EFFECTS OF LIDOCAINE AND CALCIUM ION ON THE RABBIT ATRIUM

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ABSTRACT

The effects of lidocaine: 1 x 10^{-5}M; 5 x 10^{-5}M and calcium: 1.25; 2.5 and 5.0 mM on the electrophysiological parameters of the action potential, were studied in isolated superfused rabbit atria using standard microelectrode technique.

The electrophysiological parameters studied included: membrane resting potential (M.R.P); overshoot (O.S.); action potential amplitude (A.P.); $\dot{V}$max; action potential duration (A.P.D.); membrane voltage - $\dot{V}$max relationships; the time constant (T) of recovery from inactivation of the sodium channels, and the effective refractory period (E.R.P.).

Lidocaine, 1 x 10^{-5}M, when studied in atria superfused with Krebs with 2.5 mM $CaCl_2$ did not modify significantly any of the electrophysiological parameters in consideration, except for a significant prolongation of T. At a higher concentration (5 x 10^{-5}M), the drug significantly decreased the rate of beating, O.S., and $\dot{V}$max in the right atria, but without significant changes in M.R.P. or A.P.D.

In the driven left atria, $\dot{V}$max was significantly greater at lower frequency of stimulation, and the effects of the higher dose of lidocaine were similar to those observed in the spontaneously beating preparations.

The steady-state membrane voltage-$\dot{V}$max relationship was shifted in a downward direction by lidocaine, 5 x 10^{-5}M, greater changes occurring at higher membrane potentials. The membrane voltage at which

Vmax was 50% was not modified by lidocaine. This effect is different from that observed in Purkinje fibers and ventricles. The lower dose of the drug did not shift this relationship.

The membrane responsiveness curve was also shifted in a downward direction only by the higher dose of lidocaine, and this effect was more noticeable at higher levels of membrane potentials. At lower membrane potentials, the relationship was shifted toward more negative potentials. The E.R.P. was significantly prolonged by the drug.

It was consistently observed that extrasystoles delivered at an appropriate time were able to elicit brief bursts of rhythmic activity. This dysrrhythmia was prevented by lidocaine, when used at 5×10^{-5}M.

In all the experiments, the recovery of $\dot{V}max$ was found to obey a monoexponential process, as is the case in Purkinje fibers and ventricles. τ was found to be 52 ms for a mean value of -71 mV M.R.P. Lidocaine at both concentrations significantly prolonged τ.

It was found that the duration of the premature action potentials was modified by the diastolic intervals. These changes were: 1) a significant prolongation of the overshoot, and 2) a significant shortening of A.P.D. at the 95% level as the diastolic intervals were decreased.

Lowering the external calcium concentration to 1.25 mM, caused a significant depolarization of the cells and a decrease in $\dot{V}max$. Only the overshoot was lengthened, and τ was significantly prolonged. The membrane responsiveness curve was shifted in a downward direction.

Increasing the external calcium concentration to 5.0 mM, significantly hyperpolarized the cells, increased the action potential amplitude and $\dot{V}max$, and had a dual effect upon the A.P.D., shortening it at the

zero potential and 50% levels, and lengthening it at the 95% level. τ was shortened, and the membrane responsiveness curve was shifted in an upward direction.

The recovery from inactivation of the sodium channels was a time, as well as a membrane potential dependent process. Lowering the external potassium concentration from 4.7 to 2.35 mM, significantly hyperpolarized the cells and shortened τ. The opposite effects were seen when $(KCl)_o$ was raised to 9.4 mM. Equivalent changes in the M.R.P. due to variations of the external calcium concentrations shortened τ more than variations in potassium.

The prolongation of the premature action potential duration at the zero potential level (overshoot) was less prominent at the higher calcium concentration, as the diastolic intervals approached lower values (<100ms). On the other hand, the shortening of the premature action potential duration at the 95% level was only significantly different in 5.0 mM $(Ca^{2+})_o$ and was shortened at relative long diastolic intervals (>500ms).

The effect of lidocaine, $1x10^{-5}$M on all the electrophysiological parameters studied was enhanced when the calcium concentration was 1.25 mM, and the drug was able to prevent the rhythmic activity evoked by premature stimuli. At an external calcium concentration of 5.0 mM, the effects of lidocaine, $5x10^{-5}$M, on $\dot{V}max$ and on the E.R.P. were significantly less prominent than those observed at 2.5 mM $(Ca^{2+})_o$. Lidocaine prevented the rhythmic activity only in two out of six preparations at the higher calcium concentration.

In summary, one can conclude: a) that lidocaine, within the accepted 'therapeutic' plasma concentration range of 1.2 to 5.0 x 10^{-5}M

and in the isolated superfused rabbit atria bathed with an external potassium concentration of 4.7 mM, which is close to that observed in humans, is able to prevent the supraventricular type of dysrrhythmia produced by premature extrasystoles; b) that the main effect of the drug in atrial tissue is upon the sodium channels, and mainly on the recovery process from inactivation of the channels; c) that the difference of effect of the drug on the supraventricular and ventricular type of dysrrhythmias is better explained on the basis of an interaction of lidocaine with the fast sodium channels, and not on the basis of changes in the conductance for K^+, as was suggested by Kabela (1973); d) that calcium ion is modifying the effect of lidocaine; e) that calcium ion, in atrial tissue, is interacting with the process responsible for the repolarization of the action potential; and f) that calcium ion is modifying T independently of its dependence upon variations of the membrane potential.

TO

THE MEDICAL STUDENTS OF

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INTRODUCTION

INTRODUCTION

CARDIAC ACTION POTENTIAL EFFECT OF EXTRASYSTOLES, CALCIUM ION AND LIDOCAINE ON ITS CHARACTERISTICS.

A. Cardiac Action Potential.

Studies of the electrical activity of the excitable tissues, have been done either with extracellular or with intracellular recording electrodes. Hodgkin and Huxley in 1939 using a fine microelectrode inserted inside a giant axon of squid, were able to measure the potential difference between the exterior and the interior of the nerve. Due to the anatomical differences between nerve and cardiac muscle, it was not until the development of the glass microelectrodes of Ling and Gerard in 1949 that the intracellular potentials of the heart were studied. The first report of action potentials in cardiac tissue was by Coraboeuf and Weidmann (1949) in Purkinje fibers. Woodbury and coworkers reported on the intracellular action potentials in the frog ventricle in 1950. Later, in 1951, Draper and Weidmann reported more studies on the action potential in Purkinje fibers.

These studies of the action potential in nerve and cardiac muscle up to 1949 and 1950 established the important concepts of the ionic hypothesis to explain the events in the action potential, emphasizing the specific changes of permeability for sodium and potassium ions. These ideas were proven and given quantitative basis by a new type of experimental procedure developed by Marmot, Cole, and by Hodgkin and Huxley, during the years 1947 to 1959. The method is known as the voltage clamp, and it means to control the voltage across the cell

membrane, allowing the measurement of the current produced through the membrane. With the aid of these two techniques, we understand the action potential as a phasic and repetitive electrical event, in which we can distinguish five phases. The upstroke or phase 0 corresponds to a sudden depolarization of the cell membrane, due to the changes in the transmembrane ionic currents. Inwards currents correspond to an entry of positive charges into the cell, and outward currents correspond to cations leaving the cell or anions entering the cell. When the sum of these currents equals zero, the cell is in the resting state. In the active state of the cardiac cell, the sum of inward currents is greater than the outward currents, and an action potential starts. In Purkinje fibers and in ventricles, sodium ion is responsible for the inward current, whereas in atrial cells, there is evidence that both sodium and calcium ions are responsible for the upstroke (Ruiz-Ceretti and Ponce-Zumino, (1976). The equilibrium between the inward and outward currents in the cell membrane, is broken due to changes in the conductance of the membrane. This change of conductance is voltage and time dependent and result from the opening of membrane channels which are controlled by gating mechanism. In other words, the configuration of the channels change due to the stimuli. Hille, 1978, has postulated the existence of "Voltage sensors", attached to or within the membrane, on which the electric field acts, and the electric work done on them is the energy injected into the gating process.

Our understanding of the behaviour of the sodium channels originated with the work of Hodgkin and Huxley (1952). They postulated

that each sodium channel of the squid axon membrane is controlled by two gates, a Na activation gate (m) and a Na inactivation gate (h). The activation gate is closed at the resting state of the membrane, and opens quickly after depolarization. The inactivation gate has slower kinetics than the activation gate, and has an opposite voltage dependence: it is open at the resting state of the membrane, and closes slowly during the depolarization. They also proposed a model in which three "m gating particles" in each sodium channel, during the depolarization process, must undergo independent but identical transitions to activate the channel, and at the same time one "h gating particle" undergoes a single transition to inactivate it. In this model the activation and inactivation gates can open and close regardless of the condition of the other gate. The mathematical statement for this model is: $g_{Na}=\bar{g}_{Na}m^3h$. Recent work done by Benzanilla and Armstrong (1977), has demonstrated that in squid axons treated with pronase, which selectively destroys inactivation, there was a lag of hundred microseconds in the onset of inactivation. They also demonstrated that previous hyperpolarization of the membrane up to 140mV, before depolarization, both the activation and inactivation are delayed suggesting that the channels must open before they can be inactivated. They proposed a model for the sodium channel which has voltage-dependent transitions between the closed and open states of the channels, and a voltage-independent transition between the open and the inactivated state. In cardiac tissue, the electrical activity of the Purkinje fibers, as well as the ventricle, have been reconstructed by computer, using the modified mathematical model proposed

by Hodgkin and Huxley, (McAllister, Nobel, and Tsien; 1975. Beeler and Reuter, 1977).

The first phase of the action potential is called Rapid Repolarization. This phase is pronounced in Purkinje fibers and is partly the result of inactivation of g_{Na}, and of another current. This current is an outward current and has been called the "chloride" current or ICl (Dudel et al 1967); the "positive dynamic" current (Peper and Trautwein, 1968) and most recently Iq_r (McAllister et al, 1975). It has been accepted since the work of Dudel and coworkers in 1967, that Iq_r is due to the inward movement of chloride ions and is largely responsible for the initial rapid phase of repolarization of the action potential. Recently, Kenyon and Gibbons, (1977) have reported results of experiments done in sheep Purkinje fibers, superfused with normal Tyrode and low-chloride solutions, and the adjustment necessary to keep the calcium activity equal to the control situation. They concluded that "if a time and voltage-dependent chloride current exists in sheep Purkinje fibers, it plays little role in generating phase 1 of the action potential".

Phase 2 of the action potential curresponds to the plateau. In contrast to the phase zero of the action potential, during the plateau phase, the conductance of the membrane is very low (Eyster and Gilson: 1947; Weidmann, 1951). There is evidence which supports the idea that the net currents involved during phase 2 are small, and result from the equilibrium of the inward and outward currents. The inward current which plays an important role during the plateau phase have

been identified as the "secondary current" I_{s1} (Johnson and Lieberman, 1971) and is mainly carried by calcium and sodium ions. (Reuter, 1968; Vitek and Trautwein, 1971). The outward currents are time dependent, and the potassium ion, perhaps together with other ions Na^+, plays the important role. These time dependent outward currents have been named i_x and because i_x can be separated into two first order mechanisms, it has been divided in I_{x1} and i_{x2} respectively. This time dependent current, together with the inactivation of the secondary current is responsible for the termination of the plateau. The relative importance of these two currents varies among the different parts of the heart. Thus, in ventricular muscle, the Ca^{2+} inactivation process is slow ($\tau \simeq$ 400 Ms. Beeler and Reuter, 1970). In the Purkinje fiber, the Ca^{2+} inactivation process is much faster (τ= 50 Ms; Vitek and Trautwein; 1971); and in the atrium is even faster (τ = 22 Ms; Rougier, et al; 1969). It is clear that in those action potentials of short duration (<300 Ms), as is the case of atrium, the inactivation of the secondary current is the important process to control the duration of the action potential; whereas in the action potential of longer duration, as in the case of Purkinje fibers, the activation of the potassium current is more important (Vassalle, 1966; McAllister, 1975).

Phase 3 of the action potential corresponds to the final repolarization of the action potential, and is a process which has been called regenerative with a threshold for all or nothing repolarization. (Vasalle, 1966; Noble, 1975). Besides the currents described in the

plateau phase, the repolarization process is thought to consist of a voltage dependent, virtually "all or none" K^+ current which has been designated iK_1. (McAllister and Nobel, 1966; McAllister, et al, 1975).

Phase 4 of the action potential is the pacemaker process. This process has been well studied in the Purkinje fibers. Essentially, it is due to the participation of two types of currents: a) The time independent background inward current, carried by Na^+ and Ca^{++} ions, and b) The time dependent deactivation of the potassium current iK_2 (Noble and Tsien, 1968; McAllister et al, 1975; Vassalle, 1966; Vassalle, 1977).

In the sinus node, the mechanism for the automaticity is believed to differ from Purkinje fibers. In this dominant pacemaker, the slope of the slow diastolic depolarization is steeper than in Purkinje fibers, and the upstroke of the action potential originates at more positive potentials. This higher level of firing has been explained by assuming that there is no iK_2 in the S-A node (Vassalle, 1978). Another possible explanation is the fact that in the S-A node the background sodium current is larger than in Purkinje fibers (Trautwein and Kassebaum, 1961). There is evidence which support the fact that the S-A potentials have little sensitivity for TTX (Lenfant, et al, 1968), but are sensitive to variation of calcium (Seinfen et al, 1964) and to compounds which interfere with the kinetics of the secondary current (Zipes and Fischer, 1974). If one accepts the assumption that iK_2 is absent in the S-A node, then it is possible to think of another type of current as being responsible for the

diastolic depolarization. Vassalle (1978) has pointed out the possibility that the current responsible for the pacemaker activity is the deactivation of the i_{x1}. This deactivation together with the sodium-calcium background current, would lead to a net inward current and therefore the diastolic depolarization. It is possible to conclude that the electrical phenomena of the cardiac tissue are essentially due to the operation of voltage and time dependent conductances of the membrane for several ions.

B. Membrane Voltage-$\dot{V}$max Relationships

1. Concept of $\dot{V}$max

In studies of the electrical events in excitable tissues, interest has been concentrated on the determination of $\dot{V}$max, and the relationship between $\dot{V}$max and the membrane potential. The term $\dot{V}$max refers to the maximum rate of change of the membrane potential during phase zero of the action potential. The measure and development of this concept started with the experiments of Hodgkin and Huxley, 1949, who postulated that the rate of rise of the action potential in nerve, is determined by the rate of entry of sodium, and that it was proportional to the external sodium concentration. They also established the quantitative basis of this concept and demonstrated that the maximum rate of rise of the action potential was proportional to the ionic current entering the membrane, carried by sodium ions. Brady and Woodbury in 1960 demonstrated in the frog ventricle that the overshoot of the membrane and the maximum rate of depolarization of the action potential, were proportional to the external sodium concentration. Weidmann (1955) also demonstrated that in the Purkinje fiber, "the rate of rise and the overshoot are indicative of the ability of the surface membrane to undergo an increase in the

sodium permeability. Recently, Ruiz-Ceretti and Ponce-Zumino (1976) demonstrated that the rate of rise of the upstroke in atrial cells, is dependent on both sodium and calcium ions.

The study of the relationship between the membrane potential and the conductance of the nerve membrane for sodium ion was given by Hodgkin and Huxley in 1952. They used the double step voltage clamp method and demonstrated that a steady depolarization of 10 mV reduced the sodium current, associated with a sudden depolarization of 45 mV, to about 60%; whereas, a rise of 10 mV increased the sodium current by about 50%. Another interesting result was the demonstration of the influence of the membrane potential on the steady level of inactivation of the sodium current. They demonstrated that even at the resting potential, the inactivation was about 40%. Weidmann (1955) demonstrated that in the calf and sheep Purkinje fiber, the membrane potential also plays an important role in the control of the conductance for sodium ions. He also demonstrated, using the double step voltage clamp method, the influence of the membrane potential on the inactivation-reactivation process of the sodium ions. This dependence of the sodium current upon the membrane potential can be seen in certain species like frog, cat, or rabbit. Woodbury et al, 1951, reported low values of the transmembrane potential (64.5 mV) in the frog ventricle and also reported low values of the maximum rate of depolarization, and its dependence on the temperature. Burger et al, 1953 also reported low values of the resting potential in the cat ventricle. They hyperpolarized the membrane with acetylcholine or carbamyl-

choline and observed an increase in the phase zero of the action potential. Further evidence supporting this relationship is the work done by Kreitner, 1975. He demonstrated that in the rabbit sino-atrial nodal cell, the value of the transmembrane potential is low (-33 mV) and consequently the rate of rise of phase zero is also low, and that hyperpolarization of the cell with carbamylcholine up to 40 mV, increased $\dot{V}max$ in those cells.

2. Steady-State Membrane Voltage-$\dot{V}max$ Relationship.

The relationship between membrane potential and sodium current found in nerve by Hodgkin and Huxley, and in Purkinje fibers by Weidmann, is S-shaped. I shall comment more about this relationship later. The advantage of the double step voltage clamp method is that it allowed the study of the response of the membrane to a second step voltage, previously conditioned by a first voltage step, in which its magnitude or/and its duration could be controlled. When the time of the conditioning voltage step was long enough to allow a maximum response, for each second step voltage, the voltage-current relationship is known as the steady-state relationship. Another approach to study the voltage-current relationship, or the voltage $\dot{V}max$ relationship with a minimum influence of the time factor, (steady-state), is done by depolarizing the cell membrane with KCl (Strauss and Bigger, 1972; Gettes and Reuter, 1974).

3. "Membrane Responsiveness Curve"

Another way of studying the relationship of voltage and $\dot{V}max$ is to determine the values of $\dot{V}max$ of an extra stimulus interpolated at intervals from an infinite time to the absolute refractory period of the fiber. The relationship obtained in this way is called the "membrane responsive-

ness curve" (Singer et al, 1967; Strauss et, 1972). Both the voltage and the time dependent factors which determine $\dot{V}max$ are present in the membrane responsiveness curve. Nevertheless, this is a quite accepted method of studying the voltage-$\dot{V}max$ relationship; it should be considered only as an approximation of the inward current, expressed as $\dot{V}max$, due to some errors of the method, (Fozzard, 1977) among which are: a) No uniformity of the membrane voltage, because conduction effects are unavoidable in a large piece of tissue, b) $\dot{V}max$ is not a good index of the maximal inward current because part of the current is used to excite the adjacent membrane passively, c) The recovery process is not instantaneous, but time is required for recovery at any given voltage, and as a consequence, the values of $\dot{V}max$ are less than those that would be found at any voltage level, if time for full recovery were allowed. Chen and Gettes (1976) have demonstrated in papillary muscle that the "membrane responsiveness curve" is rate-dependent. Pasmooij and coworkers (1976) also demonstrated this rate dependence in atrial cells. This rate dependence of the $\dot{V}max$-voltage relationship has been explained by these authors as a consequence of the time for the recovery from inactivation of the sodium system (Chen and Gettes, 1976), and as a possible role of an electrogenic sodium-potassium pump in the atrial cells (Pasmooij and coworkers, 1976).

C. Recovery Process of $\dot{V}max$.

Hodgkin and Huxley (1952) demonstrated that the recovery process of inactivation of the sodium system, was voltage and time dependent, and that the time constants of inactivation and of recovery from inactivation were short and of the same order of magnitude. Weidmann, in his work (1955) using the Purkinje fiber and the double step voltage clamp demon-

strated that the relationship between $\dot{V}max$ and voltage, was similar to that of nerve, and that the time constant for inactivation and recovery from inactivation, were similar (20 ms) when the membrane voltage was -90 mV. He also demonstrated that the recovery of $\dot{V}max$ from inactivation of the sodium system was a monoexponential function, and that the time constant of the process was voltage dependent being 1.4 ms when the membrane potential was held at -106 mV, 5.0 ms at -90 mV, and 10.5 ms at -80 mV. The evidence of the time factor in the recovery from inactivation of the sodium system was given by Strauss and Bigger, 1972 and was done in atrial cells. They demonstrated with the method of late interpolated extrasystoles, that even at completed repolarization of the membrane, the value of $\dot{V}max$ in the extra action potentials was dependent upon the diastolic intervals (the time between the completed repolarization of the basic action potential and the phase zero of the extra action potentials). Furthermore, they compared the "membrane responsiveness curve" and the $\dot{V}max$-voltage relationship obtained in the steady-state condition (depolarization with KCl), and found that at same levels of voltage, $\dot{V}max$ was less when studied in the membrane responsiveness curve, than in the steady-state condition. Later Gettes and Reuter (1974), demonstrated in Purkinje fibers and ventricular muscle fibers that recovery from inactivation of the sodium system is a voltage and time-dependent process, and that it is a slower process than that of inactivation. Therefore, we accept that the sodium carrying system in cardiac tissue undergoes an activation process during phase zero of the action potential, then an inactivation of the system occurs and

consequently a recovery from inactivation, which is a voltage and time dependent process. Strauss and Bigger, (1972) found that the recovery of $\mathring{V}max$ from inactivation was a monoexponential process, even though they did not calculate the time constant of the process. Hass et al (1971) using the voltage clamp technique in frog atria, demonstrated that the time constant for the recovery of $\mathring{V}max$ from inactivation was prolonged (>100 ms). In ventricle and Purkinje fibers, Gettes and Reuter found values of less than 20 ms at a resting potential of about -80 to -90 mv.

D. Effects of Extrasystoles on the shape and duration of the action potential.

Together with the changes in $\mathring{V}max$, changes in the configuration of the action potential also reflect the dynamic of the action potential. Changes in the shape of the action potential have been studied in two ways: a) changes in the heart rate. This can be done by changing the cycle time of stimulation in an electrically driven preparation. Every cycle length is studied after a period of quiescence. b) Intercalated extrasystoles. This is done by delivering an extra-stimulus after complete or partial repolarization, at different intervals of time. Trautwein et al, 1952 and 1954, demonstrated in two different species that the duration of the action potential was decreased by higher frequency of stimulation. Hoffman and Suckling (1954), demonstrated in the papillary muscle of the dog, that the influence of heart rate on the action potential was mainly in the duration of the plateau phase of the action potential. They found a

relationship between the heart rate and the duration of the plateau, from 60 to 300 beats per minute; the faster the rate, the shorter the duration. They also concluded that this linear relationship remains constant regardless of the method used (extrasystoles or sudden changes in cycle length). Another observation was that the resting potential and the overshoot were relatively insensitive to changes in the cycle length. Later in 1955 Carmeliet studied the relationship between frequency of stimulation and action potential duration in the pig ventricle. He found a monoexponential relationship and postulated a mathematical formula to find the action potential duration.

In 1960, Gibbs and Johnson emphasized that it was important to study the changes in the shape and area of the action potential in addition to the changes in the duration. They studied the influence of frequency on these parameters, using rabbit ventricular muscle. They showed that the area and the duration of the action potential were maximum at a range of frequency from 1 to 3 sec^{-1} and that changes in frequency, below or above this range, produced a decrease in the area of the action as well as the duration. Another observation from this work is that the changes in the area of the action potential, at high frequencies of stimulation is due mainly to the fact that the action potentials originated at lower "take off" potential, without any change in the level of the plateau. On the other hand, the decrease in the area at low frequencies was caused largely by a drop in the height of the plateau. They also presented the results of change of area of the action potential due to the interpolation of an extrasystole. With this

technique, the area of the action potential of the extrasystole was longer. Higher frequency of stimulation previous to the extrasystole caused these to have smaller action potentials. Carmeliet (1958) using frog ventricular tissue, found a decrease in the area of the action potential originated by an extrastimulus. This difference could be due to species used. Later, Gibbs, Johnson and Tille, (1963, 1964) developed and proved a mathematical model to predict the changes in the area of the extra action potential. Edmons and coworkers (1966) reported changes only in the configuration of the canine ventricular action potential without any change in the total duration after abrupt changes in the cycle length of stimulation. Greenspan and coworkers (1966) demonstrated that change in shape but not in total duration of the action potential was also seen in atrial cells, but not in Purkinje fibers. Miller and coworkers (1971) also pointed out the difference of the changes of shape of AP between Purkinje fibers and venticle.

A study of the variation of the action potential shape and duration in atrial cell was reported by Tanaka and coworkers (1967). They used a rabbit right, but non-beating, atria. They found that a certain type of atrial cell with a pronounced phase one of repolarization, and a plateau phase, developed at lower potentials than that observed in Purkinje fibers or ventricles. When they changed the frequency of stimulation after a quiescent period of relatively long duration (10 to 1 minute), they observed that the phase one and two of these fibers did not change, but the duration at the plateau level and its height were longer and higher respectively, at lower frequencies of

stimulation. Later, in 1971, Saito demonstrated in the same preparation that the addition of TTX or the lack of sodium in the solution did not modify the changes in the plateau seen with the changes of frequency; on the other hand, the addition of TEA enhanced this frequency dependent effect on the plateau. Acetylcholine abolished the plateau phase of these fibers and suppressed the changes of the action potential shape. He also tested the effect of calcium ion, and observed that both an increase and a decrease of the calcium concentration shortened the plateau in the premature action potentials. He concluded that these changes in the action potential were due to a decrease in the potassium conductance. Saito (1972) also studied the effect of depolarizing and hyperpolarizing currents on the properties of the membrane in atrial cells. He concluded that changes in the plateau configuration of the action potential at different intervals may be explained by a progressive enhancement of the decrease in the conductance of the membrane for potassium ion. Hoffman and Suckling (1954) observed that the changes in the duration of the action potential were more prominent with extrasystoles than those observed with changes in the cycle length.

Gettes and coworkers (1972) compared premature and nonpremature action potentials in Purkinje and ventricular fibers of the pig. They found that in both structures, there were two factors to consider, a) a cycle dependent modification of the duration and b) a cycle independent factor, which was dependent upon the coupling interval of the extrasystole, the duration of the preceding action potential, and of the "take-off" potential. They also showed differences between Purkinje

and ventricular fibers. These differences were: a) In Purkinje fibers, the shortening of the premature action potentials was greater that observed due to the cycle length dependent factor; b) In Purkinje fibers, the shortening of the premature action potentials was greater the shorter the coupling intervals, whereas in ventricles they observed a lenthening of the premature action potentials up to a coupling interval of 26 ms; c) always, the earliest premature action potential was shorter in Purkinje fibers than in ventricles. The mechanisms mediating this change in the configuration of the action potential due to the extrasystoles, are different among the structures of the heart. Hauswirth and coworkers (1972) demonstrated in the Purkinje fibers, that the time constant of the decay of duration of the action potentials produced by shortening the cycle length of stimulation was 200 ms, and that this time constant corresponds to the time constant of the decay of the outward current i_x carried mainly by K^+. They concluded that this current may be responsible for the variation of the duration of the action potential. They also pointed out that an inward current may be involved in the process. Gettes and Reuter (1974) demonstrated in ventricular tissue that the plateau duration of the premature action potential was determined by the kinetics of reactivation of the secondary calcium inward current. Hiraoka and Sano (1978), using the single sucrose-gap voltage clamp technique, demonstrated that there was an increase of the secondary inward current in ventricular tissue which was parallel with the increase of the duration of the action potentials at shorter intervals. This current was blocked by Mn^{++} ions. The importance of these changes

in duration or in the total area of the action potentials due to prematurity, have been related to a possible role in the genesis of reentrant arrhythmias (Gettes et al 1972), and have been correlated with the changes in the time to peak of the contraction in hearts of different species (Anderson and Johnson, 1976).

