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

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