THE RELATION OF $\dot{V}_{max}$ TO I_{Na}, $\bar{G}_{Na}$, AND h_∞ IN A MODEL OF THE CARDIAC PURKINJE FIBER

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ABSTRACT The inward sodium current in cardiac muscle is difficult to study by voltage clamp methods, so various indirect experimental measures have been used to obtain insight into its characteristics. These methods depend on the relationship between maximal upstroke velocity of the action potential ($\dot{V}_{max}$) and the sodium current (I_{Na}), usually defined in terms of the Hodgkin-Huxley model. These relationships were explored using an adaptation of this model to cardiac Purkinje fibers. In general $\dot{V}_{max}$ corresponded to I_{Na}, and it could be used to determine the relationship of membrane potential to $\bar{G}_{Na}$ and h_∞. The results, however, depended on the method of stimulation of the action potential, and an optimal stimulation method was determined. A commonly used experimental technique called "membrane responsiveness" was shown to distort seriously the properties of steady-state gating inactivation that it is supposed to measure. Estimation of the changes in maximal sodium conductance, such as those produced by tetrodotoxin (TTX), would be accurately measured. Some experimental results have indicated a voltage-dependent effect of TTX. Characteristics of the measures of TTX effect under those conditions were illustrated. In summary, calculations with a model of the cardiac Purkinje fiber action potential provide insight into the accuracy of certain experimental methods using maximal upstroke velocity as a measure of I_{Na}, and cast doubt on other experimental methods, such as membrane responsiveness.

INTRODUCTION

The activated sodium current is by far the largest ionic current in many excitable tissues, and is the source of the depolarizing current responsible for excitation and conduction. This current has been carefully studied in many tissues by the voltage clamp technique. In cardiac tissues, however, the sodium current is not fully controllable, and voltage clamp studies have not provided much quantitative information about sodium current (Weidmann, 1955; Dudel and Rüdel, 1970; Schoenberg and Fozzard, 1979). Consequently, alternative ways to study sodium current would be helpful. One way of obtaining information on the sodium current, I_{Na} (see Table I) and thereby on its component properties is with $\dot{V}_{max}$ values. In the uniform membrane case the first time derivative of voltage, $\dot{V}$, is proportional to the net ionic current, I_i, at all times. Many experimentalists use $\dot{V}_{max}$ as a direct indication of I_{Na} and draw conclusions based on comparisons under different conditions (Gettes and Reuter, 1974; Weidmann, 1955; Baer et al., 1976; Chen et al., 1975). The required assumption is that I_{Na} is so much greater than the other currents at the time of $\dot{V}_{max}$ that the difference in I_i from I_{Na} is not significant.

This study simulates these experimental techniques, employing a mathematical model of the Purkinje fiber membrane. The sodium current is modeled by the equation $I_{Na} = \bar{G}_{Na} \cdot$

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TABLE I

LIST OF SYMBOLS

V	Membrane potential.
$\dot{V}$	First derivative of potential with respect to time (dV/dt).
$\dot{V}_{max}$	Maximum value of $\dot{V}$ during action potential upstroke.
V_{half}	Conditioning potential at which $\dot{V}_{max}$ is one-half of the greatest $\dot{V}_{max}$.
$\overline{V}$	Membrane potential at which $\dot{V}_{max}$ occurred.
$\overline{V}_V$	$\overline{V}$ for upstroke from conditioning potential of V mV; as $\overline{V}_{-100}$ is $\overline{V}$ for upstroke from −100 mV conditioning potential.
V_{base}	Base holding potential before start of conditioning step.
V_{peak}	Maximum membrane potential reached in action potential upstroke.
E_{Na}	Sodium equilibrium potential.
$(V - E_{Na})$	Electrochemical sodium driving force across membrane.
I_{Na}	Sodium current crossing membrane per unit area.
I_{K2}	Potassium current responsible for the spontaneous pacemaker behavior of the MNT model.
I_{x1}, I_{x2}	Outward currents, largely potassium, responsible for repolarization from the plateau of an action potential in the MNT model.
I_i	Net ionic current crossing the membrane per unit area, equal to the sum of the currents through the individual channels.
$\overline{G}_{Na}$	Maximal conductance of the sodium channel.
m	Sodium channel activation variable; voltage and time dependent.
h	Sodium channel inactivation variable; voltage- and time-dependent.
h_∞	Steady state h-value, a function of potential.
τ_h	Time constant for rate of change of h-variable from one value to another, a function of the membrane potential.