E. PAROXYSMAL TACHYCARDIA DUE TO EARLY EXTRASYSTOLES.

1. Reentry

One of the effects observed when an extrasystole is delivered at a certain specific time during the action potential, is that it is able to induce a burst of rapid repetitive activity (rhythmic activity). This event is said to happen when an extra stimulus reaches the cell during the "vulnerable" phase. Sano and Scher (1964) using extracellular recordings demonstrated the production of atrial fibrillation using repetitive electrical stimuli at different times during the cardiac cycle of the dog. This rhythmic activity has also been studied with microelectrodes (Müller, 1965). Two mechanisms have been postulated to explain the rhythmic activity: a) reentry and b) ectopic foci.

The term reentry was used by Schmitt and Erlanger (1928-29). It is known that the heart muscle is able to conduct the wave of excitation with equal facility in either direction. Nevertheless, Erlanger (1906) demonstrated that strips of heart muscle were able to lose their homogeneous condition of conduction. Schmitt and Erlanger (1928) used ventricular strips of the turtle and arranged the preparation in a 5 compartment chamber. In certain chambers they stretched, applied

depressants, cooled or raised the K^+ and then studied the conduction in both directions, and also studied the contraction in each chamber. They found that the injured segment presented different states of conduction; a) When the rate of conduction was equal in both directions, they called this state isodromic; b) when it was faster in one direction, was heterodromic; c) monodromic was the state of conduction in only one direction, and block in the other; d) the adromic state was a complete block in either direction. They also found that "under certain circumstances a second response of one side may follow the response of the other side in the absence of a second artificial stimulation". To this state of return or reentry, they called it the opisthodromic state. Their conduction study supported the idea that the phenomenon of opisthodromia (reentry) is possible when a monodromic condition exists in some fibers, and a heterodromic state in the adjacent fibers.

The two conditions for the phenomenon of reentry postulated by Schmitt and Erlanger in 1928 have been demonstrated by Cranefield, Klein and Hoffman (1971). They used long bundles of canine Purkinje fibers, on which was possible to depress part of the segment - by using high potassium concentration. They demonstrated that slow conduction and unidirectional block in the depressed segment permitted reentry.

The phenomenom of reentry has also been applied to explain paroxysmal atrial tachycardia. The first attempt to explain the phenomenon was that of the description of the circus movement by Mines (1913). The first mention of circus movement was done by McWilliam in 1887.

Later Iliescu and Sebastiani (1923) suggested that paroxysmal supraventricular tachycardia could be explained with this theory. Bigger and Goldreger (1970), studied paroxysmal supraventricular tachycardia in six patients. Their electrophysiological study, obtained by intracavitary atrial electrogram, and surface electrocardiogram allowed them to conclude that this paroxysmal supraventricular tachycardia was due to reentry, utilizing the A-V conducting system. Bigger and Goldreger (1970) also pointed out that "supraventricular tachycardia always began with an atrial premature depolarization", and that this atrial premature depolarization always showed prolonged A-V conduction.

Studies in vitro, using short strips of left atrial tissue, have been done by Allessie and coworkers (1973, 1976, 1977). They studied the production of the paroxysmal tachycardia by sending an extrastimulus during the vulnerable period of the cells. Their technique of recording simultaneously more than 100 intracellular and extracellular events, allowed them to conclude that there is an unidirectional block and that the premature beat can force the impulse to conduct in a cicuitous route. They have postulated the model of the "leading circle concept" to explain the circus movement that is seen in short pieces of tissue, without any anatomical obstacle. The concept of the circus movements since it was postulated, requires an anatomical obstacle to sustain the arrhythmia. In the case of short strips of tissue such an anatomical obstacle does not exist. Allessie and coworkers (1977) in their experiments, demonstrated that in those fibers approaching the central area of the circuit, the wave of excitation (called by

then the "centripetal wavelet") progressively lost its "stimulating efficiency" until it was unable to stimulate the fiber ahead. This loss of "stimulating efficiency" was demonstrated by showing that the amplitude, rate of rise of phase zero, and action potential duration, of the fibers from the periphery toward the center, gradually decreased. In other words, the centripetal wavelet is conducted with decrement. They concluded that in the center of this vortex, the centripetal wavelets will collide, and therefore there will be a functional obstacle to the wavelet to get the other side of the pathway. Apparently, there are conditions which fulfill the requirements for the phenomenon of opisthodromia (reentry) a) there is a decremental conduction, from one point of the periphery to the center; b) there is a monodromic state (unidirectional block), from one point of the periphery to any other point of the periphery passing through the center; c) block in the center. One consideration of the model is that the original basic stimulus, showed a concentric spread, whereas the premature impulse was converted in a circus movement. Regardless of the explanations for the decremental conduction and the block in the center their work sustained the phenomenom of reentry as the mechanism to explain the paroxysmal atrial tachycardia produced by an extrasystole.

There is evidence that the sustained rhythmic activity in atria may be mediated by the specialized conducting pathways in this structure. Montgomery and Dresel (1974) demonstrated transient arrhythmia in the isolated dog heart preparation when an extrastimulus

was delivered close to the refractory period. They also demonstrated that acetylcholine increased the variability of the conduction time of the extrastimulus. They explained this variability by the different effect of the drug on refractoriness between atrial muscle and the specialized conducting pathway in this structure. They concluded saying: "It would appear possible, therefore, that junctions of atrial muscle and conducting system fibers may be involved in atrial reentry". Recently, Pastelin and coworkers (1978) have presented evidence in the canine heart that the surgical section of the middle and posterior internodal pathways prevents the establishment of flutter in atria.

2. Ectopic Foci.

Another theory to explain the sustained rhythmic activity seen in cardiac tissue, is that of the automatic ectopic pacemaker. Automaticity is the term applied to describe the ability of certain cardiac fibers to generate an impulse of their own. This property is characteristic of the specialized cells of the heart S-A node, intra atrial conducting system, A-V node, His bundle, bundle branches, and peripheral Purkinje fibers. The automatic cells, gradually lose their resting potential level, to reach a threshold potential, and then generate an action potential. The gradual decrease in the resting potential is called diastolic depolarization.

Besides this normal automaticity, controlled by the S-A node pacemaker, there is another type of change in the membrane resting potential which is different from that of normal diastolic depolarization.

These changes in the resting potential has been called oscillatory changes. The evidence for these oscillatory changes are found since 1943 in the work done by Bozler. He observed that in injured hearts of the turtle, dog and rabbit besides the normal automaticity, there were oscillations of increasing magnitude which were able to take command of pacemaker activity. He also demonstrated that these oscillations were modified by acetycholine and adrenaline. He concluded that the oscillations could explain the paroxysmal tachycardia, and the coupled extrasystoles observed in the heart.

In 1969, Brown and Noble, using a double sucrose gap technique demonstrated that in the quiescent atria of the frog (Rana ridibunda) depolarizing currents of relatively low magnitude (0.12 - 0.34 μA), and several seconds duration (3 to 10 s) were able to induce pacemaker activity in this tissue. The maximum activity was seen at -50 mV depolarization level. The results of the voltage clamp studies done by these authors demonstrated that this repetitive activity could be correlated with a component of inward current which was insensitive to tetrodotoxin (TTX).

Trautwein, (1970) also have presented results in the Purkinje fibers, showing this spontaneous activity during the repolarization process, and approximately at the plateau level. He pointed out the possibility that because at this potential level the sodium carrier system should be inactivated, this inward current ought to be carried through the slow channels, and that the calcium ion may play an important role. He also pointed out that such spontaneous activity was

more easily seen in preparations with low oxygen tension, low potassium concentration or elevated CO_2 tension.

From these studies it seems clear that the secondary current plays an important role in this type of arrhythmia; but not only the slow channels are of importance, because it has also been demonstrated that aconitine is able to produce this type of arrhythmia and that its effect is not seen when the cells are pretreated with TTX, a substance capable of blocking the fast sodium system. (Schmidt, 1960).

There is evidence also, that cardiac glycosides are able to cause cardiac arrhythmias by increasing the automaticity of Purkinje fibers. Ferrier and coworkers (1973) demonstrated that in the Purkinje fiber, toxic doses of acetylstrophantidin, increased automaticity of the tissue. They observed that after the last driven response, there was development of rhythmic activity due to subthreshold oscillations of the membrane. They called these oscillations "Transient depolarizations". They did not observe such transient depolarizations in muscle. In further studies, they demonstrated that high external calcium concentrations (12.5 mM) also produced the transient depolarization and that the ion potentiated the effect of acetylstrophanthidin. When the preparation was bathed in low calcium concentration, or in the presence of Mn^{++} ions, the transient depolarization seen with the cardiac glycoside were reversed. They concluded that the enhancement of this rhythmic activity was a different process from that of the normal diastolic depolarization, and that these transient depolarizations responsible for this increase in automaticity, were caused by

the transient increase of Ca^{++} influx.

Further evidence that the secondary current is involved in this type of sustained rhythmic activity is the work done by Cranefield and Aronson (1974). They demonstrated that canine cardiac Purkinje fibers, exposed to sodium-free high calcium (16.2 mM) media were able to show spontaneous activity after a quiescent period. Aronson and Cranefield (1974) also pointed out that in the Purkinje fiber, it was possible to demonstrate two states of the membrane potential in which automaticity could be seen. When the resting potential was about -90 mV, the rhythmic activity produced by ouabain had the characteristic of the normal diastolic depolarization, whereas when the membrane potential was about -50 mV, the rhythmic activity was of the type produced by the secondary current.

Recently, Lederer and Tsien (1976), have demonstrated with the voltage clamp that the transient depolarization produced by cardiac glycosides were followed by a transient inward current. This current appeared superimposed upon the normal pacemaker mechanism (the decay of the potassium current i_{K2}), and also could be recorded at holding potentials positive to -55 mV, which is a voltage outside the range where 1_{K2} occurs.

Oscillatory afterpotentials have also been demonstrated in atrial tissue. Hashimoto and Moe (1973) presented evidence that in the atrial specialized conduction fibers (plateau fibers), acetylstrophanthidin ($1\text{-}3 \times 10^{-7}$ g/ml) produced the transient depolarization observed by Ferrier and coworkers (1973). Saito and coworkers (1978)

have presented results using atrial "plateau" fibers, which indicate that sustained rhythmic activity was produced by oscillatory potentials and this it could not be explained by the phenomenon of opisthodromia (reentry). He did not use cardiac glycosides.

In ventricular tissue this type of automaticity have been demonstrated by using depolarizing currents (Katzung, 1974). He also demonstrated that in the guinea pig papillary muscle, calcium and sodium ions play an important role in the genesis of this sustained rhythmic activity induced by depolarizing pulses (Katzung, 1974).

It has also been demonstrated, that besides the specialized system and the working muscles, other structures are able to generate ectopic foci. Wit and Cranefield (1976) presented results showing that in the anterior mitral valve leaflet of the monkey heart, the action potential is followed by an after hyperpolarization and that catecholamines cause a delayed afterdepolarization. Oscillations in membrane potential were enhanced at lower cycle lengths or by prematurity, and that these conditions are able to induce sustained rhythmic activity. They also demonstrated that the amplitude of the oscillations were little affected by TTX, but verapamil abolished the delayed afterdepolarization produced by catecholamines. This sustained rhythmic activity cannot be explained by reentry. Therefore, the evidence presented is consistent with the fact that both phenomena opisthodromia (reentry) and ectopic foci are possible in the heart. What is common in both explanations is the fact that the secondary current plays an important role in the ectopic foci theory, as well as in

reentry. (Cranefield, 1975; Hiraoka and Sano, 1976). Cranefield (1975) has pointed out that the phenomenon of reentry is favoured by the presence of "slow response" action potentials, and as a consequence slow conduction velocity. Recently Brennan and coworkers (1978) have demonstrated that the slow response action potentials can be generated by two mechanisms: a) action potentials generated by the secondary current, and b) action potentials generated by depressed fast sodium current. The common characteristic is a slow velocity for the propagation of the impulse, condition which favor the phenomenon of opisthodromia.

F. Effects of Calcium Ion on Cardiac Action Potential

1. Effects on the shape of the action potential.

The changes in the action potential characteristics due to variation of the external calcium concentration are well known since the work of Hoffman and Suckling (1956). Studies done by Weidman (1955) in Purkinje fibers had demonstrated that variation of the calcium concentration (four fold decrease or four fold increase) did not affect markedly the size or shape of the action potential, the maximal diastolic membrane potential or the membrane resistance, but did increase the threshold for the action potential "take off". Ware and coworkers (1955) saw changes in the membrane resting potential, overshoot, or duration of the action potential of the frog heart when the Ringer perfustion solution was changed from normal to calcium free. Hoffman and Suckling confirmed Weidman's observation that the configuration of the action potential in the Purkinje fibers was less

affected by changes in the external calcium concentration, whereas the ventricles and the auricles showed prominent changes in the duration of the action potential. Low values of calcium prolonged the action potential, at the plateau level, in ventricles, but had little effect in atrial cells. On the other hand, an increase in the external calcium concentration decreased the duration of the action potential in both structures. Temte and Davis et al (1967) restudied the problem and showed that when the frequency of stimulation of Purkinje fibers is kept constant, low external (Ca^{2+}) prolonged the AP, whereas an increase in the external calcium concentration decreased the duration.

Klein and Holland (1959) studied the effect of calcium concentration on $^{42}K^{+}$ fluxes and action potentials in rabbit atria. They emphasized the effect of calcium on two phases of the duration: phase A, which corresponded in their study to the duration from the initiation of the depolarization (phase zero of the action potential) to the second inflexion point of the repolarization process; and phase B which was considered by them as the duration of a negative afterpotential, and was measured from the second inflexion point to the end of repolarization process. They found that higher external calcium concentration showed a biphasic effect on duration of the action potential, shortening of phase A and lengthening of phase B.

Recently, Isenberg (1975) increased the intracellular calcium concentration by injecting Ca^{2+} iontophoretically in short Purkinje fibers, demonstrated a hyperpolarization of the membrane and a

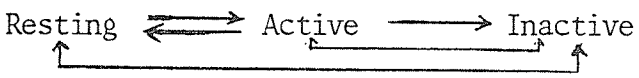
shortening of the action potential. He suggested that such an increase of the intracellular calcium concentration increased the potassium conductance, gK. Kass and Tsien (1976) studied the effect of the external concentration in Purkinje fibers using the voltage clamp technique and found that higher calcium concentration increased the plateau level and that this alteration of the plateau level accelerated time and voltage dependent current changes which triggered the repolarization process. The main current affected was the iK_1 or background K^+ current, and as a consequence, the action potential was shortened. In the ventricular tissue, Bassingthwaighte and coworkers (1976) have also demonstrated shortening of the action potential duration and the increase of the time independent outward current as a consequence of an increase in the intracellular calcium content. What is common in these two structures (Purkinje fibers and ventricles) is a reduction of the action potential duration, whereas in atrium there is a biphasic change in the duration when the external calcium concentration is increased; a shortening of phase A and a prolongation of phase B (Klein and Holland, 1959). The lengthening of phase B by higher calcium concentration is similar to that seen with low K^+ concentration.

The external calcium concentration also influences the changes in the action potential configuration related to the frequency of stimulation. Reiter and Stickel (1968) demonstrated that in the papillary muscle of the guinea pig that the action potential duration increased in the range of frequency of stimulation from 0.004/sec. to 0.125/sec. when the external calcium concentration was between 1.2 and 2.4 mM.

When the external calcium concentration was 4.8 mM the prolongation of the action potential at the low frequency range was minimal. Bass (1975) also demonstrated that the changes in the area of the action potentials produced by extrasystoles were greater in low external calcium concentration (0.625 mM), and were decreased at a concentration of 7.5 mM.

2. Effect on the recovery of $\dot{V}max$.

The effect of calcium ion on the sodium carrier system was first studied by Weidmann (1955), using Purkinje fibers. He observed that a four-fold increase in the external calcium concentration did not increase the maximum value of $\dot{V}max$, but shifted the voltage - $\dot{V}max$ relationship toward the left on the voltage axis. He explained this effect of calcium ion by assuming three different states in the sodium carrier system: a) resting state, b) an active state and c) an inactive state. He assumed the system in equilibrium by the reaction:



Calcium ion shifted the reaction to the left, favoring the resting state and therefore more "carrier units" will be available at lower membrane voltages, and therefore higher values of $\dot{V}max$ than in normal calcium concentration. Beeler and Reuter (1970) also demonstrated in the ventricle that higher external calcium concentration shifted the Vh value (voltage for half inactivation) of the sodium system toward less negative potentials. Gettes and Reuter (1974) have demonstrated in Purkinje fibers and ventricles, and Haas (1971) in atrium, that the recovery process from inactivation of the sodium system is slower than

the inactivation. Gettes and Reuter (1974) demonstrated that higher external calcium action hastened the recovery from inactivation in Purkinje fibers and in ventricle.

G. Effects of Lidocaine on Atrium

1. Lidocaine as antiarrhythmic agent.

Lidocaine was synthesized in 1943 (cited by Collinsworth et al, 1974) and introduced as a local anesthetic agent in 1948 (Ritchie and Cohen, 1974). The first use of the drug as an antiarrhythmic was reported in 1950, (Southworth et al) for the treatment of a ventricular fibrillation precipitated by cardiac catheterization. Since that time up to the present, the drug is still one of the most used for the treatment of the ventricular type of arrhythmias, particularly those associated with myocardial infarction, and cardiovascular surgery. (The Medical Staff Conference, 1976; Harrison et al, 1963). In contrast to the well defined effect of the drug in the ventricular type of arrhythmias, its effect on the supraventricular type is much less prominent. The reason for this lack of effect is not known.

2. Electrophysiological Effects of Lidocaine.

a) On the shape of the action potential.

One characteristic observed with the drug is that its effects are different among the structures of the heart. Lieberman and coworkers (1968) demonstrated that lidocaine affected the pacemaker activity differently. They used dogs with complete atrioventricular block done by injection of formaldehyde into the bundle of His. They observed that the atrial rate was altered very little, whereas the ventricular

rate was reduced. Another important difference of effect is that lidocaine is able to shorten the action potential in Purkinje fibers and ventricular tissue, but not in atria. (Davis and Temte, 1969; Bigger and Mandel, 1970; Mandel and Bigger, 1971; Singh and Vaughan Williams, 1971). Bigger and Mandel (1970) demonstrated that the shortening of the action potential in Purkinje fibers due to lidocaine was dose-dependent and also that this shortening was greater at longer cycle length of stimulation. They concluded that lidocaine may increase the potassium conductance in the Purkinje fibers. Later, Arnsdorf and Bigger (1972) demonstrated an increase in the membrane conductance for potassium ion. Another important observation, related to the effect of lidocaine, was made by Kabela (1973). He used cardiac tissues of the dog and preloaded them with $^{42}K^{+}$, and then studied the effect of lidocaine. He observed that lidocaine significantly enhanced the efflux of $^{42}K^{+}$ from ventricle strips and Purkinje fibers, but did not accelerate the efflux of $^{42}K^{+}$ from atrial tissue. He concluded that lidocaine increases the potassium conductance and, therefore, the explanation for the shortening of the action potential, the antiarrhythmic effect of the drug, and the lesser effect of the drug in the supraventricular type of arrhythmias.

b) Effect on the Membrane Responsiveness and Steady-state. Influence of Potassium Ion.

The studies done by Davis and Temte (1969) and by Bigger and Mandel (1970) had demonstrated that lidocaine at 1×10^{-5}M, did not affect the maximum rate of rise of phase zero, nor change the membrane responsiveness curve but did decrease the action potential duration and the effective

refractory period in Purkinje fibers. The same effect was observed in ventricular muscle. They also observed that the effective refractory period was longer than the action potential duration and that this effect was more prominent in ventricular tissue. Their conclusion was that lidocaine did not reduce the availability of the sodium carrier system. Mandel and Bigger (1971) also demonstrated that in atrial tissue lidocaine concentrations in the range of the therapeutic usage did not affect the maximum rate of rise of phase zero and did not modify the membrane responsiveness curve. They concluded that this lack of effect may explain why the drug is not effective in the treatment of the supraventricular arrhythmias. These results were challenged by Singh and Vaughan Williams (1971). These investigators reexamined the problem in the atrial tissue of the rabbit. The basis for their study was the fact that in previous studies (Bigger and Mandel, 1970; Mandel and Bigger, 1971; Davis and Temte, 1969) the tissues were studied with an external potassium concentration in the range of 2.7 to 3 mM. Singh and Vaughan Williams (1971) pointed out that these were low potassium concentration because the average potassium concentration in humans is 5.0 mM. They used lidocaine at different concentrations (3.73 to 112 10^{-5}M) and two potassium concentrations (3.0 and 5.6 mM). They demonstrated that the effect of the drug on the maximum rate of depolarization ($\dot{V}max$) was enhanced in the higher potassium concentrations. Similar effects were seen in ventricle strips. The action potential duration of atrial cells was not changed by lidocaine at any of the potassium concentrations, whereas it was shortened in ventricles. They explained this effect of lidocaine by saying that low potassium concentration

hyperpolarizes the membrane, and as a consequence the maximum rate of depolarization also increases, and therefore less effect would be seen with drugs that interfere with the sodium carrier system. However, even at a higher external potassium concentration (5.6 mM), the minimum dose of lidocaine (11.2×10^{-5}M) at which Singh and Vaughan Williams (1971) observed a significant decrease of $\dot{V}max$ in atria is higher than the normal therapeutic range (1.2 to 6×10^{-5}M). The influence of potassium concentration has also been studied in Purkinje fibers by Obayashi and coworkers (1975). They found that when the potassium concentration was 6.0 mM, lidocaine decreased $\dot{V}max$. Pamintuan and coworkers (1970) demonstrated that the antiarrhythmic effect of lidocaine was enhanced by higher potassium concentration. Recently, Saito and coworkers (1978) have demonstrated that hyperkalemia enhanced the effect of lidocaine on intraventricular conduction time of the dog.

The evidence presented show clearly that potassium ion is involved in the complex effect of lidocaine. There are other experiments which also demonstrate that the effect of lidocaine is mainly related to the sodium system.

Chen and coworkers (1975) studied the effect of lidocaine (1.72 to 6.87×10^{-5}M) in the papillary muscle of guinea pig. They demonstrated that lidocaine decreased $\dot{V}max$ at these concentrations, and that its effect was more prominent when the membrane potential was less negative. In their study, the membrane potential was made more positive by increasing the external potassium concentration. They also showed that this effect of lidocaine was due to change in the membrane potential

as a consequence of increasing the external potassium concentration, and not due to potassium itself. This was proven by injecting hyperpolarizing or depolarizing currents into the preparation; a hyperpolarization of the membrane restored the depressed $\dot{V}max$ due to lidocaine eventhough the external potassium concentration was elevated. They also demonstrated that the membrane responsiveness curve was shifted toward more negative values on the voltage axis. Rosen and coworkers (1976) did experiments in canine Purkinje fibers, with special emphasis upon the potassium concentration and the therapeutic range of lidocaine. It was also evident that the drug decreased $\dot{V}max$, conduction, and depressed the membrane responsiveness. They also confirmed the previous work done by Singh and Vaughan Williams (1971) showing that lidocaine is acting on the sodium carrier system.

G. Effect on the Recovery of $\dot{V}max$. Influence of Heart Rate.

Lidocaine also affects the recovery process of the sodium system. Gettes and Reuter (1974) demonstrated in the Purkinje fibers, and Haas (1971) in frog atrial tissue, that the recovery from inactivation of the sodium system was a slower process than that of inactivation. Weld and Bigger (1975), using the voltage clamp technique, demonstrated in Purkinje fibers that lidocaine markedly slowed the recovery from inactivation of the sodium system. Chen and coworkers (1975), using the technique of interpolated extrasystoles, studied the recovery of $\dot{V}max$ in guinea pig papillary muscle. They demonstrated that lidocaine significantly prolonged the time constant of the recovery process of the sodium system. This effect of the drug on the process of recovery from inactivation may explain the rate-dependent effect of lidocaine

on Vmax pointed out by Tritthart and coworkers (1971) and also demonstrated by Chen and coworkers (1976) as well as the greater effect of the drug on atrial conduction time at faster frequency of stimulation demonstrared by Man and Dresel (1977) in the canine heart.

H. STATEMENT OF THE PROBLEM

As has been pointed out previously, the effect of lidocaine is well known in Purkinje fibers and ventricle, whereas in atrial tissue the results have reported lack of effect, or the effect of the drug has been obtained with drug concentrations higher than those used in the therapeutic range. There are clinical reports which indicate that the drug has shown effectiveness in certain supraventricular types of arrhythmias (Malach, M. 1969; Josephson and coworkers, 1976). Therefore, we decided to reexamine the problem studying the electrophysiological effects of lidocaine in atrial tissue, within a therapeutic range, and an external potassium concentration close to the normal human values, and its effect upon an atrial arrhythmia.

The questions addressed were:

1. Is there any effect of lidocaine on the electrophysiological characteristics of the atrial tissue at concentrations from 1 to 5 x 10^{-5}M?
2. Is the drug antiarrhythmic at these concentrations?
3. If there is any antiarrhythmic effect what would be the possible mechanism?

I have also pointed out that the drug does not modify the membrane conductance for potassium ion in the atrial tissue (Kabela, 1973). This difference of effect has been taken as the main cause for the lack of

effectiveness of the drug in the supraventricular type of arrhythmias. Recent reports indicate that calcium ion is involved in the dynamic process of the atrial action potential. Therefore, we also studied the effect of lidocaine at different external calcium concentrations.

METHODS

METHODS

A. THE ISOLATED SUPERFUSED RABBIT ATRIUM

1. Surgical Procedure

The experiments were carried out in atria from New Zealand rabbits, of either sex, weighing 2.5 to 4.3 Kg. The animals were killed by cervical dislocation. The chest was opened by a medial incision, and the heart removed as soon as possible (1 to 2 minutes) and placed in ice-cold Krebs solution (4 to 5°C). Under constant aeration with a mixture of 95% oxygen and 5% CO_2, either the left or the right atrium was dissected from the heart, everted and fixed with four stainless steel pins to the bottom of a 20 ml capacity plexiglass chamber. The tissue was superfused continuously by means of gravity and the overflow removed with suction.

2. Superfusion Solutions

The solutions used were Krebs solution at different calcium concentrations and Krebs with lidocaine. The Krebs solution considered normal, which was taken as control perfusate had the following composition, expressed in millimoles. NaCl:118.05; KH_2PO_4:1.175; $MgSO_4 \cdot 7H_2O$:1.176; KCl:4.7; $NaHCO_3$:26.2: $CaCl_2 \cdot 6H_2O$:2.5; Glucose:11.2. The pH was 7.4. The $CaCl_2$ was decreased to 1.25 mM, or increased to 5.0 mM, and these solutions are considered as low calcium Krebs solution, and high calcium Krebs solution respectively. Powdered lidocaine hydrochloride (xylocaine-hydrochloride monohydrate; Astra Pharmaceutical Products, Inc.) was dissolved in Krebs solution at the three calcium

concentrations to obtain a final concentration of 1×10^{-5}M or 5×10^{-5}M. Krebs solutions with the drug, are considered as treatment perfusate. The everted, fixed atrial tissue which I shall call the preparation, was superfused at a constant rate of 10 ml/min. with the above mentioned solutions, at a temperature of 30^{o} C ± 0.5^{o} C, kept in this range by means of an electrical heater controlled thermostatically with a telethermometer. Whenever Krebs solution was changed from one calcium concentration into another, a minimum perfusion time of 30 minutes was allowed before any estimations of the electrophysiological parameters were done, and when lidocaine was in the perfusate, at least 15 minutes were taken as the minimum time for the drug to reach its steady-state effect. In Figure 1, I have drawn a schematic diagram of the perfusion set-up and the electronic units used during the experiments.

