducting membrane to suffer a progressive diminution until it passed the critical point and an impulse was then setup. Several groups, including Eccles and Hoff (1934) and Rijlant (1932) using extracellular electrodes were unable to demonstrate such an event. Goldenberg and Rothberger (1936) and Bozler (1942-43) were the first to record spontaneous diastolic depolarization at the pacemaker site using extracellular electrodes.

With the introduction of intracellular recording by Ling and Gerard (1949), it became possible to directly record slow diastolic depolarization in pacemaker tissue. The pacemaker potential was first recorded intracellularly in spontaneously discharging Purkinje fibers by Draper and Weidmann (1951), in frog sinus venosus by Trautwein and Zink (1952), and in the sinus region of turtle hearts by Brady and Hecht (1954). The first intracellular recording of the pacemaker potential in mammalian sinoatrial node was made by West (1955) using the "floating microelectrode" of Woodbury and Brady (1956). Thus, the "inner stimulus" postulated by Engelmann was verified some 50 years later by intracellular recording techniques.

By this time the principles of neurohumoral transmission had been firmly established, although there was some early confusion as to whether "sympathin I" or "sympathin E" was the major neurotransmitter in the sympathetic nervous system. Several groups had measured the concentrations of norepinephrine in the heart and in 1956 von Euler showed that sympathetic denervation markedly reduced the norepinephrine content of the heart and concluded that norepinephrine in the heart is associated with adrenergic nerve endings. It had long been known that electrical stimulation of the sympathetic nerves to the heart accelerated the heart rate and enhanced myocardial contractility in the intact animal. It was not surprising then that in some of the first studies which had successfully recorded the intracellular pacemaker potential associated with cardiac automaticity, the effects of sympathetic stimulation and exogenous catecholamines were examined.

Hutter and Trautwein (1956) recording electrical activity in the sinus venosus of frog showed that stimulation of the vagosympathetic trunk accelerated the rate of the pacemaker potential when atropine was used to block vagal activity. It appeared that the increased slope of the pacemaker potential could account for the positive chronotropic effect observed, although epinephrine was also observed to increase the overshoot and the rate of rise of spontaneous action potentials. West, Falk, and Cervoni (1956) studied the effects of applied epinephrine on spontaneous pacemaker activity in the rabbit sinoatrial node. Epinephrine accelerated the rate primarily by increasing the slope of the pacemaker potential. In excised Purkinje fibers, which often show spontaneous pacemaker

activity, epinephrine was found to increase the spontaneous rate primarily by enhancing the slope of the pacemaker potential (Trautwein, 1963), although Otsuka (1958) also observed that epinephrine produced a negative shift in the maximum diastolic potential.

These early studies established that slow diastolic depolarization is the unique property of pacemaker cells which prevents the maintenance of a steady resting potential and thus represents the "inner stimulus" which produces spontaneous rhythmicity. Although investigators found somewhat variable effects, the most consistent effects of sympathetic stimulation or exogenously applied catecholamines on pacemaker activity was to increase the slope of the pacemaker potential and thus accelerate the spontaneous rate. More recent studies have shown that the chronotropic effects of drugs in cardiac tissue can also be mediated by changes in the maximum diastolic potential or in the threshold potential (Hoffman and Singer, 1967). Tsien, Giles, and Greengard (1972) have recently implicated changes in action potential duration as a contributing factor in the chronotropic action of catecholamines in Purkinje fibers.

1.3 Ionic Mechanisms of Pacemaker Activity and the Effects of Catecholamines

The mechanisms responsible for the pacemaker potential were of considerable interest to early investigators, however, until a suitable cardiac preparation was available for voltage clamping, such mechanisms could only be speculated upon. Such speculations included (i) a decrease in potassium permeability, (ii) an increase in sodium permeability, (iii) a reduction in activity of the sodium pump, or (iv) combinations of i-iii (Weidmann, 1956; Trautwein, 1957; Dudel and

Trautwein, 1958; Hoffman and Cranefield, 1960). Similarly, early speculations on the mechanisms by which sympathetic stimulation or added catecholamines increased the slope of the pacemaker potential revolved around changes in (i) sodium permeability or (ii) potassium permeability (Hutter and Trautwein, 1956; Hutter, 1957; Trautwein, 1963).

1.3.1 Ionic Mechanisms Underlying Pacemaker Activity in Purkinje Fibers

In 1964, Deck, Kern, and Trautwein described a short Purkinje fiber preparation which was suitable for voltage clamp analysis of slow currents. By using a double-microelectrode voltage clamp technique, Deck and Trautwein (1964) and Vassalle (1966) showed that a decreasing outward current which reversed in direction near the estimated potassium equilibrium potential occurred during the pacemaker potential in Purkinje fibers. They concluded that the pacemaker potential, which occurs over the range of maximum diastolic potentials of -90 to -60 mV, results from a decreasing potassium current superimposed upon a background inward current, which depolarizes the cell to threshold, initiating another cycle.

Noble and Tsien (1968) analyzed the kinetic and time dependent properties of the outward current responsible for the pacemaker potential in Purkinje fibers. They names this current i_{K2}, and found that a Hodgkin-Huxley gating variable, s, governs the activation and deactivation of i_{K2} over the same range of potentials in which spontaneous activity occurs. Interestingly, the activiation of s is fast at depolarized potentials, which means that s is fully activated within a few milliseconds of the upstroke of the action potential. However, due to the inward rectifying

properties of this channel, no current flows until the cell has repolarized to more negative potentials. This means that the potassium current underlying spontaneous activity in Purkinje fibers plays no role in the repolarization of the action potential. Noble and Tsien's detailed analyses also demonstrated the existence of two additional time and voltage dependent outward currents, i_{X1} and i_{X2}, and one time independent outward current, i_{K1}. The two former currents appeared to be less selective for potassium since their reversal potentials were somewhat positive to the estimated potassium equilibrium potential.

1.3.2 Ionic Mechanisms of Catecholamines in Purkinje Fibers

Catecholamines usually produce two major effects upon the pacemaker activity of Purkinje fibers. There is an increased rate, due primarily to an increase in the slope of the pacemaker potential, and the maximum diastolic potential becomes more negative. Hauswirth, Noble, and Tsien (1968) and Tsien (1974) examined the effects of epinephrine on the pacemaker current, i_{K2}, of Purkinje fibers. Epinephrine was shown to cause a voltage shift of the steady-state gating variable, s_{oo} some 30 mV in the depolarizing direction (Figure 1-1). This produces a change in the threshold for activation of i_{K2} from about -90 mV to -80 mV, and the decay of i_{K2} is accelerated over the pacemaker range. An accelerated decay of i_{K2} unmasks background inward current more rapidly, which increases the slope of the pacemaker potential. No changes in the rectification of the i_{K2} channels or in the background inward current were observed. Hauswirth et al (1969) showed by computer calculations that a shift in the activation curve for i_{K2} can indeed

produce an increase in the rate of diastolic depolarization.

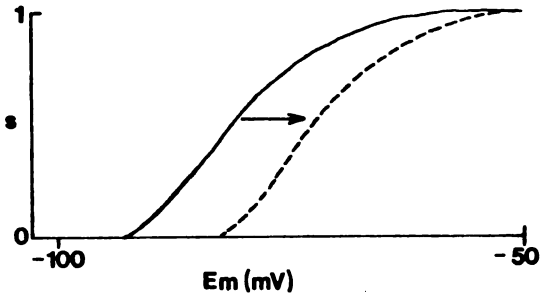


Figure 1-1

Positive shift in activation of s, produced by epinephrine in sheep Purkinje fibers. (Adapted from Brown, McNaughton, Noble, and Noble, 1975.)

These studies, however, failed to account for the increased negativity of the maximum diastolic potential seen in the presence of epinephrine. This effect can be accounted for by the results of ionic flux experiments conducted by Vassalle and Barnabei (1971), Vassalle et al (1972), and Borasio and Vassalle (1974). These experiments suggested that catecholamines can stimulate an electrogenic Na^+, K^+-pump. More recently, Cohen, Eisner, Giles and Noble (1977) and Cohen, Eisner, and Noble (1978) have suggested that the ability of epinephrine to shift the maximum diastolic potential to more negative levels may be the result of a change in the potassium equilibrium potential consequent to Na^+, K^+-pump stimulation. Since i_{K2} in Purkinje fibers is highly potassium selective, changes in the reversal potential of this current were used as an index of changes in the potassium equilibrium potential. Epinephrine consistently produced a negative shift of some 10 mV in the potassium

equilibrium potential thus measured. Furthermore, computer simulation of this effect suggested that such a shift would contribute to the shift in the s_{oo} activation curve.

1.3.3 Ionic Mechanisms Underlying Pacemaker Activity in Frog Atrial Trabeculae

Despite the elegant work on Purkinje fiber pacemaker mechanisms, there are numerous electrophysiological differences between pacemaking as it occurs in Purkinje fibers over a range of potentials of -90 to -60 mV, and that which has been observed to occur in the sinoatrial node over a range of -60 to -40 mV. The different ranges of potentials for pacemaking in the two preparations are believed to correspond to different inward regenerative currents responsible for the upstroke of the action potentials. A fast sodium current is involved in Purkinje fibers, whereas automaticity over less negative potentials is believed to involve a slow (Ca^{2+}/Na^{+}) current (see Wit, Rosen, and Hoffman, 1974). Also, different responses of the two tissues to a variety of experimental manipulations suggests that the ionic events underlying pacemaker activity in the two regions may be quite different. However, the anatomical complexity, small cell size, and contractile activity of the sinus region have prevented the successful voltage clamp analysis of the ionic currents underlying spontaneous activity, until very recently. For these reasons, several groups have attempted to analyze by voltage clamp the time and voltage dependent conductance changes responsible for pacemaker activity in atrial fibers, a tissue in which pacemaking closely resembles that of the sinoatrial node.

Brown and Noble (1969) demonstrated that frog atrial trabeculae undergo repetitive activity over the ranges of -60 to -30 mV in the presence of constant depolarizing currents. A double sucrose gap voltage clamp was applied to frog atrial trabeculae by Brown and Noble and two components of delayed rectification were found which closely resembled the i_{X1} and i_{X2} delayed outward currents described by Noble and Tsien in Purkinje fibers. Brown, Clark, and Noble (1972) analyzed the pacemaker current in this tissue and found an activation range of -50 to -40 mV, with a reversal potential of about -70 mV, indicating similarities to i_{X1} of Purkinje fibers. Brown, Clark, and Noble (1976a, 1976b) were able to resolve the outward currents in frog atrial trabeculae. By semi-logarithmic analysis of positive current decay tails, two outward conductance components, $i_{X\ fast}$ and $i_{X\ slow}$, were separated from a third interfering component, presumably due to potassium accumulation. The pacemaker current and $i_{X\ slow}$ were shown to have similar reversal potentials between -85 and -65 mV, and similar time constants of about 900 msec. at -40 mV.

1.3.4 Ionic Mechanisms of Catecholamines in Frog Atrial Trabeculae

Following the initial work on outward currents in frog atrial fibers in 1969 and 1972, Brown and Noble (1974) analyzed the effects of epinephine upon currents underlying the pacemaker potential in this preparation. Epinephrine increased the pacemaker rate induced by depolarizing currents, primarily by increasing the slope of the pacemaker potential. In addition, when compared at the same levels of depolarizing current, automaticity occurred from a more negative maximum diastolic potential in the presence of epinephrine;

an effect very similar to the hyperpolarizing effects seen in Purkinje fibers. Experiments revealed that unlike the ability of epinephrine to shift the voltage dependence of the activation of i_{K2} as in Purkinje fibers, epinephrine greatly increased the magnitude of the i_X outward currents in atrial trabeculae (see Figure 1-2).

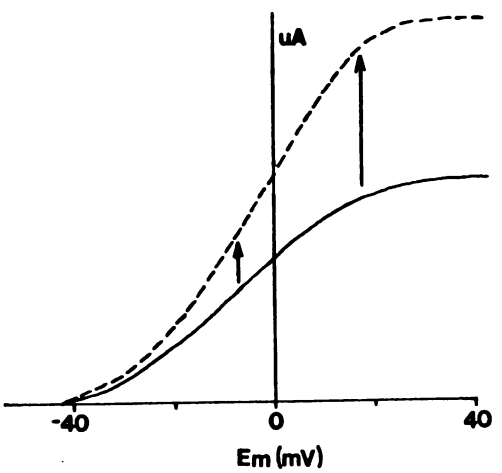


Figure 1-2

Increase in the magnitude of i_X currents produced by epinephrine in frog atrial trabeculae. (Adapted from Brown and Noble, 1974.)

This effect is sufficient to account for the hyperpolarizing actions of epinephrine, however, increased outward currents by themselves would appear to produce a decrease in the slope of the pacemaker potential. Therefore, epinephrine must be affecting some other conductance mechanism in addition. As shown in Figure 1-3, epinephrine was found to also substantially increase the magnitude of the slow inward (Ca^{2+}/Na^{+}) current, which presumably overrides the effect on outward currents, resulting in an acceleration of the diastolic depolarization rate.

The ability of catecholamines to enhance calcium conductance of cardiac cell membranes via interaction with the beta-receptor has received considerable support from a large variety of studies. Flux measurements by Reuter (1964, 1965), Meinertz et al (1973), and others have shown that catecholamines greatly enhance the uptake of calcium into cardiac cells. In addition, numerous voltage clamp studies have documented the ability of catecholamines to enhance the slow inward current (Reuter, 1967, 1974; Vassort et al, 1969). Reuter and Scholz (1977b) have carried out the most extensive voltage clamp analysis of the effects of adrenaline upon the slow inward current in cardiac muscle. They showed that adrenaline greatly increases the limiting calcium conductance ($\bar{G}s$) without affecting the kinetics, reversal potential, or ion selectivity of the calcium conductance channels. They hypothesized that adrenaline increases the limiting calcium conductance by increasing the number of functional channels, a suggestion also recently put forward by Sperelakis and Schneider (1976).

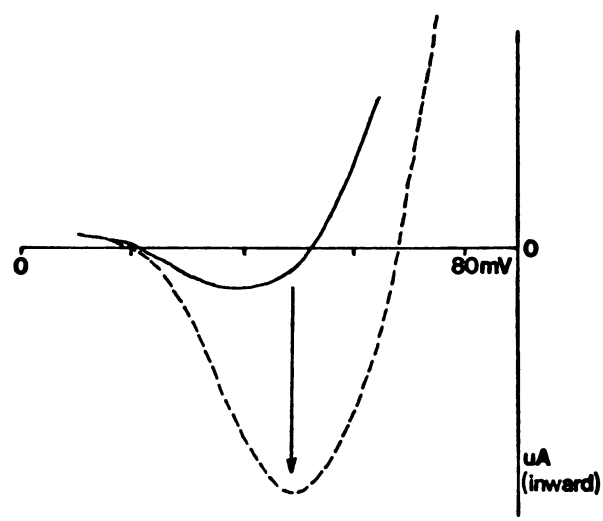


Figure 1-3

Increase in slow inward (Ca^{2+}/Na^{+}) current in frog atria produced by epinephrine. (Adapted from Brown and Noble, 1974.)

Recently, Seyama (1976) and Noma and Irisawa (1976a, 1976b) have successfully applied a voltage clamp technique to rabbit sino-atrial node and Brown, Giles, and Noble (1976, 1977) have successfully voltage clamped the frog sinus venosus. In rabbit sino-atrial node, the presence of a single slow inward current and an outward current activated over a range of -50 to +20 mV, with a reversal potential near the estimated K^+ equilibrium potential were demonstrated. In frog sinus venosus, the activiation of a slow inward current was shown to interact with the decay of an outward current to produce the pacemaker potential. The effects of catecholamines upon pacemaker activity in either of these preparations, however, has not been studied to date.

1.3.5 Receptor Mediation of the Effects of Catecholamines on Pacemaker Activity

Adrenergic receptors were originally categorized into alpha (α) and beta (β) receptor subtypes, based upon the relative responsiveness to a selected series of catecholamines and blocking agents in a variety of tissues by Ahlquist (1948; 1967). In the heart, the rank order of potency for producing positive inotropic and chronotropic effects was isoproterenol > epinephrine > norepinephrine. This was interpreted to mean that there was a predominence of adrenergic receptors of the beta subtype in the heart. This evidence was further substantiated by later studies (Furchgott, 1967) and led to the generally accepted belief that adrenergic receptors in the heart are exclusively of the beta subtype. This hypothesis has, however, recently been challenged by a variety of studies which purport to demonstrate alpha-mediated effects in the heart.

Many studies which have examined the effects of catecholamines in the heart have made the assumption that the observed effects were due to beta-receptor stimulation rather than using selective agonists and antagonists to specifically verify receptor mediation. Among the effects which have been verified to be true beta effects (since they are blocked by beta-antagonists) are a distinct positive inotropic effect, stimulation of the slow inward current, and the previously described effects of catecholamines upon the i_{K2} pacemaker current in Purkinje fibers and upon i_{X1} pacemaker current in frog atrial trabeculae. Another effect of catecholamines which might be due to beta-receptor stimulation is the increased negativity of the maximum diastolic potential seen in Purkinje fibers which has been attributed to a stimulation of the Na^+, K^+ exchange pump. Such an effect has been seen in the presence of epinephrine (Otsuka, 1958) and norepinephrine (Vassalle and Barnabei, 1971), but has not been conclusively shown to be mediated by beta-stimulation.

The existence of myocardial alpha-receptors has been verified both by studying relative potencies of agonists and by the use of alpha-antagonists. A direct positive inotropic effect mediated by alpha-receptor stimulation was first demonstrated by Govier et al (1966, 1968) in guinea pig left atria. A similar effect was reported in rabbit left atria by Benfey and Varma (1967), Benfey and Carolin (1971), and Par and Urquilla (1972). However, several studies have attributed the positive inotropic effects of phenylephrine in rabbit atria to stimulation of beta receptors (Yoo and Lee, 1970; Wagner and Schümann, 1973; Wagner and Reinhardt, 1974) and to a lesser extent to an indirect action (Wagner and

Schümann, 1973; Wagner and Reinhardt, 1974). In ventricular tissue, positive inotropic effects of alpha-stimulation appear to be less controversial. Positive inotropic effects have been demonstrated in rat ventricular strips by Wenzel and Su (1966), in rabbit papillary muscles by Schümann, Endoh, and Wagner (1974) and Endoh and Schümann (1975) and Endoh et al (1976), and in cat papillary muscles by Rabinowitz et al (1975). In addition to inotropic effects, alpha-stimulation has been reported to rpoduce a variety of electrophysiological effects upon the cardiac action potential (Pappano, 1971; and Giotti et al, 1973) and to produce chronotropic effects in a variety of cardiac preparations (James et al, 1968; Rosen et al, 1977). No voltage clamp analysis of the effects of alpha-stimulation upon ionic currents underlying the action potential or automaticity have yet been performed.

In relation to alpha-mediated effects of adrenergic amines in cardiac tissue, new research on the modulation of the release of endogenous neurotransmitters promises to add substantially to our understanding of the role that alpha-adrenoceptors play in modulating contractility and pacemaker activity in the heart. Starke (1971) proposed that adrenergic nerve terminals posses alpha-adrenoceptive sites, the reaction of which with alpha-agonists inhibits norepinephrine release, and with alpha-antagonists enhances norepinephrine release. Several comprehensive reviews on the hypothesis of alpha-modulation of norepinephrine release have recently been published (see Langer, 1974; Stjärne, 1975; Starke, 1977).

1.4 Effect of Electric Current on Cardiac Tissue

1.4.1 Pacemaker Activity and Electrical Current

As previously mentioned, Foster as long ago as 1869 showed that the application of high frequency current pulses produced rhythmic activity in quiescent frog ventricle. Later, Foster and Dew-Smith also found that the application of constant current also produced rhythmic activity in snail hearts (1875) and in tortoise ventricles (1876). More recently, Trautwein and Kassebaum (1961) observed that automaticity of the rabbit sinus node and the rate of spontaneously beating sheep Purkinje fibers was enhanced by depolarizing current pulses and depressed by hyperpolarizing current pulses of 100 msec. duration or longer. Quiescent Purkinje fibers were induced to rhythmic activity by long cathodal current pulses. Reuter (1965, 1966) observed that when quiescent sheep and calf Purkinje fibers, soaked in sodium-free solutions, were stimulated by constant current pulses, non-propagated regenerative membrane depolarizations occurred. This regenerative behavior appeared to be dependent upon the presence of extracellular calcium. The same was observed by Reuter and Scholz (1968) in ventricular myocardial fibers. The induction and modulation of automaticity by long duration current pulses has also been observed in Purkinje fibers by Imanishi (1971), Cranefield (1975), Brennan et al (1978), in sinus nodal fibers by Lentant et al (1972), in frog atrial cells by Brown and Noble (1969), and in ventricular myocardial cells by Katzung (1974a, b), Surawicz and Imanishi (1974), and Arita et al (1976).

Katzung and Morgenstern (1977) using a single sucrose gap voltage clamp technique in cat ventricular myocardial cells analyzed the ionic currents underlying pacemaker activity induced by depolarizing

currents. They found that a time-dependent outward current was activated by ordinary action potentials and by clamps to the plateau levels which deactivates slowly over the pacemaker range of potentials of -65 to -30 mV. The activation curve for this current is shown below in Figure 1-4. This current is very similar to the pacemaker current described by Brown, Clark, and Noble in frog atrial trabeculae and to the i_{X1} current described in Purkinje fibers, except that it appears to have a somewhat greater selectivity for potassium ($E_{rev} = E_K + 6$ mV).

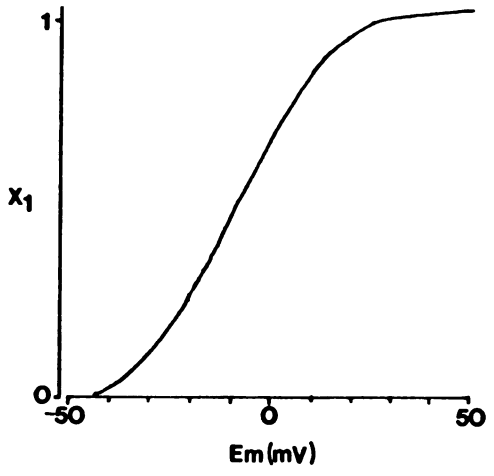


Figure 1-4

Activation curve for pacemaker current gating variable in cat ventricular myocardium. (Adapted from Katzung and Morgenstern, 1977.)

Automaticity of the type induced by depolarizing currents requires (i) a slow depolarizing process which prevents the cell from maintaining the maximum diastolic potential, resulting in the pacemaker potential, (ii) a more rapid depolarizing process (spike), and (iii) a repolarizing process which returns the cell to its maximum

diastolic potential. Investigations on the kinetic and voltage-dependent properties of a declining outward current underlying pacemaker activity relate to (i). Although it appears to be the time-dependent decline of an outward current which determines the slope of the pacemaker potential, it is the presence of some background inward current which enables the cell to depolarize. Regenerative, time-dependent inward currents are involved in the spike process (ii).

Katzung (1975) investigated the role of inward currents in (i) the depolarizing process and in (ii) the spike process in depolarization-induced ventricular automaticity. Reduction of either Ca++ or Na+ in the external perfusate reduced the slope of the pacemaker potential, suggesting a contribution of both ions to the background inward current during diastolic depolarization. However, indirect effects due to the reduction of either ion could not be eliminated as the cause of the decrease in the slope of the pacemaker potential. Much stronger evidence for the participation of both Na+ and Ca++ in the regenerative spike process was obtained. Regenerative action potentials over the more negative range of maximum diastolic potentials appeared to be dependent upon activation of the rapid inward Na+ current, whereas regenerative action potentials over the less negative range of maximum diastolic potentials appeared to be dependent upon activation of the slow (Ca++/Na+) current. These results were also supported by later studies which showed that verapamil was more selective in abolishing automaticity over the less negative range of maximum diastolic potentials (Grant and Katzung, 1976), whereas lidocaine and tetrodotoxin were more

selective in abolishing automaticity over the more negative range of maximum diastolic potentials (Katzung, 1974a).

1.4.2 Endogenous Catecholamines and Electric Current

Isolation of the heart, or portions thereof, obviously entails surgical interruption of the nerve supply to the heart. Does this necessarily mean complete interruption of the innervation apparatus in all tissues studied *in vitro*? Total intrinsic dennervation experiments *in situ* have demonstrated that depletion of endogenous catecholamines requires up to three to four days, presumably due to neuronal degeneration (Cooper *et al*, 1961). Non-refrigerated heart tissue four hours after excision from the body shows only about 50% depletion of endogenous catecholamine content and the perfusate from isolated cat and rabbit heart preparations has been shown to contain positive inotropic capability (Hoffman *et al*, 1945). According to Cooper (1965), "the reactions of the isolated or decentralized heart might not be properly interpreted only in terms of 'intrinsic' muscular responses. The relation of the catecholamine containing terminal innervation apparatus to the myofibrillar structures must be taken into serious consideration."

In support of this view is another frequently observed, but often ignored, effect of the application of electric current to cardiac preparations. Numerous *in vitro* studies, using a variety of electrical stimulation techniques have reported the stimulation-induced release of endogenous neurotransmitters from intramural nerve endings residing in close proximity to myocardial cells. Among the earliest demonstrations of such a phenomenon in vertebrate hearts were the

experiments by Knowlton (1941/42, 1943/44). The application of weak interrupted current of frequencies up to 50 per second to spontaneously beating double auricle preparations from turtle hearts, slowed or even arrested the spontaneous rate, with an accompanying reduction in contractile force. Upon cessation of the weak current, there was frequently a supranormal period of recovery. Knowlton found that atropine blocked and eserine potentiated the depressant effects of interrupted current and concluded that the current stimulated the release of acetylcholine from vagal fibers. In a later study, Knowlton showed that single induction shocks of sufficient intensity could slow or arrest spontaneous activity by liberation of acetylcholine. Nelemans (1951) found that faradization of frog ventricles produced an initial depression of contractile force followed by an augmentation. Aware that Starling (1933) had shown that simultaneous stimulation of the vagus and accelerator nerves produced an initial negative chronotropic and inotropic effect, followed by a positive effect Nelemans considered the possible release of both acetylcholine and "sympathin" by faradization. The initial depression in contractility was blocked with atropine, and in frogs which had been surgically denervated for at least a month, no secondary augmentation of contractile force was ever observed. Nelemans was, therefore, among the first to demonstrate stimulation-induced release of endogenous catecholamines.

Since these early studies, stimulation-induced release of both acetylcholine and catecholamines has been observed frequently. Ursillo (1958, 1961) demonstrated the phenomenon in cat and rabbit atria using stimuli which were subthreshold for myocardial cells,

whereas Whalen (1958) and Whalen et al (1958) demonstrated a similar phenomenon in cat papillary muscles and atria by suprathreshold intensity stimuli, One of the difficulties in establishing that the secondary augmentation of rate and/or contractility was due to release of endogenous catecholamines was the unavailability of specific beta-antagonist drugs at this time. One of the first such specific antagonists synthesized was dichloroisoproterenol (DCI) and Furchgott et al (1958) were quick to demonstrate that both reserpine pretreatment or perfusion with DCI abolished the secondary augmentation of contractility produced by suprathreshold stimulation of cat papillary muscles. Brady et al (1960) demonstrated that high intensity pulses applied to cat papillary muscles during the absolute refractory period produced an initial depression of contractility followed by an increase. The secondary effect could be abolished by application of DCI or by pretreatment with reserpine. A somewhat different method of evaluating release of autonomic mediators by electric current was used by Azuma et al (1961). They recorded the intracellular action potentials of both atria and ventricular fibers and showed that elevation of the intensity of the driving stimulation above threshold produced a shortening of the action potential duration quite similar to the effects observed upon application of acetylcholine. In the presence of atropine, a prolongation of the action potential duration and increased amplitude of the action potential overshoot was seen upon increasing the stimulus intensity. Because these effects mimicked the effects of applied catecholamines, they hypothesized that increasing the intensity of the stimulus elicited release of both acetylcholine and catecholamines from intramural nerve endings.

The most thorough investigation of stimulation-induced release of endogenous neurotransmitters has been carried out by West and his collaborators. West (1961) showed that repetitive electrical stimulation of appropriate intensity and frequency produced membrane hyperpolarization and arrest of a spontaneously beating rabbit sinoatrial node, followed by resumption of spontaneity at an accelerated rate. Amory and West (1962) pharmacologically evaluated this phenomenon and concluded that electrical stimulation elicited the release of both acetylcholine and norepinephrine from intramural nerve endings, which respectively produced the initial membrane hyperpolarization and arrest and the subsequent enhanced rate. Vincenzi and West (1963) further analyzed parameters of the electrical stimulation procedure necessary to elicit release. Strength-duration relationships were defined, and it was shown that rabbit SA node, AV node, and left atrium demonstrated "electrorelease" at intensities which were subthreshold for myocardial excitation. Electrical stimulation of cat papillary muscles elicited release of both acetylcholine and norepinephrine which produced a biphasic inotropic effect. It was also found that extracellular stimulation using silver or platinum electrodes was considerably more effective in producing electrorelease than stimulation via an intracellular microelectrode and that a directional pathway for electrorelease appeared to exist. Vincenzi and West (1965) later studied the calcium-dependence of the electrorelease phenomenon in isolated SA node-right atrial preparations.

Despite the variable stimulus techniques and protocols which have been used, the variety of cardiac preparations which have been

examined and shown capable of stimulation-induced release of autonomic mediators suggests that in vitro cardiac preparations cannot be considered completely devoid of neuronal influences. Indeed, the experiments by West and others might suggest that all electrical stimuli sufficient to excite myocardial cells, are capable of eliciting some release of endogenous neurotransmitters. It is interesting to note the similarity between the biphasic inotropic and chronotropic responses to electrical stimulation observed in many of these studies, to the biphasic contractile responses to weak interrupted current and single induction shocks observed by Gaskell back in 1884 in tortoise hearts.

1.5 Possible Mechanisms Underlying Automaticity at Depolarized Potentials

1.5.1 The Pacemaker Potential

The ionic mechanisms underlying pacemaker activity in cardiac cells have been extensively reviewed by several authors (McCann, 1972; Noble, 1975; Vassalle, 1977). In terms of ionic theory, the only requirement necessary for diastolic depolarization to occur is that the net outward balance of ionic currents which predominates during repolarization be reversed so that a net inward balance of ionic currents occurs. The development of a net inward balance of ionic current could conceivably result from either an increasing inward current or a declining outward current. The concensus to date in nearly every cardiac pacemaker preparation studied appears to be that the pacemaker potential arises as a result of the deactivation of an outward current, which occurs in the presence of some constant background inward current (Noble, 1975).

The elucidation of such a mechanism was first made in cardiac Purkinje fibers and was previously discussed in Section 1.3.1. The evidence for such a mechanism in this preparation is based primarily on (i) the measurement of a progressive increase in membrane slope resistance during the pacemaker potential (interpreted as a decrease in membrane conductance) (Weidmann, 1951; Vassalle, 1966), (ii) the demonstration that an outward current which reverses in direction near the estimated K+ equilibrium potential, deactivates over the same potential range over which pacemaker activity occurs (Vassalle, 1966; Noble and Tsien, 1968) (iii) the demonstration that the kinetics of this potassium current (i_{K2}) are of similar magnitude to the kinetics of the increase in net inward current underlying the pacemaker potential (Noble and Tsien, 1968),(iv) that augmentation of potassium permeability by elevated K_o, decreases the slope of the pacemaker potential (Vassalle, 1965; 1966), (v) that the mechanism of a chronotropic agent, like catecholamines, can be entirely explained in terms of changes in I_{K2} (Hauswirth, Noble, Tsien, 1968; Tsien, 1974), (vi) that pacemaker activity could be accurately simulated by a computer model incorporating the decay of an outward K+ current superimposed upon a steady background inward current (Hauswirth et al, 1969). The nature of the time-independent background current has not been chanracterized but was assumed to represent residual sodium current remaining after steady-state inactivation (Weidmann, 1955b).

The mechanism of the Purkinje fibers pacemaker potential which is based upon the decay of i_{K2} appears to be unique since i_{K2} has not been identified in any other cardiac preparations examined. Another

outward current which is apparently common to all cardiac tissues, with minor qualitative differences, has been recently identified by the application of voltage clamp techniques. This current is similar to the i_{X1} outward current originally characterized in Purkinje fibers (Noble and Tsien, 1969) and involved in the repolarization of the Purkinje fiber action potential. Thus a similar current has been identified in frog atrial trabeculae (Ojeda and Rougier, 1974; Brown, Clark and Noble, 1976), cat ventricular myocardial cells (McGuigan, 1974; Katzung and Morgenstern, 1976; MacDonald and Trautwein, 1978), mammalian sinus nodal cells (Noma and Irisawa, 1976b) and frog sinus venosus (Brown, Giles, and Noble, 1977, 1978). The role of this current in induced pacemaker activity in frog atrial trabeculae and ventricular myocardial cells was previously discussed in Sections 1.3.4 and 1.4.1 and the properties of this current and its role in pacemaker activity in all of the above cardiac cells have recently been reviewed by Katzung (1978). With the exception of sinus node or sinus venosus cells which are spontaneously active, atrial trabeculae and ventricular myocardial cells can be induced to pace by the application of depolarizing currents. In addition to having a similar outward current, all of these preparations demonstrate either spontaneous or induced pacemaking over a similar range of maximum diastolic potentials (with minor variations), -65 to -30 mV. Nearly all studies which have attempted to analyze by voltage clamp technique the mechanisms underlying pacemaker activity in these various preparations have generally modelled the phenomenon based on the mechanisms previously determined in Purkinje fibers, namely: the progressive decay of an outward current superimposed upon a constant background inward current. It might, therefore, be useful to consider how well this

form of pacemaker activity meets the same criteria previously established for the Purkinje pacemaker potential.

To my knowledge, measurement of the membrane slope resistance during the course of the pacemaker potential in sinoatrial nodal cells has only been reported by Seyama (1976). Interruption of the pacemaker potential in Purkinje fibers by a voltage clamp was previously shown by Vassalle (1966) to result in a slow increase in inward current. It was the kinetics of this net inward current which corresponded to the kinetics of the decay of i_{K2}. Measurement of membrane slope resistance during the entire course of the pacemaker potential revealed that a progressive <u>increase</u> in membrane slope resistance accompanied the increase in net inward current. This certainly implicated a decrease in outward conductance during the pacemaker potential. In rabbit sinus node, Seyama found a slow development of inward current upon interruption of the pacemaker potential which was accompanied by a <u>decrease</u> in membrane slope resistance. This suggested that during the pacemaker potential an inward current is partially or totally responsible for development of the pacemaker potential. Membrane slope resistance measurements were not made in the analysis of pacemaker activity induced in frog atrial trabeculae. In ventricular myocardial cells induced to pace (Katzung, 1975), membrane slope resistance measurements were made only for automaticity arising from more negative maximum diastolic potentials. These measurements revealed an increase in membrane slope resistance <u>at the potentials measured</u>. Imanishi and Surawicz (1976) measured membrane slope resistance during the entire course of the pacemaker potential in guinea pig

ventricular myocardial cells induced to pace by applied current. They found for automaticity arising from apparently less negative maximum diastolic potentials (< -30 mV) that an increase in membrane resistance occurred during the first half of the pacemaker potential, which changed to a decrease in membrane resistance during the latter portion of the pacemaker potential. Studies on pacemaker activity which occurs over a range of potentials corresponding to i_{X1} currents, then, have not as consistently shown an increase in membrane slope conductance during the pacemaker potential as has been shown for i_{K2} related diastolic depolarization in Purkinje fibers.

In tissues which are either spontaneously active or can be induced to pace over the general range of potentials of -65 to -30 mV, an outward current which deactivates over the same general range of potentials has been demonstrated. In sinus node and sinus venosus this current behaves as a relatively pure potassium electrode and is, therefore, probably a pure potassium current. In frog atria and ventricular myocardial cells, this current reverses at potentials slightly positive to the estimated potassium equilibrium potential. This might result from either wide ion selectivity of the channel or interference in analysis of this component by some inward current. It has been suggested (Katzung and Morgenstern, 1977) that a reversal potential positive to the resting potential explains why this current does not produce diastolic depolarization at the resting potential. Interestingly, in mammalian ventricular myocardial cells, there appears to be some species specificity of i_{X1}. It has been demonstrated in cat and dog (Beeler and Reuter,

1970) and guinea pig ventricular myocardium (Ochi, 1970), however results in sheep, calf, and rabbit ventricle have been negative or equivocal (Giebisch and Weidmann, 1971; Harrington and Johnson, 1973; McGuigan, 1974; Trautwein and McDonald (1978).

The kinetics of the various i_{X1}-type currents have been somewhat variable between different preparations, however, they all seem to be of the same general order of magnitude (125-400 msec. at -70 mV). This is much faster than the kinetics of the i_{K2} current described in Purkinje fibers, but does correspond to the faster pacemaker rates observed in these preparations in comparison to Purkinje fibers. It is important to note that none of the investigations on either spontaneous or induced pacemaker activity over the range of -65 to -30 mV have measured the kinetics of the development of the pacemaker potential. When clamps are applied during the terminal phase of repolarization or at the maximum diastolic potential (Brown, Clark, and Noble, 1976), the resulting outward currents which have been measured cannot be considered the same current changes which occur later during the pacemaker potential. Thus the changes in net ionic current which occur during the pacemaker depolarization have not been measured and, therefore, cannot be compared to the kinetics of the various i_{X1} currents which have been measured in the different preparations. It is surprising that the pacemaker potential has not been interrupted at various points during its progression by voltage clamp and the time course of development of net inward current measured. Measurement of the kinetics of the change in net inward current would also demonstrate whether it increases as a single exponential, as in Purkinje fibers, or whether a multiple exponential process is involved.

The rate of diastolic depolarization of Purkinje fibers has been shown to decrease as K_0 is increased to 7.1 mM eventually leading to complete quiescence (Brooks and Lu, 1972; Vassalle, 1965). This effect has been shown to be due to an increase in potassium conductance. In mammalian sinoatrial node, the slope of the pacemaker potential and consequently the pacemaker rate has been actually observed to increase as K_0 is raised as high as 10.8 mM (Lu, 1970), clearly the opposite of that observed in Purkinje fibers. In cat and guinea pig myocardial cells (Katzung and Morgenstern, 1977; Imanishi and Surawicz, 1976) the effects of elevated K_0 were not clear cut. It appeared that automaticity from more negative maximum diastolic potentials was more attenuated than automaticity arising from less negative maximal diastolic potentials, however at $K_0 \geq$ 10mM, most automaticity was suppressed.