$m^3 \cdot h \cdot (V - E_{Na})$. In many experiments under different conditions, it is usually assumed that the upstrokes are uniform so that only differences in the pre-stimulation state cause variations in $\dot{V}_{max}$. If $\overline{G}_{Na}$, m, and $(V - E_{Na})$ are unchanged, then changes in $\dot{V}_{max}$ are due to changes in h. When a change in $\overline{G}_{Na}$ is considered, variation in h is eliminated in conditions where h is assumed to have varied to the same value (Baer et al., 1976).

The suggestion by Baer et al. (1976) that the reduction of $\overline{G}_{Na}$ after exposure to tetrodotoxin (TTX) is voltage-dependent has led to a recent discussion about the meaning of $\dot{V}_{max}$ measurements (Cohen and Strichartz, 1977; Hondeghem, 1978; Strichartz and Cohen, 1978). The purpose of this study is to simulate several experimental techniques and examine the validity of the assumptions made.

METHODS

The cardiac membrane model proposed by McAllister et al. (1975) (MNT model) for the Purkinje fiber was utilized for these simulations of the uniform membrane case. The symbols frequently used in this discussion are defined in Table I.

The MNT model simulates a spontaneously active membrane. To produce a quiescent membrane for this study, the pacemaker current's (I_{K2}) time dependence was eliminated by setting its gating variable, s, at 1.0. The quiescent membrane had a resting potential of approximately −88 mV.

The programs, written in FORTRAN, were run on either a PDP 11/40, or a DEC-20 system (Digital Equipment Corp., Maynard, Mass.). The programs used a Runge-Kutta numerical integration method, with a variable time increment internally adjusted to give a constant degree of accuracy (membrane potential with the acceptable time step was within 10^{-5} mV of the membrane potential, computed with the time step cut in half) while decreasing the run time required per simulation. The conditioning steps before the upstroke were effected by simulation of a perfect voltage clamp

step to the conditioning potential for the specified length of time. Action potentials were elicited by constant current pulses. Further details will be introduced in the appropriate sections.

RESULTS

Use of $\dot{V}_{max}$ *to Imply Value of* h

VARIATION IN CONDITIONING PULSE DURATION The earliest experimental studies providing information on the cardiac sodium channel inactivation process were those of Weidmann (1955). Consequently, we simulated his experiments. The simulation was different, in that he used a central region of a longer Purkinje fiber to voltage clamp and stimulate, rather than the idealized uniform membrane of these stimulations. Beginning from a resting potential of −87.9 mV, conditioning voltage steps were executed with durations of 50, 100, and 200 ms. The action potential was initiated at the end of the voltage step by a 4-μs current pulse of the same intensity for all trials. This method was chosen because it is almost the same as an instantaneous depolarization of fixed magnitude, often used in simulations of a uniform membrane (Hodgkin and Huxley, 1952; McAllister et al., 1975; Cohen and Strichartz, 1977). As expected, in comparison to the calculated curve, the increasing durations gave increasingly better approximations of h_∞ (Fig. 1).