B. STIMULATION AND RECORDING

1. Transmembrane Potentials

Transmembrane potentials were obtained with glass micropipettes, pulled in a vertical puller, containing a few glass fibers in order to facilitate direct filling with 3M KCl, and having tip resistances from 15 to 30 mΩ. The electrical activity was obtained differentially by having one micropipette impaled intracellularly, and another in the perfusion solution, close to the intracellular one. The plexiglass chamber was grounded to a common ground through a silver-silver chloride plate attached to the bottom of the chamber. The micropipettes were connected through silver-silver chloride wires to a high impedance ($10^{12}\Omega$) amplification system, with variable capacity feedback (Frederick

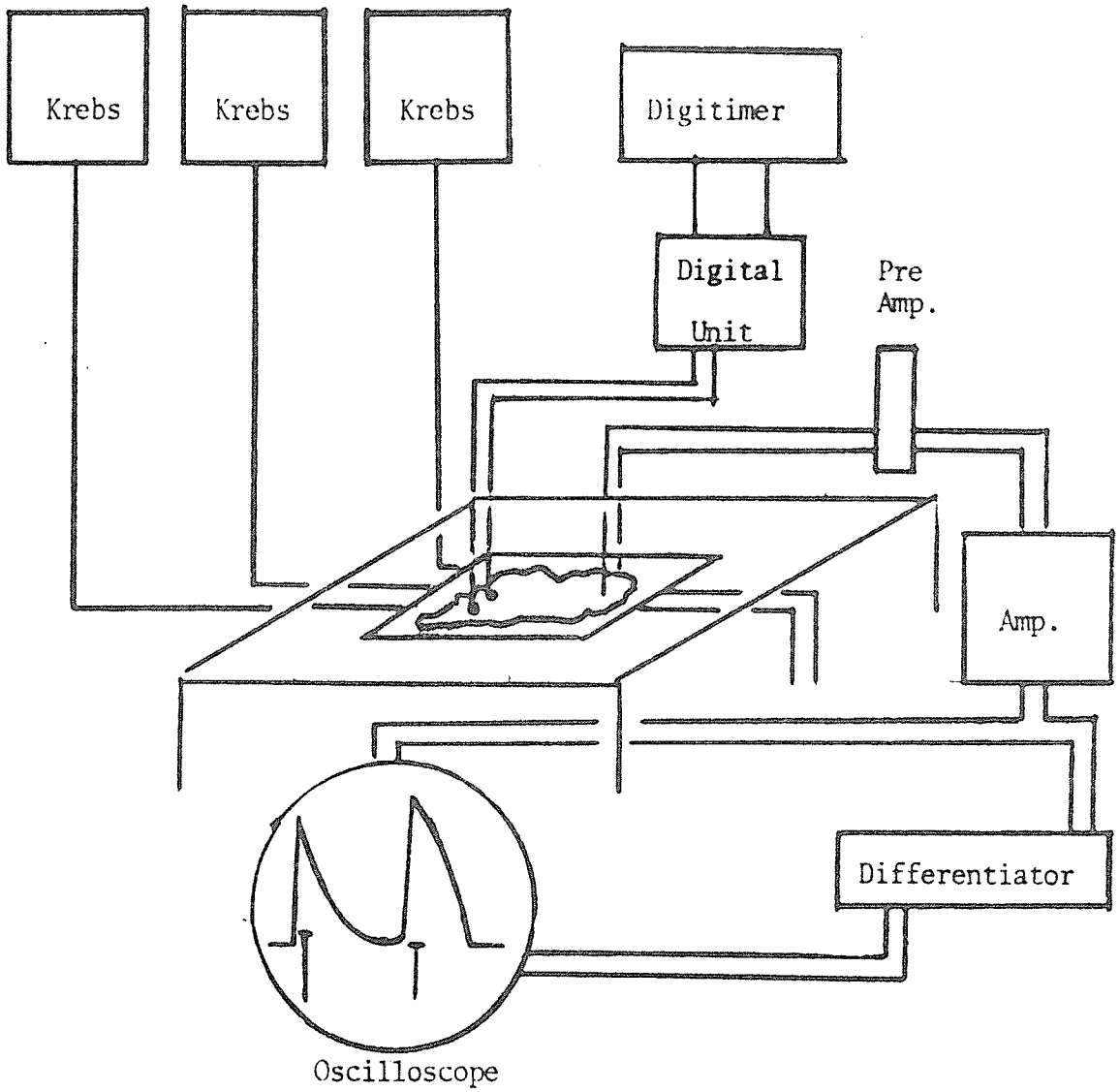


Fig. 1 - Schematic diagram of the superfusion set-up and the electronic units for the isolated superfused rabbit atria.

Haer Co.), consisting of a miniature preamplifier, a band pass amplifier, and an artefact suppressor. The frequency response of the entire system, through a 20 MΩ resistance was linear flat to 8 KHZ. The system was calibrated with a 10 mV signal, and its output displayed on one channel of a dual beam oscilloscope (Tetronix 502, or 502A). The signal was also diverted to the input of a differentiating circuit, and then back to the second channel of the oscilloscope for the display of $\dot{V}max$. The time constant of the differentiating circuit was 30 μs, and was calibrated with saw-tooth waves, showing linearity from 10 to 1000 HZ. Figures 1A, 1B, and 1C show the schematic diagram of the circuits for the obtention of $\dot{V}max$. We used a differentiator coupled to a peak detector circuit to calculate the rate of rise of phase zero of the action potential. The dead time for the peak detector was 45 ms, and it was able to hold the peak of the differentiated signal for 5 ms. In Table 1 are given the details for the integrated circuits used, as well as the pins connecting to the power supply.

2. Stimulation of the Left Atrium

The left atrial preparation was driven at fixed cycle length of 2000 ms through a silver-silver chloride teflon-coated bipolar electrode with the tips one mm apart. A digital unit (Pulsar 6, Frederick Haer Co.) delivered anodal pulses of 2 ms duration, and four times the threshold intensity to the preparation. The intracellular microelectrode was impaled at a distance no further than 2 mm from the stimulating electrode.

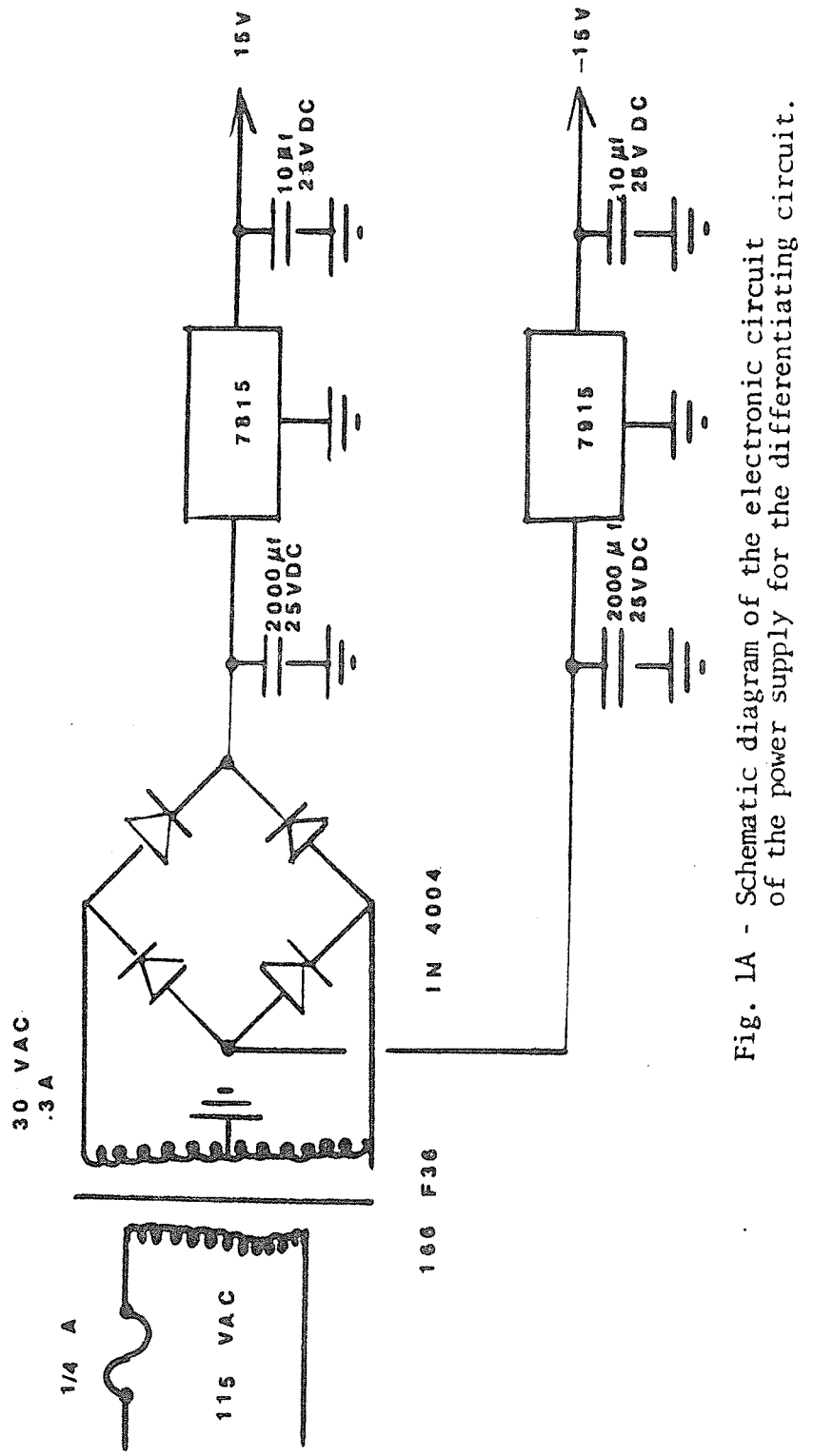


Fig. 1A - Schematic diagram of the electronic circuit of the power supply for the differentiating circuit.

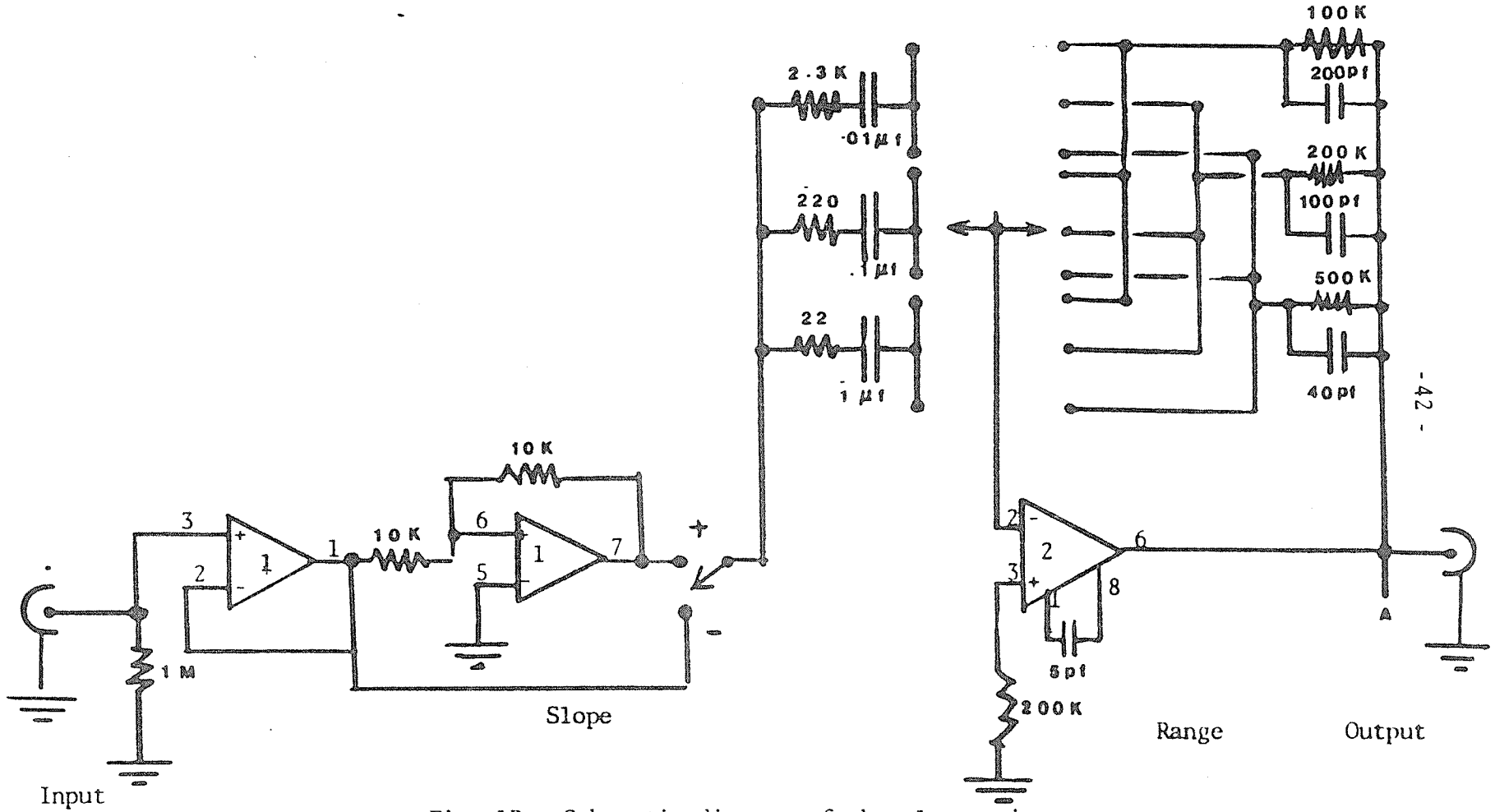


Fig. 1B - Schematic diagram of the electronic circuit for the differentiator.

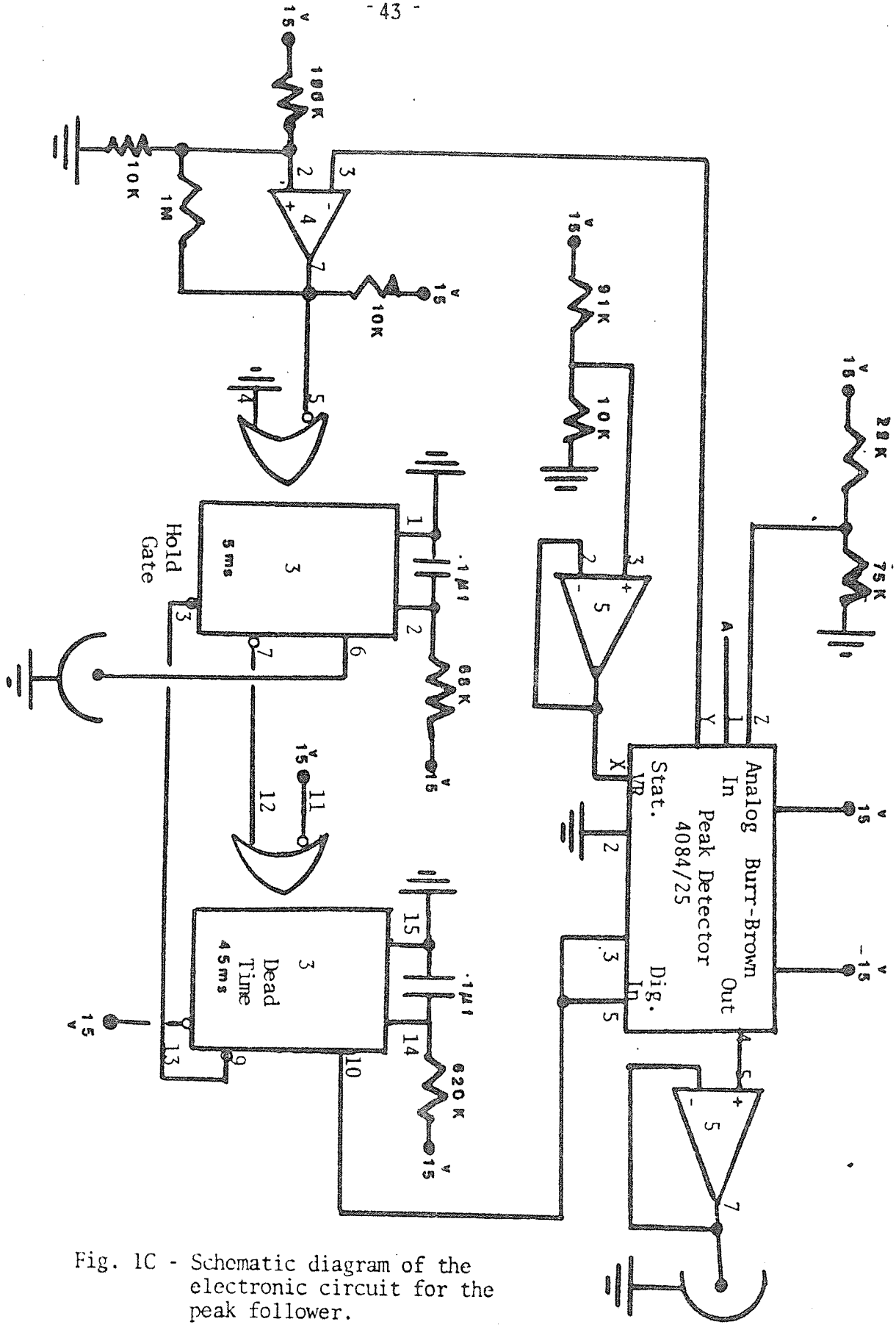


Fig. 1C - Schematic diagram of the electronic circuit for the peak follower.

TABLE 1

Integrated Circuit (I.C.) and Pin Connection Data for Figures 1A,1B, and 1C.

I.C. Number	Type	+ 15V	GND	- 15V
1	75458	8	-	4
2	LM308	7	-	4
3	MC14528	16	8	-
4	LM311	8	1,4	-
5	75458	8	-	4

C. MEASUREMENTS AND STATISTICAL ANALYSIS

The atrial action potentials and $\dot{V}max$ were photographed from the oscilloscope screen with a Shackman 35 mm oscilloscope camera using Kodak film. The films were developed as suggested by the manufacturer. The negatives were projected through a viewer, (Dagmar Super, Model 35 · magnification = 3x), and the enlarged images were measured with a ruler and the calculations were made with a Texas Instrument calculator. The comparisons between control and treatment, were done by student's t-test, simple or paired, and the level accepted for significance was $P<0.05$.

D. PROTOCOLS

1. Voltage-$\dot{V}max$ Relationships

These relationships were studied by two methods: the steady-state condition and the membrane responsiveness.

a. Steady-state Condition

The study of the relationship between $\dot{V}max$ and membrane voltage in this condition, was done by brief depolarization of the membrane with 75 to 100 µl of 3 M KCl, added to the chamber under continuous perfusion, the preparation being driven at a constant cycle length of 2000 ms. Each addition of KCl allowed recording of - action potentials at membrane potentials of - 80 mV to -45 mV.

b. Membrane Responsiveness

The preparations were driven at a constant cycle length of 2000 ms, and following every 10 regular stimuli, an extrastimulus (early extrasystole) of the same characteristics (2 ms duration, and 4 times threshold intensity) as the basic stimulus, was delivered with a

progressive shortening of the diastolic interval, decreased stepwise toward the effective refractory period. The diastolic interval was controlled with a Digitimer Unit, which triggered the Pulsar 6, at the precise time chosen by the investigator. For the determination of the membrane responsiveness curve, the diastolic intervals were decreased by 2 to 5 ms steps.

c. Determination of T

T was determined by interpolating early extrasystoles, as in the determination of the membrane responsiveness curve, but at diastolic intervals beginning at 750 ms after the basic stimulus and decreased stepwise toward the 95% repolarization level of the basic action potential. This point is referred to as the zero time. Some experiments were done with the micropipette in the same cell through the whole experiments. In others, it was necessary to reimpale. This was true especially in those experiments in which high calcium solutions were used and the preparations contracted very strongly. In these circumstances, the membrane responsiveness was redetermined either in the same fiber or in a fiber with characteristics which were as close as possible to the previous cell.

RESULTS

RESULTS

A. INFLUENCE OF HEART RATE ON $\dot{V}$MAX

The influence of heart rate on $\dot{V}$max has been well documented. Yamagishi and Sano (1967) reported that in rabbit atrium there was a linear relationship between $\dot{V}$max and frequency in the range of 4 to 12 H_Z. A similar relationship was also shown by Viersma et al (1968) in rabbit atrium. The results presented in Table 2 demonstrate the influence of rate of stimulation in driven left atria. In Figure 2, I have plotted the values of $\dot{V}$max against the frequency of stimulation. This figure shows a linear relationship. The effect of lidocaine, at 5×10^{-5}M (Table 2) on $\dot{V}$max, was in an opposite direction to that expected on the basis of a change in frequency due to the drug. This suggested that the drug is acting on different mechanisms: that which controls the sinus node pacemaker activity, and the one which controls the maximum rate of rise ($\dot{V}$max).

B. EFFECTS OF LIDOCAINE IN SPONTANEOUSLY BEATING RIGHT ATRIA

1. Effect on $\dot{V}$max and on Frequency

Early work done by Kao and coworkers (1959) in the dog demonstrated that lidocaine (1-2 mg/Kg) was able to increase the heart rate and the cardiac output. They demonstrated that this increase of rate was mediated centrally, and through the vagus reflex. On the other hand, Lieberman and coworkers (1968) demonstrated that the drug at a dose of 5 mg/Kg body weight, significantly decreased the heart rate. They also demonstrated that the drug was able to oppose the effect of vagal stimulation on the heart rate. Clinical studies

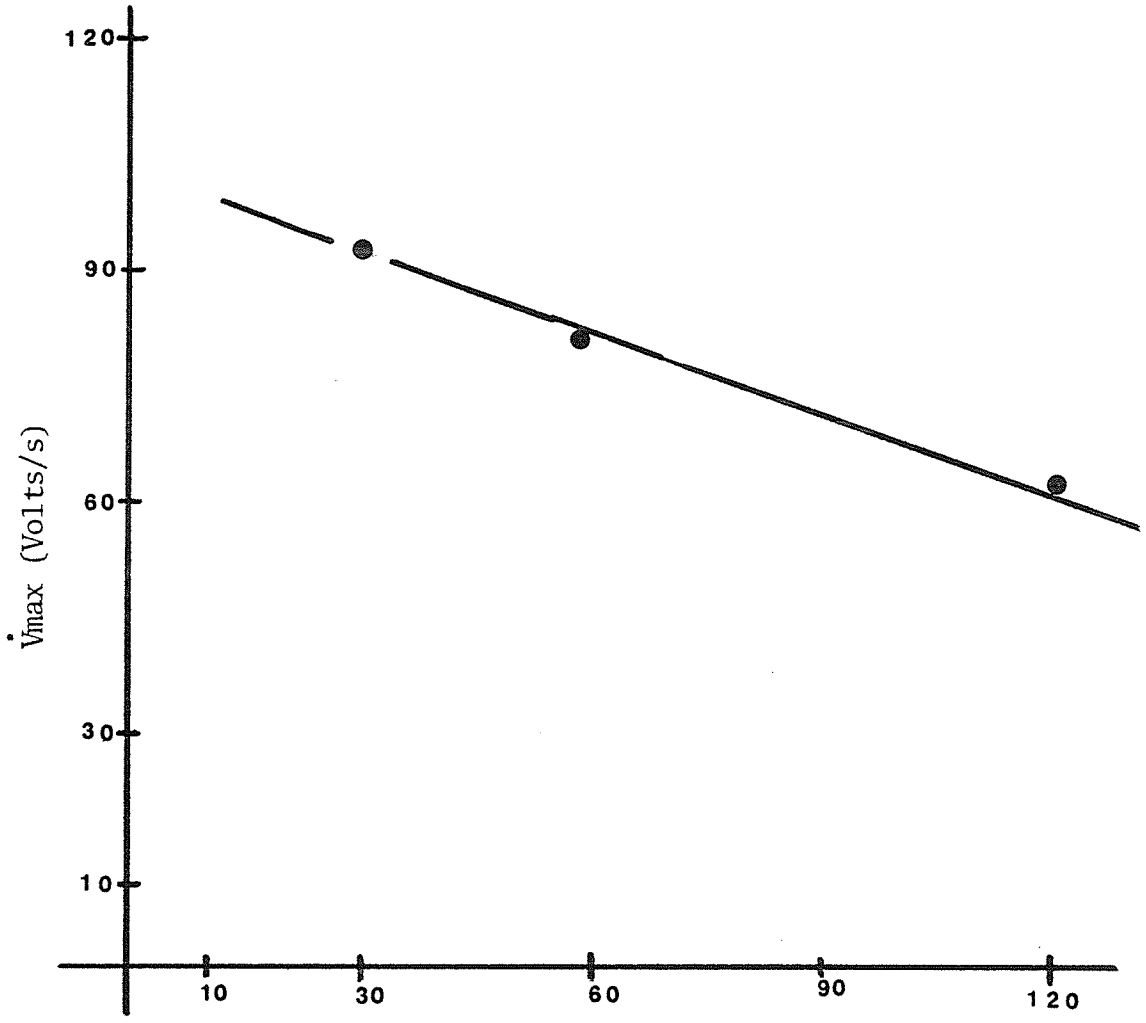


Fig. 2

Relationship Between Frequency of Stimulation and $\dot{V}$max.
Electrically Driven Left Atria.
Number of Preparations: 11, 7, and 5 Respectively.

TABLE 2

Effect of Lidocaine (5×10^{-5}M) on Rate and $\dot{V}max$ of Action Potentials in Spontaneously Beating Right Atria Compared with the Effect of Rate on $\dot{V}max$ in Electrically Driven Left Atria.

	N	n	RATE Beats/ min	$\dot{V}$MAX Volts/s
Right Atria				
Control	4	27	130±6.4	53.0±2.5
Lidocaine	4	50	104±4.6*	27.6±0.9*
Recovery	4	31	114±5.6	55.0±3.0
Driven Left Atria	5	31	120	60.9±3.5
(No Drug)	7	41	60	79.0±5.1*
	11	53	30	91.4±4.1*

*$P<0.01$. Values are means ± S.E. N = Number of Preparations; n = number of measurements.

have demonstrated that the effect of lidocaine on heart rate is not significant, (Jewitt and coworkers, 1968; Cullhed, 1969). Nevertheless, there are clinical reports which indicate that lidocaine significantly increased the heart rate in patients with sinus bradycardia (Ryden and coworkers, 1972), and in patients with normal or abnormal sinus node (Dhingra et al 1978). In our experiments, we found a significant decrease in heart rate in spontaneously beating right atria. Table 2 shows the mean values and the standard errors of the heart rate, as well as those of $\dot{V}max$. Lidocaine, $5 \times 10^{-5}M$, slowed the heart rate by approximately 20%. This effect of the drug was only partially reversed by washing the preparation with drug-free solution. $\dot{V}max$ was also significantly decreased by lidocaine, but was fully recovered after washing out the drug. Our results are in contrast with those reported by Mandel and Bigger (1971). They studied the effect of lidocaine in spontaneously beating atrial preparations in a concentration range of 1×10^{-7} to $5 \times 10^{-4}M$. They concluded that "lidocaine concentrations below $1 \times 10^{-4}M$ had no significant effect on the spontaneous firing rate or action potential configuration of S-A nodal cells". The reason for the discrepancy between their and our results, may be in the fact that we used an external potassium concentration of 4.7 mM, which is within the range of that reported for normal humans, whereas they used low potassium concentration in the perfusate. Our results on $\dot{V}max$, are in agreement with those reported by Singh and Vaughan Williams (1971). These authors reported a significant decrease of $\dot{V}max$ with lidocaine at a

concentration of 11.2 μg/ml, which is considered in the range of toxic effect of the drug; their results were done in 6 mM external potassium concentration. We have presented significant results which correlated well with both conditions: a) potassium concentration close to the human plasma concentration, and b) lidocaine concentration within the normal accepted therapeutic range.