Of the preparations in which i_{X1}-type currents have been characterized, only in frog atrial fibers have the effects of catecholamines been analyzed by voltage clamp techniques. These mechanisms were described in Section 1.3.4. In this preparation, the ability of catecholamines (via beta-adrenoceptor stimulation) to accelerate the pacemaker rate could clearly <u>not</u> be explained solely by modifications of an outward current. In fact, the effects of epinephrine on the i_{X1} outward current were clearly in the wrong direction of that expected to produce acceleration, if the mechanism of the pacemaker potential is a decay of outward current superimposed upon a constant background inward current. The demonstration that epinephrine also greatly enhanced the slow inward current is strong evidence that the slow inward current is involved in the autonomic

mechanisms modulating the pacemaker potential in frog atria. In sinoatrial node, it has long been recognized that the most characteristic effect of catecholamines in producing their chronotropic action is the acceleration of the pacemaker potential. If the i_{X1}-type outward current in sinoatrial node reacts to catecholamines in a manner similar to i_{X1} of frog atria, then clearly another current must be involved in the pacemaker potential mechanism in sinus nodal cells, and in the acceleration produced by catecholamines. Voltage clamp analysis of catecholamine effects in sinus node cells or myocardial cells have not yet been performed.

Computer reconstruction of pacemaker activity in preparations in which i_{X1}-type currents have been identified has only been done for ventricular myocardial cells, induced to pace by applied depolarizing currents (Beeler and Reuter, 1977). In this model, the pacemaker potential at less negative maximum diastolic potentials was simulated by both deactivation of i_{X1} and activation of the slow inward current.

Some injustice probably arises as a result of grouping together the different preparations in which i_X-type outward currents have been identified and considering the mechanisms underlying the pacemaker potential in these preparations. This does not deny that real differences exist between these different preparations or that different ionic mechanisms might be responsible for pacemaker activity in the different preparations. It is clear, however, that these preparations which all have i_{X1}-type outward currents, which are activated over similar potential ranges in which pacemaker activity occurs, do not presently meet the same criteria which supports the

Purkinje fiber model: a progressive decline of an outward current which unmasks a constant background inward current.

Another important difference between these preparations and the Purkinje fiber model relates to the fact that i_{X1}-type outward currents are definitely involved in the repolarization of the action potential, whereas i_{K2} in Purkinje fibers plays relatively little role in repolarization, due to the inward rectifying properties of this channel over the plateau range of potentials. This means that in Purkinje fibers, changes in the duration of the action potential are independent of changes in the pacemaker potential (Noble and Tsien, 1972). In preparations which show pacemaker activity in which i_{K2} does not exist, changes in the duration of the action potential could be either: (i) dependent upon changes in the pacemaker potential, if the decay of i_{X1} is a major determinant of the pacemaker potential, or (ii) independent of changes in the pacemaker potential, if other currents underly the pacemaker potential.

1.5.2 Involvement of Inward Current(s)

It is generally agreed that two distinct inward currents contribute to the development of the action potential in most cardiac tissues (Reuter, 1973; Coraboeuf, 1978). A fast tetrodotoxin-sensitive inward sodium current, very similar to that found in nerve, is responsible for the initial rapid phase of depolarization, and a second inward current which is sensitive to manganese and carried by calcium and/or sodium is responsible for the action potential plateau. Previous experiments studying depolarization induced ventricular automaticity in myocardial cells (Katzung, 1975; Grant and

Katzung, 1976; Katzung, 1974a) have established that regenerative action potentials over more negative maximum diastolic potentials are dependent upon activation of the fast inward sodium current, whereas regenerative action potentials over less negative maximum diastolic potentials, where the fast sodium current is nearly completely inactivated, are dependent upon activiation of the slow inward current.

The threshold for slow inward channel activation has been quite variable between the different cardiac preparations which have been studied. Thus Ochi (1970) in guinea pig myocardium reported a threshold value of about -40 mV, whereas McDonald and Tratuwein (1978) reported a threshold value of about -45 mV in cat myocardium, and Reuter (1973) showed the activation range of the slow inward current in sheep and guinea pig myocardium to begin at about -55 mV. The reason for these differences are not readily apparent but could result from different experimental conditions (e.g., different holding potentials) or species differences. A previous study of induced pacemaker activity in frog atria (Brown, Clark, and Noble, 1976) excluded the possible participation of slow channel activation in pacemaker activity, since the observed range of maximum diastolic potentials (rather than thresholds for regenerative activity) were negative to the slow inward channel threshold.

In relation to the possible overlapping participation of both inward currents in depolarization induced ventricular automaticity, the voltage dependency of the steady-state activation and inactivation variables regulating both the fast and slow inward currents are illustrated in Figure 1-5.

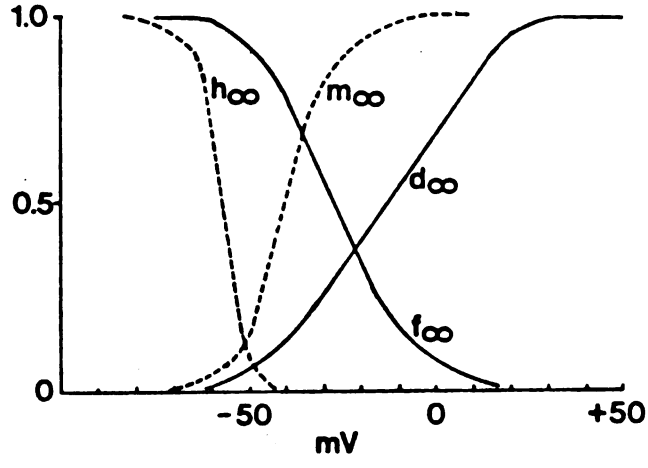


Figure 1-5

Steady-state conductance variables which determine activation (m_{00}) and inactivation (h_{00}) of the rapid inward sodium current and activation (d_{00}) and inactivation (f_{00}) of the slow inward current. The conductance variables, h_{00}, d_{00}, and f_{00} were obtained from voltage clamp experiments in sheep and guinea pig ventricular trabeculae and adapted from Reuter (1973) (Figure 6). The activation variable m_{00} is adapted from Beeler and Reuter (1977). Due to the limitations of voltage clamp techniques in cardiac tissue, m_{00} was formulated according to determinations made in squid axon (Hodgkin and Huxley, 1952).

These data from sheep and guinea pig ventricular myocardium support the possibility that such an overlap contributes to the regenerative activity produced by depolarizing currents in ventricular myocardium. This diagram also illustrates a possible source of background inward current which could contribute to the development of the pacemaker potential over the range of maximum diastolic potentials of -55 to -30 mV. There is considerable overlap of the voltage dependency of the activation and inactivation variables, d_{00} and f_{00}, which control the slow inward current. Reuter and Scholz (1977a) demonstrated that a considerable fraction of the slow inward current in cat and

cow myocardial cells is not inactivated even during long-lasting voltage clamp pulses to this range of potentials. Similar results were observed in cat myocardial cells by Trautwein et al (1975). This range of potentials comprises almost the entire range of MDPs over which induced pacemaker activity appears to occur in ventricular myocardial cells. It could be speculated that such an incompletely inactivated slow inward current might contribute to the development of the pacemaker potential over this range (as a time-dependent or independent component). As such, this component might (i) demonstrate sensitivity to both sodium and calcium concentration alterations and (ii) be sensitive to beta-adrenoceptor modulation.

1.5.3 Summary

The ionic mechanisms underlying the pacemaker potential in cardiac Purkinje fibers have been well characterized and a variety of evidence supports the hypothesis that slow diastolic depolarization results from the time-dependent deactivation of an outward current (i_{K2}) in the presence of some background inward current. For automaticity in which an outward current of the i_{X1} type has been demonstrated, the evidence supporting a similar mechanism is clearly incomplete at this time. Since pacemaker activity in these tissues, either spontaneous or electrically induced, occurs over a potential range in which inward time-dependent currents are activated, the possibility exists that inward currents are fundamentally involved in the development of the pacemaker potential in these preparations.

The possible involvement of the slow inward current in controlling the pacemaker potential has been previously suggested by Lenfant et al (1974) in frog atrial fibers and in mammalian sinus node by Vassalle (1977), Seyama (1976), and MacKaay et al (1978). Recent voltage clamp analysis of the ionic currents underlying spontaneous activity in frog sinus venosus by Brown, Giles, and Noble (1977) have provided the first voltage clamp evidence that activation of the slow inward current occurs during the latter phase of the pacemaker potential. They suggested that an intervention which would block the slow inward current like Ca++ substitution by manganese, would eliminate pacemaker activity by blocking the regenerative current responsible for the spike and also by reducing the slope of the pacemaker potential, resulting in an oscillation.

2.0 THE RESEARCH PROPOSAL

The ability of constant depolarizing current to elicit automaticity in otherwise quiescent cardiac preparations has been observed by a considerable number of investigators (Trautwein and Kassebaum, 1961; Brown and Noble, 1969; Katzung, 1974a, 1974b). One of the most extensively studied preparations has been ventricular myocardial cells of guinea pig and cat papillary muscles. Previous studies of this phenomenon (Katzung, 1975) have established a unique property of the type of automaticity induced in this preparation by applied depolarizing currents. Alterations in applied current intensity have been shown to produce automaticity over a wide range of maximum diastolic potentials (-70 to -20 mV). Over this range of potentials, the induced automaticity appears to be dependent upon two different inward regenerative currents, the potential range of which overlaps. Thus, this preparation offers the unique opportunity to study two distinct forms of cardiac automaticity; one which is dependent upon a fast sodium regenerative inward current over more negative maximum diastolic potentials; the other which is dependent upon a slow (Na^+/Ca^{++}) regenerative inward current over less negative maximum diastolic potentials. Two distinct forms of induced automaticity dependent upon different inward regenerative currents might represent different contributions to the development of clinical arrhythmias, and different sensitivities to the actions of antiarrhythmic drugs.

2.1 The Effects of Exogenous Sympathomimetic Amines upon Ventricular Automaticity

Despite the long held belief that the reactions of the heart to catecholamines are mediated exclusively by reaction with beta-adrenoceptors, recent studies have suggested that catecholamines can interact with myocardial alpha-adrenoceptors to produce inotropic effects (Wagner and Brodde, 1978), and various electrophysiological effects including chronotropic actions (James et al, 1968; Rosen et al, 1977). Because of this recent new evidence, I asked the following question:

> "Is the response of electrically induced ventricular automaticity to catecholamines mediated exclusively via interaction with myocardial beta-adrenoceptors, or is there additionally a response mediated via interaction with myocardial alpha-adrenoceptors?"

The strategy used to evaluate this question has been to determine the effect of three sympathomimetic amines upon ventricular automaticity induced by depolarizing currents. The three agents used were isoproterenol, norepinephrine, and phenylephrine. These agents were chosen because the relative potency for beta-adrenoceptor activation is considered isoproterenol > norepinephrine > phenylephrine, and for alpha-adrenoceptor potency, norepinephrine > phenylephrine > isoproterenol (Ahlquist, 1948; 1958; Furchgott, 1967). To firmly establish receptor mediated responses, relative potencies of these agonists should be established and the response to an agonist should be antagonized by specific antagonists (Furchgott, 1967, 1972). It is rarely possible to obtain complete dose-response curves for agents with positive inotropic properties in studies in which the myocardial transmembrane potential is measured with an intracellular microelectrode. I, therefore, attempted to semi-quantitatively distinguish between

alpha- and beta-adrenoceptor responses by comparing the effects of one or two doses of each agonist. Thus, a mixed-agonist like norepinephrine may be compared to the relatively pure beta-agonist, isoproterenol, and to the predominantly alpha-agonist, phenylephrine. The other important criterion for establishing specific receptor mediation of a response is the ability of specific antagonists to block the action of an agonist. Specific antagonists of beta-adrenoceptors were evaluated in this study and some preliminary experiments with alpha-antagonists were carried out.

2.2 The Effects of Endogenous Catecholamines upon Ventricular Automaticity

Considerable evidence indicated that *in vitro* cardiac preparations are influenced by endogenous neurotransmitters (Section 1.4.2). Although myocardial stores of catecholamines are quite high in comparison to levels of catecholamines found in other organ systems throughout the body (von Euler, 1956; Holzbauer and Sharman, 1972), their physiological significance is obscure. This is due in part to an incomplete anatomical portrayal of the terminal distribution of sympathetic nerve endings throughout the myocardium and of the nature of the neuromuscular relations between postganglionic sympathetic nerve terminals and their myocardial effector cells (see Discussion, Section 7).

The arrhythmogenic potency of catecholamines has been known for a considerable time and their ability to induce automaticity in normally quiescent cardiac preparations has been documented recently (Yeh and Lazzara, 1977). Two parallel lines of research on the effects of electric current on cardiac tissue have demonstrated that

electric current can (i) induce automaticity in quiescent cardiac preparations, and (ii) elicit the release of endogenous neurotransmitters ("electrorelease"). Although the stimulus parameters used to produce these two effects have generally been different (constant depolarizing currents for (i) and high frequency pulses for (ii)), it is reasonable to consider that constant depolarizing currents which elicit automaticity could also elicit the release of endogenous catecholamines, which might influence the observed automaticity. Therefore, I asked the following question with regard to depolarization induced ventricular automaticity in guinea pig myocardium:

> "Is the phenomenon of electrically induced ventricular automaticity influenced by or modulated in any way by the presence of endogenous catecholamines?"

Two major pharmacological approaches were used to evaluate this question. The first approach deals with the pharmacological demonstration that endogenous catecholamines are indeed present in the tissue under in vitro conditions and are capable of influencing the phenomenon under study, namely intracellularly recorded ventricular automaticity. To this end the effects of an indirectly acting sympathomimetic amine, tyramine, upon depolarization induced ventricular automaticity were studied.

The second major pharmacological approach used was the evaluation of the effects of various sympatholytic agents upon depolarization induced ventricular automaticity. Does the elimination of the influence of endogenous catecholamines produce any change in the observed phenomenon? Three different pharmacological interventions were used to accomplish this: the effects of (i) 6-hydroxydopamine pretreatment, (ii) in vitro reserpine (two agents known to prevent

the influence of endogenous catecholamines by a presynaptic mode of action (Shore, 1972; Tranzer and Thoenen, 1967), and (iii) beta-adrenergic antagonists (which act via a postsynaptic mode of action) were investigated.

3.0 METHODS

3.1 Preparation and Experimental Setup

Guinea pigs weighing 250-350 grams were stunned by a blow to the head, exsanguinated, and the hearts were removed. Right ventricular papillary muscles, 4-6 mm in length and .7 - 1.0 mm in diameter, were isolated and mounted in a three compartment single sucrose gap chamber (Beeler and Reuter, 1970) illustrated in Figure 3-1. This was accomplished by pulling the fibers through snugly fitting holes in two rubber membranes bounding a sucrose gap of 2 mm width. Care was taken to insure that less than 1.0 mm of the preparation protruded into the test chamber (bottom chamber, Figure 3-1). Only those preparations which were undamaged (except at their cut ends) and had no side branching over their entire lengths were utilized.

Initially all three compartments were perfused with Tyrode solution (for composition of solutions, see Section 3.3) and the preparations were allowed to recover for approximately 30 minutes. During this period the preparations were driven at a rate of 1 Hz. After recovery, the center compartment was perfused with isotonic sucrose solution, containing $CaCl_2$ (New and Trautwein, 1972; Kléber, 1973), and the posterior compartment was perfused with Tyrode solution in which NaCl had been replaced by KCl, in order to reduce the input resistance of the preparation and eliminate membrane activity in the posterior compartment. Tyrode solution was saturated with 95%

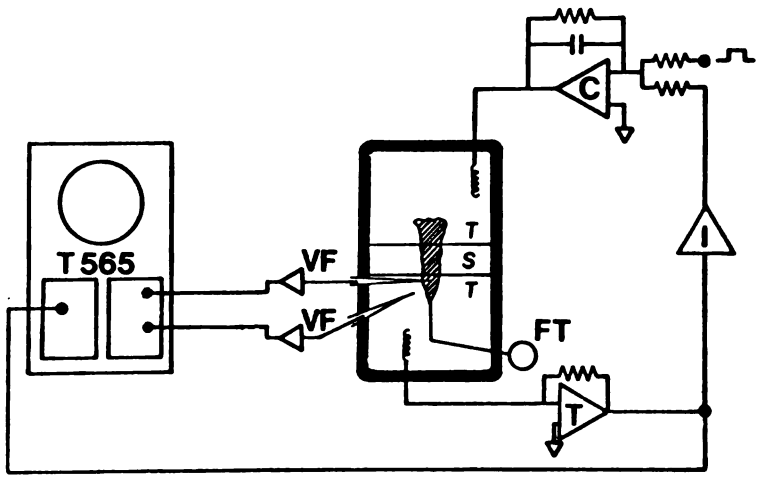


Figure 3-1

The experimental setup. VF = matched voltage followers; T565 = Tektronix 565 oscilloscope with 3A3 differential amplifier; T = current-to-voltage transducer; I = inverter; C = clamp amplifier; FT = Grass FT 03 force displacement transducer. The posterior and anterior compartments of the chamber were perfused with Tyrode solution (T), the middle compartment with isotonic solution (S), containing 3 x 10^{-5}M $CaCl_2$.

oxygen, 5% CO_2; sucrose solution with 100% oxygen. The temperature was monitored with a thermistor (Yellow Springs Inst. Co.) and maintained at between 35-37° C.

The transmembrane potential was measured differentially between an intracellular microelectrode (10-35 M ohms resistance) filled with 3M KCl, and an extracellular Ag-AgCl electrode (E 201, In Vivo Metric Systems, Redwood Valley, California), located in the test compartment near the surface of the preparation. Both electrodes were coupled to matched voltage followers (VF). The microelectrode preamplifier had a high input impedence (10^{13} ohms) and input capacity neutralization (Winston Electronics Co.). The transmembrane potential difference was amplified by a vertical amplifier (3A3, differential input) of an oscilloscope (Tektronix 565) and displayed. The transmembrane potential was electronically differentiated, filtered, and displayed on a separate beam of the oscilloscope. Schematic diagrams of the differentiator-filter-beam intensifier appear in Appendix I. The differentiator could be switched to two separate gains, which provided a linear range from 1 - 600 Volts/sec. A silk thread was tied to the chorda tendineae extending from the tip of the papillary muscle in the test chamber and attached to a force transducer (Grass FT-03). This signal was amplified by a Grass Polygraph Preamplifier (Model 70 AC) and displayed on a separate beam of the oscilloscope in most experiments.

An operational amplifier (Philbrick ML100, C in Figure 3-1) provided constant current pulses which were passed through the preparation via Ag-AgCl electrodes located in the anterior (test) and posterior chambers. The electrode in the test chamber was connected to a

current-to-voltage transducer (T), the voltage output of which was proportional to the total current transversing the sucrose gap (see Appendix III). This output voltage was compared with command pulses from a Grass (S44) Stimulator, through summing resistors at the input of the clamp amplifier. The output of the clamp amplifier was connected to the Ag-AgCl electrode in the posterior chamber. The total transgap current was displayed on a separate beam of the oscilloscope. The oscilloscope traces were photographed with a kymographic cameria (Grass Instruments Co., C4). A digital counting module with L.E.D. readout, activated by the camera shutter switch, provided a convenient method for numbering all photographic frames.

3.2 Experimental Protocol

The preparation was driven at a rate of 1 Hz with a stimulus 1.5 times threshold and duration of 2.5 msec. An equilibration period of one hour was usually allowed once the center compartment was perfused with isotonic sucrose while a stable impalement was established. An impalement was considered stable if there was a stable resting potential for at least 15 minutes. Control action potentials were recorded and then the drive rate was reduced to .05 Hz. Constant current depolarizing pulses of 1.5 second duration of variable intensities were then applied to induce automaticity. Increments in current intensity were chosen which produced 1-2 mV changes in maximum diastolic potential (MDP). The survey of automaticity was terminated when responses at the least negative range of MDP showed only damped oscillations. The basic drive rate of 1 Hz (2.5 msec. duration) pulses was then resumed and superfusion with drug-containing Tyrode solution begun. After a variable period of drug exposure

(depending upon the drug - see Results), automaticity was surveyed in the same manner. Only those experiments in which a single impalement was maintained through the control and drug exposure periods are reported in this study.

The variables used to characterize automaticity in this study and their method of measurement are described in the initial portion of Section 4.

3.3 Statistical Analyses

All variables used to characterize automaticity were compared as differences between means under control conditions and in the presence of a drug by an unpaired students t-test (two sided). In all cases where more than one experimental condition was compared to control, all variables were evaluated using the multiple comparison test of Dunnett (1955, 1964). Differences between means were considered significant if $p < .05$.

3.4 Solutions and Drugs

Tyrode solution of the following composition (mM) was used in all experiments: NaCl 137, $NaHCO_3$ 11.9, NaH_2PO_4 .4, KCl 5, $CaCl_2$ 1.8, $MgCl_2$ 1.1, and glucose 5.5. Isotonic sucrose solution had a composition (mM) of: sucrose 300, glucose 5.5, and $CaCl_2$ .03.

The following drugs were used in this study: l-norepinephrine bitartrate (generously supplied by Sterling-Winthrop Research Institute), d, l-isoproterenol hydrochloride (Aldrich Chemical Co.), l-phenylephrine hydrochloride (K and K Labs, Inc.), tyramine hydrochloride (Aldrich Chemical Co.), 6-hydroxydopamine hydrobromide (Regis Chemical Co.), reserpine (Ciba-Geigy Corp.), metoprolol

hydrochloride (H93/26) (generously supplied by Ciba-Geigy Corp.), and d-propranolol hydrochloride and l-propranolol hydrochloride (generously supplied by Ayerst Lab., Inc.). All drugs used *in vitro* were made up fresh in stock solutions and diluted with Tyrode's solution before use. In order to prevent deterioration of norepinephrine and isoproterenol due to oxidation, the final solutions were kept in an ice bath and warmed to 37°C immediately prior to entry into the anterior chamber, and only plastic connections and tubing were used in the perfusion system (Nathan and Beeler, 1975). With the exception of isoproterenol, phenylephrine, and tyramine (concentrations expressed in terms of salts), all concentrations in the text refer to the base.

For animals which received intraperitoneal injections of 6-hydroxydopamine, 150 mg/kg of drug was dissolved in a 1 ml solution of .1% ascorbic acid in order to retard auto-oxidation, and injected at 48 and 24 hours prior to sacrifice. For intravenous pretreatment, 250 mg/kg of 6-hydroxydopamine was dissolved in a .5 ml solution of .1% ascorbic acid, and injected using a 30 gauge needle into the exposed jugular vein, in animals under halothane-oxygen anesthesia, 24 hours prior to sacrifice.

3.5 Measurement of Endogenous Catecholamine Content

In a number of experiments after the papillary muscles were removed, the right ventricular wall was excised immediately. The wall specimen was then frozen in dry ice, weighed, and then stored for up to 30 days at -30°C for later determination of endogenous catecholamine levels. Norepinephrine was extracted and assayed flurometrically using the method of McCaman *et al* (1973), with

the following increase of reagent volumes. Tissues weighing up to 300 mg were homogenized in 2.0 ml of cold 0.3 N perchloric acid using a Polytron PT10 and centrifuged cold at 36,000 x g for ten minutes. An 800 ul supernatant aliquot was transferred to tubes containing 2.0 ml of 0.25 M Na_2HPO_4 (containing 7 mM EDTA) and 1.2 ml of $CHCl_3$ containing 0.1 N diethylhexylphosphoric acid (Sigma Chemical Company, St. Louis, Missouri). After shaking and centrifugation, the top aqueous phase was aspirated and 1.0 ml of the organic phase was transferred to tubes containing 2.0 ml of n-heptane and 500 µl of 0.2 N HCl. After shaking and centrifugation, the top organic phase was aspirated. A 200 µl aliquot of the acid extract was transferred to a glass tube containing 800 ul of 0.2 N sodium acetate (containing 7 mM EDTA).

Hydroxyindole product of norepinephrine was developed after the addition of 50.0 µl each of the iodine reagent, alkaline sulfite, and 5 N acetic acid, at three minute intervals with mixing (all reagents made according to McCaman, et al, 1973). The tubes were then heated in a drying oven at 100°C for five minutes and cooled to ambient temperature. The fluorescent intensity of norepinephrine was read at 392/485 nm on an Aminco-Bowman Spectrophotofluorometer, uncorrected. Tissue amine concentrations were derived from internal standards added to tissue homogenates processed concurrently. "Reversed" blank readings were subtracted from the catecholamine readings to obtain net fluorescent intensities. The amount of norepinephrine which corresponded to the intensity of blank readings was 20 ng. Average norepinephrine recovery averaged about 10%.

In only about 20% of the muscles examined electrophysiologically

could a complete survey of automaticity be made. The remainder either failed to completely recover from the dissection and mounting procedures or contracted so vigorously that a stable impalement could not be maintained long enough to complete a survey of automaticity. Therefore, a considerable number of guinea pig right ventricular catecholamine assays were performed, in which the corresponding electrophysiological data on automaticity were not obtained. These experiments afforded an opportunity to verify that the length of storage of the tissue did not influence the absolute amount of endogenous norepinephrine assayed. Appendix II shows a considerable variability in norepinephrine content between different animals, however, there was no relationship between the amount of time the frozen tissue was stored and the absolute levels of norepinephrine measured.

4.0 RESULTS - EXOGENOUS CATECHOLAMINES

4.1 Methodological Considerations in the Study of Ventricular Automaticity

4.1.1 An Example of Depolarization - Induced Ventricular Automaticity

A representative example of ventricular automaticity which can be induced by depolarizing currents under control conditions is shown in Figure 4-1. The top trace is zero membrane potential, the third trace is the differentiated upstroke velocity ($\dot{V}$), and the bottom traces are time markers representing 200 msec. in all panels. In panel a, a brief stimulus (2 msec. duration) elicits a single action potential. In panel b, a current pulse of 1.5 second duration elicits a single action potential which repolarizes to a maximum diastolic potential of only -60 mV (because of the continuing depolarizing pulse), whereupon slow diastolic depolarization ensues, resulting in a second and third action potential. Upon termination of the current pulse, the transmembrane potential immediately repolarizes to a value close to the resting membrane potential. In panel c, a slight increase in current intensity causes automaticity to occur from a slightly less negative maximum diastolic potential (-45 mV). A further increment in current intensity in panel d produces automaticity at an even less negative maximum diastolic potential (-32 mV).

It is apparent from this representative example that ventricular automaticity occurs over a range of maximum diastolic potentials, the value of which can be directly controlled by modulation of

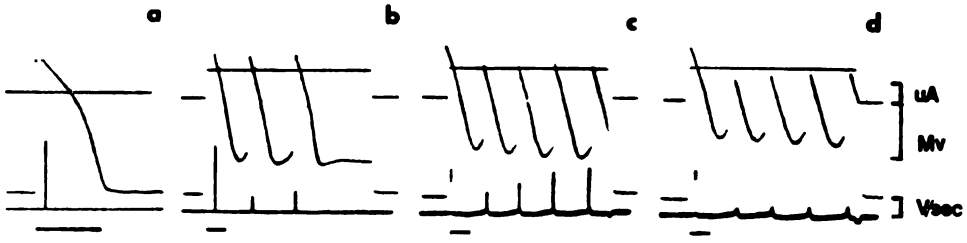


Figure 4-1

Electrically induced ventricular automaticity under control conditions. In each panel, the top trace is imposed current (I). The initial level of this trace also serves as the zero reference for membrane potential. The second trace is the transmembrane potential. The third trace is the first time derivative ($\dot{V}$) of the transmembrane potential. The bottom traces are time markers representing 200 msec. in each panel. Panel a is the action potential elicited by a brief (2 msec.) stimulus; b, c, and d, automaticity during prolonged (1.5 msec.) constant current pulses at maximum diastolic potentials (MDPs) of -60, -45, and -32 mV respectively. I calibration is 10 μA for a-d; $\dot{V}_{MAX}$ calibration is 100 V/sec for a and b, 10 V/sec for c and d; voltage calibration is 50 mV for a-d.

the intensity of applied current. Careful inspection also reveals that certain characteristics of this form of automaticity appear to vary in relation to the particular level of the maximum diastolic potential. For example, the rate seems to be slightly faster in panel c compared to panels b and d and the overshoots of the automatic action potentials appear to decrease as the maximum diastolic potential becomes less negative.

4.1.2 Variables used to Characterize Drug Effects

Figure 4-2 illustrates five variables and the methods of measurement which were used to characterize the single action potential elicited by a brief stimulus. The action potential duration (APD) was measured as the time in msec. from the initial onset of Phase 0 to 98% repolarization. The action potential duration was also measured at zero membrane potential in msec. (APD_0). The action potential overshoot (OS) was measured in mV as was the resting membrane potential (V_R). The differentiated maximum upstroke velocity of Phase 0 of the action potential ($\dot{V}_{MAX}$) was measured in volts/sec. Figure 4-3 illustrates eight variables and their method of measurement, which were used to characterize depolarization-induced automaticity. These variables were selected because the chronotropic effects of drugs in cardiac tissue have been shown to be mediated by changes in these variables (see Section 1.2). Action potential duration (APD) was defined as the time in msec. from the onset of Phase 0 of the first elicited action potential to the time the first maximum diastolic potential is reached. The inter-spike interval (ISI, reciprocal of rate) was characterized as the time in msec. between the spike of the first elicited

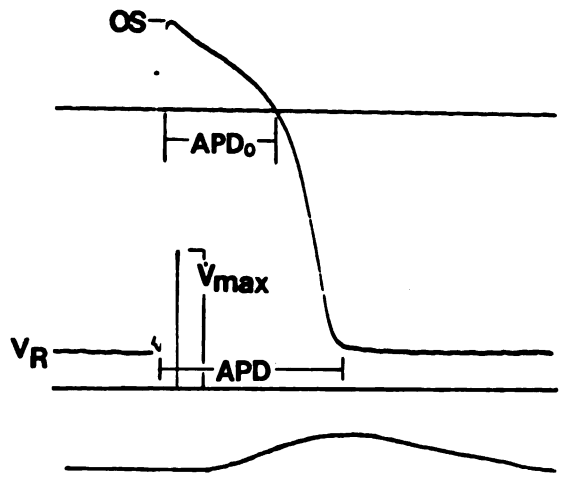


Figure 4-2

Action potential (AP) variables measured. Traces are the same as in Figure 2, except that bottom trace is contractility in this figure. OS = overshoot of AP, measured in mV. V_R = Resting membrane potential, measured in mV. ADP = AP duration to complete repolarization, measured in msec. APD_0 = AP duration at 0 mV level, measured in msec. V_{MAX} = differentiated maximum upstroke velocity of AP, measured in V/sec.

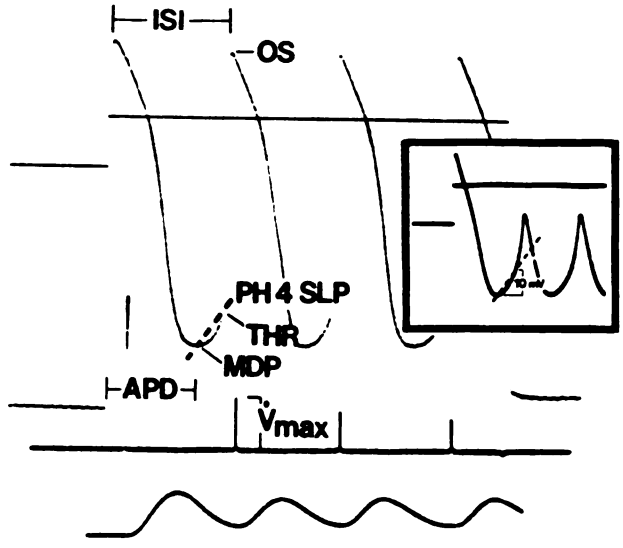


Figure 4-3

Variables measured to characterize automaticity. Traces are the same as in Figure 2, except that the bottom trace is contractility in this figure. ISI = inter-spike interval (reciprocal of rate) measured in msec. between spike of first elicited AP and first automatic AP. OS = overshoot of first automatic AP, measured in mV. APD = AP duration measured in msec. as the time from the upstroke of the first elicited AP to the time that the first MDP occurred. MDP = maximum diastolic potential measured in mV. THR = threshold potential of first automatic AP measured in mV, as described in text. PH4 SLP = Phase 4 diastolic depolarization rate measured in mV/sec., as described in text. Inset illustrates method of measuring PH4 SLP when THR not readily apparent. $\dot{V}_{MAX}$ = differentiated upstroke velocity of first automatic AP, measured in V/sec.

action potential and the spike of the first automatic action potential, or the most positive potential reached, in the case of an oscillation. Overshoot (OS) of the first automatic action potential was measured in mV, as was the maximum diastolic potential (MDP). The threshold (THR) potential was measured as the potential in mV, at which the trace abruptly disappeared (Weidmann, 1955). Phase 4 diastolic depolarization rate (PH4 SLP) was calculated by dividing the difference in potential between the maximum diastolic potential and the threshold potential by the time interval between them. This rate was expressed in mV/sec. In some preparations, automaticity over less negative maximum diastolic potentials was not characterized by a potential at which the trace abruptly disappeared (see inset). In these cases, the slope of depolarization between the maximum diastolic potential (MDP) and a point 10 mV positive to this was calculated. Current intensity was measured in μA and the differentiated upstroke velocity of the first automatic action potential was measured in V/sec. The bottom traces in Figures 2 and 3 are contractile force and were not calibrated. Any effects on contractility were considered only as a qualitative change.

4.1.3 Ventricular Automaticity as a Function of Maximum Diastolic Potential

Since the maximum diastolic potential is controlled by the intensity of applied depolarizing current, it might seem that many of the variables influencing automaticity are a function of the applied current. This raises the question of whether studying these variables as a function of applied current would give reproducible results between different preparations.

As shown in Figure 3-1, the current-to-voltage transducer measures the total amount of current which traverses the sucrose gap and reaches the anterior, test chamber. New and Trautwein (1972) showed that this total current is actually composed of two components (see Appendix III), (i) a current which traverses intracellular pathways and displaces the true membrane potential of a cell in the test compartment, and (ii) a current which traverses an extracellular pathway (shunt current) and does not contribute to the displacement of the true membrane potential. The ratio of intracellular resistance to extracellular resistance then is the major factor which determines the fraction of current which traverses the shunt pathway. New and Trautwein found a considerable variability in this ratio between different preparations. My own experiments attest to the variability in the amount of total current required to depolarize to various maximum diastolic potentials between different preparations. Grant and Katzung (1976) compared the reproducibility between preparations of measuring phase 4 slope as a function of applied current versus measuring phase 4 slope as a function of maximum diastolic potential. More consistent results were obtained using the latter method. The reason for this is that the true maximum diastolic potential is proportional to the membrane resistance and is not influenced by variability in the ratio of intracellular resistance to extracellular resistance between different preparations. Another consideration is that most of the ionic currents underlying the potential changes which occur during automaticity are voltage dependent, therefore, drugs might be expected to modify particular currents and thereby produce different effects at different potentials. For these reasons,

the variables used to quantitate automaticity in this project were studied as a function of maximum diastolic potential and the effects of drugs on these variables were compared at similar maximum diastolic potentials in the absence and presence of drug.

The variability between preparations in the amount of current required to depolarize the membrane to various potentials posed two additional problems: (i) comparisons at similar maximum diastolic potentials between different preparations, or in the same preparation during different experimental conditions, and (ii) the influence of the series resistance upon measurement of the maximum diastolic potential. The first of these will be considered here and the second in Section 4.1.4. The variability in the increments of applied current to step to different maximum diastolic potentials made it difficult during the course of an experiment to always compare automaticity at identical maximum diastolic potentials between different preparations, or in the same preparation in the absence and presence of drug. Also, since automaticity occurs over a range of maximum diastolic potentials which is a continuum, a choice had to be made of maximum diastolic potentials to be used for comparison purposes. Since automaticity typically may occur over a range of maximum diastolic potentials of -70 to -20 mV, it was decided to sample five to six values of maximum diastolic potential for each decade of maximum diastolic potential. From these five or six values of maximum diastolic potential, the variables used to quantitate automaticity were measured and a mean value for each was calculated for each decade. When this waa done in several papillary muscles, some 15-30

observations were collected for each variable in each decade of maximum diastolic potential, and a mean value calculated for that decade under each experimental condition.

In order to understand the relationship between the variables used to quantitate automaticity and maximum diastolic potential under control conditions, data were compiled from 25 experiments in which the entire range of automaticity could be sampled in each impalement. Table 4-1A summarizes the average electrophysiological parameters of the single action potential elicited by a brief stimulus in 25 preparations. The data presented are means and standard errors. These values are all of a magnitude characteristic of ventricular myocardial cells. There was considerable variability in the most negative maximum diastolic potential in which automaticity occurred, a range of -77 to -52 mV. The mean value was -67.2 mV. 9 out of 25 preparations had automaticity which commenced in the -79 to -70 mV decade of MDP. In 13 of 25, automaticity commenced in the -69 to -60 mV decade of MDP. In order to study the positive limit of automaticity, the MDP at which the overshoot of the first automatic action potential declined to 0 mV was measured (in most instances this could be considered an oscillation). The mean of the less negative limit of automaticity was -22.6 mV with a range from -46 to 0 mV. In 17 of 25 preparations, automaticity was present into the -29 to -20 mV range and in 23 of 25 preparations, automaticity was present into the -39 to -30 mV range.

Tables 4-1. Summary of action potential variables (A) and automaticity variables as a function of maximum diastolic potential (B) in 25 control experiments. Definitions and abbreviations of each of the variables are the same as in Figures 4-2 and 4-3. Each value represents the mean and standard error. Below each value in B are the number of observations made over a particular decade of maximum diastolic potential. The estimated voltage error due to R_s was calculated as described in the text.

A

V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)	MDP** (mV)
-89.0 ± .3	278.6 ± 10.5	29.6 ± .4	178.2 ± 4.6	78.0 ± 4.3	-67.2 ± 1.3	-22.6 ± 2.6

MDP* = Most negative MDP for automaticity.
MDP** = Least negative MDP for automaticity.

B

Variable	-79 -70	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
Inter-Spike Interval (msec)	469.3 ± 19.4 28	436.5 ± 5.5 147	418.7 ± 6.0 132	460.3 ± 8.7 114	494.4 ± 9.4 112	503.4 ± 13.2 77
AP Duration (msec)	329.2 ± 5.9 28	338.1 ± 3.4 147	341.3 ± 4.4 132	353.3 ± 6.6 114	357.3 ± 6.7 112	357.7 ± 8.2 77
Threshold (mV)	-63.9 ± .9 28	-53.2 ± .4 147	-42.8 ± .4 132	-28.8 ± .5 103	-16.0 ± .8 83	-9.1 ± .8 40
PH 4 Slope (mV/sec)	81.9 ± 9.2 28	142.4 ± 4.3 147	201.1 ± 4.2 132	202.2 ± 5.0 114	167.7 ± 4.8 112	144.4 ± 6.1 77
Overshoot (mV)	28.8 ± 1.6 28	34.1 ± .7 147	31.0 ± .8 132	26.5 ± 1.1 114	19.0 ± 1.7 112	9.4 ± 1.9 90
$\dot{V}_{MAX}$ (V/sec)	204.0 ± 5.4 28	110.1 ± 4.7 147	28.4 ± 2.1 123	10.5 ± .6 93	6.4 ± .3 85	4.1 ± .4 42
Mean MDP per decade (mV)	-72.6 ± .4	-64.2 ± .2	-54.8 ± .4	-44.9 ± .3	-34.8 ± .3	-25.0 ± .3
Est. R_g Error (mV)	5.2	5.6	5.8	6.0	7.2	8.6

Table 4-1B summarizes the relationship between the variables used to characterize automaticity and maximum diastolic potential. The values are means and standard errors. Immediately below each value is the number of observations. It can be seen that the interspike interval reaches a minimum in the range of -59 to -50 mV and then increases over the less negative range. There appears to be a slight prolongation of action potential duration as MDP becomes less negative. Phase 4 slope can be seen to increase and reach a maximum from -59 to -40 mV and then fall off again. This is the same range of MDP in which the rate is the maximum. There is a progressive decrease in both overshoot and $\dot{V}_{MAX}$ of the automatic action potentials as MDP becomes less negative. Note that the mean MDP per decade of MDP is very near the midpoint of each decade in every case except the decade of -79 to -70. The mean MDP for this decade is -72.6 mV which indicates that MDPs in this range are clustered in the lower end, which would be expected near the most negative limit of automaticity.