2. Effects of Lidocaine on the Action Potential Characteristics

The effects of lidocaine, 5×10^{-5}M, on the membrane resting potential (M.R.P.) action potential overshoot (O.S.), and action potential duration (A.P.D.), are presented in Table 3. The results are the means and S.E. The drug decreased significantly only the action potential overshoot. In contrast to its effect on Purkinje fibers or ventricle, lidocaine at this concentration did not decrease the action potential duration at any of the three levels of repolarization (at the end of the overshoot or 0 level, 50 and 100%) at which it was studied. These results on AP duration are in agreement with those of Singh and Vaughan Williams (1971).

C. EFFECTS OF LIDOCAINE ON THE ACTION POTENTIAL CHARACTERISTICS OF DRIVEN LEFT ATRIUM

Figure 2 shows a linear relationship between $\dot{V}$max and frequency of stimulation. The basic stimulating frequency chosen was that at which $\dot{V}$max was maximum. Table 4 shows the mean values and their S.E. of M.R.P., action potential overshoot, $\dot{V}$max, and action potential duration of left atrial cells, driven at 30 pulses per minute. The results are comparable to those obtained with the spontaneously beating preparations. The drug (5×10^{-5}M) decreased significantly

TABLE 3

Effects of Lidocaine 5 x 10^{-5}M on M.R.P., Overshoot and Duration of the Spontaneously Beating Right Atria.

	M.R.P. -mV	A.P. overshoot mV	Duration, ms		
			Zero Level	50% Repolar-ization	100% Repolar-ization
Control	61.7±1.0	14.7±0.7	29.9±2.0	57.0±4.2	196±9.8
Drug	60.8±1.1	11.5±0.5*	26.5±1.6	54.0±3.1	217±13.5
Recovery	62.9±0.9	13.8±0.5	23.0±1.7	48.0±3.5	197±11

* $P<0.01$
N = 4 (Rabbits)
n = 21-54 (observations)

TABLE 4

Effect of Lidocaine, 5 x 10^{-5}M on M.R.P. Overshoot, $\dot{V}$max and Duration of the Driven (30 Pulses-min.) Left Atria.

	M.R.P. -mV	A.P. Overshoot mv	$\dot{V}$max V/s	Duration, ms		
				Zero Level	50% Repol.	100% Repol.
Control	68.6±0.8	15.9±0.9	82.7±4.6	6.2±0.8	21.5±1.7	206±4.2
Drug	67.1±1.5	8.9±0.85*	74.3±2.7*	3.8±0.9	17.2±1.6	220±6.8
Recovery	71.8±0.7	10.3±1.2	90.7±6.2	4.3±1.3	17.2±1.9	239±5.9

*P<0.01
N = 6 Rabbits
n + 27-39 Observations

$\dot{V}$max and overshoot, without having any significant effect on the membrane resting potential, and AP duration.

Lidocaine, at a concentration of 1 x 10^{-5}M, did not modify significantly any of the electrophysiological characteristics of the action potentials. Table 5 shows the data.

D. EFFECT OF LIDOCAINE ON STEADY-STATE AND MEMBRANE RESPONSIVENESS RELATIONSHIPS

To investigate further the effects of the drug on atrial cells, we studied the relationships between the membrane potentials and $\dot{V}$max, by means of the two most commonly used techniques; depolarization with KCl, and the interpolated premature stimuli (early extrasystoles). I have previously mentioned that the effect of the drug on this relationship has been appropriately studied in ventricle (Chen et al, 1975) and Purkinje fibers (Rosen, et al, 1976; Bigger, 1970). In these structures, lidocaine has shifted the relationship along the voltage axis, toward a more negative value. Our results, on the steady-state condition, differ from those tissues.

1. Steady-state Relationship

Table 6 and Figure 3, represent the summarized results of eight experiments carried out in left atria, driven at a constant rate of 30 pulses/minute, in which the membrane voltages and $\dot{V}$max were measured after brief depolarization with 75 to 100 µl 3M KCl, before and after lidocaine at 5 x 10^{-5}M. In the abscissa of the graph, the membrane resting potentials have been grouped intervals of 10 mV, and the horizontal bars of each point, represents the S.E. of the means of each group. The ordinate represents the percentage decrease from

TABLE 5

Effects of Lidocaine 1 x 10^{-5}M on M.R.P., Overshoot, $\dot{V}$max and Duration of Electrically Driven (30 pulses/min.) Rabbit Left Atria

	M.R.P.	A.P. Overshoot mV	$\dot{V}$max V/s	Duration, ms		
				Zero Level	50% Repol.	100% Repol.
Control	77.8±1.6	18.6±0.9	105±.07	6.9±0.8	26.6±1.6	234±3.3
Drug	79.6±0.9	20.4±0.8	100.6±5.8	10.2±0.9	31.4±0.7	241±2.3
Recovery	78.6±0.8	16.8±1.2	94.0±4.3	8.6±0.95	27.0±0.8	242±5.6

N = 5 Rabbits
n = 17 to 19 Observations

TABLE 6

Effect of Lidocaine 5×10^{-5}M on the Steady-State Voltage-$\dot{V}$max Relationship

	Control		Drug	
Peak Values	94±3.6 V/s	-71.4±0.41 mV	68.57±2.9 V/s**	-71.4±0.72 mV
	% $\dot{V}$max	M.R.P. -mV	% $\dot{V}$max	M.R.P. -mV
	81.5±2.17	68.7±0.60	59.1±3.32**	68.2±0.71
	55.4±5.66	62.2±0.30	40.3±2.96*	63.2±0.59
	30.6±2.61	52.4±1.30	23.0±2.91(N.S.)	54.4±0.96

N = 8 Rabbits
n = 23-40 Observations
** P<0.01
* P<0.05
N.S. Not Significant

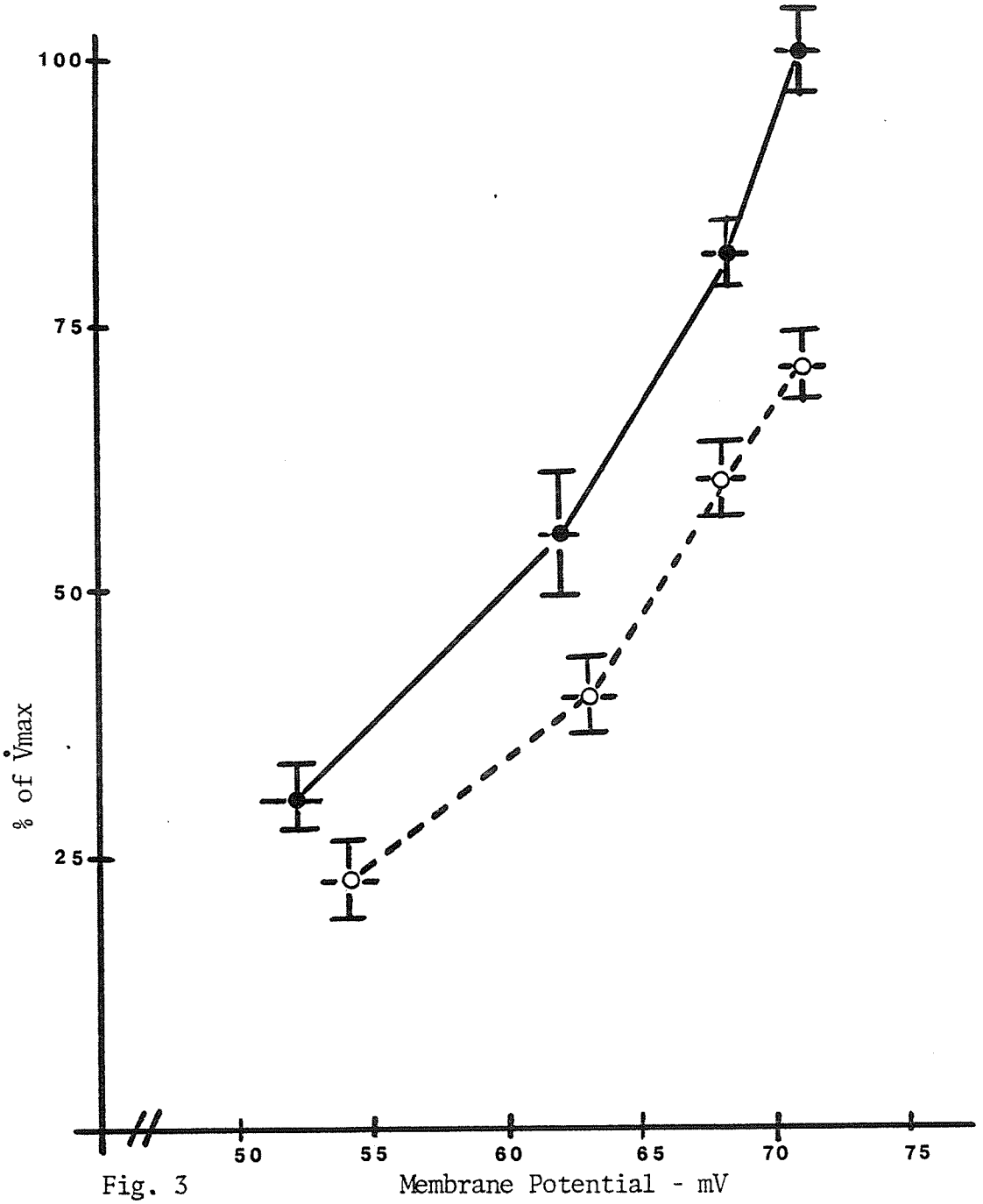


Fig. 3

Steady-state Relation of the Membrane Potential to $\dot{V}max$. Left Atria. ● Control ○ Lidocaine $5x10^{-5}$M. Preparations = 8

peak $\dot{V}max$ at each mean voltage, and the vertical bars of the points, represent the S.E. of the means of the percentage of peak $\dot{V}max$. The figure shows three facts: a) The drug did not shift the relationship on the voltage axis; b) The values of $\dot{V}max$ were significantly decreased at all the voltages, except the last group in the range of -50 to -55 mV; c) The tendency of the curves to converge toward more positive voltages; this indicates that the maximum effect of the drug is obtained at higher values of membrane potentials. Therefore, the relationship may be described as a downward shift, rather a shift to the right. This effect of lidocaine is different from that seen in Purkinje fiber and ventricles. The values of the voltages at which $\dot{V}max$ was decreased by 50% from the peak value of each experiment are shown in Table 7, before and after treatment with lidocaine, 5×10^{-5}M. This measurement of half inactivation of $\dot{V}max$, is analogous to but different from the term Vh (Weidmann, 1955), a value which can only be determined if the cells were hyperpolarized in order to obtain the maximum value of $\dot{V}max$. Nevertheless, this measurement gives an estimation of membrane voltage - $\dot{V}max$ relationship at a reference point. The results presented in Table 6 give a mean value of -59.±2.4 mV for control, and -61.5±2 mV for the treatment with lidocaine; these two values are not significantly different.

The effect of lidocaine at 1×10^{-5}M in the steady-state conditions, are given in Tables 8 and 9 and in Figure 4. The drug at this concentration did not modify the relationship; this differs from its effect in Purkinje fibers and ventricles. Our results in atria indicate that

TABLE 7

Effect of Lidocaine 5 x 10^{-5}M on the Steady-state Vm-$\dot{V}$max Relationship at 50% of $\dot{V}$max.

Peak $\dot{V}$max V/s		Membrane Potential at 50% $\dot{V}$max -mV	
CONTROL	DRUG	CONTROL	DRUG
56	48	60	62
79	43	62	63
103	78	61	51
103	49	62	63
97	80	65	70
119	108	64	66
88	59	58	62
76	25	43	55
N = 8	8	8	8
$\bar{x}$ = 90.1	62.5	59.4	61.5
S.D.M. ±6.9	±8.9	±2.5	±2.1

TABLE 8

Effect of Lidocaine, 1×10^{-5}M on the
Steady-state Membrane Voltage - $\dot{V}$max Relationship

	Control		Drug	
Peak Values	V/s 105.8±5.0	-mV 79.6±2.92	V/s 100.6±5.84	-mV 78.8±2.33
	%$\dot{V}$max	M.R.P. -mV	% $\dot{V}$max	M.R.P. -mV
	77.0±5.42	77.87±.83	67.33±4.6	78.2±.46
	53.1±6.64	71.3±.47	46.0±7.5	71.44±.52
	44.62±7.48	67.62±.46	41.33±5.92	66.55±.44
	27.69±3.19	61.92±.41	27.85±4.57	61.42±.57
	12.25±2.52	57.5±.64		

N = 5 Rabbits
n = 8 - 14 Observations

TABLE 9

Effect of Lidocaine 1×10^{-5}M on the Steady-state Vm - $\dot{V}$max Relationship at 50% $\dot{V}$max.

PEAK $\dot{V}$MAX VOLTS/S		Vm AT 50% $\dot{V}$MAX - mV	
CONTROL	DRUG	CONTROL	DRUG
105	95	70	75
102	105	72	73
102	95	67	70
125	121	66	63
95	87	68	67
N = 5	5	5	5
$\bar{x}$ + 105.8	100.6	68.6	69.6
S.D.M. ± 5.07	±5.84	±1.08	±2.1

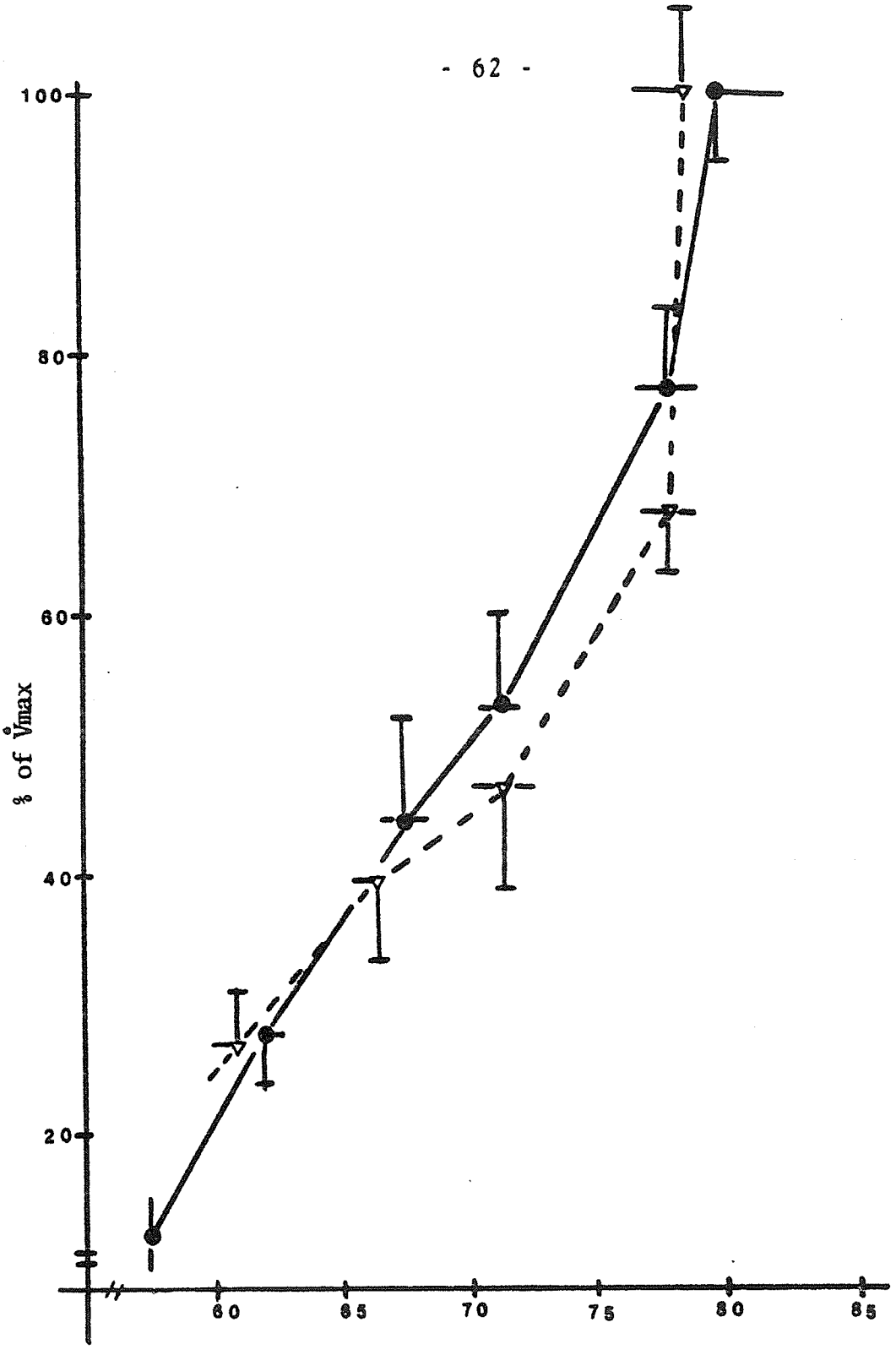


Fig. 4

Steady-state Relation of the Membrane Potential to $\dot{V}$max Left Atria. ● Control. ▽ Lidocaine 1×10^{-5}M. Number of Preparations = 5.

they are less sensitive to the action of lidocaine.

2. 'Membrane Responsiveness'

Straus and Bigger (1972) demonstrated in atrial cells that the membrane voltage-$\dot{V}$max relationship was different according with the method used. They demonstrated that with the method of the interpolated stimuli ('early extrasystole'), $\dot{V}$max, compared at the same voltage level was less than that observed at the steady-state condition. We had similar results. Figure 5 shows the membrane voltage-$\dot{V}$max relationship done by the two methods mentioned before in the absence of drug. In this experiment, both curves were obtained with the microelectrode in the same cell. This experiment demonstrates that the dependence of $\dot{V}$max is given by two factors: voltage and time. In the steady-state relationship (O) at a resting potential of -85 mV, $\dot{V}$max is 330 volts/s; whereas at the same voltage level, in the 'membrane responsiveness', $\dot{V}$max is 270 volts/s. This difference represents a decrease of 12% in $\dot{V}$max. This percentage increases as the membrane potential is more positive. For example, at -80 mV, $\dot{V}$max is 19% less in the 'membrane responsiveness' than in the steady-state; and at -70 is 50%. Another important result is that in the steady-state relationship the tail of the curve reaches a value of -50 mV, whereas, in the 'membrane responsiveness' there is not such a tail, and the cell did not respond to a stimulus beyond -67 mV. Therefore, there is a shift along the voltage axis of 14 mV toward the right. Gettes and Reuter (1974) reported a similar shift in guinea pig papillary muscle. They explained the decrease of $\dot{V}$max due to the 'early extrasystoles', as a result of changes in the sodium conductance. This shift toward the right in the

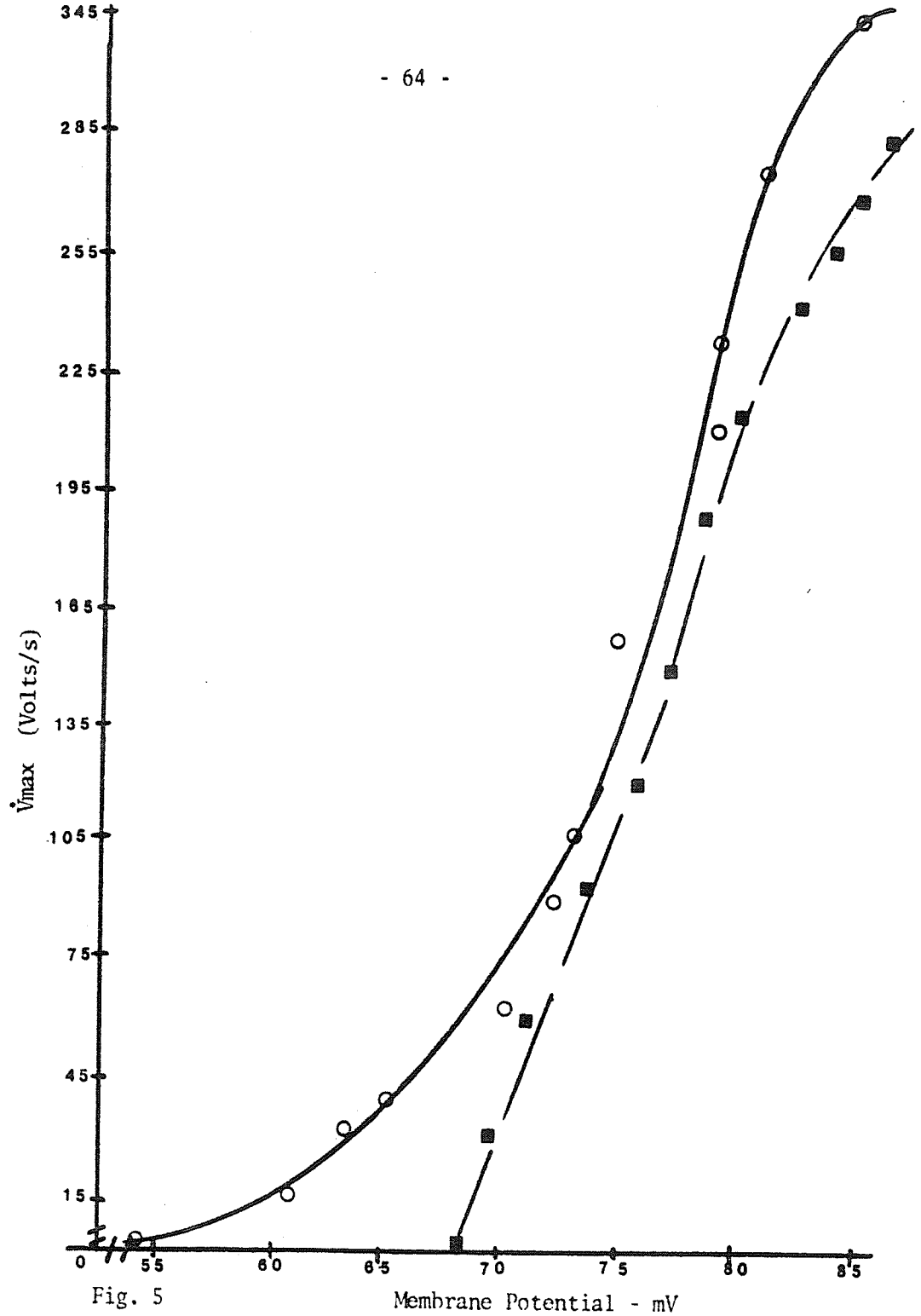


Fig. 5

Comparison of the Voltage - $\dot{V}$max Relationships.
○ Steady-state. ■ Responsiveness Curve.
One Preparation. Microelectrode in the same Cell.

'membrane responsiveness' curve in comparison with the steady-state relationship also indicates that the recovery process of the sodium channels is not an instantaneous process, and a certain time is required to a full recovery of the system. The effect of lidocaine 5×10^{-5}M on the 'membrane responsiveness' is illustrated in Figure 6. The graph shows the effect of the drug mainly on $\dot{V}max$, this effect being greater at higher values of voltage. The membrane responsiveness differs from that of steady-state condition in the way that the curve does not converge at lower voltage values. In Table 10, I have presented the values of $\dot{V}max$ and membrane voltage at three different levels; a) peak $\dot{V}max$, b) when $\dot{V}max$ was 40 to 50% of peak, and c) $\dot{V}max$ at the minimum interval at which an extrasystole was able to induce a response. The data shows that lidocaine 5×10^{-5}M, significantly decreased peak $\dot{V}max$, without a significant change in the membrane resting potential; at half inactivation, $\dot{V}max$ was also significantly decreased by lidocaine without a significant change of membrane potential at which this occurred; at the minimum interval for a response to an extrasystole, it is clearly evident that the effective refractory period (E.R.P.) in control condition, occurred at lower membrane potential than that of the treated cells; on the other hand, at this level, $\dot{V}max$ was not different from treatment. This indicates a shift toward the right at this low potential. This is supported by data shown in Figure 6. This fact, together with the lack of effect of the drug on the action potential duration, indicated that lidocaine prolonged the effective refractory period. In the control condition,

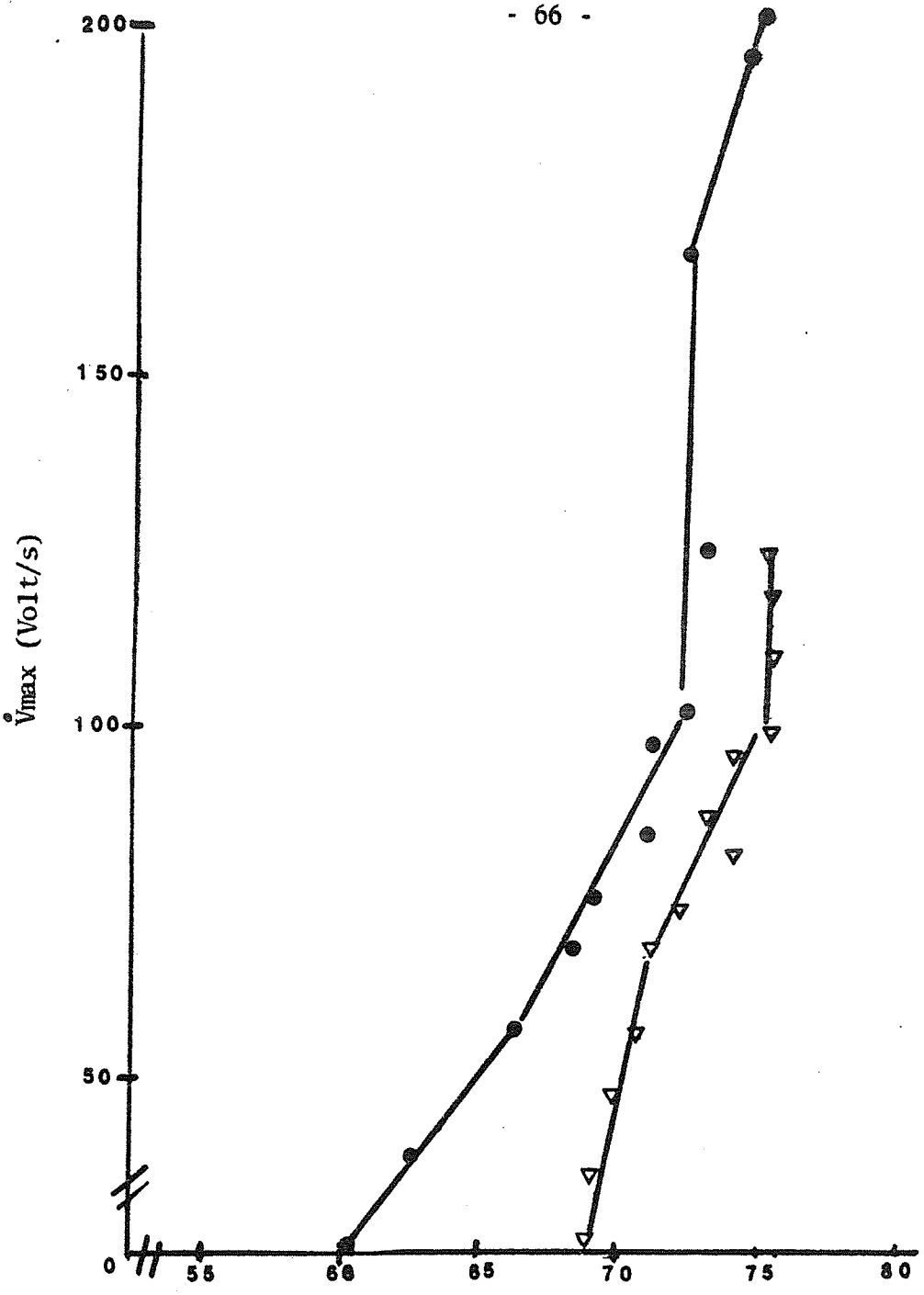


Fig. 6 'Membrane Responsiveness Curve". ● Control ▽ Lidocaine 5×10^{-5}M. One Preparation. Microelectrode in the same Cell.

TABLE 10

Effect of Lidocaine, 5×10^{-5}M on Membrane Potential and $\dot{V}max$ at Three Levels: a) At Peak Values
b) 40 to 50% of Peak $\dot{V}max$
c) At the Effective Refractory Period (E.R.P.)

CONTROL				LIDOCAINE			
Peak Values: 71.9±0.9mV; 136±9.2 V/s				Peak Values: 71.8±8±0.9mv; 81±7.1 V/s			
40-50%		E.R.P.		40-50%		E.R.P.	
mV	$\dot{V}max$	mV	$\dot{V}max$	-mV	$\dot{V}max$	-mV	$\dot{V}max$
65.1	75	56.7	19	70	62	68.6	47
61.9	58	54.3	16	69	37	65.8	10
75.3	99	73.3	74	77.9	58	75.9	25
67.9	72	60	25	71.6	64	70	56
67.3	46	57	7	68.6	40	63.7	14
63.2	103	51	34	60.6	37	54.6	12
63.3	90	54.7	12	66.6	75	60	30
64.3	75	62	41	71.3	49	69.3	38
68.6	79	67.3	58	72.6	56	70	44
67.3	75	64	64	69.9	62	66	10
64.6	77	61	32	66.6	55	64	27
63.9	99	60.6	74	65.3	74	63.3	27
63.3	47	61.9	7	61.3	16	60	9
58.8	61	55	15	60	67	55.6	8
N =							
14	14	14	14	14	14	14	14
Mean =							
65.34	75.42	59.91	34.14	67.95	53.71	64.77	25.5
S.E. =							
±1.03	±4.85	±1.55	±6.48	±1.34	±4.38	±1.57	±4.25
A	B	C	D	a'	b'	c'	d'

A vs a' t = 1.54 P>0.05**
B vs b' t = 3.32 P<0.01*
C vs c' t = 2.20 <P 0.05*
D vs d' t = 1.11 P>0.05

the mean value of the effective refractory period, (defined as the minimum interval at which an extrasystole is able to elicit a response) was 99.8±6.69 ms, whereas, that of the treated cells was 156.25±11.69. ($P<0.001$).

We observed consistently that repetitive activity of the atrial cells occurred after an extrastimulus which approached the effective refractory period. Figure 7 illustrates the intracellular recording of such a paroxysmal tachycardia. Lidocaine, at 5×10^{-5}M, was able to prevent the occurrence of this repetitive activity in 12 out of 14 experiments.

The effects of lidocaine, 1×10^{-5}M on the membrane responsiveness are presented in Table 11, and illustrated in Figure 8. We observed that in some experiments the drug shifted the 'membrane responsiveness' curve in a downward direction, and a slight shift toward more negative voltages at low membrane potentials. This effect is illustrated in Figure 8. Nevertheless, the overall values of 10 experiments, summarized in Table 11, demonstrate that lidocaine at 1×10^{-5}M does not modify the values of $\dot{V}$max at any of the levels of membrane potential compared. Also, the shift toward the right along the voltage axis observed with the high concentration of the drug at the E.R.P., was not significantly different from control condition. This fact correlates well with the lack of effect of the drug at 1×10^{-5}M upon the E.R.P. The mean value of the E.R.P. in control condition was 95.7±7.1 ms and that of the treated atrial cells was 113.8±11.39 ms. It was also observed that at this concentration the drug prevented the paroxysmal atrial tachycardia due to the early extrasystoles in only two of 12 experiments.

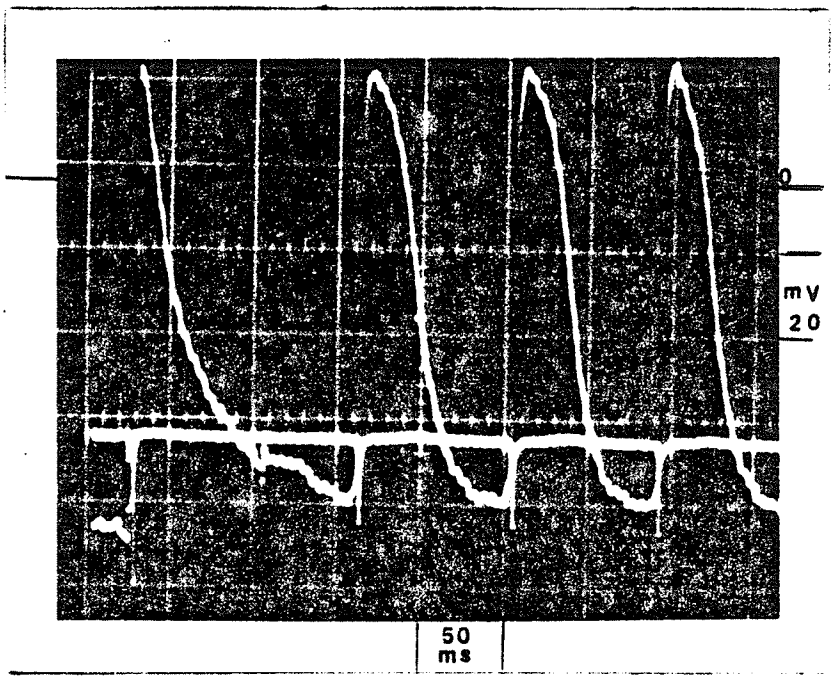


Fig. 7 Intracellular Recording of a Burst of Sustained Rhythmic Activity Triggered by an Early Extrasystole. Rabbit Left Atrium.

TABLE 11

Effect of Lidocaine 1 x 10^{-5}M on Membrane Potential and $\dot{V}$max at Three Levels: Peak Values, 40-50% of Peak $\dot{V}$max, and at the Effective Refractory Period (E.R.P.)

CONTROL				LIDOCAINE 1 x 10^{-5}M			
Peak Values: -76.78±1.16 mV; 115±11.0 V/s				Peak Values: -76.6±1.15 mV; 110±10.12			
40-50%		E.R.P.		40-50%		E.R.P.	
-mV	V/s	-mV	V/s	-mV	V/s	-mV	V/s
64.6	42	63.3	32	70	31	68	24
66.6	47	65.1	18	64	40	62	16
68.6	43	63.7	33	66.5	45	56	12
72.1	51	70	31	72.8	53	70.7	35
61.9	37	58.6	15	69	33	61.3	9
68.6	46	65.8	24	70	47	67.2	29
70	56	65	10	70	55	67	15
70.0	62	65.8	22	71.4	53	66.5	25
76.6	86	72.0	43	77	77	74	32
69.0	58	67.2	28	71.4	60	67.9	24
N=							
10	10	10	10	10	10	10	10
$\bar{x}$ =							
68.8	52.8	65.6	25.6	70.21	49.4	66.1	22.1
S.D.M. =							
±1.26	±4.43	±1.16	±3.09	±1.10	±4.28	±1.61	±2.76
A	B	C	D	a	b	c	d

P: N.S. for all the comparisons

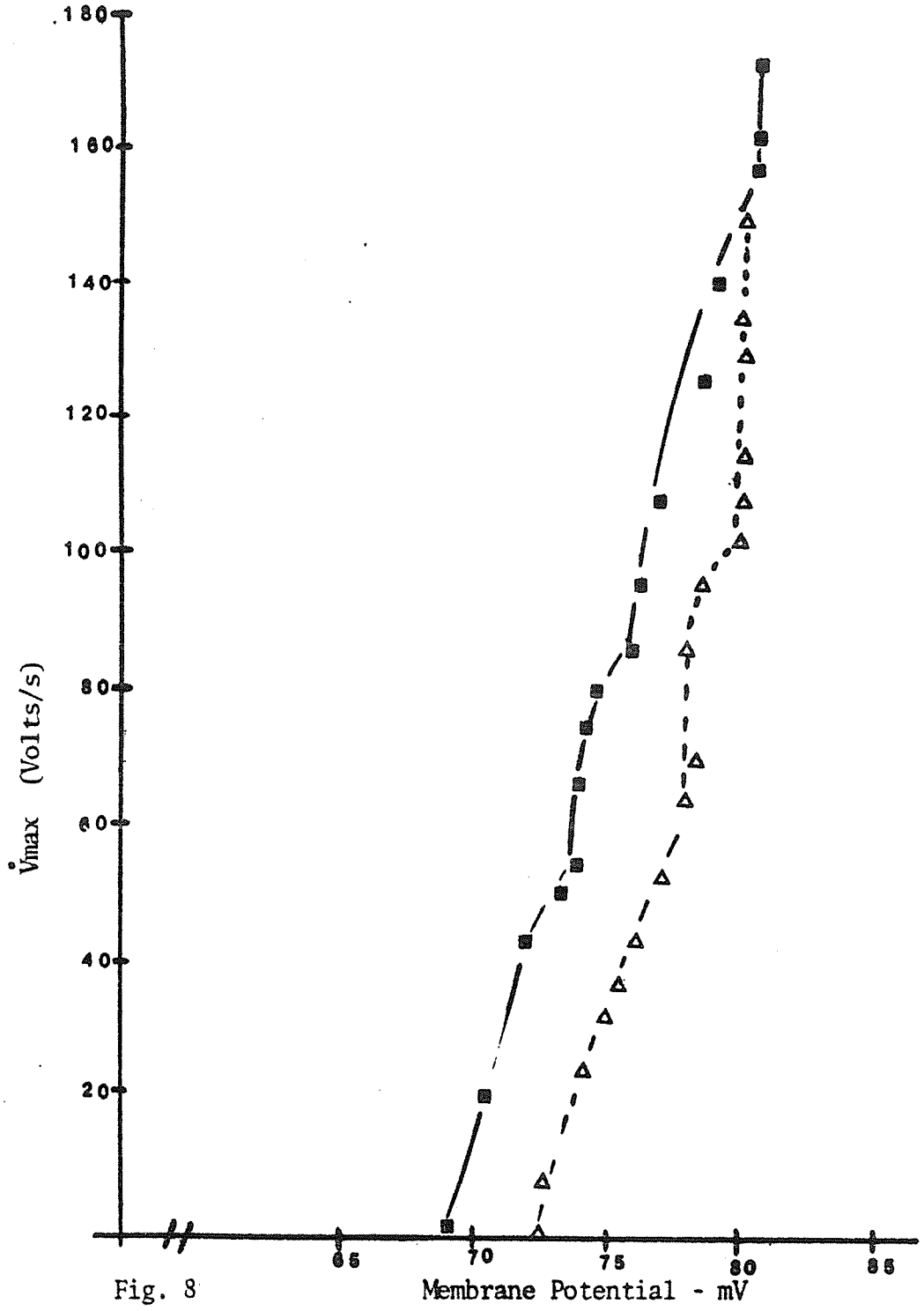


Fig. 8

"Membrane Responsiveness Curve". ■ Control
△ Lidocaine $1x10^{-5}$M. One Preparation.
Microelectrode in the same Cell.

The results presented up to this point demonstrate that: a) lidocaine at a concentration of 5×10^{-5}M, is able to prevent the electrically induced paroxysmal tachycardia; b) the main effect of the drug is on the overshoot of the action potential, and on $\dot{V}max$; c) the drug does not modify the action potential duration; d) the membrane voltage-$\dot{V}max$ relationship in the steady-state condition is shifted downward, and demonstrating that its maximum effect on $\dot{V}max$ is at higher values of membrane potential; e) the membrane responsiveness also demonstrated a downward shift at higher voltage levels, and a shift toward the right at lower membrane potentials; f) lidocaine at 5×10^{-5}M, significantly prolonged the E.R.P. These results suggested that lidocaine may be affecting the recovery from inactivation of the sodium channels.

E. TIME DEPENDENCE OF RECOVERY OF SODIUM CHANNELS

The recovery of the sodium channels have been studied in nerve (Hodgkin and Huxley, 1952) and in Purkinje fibers (Weidmann, 1955), and in frog atrial cells (Haas et al 1971) by the double pulse voltage clamp technique. Recent reports presented by Gettes and Reuter (1974) in Purkinje fibers and ventricles, and by Chen et al (1975) in ventricles have demonstrated that the kinetics of recovery of the sodium channels could be studied by the method of interpolated extrasystoles (Straus and Bigger, 1972). We used the last method in order to study the effect of lidocaine on the recovery of $\dot{V}max$. These results are presented in Table 12 and illustrated in Figure 9 and 10. We studied the relationship between $\dot{V}max$ of the extrasystoles and the diastolic interval. The diastolic interval was taken to begin at 95%

repolarization (zero time) of the basic action potential, to the phase zero of the action potential generated by the extrasystoles. The 95% repolarization level of the basic action potential was taken as a reference point (zero time) for determination of T, in order to have a more precise point to measure the diastolic intervals. This was due to the fact that in atrial cells the late repolarization is a downward concave shape process, in contrast to the sharp ending in Purkinje fibers and ventricles. Figure 9 shows a monoexponential relationship from an infinite time toward the approach of zero time. This relationship could be studied by the formula $Y_t = Y_o(1-e^{-k/t})^T$. The time constant (T) is defined as the time required for Y to decrease 1/e of its original value at the zero time. In the abscissa of Figure 9 are represented the diastolic intervals in ms, and on the ordinate the values of $\dot{V}max$ of the action potential induced by the extrasystoles. Each point in the graph represent the mean values, and the vertical and horizontal bars, the standard error of $\dot{V}max$ and diastolic interval respectively. The value of T found in the control situation was 52 ms, (for a mean M.R.P. of -71 mV). This value of T is different from that obtained by Haas and coworkers (1971) for frog atrial tissue. They reported values of T in the range of 100 to 600 ms. The difference in values could be explained by the species studied, and mainly for the fact that Haas and coworkers did the experiments at temperatures of 4 to 7°C, whereas our temperature was kept in the range of 30 ± 0.5°C. The value of 52 ms for T in our atrial cells, at a membrane potential

TABLE 12

Effect of Lidocaine 5×10^{-5} M on the Time Dependence of $\dot{V}max$

CONTROL		LIDOCAINE 5×10^{-5} M	
Diastolic Interval ms	$\dot{V}max$ V/s	Diastolic Interval ms	$\dot{V}max$ V/s
554±7.5	133±8.5	545±7.5	107±15.1
455±8.0	132±8.6	441±9.8	104±12.0
362±5.6	133±8.8	352±5.6	102±12.2
267±5.7	133±8.9	249±9.3	97±12.2
164±5.2	131±9.3	145±8.7	82±6.9
74±5.0	124±10.1	55±11.3	61±15.4
19±5.0	106±12.8	5±8	18±2.5
0	92±5.0	0	14±3.4

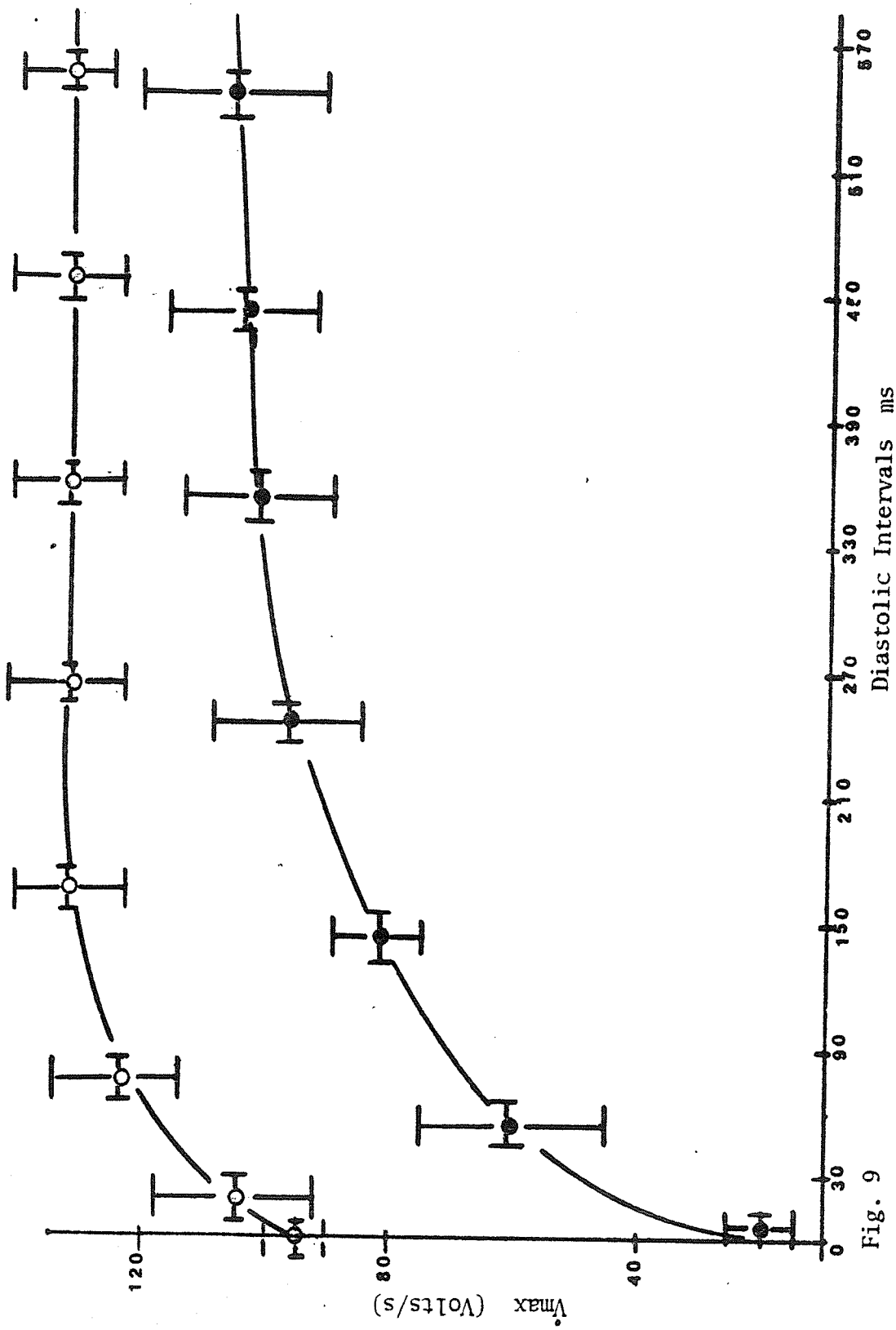


Fig. 9 Time Dependence of Recovery of $\dot{V}$max. ○ Control. ● Lidocaine 5x10-5M. Number of Preparations = 6.

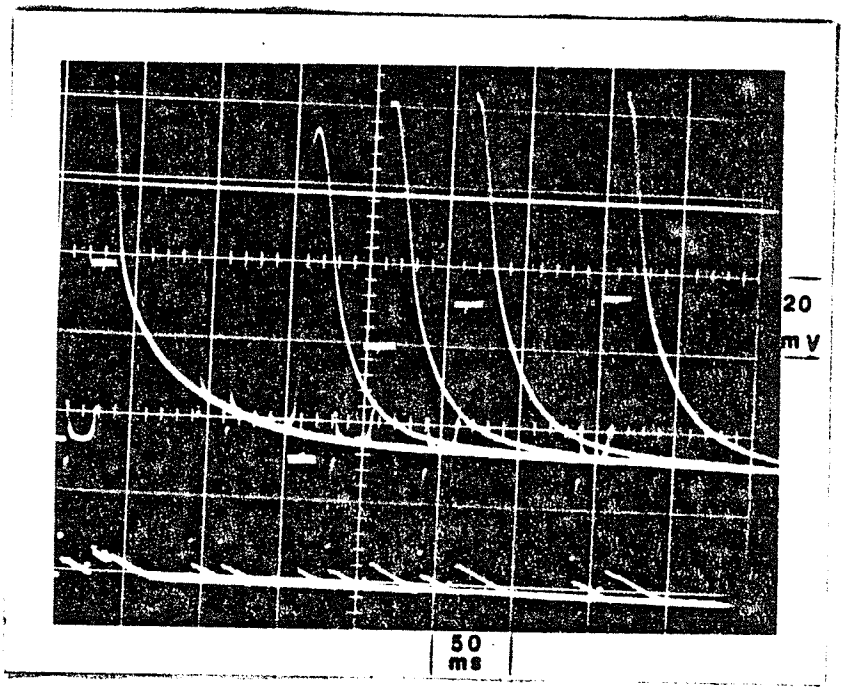


Fig. 10 Intracellular Recording of Rabbit Left Atrium Action Potentials. Influence of the Diastolic Intervals on A.P.D. and $\dot{V}max$.

of - 71 to -72 mV, is well correlated with the results obtained for ventricles and Purkinje fibers in a membrane potential range of -68 to -76 mV (Gettes and Reuter, 1974).

Lidocaine, at 5 x 10^{-5}M prolonged T from 52 ms to 100 ms (six preparations). This result supports the suspected effect upon the recovery from inactivation of sodium system. Lidocaine, at 1 x 10^{-5}M prolonged T, even though it was not antiarrhythmic at this concentration; this effect of the low concentration of the drug is illustrated in Figure 11. The effect of the lower dose of the drug on T in this experiment was greater than the mean values obtained with lidocaine at 5 x 10^{-5}M. This example shows the variability of the preparations, because the mean value of T after treatment with lidocaine 1 x 10^{-5}M in twelve preparations was 79±7 ms. This effect of lidocaine on T is similar to those reported for ventricles (Chen et al 1975) and for Purkinje fibers (Weld and Bigger, 1975).

F. EFFECT OF CALCIUM ION ON LEFT ATRIA

1. Introduction

To study further the different effect of lidocaine in the supra-ventricular type of arrhythmias, we investigated the possibility that calcium ion might be involved. The idea of the possible role of calcium came from four different lines of evidence: 1. Our observation that adrenaline, at high concentration, was able to reverse the effect of lidocaine on Vmax; 2. The evidence presented by Silva Graca and Van Zwieten (1972), showing that lidocaine at 5 x 10^{-5}M significantly reduced the rate of calcium exchange in beating guinea pig atria;

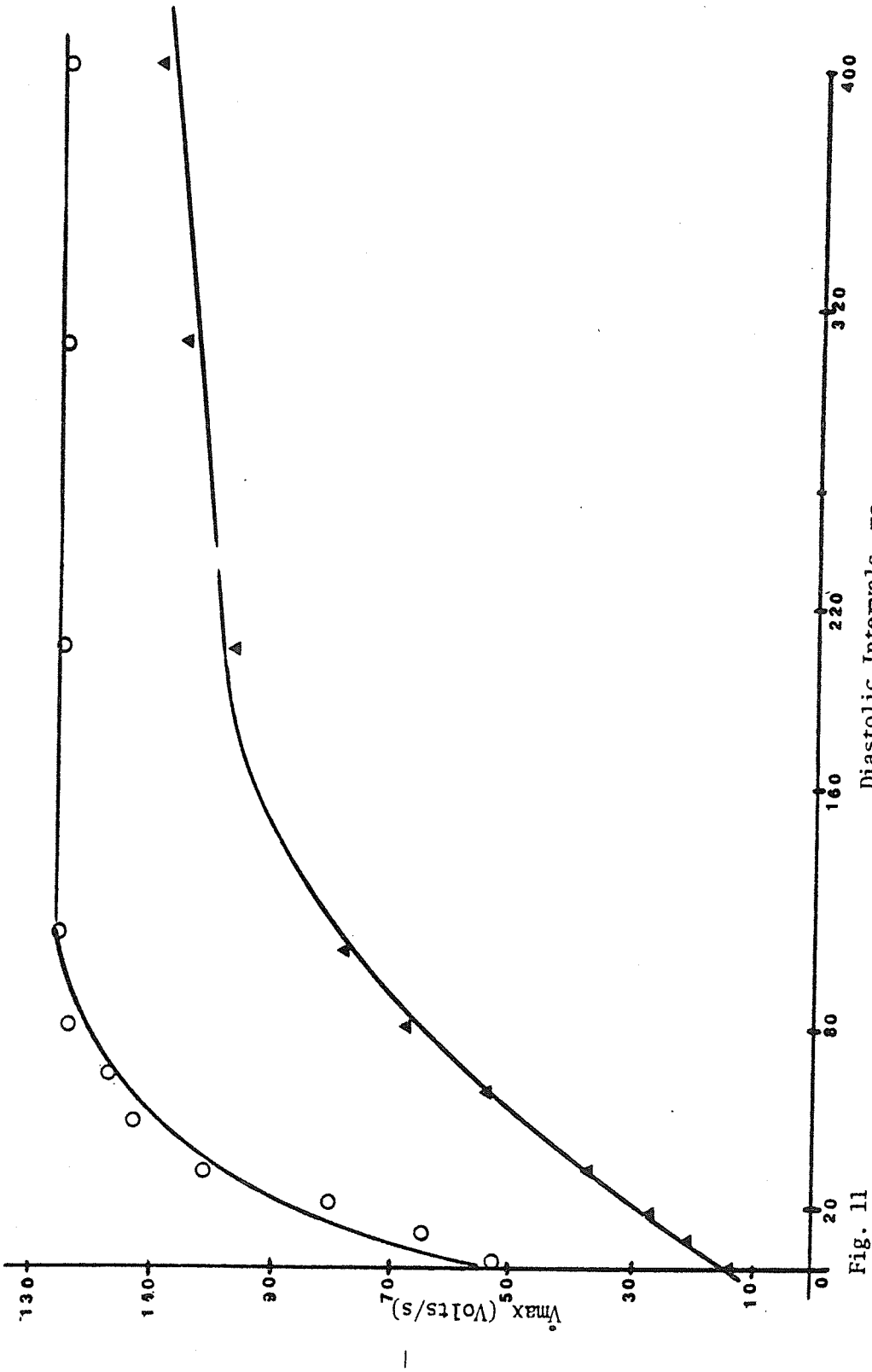


Fig. 11

Time Dependence of Recovery of $\dot{V}_{max}$. O Control ▲ Lidocaine $1x10^{-5}$M
One Preparation. Microelectrode in the same Cell.

3. The work of Fukuda (1975) which showed that in three different species, the total and "cellular" calcium content of the atria were higher than in the ventricles; 4. The evidence recently presented by Ruiz-Ceretti and Ponce-Zumino (1976), showing that the upstroke of the atrial action potential had two different components, one of them being dependent on the external calcium, and the other on the external sodium concentration.