4.1.4 The Influence of Series Resistance upon Accurate Measurement of the Transmembrane Potential

The transmembrane potential of an individual cell is by definition the difference in potential between the interior of the cell and the immediately adjacent exterior of the cell. In practice, this entails measurement of the intracellular potential with an intracellular microelectrode and measurement of the extracellular potential with a reference electrode. It is quite impossible to place the reference electrode in a vicinity immediately adjacent to the impaled cell in a multicellular, small cell preparation like cardiac muscle. The reference electrode is usually positioned

near the surface of the preation (in differential recording). Therefore, a considerable distance and quantity of cellular and non-cellular structures exist between the immediate exterior of the impaled cell and the reference electrode. These structures together with the resistivity of the bulk solution make up a considerable resistance, which has been shown by Beeler and Reuter (1970) to be in series with the true membrane resistance in dog papillary muscles. The existence of this series resistance (Rs) presents major problems in attempts to voltage clamp multicellular preparations (Attwell and Cohen, 1977; Fozzard and Beeler, 1975) and also would be expected to introduce errors in the measurement of transmembrane potentials during the passage of applied current across the single sucrose gap. An equivalent circuit of the single sucrose gap including Rs is illustrated in Appendix III. Also presented are some simple calculation which demonstrate the potential errors which can result during the passage of current. Therefore, it was considered important to determine the magnitude of Rs-related voltage errors.

The series resistance can be measured by applying short duration rectangular current pulses and measuring the resulting voltage deflections as shown in Figure 4-4. Simple application of Ohm's law will produce the appropriate value for the series resistance. In some preparations, a slight capacitive discharge appeared on the voltage trace as in Figure 4-4b. This was assumed to result from the discharge of some capacitance in the sucrose region (McGuigan, 1974) and the voltage level after the capacitive discharge was extrapolated back to time zero. This method of

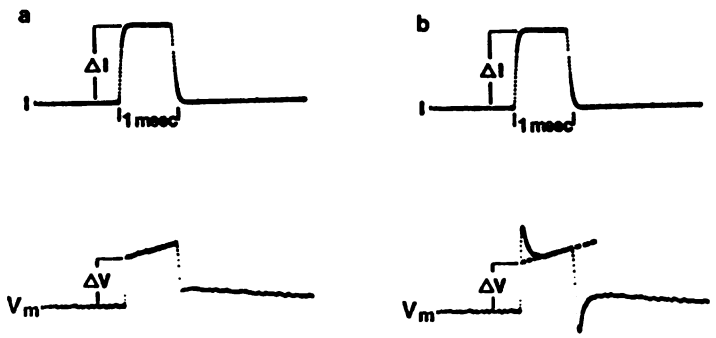


Figure 4-4

Method of series resistance (Rs) measurement. 1 msec. duration current pulses are applied (upper traces) and the resulting voltage deflections (lower traces) are measured. Measurement of Δ V in the absence of a capacitive component is shown in a; measurement of Δ V in the presence of a capacitive component is shown in b. The voltage step (Δ V) is extrapolated to zero time (the start of the pulse). Rs is then obtained from Ohm's law, Rs = Δ V/ Δ I.

measuring Rs is a relatively simple procedure, but was not performed in many of the experiments in this study. The results of a survey of Rs determinations are shown in Table 4-2. In each of 21 guinea pig papillary muscles, three estimates of Rs were made and a mean Rs for each muscle was calculated.

Rs ranged from a value of 844-182 Ω, with a mean value of 398.9 Ω. This value compares favorably with Rs determinations made in dog papillary muscles (580 Ω, Beeler and Reuter, 1970) and in cat papillary muscles (200-600 Ω, New and Trautwein, 1972; 440 Ω, McDonald and Trautwein, 1970).

Significant errors in voltage measurements arising from IR drops across Rs can probably be caused by (i) a large applied current across a relatively small Rs, or (ii) a small current applied across a large Rs. This can be readily seen from the equivalent circuit diagram in Appendix III and can also be demonstrated by two examples.

Figure 4-5 compares two impalements from two different papillary muscles which represent extremes in terms of Rs. Panels b and e are compared at a MDP of -60 mV and panels c and f are compared at a MDP of -40 mV. Very similar magnitudes of applied current (24 μA for 8-09-78 and 26.5 μA for 7-24-78) were required to achieve -40 mV. Notice that the overshoot of the first automatic action potential for 7-24-79 is +52 mV. In this muscle, at this MDP, the IR drop across the Rs of 844 Ω was calculated to be 22.4 mV. This large error most likely produces the accentuated overshoot and also an apparent MDP much less negative than it really is.

Experiment	R_1	R_2	R_3	R_{AVE}
7-17-78 #2	300	300	300	300
7-28-78	400	400	400	400
8-13-78	300	300	300	300
8-11-78	300	300	350	317
8-9-78	182	182	182	182
9-6-78	200	200	225	208
9-5-78	381	415	400	399
8-29-78	500	500	500	500
7-24-78 #1	850	833	850	844
7-24-78 #2	300	300	367	322
8-30-78	600	650	600	617
7-18-78	600	650	600	617
7-17-78 #1	250	250	250	250
7-20-78	200	233	200	211
5-30-78	214	300	233	249
7-15-78	400	400	400	400
6-5-78 #1	700	850	833	794
6-5-78 #2	280	260	200	247
6-8-78	400	350	300	350
7-10-78 #2	556	577	500	544
5-31-78	300	333	346	326

Range = 182-844 ohm
Mean = 398.9 ohm $\pm$ 41.0
N = 21

Table 4-2. Survey of series resistances (R_s) in 21 guinea pig right ventricular papillary muscles. Three determinations in each muscle were made as described in the text and an average for each muscle calculated.

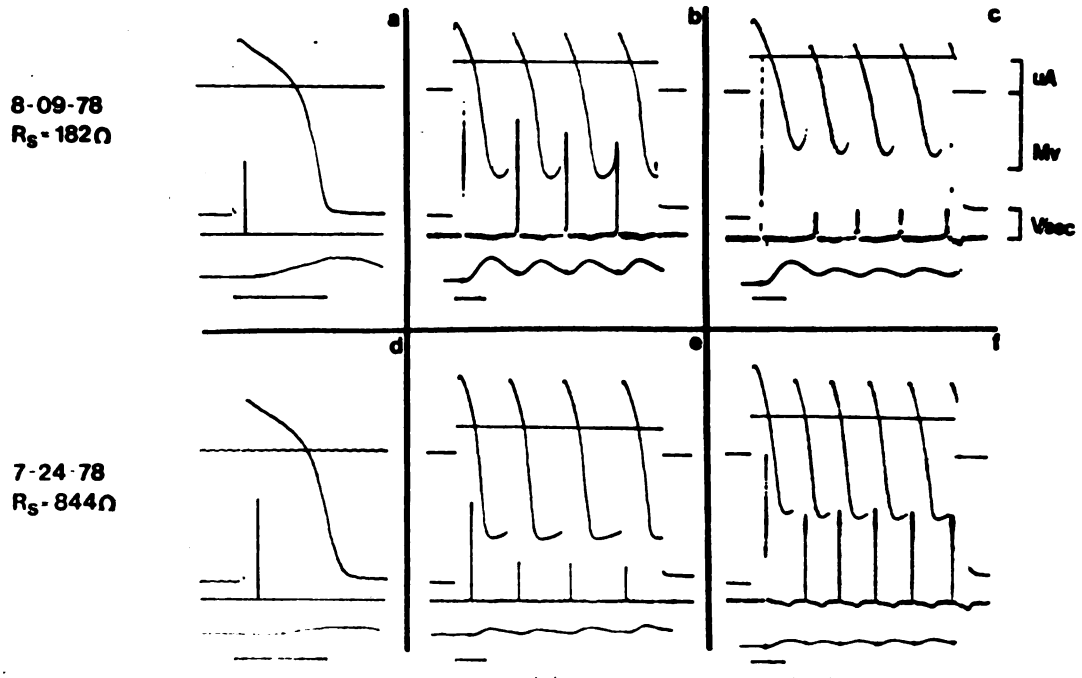


Figure 4-5

Automaticity from two papillary muscles with extreme values of Rs compared at similar MDPs. In each panel, the top trace represents the zero reference for membrane potential, and current (I). The second trace is the transmembrane potential. The third trace is the first time derivative ($\dot{V}$) of the transmembrane potential. The fourth trace is contractility, and the bottom traces are time markers representing 200 msec. in each panel. a and d are action potentials elicited by a brief stimulus (2 msec.); b and e, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -60 mV; c and f at MDPs of -40 mV. I calibration is 20 μA for all panels; 10 V/sec. for b, c, and f; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, d, and e; voltage calibration is 50 mV for all panels. Notice large overshoots during activity in f compared to c.

In contrast, the overshoot of the first automatic action potential for the muscle of 8-09-78 is 31 mV, with a calculated IR drop across an Rs of 182 Ω of only 4.3 mV. The difference in overshoot between the two muscles at an MDP of -40 mV is 21 mV; a value very close to the difference of 18.1 mV in IR drop across the respective Rs for each muscle.

Figure 4-6 compares two impalements from two different papillary muscles which have similar Rs but have very different magnitudes of applied current. For the muscle of 7-28-78, 14.8 μA of current was applied to depolarize to -40 mV. The overshoot of the first automatic action potential was 30 mV, with a calculated IR drop across Rs of 5.9 mV. In 7-15-78, an overshoot of 50 mV occurred, but the amount of current to depolarize to -40 mV was 31 μA. This resulted in a calculated IR drop of some 12.4 mV.

These examples illustrate how a large voltage error can result from either (i) a large current applied across a relatively small Rs, or (ii) a small current applied across a large Rs. In order to minimize such errors in measured voltages in these experiments, preparations were rejected if extremely large currents were required to depolarize to less negative MDPs, i.e., > 30 μA, and if overshoots at less negative MDPs ever exceeded 35 mV. These criteria will help to insure the elimination of experiments in which large errors in measured voltages are likely to occur, but smaller voltage errors probably occur in the remaining preparations and might exert an influence upon the measured variables used to quantitate automaticity and their relationship to maximum diastolic potential.

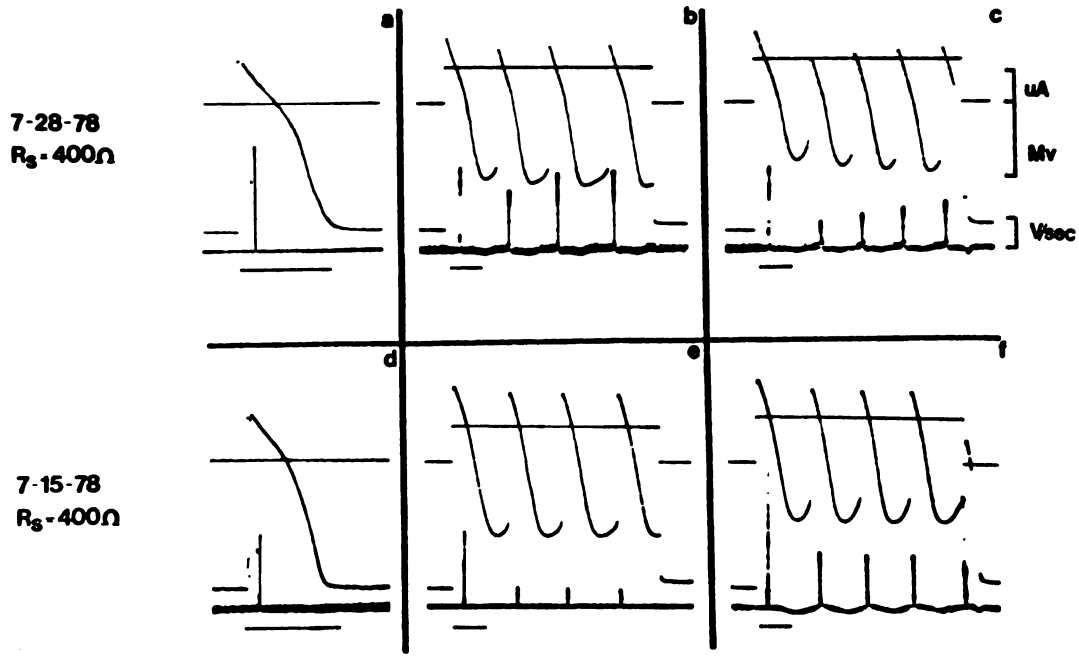


Figure 4-6

Automaticity from two papillary muscles with similar values of Rs, but different I magnitudes, compared at similar MDPs. Traces are as in Figure 2. a and d are action potentials elicited by a brief stimulus (2 msec.); b and e, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -54 mV; c and f at MDPs of -40 mV. I calibration is 10 μA for a-c, 20 μA for d-f; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, d, and e; 10 V/sec. for b, c, and f; voltage calibration is 50 mV for all panels. Notice large overshoots during activity in f compared to c.

In order to estimate the possible influence of such errors, the average magnitudes of current required to depolarize the 25 control preparations presented in Table 4-1 were tabulated by decade of MDP and the potential error that would result due to an IR drop across an average Rs of 400 Ω was calcualted. The average amount of current increased from 13 μA to 21.4 μA over the range of MDPs from -79 to -20 mV. The calculated error, as shown in Table 4-1B, ranged from 5.2 mV to 8.6 mV over the entire range of automaticity. This would mean that all of the true MDPs were (possibly) 5.2 to 8.6 mV more negative than measured, as were the other two voltage measurements, overshoot and threshold.

4.2 Effects of Isoproterenol on Depolarization Induced Ventricular Automaticity

The effects of 10^{-6}M isoproterenol, a pure beta-agonist, were examined in eight experiments. One experiment is illustrated in Figure 4-7. The effects of isoproterenol were of extremely rapid onset, usually within minutes of entry into the test chamber. Contractility increased to such an extent that it was necessary to study automaticity at the very onset of effects and therefore a steady-state was never achieved. There was an increase in overshoot and of the action potential duration of the action potential elicited by a brief stimulus (panel e). In the case of automaticity arising from more negative maximum diastolic potentials (panels b and f) the rate was increased as was overshoot of automatic APs. There appeared to be a relatively large decrease in action potential duration without much effect on phase 4 slope. No effect on threshold potential was observed. There was a slight increase in the amount of current required to depolarize the cell to the same MDP in the presence of isoproterenol. In the case

of automaticity arising from less negative MDPs (panels c and d), the effects were the same, with a slightly larger increase in overshoot of automatic APs and a much larger increase in the amount of current required to depolarize the cell to the same MDP in the presence of isoproterenol. In addition there appeared to be an increase in the upstroke velocity of APs arising from less negative MDPs. A comparison of automaticity at the same magnitude of applied depolarizing current (panels d and h) shows that in the presence of isoproterenol, automaticity arose from a more negative MDP.

The mean effects of isoproterenol in a group of four muscles, in which the impalement could be maintained throughout the experiment, is summarized in Tables 4-3. The major effects upon the AP elicited by a brief stimulus were an increase in overshoot and a slight increase in AP duration at both 0 mV and to full repolarization. The most negative limit for automaticity appeared to occur from a slightly more negative MDP in the presence of isoproterenol. The effects upon automaticity are presented in Table 4-3B. There was a significant increase in rate over the entire range of MDPs, accompanied by a decrease in AP duration. There were no consistent effects upon threshold or overshoot, while phase 4 slope was increased, especially over the less negative range of MDPs. Effects on $\dot{V}_{MAX}$ were not significant.

In all four experiments, an increase in the magnitude of depolarizing current required to depolarize to equivalent MDPs, occurred in the presence of isoproterenol. The average increase in current per decade of maximum diastolic potential is shown in Table 4-4. This effect, which is probably due to a decrease in relative membrane

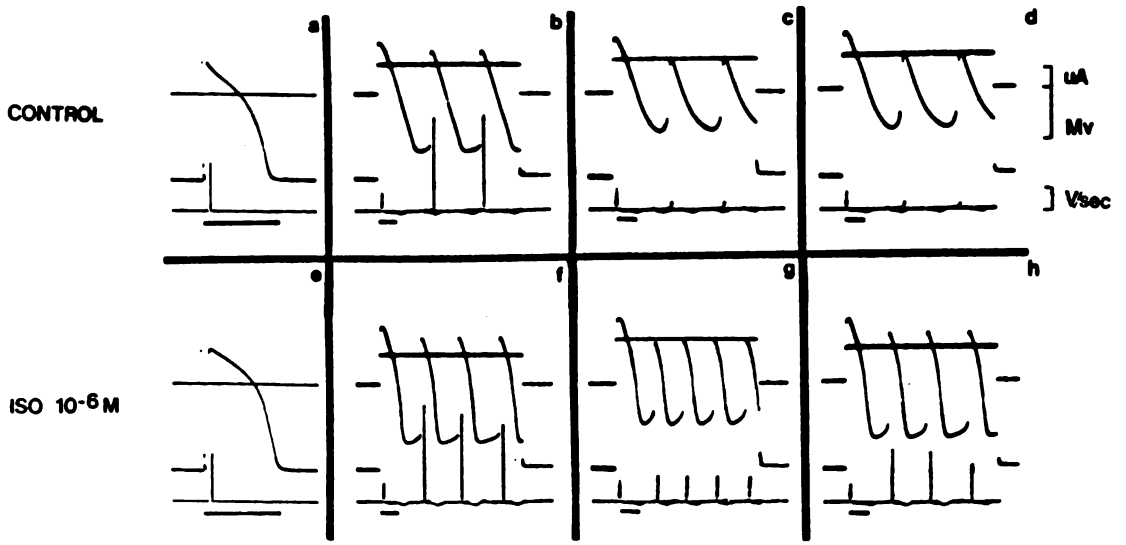


Figure 4-7

The effect of isoproterenol on ventricular automaticity. The traces ar as in Figure 2. a and e are action potentials elicited by a brief stimulus (2 msec.); b and f, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -58 mV; c and g at MDPs of -39 mV; d and h are compared at similar current magnitudes (for panel d, MDP = -39, for panel h, MDP = -55). I calibration is 20 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a and e, 10 V/sec. for b, c, d, f, g, and h. Voltage calibration is 50 mV for all panels- Panels a-d are control responses; panels e-h were obtained during superfusion with 10^{-6}M isoproterenol.

resistance, could certainly distort the measurement of the various voltage parameters (including MDP) as a result of a larger IR drop across the series resistance. The amount of such an error would depend upon the value of Rs and the magnitude of change in current. It can be seen that in the experiment illustrated in Figure 4-7 (5-06-77), the largest change in applied current occurred. Although Rs was not measured in this muscle, such a potential error was probably the largest in this impalement. The experiment of 1-31-78 is illustrated in Figure 4-8. In this muscle, Rs was measured to be 685 Ω. The magnitude of current change produced in this impalement by isoproterenol was smallest in this muscle, as seen in Table 4-4. At a MDP of -35 mV (panels c and f, Figure 4-8), the IR drop across Rs due to the change in current was only 1.1 mV. Notice in this experiment, there was a clear cut increase in phase 4 slope but relatively little effect upon overshoot of the automatic APs in the presence of isoproterenol. This suggests that in the experiment of Figure 4-7 the increase in overshoot at the less negative MDPs was probably a manifestation of a voltage error, due to Rs.

It seems quite possible that many of the effects of isoproterenol upon automaticity in the four experiments summarized in Table 4-3 may in fact be confounded by Rs errors. This would be especially true in the less negative range of MDPs, where the greatest changes in applied currents are produced by isoproterenol. It was, therefore, important to attempt corrections for such errors. This was simple for two of the experiments, in which Rs was measured. In the other two experiments, although Rs was not measured, a value for Rs of 800 Ω was used to calculate "worst case" errors due to the

Tables 4-3. Summary of the effects of 10^{-6}M isoproterenol upon action potentials (A) and upon automaticity (B) in a group of muscles. Definitions and abbreviations of each of the variables were described in Figures 4-2 and 4-3. In this table and in all subsequent tables and graphs, the following symbols represent levels of significance evaluated by the statistical tests described in Chapter 3: *$p < .05$; **$p < .01$; ***$p < .001$.

A

	V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)
Control	-88.8 ± .5	278.8 ± 30.2	28.5 ± 1.3	176.3 ± 8.8	78.3 ± 6.3	-62.3 ± 2.7
10^{-6} M ISO	-87.8 ± 1.1	250.0 ± 43.0	30.1 ± 1.3	187.5 ± 13.2	99.3 ± 15.5	-65.0 ± 2.1

B

Variable	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
Inter-Spike Interval (msec)					
Control	436.7 ± 26.7 (9)	400.7 ± 20.3 (14)	446.8 ± 26.8 (14)	506.4 ± 22.4 (14)	464.2 ± 38.9 (6)
10^{-6}M ISO	345.7 ± 18.8** (11)	287.3 ± 14.4*** (15)	307.7 ± 12.6*** (15)	343.6 ± 15.6*** (11)	367.5 ± 18.1 (4)
AP Duration (msec)					
Control	332.8 ± 11.5 (9)	320.4 ± 16.8 (14)	321.4 ± 20.9 (14)	332.7 ± 23.9 (14)	294.3 ± 24.9 (6)
10^{-6}M ISO	243.3 ± 12.2*** (11)	214.3 ± 8.0*** (15)	216.0 ± 8.9*** (15)	233.2 ± 13.9** (11)	235.0 ± 21.8 (4)
Threshold (mV)					
Control	-50.8 ± 1.3 (9)	-40.8 ± 1.2 (14)	-27.3 ± 2.3 (12)	-7.7 ± 2.7 (9)	
10^{-6}M ISO	-51.6 ± 1.3 (11)	-40.1 ± 1.0 (15)	-25.8 ± 2.3 (13)	-12.6 ± 1.7 (9)	
PH 4 Slope (mV/sec)					
Control	162.8 ± 31.9 (9)	193.6 ± 16.8 (14)	195.7 ± 18.4 (14)	145.0 ± 16.1 (14)	116.0 ± 9.3 (5)
10^{-6}M ISO	140.9 ± 26.2 (11)	230.0 ± 19.9 (15)	233.3 ± 11.2 (15)	219.1 ± 14.7** (11)	148.8 ± 13.9 (4)
Overshoot (mV)					
Control	29.7 ± 3.1 (9)	30.5 ± 2.9 (14)	25.5 ± 3.4 (14)	16.2 ± 4.9 (16)	-.6 ± 5.9 (8)
10^{-6}M ISO	33.8 ± 3.5 (11)	29.3 ± 3.5 (15)	23.3 ± 4.4 (15)	14.2 ± 7.3 (12)	13.0 ± 9.8 (5)
$\dot{V}_{MAX}$ (V/sec)					
Control	88.7 ± 15.2 (9)	30.5 ± 5.5 (11)	15.8 ± 3.3 (8)	9.4 ± 2.2 (8)	
10^{-6}M ISO	65.2 ± 7.7 (11)	29.2 ± 6.1 (13)	17.8 ± 3.1 (9)	8.0 ± 3.2 (6)	

Experiment	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
5-06-77	-1.0 uA	+2.5 uA	+6.5 uA	+14.9 uA	+25.9 uA
1-04-78	+.2 uA	+1.5 uA	+3.5 uA	+4.9 uA	
1-31-78	+.6 uA	+.3 uA	+.8 uA	+1.4 uA	+2.0 uA
2-16-78	+.9 uA	+1.7 uA	+1.6 uA	+3.1 uA	+4.2 uA

Table 4-4. Change in magnitude of applied depolarizing current produced by 10^{-6}M isoproterenol in four experiments. The changes in applied current were averaged by decade of maximum diastolic potential.

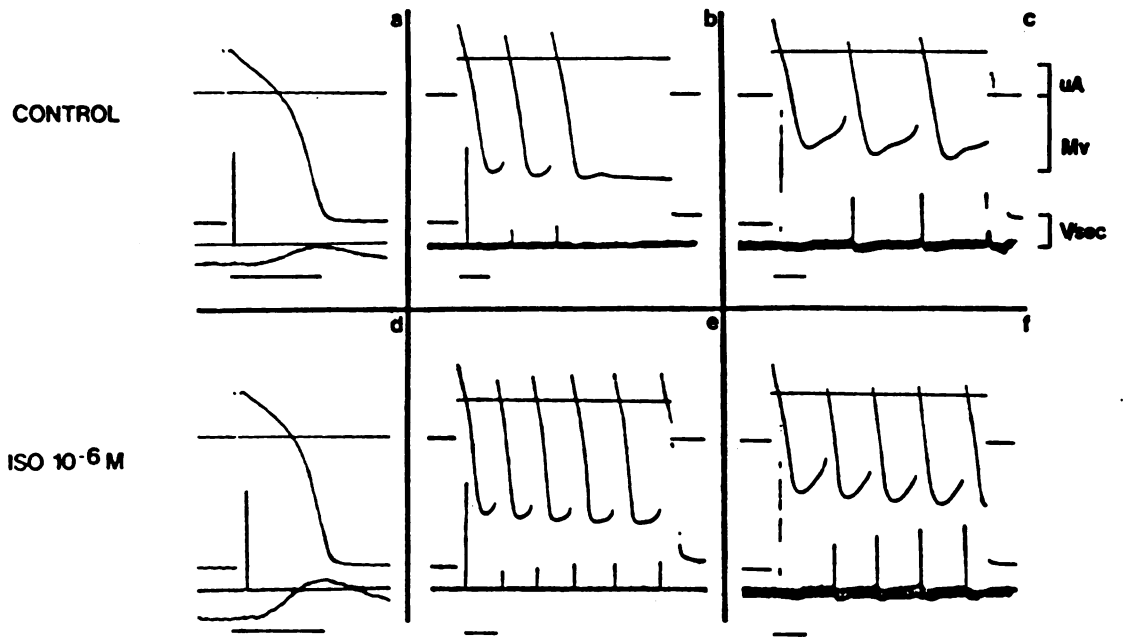


Figure 4-8

The effects of isoproterenol in a different experiment. The traces are as in Figure 2. a and d are action potentials elicited by a brief stimulus (2 msec.); b and e, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -55 mV; c and f at MDPs of -35 mV. I calibration is 10 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, b, d, and e; 10 V/sec. for c and f. Voltage calibration is 50 mV for all panels. Panels a-c are control responses; panels d-f were obtained during superfusion with 10^{-6}M isoproterenol.

Variable	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
PH 4 Slope (mV/sec)					
Control	162.8 ± 31.9 (9)	193.6 ± 16.8 (14)	195.7 ± 18.4 (14)	145.0 ± 16.1 (14)	116.0 ± 9.3 (5)
10^{-6} M ISO	140.9 ± 26.2 (11)	232.1 ± 16.2 (19)	221.7 ± 11.1 (18)	214.2 ± 21.2* (6)	135.0 ± 25.0 (2)
Overshoot (mV)					
Control	29.7 ± 3.1 (9)	30.5 ± 2.9 (14)	25.5 ± 3.4 (14)	16.2 ± 4.9 (16)	-.6 ± 5.9 (8)
10^{-6} M ISO	33.5 ± 3.5 (11)	27.5 ± 3.1 (19)	17.1 ± 4.4 (18)	12.1 ± 8.4 (6)	-5.4 ± 5.9 (3)

Table 4-5. Effects of 10^{-6}M isoproterenol upon phase 4 slope and overshoot after corrections for voltage errors due to R_s. Method of correcting for R_s errors is described in the text.

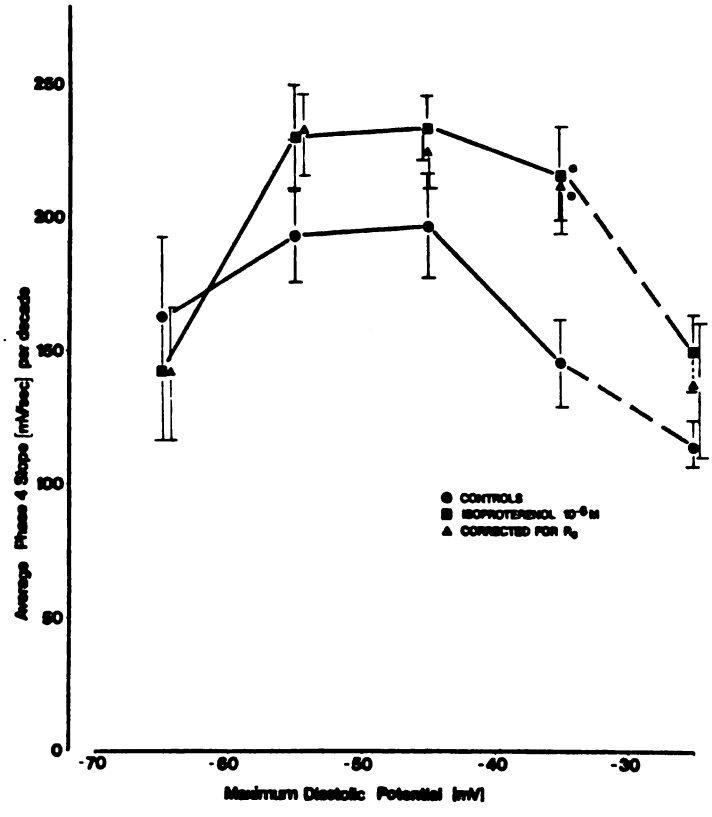


Figure 4-9

Effects of isoproterenol on phase 4 depolarization rate. The curves show mean phase 4 slope and standard errors per decade of MDP for automatic activity in a group of muscles. All values are plotted at the midpoint of each decade of MDP. *p < .05, **p < .01, ***p < .001. Not all preparations exhibited automaticity over the extreme decades of MDP (i. e., -79 to -70 and -29 to -20). In these cases means are connected with interrupted lines.

IR drop across Rs. This value for Rs was close to the largest value measured in the survey of 21 papillary muscles reported in Table 4-2. The corrected values for phase 4 slope and overshoot are presented in Table 4-5. It can be seen that after appropriate corrections in all voltage measurements are made for IR drops across Rs, an increase in phase 4 slope over the less negative range of MDP occurs in the presence of isoproterenol. There was relatively little effect in overshoot of automatic APs. Although the apparent decrease in overshoot from -59 to -20 mV (not significant) suggests that these figures may be "over corrected". The effects of isoproterenol on phase 4 slope corrected for Rs errors are summarized in Figure 4-9.

4.3 Effects of Phenylephrine on Depolarization Induced Ventricular Automaticity

The effects of two concentrations of phenylephrine were examined in seven experiments. A typical experiment is illustrated in Figure 4-10. Phenylephrine had little effect upon the action potential elicited by a brief stimulus, after 30 minutes superfusion. There was no effect upon the negative MDP limit for automaticity by phenylephrine. The major effects on automaticity were an increased rate and decreased $\dot{V}_{MAX}$ for automaticity arising from more negative MDPs (panel e) and a decrease in overshoot of automatic APs arising from less negative MDPs. The electrophysiological effects appeared to be slow and progressive in onset, and a progressive increase in contractility often precluded the study of automaticity after superfusion times longer than 30 minutes. In two experiments, 30 minutes of drug-free washout with Tyrode solution failed to restore automaticity back to control levels.

The mean effects of two concentrations of phenylephrine in a group

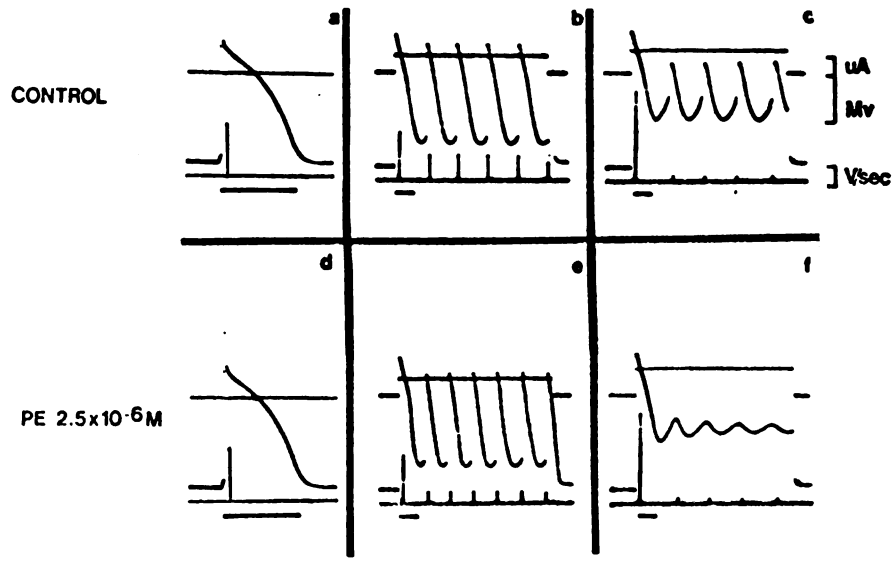


Figure 4-10

The effects of phenylephrine on depolarization-induced ventricular automaticity. The traces are as in Figure 4-1. a and d are action potentials elicited by a brief stimulus (2 msec.); b and e, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -71 mV; c and f at MDPs of -44 mV. I calibration is 5 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, b, d, and e; 10 V/sec. for c and f. Voltage calibration is 50 mV for all panels. Panels a-c are control responses; panels d-f were obtained during superfusion with 2.5 x 10^{-6}M phenylephrine.

Tables 4-6. Effects of two concentrations of phenylephrine upon action potentials (A) and automaticity (B) in a group of muscles. Definitions and abbreviations of the variables were described in Figures 4-2 and 4-3. * $p<.05$, ** $p<.01$, *** $p<.001$. No R_s-voltage corrections.

A

	V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)
Control	-87.7 ± .9	265.0 ± 37.5	29.7 ± .9	183.3 ± 28.5	66.7 ± 8.8	-73.7 ± 2.4
5×10^{-7} M PE	-87.7 ± .3	268.3 ± 27.7	29.3 ± .7	188.3 ± 41.5	62.7 ± 11.2	-75.7 ± 1.7
2.5×10^{-6} M PE	-86.7 ± 1.7	253.3 ± 29.1	28.3 ± .9	186.7 ± 39.8	61.7 ± 9.3	-73.7 ± 1.5

B

Variable	-79 -70	-69 -60	-59 -50	-49 -40	-39 -30
Inter-Spike Interval (msec)					
Control	438.9 ± 10.4 (9)	393.3 ± 13.2 (18)	385.6 ± 16.8 (16)	453.6 ± 17.1 (14)	484.6 ± 17.8 (12)
5×10^{-7}M PE	430.5 ± 12.1 (11)	352.8 ± 15.8 (16)	385.0 ± 27.4 (10)	447.9 ± 12.6 (14)	463.1 ± 15.6 (8)
2.5×10^{-6}M PE	381.0 ± 18.7* (10)	354.5 ± 17.5 (19)	386.4 ± 23.9 (14)	452.1 ± 17.8 (14)	434.4 ± 19.8 (8)
AP Duration (msec)					
Control	337.2 ± 10.2 (9)	308.6 ± 11.3 (18)	306.9 ± 10.6 (16)	312.5 ± 13.3 (14)	310.4 ± 11.6 (12)
5×10^{-7}M PE	304.1 ± 17.3 (11)	282.8 ± 14.0 (16)	294.5 ± 23.7 (10)	289.3 ± 14.8 (14)	288.6 ± 23.7 (8)
2.5×10^{-6}M PE	251.5 ± 13.3** (10)	277.1 ± 14.1 (19)	280.7 ± 18.0 (14)	270.4 ± 12.7 (14)	247.9 ± 13.5 (8)
Threshold (mV)					
Control	-64.0 ± 1.3 (9)	-54.7 ± .9 (18)	-42.1 ± 1.7 (15)	-20.0 ± 2.3 (7)	
5×10^{-7}M PE	-63.0 ± .4 (11)	-56.2 ± .6 (16)	-40.8 ± 2.3 (9)	-17.7 ± 3.6 (6)	
2.5×10^{-6}M PE	-62.3 ± .7 (10)	-55.1 ± .9 (19)	-37.1 ± 2.5 (11)	-18.3 ± 1.5 (4)	
PH 4 Slope (mV/sec)					
Control	115.0 ± 19.8 (9)	176.7 ± 16.2 (18)	237.5 ± 11.6 (16)	158.6 ± 11.3 (14)	116.3 ± 8.6 (12)
5×10^{-7}M PE	101.8 ± 9.0 (11)	159.1 ± 11.6 (16)	227.5 ± 10.2 (10)	156.4 ± 12.3 (14)	101.4 ± 4.6 (8)
2.5×10^{-6}M PE	98.0 ± 12.3 (10)	167.6 ± 12.4 (19)	218.1 ± 8.9 (14)	126.4 ± 12.6 (14)	98.3 ± 2.5 (6)
Overshoot (mV)					
Control	28.1 ± 2.7 (9)	25.7 ± 1.2 (18)	16.5 ± 1.6 (16)	9.3 ± 1.7 (14)	-9.5 ± 3.1 (12)
5×10^{-7}M PE	27.1 ± 2.3 (11)	24.2 ± 2.2 (16)	13.6 ± 2.2 (10)	-1.4 ± 3.5* (14)	-23.3 ± 1.0** (9)
2.5×10^{-6}M PE	24.3 ± 1.5 (10)	20.6 ± 1.2* (19)	11.4 ± 1.6* (14)	-10.4 ± 3.8** (14)	-24.4 ± 1.6** (9)
$\dot{V}_{MAX}$(V/sec)					
Control	214.4 ± 9.3 (9)	103.1 ± 4.0 (18)	11.5 ± 2.6 (12)	4.5 ± 1.0 (5)	
5×10^{-7}M PE	183.6 ± 8.6* (11)	74.8 ± 11.0 (16)	7.1 ± 1.3 (8)	4.4 ± 1.0 (5)	
2.5×10^{-6}M PE	171.5 ± 8.3* (10)	74.4 ± 10.6 (18)	6.6 ± 1.1 (11)	5.0 ± 1.0 (2)	

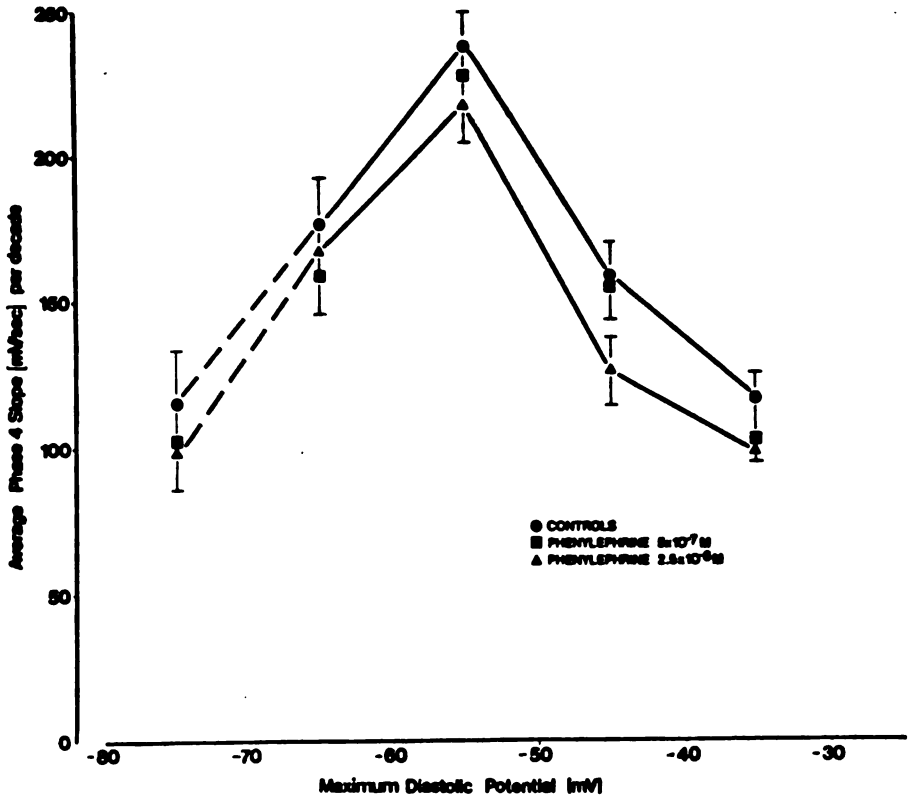


Figure 4-11

Effects of phenylephrine on phase 4 depolarization rate. The curves show mean phase 4 slope and standard errors per decade of MDP for automatic activity in a group of muscles. All values are plotted at the midpoint of each decade of MDP. No Rs voltage corrections. *$p < .05$, **$p < .01$, ***$p < .001$.

of muscles are summarized in Table 4-6. Very little effect on the AP elicited by a brief stimulus was seen. There was a slight decrease in AP duration at 0 mV and some reduction of $\dot{V}_{MAX}$. For automaticity arising from more negative MDPs, a dose-dependent increase in rate and decrease in AP duration occurred. There was a slight reduction in overshoot of automatic APs and a significant decrease in $\dot{V}_{MAX}$. There was little effect on phase 4 slope or threshold. For automaticity arising from less negative MDPs, there was little effect upon rate, AP duration, threshold or phase 4 slope. There was a significant dose-dependent decrease in overshoot of automatic APs. This effect actually abolished regenerative responses over the least negative MDP ranges and made difficult the measurement of many of the variables over this range. Measurement of overshoot under these conditions actually represented the overshoot of oscillations rather than regenerative responses. There were no consistent effects of phenylephrine upon the magnitude of current to achieve particular MDPs (i.e., relative membrane resistance), therefore, no corrections for Rs-related voltage errors were made. The effects of phenylephrine on phase 4 slope are summarized in Figure 4-11.

4.4 Effects of a Mixed Adrenergic Agonist, Norepinephrine, on Depolarization Induced Ventricular Automaticity

The effects of two concentrations of norepinephrine were examined in six experiments. A typical experiment is shown in Figure 4-12. The effects of norepinephrine were of relatively rapid onset, usually within 10-15 minutes. Because of progressively increasing contractility, it was necessary to study automaticity at the onset of effects and therefore a steady-state was never achieved. Norepinephrine had little effect upon the action potential elicited by a

brief stimulus. A dose-dependent increase in rate and decrease in AP duration were observed at more negative MDPs. There was also a dose-dependent increase in phase 4 slope at intermediate MDPs (panels g and k). For automaticity arising from less negative MDPs, there was an attenuation of overshoot for automatic APs at the lower dose, without much effect on rate. At the higher dose, the attenuated overshoot actually led to abolition of automaticity (panel l).

The mean effects of two concentrations of norepinephrine in a group of muscles are summarized in Table 4-7. Very little effect upon the AP elicited by a brief stumulus was seen. For automaticity arising from more negative MDPs, there was an increased rate (decreased interspike interval), decreased AP duration, and a slight increase in phase 4 slope, which however, was not significant. There was in addition a slight dose-dependent decrease in overshoot of automatic APs, and no significant effect on threshold or $\dot{V}_{MAX}$. For automaticity arising from less negative MDPs, there was a dose-dependent increase in rate, decrease in AP duration, and decrease in phase 4 slope. There was also a significant dose-dependent decrease in overshoot of automatic APs which could be responsible for the attenuation of automaticity that was observed at the least negative MDPs. There were no consistent effects on membrane resistance produced by norepinephrine. The effects of norepinephrine on phase 4 slope are summarized in Figure 4-13.

Although the results of Table 4-7b and Figure 4-13 indicate that norepinephrine produced a decrease in phase 4 slope over the less negative range of MDP, it can be argued that this effect was the result of utilizing two measurement methods. In the controls,

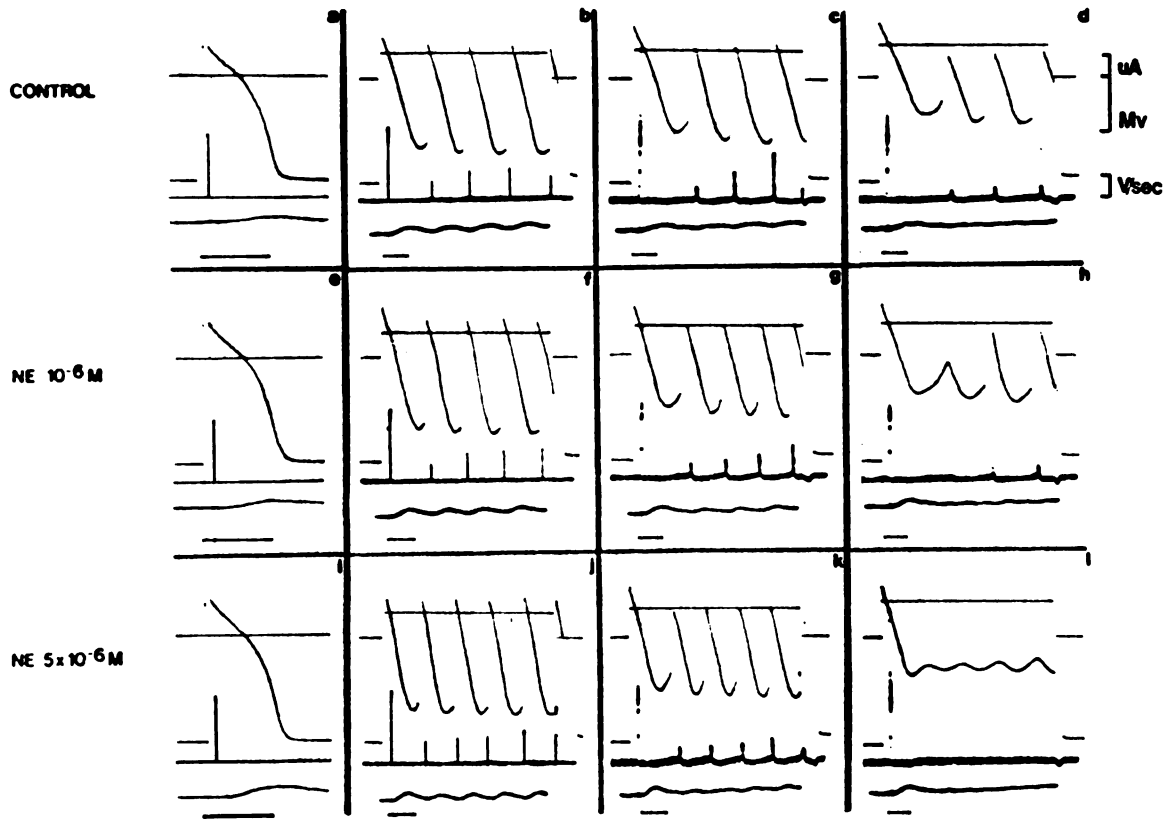


Figure 4-12

The effects of norepinephrine on depolarization induced ventricular automaticity. Traces are the same as in Figure 6. a, e, and i are action potentials elicited by a brief stimulus (2 msec.); b, f, and j, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -60 mV; c, g, and k at MDPs of -45 mV; d, h, and 1 at MDPs of -30 mV. I calibration is 10 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, b, e, f, i, and j; 10 V/sec. for rest of panels. Voltage calibration is 50 mV for all panels. Panels a-d are control responses; panels e-h were obtained during superfusion with 10^{-6}M norepinephrine; panels i-l were obtained during superfusion with 5×10^{-6}M norepinephrine.

Tables 4-7. Effects of two concentrations of norepinephrine upon action potentials (A) and upon automaticity (B) in a group of muscles. Definitions and abbreviations of each of the variables were described in Figures 4-2 and 4-3. * $p<.05$, ** $p<.01$, *** $p<.001$. No R_s-voltage corrections.

A

	V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)
Control	-88.7 ± 1.9	270.0 ± 17.3	30.3 ± .33	181.0 ± 8.0	90.7 ± 8.2	-67.7 ± 2.3
10^{-6} M NE	-89.3 ± 1.2	268.3 ± 22.1	28.7 ± 1.3	176.7 ± 8.8	85.0 ± 7.6	-69.0 ± 2.1
5 x 10^{-6} M NE	-89.0 ± .6	296.7 ± 16.7	29.3 ± 1.5	183.3 ± 23.2	83.3 ± 6.0	-70.7 ± 2.2

B

Variable	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
Inter-Spike Interval (msec)					
Control	445.6 ± 7.4 (19)	446.3 ± 6.0 (15)	505.9 ± 13.1 (11)	532.5 ± 15.3 (12)	580.0 ± 29.3 (9)
10^{-6}M NE	401.3 ± 7.7** (19)	409.6 ± 8.3** (13)	462.3 ± 6.8** (13)	508.9 ± 18.1 (14)	558.3 ± 15.8 (6)
5 x 10^{-6}M NE	352.0 ± 7.3** (20)	340.7 ± 10.1** (14)	381.2 ± 8.7** (13)	428.2 ± 9.1** (14)	
AP Duration (msec)					
Control	344.7 ± 2.7 (19)	376.3 ± 4.3 (15)	398.2 ± 4.3 (11)	401.5 ± 8.4 (13)	395.5 ± 8.1 (11)
10^{-6}M NE	318.4 ± 5.3** (19)	343.5 ± 7.2** (13)	333.5 ± 8.7** (13)	324.6 ± 7.5** (14)	360.6 ± 11.4** (9)
5 x 10^{-6}M NE	275.0 ± 8.4** (20)	284.3 ± 9.2** (14)	282.3 ± 10.0** (13)	267.5 ± 7.0** (14)	257.5 ± 7.5 (2)
Threshold (mV)					
Control	-53.1 ± .7 (18)	-44.5 ± 1.2 (13)	-29.0 ± 2.0 (14)	-12.5 ± 2.2 (3)	
10^{-6}M NE	-54.3 ± .9 (19)	-44.5 ± 1.1 (13)	-21.0 ± 2.0** (14)	-7.7 ± 2.6 (3)	
5 x 10^{-6}M NE	-54.1 ± .7 (19)	-44.1 ± 1.0 (14)	-23.4 ± 1.8 (13)		
PH 4 Slope (mV/sec)					
Control	136.7 ± 8.4 (18)	188.0 ± 8.0 (15)	205.9 ± 12.0 (11)	174.6 ± 16.3 (13)	105.6 ± 11.9 (8)
10^{-6}M NE	147.6 ± 7.9 (19)	205.4 ± 9.7 (13)	223.9 ± 12.4 (13)	112.1 ± 13.1** (14)	115.0 ± 11.9 (4)
5 x 10^{-6}M NE	167.0 ± 11.4 (20)	233.2 ± 6.8** (14)	250.4 ± 12.0* (13)	110.8 ± 13.5** (13)	
Overshoot (mV)					
Control	36.8 ± .9 (18)	31.0 ± 1.1 (15)	27.1 ± .9 (11)	21.5 ± .7 (13)	-.6 ± 3.1 (11)
10^{-6}M NE	34.6 ± .6* (19)	28.1 ± 1.2 (13)	22.0 ± .9** (13)	4.0 ± 3.1** (14)	-11.6 ± 3.7* (9)
5 x 10^{-6}M NE	31.5 ± .8** (20)	22.7 ± 1.4** (14)	15.5 ± 1.5** (13)	-11.5 ± 3.4** (14)	-21.6 ± .5** (7)
$\dot{V}_{MAX}$ (V/sec)					
Control	133.3 ± 9.7 (16)	31.5 ± 5.1 (13)	8.5 ± 1.3 (10)	4.1 ± .4 (9)	
10^{-6}M NE	125.8 ± 10.7 (15)	27.9 ± 4.2 (10)	6.6 ± .3 (10)	4.3 ± .7 (3)	
5 x 10^{-6}M NE	119.3 ± 15.2 (15)	27.0 ± 5.9 (11)	5.5 ± .7 (10)		

there was usually a distinct potential where the trace disappeared (threshold). Thus, the usual method of measuring phase 4 slope, i.e., division of the difference in potential between MDP and threshold by the time interval between them, could be used. In the presence of norepinephrine, however, the automatic APs in the less negative range were depressed in amplitude and no clear potential where the trace disappeared (threshold) was observed. This necessitated using the modified method of measuring phase 4 slope, i.e., taking a point 10 mV positive to the MDP as the threshold. A comparison between these two types of measurement in the absence and presence of norepinephrine at similar MDPs is shown in Figure 4-14. In 1, phase 4 slope is calculated in the usual way in the control. In 2, because there is no apparent threshold potential in the presence of norepinephrine, the modified measurement method must be used. It can be seen that the apparent decrease in phase 4 slope in 2, results most likely from the modified technique of measurement, rather than a real decrease in diastolic depolarization rate. A decrease in the diastolic depolarization rate would be expected to reduce the rate (lengthen the inter-spike interval), which was not observed in the presence of norepinephrine.

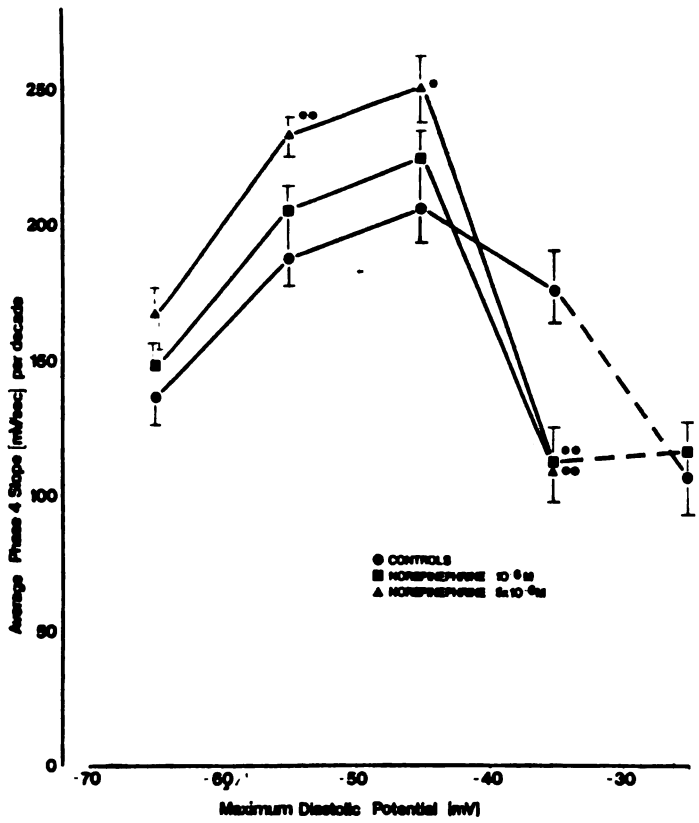


Figure 4-13

Effects of norepinephrine on phase 4 depolarization rate. The curves show mean phase 4 slope and standard errors per decade of MDP for automatic activity in a group of muscles. All values are plotted at the midpoint of each decade of MDP. No Rs voltage corrections.
*p < .05, **p < .01, ***p < .001.

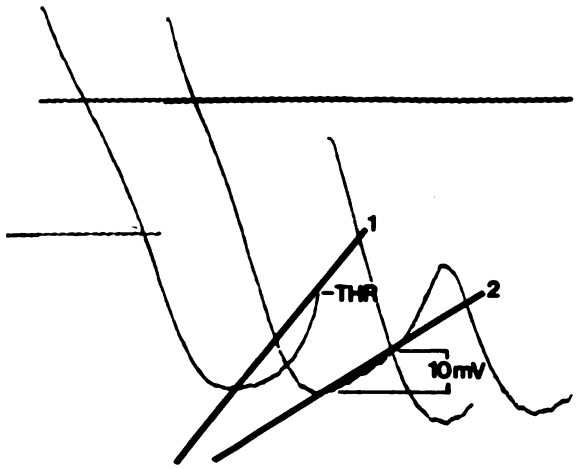


Figure 4-14

A comparison of the methods of measuring phase 4 slope in the control (1) and in the presence of 10^{-6}M norepinephrine (2). Traces are superimposed, but the trace in the presence of norepinephrine lags the control by 300 msec. In 1, the usual method of measuring phase 4 slope is used, i.e., division of the difference in potential between MDP and threshold by the time interval between them. In 2, no apparent threshold is evident and, therefore, a point 10 mV positive to MDP is arbitrarily taken as the threshold potential.

5.0 DISCUSSION - EXOGENOUS CATECHOLAMINES

5.1 Methodology

An important question related to the experimental approach used in this study relates to the multicellular nature of the preparation used. Is it likely that the automaticity recorded by the intracellular microelectrode actually originates in the individual cell impaled or might it actually originate in Purkinje cells or in nearby ventricular cells and spread to the cell being studied? Anatomical studies of mammalian papillary muscles have shown that the majority of Purkinje cells terminate in the base of the papillary muscle and are absent or sparse 1-2 mm from the apex of the muscle (Draper and Mya-Tu, 1959; Hondeghem and Stroobandt, 1974). The use of Tyrode solution in which NaCl is replaced with KCl in the posterior compartment of the sucrose gap chamber reduces the input resistance of the tissue in that chamber by depolarizing most of the cells which comprise the base of the papillary muscle. This procedure then eliminates possible contributions from the majority of Purkinje cells in the muscle. In addition, no muscles used in this study ever exhibited automaticity in the absence of applied depolarizing currents across the sucrose gap, once the posterior compartment contained KCl-Tyrode solution and the middle compartment contained isotonic sucrose solution. Spontaneous automaticity in Purkinje fibers has been observed to occur over a range of maximum diastolic potentials of -90 to -60 mV (Noble and Tsien, 1968). In the present study, the mean maximum diastolic potential for the most

negative limit for automaticity was -67.2 mV. Therefore, automaticity was elicited at potentials which were generally positive to the range in which Purkinje fibers characteristically generate spontaneous activity.

The question of the possible interactions between neighboring myocardial cells in the tissue extending into the test chamber relates to the more general problem of spatial homogeneity. Grant (1975) previously considered the problem of spatial non-uniformity as a possible contribution to diastolic depolarization induced by depolarizing currents in guinea pig papillary muscles. Based upon the theoretical analysis of a cable model by Hodgkin and Rushton (1946), he concluded that during diastolic depolarization, activity in a neighboring cell would be recorded as a voltage displacement convex upwards. Based upon this, only those preparations which exhibited diastolic depolarization as either rectilinear or concave upward voltage deflections, over the entire range of maximum diastolic potentials, were used in this study. Preparations in which any observed evidence of cell-to-cell electrotonic interactions, such as notches or diastolic convex-upward voltage deflections, were rejected outright.

Noble (1975) in an evaluation of the various voltage clamp techniques which have been applied to multicellular cardiac preparations has attributed spatial non-uniformity to three primary causes: (i) the length of the fiber, (ii) boundary regions between sucrose and ion solutions in sucrose gap techniques, and (iii) the resistance of the narrow clefts between cells and the extracellular fluid.

Ventricular trabeculae and papillary muscles are usually considered to be composed of short cylindrical cells 10-15 u in diameter and approximately 100 u in length, which are arranged into long columns which extend the length of the preparation. Preliminary attempts to model the spread of electric currents according to one-dimensional cable analysis in ventricular preparations were unsuccessful (Tarr and Sperelakis, 1964). It was shown by Kamiyama and Matsuda (1966) in dog ventricular muscle that polarization using an intracellular microelectrode was ineffective in producing electrotonic potentials; however, electrotonic current spread could be produced by application of current across the entire diameter or cross section of one end of the muscle, which was isolated from the remainder of the muscle by a thin celluloid plate. Bar and Dewey (1968) suggested that the difficulty in producing electrotonus with point source stimulating electrodes arises as a result of the three-dimensional geometry of many cardiac preparations. However, if two intracellular current electrodes are used or if current is passed through the tissue through a single sucrose gap, one-dimensional cable-like spread of current results. The three-dimensional cable properties of the papillary muscles used in this study probably minimizes possible electronic interactions occurring between cells.

The ability to inject current uniformly across the entire cross sectional area of the muscle is one of the distinct advantages of utilizing a single sucrose gap technique (Fozzard and Beeler, 1975). According to linear cable theory, the decay of potential with distance along the fiber will depend upon the space constant of the preparation. For a distance of one space constant, the potential

will fall to about 37% of its original value. However, Jack, Noble, and Tsien (1975) have shown that the presence of a high resistance at the end of the fiber (apex of muscle) will decrease the rate of potential decay. It has been estimated that in such a fiber, one space constant in length, the potential will decline to only about 66% of its original value (op. cit., p. 75). This represents another advantage of using a single sucrose gap to pass current. It can be seen that minimizing the length of tissue which extends into the test chamber will minimize voltage non-uniformity, which arises as a result of the cable properties of the preparation. In all of the experiments reported in this study, less than 1 mm of tissue extended in to the anterior test chamber. The value of λ (space constant) in ventricular muscle has been estimated to be 1.1 - 1.3 mm in dog (Kamiyama and Matsuda, 1966; Inui and Imamura, 1976), .96 mm in sheep and cow (Weidmann, 1970). λ for guinea pig papillary muscle has been estimated to be .62 mm (Ochi and Hashimoto, 1978), a value somewhat lower than that found in other mammalian ventricular fibers.

A disadvantage of utilizing a single sucrose gap is that the boundaries between the sucrose and ion solutions are not distinct and probably vary between preparations depending upon the sealing achieved by the rubber partitions used to separate the chambers and diffusion within the intracellular space of the muscle. Mixing of sodium and sucrose ions in the extracellular space at a sucrose-tyrode interface has been observed by Kléber (1973). Calculations by Atwell and Cohen (1977) have shown that such mixing might increase the transgap leakage current (Ieg in Appendix III).

The effects of mixing of solutions at the tyrode-sucroce interface upon the spatial uniformity of the preparation in the test chamber in the present experiments are probably minimal, since close to 1 mm of tissue extended into the test compartment. The smaller the test gap, the larger the proportion of tissue that is in a diffuse boundary region and the greater the contribution to spatial non-uniformity from this source (Noble, 1975).

It would certainly seem that there is a trade-off between the spatial non-uniformity arising as a result of sucrose-tyrode boundaries. The shorter the test strip, the less potential decay due to linear decrement, however, the greater the proportion of tissue is in a sucrose boundary region. This certainly indicates that in a single sucrose gap, shortening of the tissue in test compartment below a certain value will not necessarily ensure better spatial uniformity. Spatial uniformity can be assessed experimentally by multiple simultaneous impalements. Although spatial uniformity was not assessed in the experiments completed in this study, New and Trautwein (1972) found rather good spatial distribution of potential in cat trabeculae and papillary muscles using a single sucrose gap. Reuter and Scholz (1977a) have had similar successful results with cow and cat ventricular trabeculae and papillary muscles, and Katzung and Morgenstern (1977) in cat and guinea pig papillary muscles.

The resistance of the narrow clefts between cells and the extracellular fluid (Rs) probably makes the most significant contribution to spatial non-uniformity in the constant current experiments carried out in this study. Indeed, Fozzard (1966), Beeler and

Reuter (1970), and Tarr and Trank (1971) have shown that in a single sucrose gap the intracellular potential measured includes a component due to a voltage drop across the series resistance (Rs) during the passage of constant current. In the present experiments, attempts were made to evaluate the degree of error introduced by Rs in the transmembrane potential measurements made. Several examples were presented, which demonstrated that large potential errors could arise as a result of (i) a large applied current across a relatively small Rs, or (ii) a small current applied across a large Rs. Large errors were eliminated in the present experiments by excluding preparations in which large applied currents were required for depolarization or preparations which exhibited abnormally large overshoots over the less negative range of potentials.

The magnitude of small voltage errors in the preparations utilized in this study were estimated based upon an average Rs, determined from a survey of Rs in 21 guinea pig papillary muscles (Table 4-2) and the known amount of current to depolarize the cells. This estimated potential voltage error ranged from 5.2 mV over the -79 to -70 mV decade of maximum diastolic potentials to 8.6 mV over the -29 to -20 mV decade of maximum diastolic potentials. These voltage errors, although having the effect of shifting all of the variables to less negative MDPs and increasing the magnitudes of overshoot and threshold somewhat, do not appear to distort the basic relationship between each variable and MDP. The most striking effect is probably over the decade of MDP of -29 to -20 mV. The most positive limit for automaticity calculated in Table 1A is more likely to be at an MDP of -30 mV rather than the calculated mean of -22.6 mV.

It was considered important in this study of drug effects upon automaticity to concentrate on making corrections for voltage errors across Rs which arise as a result of drug actions, which alter the relative membrane resistance. Not taking such errors into consideration could qualitatively influence the observed effects that some drugs have on automaticity.

5.2 The Three Adrenergic Agonists and Depolarization Induced Ventricular Automaticity

Furchgott (1967) proposed a general definition of a beta-adrenoceptor as a receptor which mediates a response pharmacologically characterized by (i) a relative potency series in which isoproterenol > norepinephrine > phenylephrine; and (ii) a susceptibility to specific blockade by beta-antagonists at relatively low concentrations. An alpha-adrenoceptor as proposed to Furchgott (1967) was a receptor which mediates a response pharmacologically characterized by (i) a relative potency series in which norepinephrine > phenylephrine > isoproterenol; and (ii) a susceptibility to specific blockade by alpha-antagonists at relatively low concentrations. In the present experiments, the inotropic effects of the three sympathomimetic amines precluded the determination of the relative potencies of these agents in modulating depolarization induced ventricular automaticity. Partial fulfillment of (i) was accomplished by comparing the effects of one or two doses of each of the agents on the important variables underlying ventricular automaticity. By comparing the effects of the agents on these variables, some insight into the mechanism of adrenoceptor-mediated chronotropic effects was obtained.

5.2.1 Isoproterenol

10^{-6}M isoproterenol consistently produced a positive chronotropic

effect over the entire range of maximum diastolic potentials examined. The analysis of the effects on the variables used to characterize ventricular automaticity was complicated by the decrease in relative membrane resistance which invariably occurred in the presence of isoproterenol. After corrections were made for voltage errors occurring as a result of the increased magnitude of current across the series resistance, changes in the individual variables could be examined as a function of maximum diastolic potential. There was a decrease in action potential duration over the entire range of MDPs, without any significant effects upon threshold, overshoot, or $\dot{V}_{MAX}$ of automatic action potentials. Isoproterenol increased phase 4 slope at all MDPs less negative than about -55 mV, but surprisingly had little effect upon this variable at more negative potentials.

These results suggest that the positive chronotropic effect of beta-stimulation may be caused by a different mechanism over the MDP range of -69 to -55 mV, compared to MDPs less negative than -55 mV. It would appear that the substantially reduced action potential duration over the negative potentials is the cause rather than the result of the increased rate since no change in phase 4 slope occurred. The action potential elicited by a brief stimulus was, however, slightly prolonged by isoproterenol as was action potential duration at 0 mV. Previous studies (Hoffman and Cranefield, 1960; Reuter, 1974; Braveny, Simurdova, and Sumbera, 1974) have demonstrated that beta-stimulation can slightly reduce or slightly prolong the duration of the ventricular myocardial cell action potential. The dramatic shortening of action

potential duration during automaticity might therefore appear puzzling.

In Purkinje fibers, action potential repolarization is believed to be determined primarily by activation of the i_{X1} outward current and inactivation of the slow inward current (Noble and Tsien, 1972). In ventricular myocardial cells a similar mechanism is believed to account for the repolarization process (Beeler and Reuter, 1977), however the role of i_{X1} may be minimal in sheep and calf ventricular myocardium (McGuigan, 1974). Atrial action potentials which generally are shorter in duration than either myocardial or Purkinje fiber action potentials are believed to be more dependent upon inactivation of the slow inward current for repolarization, since less outward current is probably activated during the shorter duration (Noble and Tsien, 1972). For action potentials of longer duration, as in ventricular myocardium, there is probably more complete activation of outward current, which contributes substantially to phase 3 repolarization. In the present study, it was shown (Table 4-3a) that the mean duration of the action potential elicited by a brief stimulus under control conditions was 176 msec. During depolarization induced automaticity (Table 4-3b), the mean action potential duration under control conditions ranged from 294 to 332 msec. This considerable prolongation of action potential duration during constant current application might be considered to be more dependent upon activation of outward current than upon inactivation of the slow inward current.

Since beta-stimulation is known to enhance the slow inward current (Reuter, 1967; Reuter and Scholz, 1977) and also to enhance the

magnitude of the outward plateau current, i_{X1} (Brown and Noble, 1974; Tsien et al, 1972), it can be seen that isoproterenol could produce opposing effects upon plateau amplitude and on repolarization time of the action potential elicited by a brief stimulus. In the present experiments, isoproterenol caused a slight prolongation of AP duration to complete repolarization as well as AP duration at 0 mV of the action potential elicited by a brief stimulus. This most likely suggests that enhancement of the slow inward current in the presence of isoproterenol predominates in determining the onset of repolarization. However, during automaticity when the action potential duration is prolonged by the applied current and i_{X1} is the more important factor in controlling the onset of repolarization, isoproterenol greatly decreases action potential duration by enhancing i_{X1} (especially over the plateau range of potentials). Alteration in action potential duration as a mechanism for chronotropic effects has been previously shown in Purkinje fibers by Tsien, Giles, and Greengard (1972). This study specifically attributed part of the chronotropic effects of beta-stimulation and cAMP administration to reduced action potential duration arising from enhanced magnitude of i_{X1} outward current.

The lack of effect of isoproterenol upon phase 4 slope at MDPs of -69 to -55 mV (Figure 4-9) might be interpreted in at least two ways. Isoproterenol probably produces a significant enhancement of i_{X1} and since no change in the diastolic depolarization rate occurs, the decay of i_{X1} over this potential range may not be an important factor responsible for the pacemaker potential. Alternatively,

previous studies (Brown and Noble, 1974; Tsien et al, 1972) have shown that beta-stimulation produces a greater enhancement of i_{X1} at depolarized potentials (see Figure 1-2) than at more negative potentials. Such an effect might be expected to dramatically influence the rate of repolarization over plateau potentials without changing the magnitude of i_{X1} over more negative potentials, where the decay of i_{X1} might be involved in development of the pacemaker potential. The lack of effect of isoproterenol on phase 4 slope at these potentials implies that the slow inward current is not involved in the pacemaker potential at this potential range. In addition, this range of MDPs is generally more negative than the range of potentials over which slow inward current is activated (see Section 1.5.2).

For automaticity at MDPs less negative than -55 mV, isoproterenol produced an increase in the diastolic depolarization rate (Figure 4-9). Over this potential range both enhanced diastolic depolarization rate and reduction in action potential duration are primary factors responsible for the positive chronotropic effect. The increase in diastolic depolarization rate by isoproterenol over this potential range might be interpreted in two ways. Brown and Noble (1974) considered that an enhancement of slow inward current by epinephrine in frog atria predominates over the enhancement of i_X outward currents, since the latter effect by itself would reduce the slope of the pacemaker potential. A similar interpretation could be made in the present experiments. However in light of the present evidence which supports a role for i_{X1} in the pacemaker potential (see Section 5.2.1), another possibility

must be considered. I_{X1} may be more instrumental in determining the time course of the terminal portion of repolarization and hence the maximum diastolic potential than in the kinetics of the pacemaker potential. If the pacemaker potential is more dependent upon the activation of the slow inward current, then the ability of isoproterenol to enhance the pacemaker potential can be readily understood. Regardless of which mechanism is correct, the ability of isoproterenol to enhance the pacemaker potential over MDPs less negative than -55 mV, strongly suggests that activation of the slow inward current is involved in the development of the pacemaker potential over this range of MDPs.

The increased amount of current required to depolarize the cells to the same maximum diastolic potentials in the presence of isoproterenol (Table 4-4) was also observed by Brown and Noble (1969) in frog atria in the presence of epinephrine. It was attributed to a beta-action of epinephrine and explained as the result of an enhancement of i_{X1}. When automaticity is compared at equal magnitudes of applied current in the control and in the presence of isoproterenol, a hyperpolarization of the MDP occurs in the presence of isoproterenol (Figure 4-7). In spontaneously discharging cardiac Purkinje fibers, a similar hyperpolarization of the maximum diastolic potential by epinephrine has been observed (Hauswirth *et al*, 1968; Tsien, 1974). This has been attributed to stimulation of an electrogenic pump (Vassalle and Barnabei, 1971; Borasio and Vassalle, 1974). Cohen *et al* (1978) have suggested that such pump stimulation actually shifts the potassium equilibrium potential to more negative levels by reducing the extracellular

potassium concentration in the clefts just outside the cell. Such an effect might be produced by isoproterenol in the present experiments. In this regard, it is interesting to note that Tsien et al (1972) in addition to showing that beta-stimulation enhanced the magnitude of i_{X1}, also suggested that the selectivity for potassium was improved by beta-stimulation. If such is the case, the reversal potential for i_{X1} would be expected to be shifted to a more negative potential. Some support for this was obtained in the present experiments by the negative shift of the most negative MDP at which automaticity occurred in the presence of isoproterenol (Table 4-3a).

It is well established that beta-stimulation does not affect the rapid inward sodium current of cardiac membranes and thus does not influence $\dot{V}_{MAX}$ of action potentials which are dependent primarily upon the influx of sodium (Hoffman and Singer, 1967; Kassebaum and Van Dyke, 1967). The absence of any effect of isoproterenol upon $\dot{V}_{MAX}$ of automatic action potentials arising from more negative MDPs in this study (Table 4-3b) supports these previous observations. Katzung (1975) demonstrated that during depolarization induced automaticity in guinea pig myocardium, overshoot of automatic action potentials arising from all MDPs and $\dot{V}_{MAX}$ of automatic action potentials arising from less negative MDPs were dependent upon variations in extracellular calcium concentration. The amplitude of the action potential elicited by a brief stimulus in guinea pig ventricular myocardium has also been shown to be somewhat calcium dependent (Stanley and Reiter, 1965). It was therefore surprising that in the present study isoproterenol did not affect

overshoot or $\dot{V}_{MAX}$ of action potentials arising from less negative maximum diastolic potentials. On the other hand, the overshoot and plateau of the action potential elicited by a brief stimulus were elevated by isoproterenol, which is consistent with an enhanced slow inward current (Reuter, 1974). It is probable that over the less negative range of MDPs, time-dependent outward current (which is activated above -50 mV) contributes substantially to the net current balance early in the plateau which determines the overshoot potential during automaticity. Even a small residual g_k would be expected to contribute substantial outward current as the membrane potential shoots to positive potentials, where an appreciable driving force for potassium exists and inward rectification is not prominent. The amount of this potassium current might be negligible under control conditions, but significant under the influence of beta-stimulation, which has been shown to dramatically enhance i_{X1} current, especially at positive potentials. Such an effect might also be expected to counter any increase in $\dot{V}_{MAX}$ produced by isoproterenol over less negative potentials. Imanishi and Surawicz (1976) reported that isoproterenol increased amplitude and $\dot{V}_{MAX}$ of automatic action potentials induced by depolarizing currents in guinea pig ventricular myocardium. They did not, however, correct for potentially large voltage errors due to a greater amount of current across the series resistance in their preparations. Indeed, as illustrated in Figure 4-7, if such voltage errors are not compensated for, isoproterenol appears to enhance overshoot and $\dot{V}_{MAX}$ of automatic action potentials, especially over the less negative range of potentials.

5.2.2 Phenylephrine

Phenylephrine produced a dose-dependent increase in rate at the more negative MDPs (Table 4-6b). This increase in rate was of considerably smaller magnitude than the chronotropic effect produced by isoproterenol over the same range. There is in addition, a decrease in action potential duration, slight decrease in overshoot, and a significant reduction in $\dot{V}_{MAX}$ of automatic action potentials arising at more negative MDPs. The decrease in $\dot{V}_{MAX}$ at these potentials might indicate interference with the rapid inward sodium conductance, which underlies spike activity over this range. Reinhardt and Wagner (1974) have demonstrated in electrically driven guinea pig atria that phenylephrine, in addition to possessing alpha-agonist properties, behaves as a partial beta-agonist and partial beta-antagonist at higher concentrations. It was also found by tests on rabbit cornea that phenylephrine possessed local anesthetic activity of approximately twice the magnitude of the beta-antagonist, propranolol. The ability of phenylephrine to depress $\dot{V}_{MAX}$ is consistent with a local anesthetic property of the drug. The fact that phenylephrine did not alter phase 4 slope or thresold over this range, suggests that the reduction in action potential duration of automatic action potentials was the cause rather than the result of the slight chronotropic effect.

Over less negative MDPs, phenylephrine severely depressed the overshoot of automatic action potentials without appreciably affecting the rate, phase 4 slope, or threshold. This effect upon overshoot appeared to be dose-dependent and at the higher

concentration (2.5×10^{-6}M) actually abolished regenerative activity at the least negative MDPs.

It is difficult to explain the underlying mechanisms of these effects of phenylephrine. No voltage clamp analysis of the effects of phenylephrine upon individual cardiac ionic currents has been reported, and in fact, there is a paucity of data regarding electrophysiological effects of alpha-adrenoceptor stimulation in the heart in general. The best characterized electrophysiological effects of alpha-stimulation in the heart are changes in action potential duration and functional refractory period. Govier (1966, 1967) demonstrated that beta-stimulation shortens and alpha-stimulation lengthens the refractory period in guinea pig atria and ventricles. These conclusions were based upon the orders of potency of a series of amines and the specific blockade of the effects by the appropriate blocking agents. Pappano (1971) showed that small concentrations of epinephrine increased the action potential duration of guinea pig left atria, which was insensitive to blockade by propranolol, but sensitive to blockade by phentolamine. Very similar effects were observed by Giotti et al (1973) in sheep Purkinje fibers. None of these studies, however, advanced any hypotheses concerning the possible mechanisms underlying alpha-stimulation. It is noteworthy that in the present experiments only minor effects upon the action potential elicited by a brief stimulus were produced by phenylephrine.