Thus changes in the external calcium concentration might modify the effect of lidocaine on $\dot{V}max$ and the recovery from inactivation of the sodium system of the rabbit atria. If this were true, then decreasing the external calcium concentration should enhance the effect of the drug, whereas an increase would antagonize its effect. The results presented in this section confirm this hypothesis.

2. Effects of Calcium Alone

a) On the Characteristics of the Action Potential

The effects of changes in the external calcium concentration on the characteristics of the action potential are presented in Tables 13 and 14. The tables show the mean differences ($\bar{D}$) and their standard deviations (S_D) of the paired values, either from 2.5 mM $Ca^{2+}{}_o$ to 1.25, or from 2.5 to 5.0 mM. Lowering the external calcium concentration caused a small but significant depolarization of the cells and a decrease in $\dot{V}max$, but no significant changes in the amplitude or the effective refractory period. The action potential duration was prolonged at the zero potential level, but there was no significant change at 50 and 95% repolarization levels. The low calcium concentration significantly prolonged the time constant (τ) for the recovery from inactivation of

TABLE 13

Effect of 1.25 mM $(Ca^{2+})_o$ on the Electrophysiological Parameters of Rabbit Atria

	Resting Membrane Potential -mV	Overshoot mV	Amplitude mV	Duration ms			$\dot{V}_{max}$ V/s	T ms	Effective Refractory Period ms
				Zero Level	50% Repol.	95% Repol.			
$\bar{D}$	4.11	1.62	5.72	-5.83	-3.77	23.5	15.33	-15.5	11.92
S_D	4.61	6.57	9.33	4.01	6.35	36.83	11.61	11.1	32.63
t	2.96	0.81	2.03	4.81	1.96	2.11	4.37	4.63	1.21
P	<.02	NS	NS	<.001	NS	NS	<.01	<.001	NS

$\bar{D}$: mean difference

S_D: standard deviation of the mean difference

Degrees of freedom = 11

TABLE 14

Effects of 5.0 mM $(Ca^{2+})_o$ on the Electrophysiological Parameters of Rabbit Atria

	Resting Membrane Potential -mV	Overshoot mV	Amplitude mV	Duration ms			$\dot{V}_{max}$ V/s	T ms	Effective Refractory Period ms
				Zero Level	50% Repol.	95% Repol.			
$\bar{D}$	-3.39	-5.01	-8.33	3.65	7.15	-30.68	-43.18	22.93	2.18
S_D	3.14	6.65	7.61	3.17	6.11	23.27	35.8	21.24	36.74
t	4.18	2.92	4.25	4.5	4.55	5.10	4.66	4.18	0.29
P	<.01	<.02	<.01	<.001	<.001	<.001	<.001	<.01	NS

$\bar{D}$: mean difference

S_D: standard deviation of the mean

degrees of freedom = 15

the sodium system. The modification of τ will be discussed in more detail later. Increasing the calcium concentration significantly hyperpolarized the cells, increased the action potential amplitude and $\dot{V}max$, and had a dual effect upon the action potential duration, shortening it at the zero potential and 50% repolarization levels, and lengthening it at the 95% level. This biphasic effect on the duration is in agreement with that reported by Klein and Holland (1959). Our results of higher external calcium concentration on $\dot{V}max$ are in contrast to those presented by Weidmann (1955) and Temte (1967) for Purkinje fibers; in this tissue, an increase in the external calcium concentration to two or four times the normal value produces a decrease rather than an increase in $\dot{V}max$. Our results are in agreement with the results presented by Ruiz-Ceretti and Ponce-Zumino (1976), suggesting that during the phase zero of the atrial action potential, calcium and sodium enter the cell; $\dot{V}max$ should increase as a consequence.

b) On the Membrane Responsiveness Curve

The effect of increasing the calcium concentration on the membrane responsiveness is illustrated in Figure 12. This figure shows a representative experiment, in which the microelectrode was kept in the same cell during the perfusion with 5.0 mM Ca^{++}. The relationship is shifted in an upward direction and to the left at the higher calcium concentration; the figure also shows the hyperpolarization of the cell and convergence of the curves at the lower membrane potentials. This same pattern of relationship in the high calcium concentration was observed in twelve preparations. This relationship is different from

that observed in Purkinje fibers (Weidmann, 1955). (See Discussion) The effective refractory period was unchanged at the high calcium concentration; this fact is well correlated with the convergence of the curve at lower membrane potentials.

The effect of 1.25 mM Ca^{2+}_o on the "membrane responsiveness" curve is presented in Figure 13. Each point of the graph shows the mean values and the S.E. of seven preparations. The observations of the membrane potentials were grouped in 5 mV intervals. The figure demonstrates; a) there is a significant depolarization of the cells on lowering the external calcium concentration; b) the value of $\dot{V}max$ is significantly decreased; c) the decrease in $\dot{V}max$ is greater than that expected at similar lower membrane potentials, compared with control situation; d) the relationship may be described as a downward shift.

c. On Recovery of $\dot{V}max$

Gettes and Reuter (1974) demonstrated that an increase in the external calcium concentration shortened the time constant (τ) of the recovery from inactivation of the sodium system in Purkinje fibers as well as in ventricles. Our results in atrial cells are shown in Figure 14A. The abscissa of the figure represents the variation of the external calcium concentration in mM, and the ordinate the time constant (τ) of the recovery from inactivation of the sodium system. This figure shows that the higher the external calcium concentration, the shorter the recovery process is, and that this relationship is a linear one in the range of concentrations tested.

Davis (1967) and Weidmann (1955) showed that an increase in the

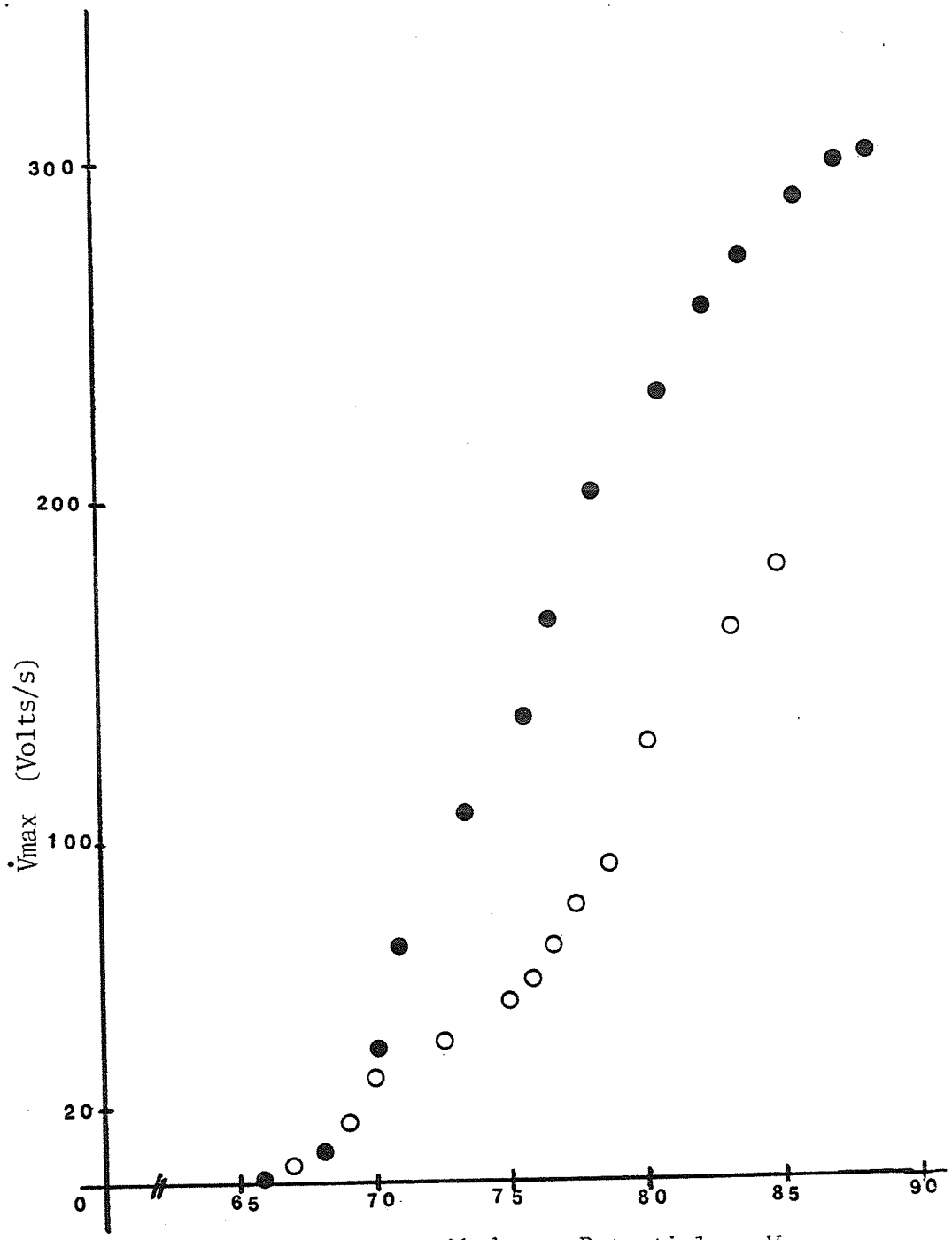


Fig. 12 "Membrane Responsiveness Curve". ○ Control. ● 5mM$(Ca^{2+})_o$. One Preparation. Microelectrode in the same Cell.

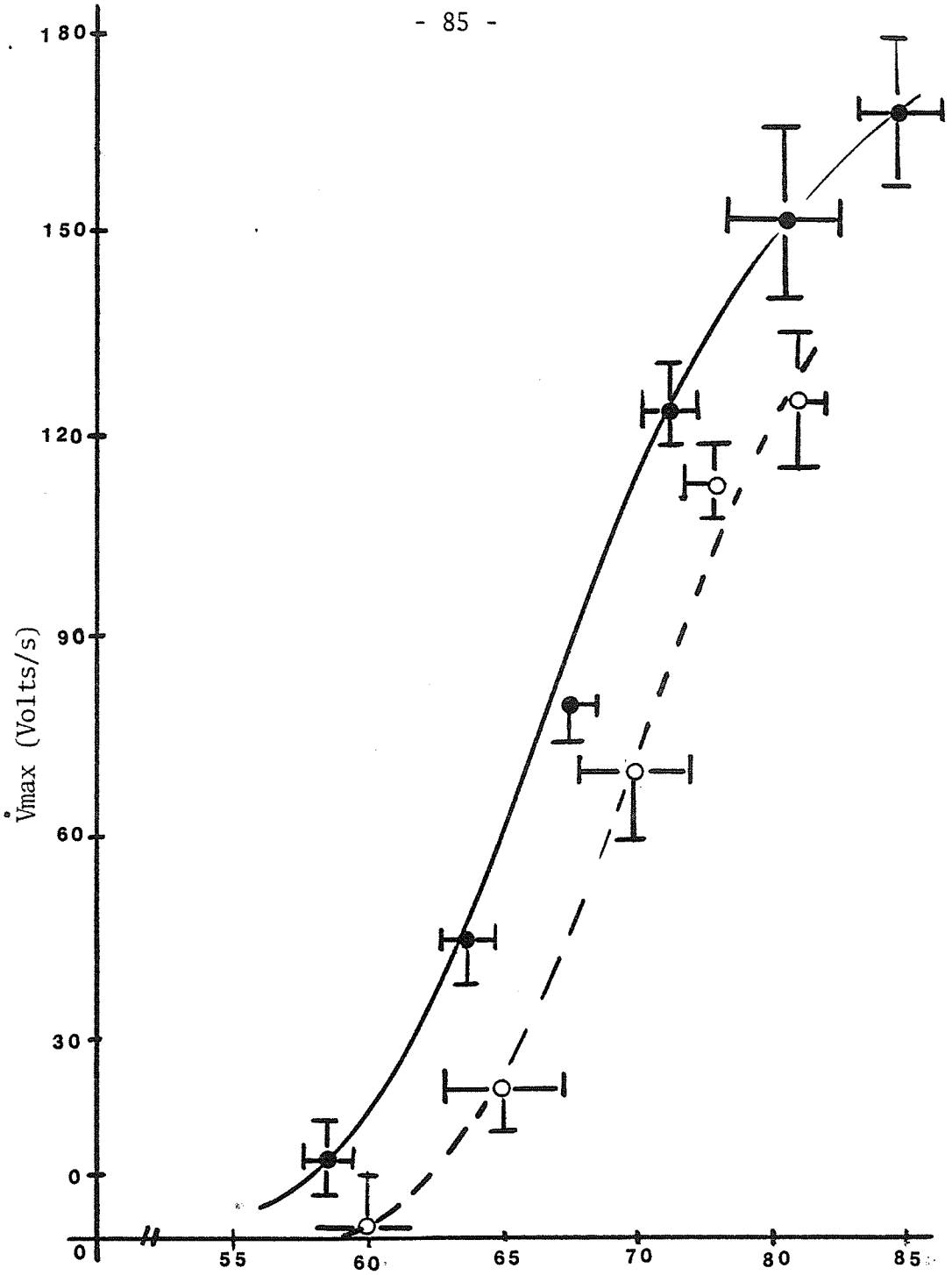


Fig. 13 "Membrane Responsiveness Curve" ● Control.
○ 1.25 mM$(Ca^{2+})_o$. Number of Preparations = 7

external calcium concentration does not change significantly the membrane resting potential in Purkinje fibers. Weidmann (1955) demonstrated in Purkinje fibers, that the time constant (T) for the recovery from inactivation of the sodium system was voltage dependent. This was corroborated by Gettes and Reuter (1974), whose study of the effect of Ca^{2+} on T did not require them to take into account the M.P. In our experiments in atrial cells, variations in the external calcium concentration did cause significant changes in the membrane potential. We therefore compared the relationship between T and the changes in the membrane potential due to variations in the external calcium concentration with the equivalent changes in M.P. induced by variations in K^+ concentration. Such a relationship is presented in Figure 14B. The curve drawn through the crosses represents the mean of 12 to 16 preparations at external calcium concentrations of 1.25, 2.5, and 5.0 mM respectively. The results show a curvilinear relationship with a downward concavity, and demonstrate that the recovery from inactivation of the sodium system is also a voltage dependent process.

In order to test the degree of influence of the calcium ion itself upon the variation of T, experiments were done in which the membrane resting potential was changed by adding KCl and T determined. The results of such experiments are also presented in Figure 14B. This study was done in seven preparations, in which the external potassium concentration was either increased or decreased from the value of 4.7 mM KCl, considered as control condition. T was first determined at this normal potassium concentration, and the the $(KCl)_o$ was raised to 9.4 mM, and after an equilibration time of 10 to 15 minutes, T was again

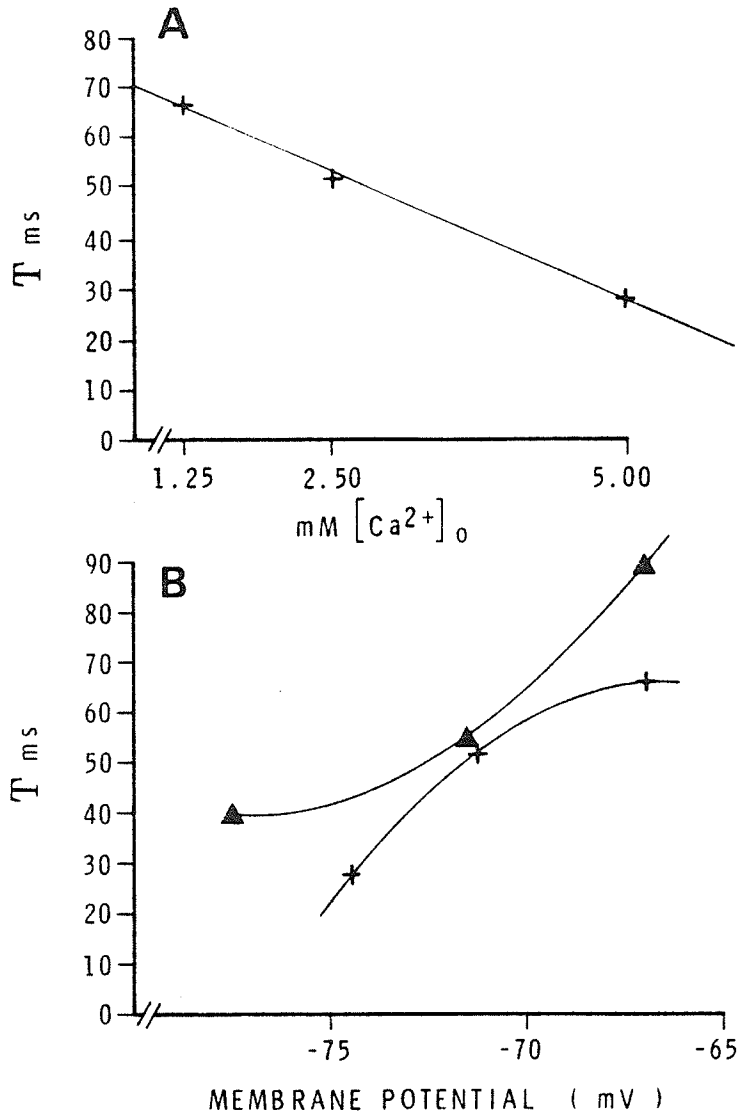


Fig. 14 A Relationship between the External Calcium Concentration and T.

Fig. 14 B Relationship between T and Variation of the Membrane Potential due to Changes in the External Ca^{2+} (+) or KCl (▲).

determined; then $(KCl)_o$ was dropped to 2.35 mM, and T determined after the equilibration time. The membrane potential and T, at 4.7 mM $(KCl)_o$ in this group of experiments were similar to those in which $(Ca^{2+})_o$ and $(KCl)_o$ were 2.5 and 4.7 mM respectively. At 9.4 mM $(KCl)_o$, the mean values of T and membrane potential were 93 ± 7.67 ms and -62 ± 1 mV, whereas at 2.35 mM $(KCl)_o$, T was 40 ± 4 ms and the membrane potential was -77.5 ± 2.mV. We found a curvilinear relationship between T and the membrane potential with an upward concavity. This indicates that at equivalent low resting potentials, the prolongation of T is less prominent when the external calcium concentration is lowered to half its normal value than when an increase in the external potassium concentrations occurs. Also, the shortening of T is greater, with the hyperpolarization produced with higher external calcium. If the role of calcium in shortening T were only due to the hyperpolarization of the cells, then we would have expected further shortening of T with the lower external potassium concentration (2.35 mM), because the cells were more hyperpolarized at this potassium concentration but T was significantly longer than that obtained with 5.0 mM Ca^{++} and less degree of hyperpolarization.

d. On the Paroxysmal Tachycardia

In our experiments, during the perfusion with high calcium concentration, the paroxysmal atrial tachycardia was seen with an interval of 10 to 15 ms earlier than control condition, indicating that the mechanism for the production of the tachycardia was facilitated by the ion. Also it was observed that 5 preparations developed spontaneous beats in the presence of higher calcium concentration, which indicates

an enhancement of automaticity. Similar enhancement of automaticity was observed in 3 tissues bathed in 2.35 mM of KCl.

3. INTERACTION OF LIDOCAINE AND CALCIUM

The effect of lidocaine, at two concentrations 1×10^{-5}M and 5×10^{-5}M was compared at three calcium concentrations: 1.25 mM, 2.5 and 5.0 respectively. The comparison of the effect of lidocaine at 1×10^{-5}M between 1.25 and 2.5 mM $(Ca^{2+})_o$ is presented in Tables 15 and 16. These tables show the mean differences ($\bar{D}$) and their standard deviations(S_D) of the paired values of control and treatment, at the two different calcium concentrations. As I have mentioned before, the lower concentrations had no significant effect upon the action potential when the calcium concentration of the medium was 2.5 mM, but T was prolonged without a concomitant prolongation of the effective refractory period by the drug.

When the calcium concentration was lowered to 1.25 mM, lidocaine significantly decreased the overshoot of the action potential, the total amplitude, and $\dot{V}max$ without having a significant effect upon the membrane potential or the duration of the action potential at 50 and 95% levels. However, the drug significantly shortened the duration at the zero potential. The recovery from inactivation of the sodium system was greatly slowed by the drug at this low calcium medium, and T could only be determined in four out of eleven experiments. This was due to the significant prolongation of the effective refractory period beyond the time to 95% repolarization of the basic action potential, which was chosen as the zero time for the determination of T. Figure 15 shows the slowing of recovery of $\dot{V}max$ in a preparation in which the effective

TABLE 15

Effects of Lidocaine (1×10^{-5} M) on the Electrophysiological Parameters of Rabbit Atria Bathed in 1.25 mM $(Ca^{2+})_o$

	Resting Membrane Potential -mV	Overshoot mV	Amplitude mV	Duration ms			$\dot{V}_{max}$ V/s	τ ms	Effective Refractory Period ms
				Zero Level	50% Repol.	95% Repol.			
$\bar{D}$	1.55	7.69	9.13	5.49	-0.5	10.18	35.27	35.5	-42.81
S_D	2.36	6.30	7.43	6.50	8.51	16.43	15.78	39.67	39.43
t	2.08	3.86	3.88	2.67	0.18	1.95	7.06	1.54	3.43
P	N.S.	<.01	<.01	<.05	N.S.	N.S.	<.001	N.S.	<.01
df	10	10	10	10	10	10	10	3	10

$\bar{D}$: mean difference
S_D: standard deviation of the mean
degrees of freedom = 10 except for τ (df=3)

TABLE 16

Effect of Lidocaine (1×10^{-5} M) on the Electrophysiological Parameters of Rabbit Atria Bathed in 2.5 mM $(Ca^{2+})_o$

	Resting Membrane Potential -mV	Overshoot mV	Amplitude mV	Duration ms			$\dot{V}$max V/s	τ ms	Effective Refractory Period ms
				Zero Level	50% Repol.	95% Repol.			
$\bar{D}$	0.17	2.94	2.58	0.0	0.15	0.95	4.9	-29	9.08
S_D	0.35	6.01	5.79	3.11	4.01	11.4	8.0	21.27	22.96
t	1.63	1.62	1.48	--	0.12	0.27	2.0	4.52	1.31
P	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	N.S.	<.01	N.S.

$\bar{D}$: mean difference

S_D : standard deviation of the mean

degrees of freedom = 11

refractory period was less than the duration of the action potential. Figure 15B illustrates the result obtained in a preparation in which the drug greatly prolonged the effective refractory period.

The effect of lidocaine (1×10^{-5}M) in the low calcium concentration on the 'membrane responsiveness curve' is illustrated in Figure 16. The figure is a representative of four experiments. In this low calcium concentration, the drug is shifting the relationship in a downward direction, mainly at more negative potentials. For instance, at a level of -74 to -75 mV, the values of $\dot{V}max$ after the treatment with the drug are lower, eventhough the M.P. is the same. This fact shows that the time dependent factor of $\dot{V}max$ has been affected. Also it is evident that the treated cell did not respond to the electrical stimulation, beyond -64 mV membrane potential, as a consequence the E.R.P. was prolonged by lidocaine. The effect of the lower concentration of lidocaine in 1.25 mM $(Ca^{2+})_o$ on the membrane responsiveness curve is different than that observed with the higher dose of lidocaine has more effect $(Ca^{2+})_o$. In the lower calcium concentration, lidocaine has more effect upon the time component of the recovery of $\dot{V}max$. In seven experiments, the cells did not respond to the electrical stimulation beyond -70 mV and τ could only be determined in four experiments. The paroxysmal tachycardia was prevented in nine out of eleven experiments.

Due to the variability of $\dot{V}max$ among the preparations, we performed a covariance analysis with lidocaine 1×10^{-5}M, at the two calcium concentrations. Figure 17 shows the results of such study. In the abscissa we have represented the values of $\dot{V}max$ of all the experiments at 2.5 mM $(Ca^{2+})_o$, as well as the values at

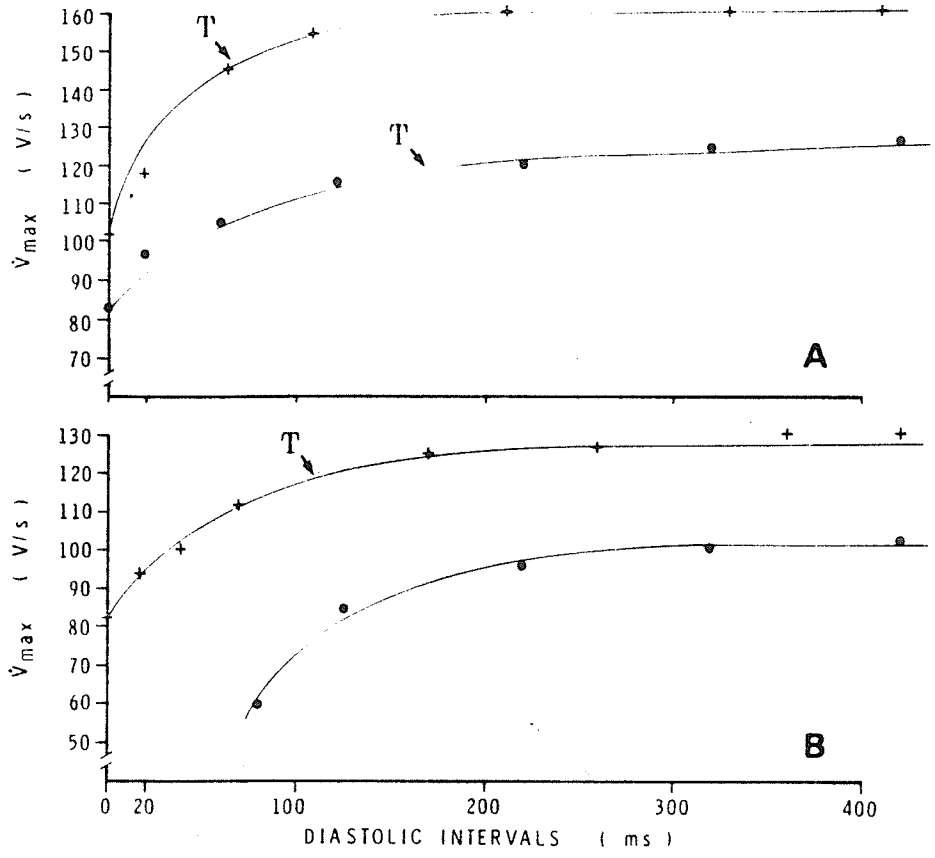


Fig. 15A and 15B

Effect of Lidocaine $1x10^{-5}$M (●) on the Time Dependence of Recovery of Vmax. 1.25 mM $(Ca^{2+})_o$. + Control. Two Preparations. Microelectrode in the same Cell.