Chronotropic responses to alpha-adrenoceptor stimulation have been reported; however, the results have been conflicting. Parr and

Urquilla (1972) found that positive chronotropic effects of epinephrine and phenylephrine in spontaneously beating rabbit atria were blocked by beta-antagonists but not by alpha-antagonists. Chiba (1977) reported that phenylephrine produced a biphasic chronotropic effect on the blood-perfused canine sinus node: an initial positive chronotropic effect which could be blocked by beta-antagonists, and a secondary negative chronotropic effect which could be blocked by alpha-antagonists or atropine. The latter effect has been attributed to stimulation of alpha-adrenoceptors located on cholinergic nerve terminals (Hashimoto et al, 1970; Chiba et al, 1974). James et al (1968) found a direct negative chronotropic effect of methoxamine in canine sinus node which was not affected by atropine, but was blocked by alpha-antagonists. Nakashima et al (1973) observed that methoxamine produced both positive and negative chronotropic effects in rat atria, mediated through alpha-adrenoceptors. Rosen et al (1977) reported that phenylephrine produced both positive and negative chronotropic effects upon spontaneous canine Purkinje fibers; however, only the negative effect was attributable to alpha-adrenoceptor stimulation. None of these studies provided hypotheses regarding the mechanisms responsible for alpha-adrenergic chronotropic effects, and it is clear that some of the confusing results obtained probably arise from the beta-agonist and -antagonist properties of the alpha-agonists examined. Several authors subscribe to the belief that alpha-adrenoceptor stimulation in the heart only evokes inotropic responses whereas chronotropic responses are due to beta properties of the alpha-agonists examined (Parr and Urquilla, 1972; Wagner and Reinhardt, 1974).

Another electrophysiological effect which is possibly influenced by alpha-adrenoceptor stimulation is the so-called "slow response" originally described by Engstfeld, Antoni, and Fleckenstein (1961) and later extensively studied by Cranefield, Wit, and Hoffman (1972). This type of response is characterized by a relatively slow upstroke velocity and low overshoot and has been shown to depend upon activation of the slow inward current (Pappano, 1970; Carmeliet and Vereecke, 1969). The slow response has been produced by a variety of experimental manipulations. The most common is the use of elevated extracellular K+ to inactivate the rapid inward sodium current. The production of slow action potentials is facilitated by the addition of catecholamines (or other agents known to enhance the slow inward current) and is blocked by beta-antagonists (Pappano, 1970). Cranefield, Hoffman, and Wit (1971) reported that methoxamine blocked conduction is partially depolarized canine Purkinje fibers which is presumably dependent upon slow channel activation. Since this effect was seen in the presence of beta-antagonists and could be prevented by alpha-antagonists, they attributed the effect to stimulation of alpha-adrenoceptors. A conflicting report by Miura et al (1978) purports to demonstrate that phenylephrine restores slow response action potentials in partially depolarized rabbit papillary muscles. This effect was not influenced by the presence of beta-antagonists at the concentrations examined, but was prevented by an alpha-antagonist. These authors could not explain the discrepancy between their results and those reported by Cranefield et al (1971). The results of Miura et al should be cautiously interpreted since their studies showed clearly that phenylephrine in

addition to stimulating slow responses produced an increase in inotropic relaxation rate, an effect which has been attributed to beta-stimulation (Kavaler and Morad, 1966; Graham and Lamb, 1968). Available evidence indicates that alpha-mediated inotropic effects are not accompanied by changes in the rate of relaxation (Ledda et al, 1975; Meinertz et al, 1978).

The severe attenuation of automaticity over less negative MDPs observed in the presence of phenylephrine in this study resembles the results of Cranefield, Hoffman, and Wit (1971). They attributed the block of conduction in partially depolarized Purkinje fibers to depression of the slow inward current. Since automaticity over less negative MDPs, which is induced by depolarizing currents is analogous to the slow response (Beeler and Reuter, 1977), a similar interpretation could be made. However, if phenylephrine attenuates automaticity over less negative MDPs by depressing the slow inward current, a decrease in phase 4 slope over this range would also be expected (since isoproterenol clearly increased phase 4 slope over this range).

Alternatively, the effects on automaticity produced by phenylephrine at both more negative and less negative MDPs might be accounted for by an increase in outward current. With the exception of the local anesthetic effects, phenylephrine modified automaticity over more negative MDPs in a manner very similar to that produced by isoproterenol. This effect of isoproterenol was attributed to an increase in i_{X1} outward current consistent with the findings of several voltage clamp studies. Over less negative MDPs, the effects of isoproterenol were attributed more to

enhanced slow inward current than to enhanced i_{X1} outward current, since enhanced outward current would be expected to attenuate automaticity. Therefore, enhancement of outward current in the absence of any change in slow inward current might be expected to attenuate automaticity over less negative MDPs, in a manner similar to that produced by phenylephrine. Since no voltage clamp analysis of the effects of alpha-adrenoceptor stimulation on cardiac currents has yet been reported, it is not possible to specify which outward currents might be influenced. However, either enhancement of i_{X1} or enhancement of the time-independent outward component, i_{K1}, could account for the changes in automaticity produced by phenylephrine in these experiments.

5.2.3 Norepinephrine

Norepinephrine produced a dose-dependent increase in rate (decrease in inter-spike interval) at more negative MDPs (Table 4-7b). This was accompanied by a decrease in AP duration and little change in phase 4 slope over the most negative MDP decade (-69 to -60 mV). As in the case of isoproterenol, the increased rate appeared to result primarily from the decreased AP duration, since effects on phase 4 slope were minimal.

At intermediate MDPs (-59 to -40 mV), there was an increase in rate, decrease in AP duration, and a distinct increase in phase 4 slope. This suggests that at intermediate MDPs, changes in phase 4 slope contribute to the chronotropic effects of norepinephrine. There were no significant effects of norepinephrine

upon threshold or $\dot{V}_{MAX}$ at these potentials. There was a significant decrease in overshoot of automatic action potentials over this MDP range.

At less negative MDPs, norepinephrine produced a dose-dependent increase in rate (decrease in inter-spike interval), a decrease in AP duration and an apparent decrease in phase 4 slope. There was also a pronounced reduction in overshoot of automatic action potentials which complicated the measurement of most of the variables in the least negative range of MDPs. Such complications especially affected the measurement of phase 4 slope in the presence of norepinephrine, and two measurement methods had to be utilized for the control and in the presence of drug. Therefore, the apparent decrease in phase 4 slope should be viewed cautiously, and might reflect measurement errors rather than a pharmacological effect. It should be noted that increase in phase 4 slope over this range is not required to explain the increased rate observed. The increase is more likely attributable to the decrease in AP duration observed.

5.3 Comparison of the Effects of the Three Sympathomimetic Amines on Ventricular Automaticity

In order to distinguish between possible alpha- and beta-mediated effects, it is useful to compare the results of norepinephrine with those obtained with isoproterenol and phenylephrine. Table 5-1 summarizes the effects of the three adrenergic agonists upon the important variables used to characterize ventricular automaticity. (Threshold is omitted since none of the drugs influenced this variable; $\dot{V}_{MAX}$ is omitted for convenience and only

phenylephrine influenced this variable.) The effects of each drug on each variable are summarized for more negative MDPs (MDPs > -55 mV) and for less negative MDPs (MDPs < -55 mV). At more negative MDPs, the three drugs produced qualitatively similar effects upon all variables except overshoot of automatic action potentials. In the case of isoproterenol this variable was not changed significantly, whereas in the case of both phenylephrine and norepinephrine overshoot of automatic action potentials was depressed. This suggests that the depression of overshoot by phenylephrine was not caused by the local anesthetic properties of the drug since norepinephrine depressed overshoot without exerting any noticable local anesthetic effects (Table 4-7b). The increased rate and decreased AP duration caused by all three drugs suggest that effects upon these variables are either (i) mediated by separate receptors with similar mechanisms of action, or (ii) mediated by the same receptor and similar mechanisms. In the case of (i), the effects on rate and action potential duration could be accounted for by an increase in outward currents, produced by all three drugs. Whether the same outward currents are increased by alpha- and beta-adrenoceptor stimulation is not known. In the case of (ii), it is possible that the increased rate and decreased AP duration are due to beta-agonist properties of phenylephrine and norepinephrine. However, the lack of effect of isoproterenol on overshoot in comparison to phenylephrine and norepinephrine tends to rule out (ii). The ability of beta-stimulation to enhance the slow inward current might be expected to counteract the effects of enhanced outward current upon overshoot of automatic action poten-

Agonist	Rate		AP Duration		PH 4 Slope		Overshoot		R_m
	>-MDP	<-MDP	>-MDP	<-MDP	>-MDP	<-MDP	>-MDP	<-MDP	
Isoproterenol	++	++	-	-	*	++	*	*	-
Phenylephrine	+	*	-	-	*	*	-	--	*
Norepinephrine	+	+	-	-	*	-(?)	-	--	*

>-MDP = More negative range of MDP.
<-MDP = Less negative range of MDP.
* = No change.
+ = Increase.
++ = Marked increase.
- = Decrease.
-- = Marked decrease.

Table 5-1. Summary of the effects of isoproterenol, phenylephrine, and norepinephrine upon important variables of depolarization induced automaticity.

tials and thus to produce little change in overshoot. In the case of norepinephrine, less enhancement of the slow inward current, might enable enhanced outward currents to reduce overshoots of automatic action potentials.

At less negative MDPs, the three drugs produced different effects upon three of the variables: phase 4 slope, overshoot, and relative membrane resistance. Isoproterenol consistently increased phase 4 slope over less negative MDPs, whereas phenylephrine did not alter phase 4 slope, and norepinephrine either decreased phase 4 slope or did not alter it. These experiments suggest that beta-stimulation causes enhancement of phase 4 slope over less negative MDPs, whereas alpha-stimulation either decreases phase 4 slope or does not affect it appreciably (further verification that the beta-adrenoceptor controls diastolic depolarization rate was obtained in experiments with beta-sympatholytic agents, see Section 6). Overshoot of automatic action potentials was not affected by isoproterenol but was depressed by both phenylephrine and norepinephrine. Isoproterenol produced a relative decrease in membrane resistance whereas both phenylephrine and norepinephrine had little effect upon this variable, which suggests that this effect is a consequence of beta-adrenoceptor stimulation.

The effects of the three drugs on phase 4 slope and overshoot at less negative MDPs can also be interpreted in terms of changes in slow inward current relative to changes in outward current. In the case of beta-stimulation, enhancement of slow inward current may be primarily responsible for the enhanced diastolic depolarzation rate, and tend to counteract any effect on overshoot by

enhanced outward currents. In the case of alpha-stimulation, a lack of effect upon the slow inward current results in no change in the diastolic depolarization rate (or enables enhanced outward current to decrease it) and enables enhanced outward current to predominate at positive potentials, reducing overshoots of automatic action potentials.

The results with norepinephrine might represent a mixture of alpha- and beta-adrenergic effects. Less enhancement of slow inward current at more negative MDPs, in the presence of enhanced outward current, could produce an increased rate by decreasing AP duration and also reduce overshoot. At intermediate MDPs, relatively more enhancement of slow inward current would produce an increase in the diastolic depolarization rate. However, at even less negative MDPs, the balance of slow inward current to outward current could change and outward current begin to predominate, severely depressing the overshoot of automatic action potentials. This effect could lead to attenuation of automaticity at less negative potentials in the case of both phenylephrine and norepinephrine. It is possible that alpha-stimulation reduces the overshoots of automatic action potentials and leads to the attenuation of automaticity at less negative MDPs by reducing the magnitude of the slow inward current, as suggested by Cranefield *et al* (1971). However, if this were true, the apparent dependence of the diastolic depolarization rate upon the magnitude of the slow inward current over less negative MDPs illustrated in the experiments with isoproterenol would predict that alpha-stimulation should decrease phase 4 slope and the rate at less negative MDPs. The results do not support such a

prediction (Table 5-1). In fact, agents which might be expected to reduce the slow inward current (beta-antagonists, Section 6.4) do reduce phase 4 slope and rate over less negative MDPs.

5.4 Conclusions Regarding the Adrenoceptors Mediating the Effects of the Three Sympathomimetic Amines

Since isoproterenol is a relatively pure beta-agonist (Innes and Nickerson, 1974; Schümann et al, 1977), it can be reasonably concluded that the actions exerted by this agent are mediated by interaction with the beta-adrenoceptor. Additional support for this conclusion arises from experiments with specific beta-antagonists (Section 6.4). Therefore, these experiments generally fulfill the two criteria of Furchgott (1967) which are fundamental in the identification of beta-adrenoceptor mediated responses. It can be concluded that the beta-adrenoceptor is involved in the modulation of ventricular automaticity and the characteristic changes in the variables underlying ventricular automaticity, which result in a positive chronotropic response to beta-stimulation, have been described.

Because phenylephrine is not a pure alpha-agonist, but is known to possess local anesthetic properties as well as beta-agonist and-antagonist properties (Reinhardt and Wagner, 1974), the effects observed in the presence of phenylephrine cannot be readily attributable to interaction with an alpha-adrenoceptor. Some similarities between the effects of phenylephrine and norepinephrine, an agent with known alpha- and beta-agonist properties, lends support to the supposition that the effects produced by phenylephrine were mediated by interaction with the alpha-

adrenoceptor and not due to secondary effects. Preliminary experiments with the alpha-antagonist, phenoxybenzamine, were performed and appeared to indicate that this agent was capable of specifically blocking the effects of phenylephrine, however, more experiments are required to document this. Therefore, the involvement of the alpha-adrenoceptor in the modulation of ventricular automaticity must be considered tentative until additional experimental evidence is obtained.

6.0 RESULTS - ENDOGENOUS CATECHOLAMINES

6.1 Effect of an Indirect Sympathomimetic Amine, Tyramine on Depolarization Induced Automaticity

The effects of 5×10^{-5}M tyramine were examined in six experiments. An example is illustrated in Figure 6-1. Tyramine produced a slight increase in overshoot of the action potential elicited by a brief stimulus (panel d) after 30 minutes superfusion. The major effects on automaticity were an increased rate and decreased AP duration for automaticity arising from more negative MDPs (panel e). For automaticity arising from less negative MDPs (panel f), there was an increased rate, decreased action potential duration and an increase in phase 4 slope, in the presence of tyramine. Tyramine also decreased relative membrane resistance, especially over the less negative range of MDPs. The increase in contractility produced by tyramine was progressive in onset and of relatively long duration.

The mean effects of 5×10^{-5}M tyramine in a group of muscles are summarized in Tables 6-1. In each experiment, tyramine increased the amount of current required to depolarize the cell. Measurement of Rs in each muscle was made and voltage corrections carried out for the IR error. The calculated values for Rs ranged from 400-500 Ω and the maximum calculated voltage error ranged from .2 mV to 2.7 mV over the -29 to -20 MDP range. The values in Table 6-1b are corrected for Rs errors. It can be seen in Table

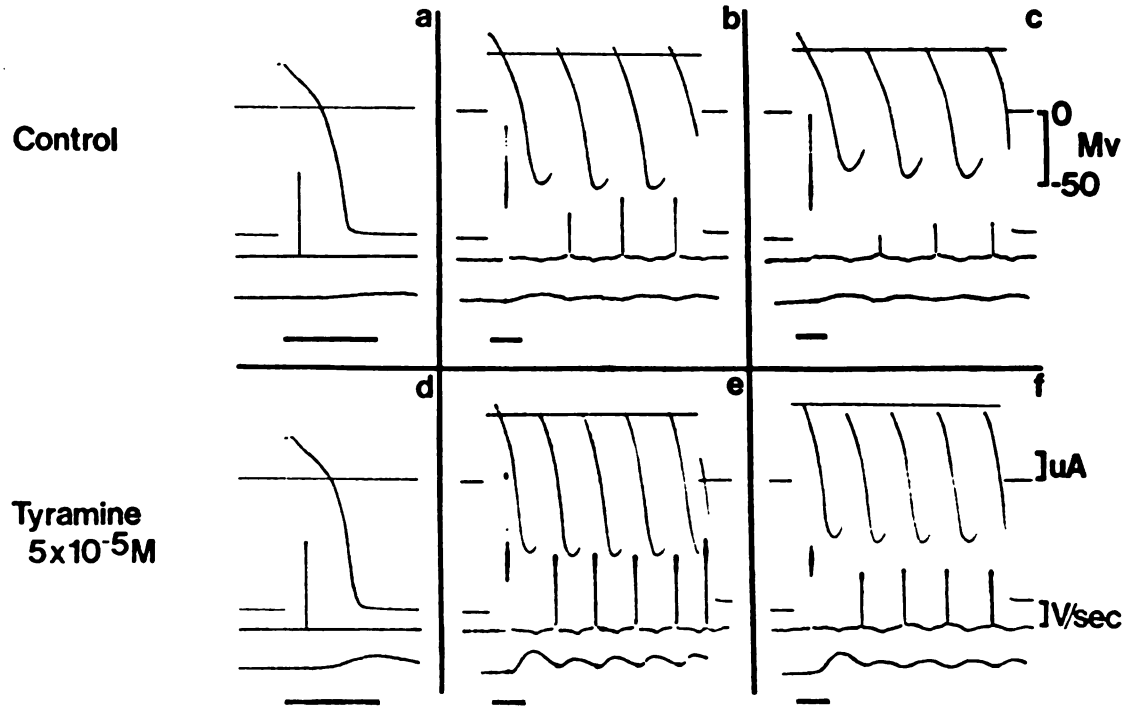


Figure 6-1

The effects of tyramine on depolarization induced ventricular automaticity. The traces are the same as in Figure 4-5. a and d are action potentials elicited by a brief stimulus (2 msec.); b and e, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -55 mV; c and f at MDPs of -45 mV. I calibration is 10 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a and d; 10 V/sec. for b, c, e, and f. Voltage calibration is 50 mV for all panels. Panels a-c are control responses; panels d-f were obtained during superfusion with 5×10^{-5}M tyramine.

Tables 6-1. Effect of 5×10^{-5} M tyramine upon action potentials (A) and upon automaticity (B) in a group of muscles. Definitions and abbreviations of each variable were described in Figures 4-2 and 4-3. Since tyramine consistently produced a decrease in relative membrane resistance, all values in this table were corrected for voltage errors due to changes in applied currents across Rs.
*p < .05, **p < .01, ***p < .001.

A

	V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)
Controls	-88.7 ± .3	233.3 ± 22.1	29.0 ± .6	186.7 ± 21.3	92.7 ± 16.9	-61.0 ± 4.9
5 x 10^{-5} M TYR	-87.7 ± 1.5	243.3 ± 31.8	29.7 ± .9	191.7 ± 24.0	99.7 ± 17.3	-64.3 ± 1.5

B

Variable	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
Inter-Spike Interval (msec)					
Control	568.1 ± 11.2 (18)	544.7 ± 4.8 (15)	596.3 ± 13.8 (19)	614.7 ± 15.4 (16)	667.9 ± 21.7 (12)
5 x 10^{-5} M TYR	454.6 ± 15.9*** (12)	426.5 ± 9.3*** (17)	437.4 ± 9.9*** (17)	465.4 ± 16.3*** (13)	451.7 ± 33.0*** (6)
AP Duration (msec)					
Control	405.6 ± 7.9 (18)	423.3 ± 13.5 (15)	462.1 ± 10.8 (19)	467.8 ± 12.9 (16)	476.7 ± 16.5 (12)
5 x 10^{-5} M TYR	331.7 ± 7.3*** (12)	339.1 ± 8.1*** (17)	341.8 ± 7.9*** (17)	347.7 ± 9.8*** (13)	327.5 ± 15.9*** (6)
Threshold (mV)					
Control	-46.2 ± .5 (18)	-39.9 ± 1.2 (15)	-29.4 ± 1.1 (19)	-17.8 ± 1.9 (16)	-7.0 ± 2.2 (12)
5 x 10^{-5} M TYR	-51.2 ± 1.5 (12)	-42.5 ± 1.8 (17)	-30.6 ± 1.4 (15)	-17.1 ± 1.9 (9)	-9.3 ± .9 (4)
PH 4 Slope (mV/sec)					
Control	132.5 ± 9.8 (18)	174.7 ± 14.0 (15)	161.8 ± 11.3 (19)	143.4 ± 7.0 (16)	112.9 ± 9.7 (12)
5 x 10^{-5} M TYR	111.3 ± 11.8 (12)	169.4 ± 19.2 (17)	217.6 ± 15.1** (17)	186.9 ± 12.0** (13)	160.0 ± 14.1** (6)
Overshoot (mV)					
Control	38.7 ± .8 (18)	38.7 ± 1.5 (15)	38.1 ± 1.7 (19)	34.3 ± 2.3 (16)	25.1 ± 4.2 (12)
5 x 10^{-5} M TYR	40.8 ± 2.0 (12)	39.4 ± 1.6 (17)	34.8 ± 2.3 (17)	30.1 ± 3.5 (13)	25.7 ± 9.7 (6)
$\dot{V}_{MAX}$ (V/sec)					
Control	52.2 ± 4.1 (18)	19.2 ± 2.1 (15)	9.7 ± 1.2 (19)	6.3 ± .7 (14)	4.3 ± .8 (7)
5 x 10^{-5} M TYR	93.0 ± 10.3*** (12)	35.7 ± 5.6* (17)	14.1 ± 2.2 (17)	9.6 ± 2.1 (13)	9.2 ± 2.5 (6)

6-1a that the major effects of tyramine on the action potential elicited by a brief stimulus included a slight increase in overshoot and a slight prolongation of action potential duration at 0 mV. With long current pulses (Table 6-1b), tyramine increased rate and decreased AP duration over the entire range of MDPs. There was a significant increase in phase 4 slope, especially over the less negative range of MDPs. There were minimal effects on threhold and overshoot. The effects of tyramine on mean phase 4 slope are summarized in Figure 6-2. It is particularly interesting that with regard to nearly every variable measured, tyramine produced effects similar to those of isoproterenol.

The results in Table 6-1b indicate that tyramine produced a significant increase in $\dot{V}_{MAX}$ over the decades of -69 to -60 and -59 to -50 mV. As can be seen in Table 6-1a, the most negative threshold for automaticity in the presence of tyramine was shifted from a mean MDP of -61 mV to -64 mV. In one experiment this negative threshold was actually shifted from -52 mV to -62 mV by tyramine. This negative shift in the threshold MDP for automaticity probably accounts for the increase in $\dot{V}_{MAX}$ seen at MDP decades of -69 to -60 and -59 to -50, since the average MDP per decade was more negative in this range in the presence of tyramine.

6.2 Effect of 6-Hydroxydopamine Pretreatment on Depolarization Induced Automaticity

6.2.1 Intraperitoneal Administration of 6-Hydroxydopamine

In initial experiments with 6-hydroxydopamine (6-OHDA), guinea pigs were administered two intraperitoneal (i.p.) injections of 150 mg/kg of 6-OHDA at 48 and 24 hours prior to sacrifice. This

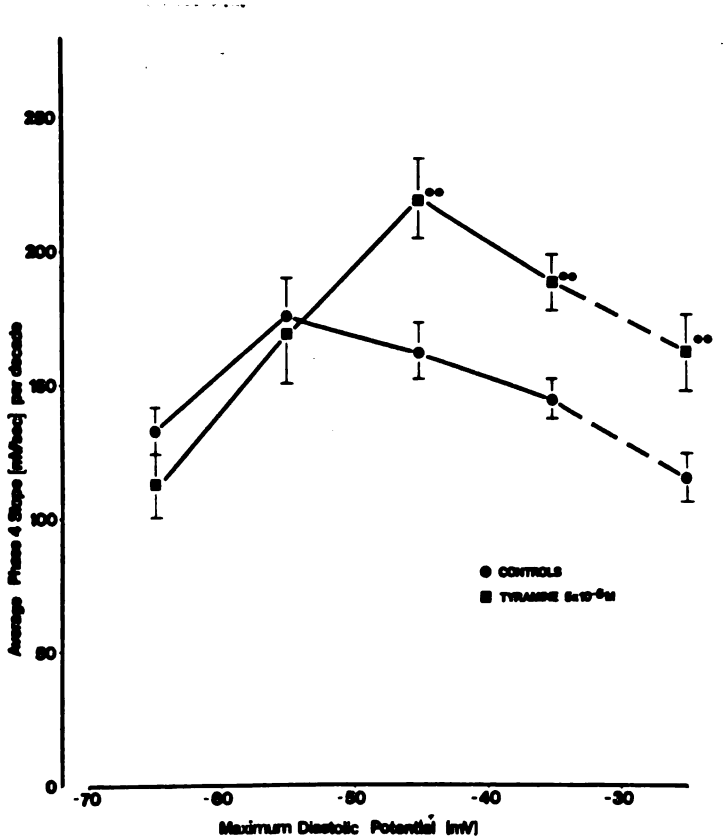


Figure 6-2

Effects of 5 x 10^{-5}M tyramine on phase 4 depolarization rate. The curves show mean phase 4 slope and standard errors per decade of MDP for automatic activity in a group of muscles. All values are plotted at the midpoint of each decade of MDP. All values of MDP corrected for Rs voltage errors as described in the text. *p < .05, **p < .01, ***p < .001.

dose was well in excess of doses used by Berkowitz, Spector, and Tarver (1972) to produce up to 95% depletion of norepinephrine stores in guinea pig whole hearts. One of the first experiments on automaticity in a right ventricular papillary muscle from one of these pretreated animals revealed that automaticity at more negative MDPs resembled that seen in muscles from control animals. However, automaticity over the entire range of MDPs less negative than about -55 mV was absent, as illustrated in Figure 6-3. This experiment was repeated several times but the results were inconsistent. In muscles from some pretreated animals, automaticity over the less negative range of MDPs was absent and in muscles from other, similarly pretreated animals, automaticity was present over the less negative range of MDPs and resembled that seen in control muscles.

The most obvious explanation for these inconsistent electrophysiological results was that the degree of norepinephrine depletion might be inconsistent. Therefore, a sensitive fluorimetric biochemical assay (McCaman et al, 1973) for measuring levels of endogenous norepinephrine was used in an attempt to verify the consistency of depletion. In six control animals, the right ventricular wall was removed and assayed fluorimetrically for norepinephrine content. The results of this assay are presented in Table 6-2. Ten animals were then pretreated with the same i.p. dose of 6-OHDA and the right ventricular walls from these animals were assayed for norepinephrine content. It can be seen in Table 6-2 that the mean norepinephrine levels in these animals was .26 ug/g WWT. This represented about 88% depletion of endogenous norepinephrine in hearts from pretreated animals, compared to controls. However,

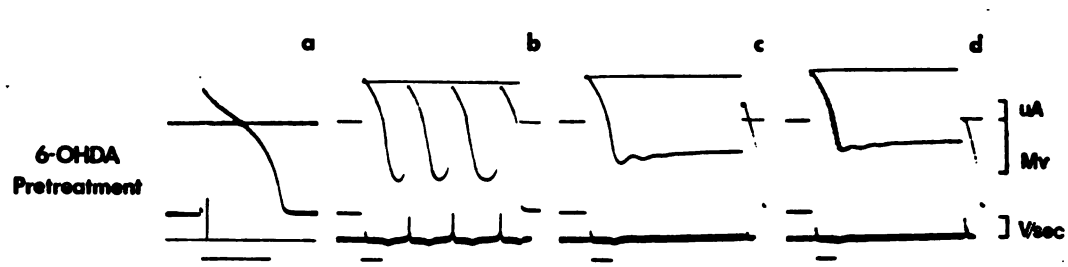


Figure 6-3

Activity during prolonged current pulses in a papillary muscle from an animal administered two intraperitoneal doses of 6-OHDA (150 mg/kg), 48 and 24 hours prior to sacrifice. The traces are the same as in Figure 4-1. a is the action potential elicited by a brief stimulus (2 msec.); b, activity during prolonged (1.5 sec.) constant current pulse at MDP of -60 mV, c at MDP of -41 mV, and d at MDP of -30 mV. I calibration is 10 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a; 10 V/sec. for b-d. Voltage calibration is 50 mV for all panels.

	Date	ug/g wwt
Controls	9-17-77	1.92
	9-19-77	2.85
	9-22-77	2.34
	9-26-77	2.33
	3-14-78	2.02
	3-23-78	1.89
		2.23 ± .15
6-OH Dopamine[a]	5-26-77[b]	.13
	6-10-77[b]	.06
	7-21-77[b]	.60
	8-25-77	.31
	9-02-77[b]	.12
	9-09-77	.46
	9-16-77	.16
	2-21-78	.10
	2-27-78	.23
	3-02-78[b]	.47
		.26 ± .06
		($p < 0.01$)

a = 150 mg kg i.p. -24/48 hrs. before experiment.

b = automaticity absent over less negative MDPs (-55 to -30 mV).

Table 6-2. Right ventricular norepinephrine content of guinea pigs which received no pretreatment (controls) and which received two intraperitoneal doses of 6-hydroxydopamine. Norepinephrine assays were performed as described in the methods. Half of the muscles from pretreated animals displayed activity similar to that illustrated in Figure 6-3. The remainder displayed automaticity very similar to that observed in control muscles.

as indicated in the table, in only half of these muscles from pretreated animals were there any characteristic differences in automaticity. Comparing the levels of norepinephrine in muscles in which automaticity was absent over less negative MDPs to ventricular levels of norepinephrine of muscles in which automaticity was present, showed no consistent differences. It was concluded from these experiments, therefore, that the inconsistent electrophysiological results were not due to inconsistencies in the degree of depletion of endogenous norepinephrine.

In two of the muscles from animals pretreated with i.p. 6-OHDA which had automaticity resembling that in control muscles, the effects of isoproterenol were investigated. One such experiment is illustrated in Figure 6-4. The norepinephrine content of the right ventricle from which this muscle had been removed was .23 ug/g WWT. Automaticity was present at both negative MDPs (panel b, MDP = -58 mV) and at less negative MDPs (panel c, MDP = -30 mV). The superfusion of 10^{-6}M isoproterenol produced an extraordinary chronotropic effect. The rate increased an average of 62% over the entire range of MDPs. This contrasts with an average rate increase produced in a group of control muscles by 10^{-6}M isoproterenol of 37%. The rate shown in panel f in the presence of isoproterenol is by far the highest observed under any experimental conditions during the course of this project. Translated to beats per minute, it would correspond to an astounding 480 beats per minute. These results suggest that in those muscles which were depleted of endogenous norepinephrine but exhibited automaticity resembling control muscles, super-

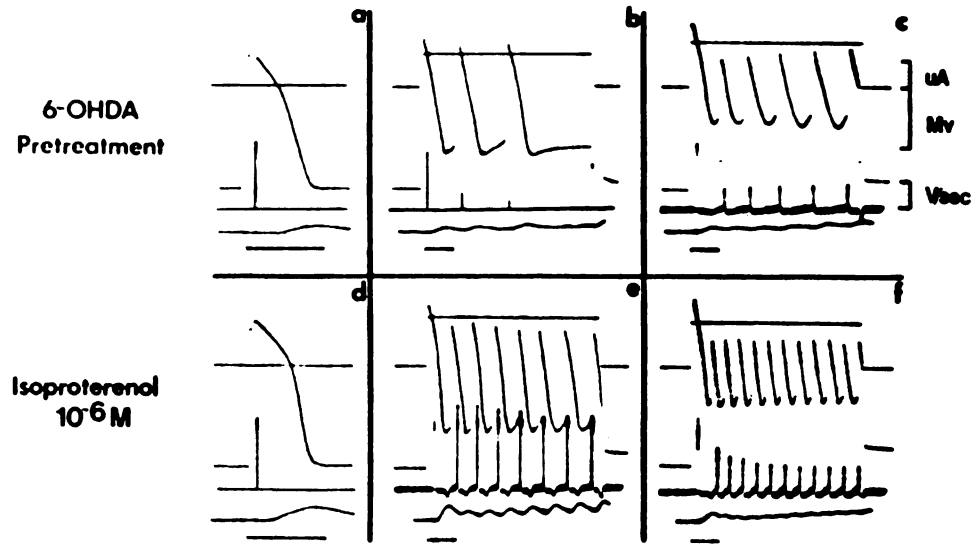


Figure 6-4

The effect of isoproterenol on automaticity in a papillary muscle from an animal pretreated with two intraperitoneal injections of 6-OHDA (150 mg/kg). The traces are the same as in Figure 4-5. a and d are action potentials elicited by a brief stimulus (2 msec.); b and e; automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -58 mV; c and f at MDPs of -30 mV. I calibration is 10 μA for a-e; 20 μA for f; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, b, and d; 10 V/sec. for c, e, and f. Voltage calibrattion is 50 mV for all panels. Panels a-c are control responses in a muscle from an animals pretreated with 6-OHDA (i.p.); panels d-f were obtained during superfusion with 10^{-6}M isoproterenol.

sensitivity to beta-stimulation might have developed.

6.2.2 Intravenous Administration of 6-Hydroxydopamine

In an attempt to improve the consistency of the electrophysiological effects of 6-hydroxydopamine pretreatment, four guinea pigs were administered a single intravenous dose of 6-OHDA, 250 mg/kg, through the jugular vein, 24 hours prior to sacrifice. In muscles from all four of these animals, automaticity was present over the more negative range of MDPs, but absent at MDPs less negative than -55 mV. One such experiment is illustrated in Figure 6-5. The action potential elicited by a brief stimulus appeared to have a depressed overshoot and reduced action potential duration at 0 mV. Automaticity at more negative potentials (panel b, MCP = -72 mV) appeared similar to that observed in control muscles, except for a slight depression of overshoot and $\dot{V}_{MAX}$ of automatic action potentials. Automaticity at less negative MDPs was absent (panel c, MDP = -55 mV; panel d, MDP = -40 mV). In this particular experiment, superfusion with 5×10^{-6}M norepinephrine restituted automaticity over the entire range of MDPs. This dose is similar in magnitude to the threshold dose of norepinephrine which produced a chronotropic effect in a group of control muscles (Section 4.4).

Biochemical assay of the norepinephrine levels of the right ventricles of these four pretreated animals revealed over 95% depletion of endogenous norepinephrine in comparison to controls, as shown in Table 6-3. This appeared to be a slightly greater degree of depletion than that observed after i.p. administration

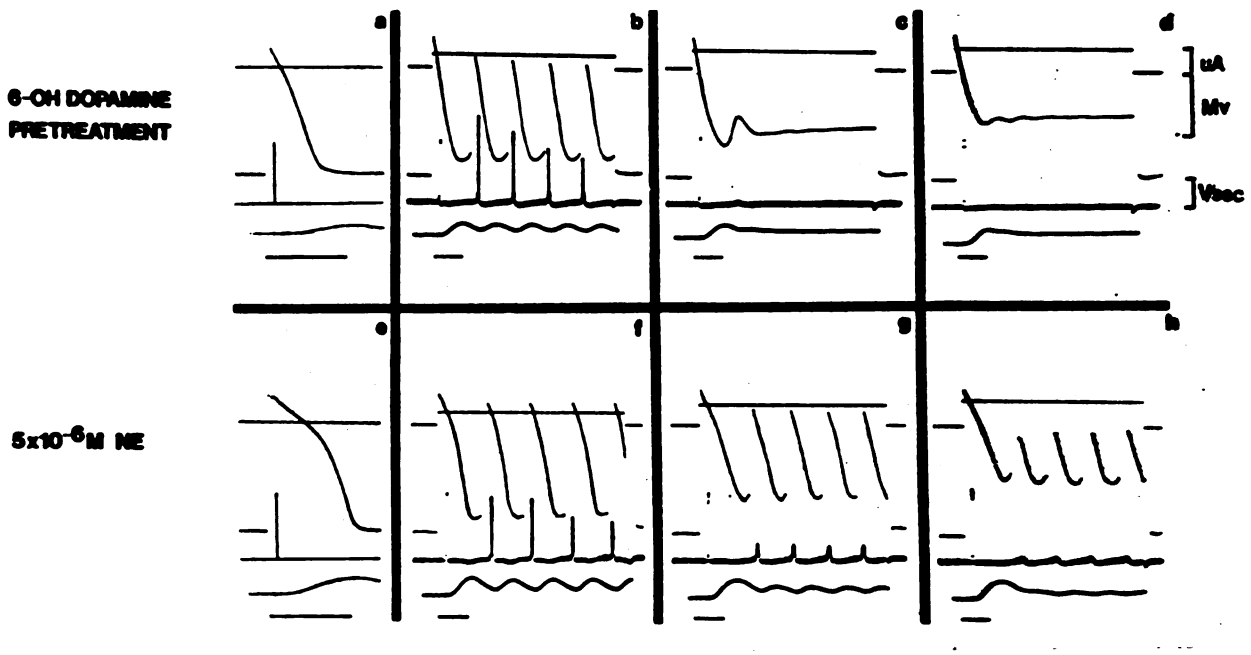


Figure 6-5

Activity during prolonged current pulses in a muscle from an animal pretreated with intravenously administered 6-OHDA (250 mg/kg). The traces are the same as in Figure 4-5. a and e are action potentials elicited by a brief stimulus (2 msec.); b and f, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -72 mV; c and g at MDPs of -55 mV; d and h at MDPs of -40 mV. I calibration is 10 μA for all panels; V_{MAX} calibration is 100 V/sec. for a and e; 10 V/sec. for all other panels. Voltage calibration is 50 mV for all panels. Panels a-d are control responses in a muscle from an animal pretreated with 6-OHDA (i.v.); panels e-h were obtained during superfusion with 5 x 10^{-6}M norepinephrine.

of 6-OHDA, although the difference was not significant. However, the variability in degree of depletion was considerably reduced.

A comparison of the action potentials elicited by a brief stimulus and automaticity between the four muscles from animals which received intravenous 6-OHDA and the six muscles from untreated control animals (same controls as in Table 6-3) is summarized in Tables 6-4. In the pretreated group, the action potentials elicited by a brief stimulus appeared to have a slighly depressed overshoot and reduced action potential duration at 0 mV, in comparison to controls. For automaticity arising from more negative MDPs, the rate appeared to be slightly faster and action potential duration was slightly shorter in pretreated muscles. There was additionally a significant reduction in AP overshoot and in $\dot{V}_{MAX}$ for automatic APs arising from more negative MDPs. For less negative MDPs, automaticity was absent at all potentials less negative than -55 mV. This precluded the measurement of most of the variables over this potential range for the pretreated muscles. However, a significant decrease occurred in phase 4 slope in the least negative decade of MDP (-59, -50 mV) which could be measured. The severely reduced overshoots measured in the less negative range of MDPs for pretreated muscles reflects the absence of regenerative activity for this potential range. A comparison of the mean phase 4 slope per decade of MDP for the six controls and the four pretreated muscles is shown in Figure 6-6.

	Date	ug/g wwt
Controls	9-17-77	1.92
	9-19-77	2.85
	9-22-77	2.34
	9-26-77	2.33
	3-14-78	2.02
	3-23-78	1.89
		2.23 ± .15
6-OH Dopamine[a]	4-29-78	.09
	5-03-78	.07
	5-09-78	.15
	5-23-78	.07
		.10 ± .02
		($p < 0.01$)

a = 250 mg/kg i.v. - 24 hr. before experiment.

Table 6-3. Right ventricular norepinephrine content of guinea pigs which received no pretreatment (controls) and which received a single intravenous dose of 6-hydroxydopamine. Norepinephrine assays were performed as described in the methods. All of the muscles from this group of i.v. pretreated animals displayed automaticity only over more negative MDPs (e.g., MDP > -55 mV).

Tables 6-4. Effect of intravenous 6-hydroxdopamine pretreatment upon action potentials (A) and automaticity (B) in comparison to control muscles (muscles are the same as in Table 6-3). Definitions and abbreviations of each variable were described in Figures 4-2 and 4-3. The absence of automaticity over less negative MDPs in pretreated muscles precluded the measurement of most of the variables over this range. * $p<.05$, ** $p<.01$, *** $p<.001$. No R_s-voltage corrections.

A

	V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)
Control	-89.7 ± .6	291.7 ± 26.7	31.0 ± .7	173.0 ± 7.9	85.3 ± 7.5	-68.7 ± 1.7
6-OHDA	-87.5 ± 1.2	268.8 ± 12.0	23.3 ± 2.0	141.3 ± 19.8	37.5 ± 4.3	-70.1 ± 1.9

B

Variable	-79 -70	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
Inter-Spike Interval (msec)						
Control	620.0 ± 88.4 (4)	431.6 ± 4.8 (40)	414.4 ± 5.6 (32)	429.5 ± 13.2 (28)	458.1 ± 14.6 (31)	462.6 ± 23.7 (21)
6-OHDA	402.9 ± 17.2*** (14)	394.2 ± 6.9*** (30)	418.3 ± 19.3 (9)	-	-	-
AP Duration (msec)						
Control	342.5 ± 8.5 (4)	340.4 ± 4.4 (40)	347.3 ± 4.6 (32)	342.8 ± 8.2 (28)	347.3 ± 8.8 (31)	342.8 ± 11.6 (21)
6-OHDA	269.3 ± 11.0** (14)	296.3 ± 6.9*** (30)	289.0 ± 10.6*** (9)	-	-	-
Threshold (mV)						
Control	-56.3 ± 1.4 (4)	-52.8 ± .9 (40)	-43.1 ± .8 (32)	-28.9 ± .9 (26)	-17.4 ± 1.4 (23)	-10.4 ± 1.7 (10)
6-OHDA	-61.5 ± .3*** (14)	-51.6 ± .9 (30)	-42.3 ± 1.1 (9)	-	-	-
PH 4 Slope (mV/sec)						
Control	77.5 ± 15.6 (4)	146.6 ± 6.5 (40)	221.7 ± 6.7 (32)	227.5 ± 11.8 (28)	180.8 ± 9.9 (31)	168.1 ± 15.5 (21)
6-OHDA	102.1 ± 7.0 (14)	158.0 ± 6.9 (30)	152.2 ± 7.4*** (9)	-	-	-
Overshoot (mV)						
Control	33.0 ± 1.0 (4)	37.5 ± 1.0 (40)	33.7 ± 1.3 (32)	28.4 ± 1.3 (28)	19.1 ± 2.4 (31)	7.5 ± 2.8 (25)
6-OHDA	12.4 ± 2.0*** (14)	8.8 ± 2.8*** (32)	-26.3 ± 4.7*** (32)	-38.1 ± .6*** (23)	-30.5 ± .6*** (20)	-25.1 ± .7*** (10)
$\dot{V}_{MAX}$ (V/sec)						
Control	175.0 ± 11.9 (4)	134.0 ± 8.6 (40)	42.7 ± 5.9 (32)	13.3 ± 1.3 (23)	6.2 ± .5 (26)	3.2 ± .3 (10)
6-OHDA	85.6 ± 19.8* (14)	45.0 ± 8.8*** (29)	16.3 ± 4.6* (9)	-	-	-

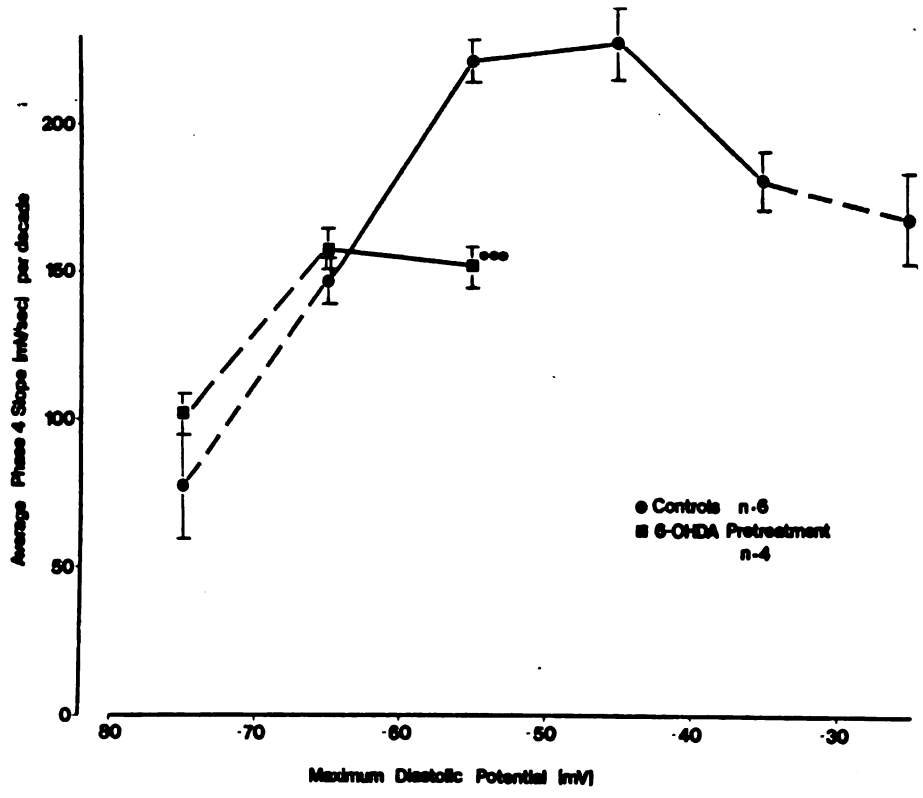


Figure 6-6

Comparison of mean phase 4 diastolic depolarization rate in six control experiments and in four experiments in which the animal had received a single intravenous injection of 6-hydroxydopamine. Controls and pretreated muscles are the same as those in Table 6-3. Because automaticity over less negative MDPs was absent in muscles from pretreated animals, phase 4 slope could not be measured over this range of potentials. The curves show mean phase 4 slope and standard errors per decade of MDP for automatic activity. All values are plotted at the midpoint of each decade of MDP. *$p < .05$, **$p < .01$, ***$p < .001$. No Rs-voltage corrections.

6.2.3 Reversal of the Effects of Catecholamine Depletion of Exogenous Norepinephrine

Restitution of automaticity over the less negative range of MDPs by norepinephrine was illustrated in Figure 6-5. In three attempts out of four muscles from pretreated animals, 5×10^{-6}M norepinephrine restored automaticity and in one of the four, 2×10^{-6}M isoproterenol was used to restore automaticity. The restitution of automaticity by 5×10^{-6}M norepinephrine in muscles from intravenously pretreated animals is summarized in Table 6-5. For automaticity arising from more negative MDPs, norepinephrine slightly slowed the rate, prolonged AP duration, and increased overshoot of automatic APs. There was no effect of norepinephrine on $\dot{V}_{MAX}$. For less negative MDPs, norepinephrine restored automaticity. This appeared to be caused by a significant increase in phase 4 diastolic depolarization rate in the decade of -59 to -50 mV. The overshoots for automatic APs were also restored toward control levels. At the concentration of norepinephrine used, no consistent effects on relative membrane resistance were observed, therefore, no corrections for Rs-related voltage errors were made. The effects of norepinephrine reversal on phase 4 depolarization rate are shown in Figure 6-7.

In two experiments, norepinephrine superfusion was followed by a 20 minute washout with standard Tyrode solution. A reexamination of automaticity showed the complete absence of repetitive activity at all potentials less negative than -55 mV.

6.2.4 The Effect of Tyramine in a Muscle Depleted of Endogenous Catecholamines

In two muscles from animals pretreated with 6-hydroxydopamine

Table 6-5. The effects of 5 x 10^{-6}M norepinephrine in three muscles from animals pretreated with intravenous 6-hydroxydopamine. In A, the effects in action potentials are summarized; in B, the effects on automaticity are summarized. Definitions and abbreviations of each variable were described in Figures 4-2 and 4-3. The absence of automaticity over less negative MDPs in pretreated muscles before addition of norepinephrine precluded the measurement of most of the variables over this range. *$p < .05$, **$p < .01$, ***$p < .001$. No Rs-voltage corrections.

Variable	-79 -70	-69 -60	-59 -50	-49 -40	-39 -30
Inter-Spike Interval (msec)					
6-OHDA	369.5 ± 7.7 (10)	378.5 ± 7.7 (17)	420.0 ± 17.6 (3)	-	-
5×10^{-6}M NE	413.8 ± 7.9*** (12)	377.0 ± 13.2 (15)	391.0 ± 15.0 (15)	403.2 ± 18.4 (14)	498.0 ± 2.5 (10)
AP Duration (msec)					
6-OHDA	249.5 ± .5 (10)	268.5 ± 1.9 (17)	268.8 ± 6.6 (4)	-	-
5×10^{-6}M NE	312.1 ± 15.2** (12)	297.0 ± 15.2 (15)	308.0 ± 16.7 (15)	305.0 ± 17.7 (14)	373.0 ± 5.2 (10)
Threshold (mV)					
6-OHDA	-61.7 ± .3 (10)	-49.2 ± 1.3 (17)	-41.0 ± 2.5 (3)	-	-
5×10^{-6}M NE	-64.3 ± 1.1* (12)	-54.3 ± 1.1** (15)	-36.3 ± 1.9 (15)	-25.6 ± 1.6 (11)	
PH 4 Slope (mV/sec)					
6-OHDA	112.0 ± 6.7 (10)	178.1 ± 9.1 (17)	131.7 ± 6.0 (3)	-	-
5×10^{-6}M NE	119.6 ± 8.9 (12)	164.3 ± 7.6 (15)	230.0 ± 8.5*** (15)	229.3 ± 13.3 (14)	109.0 ± 12.7 (10)
Overshoot (mV)					
6-OHDA	7.9 ± .3 (10)	.7 ± 3.6 (17)	-30.9 ± 5.9 (14)	-39.1 ± .7 (12)	-30.6 ± .8 (11)
5×10^{-6}M NE	20.2 ± 1.8*** (12)	20.8 ± 1.7*** (15)	10.9 ± .8*** (15)	-3.1 ± 3.0*** (14)	-23.8 ± 2.2*** (10)
$\dot{V}_{MAX}$ (V/sec)					
6-OHDA	40.8 ± 3.2 (10)	10.1 ± 1.8 (17)	6.3 ± .7 (3)	-	-
5×10^{-6}M NE	45.6 ± 14.9 (12)	28.0 ± 8.8 (15)	6.5 ± .5 (15)	3.1 ± .4 (11)	

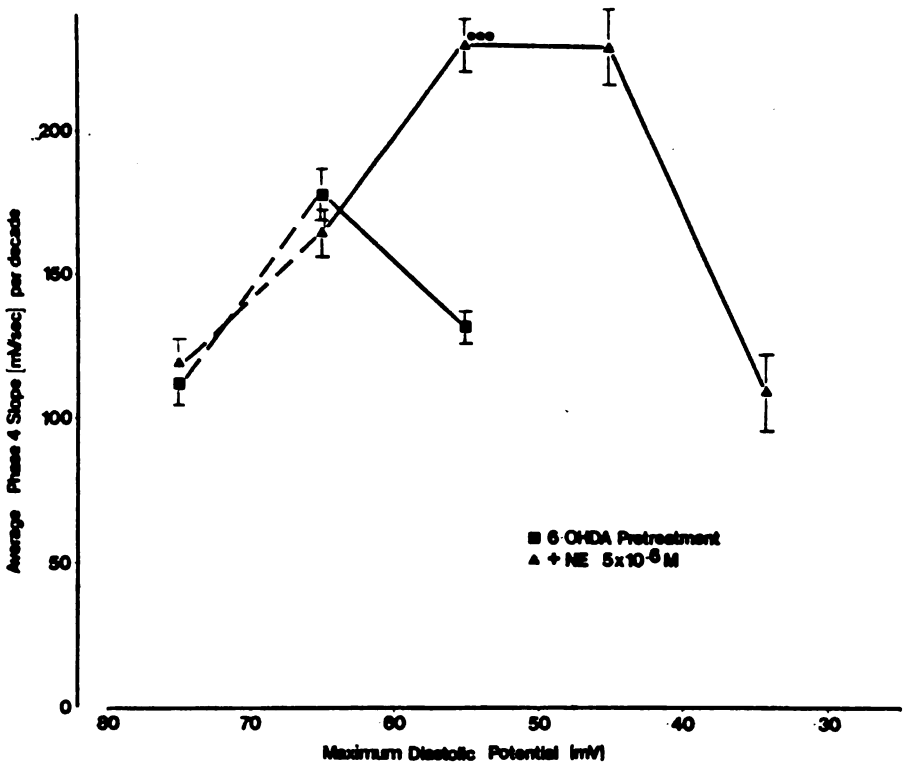


Figure 6-7

The effect of 5 x 10^{-6}M norepinephrine on mean phase 4 diastolic depolarization rate in muscles from animals pretreated with a single intravenous injection of 6-hydroxydopamine. Because automaticity was absent over less negative MDPs in muscles from pretreated animals, phase 4 slope could not be measured over this range of potentials. The curves show mean phase 4 slope and standard errors per decade of MDP for automatic activity. All values are plotted at the midpoint of each decade of MDP. *p < .05, **p < .01, ***p < .001. No Rs-voltage corrections.

(i.v.), the effects of 5 x 10^{-5}M tyramine were investigated. One such experiment is illustrated in Figure 6-8. The same concentration of tyramine which had a positive chronotropic effect in control muscles (Section 6.1, compare Figure 6-1) had no effect in two muscles from pretreated animals.

6.3 Effect of In Vitro Reserpine on Depolarization Induced Ventricular Automaticity

In three experiments, the effects of 5 x 10^{-5}M reserpine were examined. In each case, reserpine caused a progressive increase in contractility which invariably caused dislodgement of the intracellular microelectrode. After 30 minutes of superfusion with reserpine, the test chamber was superfused with standard Tyrode solution for one hour. After this hour washout, several impalements were made and automaticity examined. One experiment is illustrated in Figure 6-9. In all impalements examined after the washout period, automaticity during prolonged current pulses existed only at MDPs more negative than -55 mV. Superfusion with 3 x 10^{-6}M isoproterenol readily restored automaticity over the less negative range of MDPs. Washout of the isoproterenol again resulted in the loss of automaticity over this potential range. Since activity before and after reserpine was from different impalements, no attempts were made to quantitate the reserpine effect.

6.4 The Effect of Beta-Antagonists on Depolarization Induced Ventricular Automaticity

6.4.1 Metoprolol

The effects of several concentrations of metoprolol on ventricular automaticity were examined in two complete experiments. Figure 6-10 illustrates one such experiment. After superfusion with

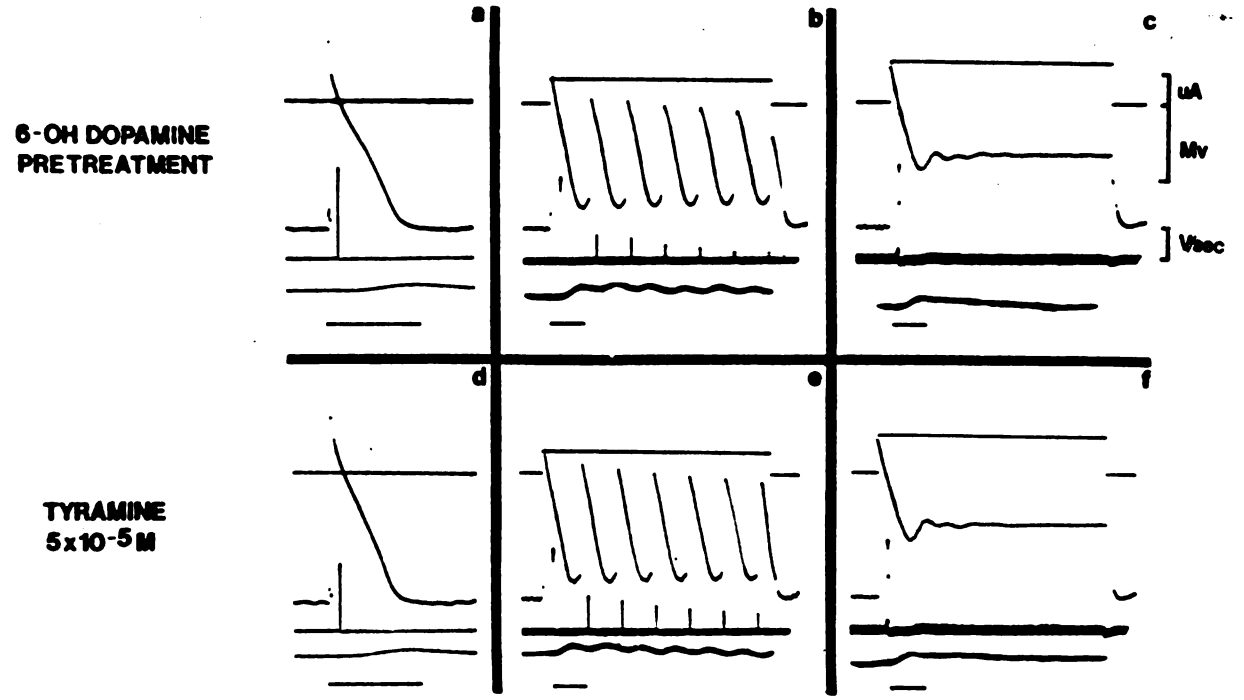


Figure 6-8

Lack of effect of 5 x 10^{-5}M tyramine in a muscle from an animal pretreated with a single intravenous injection of 6-OHDA (250 mg/kg). The traces are the same as in Figure 4-5. a and d are action potentials elicited by a brief stimulus (2 msec.); b and e, activity during prolonged (1.5 sec.) constant current pulse at MDPs of -68 mV; c and f at MDPs of -44 mV. I calibration is 10 µA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, b, d, and e; 10 V/sec. for c and f. Voltage calibration is 50 mV for all panels. Panels a-c are control responses in a muscle from an animal pretreated with 6-OHDA (i.v.); panels d-f were obtained during superfusion with 5 x 10^{-5}M tyramine.

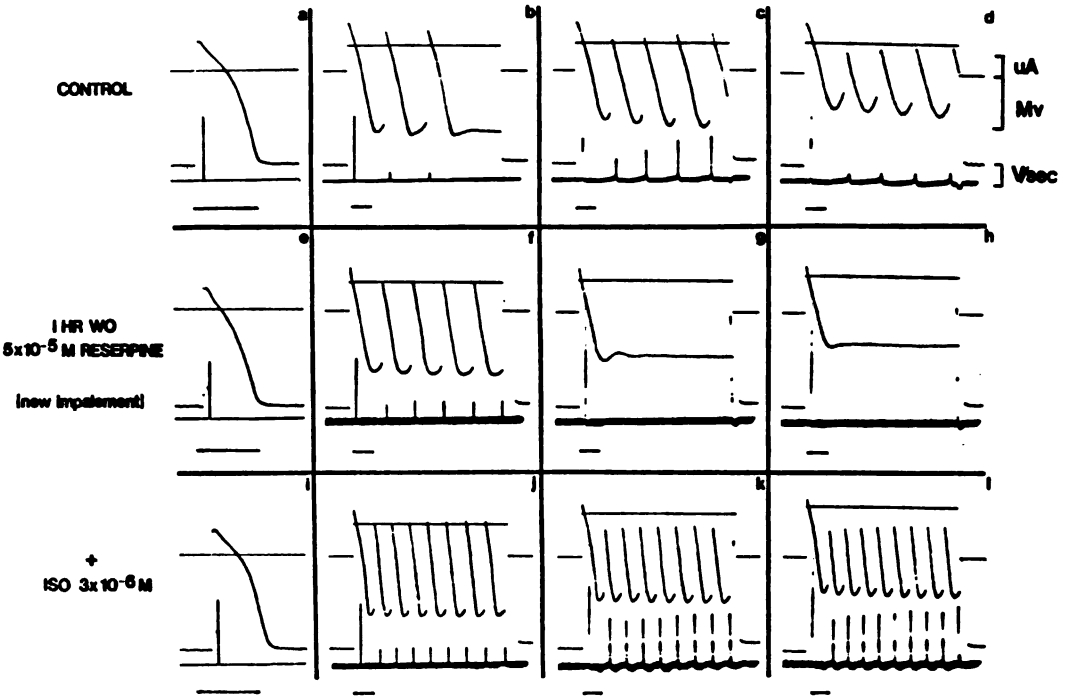


Figure 6-9

The effect of in vitro reserpine (5 x 10^{-5}M) on depolarization induced ventricular automaticity. The traces are the same as in Figure 4-1. a, e, and i are action potentials elicited by a brief stimulus (2 msec.); b, f, and j, activity during prolonged (1.5 sec.) constant current pulse at MDPs of -60 mV; c, g, and k at MDPs of -50 mV; d, h, and 1 at MDPs of -35 mV. I calibration is 10 μA for all panels; V_{MAX} calibration is 100 V/sec. for a, b, e, f, i, and j; 10 V/sec. for rest of panels. Voltage calibration is 50 mV for all panels. Panels a-d are control responses; panels e-h were obtained in a new impalement in the same muscle one hour after washout of 5 x 10^{-5}M reserpine; panels i-1 were obtained in same impalement as in e-h, during superfusion with 3 x 10^{-6}M isoproterenol.

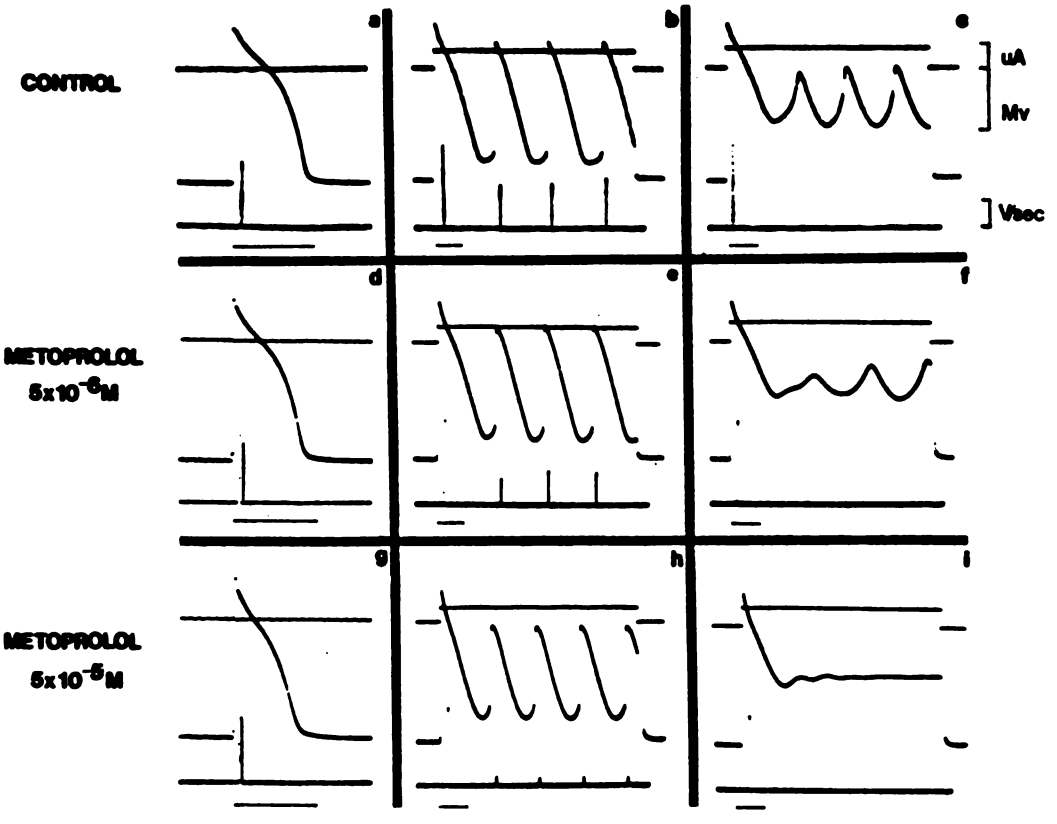


Figure 6-10

The effect of two concentrations of metoprolol on depolarization induced ventricular automaticity. The traces are the same as in Figure 4-1. a, d, and g are action potentials elicited by a brief stimulus (2.5 msec.); b, e, and h, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -70 mV; c, f, and i at MDPs of -40 mV. I calibration is 20 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, b, d, e, g, and h; 10 V/sec. for c, f, and i. Voltage calibration is 50 mV for all panels. a-c are control responses; d-f were obtained after superfusion with 5 x 10^{-5}M metoprolol for 20 minutes.

5×10^{-6}M metoprolol for 20 minutes, there was a slight reduction in overshoot and decrease in AP duration at both 0 mV and at resting potential for action potentials elicited by a brief stimulus. For automaticity arising from more negative MDPs (panel e, MDP = -70), there were minimal effects, except for a slight reduction in $\dot{V}_{MAX}$ of automatic APs. For automaticity arising from less negative potentials, there was a clear depression of overshoot of automatic APs and a reduction in phase 4 slope. At the higher concentration, metoprolol further decreased the overshoot and reduced the AP duration at 0 mV and at resting potential of action potentials elicited by a brief stimulus. However, there was no decrease in $\dot{V}_{MAX}$ at this potential. For automaticity arising from more negative MDPs (panel h), there was a depression of overshoot of automatic APs and a considerable reduction in $\dot{V}_{MAX}$. Automaticity at less negative MDPs (panel i) was suppressed by this concentration of metoprolol.

Similar resluts were seen in the other complete experiment with metoprolol. However, these two experiments did not yield enough data to analyze in a quantitative manner.

6.4.2 L-propranolol

The experiments with metoprolol revealed that this agent attenuated automaticity over less negative MDPs; however, there was also a depression of $\dot{V}_{MAX}$ at more negative MDPs. This latter effect resembles direct membrane or local anesthetic properties which have been observed with other beta-antagonists (Wit, Hoffman, and Rosen, 1975). The beta-antagonist properties of propranolol have been shown to be stereospecific whereas direct membrane

properties of this agent are not (Barrett and Cullum, 1968). In order to distinguish beta-antagonist effects from direct membrane effects, an evaluation of the optical isomers of propranolol on depolarization induced ventricular automaticity was carried out.

L-propranolol was initially examined in three experiments at concentrations of 10^{-6}M and higher. Five additional experiments at concentrations starting at 10^{-7}M were carried out. In all experiments, automaticity was examined after 30 minute superfusion with each concentration studied. Figure 6-11a illustrates a typical experiment with l-propranolol. 2 x 10^{-7}M l-propranolol had no effect upon the action potential elicited by a brief stimulus or upon automaticity arising from more negative MDPs (panel f, MDP = -55 mV), or automaticity at intermediate MDPs (panel g, MDP = -46 mV). Nor was there effects on $\dot{V}_{MAX}$ of automatic action potentials at these potentials. For automaticity arising from less negative MDPs (panel h, MDP = -37 mV), there was a reduction in rate and a reduction on phase 4 depolarization rate. 3 x 10^{-7}M l-propranolol had minimal effects upon the action potential elicited by a brief stimulus or upon automaticity at more negative MDPs (panel j, MDP = -55 mV). Automaticity was attenuated at MDPs less negative than -50 mV by the higher concentration of l-propranolol (panels k and l). L-propranolol had no effect on relative membrane resistance in this experiment.

In Figure 6-11b, the effects of 3 x 10^{-7}M l-propranolol are shown to be reversible by two concentrations of norepinephrine (same impalement as in Figure 6-11a). Concentrations of

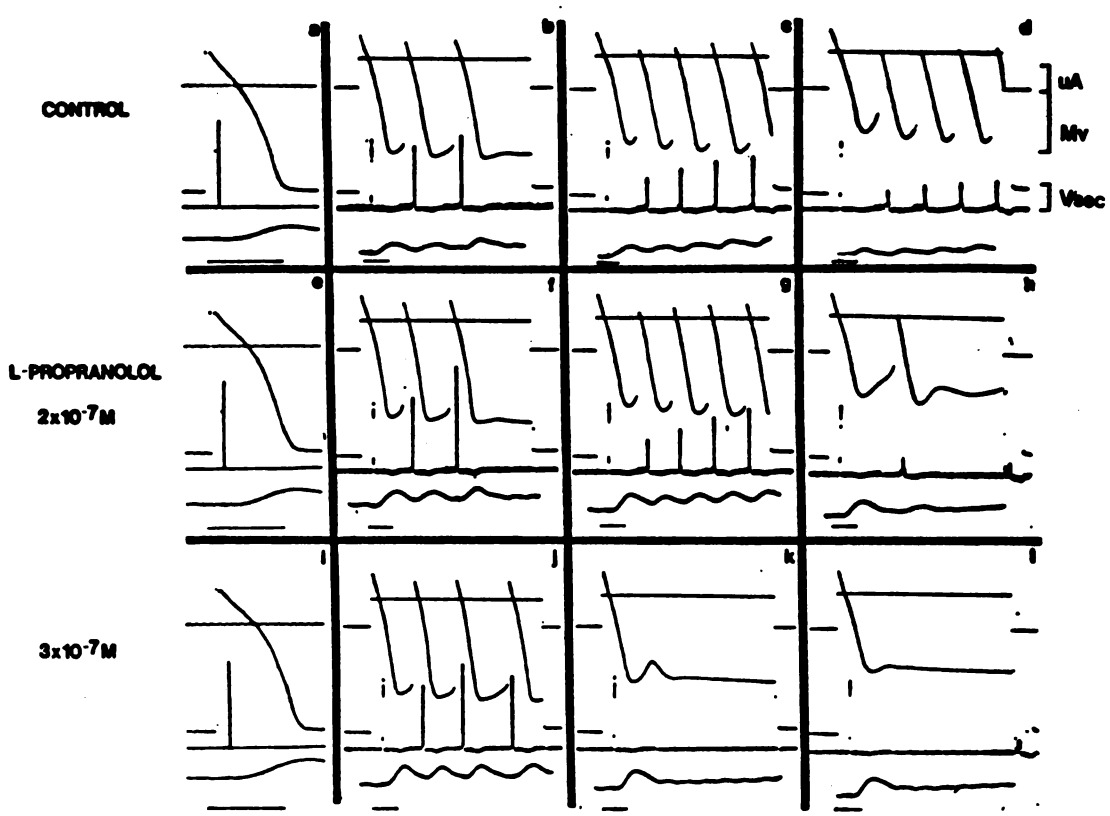


Figure 6-11a

The effect of two concentrations of l-propranolol on depolarization induced ventricular automaticity. The traces are the same as in Figure 4-6. a, e, and i are action potentials elicited by a brief stimulus (2 msec.); b, f, and j, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -55 mV: c, g, and k at MDPs of -46 mV; d, h, and l at MDPs of -37 mV. I calibration is 10 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, e, and i; 10 V/sec. for rest of panels. Voltage calibration is 50 mV for all panels. a-d are control responses; e-h obtained during superfusion with 2 x 10^{-7}M l-propranolol; i-l during superfusion with 3 x 10^{-7}M l-propranolol.

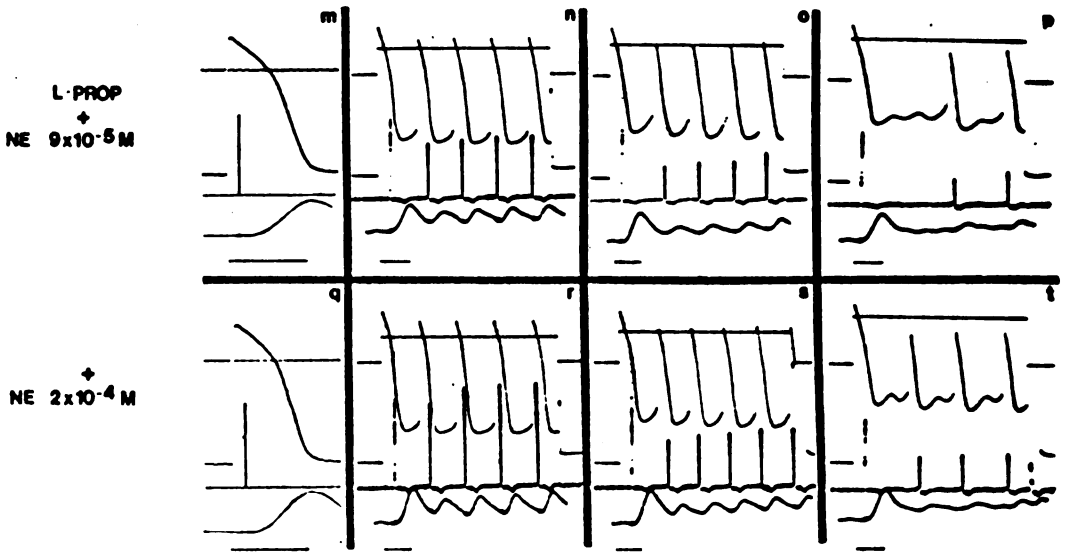


Figure 6-11b

Reversal of the effects of 3 x 10^{-7}M l-propranolol by norepinephrine. Same impalement as in Figure 6-11a. m and q are action potentials elicited by a brief stimulus (2 msec.); n and r, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -55 mV; o and s at MDPs of -46 mV; p and t at MDPs of -37 mV. I calibration is 10 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for m and q; 10 V/sec. for rest of panels. Voltage calibration is 50 mV for all panels. m-p obtained during superfusion with 3 x 10^{-7}M l-propranolol and 9 x 10^{-5}M norepinephrine; q-t obtained during superfusion with 3 x 10^{-7}M l-propranolol and 2 x 10^{-4}M norepinephrine.

norepinephrine up to 5 x 10^{-5}M had no effect on automaticity in the presence of 3 x 10^{-7}M l-propranolol. At 9 x 10^{-5}M, norepinephrine produced a large increase in contractility and restored automaticity at less negative MDPs. This concentration is approximately 90 times higher than the threshold dose necessary to produce a chronotropic effect in a group of control muscles (Section 4.4). 2 x 10^{-4}M norepinephrine produced an even greater reversal of the depressant effects of l-propranolol.

The mean effects of two concentrations of l-propranolol in a group of muscles are summarized in Tables 6-6. L-propranolol produced a slight reduction in action potentials duration at 0 mV and at complete repolarization, and in overshoot of action potentials elicited by a brief stimulus. For automaticity arising from more negative MDPs, 2 x 10^{-7}M l-propranolol slightly reduced action potential duration, phase 4 slope, and overshoot of automatic action potentials. For automaticity arising from less negative MDPs, 2 x 10^{-7}M l-propranolol produced a significant reduction in rate, action potential duration, phase 4 slope, and overshoot of automatic action potentials. 3 x 10^{-7}M l-propranolol produced qualitatively similar effects, although of greater magnitude for automatic action potentials arising from more negative MDPs. This concentration of l-propranolol abolished automaticity at nearly all potentials less negative than -50 mV. This is reflected by the severe depression of overshoots of automatic action potentials over the less negative range of MDPs. These actually reflect amplitudes of oscillatory potentials. Neither concentration of l-propranolol produced any consistent effects

Tables 6-6. The effects of two concentrations of l-propranolol upon action potentials (A) and upon automaticity (B) in a group of muscles. Definitions and abbreviations of each variable were described in Figures 4-2 and 4-3. The absence of automaticity over less negative MDPs in the presence of the higher concentration of l-propranolol precluded measurement of most of the variables over this potential range. *$p < .05$, **$p < .01$, ***$p < .001$. No Rs-voltage corrections.

A

	V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)
Controls	-90.0 ± 0	328.3 ± 26.8	29.0 ± .6	181.7 ± 10.1	69.3 ± 3.0	-66.7 ± 4.5
2 x 10^{-7}M L-Prop	-89.3 ± .7	323.3 ± 31.8	28.7 ± .7	161.7 ± 11.7	51.7 ± 11.7	-65.0 ± 3.8
3 x 10^{-7}M L-Prop	-87.3 ± 1.5	320.0 ± 35.1	27.3 ± 1.2	165.0 ± 13.2	56.7 ± 14.5	-63.0 ± 3.1

B

Variable	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
Inter-Spike Interval (msec)					
Control	359.6 ± 6.1 (14)	366.9 ± 6.5 (21)	362.3 ± 4.1 (15)	401.4 ± 13.0 (14)	427.5 ± 18.1 (14)
2 x 10^{-7}M L-Prop	367.2 ± 4.3 (16)	370.5 ± 5.5 (27)	386.2 ± 6.1** (21)	463.4 ± 16.3** (22)	-
3 x 10^{-7}M L-Prop	365.8 ± 3.3 (13)	373.0 ± 5.8 (22)	377.5 ± 4.3 (4)	-	-
AP Duration (msec)					
Control	289.3 ± 3.2 (14)	294.1 ± 1.8 (21)	289.7 ± 2.0 (15)	299.3 ± 4.1 (14)	308.0 ± 5.4 (14)
2 x 10^{-7}M L-Prop	272.5 ± 8.9 (16)	263.0 ± 6.5** (27)	273.6 ± 5.8* (21)	280.9 ± 5.2* (22)	281.0 ± 10.2* (7)
3 x 10^{-7}M L-Prop	280.0 ± 6.9 (13)	268.2 ± 5.6** (22)	268.8 ± 1.3 (4)	-	-
Threshold (mV)					
Control	-56.3 ± 1.5 (14)	-43.3 ± .9 (21)	-31.7 ± 1.0 (15)	-18.1 ± 1.6 (14)	-9.6 ± 1.6 (12)
2 x 10^{-7}M L-Prop	-52.6 ± 1.4 (16)	-41.4 ± 1.1 (27)	-27.9 ± 1.2* (21)	-15.4 ± 1.4 (21)	-
3 x 10^{-7}M L-Prop	-56.1 ± 1.5 (13)	-44.2 ± 1.2 (22)	-33.5 ± .9 (4)	-	-
PH 4 Slope (mV/sec)					
Control	129.0 ± 16.9 (14)	185.0 ± 11.6 (21)	213.3 ± 9.8 (15)	198.6 ± 5.6 (14)	158.2 ± 9.5 (14)
2 x 10^{-7}M L-Prop	127.8 ± 5.2 (16)	148.5 ± 5.9** (27)	163.3 ± 5.8** (21)	120.2 ± 4.5*** (22)	105.0 ± 5.0 (2)
3 x 10^{-7}M L-Prop	119.2 ± 7.3 (13)	145.2 ± 6.7** (22)	147.5 ± 2.5 (4)	-	-
Overshoot (mV)					
Control	34.7 ± .5 (14)	33.3 ± .9 (21)	28.5 ± .9 (15)	25.7 ± .8 (14)	16.2 ± 2.8 (14)
2 x 10^{-7}M L-Prop	26.5 ± 1.2** (16)	26.2 ± 1.6 (27)	23.3 ± 1.9 (21)	19.4 ± 2.8 (23)	-9.9 ± 4.1** (13)
3 x 10^{-7}M L-Prop	21.4 ± 1.4** (13)	15.2 ± 5.0** (25)	-18.4 ± 6.2** (18)	-28.1 ± .6** (14)	-20.3 ± .9** (10)
$\dot{V}_{MAX}$ (V/sec)					
Control	140.6 ± 17.7 (14)	26.8 ± 2.5 (21)	11.0 ± .8 (15)	6.9 ± .3 (14)	4.3 ± .6 (12)
2 x 10^{-7}M L-Prop	108.4 ± 16.9 (16)	23.2 ± 2.3 (27)	9.5 ± 1.2 (21)	6.9 ± .7 (22)	-
3 x 10^{-7}M L-Prop	151.0 ± 21.7 (13)	25.5 ± 3.1 (22)	13.3 ± .3 (4)	-	-

on relative membrane resistance in these experiments. The effects of these two concentrations of l-propranolol on mean phase 4 slope are summarized in Figure 6-12.