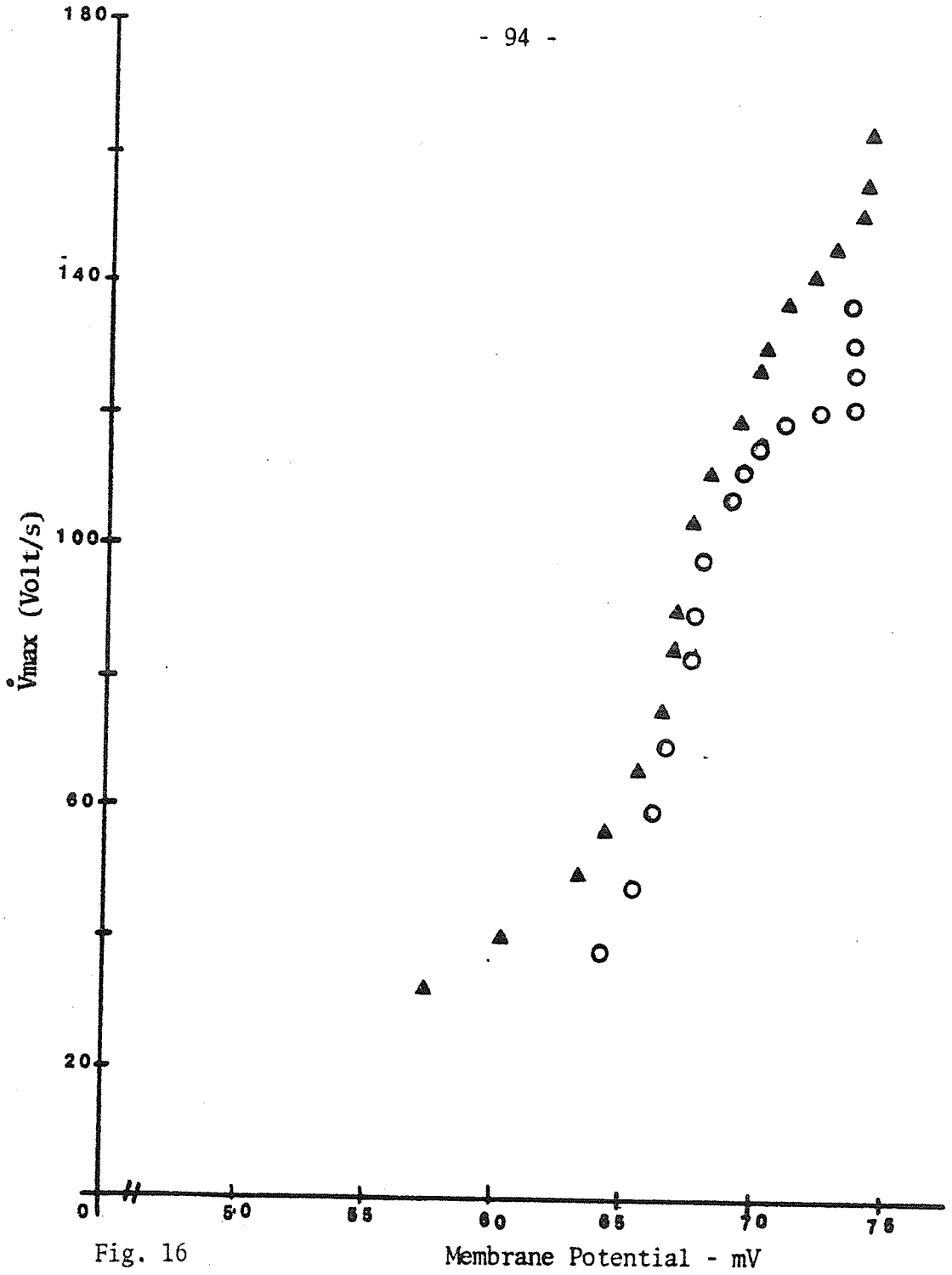


Fig. 16

"Membrane Responsiveness Curve" Effect of Lidocaine (O) $1x10^{-5}$M. ▲ Control. 1.25 mM$(Ca^{2+})_o$. One Preparation. Microelectrode in the same Cell.

1.25 mM (Ca^{2+}). In the ordinate are plotted the values of the differences in $\dot{V}max$, after the treatment with lidocaine 1 x 10^{-5}M in both calcium concentrations. These relationships indicate two facts: a) that the effect of lidocaine was independent of the values of $\dot{V}max$ previous to the treatment with the drug, because the slopes of the lines are almost zero; and b) that the effect of the drug on $\dot{V}max$ was statistically greater at the low calcium concentration than at 2.5 mM (Ca^{2+}), because the mean difference was higher at the lower calcium medium (34.7 and 4.8 respectively). These results indicate that lidocaine, at 1 x 10^{-5}M, a concentration which is effective in the Purkinje fibers, is not effective in atrium when the external calcium concentration is 2.5 mM but it becomes effective when the external calcium concentration is lowered to half the normal value.

The drug did not modify the action potential duration at 50 and 95% levels, at the low calcium media which may indicate that the K^+ conductance is not modified. The comparison of the effect of lidocaine, 5 x 10^{-5}M, at 2.5 mM and 5.0 mM of external calcium concentration is presented in Tables 17 and 18. The drug at this concentration in the high calcium produced almost the same effects as it did in the normal calcium concentration, except that the drug did not prolong significantly the effective refractory period, and only prevented the paroxysmal tachycardia in two out of six experiments. Nevertheless, the effect of lidocaine at 5 x 10^{-5}M on $\dot{V}max$ was statistically significantly greater at the normal calcium concentration, than at 5.0 mM $(Ca^{2+})_o$ ($t=3.4$; $P<0.01$; $df=18$). The effect of lidocaine at 5 x 10^{-5}M was also tested in three preparations bathed at 1.25 mM $(Ca^{2+})_o$; the drug caused toxic

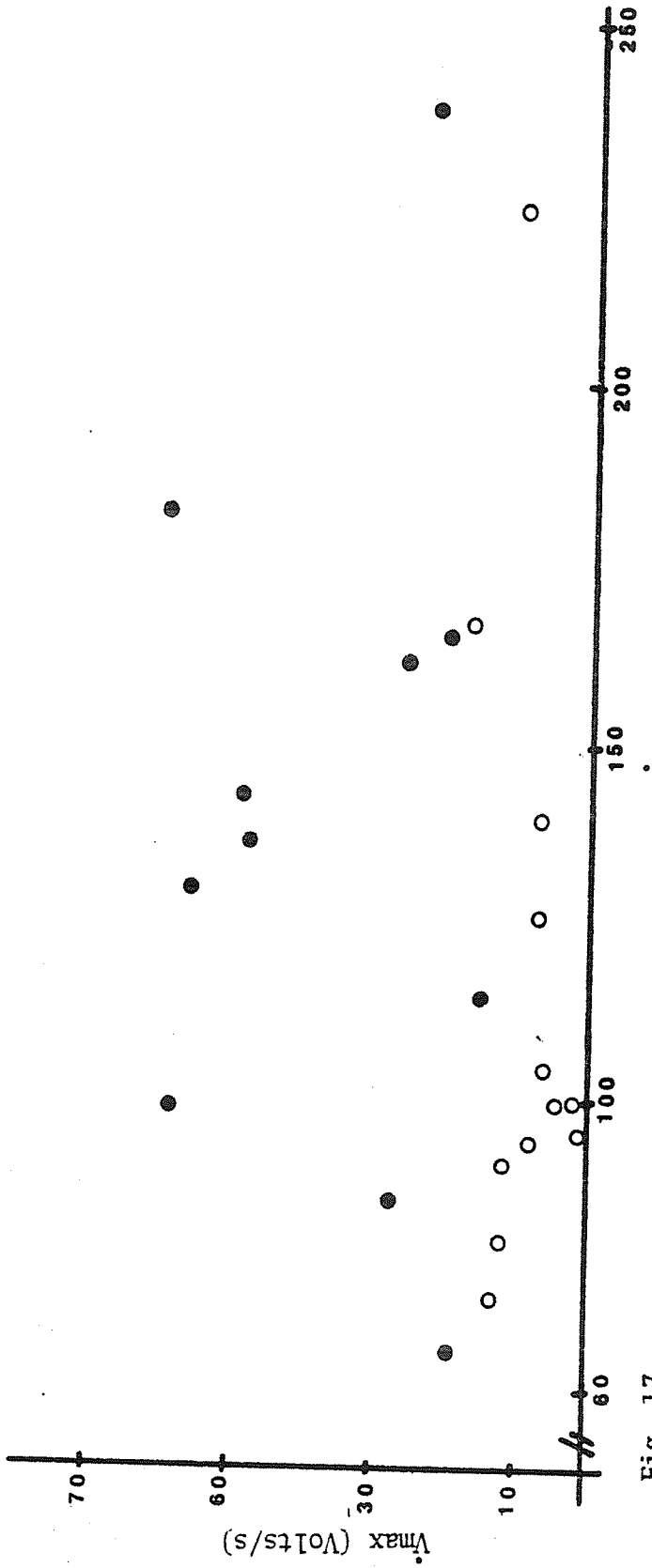


Fig. 17

Relationship between the Values of V̇max at two External Calcium Concentration: 1.25 mM (●), 2.5 mM (○) and the Difference after Treatment with Lidocaine $1x10^{-5}$M. Number of Preparations = 12.

effects represented by decrease of membrane resting potential, $\dot{V}max$, and inexcitability of the cells in all of them.

G. CHANGES IN ACTION POTENTIAL DURATION DUE TO THE EXTRASYSTOLES

The changes in the shape and duration of the action potential due to the extrasystoles is illustrated in Figure 18 and 20. Figure 18 shows the intracellular recording of two action potentials, the basic action potential (first from left to right) obtained at a constant cycle length of 2000 ms, and an action potential originated by an extrasystole delivered 230 ms after the basic action potential. The premature potential is different in shape, and the duration at the zero potential and the 50% levels is longer than those of the basic action potential. Figure 19 shows photographs of two action potentials obtained at a faster sweepspeed and superimposed. The extrasystole was delivered 750 ms after the basic one. This figure shows that the slope of the repolarization process is steeper in the basic action potential than it is in the premature action potential.

1. Effect of Calcium Ion

Figure 20 shows the relationship between the duration at zero potential level of the premature action potentials and the diastolic intervals. The ordinate of the figure represents the difference of duration (ms) between the basic action potential and the mean value of premature action potentials at each particular diastolic interval. The abscissa represents the diastolic intervals in ms. The comparisons were done at three external calcium concentrations: 1.25 mM (8 preparations), 2.5 mM (14 preparations) and 5.0 mM (5 preparations). The figure shows that the recovery of the duration at the zero potential

TABLE 17

Effect of Lidocaine (5×10^{-5}M) on the Electrophysiological Parameters of Rabbit Atria Bathed in 2.5 mM $(Ca^{2+})_o$

	Resting Membrane Potential -mV	Overshoot mV	Amplitude mV	Duration ms			$\dot{V}_{max}$ V/s	τ ms	Effective Refractory Period ms
				Zero Level	50% Repol.	95% Repol.			
$\bar{D}$	0.08	8.46	8.29	1.12	1.16	1.06	54.87	-61.66	-58.87
S_D	0.94	6.34	6.39	2.26	6.52	10.85	25.6	49.81	43.84
P	N.S.	<.001	<.001	N.S.	N.S.	N.S.	<.001	<.001	<.001
t	0.33	4.99	5.02	1.92	0.69	0.37	8.29	4.10	5.19
df	15	15	15	15	15	15	15	11	15

$\bar{D}$: mean difference
S_D: standard deviation of the mean
df: degrees of freedom = 15
Except for τ, df = 11

TABLE 18

Effect of Lidocaine (5×10^{-5}M) on the Electrophysiological Parameters of Rabbit Atria Bathed in 5.0 mM $(Ca^{2+})_o$

	Resting Membrane Potential -mV	Overshoot mV	Amplitude mV	Duration ms			Vmax	τ ms	Effective Refractory Period ms
				Zero Level	50% Repol.	95% Repol			
$\bar{D}$	-0.20	6.05	5.78	1.0	0.5	-0.33	28.5	-58.0	51.5
S_D	0.35	3.53	3.41	1.82	1.25	2.80	14.51	23.15	51.78
t	1.28	3.82	3.78	1.22	0.88	0.26	4.39	4.33	2.22
P	N.S.	.02	.02	N.S.	N.S.	N.S.	.01	.01	N.S.
df	5	5	5	5	5	5	5	3	5

$\bar{D}$: mean difference
S_D: standard deviation of the mean
df: degrees of freedom = 5
Except for τ, df = 3

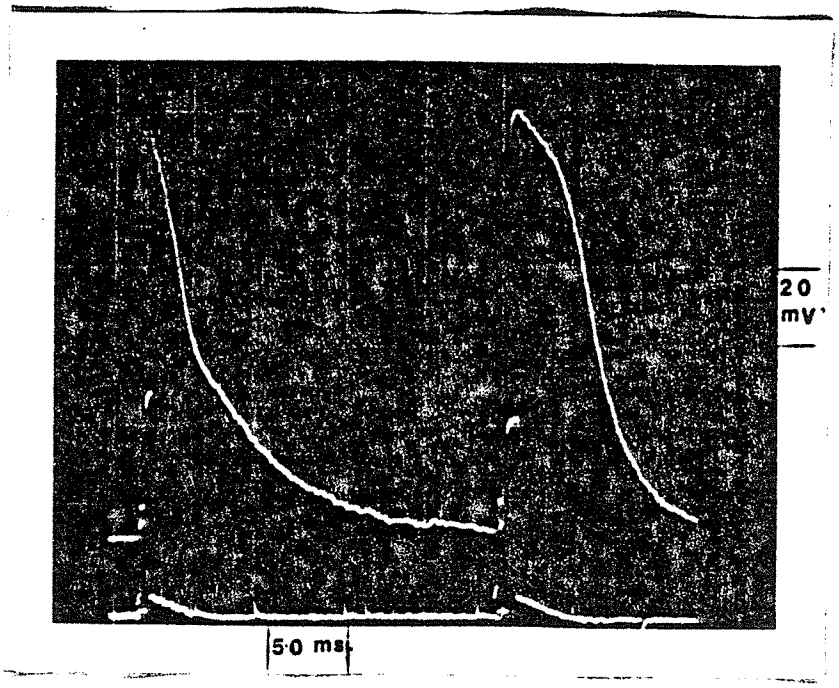


Fig. 18 Intracellular Recording of Two Atrial Action Potentials from Rabbit Left Atrium. Influence of Diastolic Interval on Shape and Duration.

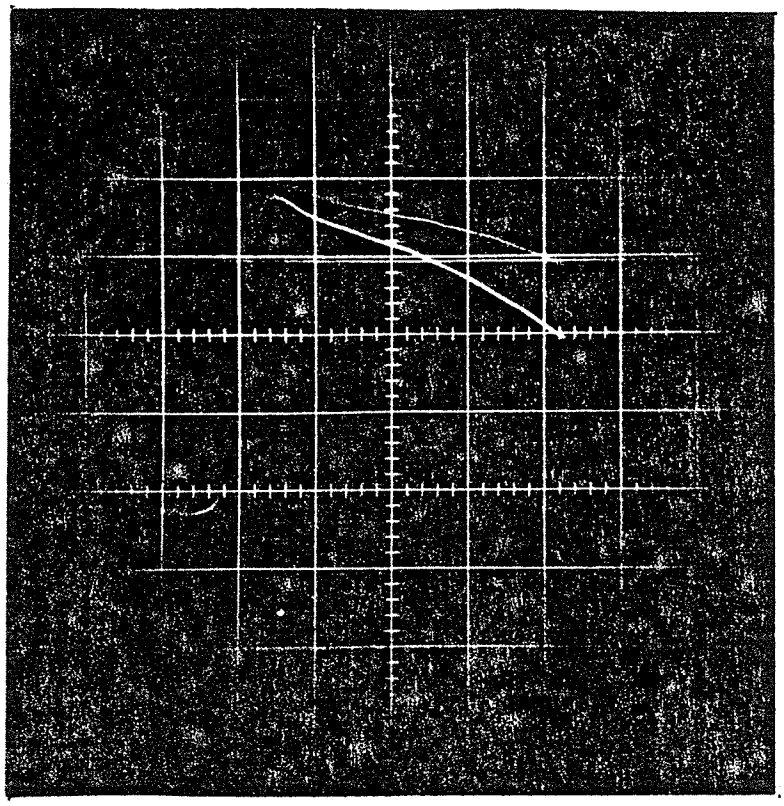


Fig. 19 Photograph of two Superimposed Action Potentials. Effect of Prematurity on the Duration at Zero Potential Level.

level is a time dependent process and because even at a diastolic interval of 600 ms the duration of the premature potentials is greater than that of the basic potential (30±4 ms vs 16±2 ms for 1.25 mM $(Ca^{2+})_o$; 19±4 ms vs 5±1 ms for 5.0 mM $(Ca^{2+})_o$. Also the difference increases as the diastolic interval shortens. This change was less prominent in the high external calcium concentration. As the diastolic interval approaches the zero time (95% repolarization of the basic potential), the difference decreases in the 2.5 mM and 1.25 mM $(Ca^{2+})_o$. In the higher calcium concentration the change was in the opposite direction.

The decline of the duration beyond the zero time indicates another factor involved in the recovery of the action potential duration. This factor corresponds to the membrane potential, because beyond the zero time the upstroke of the premature action potentials will "take off" from lower membrane potential. Gettes and coworkers (1972) demonstrated the importance of the "take off" potential and the diastolic interval, as factors to regulate the duration of the premature potentials in Purkinje fibers and in ventricle.

The changes of the duration of the premature action potentials at 95% repolarization are illustrated in Figure 21. The abscissa represents the diastolic intervals in ms, and the ordinate the difference of the duration (ms) between the basic action potential and the premature action potentials. At a diastolic interval of 600 ms, the duration of the premature action potentials was shorter than that of the basic action potential in all three calcium concentrations, but was only statistically significant at 5.0 mM $(Ca^{2+})_o$ (127 ± 8 ms vs 110 ± 7 ms

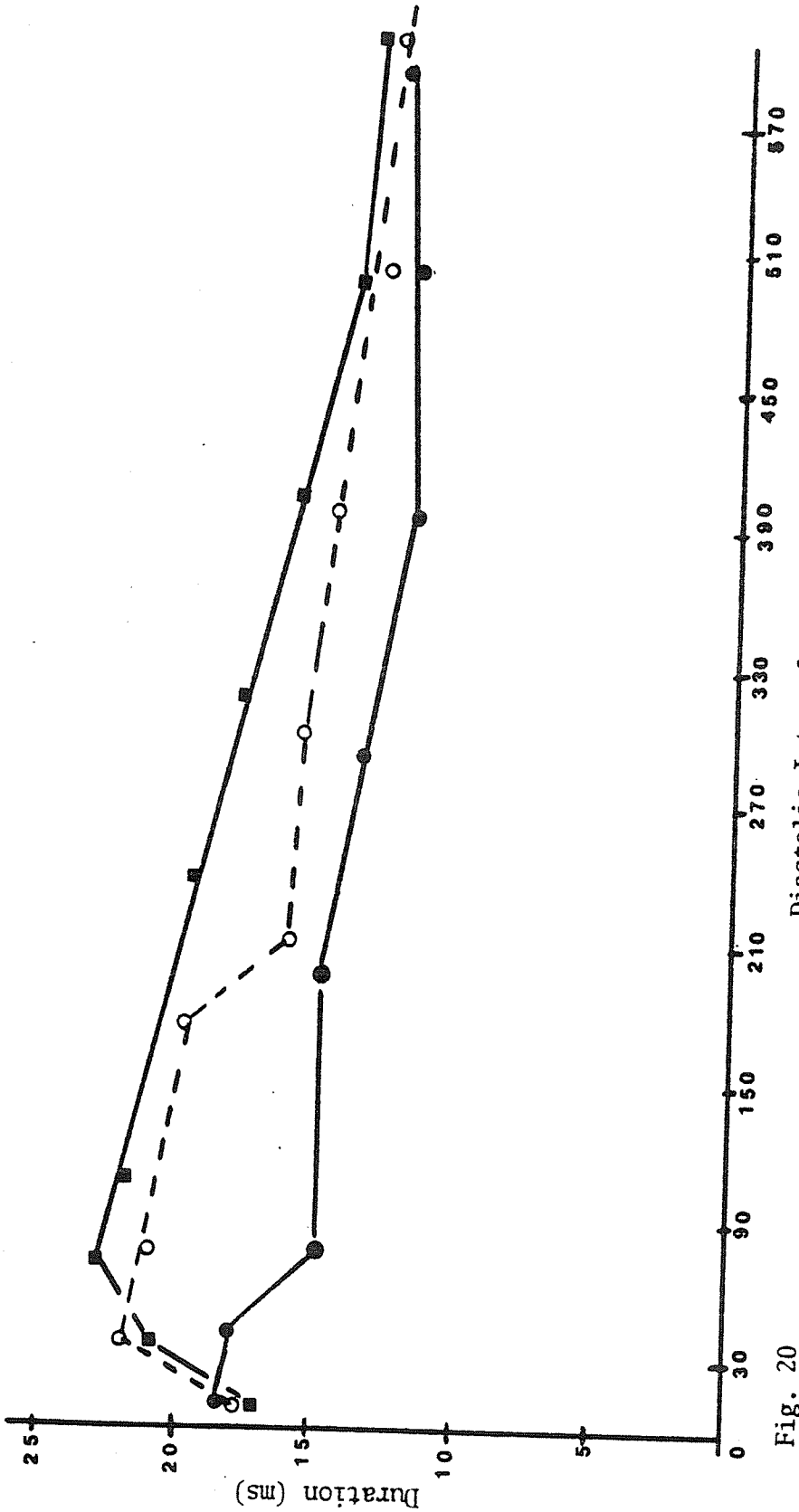


Fig. 20

Relationship between the Duration of the Premature Action Potential at the Zero Level of Repolarization and the Diastolic Intervals. ● 5 mM $(Ca^{2+})_o$; O 2.5 mM$(Ca^{2+})_o$; ■ 1.25 mM$(Ca^{2+})_o$

in 1.25 mM $(Ca^{2+})_o$; 124 ± 9 ms vs 114 ± 9 ms in 2.5 mM $(Ca^{2+})_o$; 167 ± 13 ms vs 129 ± 9 ms in 5.0 mM $(Ca^{2+})_o$ $P<0.01$. Figure 21 also shows that there is an increase of the difference in the range of 60 to 120 ms diastolic interval in comparison with the 600 ms interval. This increment in the difference was not statistically significant at the lower calcium concentration. Similar analysis applied to the 50% level of repolarization, showed that at this level the premature potential does not change with the diastolic interval.

2. Effect of Lidocaine

Lidocaine, 1×10^{-5}M, significantly decreased the premature action potential duration only at the zero potential level and only at 1.25 mM $(Ca^{2+})_o$. This is illustrated in Figure 22. The abscissa represents the diastolic interval in ms, and in the ordinate are the mean ± S.E. of the duration at the zero potential level of the premature action potentials. The observations were done in 8 preparations. The drug was able to decrease significantly the duration in all the intervals studied. It is evident that in a calcium concentration of 1.25 mM, the duration of the basic action potential was significantly prolonged at the zero potential level (Table 12). It is possible that lidocaine in the lower calcium concentration may be modifying the process which controls the early repolarization.

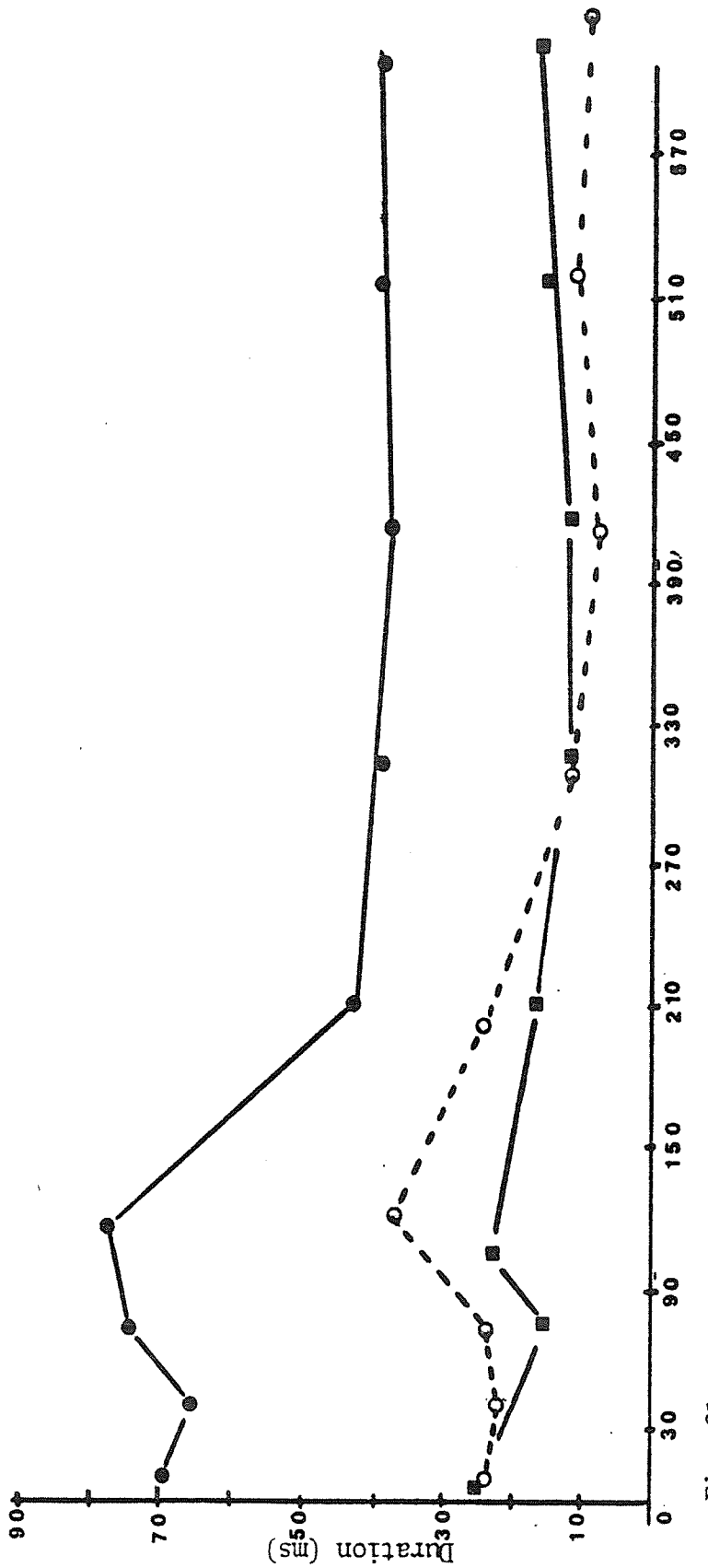


Fig. 21

Relationship between the Duration of the Premature Action Potential, at the 95% Level of Repolarization, and the Diastolic Intervals ● $5mM(Ca^{2+})_o$; ○ $2.5mM(Ca^{2+})_o$; ■ $1.25mM(Ca^{2+})_o$.