In Table 6-6b, there is some indication of a reduction in $\dot{V}_{MAX}$ at the MDP decade of -69 to -60 mV in the presence of 2 x 10^{-7}M l-propranolol. However, at the higher concentration of l-propranolol, $\dot{V}_{MAX}$ returned toward control values. This suggests that the reduced $\dot{V}_{MAX}$ observed at 2 x 10^{-7}M l-propranolol may be attributable to measurement errors rather than a true drug effect. In order to check this, mean $\dot{V}_{MAX}$ values per decade of MDP were plotted as a function of the mean MDP of the observations per decade of MDP in Figure 6-13. It can be seen that the mean MDP of observations for the decade -69 to -60 mV in the presence of 2 x 10^{-7}M l-propranolol (-63.3 mV) was actually less negative than the mean MDP of observations in the control (-64.5 mV) or in the presence of 3 x 10^{-7}M l-propranolol (-65.0 mV). Such a slight shift in a region where the sodium inactivation curve is so steep could indeed account for the apparent reduction of $\dot{V}_{MAX}$. For this reason and because the value of $\dot{V}_{MAX}$ returned to control levels at the higher drug concentration, it was concluded that at neither concentration did l-propranolol affect $\dot{V}_{MAX}$ of the action potentials. No effects of l-propranolol upon $\dot{V}_{MAX}$ of the action potential elicited by a brief stimulus were observed at these concentrations either.

In two experiments in which 10^{-6}M l-propranolol was the lowest dose of l-propranolol superfused (this data is not included in

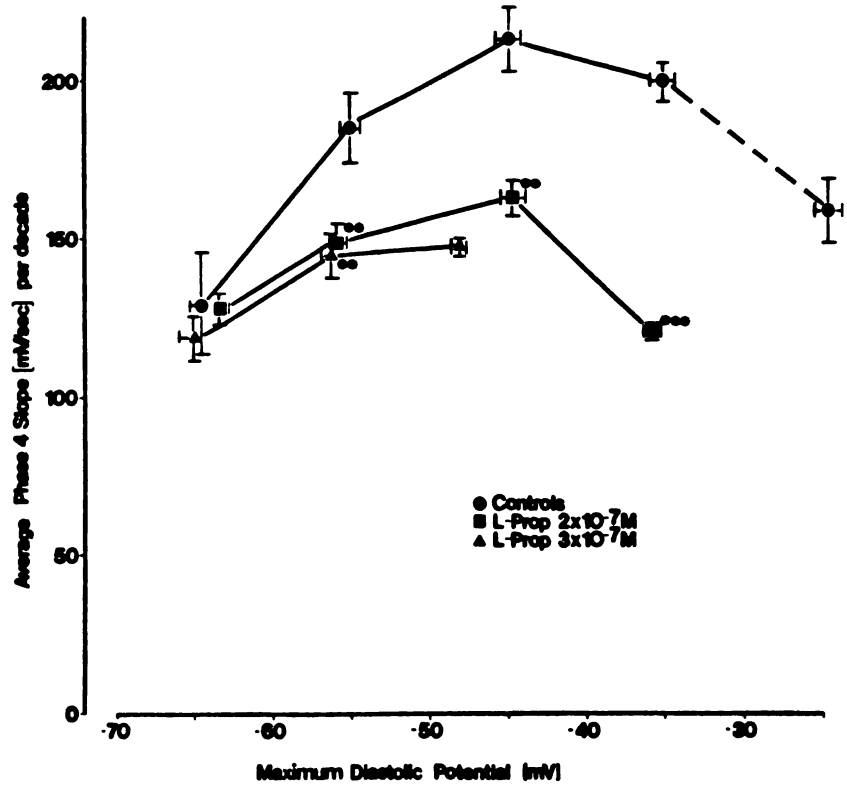


Figure 6-12

The effect of two concentrations of l-propranolol on mean phase 4 diastolic depolarization rate in a group of muscles. Because automaticity was absent over less negative MDPs in the presence of 3 x 10^{-7}M l-propranolol, phase 4 slope could not be measured over this range of potentials. The curves show mean phase 4 slope and standard errors per decade of MDP for automatic activity. In this case, all values are plotted at the mean MDP of each interval, rather than the midpoint. Standard errors for these values are also shown. *$p < .05$, **$p < .01$, ***$p < .001$. No Rs-voltage corrections.

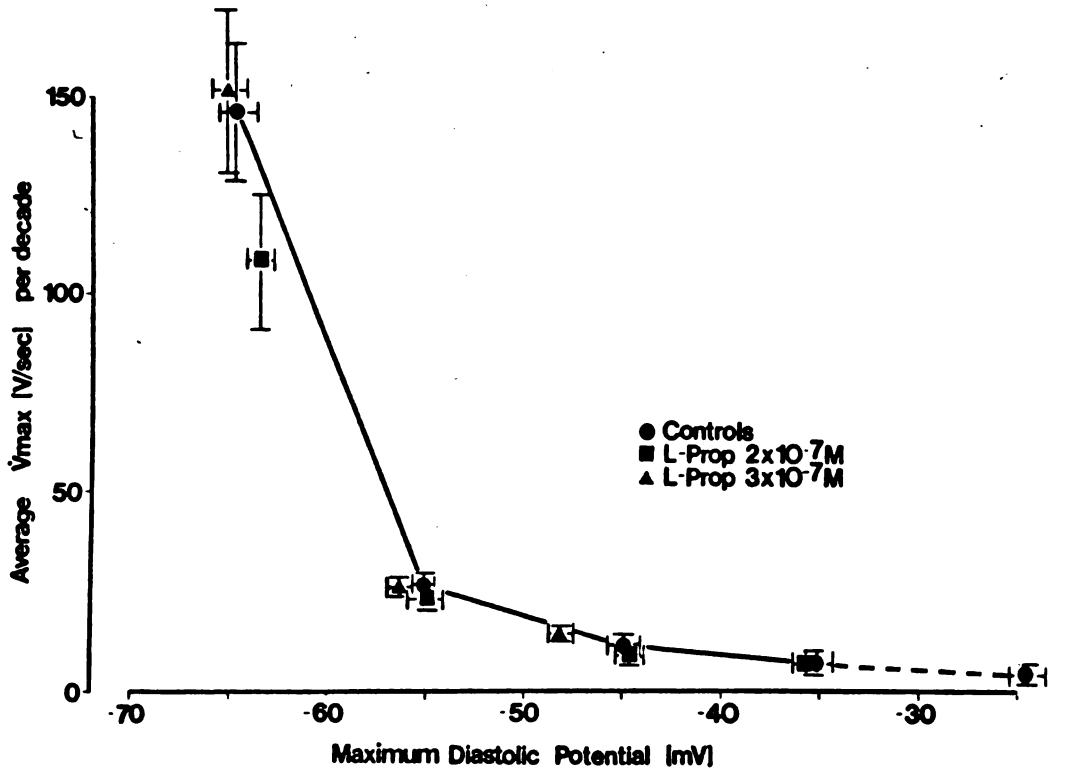


Figure 6-13

Effect of two concentrations of l-propranolol on average $\dot{V}_{MAX}$ in a group of muscles. The points show mean $\dot{V}_{MAX}$ and standard errors per decade of MDP for automatic activity. In this case, all values are plotted at the actual mean MDP of each interval, rather than the midpoint. Standard errors for these values are also shown. No Rs-voltage corrections.

Tables 6-6), qualitatively similar results on automaticity were observed as those with 3 x 10^{-7}M l-propranolol. However, in each of these high concentration experiments there was a clear cut increase in relative membrane resistance. In one of these experiments, superfusion with drug-free Tyrode solution for 45 minutes resulted in restoration of automaticity over less negative MDPs; however, the increase in relative membrane resistance was not reversed.

6.4.3 D-propranolol

The effects of 3 x 10^{-7}M d-propranolol on ventricular automaticity were observed in five experiments. In one experiment, concentrations ranging from 3 x 10^{-7}M to 6 x 10^{-6}M d-propranolol were studied. This experiment is illustrated in Figure 6-14. In all experiments, 30 minutes superfusion with each concentration was carried out before starting data collection.

3 x 10^{-7}M d-propranolol had negligible effects upon the action potential elicited by a brief stimulus and upon automaticity over the entire range of MDPs. At 3 x 10^{-6}M d-propranolol, there were negligible effects upon the action potential elicited by a brief stimulus and upon automaticity arising from more negative MDPs (panel h). There was a slight slowing of the rate, overshoot, and phase 4 diastolic depolarization rate of automaticity at less negative MDPs (panel i) at this concentration. At 6 x 10^{-6}M d-propranolol, there was a decrease in overshoot and in action potential duration at 0 mV and at resting potential. There was, in addition, a depression of $\dot{V}_{MAX}$ at this concentration

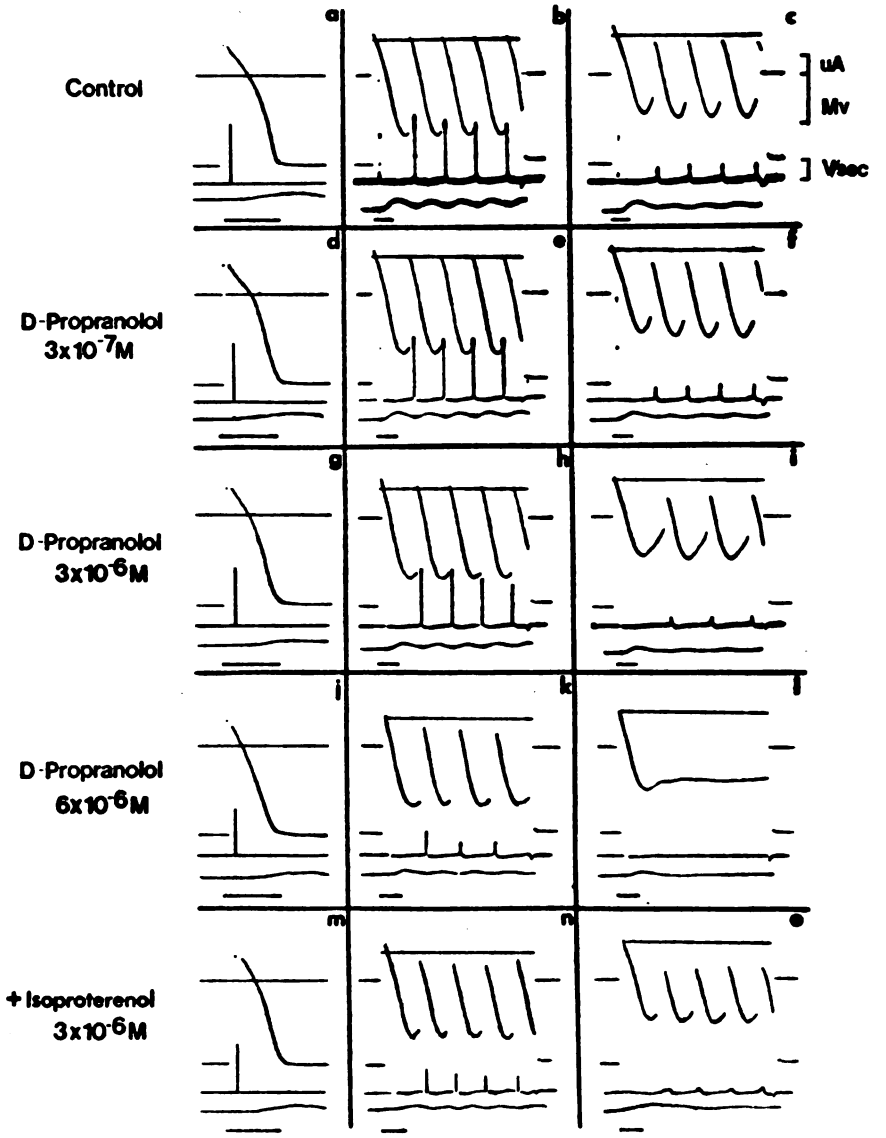


Figure 6-14

Effect of three concentrations of d-propranolol on depolarization induced ventricular automaticity. The traces are the same as in Figure 4-6. a, d, g, j, and m are action potentials elicited by a brief stimulus (2 msec.); b, e, h, k, and n, automaticity during prolonged (1.5 sec.) constant current pulse at MDPs of -60 mV; c, f, i, l, and o at MDPs of -40 mV. I calibration is 10 μA for all panels; $\dot{V}_{MAX}$ calibration is 100 V/sec. for a, d, g, j, and m; 10 V/sec. for rest of panels. Voltage calibration is 50 mV for all panels. a-c are control responses; d-f obtained during superfusion with 3 x 10^{-7}M d-propranolol; g-i obtained during superfusion with 3 x 10^{-6}M d-propranolol; j-l obtained during superfusion with 6 x 10^{-6}M d-propranolol; m-o obtained during superfusion with 6 x 10^{-6}M d-propranolol and 3 x 10^{-6}M isoproterenol.

(panel j). For automaticity arising from more negative MDPs (panel k, MDP = -60 mV), there was a slight slowing of the rate, but the major effects were a diminished overshoot and depressed $\dot{V}_{MAX}$ of automatic action potentials. Automaticity at less negative MDPs was abolished by 6 x 10^{-6}M d-propranolol (panel l, MDP = -40 mV). In the presence of 6 x 10^{-6}M d-propranolol, 10^{-6}M isoproterenol was without any effect. 3 x 10^{-6}M isoproterenol, however, restored automaticity at less negative MDPs (panel o) without reversing the depressed $\dot{V}_{MAX}$ of automatic action potentials at more negative MDPs.

The mean effects of 3 x 10^{-7}M d-propranolol in a group of muscles are summarized in Tables 6-7. This concentration of d-propranolol produced virtually no effect on the action potential elicited by a brief stimulus or upon automaticity over the entire range of MDPs. There was a significant increase in rate and reduction of action potential duration at the most negative MDPs; however, this result may be spurious iwing to the small number of observations in this MDP decade of -79 to -70 mV. The effect of 3 x 10^{-7}M d-propranolol on phase 4 slope and $\dot{V}_{MAX}$ of automatic action potentials in this group of muscles are summarized in Figure 6-15 and Figure 6-16 respectively. D-propranolol produced no consistent effects upon relative membrane resistance in any of these experiments.

Tables 6-7. The effects of 3 x 10^{-7}M d-propranolol upon action potentials (A) and upon automaticity (B) in a group of muscles. Definitions and abbreviations of each variable were described in Figures 4-2 and 4-3. *p < .05, **p < .01, ***p < .001. No Rs-voltage corrections.

A

	V_R (mV)	$\dot{V}_{MAX}$ (V/sec)	OS (mV)	APD (msec)	APD_0 (msec)	MDP* (mV)
Controls	-88.7 ± .7	270.0 ± 10.0	28.3 ± 1.2	171.7 ± 10.9	72.0 ± 14.3	-70.7 ± 2.6
3 x 10^{-7}M D-Prop	-88.0 ± 1.2	271.7 ± 9.3	28.3 ± 1.2	171.7 ± 10.9	69.3 ± 17.4	-68.7 ± 3.2

B

Variable	-79 -70	-69 -60	-59 -50	-49 -40	-39 -30	-29 -20
Inter-Spike Interval (msec)						
Control	439.3 ± 4.7 (7)	419.7 ± 3.1 (29)	402.9 ± 4.3 (19)	424.2 ± 8.0 (13)	428.8 ± 1.5 (13)	440.0 ± 2.7 (11)
3 x 10^{-7}M D-Prop	403.1 ± 2.5*** (9)	418.3 ± 7.5 (29)	400.6 ± 5.7 (18)	415.3 ± 4.6 (16)	435.4 ± 5.1 (13)	448.6 ± 3.6 (11)
AP Duration (msec)						
Control	337.1 ± 3.1 (7)	337.1 ± 2.0 (29)	335.0 ± 2.4 (19)	330.4 ± 2.6 (13)	335.0 ± 2.7 (13)	330.9 ± 4.7 (11)
3 x 10^{-7}M D-Prop	311.7 ± 2.9*** (9)	328.3 ± 3.4* (29)	326.7 ± 4.9 (18)	325.9 ± 5.2 (16)	337.0 ± 7.3 (13)	337.3 ± 7.9 (11)
Threshold (mV)						
Control	-65.1 ± .9 (7)	-56.2 ± .6 (29)	-44.4 ± 1.2 (19)	-30.5 ± .8 (16)	-19.0 ± 1.3 (13)	-8.2 ± .8 (11)
3 x 10^{-7}M D-Prop	-63.8 ± .7 (9)	-55.2 ± .4 (29)	-45.8 ± 1.3 (18)	-30.4 ± 1.1 (16)	-19.4 ± 1.3 (13)	-9.4 ± .7 (11)
PH 4 Slope (mV/sec)						
Control	83.6 ± 6.9 (7)	125.3 ± 5.6 (29)	190.5 ± 5.9 (19)	208.5 ± 11.1 (13)	198.1 ± 3.1 (13)	170.5 ± 5.4 (11)
3 x 10^{-7}M D-Prop	100.0 ± 6.9 (9)	120.2 ± 6.5 (29)	185.8 ± 5.7 (18)	219.1 ± 8.9 (16)	196.2 ± 5.1 (13)	159.1 ± 4.8 (11)
Overshoot (mV)						
Control	21.4 ± .4 (7)	32.5 ± 2.0 (29)	29.6 ± 2.7 (19)	23.2 ± 4.1 (13)	21.9 ± 5.4 (15)	19.1 ± 4.0 (13)
3 x 10^{-7}M D-Prop	20.2 ± .3* (9)	36.1 ± 2.0 (29)	30.5 ± 3.4 (18)	25.1 ± 4.8 (17)	23.6 ± 5.8 (15)	22.5 ± 5.4 (13)
$\dot{V}_{MAX}$ (V/sec)						
Control	205.0 ± 13.2 (7)	96.8 ± 8.4 (29)	21.1 ± 3.7 (19)	6.8 ± .9 (13)	6.4 ± .2 (13)	3.6 ± .4 (10)
3 x 10^{-7}M D-Prop	186.1 ± 9.9 (9)	99.2 ± 6.2 (29)	19.3 ± 2.5 (18)	9.2 ± .9 (16)	7.1 ± .4 (13)	3.6 ± .4 (11)

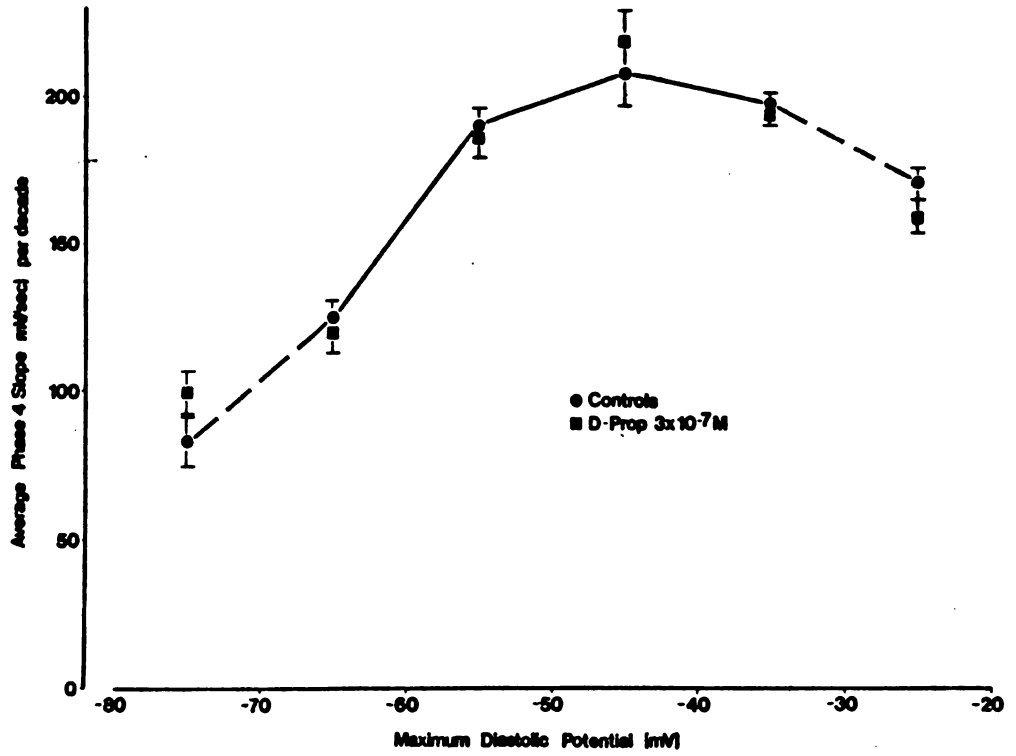


Figure 6-15

The effect of 3 x 10^{-7}M d-propranolol on mean phase 4 diastolic depolarization rate in a group of muscles. The points show mean phase 4 slope and standard errors per decade of MDP for automatic activity. All values are plotted at the midpoint of each decade of MDP. No Rs-voltage corrections.

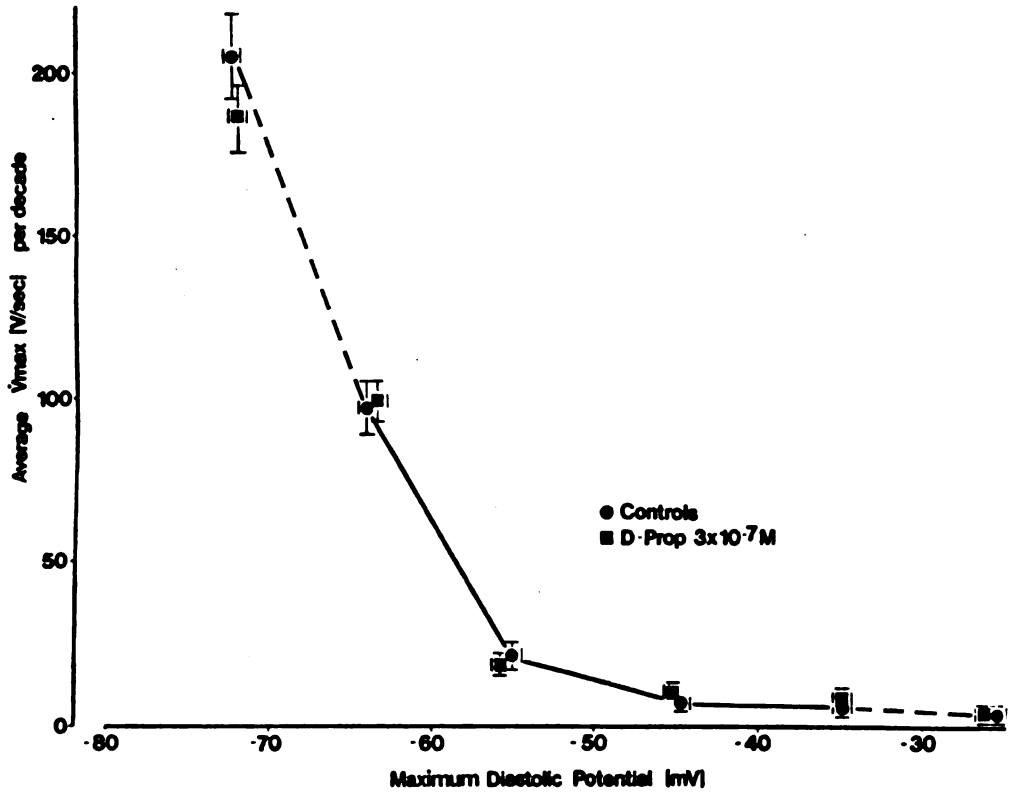


Figure 6-16

Effect of 3 x 10^{-7}M d-propranolol on average $\dot{V}_{MAX}$ in a group of muscles. The points show mean $\dot{V}_{MAX}$ and standard errors per decade of MDP for automatic activity. All values are plotted at the actual mean MDP of each interval, rather than the midpoint. Standard errors for these values are also shown. No Rs-voltage corrections.

7.0 DISCUSSION - ENDOGENOUS CATECHOLAMINES

7.1 Tyramine

Tyramine produced a positive chronotropic effect in normal muscles over the entire range of maximum diastolic potentials. In addition, there was a decrease in relative membrane resistance, especially at less negative potentials. The increased rate produced by tyramine at more negative MDPs was accompanied by a decrease in duration but little effect upon phase 4 slope, threshold, or overshoot of automatic action potentials. This suggests that the increased rate over this range of potentials was caused by primarily the decrease in action potential duration. There was an increase in $\dot{V}_{MAX}$ of automatic action potentials over this range, which probably resulted from the negative shift in the most negative MDP threshold for automaticity which occurred in several experiments in the presence of tyramine.

The chronotropic response to tyramine at less negative MDPs was accompanied by decreased action potential duration and an increase in phase 4 slope. This suggests that in contrast to the more negative MDP range, both the decrease in action potential duration and the increase in phase 4 slope contribute to increased repetition rate over this range of potentials. There were no significant effects upon threshold or $\dot{V}_{MAX}$ at these potentials.

A comparison of the effects of tyramine with those produced by

isoproterenol in Section 4.2 reveals that tyramine produced nearly exactly the same effects on every variable used to characterize ventricular automaticity. This suggests that the effect of tyramine are due to stimulation of beta-adrenoceptors. It is generally accepted that the mode of action of tyramine is indirect and caused by the release of endogenous norepinephrine from nerve endings (Muscholl, 1966; Trendelenburg, 1972). The present experiments are consistent with this indirect mode of action of tyramine. The indirect effects of tyramine upon individual ionic currents may be similar to those produced by beta-stimulation: enhanced i_{X1} outward current, enhanced slow inward current, and stimulation of an electrogenic pump.

It is possible that tyramine might be acting through direct stimulation of beta-adrenoceptors in these experiments. Some early studies suggested that tyramine exerted a direct vasoconstrictor action in vascular smooth muscle (Takenaka, 1963) and in perfused rabbit ears (Ludnena, 1963). In the heart, Crout et al (1962) showed that tyramine produced a positive chronotropic effect in guinea pig atria. It was demonstrated that this effect was indirect through the release of catecholamines from sympathetic nerve terminals by showing that the response to tyramine was abolished in animals depleted of endogenous catecholamines by acute pretreatment with reserpine. It was considered important in the present study to verify the indirect mode of action of tyramine, and a technique similar to that used by Crout et al (1962) was utilized. In several muscles which had been depleted of endogenous catecholamines by acute pretreatment of the animals with

6-hydroxydopamine, the effects of a concentration of tyramine which produced chronotropic effects in control muscles were examined. No response to tyramine was observed in any of these muscles. It seems reasonable to conclude from these results that the chronotropic effects produced by tyramine on ventricular automaticity are due to the release of endogenous norepinephrine from nerve terminals in the muscle. Indirect effects of tyramine have been previously reported in cat papillary muscles (Kaumann, 1970) and in guinea pig papillary muscles (Brasch et al, 1977).

The mechanisms by which tyramine causes the release of norepinephrine from sympathetic nerve terminals are interesting because they appear to be quite different from the mechanisms of release evoked by nerve impulses. Smith (1973) has recently reviewed the evidence which supports the hypothesis that tyramine is taken up into the nerve terminal and either displaces norepinephrine from the storage vesicles into the cytosol or increases the spontaneous release of norepinephrone from the cytosol. Tyramine-evoked release is not through exocytosis. Because tyramine-evoked release is different from the normal exocytotic processes governing impulse-evoked release, there are some interesting properties unique to the tyramine mechanism. Uptake blockers like cocaine prevent tyramine-evoked release but do not affect impulse-evoked release of norepinephrine. Tyramine-evoked release is independent of calcium, whereas impulse-evoked release is calcium dependent. It has been recently demonstrated that impulse-evoked release of norepinephrine is modulated by presynaptic alpha-adrenoceptors in a variety of systems whereas tyramine-evoked release is not (Starke, 1971; 1977).

It can be concluded, based upon the present results with tyramine, that (i) endogenous catecholamines are stored in the apex of the papillary muscles under study, (ii) release of endogenous catecholamines is functionally possible under *in vitro* conditions, and (iii) endogenous catecholamines are capable of influencing the phenomenon being examined, namely, depolarization induced ventricular automaticity.

7.2 6-Hydroxydopamine Pretreatment

7.2.1 6-Hydroxydopamine as a Denervation Tool

Perhaps the most common technique which has been used to study the influence of neuronal function upon a particular physiological response or organ system is to study the response after all possible neuronal influences have been removed (Cannon and Rosenblueth, 1937). In 1967, Thoenen and Tranzer discovered that 6-hydroxydopamine (6-OHDA) caused specific depletion and degeneration of adrenergic nerve terminals. Since this discovery, 6-OHDA has been widely used to produce chemical sympathectomy in a variety of tissues and organ systems.

Two factors are believed responsible for the neurotoxicity of 6-OHDA (Thoenen and Tranzer, 1973). 6-OHDA is accumulated intraneuronally by a neuronal uptake system. This process is probably the so-called "membrane pump" associated with adrenergic nerve terminals, originally described by Iversen (1965) as $uptake_1$ in order to distinguish it from the non-specific, non-saturable system, $uptake_2$. Chemicals known to interfere with this specific uptake system, like antidepressants and d-amphetamine, if

administered prior to 6-OHDA, prevent catecholamine depletion and subsequent neuronal degeneration. It is also believed that 6-OHDA is taken up by the amine storage vesicles once it gains access to the cell interior, probably by the reserpine-sensitive ATP-Mg dependent uptake mechanism, however blockade by reserpine has no noticeable effect upon the neurotoxity produced by 6-OHDA. This has led to the conclusion that it is 6-OHDA accumulated intraneuronally in the cytoplasm which is the prerequisite for the degenerative effects.

The second factor responsible for the neurotoxicity of 6-OHDA is the property of its susceptibility to non-enzymatic oxidation. It is this property which is associated with its cytotoxicity. Two hypotheses have been proposed for the molecular mechanisms involved in neurodegeneration. One is that it is H_2O_2 and free radicals formed during oxidation of 6-OHDA that initiate degeneration (Heikkila and Cohen, 1971). The other hypothesis is that oxidation products like a 5-hydroxy-indole derivative and/or a para-quinone derivative undergo covalent binding with certain macromolecules in the neuron, leading to degeneration (Adams *et al*, 1972).

Regardless of which hypothesis represents the true mechanism, there appears to be general agreement that there is a critical concentration of 6-OHDA which must be taken up in order for degeneration to occur (Thoenen *et al*, 1970; Sachs *et al*, 1973). This threshold concentration has been estimated from *in vitro* experiments to be 30-100 mM, and binding data of ^{3}H-6-OHDA

in mice hearts have indicated that such concentrations are reached intraneuronally by *in vivo* administration of the drug. Also consistent with the view of a threshold concentration are studies which have shown that 6-OHDA acts on adrenergic nerve terminals in an all-or-nothing manner with complete destruction of catecholamine uptake-storage sites in a proportion of nerves which depends upon the dose administered (Jonsson and Sachs, 1972).

Since in lower doses 6-OHDA is taken up and released as a false neurotransmitter, the possibility exists that it might have some direct sympathomimetic effect itself and this could account for the all-or-nothing phenomenon mentioned here. Kuchi and Shibata (1972) investigated this possibility in isolated rat atria. It was found that *in vitro* administration of 6-OHDA caused a transient positive inotropic and chronotropic response which could be prevented by prior administration of cocaine or desmethylimipramine. In animals pretreated *in vivo* with reserpine or 6-OHDA, the *in vitro* administration of 6-OHDA failed to elicit any positive inotropic or chronotropic response. They concluded that 6-OHDA lacked any direct sympathomimetic effect of its own.

There appears to be wide variability in the organ susceptibility to 6-OHDA. Several hypotheses have been advanced to account for this and most relate to differences in the blood supply to the various organs, which would determine the concentration of 6-OHDA reaching each, or to the difference in the density of innervation of organs or tissues. More densely innervated tissues would require higher doses of 6-OHDA to produce depletion and subsequent

degeneration. A recent review of the literature by Kostrezewa and Jacobowitz (1974) compares the dosage schedules of 6-OHDA and their relative effectiveness in depleting norepinephrine levels in many tissues of different species. They concluded that the decreasing order of tissue susceptibility to 6-OHDA is: cardiac ventricles = salivary gland > whole heart > spleen > vasa deferentia > major vascular arteries. The intraperitoneal route of administration also appeared to be as effective as the intravenous route, however higher doses are probably needed.

In light of the proposed free radical mechanism whereby 6-OHDA exerts its effects, the drug would be expected to be rather non-specific. The observed specificity in acting upon adrenergic terminals derives from the selective uptake into those terminals. Studies which have examined the effects of 6-OHDA upon levels of other putative neurotransmitters have verified the lack of much effect on neurons other than catecholamine and dopamine containing neurons (Kostrzewa and Jacobowitz, 1974).

Despite the specificity and effectiveness of 6-hydroxydopamine in producing sympathectomy, de Champlain *et al* (1975) have pointed out that many investigators who have used 6-hydroxydopamine misinterpreted their experimental results. This is because many of the compensatory mechanisms which develop following denervation have not been taken into account. Such compensatory mechanisms have been studied at the neuromuscular junction and many reviews have been published (see Harris, 1974; Purves, 1975; Gutmann, 1976). One compensatory response, denervation supersensitivity, which could certainly confound attempts to study muscular

responses in the absence of neuronal influences, was recognized as long ago as 1939 by Cannon in the "law of denervation": a denervated organ becomes supersensitive over time to its usual neurotransmitter. Mechanisms of denervation supersensitivity have only recently been revealed. For adrenergic systems, post-synaptic mechanisms range from changes in adrenoceptor numbers, changes in affinity of adrenoceptors for ligands (Glaubiger and Lefkowitz, 1977), to changes beyond the receptor, in ion permeabilities or adenylate cyclase (Chiu, 1978) (for review, see Fleming et al, 1973).

It is easy to understand how studies which examine muscular responses after denervation and do not take into account changes in the sensitivity of the muscle to its neurotransmitter can produce misleading results. If sympathectomy is only 90% complete and supersensitivity develops, it is conceivable that no change in the muscle response will be observed. This could erroneously be interpreted as evidence that nervous influences over the muscle are negligible. In the heart, it has been demonstrated that supersensitivity to the chronotropic effects of catecholamines can appear as early as one to three days following reserpine pretreatment (Westfall and Fleming, 1968a, b). Investigations studying the effects of denervation upon myocardial responses should, in addition to measuring the muscular response, determine the degree of sensitivity of the denervated muscle to its neurotransmitter.

7.2.2 Experimental Results Produced by 6-Hydroxydopamine Pretreatment

In initial experiments, an acute dosage regimen for the intra-

peritoneal administration of 6-OHDA was selected in order to attempt to avoid the production of denervation supersensitivity. These experiments revealed that some muscles from animals receiving two intraperitoneal injections of 6-OHDA only exhibited induced pacemaker activity over more negative MDPs (-65 to -55 mV). However, the results were inconsistent in that some of these muscles from pretreated animals, appeared to exhibit automaticity identical to control muscles, e. g., over the entire range of MDPs (-65 to -30 mV). Two possible causes for these inconsistent results were considered. Those muscles from pretreated animals which exhibited "normal" automaticity might not have achieved the same degree of depletion of endogenous catecholamines, or these muscles might have developed supersensitivity to the effects of whatever norepinephrine remained in the tissue. Either of these potential causes could be expected to mask the electrophysiological effects of acute myocardial denervation.

The first alternative was investigated by assaying endogenous norepinephrine content of the right ventricular walls from which the papillary muscles were removed. In six control animals, these assays measured mean ventricular wall norepinephrine levels of 2.33 ug/g wet weight of tissue (Table 6.3). The levels measured correspond closely to previous measurements of endogenous norepinephrine levels of guinea pig right ventricles (Angelakos *et al*, 1963, 2.21 ug/g W.W.T.; Spann *et al*, 1964, 2.15 ug/g W.W.T.; Muscholl, 1959, 1.57 ug/g W.W.T.). Muscles from ten animals pretreated with 6-OHDA (intraperitoneally) showed inconsistent electrophysiological results even though endogenous norepinephrine

levels in all ten were reduced by 73 to 96% in comparison to controls. No significant difference in the degree of depletion was observed between the two groups of pretreated muscles which exhibited differences in automaticity. It is concluded that the inconsistent electrophysiological results observed are not due to differing degrees of depletion of endogenous norepinephrine.

It two of the five pretreated muscles which exhibited "normal" automaticity, the effects of 10^{-6}M isoproterenol were examined. An extraordinarily large chronotropic effect was produced by this dose of isoproterenol (almost twice as great an increase in rate in comparison to the effects of 10^{-6}M isoproterenol in control muscles). Although complete dose-response curves should be made to establish the development of supersensitivity (Trendelenburg, 1963), these results suggest that those denervated muscles which demonstrated automaticity similar to controls, probably developed supersensitivity. This was most likely post-junctional supersensitivity, since isoproterenol is not taken up into adrenergic neurons by the specific membrane pump reuptake system (Hertting, 1964).

Supersensitivity has been observed to develop as a function of the time after denervation (Fleming et al, 1973). It is possible that the inconsistency of electrophysiological responses observed in the pretreated muscles was the result of variability in the development of supersensitivity in the muscles due to variability in the time course of depletion. It is reasonable to conclude that a considerable variability in the time course of depletion could result from differences in absorption, distribution, and

metabolism of intraperitoneally administered 6-OHDA in different animals. I, therefore, decided to use a route of administration in which the time course of depletion would be less subject to differences in absorption and distribution between animals.

Automaticity in all of the muscles from animals which received intravenous 6-OHDA was present only at MDPs more negative than -55 mV. Right ventricular norepinephrine levels were reduced by 95% in comparison to controls. The loss of automaticity at all potentials less negative than -55 mV precluded the measurement of most of the variables used to characterize automaticity over this range. However, in the least negative MDP decade in which automaticity occurred (-59 to -50 mV), there was a significant reduction in phase 4 slope in comparison to controls. The administration of 5×10^{-6}M norepinephrine restored automaticity at less negative MDPs and increased phase 4 slope over the decade of -59 to -50 mV back toward control levels. The rate (inter-spike interval) observed in the presence of 5×10^{-6}M norepinephrine was of similar magnitude to that observed in the presence of 5×10^{-6}M norepinephrine in control muscles (Section 4.4). It is concluded that these muscles, in which over 95% depletion of endogenous norepinephrine occurred, did not demonstrate any evidence of denervation supersensitivity. From these experiments, it can be concluded that removal of the influence of endogenous catecholamines results in loss of induced automaticity at all MDPs less negative than -55 mV. This was most likely caused by attenuation of the diastolic depolarization rate over these potentials. In muscles in which supersensitivity develops, this

phenomenon may not be observed since compensatory changes occur postsynaptically.

The loss of automaticity due to decreases in phase 4 slope can be attributed to the absence of endogenous norepinephrine, since exogenously administered norepinephrine reversed these effects. The phenomenon probably results from a beta-adrenolytic action, since in one experiment isoproterenol reversed the effects and phenylephrine up to 5 x 10^{-6}M was without effect. 6-OHDA pretreatment did not likely produce the electrophysiological effects through destruction of beta-adrenoceptors or beta-blockade, since a dose of norepinephrine close to the threshold dose for control muscles reversed the effects of norepinephrine depletion. It is possible that some of the other electrophysiological effects observed in pretreated muscles, e. g., reduced $\dot{V}_{MAX}$ of automatic action potentials at more negative MDPs, were independent of the depleting actions of 6-OHDA since norepinephrine did not restore these variables to control levels.

7.3 In Vitro Reserpine

Due to the inability to maintain an impalement after reserpine was added directly to the bath, only qualitative results were obtained. New impalements after reserpine was washed out and contractility had diminished revealed that automaticity over MDPs less negative than -55 mV was absent. Upon infusion with the beta-agonist, isoproterenol, automaticity was restored. After washout of the isoproterenol, automaticity was again absent over this range.

Brodie, Pletscher, and Shore (1955) were the first to discover the

amine-depleting action of reserpine. Kirshner (1962) and Carlsson *et al* (1962) formulated the hypothesis that reserpine inhibited the uptake of amines into storage vesicles by blocking a granular membrane transport pump. It is now recognized that reserpine in addition to depleting noradrenergic neurons also depletes dopaminergic and serotonergic neurons (Shore, 1972). Preliminary studies indicated that the depletion effects of reserpine outlasted the physical presence of the drug (Hess *et al*, 1956) which led to the well known "hit and run" hypothesis of reserpine action. More recent studies, however, have indicated that persistent actions of reserpine may be due to the irreversible binding of minute quantities of the drug to specific sites on amine storage vesicles (Giachetti and Shore, 1978).

The initial positive inotropic effects immediately after reserpine was added to the bath can be attributed to norepinephrine released by the depleting action of reserpine. Similar effects were observed in intact animal preparations immediately after reserpine administration, *e. g.*, tachycardia (Plummer *et al*, 1954), rises in blood pressure (Domino and Rech, 1957). The subsequent decline in contractility may reflect a diminishing concentration of endogenously released norepinephrine as the depletion progresses. However, Kirshner (1962) and von Euler (1966) found that reserpine also blocks the conversion of dopamine to norepinephrine. Therefore, the subsequent fall in contractility may reflect lowered norepinephrine levels as a result of impaired synthesis rather than or, in addition to, depletion.

However, it is also possible that the loss of automaticity at less negative MDPs results not from depletion of nor interference with the biosynthesis of catecholamines but from some non-specific myocardial depressant effects of reserpine. Some studies (Schwartz and Lee, 1960; Nayler, 1963; Wilcken et al, 1967) have reported myocardial depression and uncoupling of oxidative phosphorylation in heart mitochondria after in vivo pretreatment for several days. Others (Spann et al, 1966; Blinks and Waud, 1961) have been unable to document these effects. Such changes may actually occur after prolonged treatment but could be due to the loss of myocardial catecholamines rather than a non-specific effect of reserpine, since catecholamines are known to exert a variety of metabolic effects on cardiac muscle (see Himms-Hagen, 1972). It is interesting how closely automaticity after the action of reserpine resembles that in muscles from animals depleted of endogenous norepinephrine with 6-hydroxydopamine (compare Figures 6-5 and 6-9). The ability of in vitro reserpine to arrest spontaneously beating isolated sino-atrial node preparations has been previously interpreted as evidence that the presence of catecholamines is required for pacemaker activity in this tissue (Tuganowski et al, 1973).

7.4 Beta-Antagonists

Agents which block the effects of catecholamines at the receptor theoretically provide a much more sensitive and specific tool for the study of the effects of adrenergic deprevation on cardiac electrical activity than that provided by reserpine (Hoffman and Singer, 1967). The use of beta-antagonist agents to study the effects of adrenergic deprivation would also be expected to

circumvent problems which arise from compensatory mechanisms which develop following denervation by surgical or chemical techniques. However, studies on the mechanism of action of various beta-antagonist agents on cellular electrophysiology have been complicated by the finding that many of these in addition to possessing beta-blocking properties, also exert direct membrane effects (Wit, Hoffman, and Rosen, 1975).

Direct effects of beta-antagonists are described as local anesthetic effects or as "quinidine-like" (Abrams and Davies, 1973). Local anesthetic effects are evaluated by measuring the potency of a beta-antagonist in comparison to the potency of procaine to block excitability and/or conduction in a variety of nerve preparations (intradermal wheal method or phrenic nerve diaphram, Davis, 1970). Vaughan Williams (1966) was among the first to describe the direct membrane effects of beta-antagonists as "quinidine-like". It was found that the beta-antagonists, pronethalol and propranolol, shared the following myocardial properties with quinidine: (i) did not alter the resting potential, and (ii) greatly reduced the height and rate of rise ($\dot{V}_{MAX}$) of the action potential. Weidmann (1955b) demonstrated that $\dot{V}_{MAX}$ of phase 0 of the action potential is an index of the peak inward sodium current in cardiac tissue and this concept has been validated by other studies (Brady and Woodbury, 1960; Hondeghem, 1978). Therefore the ability of beta-antagonists to reduce $\dot{V}_{MAX}$ of phase 0 of the action potential is generally interpreted as due to interference with the inward sodium conductance. Other actions of beta-antagonists which have been found to be "quinidine-like" include reduced maximum

following frequency (Sekiya and Vaughan Williams, 1963) and negative inotropic effects (Levy, 1968).

A considerable number of agents with beta-antagonist properties have been synthesized and these agents vary considerably in beta-antagonist potency and specificity and in direct membrane properties. Abrams and Davies (1973) have compared beta-blocking potency, membrane activity, intrinsic sympathomimetic activity and tissue selectivity of a number of these agents. Pronethalol, propranolol, alprenolol, and dichloroisoproterenol (DCI) are all potent beta-antagonists possessing direct membrane effects. A few newer agents, such as practolol, sotolol, and butoxamine are potent beta-antagonists believed to possess minimal direct membrane properties (Abrams and Davies, 1973). Some of these agents however, possess some intrinsic sympathomimetic activity.

In order to evaluate the effects of adrenergic deprivation upon electrically induced ventricular automaticity using beta-antagonists, it would be useful to use an agent which is a potent beta-antagonist for myocardial beta-adrenoceptors and which is devoid of direct membrane effects. A relatively new beta-antagonist, metoprolol (H93/26), was reported to be the first "cardioselective" beta-antagonist without appreciable intrinsic sympathomimetic activity or direct membrane effects (Åblad *et al*, 1973; Åblad *et al*, 1975). This was the rationale for evaluating the effects of metoprolol upon electrically induced ventricular automaticity in the present study.

At 5×10^{-6}M, metoprolol produced a slight decrease in $\dot{V}_{MAX}$ and

overshoot of automatic action potentials arising from more negative MDPs, and a decrease in overshoot and phase 4 slope of automatic action potentials arising from less negative MDPs. At 5 x 10^{-5}M, the primary effect of metoprolol was a further reduction in overshoot and $\dot{V}_{MAX}$ of automatic action potentials arising from more negative MDPs and attenuation of automaticity over less negative MDPs. The decrease in $\dot{V}_{MAX}$ of automatic action potentials over more negative MDPs suggests interference with the rapid inward sodium current, since previous results have indicated that this current is the primary charge carrier underlying regenerative activity over this potential range (Katzung, 1974a; 1975). Such an effect is probably similar to the direct membrane effects which have been observed to occur with other beta-antagonists. However there were minimal effects of metoprolol upon $\dot{V}_{MAX}$ of the action potential elicited by a brief stimulus. This might appear inconsistent with a local anesthetic effect. It has been shown in the case of quinidine and lidocaine, two antiarrhythmic agents with potent local anesthetic properties that the decrease in $\dot{V}_{MAX}$ is enhanced in depolarized cardiac cells, in comparison to cells with normal resting potentials(Hondeghem et al, 1974; Watanabe and Dreifus, 1967; and Chen et al, 1975). Hondeghem and Katzung (1977; 1978) have recently proposed a molecular model of the interaction of antiarrhythmic agents with cardiac sodium channels which accounts for both time and voltage dependent effects. Enhanced depression of $\dot{V}_{MAX}$ in depolarized cells by various drugs with local anesthetic properties can be accounted for by the preferential binding of these agents to sodium channels in the inactivated state. The potential-dependent effects of metoprolol on

$\dot{V}_{MAX}$ are therefore consistent with a direct membrane effect of this agent.

The depressant effects of metoprolol upon automaticity arising from less negative MDPs, resembles the results observed in muscles pretreated with 6-hydroxydopamine or after in vitro reserpine. However, the evidence for a direct membrane effect of metoprolol in these experiments raised the possibility that the depressed automaticity at less negative MDPs by metoprolol is a manifestation of this direct membrane effect, rather than a beta-adrenolytic action.

In 1963, Howe reported that the l (-)-isomer of pronethalol was approximately 40 times more potent in beta-blocking activity than the d (+)-isomer. A similar evaluation of the optical isomers of propranolol, which is about ten times more potent than pronethalol in blocking beta-adrenoceptors, was made by Howe and Shanks (1966). l (-)-propranolol was found to be about 60 times more potent than d (+)-propranolol in preventing isoproterenol induced tachycardias in anesthetized cats. Since Ariens (1967) had demonstrated that local anesthetics are not usually stereospecific, these studies suggested that beta-antagonist properties of beta-antagonists might be separated experimentally from direct membrane properties by comparing the potencies of optical isomers of these drugs. Barrett and Cullum (1968) further evaluated differences in potency of the optical isomers of propranolol. They verified that l (-)-propranolol was approximately 100 times more potent in blocking isoproterenol induced positive inotropic and chronotropic effects in anesthetized rats but that the isomers were equipotent in producing negative

inotropic effects in isolated guinea pig atria and local anesthetic effects, measured on an isolated frog nerve preparation. By comparing potencies of isomers of beta-antagonists, these investigators and others (Lucchesi, 1965) attributed the blockade of ouabain induced arrhythmias by beta-antagonists to direct membrane properties. It seemed feasible to use a similar approach in order to distinguish the effects of beta-blockade from direct membrane effects exhibited by beta-antagonists (metoprolol) on electrically induced ventricular automaticity.

My experiments with l-propranolol showed that in low concentrations this agent preferentially affected automaticity arising from less negative MDPs. 2×10^{-7}M l-propranolol slowed automaticity by decreasing the diastolic depolarization rate, whereas 3×10^{-7}M l-propranolol abolished automaticity at all MDPs less negative than -50 mV. There were no significant effects on automaticity at more negative MDPs or upon the action potential elicited by a brief stimulus at these concentrations of l-propranolol. Experiments with 3×10^{-7}M d-propranolol showed no significant effects at any MDP upon automaticity, any of the variables used to characterize automaticity, or upon the action potential elicited by a brief stimulus. The differential potency exhibited by the two isomers of propranolol strongly suggest that the effects of the l-isomer are attributable to beta-blocking properties of the drug. This conclusion is further supported by (i) the observation that the suppressive effects of 3×10^{-7}M l-propranolol upon automaticity over less negative MDPs required a dose of norepinephrine, approximately 90 times higher than the threshold dose to produce a chronotropic effect in

control muscles, to restore automaticity, and (ii) the absence of any effect upon $\dot{V}_{MAX}$ at any MDP or upon $\dot{V}_{MAX}$ of action potentials elicited by a brief stimulus, by either isomer at concentrations up to 3×10^{-7}M.

In one experiment with d-propranolol, successive doses up to 6×10^{-6}M were evaluated. This experiment indicated that at 3×10^{-6}M, d-propranolol produced a noticeable reduction in phase 4 slope for automaticity arising from less negative MDPs. At 6×10^{-6}M, d-propranolol abolished automaticity at less negative MDPs and simultaneously reduced $\dot{V}_{MAX}$ of automatic action potentials at more negative MDPs and $\dot{V}_{MAX}$ of the action potential elicited by a brief stimulus. It can be tentatively concluded that the l-isomer is approximately 20 times more potent in blocking beta-adrenoceptors than the d-isomer in this preparation. With regard to direct membrane effects, other investigations have evaluated propranolol. Davis and Tempte (1968) observed a significant depression of membrane responsiveness by racemic propranolol in Purkinje fibers and ventricular myocardial cells at concentrations of about 10^{-5}M and higher. Coltart and Meldrum (1971) found 3×10^{-5}M racemic propranolol to be the lowest concentration to cause a depression of V_{MAX} of human and canine ventricular myocardial cells. Pruett *et al* (1977) observed a depression of $\dot{V}_{MAX}$ by racemic propranolol in canine Purkinje fibers at concentrations as low as 3×10^{-6}M. The observed depression of $\dot{V}_{MAX}$ by concentrations of d-propranolol of 6×10^{-6}M or higher are therefore within the concentration ranges previously reported to produce local anesthetic effects. The fact that 6×10^{-6}M d-propranolol depressed $\dot{V}_{MAX}$ and abolished

automaticity over less negative MDPs, certainly illustrates how beta-blocking properties and direct membrane effects of beta-antagonists could be difficult to distinguish, if only racemic mixtures of beta-antagonists are studied. The beta-blocking effects produced by l-propranolol in these experiments were at concentrations approximately 20 times lower than concentrations of racemic propranolol which have been shown to produce direct membrane effects in similar cardiac preparations.

Other evidence has recently become available which supports the conclusion that the ability of l-propranolol to attenuate automaticity over less negative MDPs is due to beta-blockade, rather than direct membrane effects. Automaticity over less negative MDPs can be considered to be a form of the "slow response" (Beeler and Reuter, 1977), and a few recent studies have examined the effects of known local anesthetic agents upon calcium-dependent action potentials. Josephson and Sperelakis (1976) examined the effects of lidocaine, procaine, and cocaine on slow response action potentials induced by isoproterenol in intact embryonic chick hearts and reaggregated cell cultures. All three agents blocked the induced slow responses at 10^{-3}M or higher. For lidocaine, this concentration is approximately 100 times higher than threaputic blood levels reached *in vivo* (6.0 ug/ml $\approx$ 1.2 x 10^{-5}M; Collingsworth *et al*, 1974), and thus probably represents a toxic effect rather than a pharmacological effect. Katzung (1974a) found that 5 ug/ml of lidocaine abolished electrically induced automaticity over more negative MDPs without appreciably affecting automaticity over less negative MDPs in guinea pig papillary muscles. Imanishi *et al*

(1978) in a similar preparation, found that lidocaine up to 16 ug/ml had no effect upon automaticity arising from less negative MDPs. Brennan, Cranefield, and Wit (1978) concluded that lidocaine up to 20 mg/l had no significant effect upon Purkinje fiber slow responses and Kohlhardt et al (1972) have shown that lidocaine concentrations as high as 50 mg/l have no apparent effects upon the slow inward current in cat ventricular myocardium. All these data support the conclusion that local anesthetics, at concentrations which clearly depress fast response action potentials (by interfering with inward sodium conductance), have relatively little effect upon slow inward current channels.

7.5 Conclusions Regarding the Influence of Endogenous Catecholamines on Depolarization Induced Ventricular Automaticity

In order to establish that endogenous catecholamines modulate electrically induced ventricular automaticity, the following pharmacological criteria should be fulfilled:

1. the demonstration that endogenous catecholamines are present in the tissue under in vitro conditions,
2. the demonstration that endogenous catecholamines can be released under in vitro conditions,
3. the demonstration that endogenous catecholamines are capable of influencing the phenomenon being studied,
4. that different pharmacological means of producing depletion of endogenous sympathetic neurotransmitters should all produce similar effects upon the phenomenon being studied, and should be effects on the same variables but in the opposite direction of those produced by added exogenous catecholamines,
5. that the effects of adrenergic deprivation should be

reversed by exogenous catecholamines at concentrations appropriate to the conditions used to produce adrenergic deprivation (e.g., threshold doses for reversing the effects of amine depletion and higher than threshold doses to reverse the effects of adrenoceptor blockade).

In this study, the first three criteria were fulfilled by the experiments with tyramine and the biochemical assays of endogenous catecholamine levels. Tyramine exerted its positive chronotropic effects through an indirect mechanism: via release of endogenous catecholamines. The last two criteria were fulfilled by three different pharmacological approaches used to eliminate the influence of endogenous catecholamines. Myocardial cells in papillary muscles which were chemically denervated by pretreatment with 6-hydroxydopamine and by in vitro reserpine, or which were under the influence of beta-antagonists, did not exhibit automaticity over less negative maximum diastolic potentials. The loss of automaticity over this potential range in the case of 6-hydroxydopamine pretreatment and in the presence of beta-antagonists was found to be due primarily to a reduction in the diastolic depolarization rate. Exogenously applied norepinephrine restored automaticity over this potential range primarily by enhancing the diastolic depolarization rate. In the case of 6-hydroxydopamine pretreatment and in vitro reserpine, beta-agonists restored automaticity at concentrations which were threshold for producing chronotropic effects in control muscles. In the case of beta-antagonists, a concentration of norepinephrine approximately 90 times in excess of a threshold dose was required to restore

automaticity over less negative potentials. Although it is possible that all three pharmacological interventions which eliminated automaticity over less negative potentials were mediated by non-specific effects of these drugs, such a possibility is considered remote. It is concluded that the beta-adrenoceptor under the influence of endogenous or exogenous catecholamines controls the development of the pacemaker potential during the application of depolarizing currents over a maximum diastolic potential range of approximately -55 to -30 mV.

It was previously shown that isoproterenol accelerated the diastolic depolarization rate over less negative potentials, which resulted in an enhanced rate of automaticity. A possible mechanism for this was considered to be enhanced slow inward current. The three interventions which eliminated the influence of endogenous catecholamines, reduced the diastolic depolarization rate, which could be due to a diminution of the slow inward current. Reuter and Scholz (1977) addressed the question of whether the slow inward current can be measured by voltage clamp techniques in the complete absence of endogenous or exogenous catecholamines. They found that the slow inward current could still be measured in the presence of high concentrations of beta-antagonists or in muscles pretreated with reserpine. Since the pacemaker potential is a dynamic balance of inward and outward currents, the slightest alteration of either could result in initiation and acceleration of automaticity or, conversely the attenuation of automaticity. It may be that beta-adrenoceptor regulation of ventricular automaticity at potentials less negative than -55 mV is mediated by regulation of the magnitude

of the slow inward current, and that below a certain level automaticity ceases but plateau generation and excitation-contraction coupling continue.

Postganglionic Sympathetic Nerve Terminals as a Possible Site of Endogenous Catecholamine Regulation of Electrically Induced Ventricular Automaticity

The nature of chemical neurotransmission at sympathetic neuroeffector junctions is less well understood than the processes which have been elucidated at the neuromuscular junction. Based upon morphology and light microscopic studies, Hillarp (1959, 1960) proposed the concept of the "autonomic ground plexus" to describe the innervation apparatus of smooth muscle. Postganglionic axons were believed to ramify into smaller processes which enter the plexus, where they become preterminal axons which give rise to fine anatomosing varicose fibers. Abundant varicose processes comprise the major portion of the autonomic ground plexus, from which one to several fibers appear to run together for variable distances to form an anastomosing network in close proximity to the muscle. The varicosities are believed to contain high concentrations of the neurotransmitter and appear to occur at irregular intervals in most fibers (Jacobowitz, 1974). This pattern of innervation of smooth muscle is quite diffuse in comparison to the innervation pattern of skeletal muscle, in which each effector (end plate) unit is directly innervated. Burnstock and Iwayama (1971) proposed a model of the sympathetic neuromuscular junction in which (i) transmitter is released "en passage" from large numbers of terminal varicosities, (ii) the effector is a muscle bundle and individual cells are connected by low resistance pathways (nexuses) which allow electro-

tonic spread of activity, and (iii) some muscles are directly innervated, some directly coupled, and some indirectly coupled.

In the heart, a large number of light microscopic studies have revealed the presence of extensive nerve plexuses within the myocardium (Hirsch, 1971). However the maximum resolution of light microscopy did not permit definition of precise neuromuscular relationships, or even close contacts between nerve processes and the surfaces of individual myocardial cells. Within the last 15 years, electron micrographs and new fluorescent techniques for histochemical identification of nerve processes in the heart have supported the "autonomic ground plexus" hypothesis advanced by Hillarp. Some studies (Thaemert, 1969; Yamauchi and Burnstock, 1968) have found the existence of close contacts of 60-200 A between nerve varicosities and individual myocardial cells in most regions of the heart, except in the apex. Several good reviews (Yamauchi, 1969; Yamauchi, 1973; Schiebler and Winckler, 1971) deal with the most recent literature on the fine structural innervation of the vertebrate heart. It would appear that there is sufficient evidence supporting the "autonomic ground plexus" hypothesis in the heart, and such a structure could conceivably be the source of endogenous catecholamines which appear to modulate electrically induced ventricular automaticity.

An important question related to endogenous neurotransmitter regulation of electrically induced ventricular automaticity is whether catecholamine release is elicited by the applied depolarizing current or whether there is a constant tonic influence of the sympathetic terminals upon individual myocardial cells. At this time

there is insufficient evidence supporting quantal release of norepinephrine at sympathetic nerve terminals; however it is generally believed that the processes of norepinephrine release at sympathetic nerve terminals are similar to those at the motor nerve terminal (Smith, 1973; Rolett, 1974). In support of this, Haeusler et al (1968) have shown that KCl depolarization of adrenergic nerve terminals in the isolated perfused cat heart liberates norepinephrine even in the presence of tetrodotoxin. This shows that it is depolarization per se and calcium which is required for transmitter release and invasion of the terminal by an action potential is not required (Haefely, 1972). It is quite possible that the application of constant current to induce automaticity might elicit release of norepinephrine by a similar depolarization of sympathetic terminals within the papillary muscle. Haeusler et al could measure norepinephrine released at a KCl concentration of 50 mM, which would roughly correspond to a depolarization to -30 mV in their preparation. This is certainly near the range of induced automaticity which appeared dependent upon the presence of endogenous catecholamines in this study. Muscholl et al (1975) have found that high KCl solutions induce a release of norepinephrine from perfused rabbit hearts which declines exponentially in two phases with half times of 1.59 and 16.9 minutes. Thus norepinephrine release by electrical depolarization could theoretically occur during the entire time course of the 1.5 second constant current pulses used in this study.

Alternatively, a tonic influence of sympathetic neurotransmitters upon cardiac musculature has been recently suggested by several studies. Loffelholz and Muscholl (1969), Starke and Montel (1974),

and Gothert et al (1976) have measured spontaneous efflux of norepinephrine from perfused rabbit hearts in the absence of electrical stimulation or depolarization. In addition, beta-antagonists have been shown to depress basal adenyl cyclase activity in membrane particles obtained from kitten ventricular tissue (Kaumann and Birnbaumer, 1974) and in broken cell preparations from guinea pig ventricular tissue (Krawietz et al, 1976). These authors concluded that an appreciable fraction of basal adenyl cyclase activity in these preparations was due to the presence of endogenous norepinephrine occupying beta-adrenoceptors because the beta-antagonist induced depression was stereospecific ((-) isomers 50 times more potent than (+) isomers) and absent in preparations from animals pretreated with reserpine. Spontaneous release of norepinephrine from sympathetic terminals thus might be homologous to that at the neuromuscular junction, where random release or leakage of acetylcholine produces spontaneous miniature end plate potentials (Katz, 1971; Katz and Miledi, 1977).

8.0 PHYSIOLOGICAL SIGNIFICANCE OF ELECTRICALLY INDUCED VENTRICULAR AUTOMATICITY AND REGULATION BY SYMPATHOMIMETIC AMINES

Evidence from a variety of sources is beginning to focus attention on ventricular myocardium as the site of arrhythmogenesis, especially of the early "lethal" arrhythmias which often accompany myocardial infarction (Wit and Bigger, 1975). Ischemic myocardial cells are unable to maintain normal intracellular ionic concentrations and potassium has been shown to be released into the extracellular space (Jennings et al, 1963) causing depolarization of injured cells. Boundary potentials between depolarized and normal tissue might give rise to internally generated "currents of injury". It has been demonstrated by Cohen and Kaufman (1975) that currents of injury flow during most of the cardiac cycle following acute myocardial experimental ischemia in the in situ heart. Several studies have demonstrated that such internally generated currents of injury are capable of inducing repetitive activity in nearby healthy myocardium. Mechanical or laser-induced injury to Purkinje fibers was shown by Délèze (1970) to result in initiation of repetitive activity in nearby healthy tissue. Ten Eich, Singer, and Solberg (1976) found that myocardial cells in depressed papillary muscles from dogs with acute myocardial infarctions underwent slow diastolic depolarization and repetitive firing. The application of depolarizing currents from an external source to healthy myocardial cells might therefore represent a good model for studying automaticity generated by internal currents of injury,

arising subsequent to myocardial damage. Katzung, Hondeghem, and Grant (1975) demonstrated the initiation of repetitive activity in ventricular myocardial cells of guinea pig papillary muscles in a sucrose gap chamber by an experimentally induced internal current of injury. Without any external current source the perfusion of the center chamber with isotonic potassium Tyrode solution, which depolarized cells in that chamber, produced repetitive activity in myocardial cells in the test chamber, presumably induced by the flow of current from depolarized cells to healthy cells in the test chamber.

Cardiac arrhythmias are believed to result from disorders of impulse initiation (automaticity), impulse conduction, or combinations of the two (Rosen, 1977). It is generally agreed that two distinct inward currents contribute to the action potential in most cardiac preparations (Reuter, 1973; Coraboeuf, 1978): a fast inward sodium current and a slow inward calcium/sodium current. Regenerative inward currents underlying induced automaticity in ventricular myocardial cells appear to involve these same inward currents (Katzung, 1975), the activation ranges of which are different but overlapping. Automaticity at more negative potentials exhibits a rapid upstroke velocity and a large overshoot, both of which contribute to fast conduction. Therefore automaticity occurring over more negative potentials might contribute to the development of clinical arrhythmias as a result of disorders of impulse initiation (automaticity), but would be less likely to be involved in reentry arrhythmias. Induced automaticity over less negative potentials appears similar to the slow response described

by Cranefield, Wit, and Hoffman (1972) and is characterized by a relatively slow upstroke velocity and overshoot, both conductive to slow conduction. Slow conduction and a low safety factor make conduction of slow response action potentials prone to block especially at branching points in the conduction pathway (Wit, Rosen, and Hoffman, 1974). Thus induced automaticity over less negative potentials may be a contributory cause in the development of reentry arrhythmias (Bailey et al, 1978). The existence of two distinct inward regenerative currents underlying electrically induced ventricular automaticity and cardiac excitability in general suggests that differential sensitivities to antiarrhythmic agents might occur as a function of which particular inward current generates a disturbance, and hence the particular level of maximum diastolic potential. Agents which preferentially interfere with the rapid inward sodium conductance (lidocaine, quinidine, etc.) would be expected to be particularly beneficial in cases where the disturbance arises from more negative potentials. For disturbances arising from less negative potentials in which sodium current is inactivated, agents like verapamil, D-600 or nifedipine would be expected to be particularly beneficial. The results of this study indicate that an adrenolytic intervention, like beta-antagonists, might also be beneficial at this range of potentials.

Another important precipitating factor in the genesis of arrhythmias accompanying myocardial infarction and ischemia is believed to be the release of catecholamines from ischemic myocardium (Griffiths and Leung, 1971; Wit and Freidman, 1975; Corr and Gillis, 1978). Experiments which examined the effects of catecholamines upon

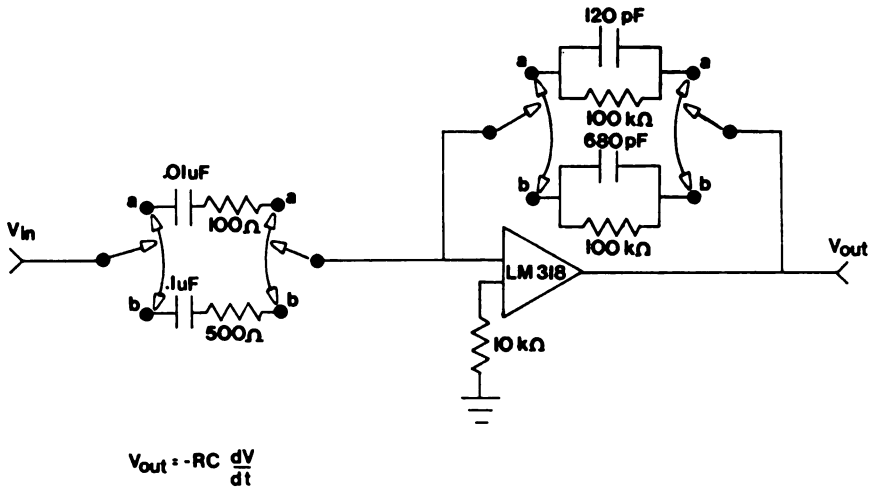
electrically induced ventricular automaticity in the present study suggest that such arrhythmias may be profoundly influenced by catecholamines and that disturbances which involve slow channel activation, may in fact be dependent upon endogenous catecholamines.

The results of this study which implicate endogenous catecholamines in the initiation and maintenance of slow channel-dependent automaticity induced by depolarizing current might lead to the more general question of the involvement of endogenous catecholamines in the initiation and maintenance of all forms of slow response electrical activity in the heart. Three experimental manipulations in addition to electrical depolarization have been used to eliminate the fast inward current in order to study slow responses in fibers which normally only show fast responses. These manipulations include the use of sodium-free medium (Cranefield, Wit, and Hoffman, 1972; Tritthardt et al, 1973; Aronson and Cranefield, 1973), elevated potassium (Vassort et al, 1969; Engstfeld et al, 1961; Pappano, 1970; Shigenobu et al, 1972), or tetrodotoxin (Shigenobu et al, 1972). Under these conditions, a strong electrical stimulus will elicit slowly rising action potentials which appear to be graded in nature and depend upon the size and duration of the stimulus. This dependence of the slow response upon the size and duration of electrical stimulus parameters is similar to the variety of electrorelease phenomena described in Section 1.4.2. Because of the variability in the induction of these slow responses by electric current, many investigators routinely add catecholamines, methyl xanthines, or other agents known to enhance the slow inward current to initiate or facilitate the production of slow responses.

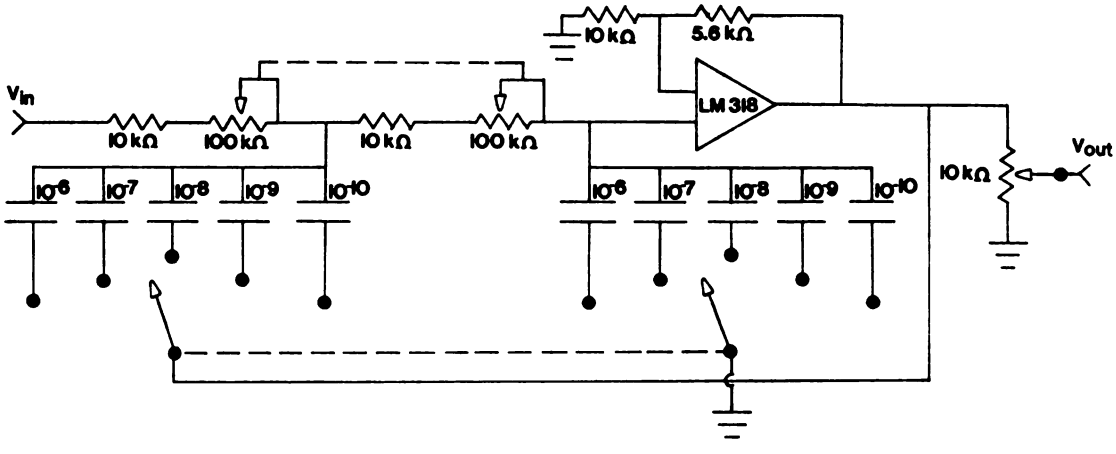
To my knowledge no studies have yet been attempted to determine the effects of beta-adrenolytic agents upon these forms of slow response activity in the absence of added exogenous catecholamines.

The slow response is believed to be characteristic of normal electrical activity of the sinus and atrioventricular nodes, and fibers of the mitral and tricuspid valves (Wit, Rosen, and Hoffman, 1974). Voltage clamp studies of the rabbit sinus node (Noma et al, 1977) and frog sinus venosus (Brown, Giles, and Noble, 1977; 1978) have identified a slow inward current which is activated during spontaneous activity. A recent hypothesis by Pollack (1977) concerning the initiation of spontaneous activity in cardiac tissue postulates (i) an obligatory role for endogenous catecholamines, and (ii) recurrent changes in intracellular levels of cAMP. Most of the evidence presented in support of this hypothesis concerns cardiac preparations exhibiting slow-channel dependent automaticity. The experiments carried out to determine the influence of endogenous catecholamines upon electrically induced ventricular automaticity in this study support the hypothesis proposed by Pollack, with regard to slow-channel dependent automaticity. However the persistence of automaticity at more negative potentials in muscles depleted of endogenous catecholamines or in the presence of beta-antagonists, clearly contradicts the hypothesis that all cardiac pacemaker activity is dependent upon endogenous catecholamines. The question of whether all slow-channel dependent electrical activity (including pacemaker activity) in the heart is dependent upon occupation of the beta-adrenoceptor is open for future investigation.

Appendix I:

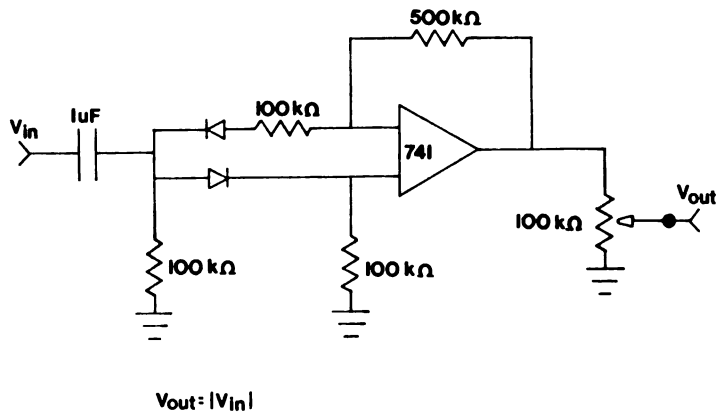


Differentiator Circuit



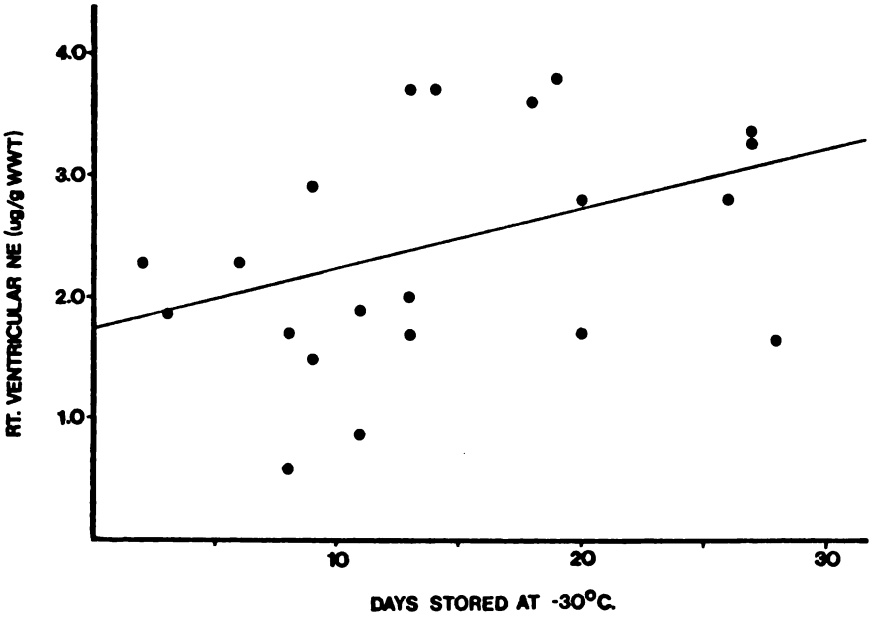
Filter Circuit

Appendix I:



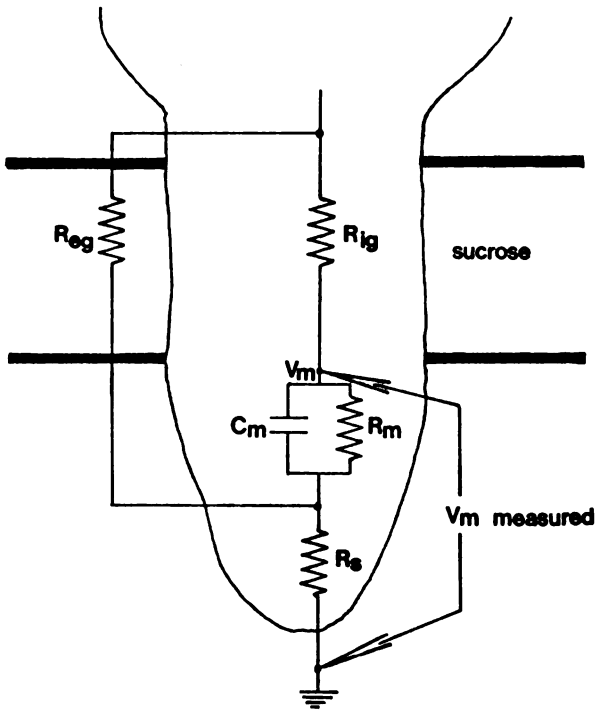
Absolute Amplifier Beam Intensifier Circuit

Appendix II:



Relationship between time of storage of right ventricles and the absolute amounts of norepinephrine measured. The line was fitted to the data using the equation $y = 1.71 + .047x$. The slope of the line was not significantly different than 0.

Appendix III:



C_M = Membrane capacitance
R_M = Membrane resistance
R_s = Series resistance
Reg = Extracellular longitudinal resistance
Rig = Intracellular longitudinal resistance
V_M = Intracellular membrane potential

Equivalent Circuit of Single Sucrose Gap

During the passage of constant current; part of the current traverses across an extracellular longitudinal pathway (across Reg) and part of the current traverses across an intracellular longitudinal pathway (across Rig). The proportion of total current traversing along these pathways is determined by the ratio of Rig to Reg.

Δ Vmeasured = Iig x R_M + I_{total} x R_s

= V_M + V_{Rs}

V_{Rs} = voltage error component in Vmeasured due to IR drop across R_s

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