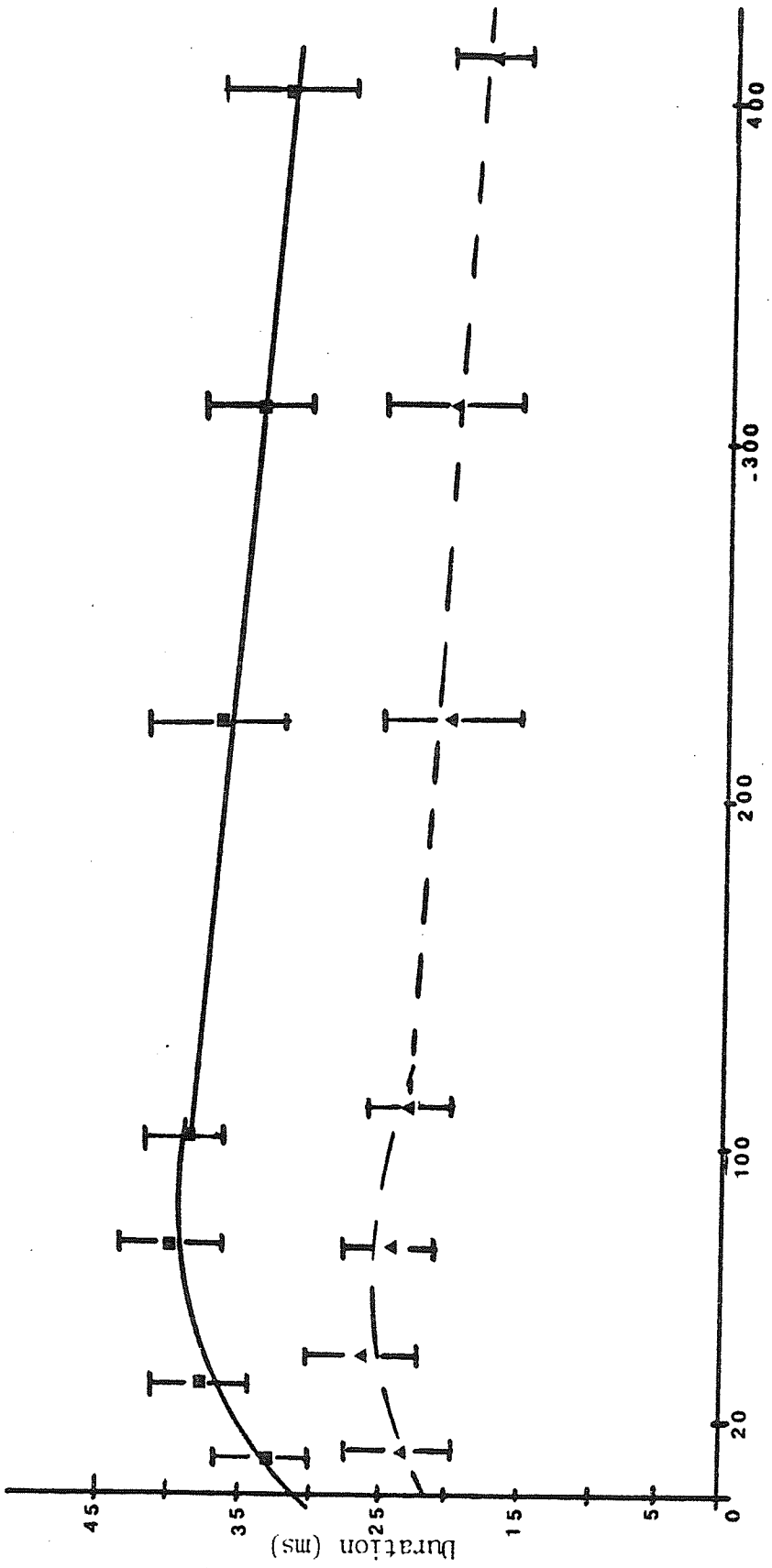


Fig. 22

Effect of Lidocaine ▲ $1x10^{-5}$M on the Duration of the Premature Action Potential at the Zero Potential of Repolarization, as a Function of the Diastolic Intervals.

DISCUSSION

DISCUSSION

A. ANTIARRHYTHMIC EFFECT OF LIDOCAINE

In order to consider the antiarrhythmic effect of lidocaine, it is necessary to remember the genesis of cardiac arrhythmias, and the electrophysiological changes of action potential characteristics induced by the drug. Lidocaine has been proven (Weld et al 1976) to be able to decrease the magnitude of iK_2 current the deactivation of which is responsible for the slow diastolic depolarization in Purkinje fiber. Therefore, because of this effect upon automaticity, lidocaine could prevent an enhancement of this process and be antiarrhythmic. Sasyniuk and Ogilvie (1975), Allen et al (1978), reported that the cells of Purkinje fibers from infarcted areas showed pacemaker activity which was suppressed by lidocaine. Previously, I have presented evidence suggesting that the ectopic foci, which may be the responsible for certain type of arrhythmias, were related to the secondary current. There is evidence to indicate that lidocaine does not modify the secondary current. (Carmeliet and Verdonck, 1974; Brennan and coworkers, 1978). Considering the lack of effect of lidocaine upon the secondary current, it is possible to consider in the reentry mechanism involved in the genesis of arrhythmias to explain the antiarrhythmic effect of the drug. It was shown by Erlanger (1906) that the heart is able to lose its relative homogeneous condition for the conduction of the impulses very easily, and that this inhomogeneity favored the phenomenon of opisthodromia (Schmitt and Erlanger, 1928). Han and Moe (1962; 1964) have stressed the importance of nonuniform recovery of excitability in the myocardium, as a factor to the genesis of reentry arrhythmias. Myerburg and coworkers (1970) demonstrated that both the action potential duration and the effective refractory period are different

through the regions of the free running Purkinje fibers, toward the ventricular muscle. They found a region of maximal action potential duration and effective refractory period, and refered to it as a "gate". Eventhough this "gate" will protect the ventricles from anterograde preexcitation, it will favor the reentry mechanism under appropriate conditions of timing and conduction velocities. Lidocaine enhances the membrane conductance for the potassium ion and as a consequence decreases the action potential durations in ventricular muscle and Purkinje fibers. Davis and Temte (1969) advanced the idea that lidocaine reduces or abolishes the normal differences in recovery of excitability in both tissues (ventricular and Purkinje fibers), and therefore may decrease the chance for reentry. Wittig and coworkers (1973) demonstrated that lidocaine produced a greater shortening of the action potential in the "gate" region. This effect of the drug contributes to decrease the degree of nonuniformity of recovery of excitability.

The well established effect of the drug on the sodium system will provide another way to explain the antiarrhythmic effect of lidocaine. The maximum rate of rise of phase zero of the action potential ($\dot{V}max$), has been related as an important index of conduction velocity. Lidocaine reduces $\dot{V}max$ and conduction velocity in ventricles as well as in Purkinje fibers. Sasyniuk et al, (1975), have pointed out that even though lidocaine decreases $\dot{V}max$ to a greater extent in areas of post-infarction damage than in normal tissue, conditions which could enhance the chance of reentry, the drug acting at higher doses is able to produce a bidirectional block, and as a consequence would abolish reentry.

We believe that in the isolated left atria, the sustained rythmic activity produced by a premature extrasystole is due to reentry. In the dog isolated heart preparation, Man and Dresel (1977) have demonstrated an increase in the intra-atrial conduction time with lidocaine, suggesting a slowing a conduction velocity in this tissue. In our experiments, the antiarrythmic effect of lidocaine could be correlated well with a decrease in $\dot{V}max$ and a prolongation of the E.R.P. $\dot{V}max$ has been taken as an important index of conduction velocity (Singer et al, 1967), even though there is evidence that support the fact that $\dot{V}max$ could be decreased without any significant change of conduction velocity (Peon et al, 1978); therefore, if lidocaine is decreasing the conduction velocity of the premature stimuli, it may reduce the chance of reentry. Our data support the idea that in atrial tissue, the effect of lidocaine is mainly upon the sodium carrier system, because the overshoot, amplitude and $\dot{V}max$ are decreased by the drug, and T is significantly prolonged. In contrast to the effect of lidocaine in shortening the action potential duration in Purkinje fibers and ventricles, in the atrial cells this is not the case. This fact allows one to deny the suggestion postulated by Kabela (1973), that the lack of efficacy of lidocaine on supraventricular arrhythmias could be explained on the basis of its lack of effect on the repolarization process. We believe that the drug at 5×10^{-5}M is efficaceous in preventing the paroxysmal atrial tachycardia, and that at lower dose (1×10^{-5}M), the difference of effect in comparison with Purkinje fibers and ventricles would be explained taking in consideration the participation of the fast sodium system. The evidence for the interaction of lidocaine with the fast sodium system was presented by Singh

and Vaughan Williams (1971) in atrial cells, and has been well documented in Purkinje fibers (Arnsdorf and Bigger, 1972), and ventricle (Chen, et al, 1975). The data presented herein support the idea that the drug at high concentration (5×10^{-5}M) is interfering with the activation process of the sodium system. The fact that lidocaine at this concentration did not shift the steady-state voltage $\dot{V}max$ relationship in the voltage-axis direction, and the fact that T was significantly prolonged, as well as the E.R.P., without a significant change in the action potential duration, indicate that the effect of the drug is mainly upon the recovery from inactivation of the sodium system. Another evidence to sustain this conclusion is the fact that in the normal Krebs (2.5 mM Ca^{++}) lidocaine, 1×10^{-5}M, only affected significantly the recovery process, and was not able to prevent the paroxysmal tachycardia. This last result allows one to conclude that the estimation of T per se, is not a good index to estimate the anti-arrhythmic effect of lidocaine, at least in this type of arrhythmia, and that it is much better to consider the action potential characteristics and other electrophysiological parameters in order to understand the antiarrhythmic effect of a drug.

B. ROLE OF CALCIUM ION ON THE ELECTROPHYSIOLOGICAL PARAMETERS OF ATRIA.

In our experiments, we found that calcium ion modified almost all the electrophysiological parameters studied. An increase in the external calcium concentration changed the duration of the action potential in a biphasic way, shortening it at the beginning of repolarization and lengthening it at the end of repolarization. Similar effects were reported by Klein and Holland (1959). This effect of calcium on the duration is unique for the atria. The effect of the

ion has been well studied in Purkinje fibers and ventricles. Hoffman and Suckling (1956) demonstrated that increasing the external calcium concentration decreases the action potential duration of ventricles and Purkinje fibers. In Purkinje fibers an increase of the external calcium concentration will raise the plateau level, and this effect is due to the role of Ca^{2+} as charge carrier (Reuter, 1973). The role of the secondary current during the plateau level, which dominates the first 200 ms of the action potential duration of Purkinje fibers is well known. An increase in the external Ca^{2+}, enhances the secondary current, and thereby the plateau level is higher (Reuter, 1967). The atrial cells that we studied were lacking a plateau, and therefore, the mechanisms for shortening the duration can not be explained on the same basis of those fibers where a plateau exists. The shortening of the action potential in ventricles and Purkinje fibers could be due to an enhancement either of the slow outward plateau current i_x (Tsien et al 1972), or the background K current i_{K1}. Epinephrine elevates the plateau level and shortens the action potential duration due to an increase in i_x (Tsien et al 1972). This same mechanism was attributed to calcium ion by Beeler and Reuter (1970) and by Kass and Tsien (1975). Recent studies have demonstrated that the mechanism is different. Isenberg (1975) demonstrated in Purkinje fibers that an increase in the intracellular calcium by iontophoresis shortened the action potential duration and hyperpolarized the cell; because of this last effect he ruled out the possibility that an elevation of the intracellular calcium concentration will increase the potassium conductance, g_K. Kass andTsien (1976) demonstrated in Purkinje fibers and with the voltage clamp technique that an increase in the external calcium

concentration enhanced the background K current i_{K1}, and therefore favored a faster repolarization of the fibers, and as a consequence, the action potential shortens. In the ventricles also there is evidence that an increase in the intracellular calcium concentration enhances the potassium conductance (Bassingthwaighte et al 1976). These authors have postulated a regulatory mechanism between intracellular calcium and potassium conductance for the control of the action potential duration. In atrial cells, the mechanism has been studied less. In our experiments, an increase of the external calcium concentration shortened the action potential at the beginning of the repolarization, and also caused hyperpolarization of the cells. We might say that gK has been enhanced, but the lengthening of the duration of the action potential at 95% repolarization, implies a more complex mechanism to explain the dual effect of an increase in the external calcium concentration. Van Der Walt and Carmeliet (1971) have reported a lengthening of Purkinge action potential after a long duration hyperpolarizing clamp pulses. Also, a decrease in the extracellular potassium concentration in our experiments hyperpolarized the cells and lengthened the duration at 95% repolarization. It may be possible to say that calcium ion is decreasing the potassium conductance at the end of repolarization in atrial cells.

Another difference that we found was the fact that an increase in the external calcium concentration increased the overshoot and hyperpolarized the cells, and as a consequence the action potential amplitude and $\dot{V}max$ were increased. These effects are again in the opposite direction with respect to Purkinje fibers. In the last tissue, Weidmann (1955) and Temte and Davis (1967) have reported a decrease in $\dot{V}max$, and no change in the membrane potential with higher calcium

concentration. Frankenhauser and Hodgkin, (1957) demonstrated that in nerve, an increase in the external calcium concentration shifted the conductance-voltage relationship along the voltage axis in a positive direction. The explanation for this phenomenon was the suggestion by Huxley that Ca^{++} might bind to the outer surface of the nerve and then produce a localized hyperpolarization, or additional electric field within the membrane; this last effect could influence the field-dependent Na and K channels. Another possible explanation has been presented by McLaughlin et al, 1971. They have done experiments with artificial phospholipid bilayer membranes, and changes in the concentration of divalent cations. They have demonstrated that when a negative charge density in the membrane is high, there is a negative potential at the surface of the membrane relative to the bulk of the solution. According to the theory of the diffuse double layer, an increase in the concentration of divalent ions to one side of the membrane, will reduce the negative surface potential on that side by a screening process. McLaughlin et al (1971) found a good correlation between the experimental data and the theoretical values, and concluded that similar mechanism may be operating in the cell membrane. In the case of atrial cells, there is evidence to believe that calcium ion is entering the cell during the upstroke of the action potential. Orkand and Niedergerke (1964) demonstrated a direct relationship between the extracellular calcium concentration and the height of the overshoot. In rabbit atria, Ruiz-Ceretti and Ponce-Zumino (1976) have demonstrated the existence of two components of the upstroke of the action potential in atrial cells. Therefore, it could be said that calcium ion enters the cell as a positive charge

carrier, and that this fact could explain the enhancement of $\dot{V}max$ seen in our experiments with high calcium concentration.

Gettes and Reuter (1974) demonstrated that calcium was able to shorten the time constant (τ) of the recovery from inactivation of the sodium system in ventricles and in Purkinje fibers. They also demonstrated that the recovery process was slower than the inactivation, and that it was dependent upon membrane potential, time and temperature. In frog atrial cells, Haas and coworkers (1971) using the voltage clamp technique, reported values of τ in the range of 100 to 600 ms. We reported here a value of 52 ms, for τ, at an average of -71 mv for the membrane potential. The difference of values could be due to the species used and mainly that our experiments were performed at 30°C, whereas Haas and coworkers (1971) used temperatures of 4 to 7°C. The results of Gettes and Reuter (1974), Haas and coworkers (1971) and our results, confirm the fact that the recovery from inactivation of the sodium channels is a slow process compared with that of inactivation. These results are in disagreement with the conclusion of Weidmann (1955) that both processes had identical short time constants. Changes in the external calcium concentration modified τ. An increase one fold in the external calcium concentration shortened it. Due to the fact that in this situation the cells were also hyperpolarized, it was necessary to evaluate if the role of calcium in shortening or prolonging τ was due only to changes in the membrane potential. Parallel experiments in which changes in the membrane potential were due to variation of the external KCl concentration, showed that calcium is modifying another parameter to induce changes in τ. Fig. 14B shows that by lowering the external potassium concentration, the degree of hyperpolarization is higher than when

the external calcium concentration is raised to 5.0 mM, but the curves relating T and M.P. diverge in this range of potentials (-77 to -75 mV) indicating that calcium ions enhances the effect of hyperpolarization upon T; whereas in the range of -62 to -65 mV, again the two curves diverge, indicating that the ions are opposing the effect of depolarization on T.

Calcium ion is also modifying the recovery of the shape and duration of extrasystoles. Figure 21 illustrates the changes in the duration of the action potential at the zero potential level. At 2.5 mM Ca^{2+}, the duration changes in a biphasic mode, it reaches a maximum prolongation at the diastolic intervals between 80 to 100 ms, and then starts shortening toward the approach of the zero time. This fact indicates that the duration of the premature action potential depends upon the diastolic interval as well as on the "take off" potential. Gettes et al (1972) reported similar dependence in the duration of premature action potential of Purkinje fibers and ventricles. The effect of lowering the external calcium concentration to half its original value significantly enhanced the effect of prematurity observed at the normal Krebs. Increasing the external calcium concentration significantly decreased the duration of the premature action potentials, and the maximum lengthening of the duration was observed at a diastolic interval of 20 to 40 ms. In cat papillary muscle, Bass(1975) has reported that a decrease in the external calcium concentration prolongs the area of the premature potential, with respect to control, whereas an increase of the external calcium concentration, decreases the area. Saito (1971 and 1972) using rabbit atria, has explained the increase in the duration of the premature action potentials as a progressive enhancement of the decrease in the conductance of the

membrane for the potassium ion. Hauswirth et al (1972) has concluded that in Purkinje fibers the changes in the duration of the premature action potential may be due to changes in the outward current i_{x1}, carried mainly by potassium ions. They also suggested the possibility of the participation of an inward current in the process. Gettes and Reuter (1974) demonstrated that in ventricular tissue, the plateau duration of the premature action potentials were determined by the kinetics of reactivation of the secondary calcium inward current. A recent report by Hiraoka and Sano (1978) supports the fact that in ventricular tissue, there is an increase of the secondary current and that these changes are parallel to the lengthening of the plateau duration of the premature action potential. Atrial cells are lacking a plateau phase, and there are not voltage clamp studies available to correlate the duration of premature action potentials and the changes in the ionic currents. Due to the nature of the biphasic change of the duration of premature potentials, it might be possible that in atrial cells more than one ionic currents are involved. If it were correct to assume that changes in the duration of the premature action potential at the zero level would reflect changes in the slow inward current, our data will support the idea that this current is enhanced by a onefold increase of the external calcium concentration, because the curves in Figure 21, at 1.25 and 5.0 mM Ca^{2+} are in opposite direction from the diastolic intervals of 80 ms toward the zero time. This fact could be correlated with the observation that the paroxysmal tachycardia was induced earlier in higher calcium concentration.

C. INTERACTION OF LIDOCAINE AND CALCIUM

Local anesthetics drugs block the excitability of nerve by acting on the sodium channels, and mainly by decreasing the amount and the rate of development of the sodium inward current (Taylor, 1959). This effect is different from that reported by Weidmann (1955) for Purkinje fibers, in which the local anesthetics drugs, besides causing the reduction of the inward sodium current, also act on the inactivation of the sodium system.

The interaction of the local anesthetic agents and the membrane receptors, is a function of the state of the voltage-dependent gates of the sodium channels. Therefore factors which are able to modify the sodium system would also affect the interaction of the drug with its receptors. For example, Strichartz (1973) has reported that in myelinated nerve, the inhibition of sodium current by quaternary derivatives of lidocaine, was favored by depolarization of the membrane. Another way of altering the sodium system has been the increase in the frequency of stimulation in a biological preparation. Courtney (1975) has demonstrated that in the myelinated sciatic nerve of the frog (Rana pipiens), the inhibition of the sodium inward current by a lidocaine derivative (GEA 968), was greater when the rate of stimulation was increased. Chen et al (1976), have demonstrated in ventricle that the effect of lidocaine on $\dot{V}max$ is also rate dependent.

It is known that calcium ions modify the sodium system in Purkinje fibers (Weidmann, 1955), and in nerve (Frankenhaeuser, 1957). The effect of calcium has been reported to influence the effect of

local anesthetic agents in excitable tissues. In 1928, Simon and Szelöczey demonstrated that cocaine hydrochloride (0.5%) was able to enhance the diffusion of calcium from the sciatic nerve of the rabbit. Aceves and Machne (1963) demonstrated in cells of isolated frog spinal ganglia that an increase in the external calcium concentration (5 to 10 fold), partially antagonized the depressed spike amplitude produced by local anesthetic agents. Blaustein and Goldman (1966), using the voltage clamp technique showed that in the lobster axon an increase in the external calcium concentration reduced the effectiveness of procaine in decreasing the sodium conductance, and that a decrease in the calcium concentration had an opposite effect.

Recent studies by Narahashi et al, (1976) and Hille (1977) have also demonstrated that the effect of local anesthetics on nerve is modified by changes in the external calcium concentration.

This effect of calcium ion apparently is not specific for the local anesthetic drugs (Seeman et al, 1974), because it also reversed the blocking effect of imipramine, chlorpromazine, TTX, hexanol, heptanol, benzyl alcohol, and barbiturates in the phrenic nerve of the rat.

In cardiac tissue, I am not aware of any study indicating modifications of the effect of local anesthetic agents by changes in the external calcium concentration. Our results indicate that similar interaction occurs in atrial tissue.

The effect of lidocaine at 1×10^{-5}M on almost all of the electrophysiological parameters studied was significantly enhanced when the external calcium concentration was lowered to half its control volume. When the drug was used in a higher concentration (5×10^{-5}M), there was a greater decrease in $\dot{V}max$ at an external

calcium concentration of 2.5 mM than at 5.0 mM $(Ca^{2+})o$.

Lidocaine has been proven to affect mainly the repolarization process in Purkinje fibers and ventricles, but also has effect upon the sodium system in those structures. Singh and Vanghan Williams (1971) demonstrated that in atrial cells, the effect of lidocaine in decreasing $\dot{V}max$ was enhanced by higher external potassium concentration.

Chen and coworkers (1975) were also able to demonstrate in ventricles, that the effect of lidocaine was enhanced by external potassium concentration, and they presented evidence which showed that this enhancement of the effect was due to changes in the membrane potential produced by the potassium and no due to the ion itself. They demonstrated that hyperpolarizing currents were able to overcome the effect of lidocaine on $\dot{V}max$. This indicates that in cardiac tissue, modification of the characteristics of the sodium system will influence the effect of lidocaine.

The results presented in Tables 13 and 14 show a significant decrease in M.P when the calcium concentration was 1.25 mM, and a significant hyperpolarization at 5.0 mM $(Ca^{2+})o$. It is known that the sodium channels are modified by changes in M.P (Weidmann, 1955), and that modifications of the sodium system are able to influence the effect of local anesthetics. These changes in the sodium channels produced by variations in the external calcium concentration might explain the enhancement of effect of lidocaine at a low calcium concentration, and the attenuation of its effect at 5.0 mM $(Ca^{2+})o$.

Another possible explanation for the modification of the effect of lidocaine is taking in consideration the fact that in atrial cells, during the upstroke of the action potential calcium ion has been

proven to participate (Ruiz-Ceretti and Ponce-Zumino, 1976).

They demonstrated that the participation of Ca^{2+} was on the second component of the upstroke seem mainly in the overshoot, which presents slower rate of rise. In other words, in atrial cells 12-13% of the upstroke of the action potential is due to the flow of ions through the "slow" channels.

There is evidence to support the fact that lidocaine does not modify the secondary current. Carmeliet and Verdonck (1974) point out that in atrial cells depolarized with KCl and treated with isoproterenol or high calcium concentration, the action potentials originated in this condition, which are due to the participation of the secondary current, were not affected by lidocaine. A recent report by Brennan and coworkers (1978) also confirms the fact that lidocaine does not modify the action potentials generated by the secondary current in Purkinje fibers. It is reasonable to think that the interaction is done at the fast sodium channels.

It is necessary to consider the site or possible sites of interaction of the drug with its receptors.

Straub (1956) ruled out that procaine was modifying the membrane permeability for potassium and sodium ions in the mylinated nerve of the frog. Feinstein (1964) demonstrated that local anesthetics are able to interact with lipids and phospholipids extracted from nerve and muscle membranes.

Hille (1977) has pointed out evidence which indicate that the site of action of the local anesthetics drugs is located inside the membrane. His evidence were: a) the effect of the local anesthetics agents are modified by changes in pH; b) the experiments with the charged N-ethyl or N-methyl derivatives of lidocaine and trimecaine;

these compounds are able to block the sodium channels when applied inside the nerve, but not when applied outside. It is accepted that local anesthetic molecules diffuse in the amine form from outside to the inside of the excitable tissues. Nevertheless, Khodorov and coworkers (1976) have suggested the existence of several types of receptor sites (at the internal opening of the sodium channels, and at the outer side of the membrane). Hille (1977) has presented a new model for the interaction of local anesthetics and its receptors, he concluded saying: "to the old question of inside vs. outside action, the answer is within the membrane". He also pointed out that the idea of the modulated receptor could be applied to other cases, like the anti-arrhythmic effect of local anesthetics on the myocardium.

Feinstein (1964) showed that local anesthetics interfere with the calcium binding by phospholipids. He suggested a competitive binding between local anesthetic and calcium. Blaustein and Goldman (1966) taking in consideration the evidence presented by Aceves and Machne (1963) that calcium ions partially antagonized the effect of local anesthetic on spinal ganglia, and their own observation on lobster axon, proposed a model in which the polar head of the phosphalipids may be oriented in several configurations, depending upon the electric field across the membrane. They assumed that one of the configurations binds calcium preferentially, and two others prefer sodium and potassium. The effect of local anesthetics would be upon the configuration which preferentially binds calcium. Suarez-Kurtz and coworkers (1970) presented results of binding studies with radiocalcium in the frog sciatic nerve, which supported the kinetic model proposed by Blaustein and Goldman (1966) for the competitive interaction between

local anesthetics drugs and calcium ions. In the model presented by Hille (1977) he postulates that the receptor for the local anesthetic agents has a voltage and time-dependent conformation. He called this model "modulated receptor", and also assumes that the sodium channel is making transitions between several states: resting, open, and inactive, and that the drug is able to react with each channel form. In this model, the binding site of the receptor is located in the channel, near the inactivation gating subunit (h), therefore, when the h gate is open binding to the receptor is not very firm, but when the gate is closed the receptor is modified and the binding is stronger.

Hille (1977) also explained that the relief of block produced by higher calcium concentration in the presence of local anesthetics is due to the relief of some of the normal resting inactivation of the sodium channels, this relief being produced by calcium ions through their effect on the electric field within the membrane.

In our experiments, the results of the calcium ions on the "membrane responsiveness curve" illustrated in figures 12 and 13 show that an increase in the external calcium concentration hyperpolarized the atrial cells, and the curve is shifted in an upward direction, and that at 50% $\dot{V}max$ the membrane voltage is shifted to more positive potentials with the high calcium concentration. Opposite results were obtained with lower calcium concentration (Figure 13). These results indicate that atrial cells behave different from Purkinje fibers. Weidmann (1955) showed in Purkinje fibers that an increase in the external calcium concentration did not enhance $\dot{V}max$, neither changed the membrane resting potential, but shifted the Vh curve toward less negative potential, indicating an effect upon the

h (inactivating gates). In atrial cells, our results may indicate that calcium ion is also altering the permeability of the membrane, in such a way that hyperpolarization occurs, and as a consequence $\dot{V}max$ will increase in response to a more negative potential across the membrane. This fact together with the shortening of the action potential in the higher calcium concentration, allows one to speculate that in atrial cells, calcium ions might be also modifing the conductance for potassium ions, as is the case in Purkinje fibers (Isenberg 1975) and in ventricles (Bassingthwaighte, 1976).

It is possible to conclude that changes in the external calcium concentration are able to modify the sodium channels in atrial tissue, and that these modification of the sodium system will be responsible for the difference of effect observed with lidocaine.

With the results of these experiments it is not possible to explain completely the difference of effect of lidocaine in the supraventricular arrhythmias and in the ventricular type. Nevertheless, Fukuda (1975)demonstrated a higher intracellular calcium content in atrial tissues than in ventricles.

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