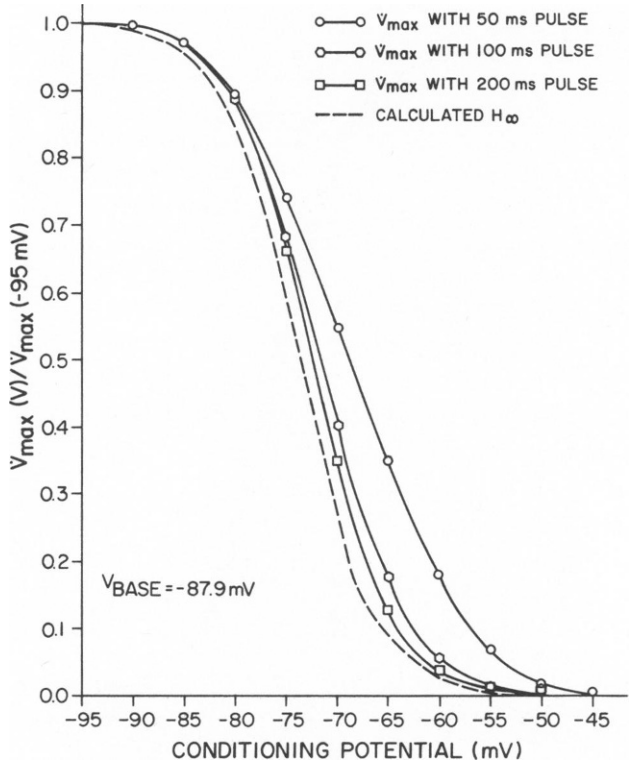

FIGURE 1 Fractional $\dot{V}_{max}$ after conditioning potential step, relative to value after −95 mV step. Conditioning steps of 50, 100, and 200 ms were simulated from a steady-state resting potential of −87.9 mV. Stimulus in all trials was 100 mA/cm^2 for 4 μs. The dashed comparison curve is the steady-state h value calculated from explicit equations in model.

VARIATION IN STIMULUS CHARACTERISTICS The method of stimulation used for the studies in the previous section occasionally produced erratic results. Depending upon the strength of the current pulse, $\dot{V}_{max}$ from -100 mV was in some cases actually less than that from less hyperpolarized potentials (Fig. 2) and other portions of the $\dot{V}_{max}$ curve could show distortions from the h_∞ curve. This type of stimulus was inadequate to handle the complete range of conditioning potentials used (-100 to about -55 mV). The reasonably good results of the previous section are partially due to a judicious choice of the stimulus strength and limitation of the range of conditioning potentials to -95 mV or less hyperpolarized. A more explicitly defined stimulus was required, and as a first attempt, the concept of constant latency was added to the 4-μs stimulus. Latency was defined as the time between end of stimulus and the moment of $\dot{V}_{max}$. The end of the current pulse was chosen for this measurement because the passive membrane charging is complete at that time. For experimental purposes the criterion of constant latency is identical if the measurement is made at the beginning or the end of the stimulating current, but latency is not defined if the action potential occurs before the end of the pulse. Constant latency has been used in some recent experiments (Gettes and Reuter, 1974; Chen et al., 1975). Trials were run for latencies of 2 and 5 ms (Fig. 2). We used resting potential as holding potential and a conditioning period of 200 ms.

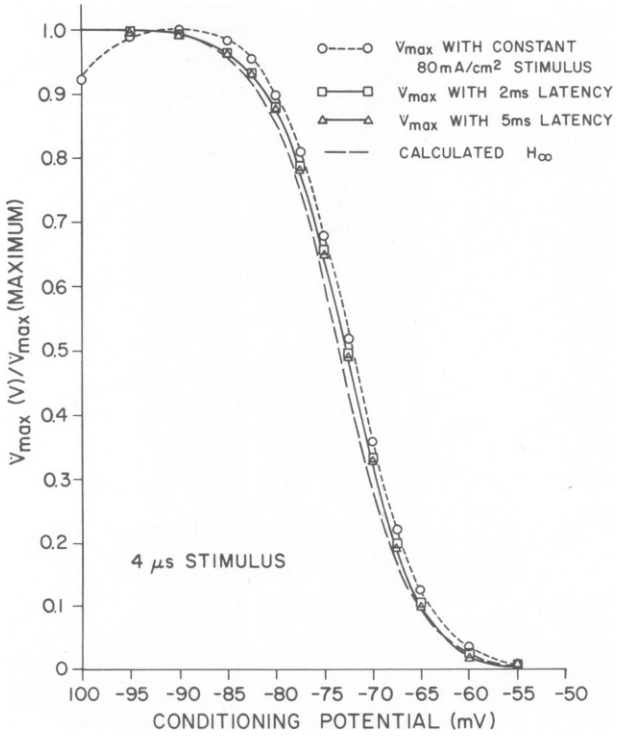

FIGURE 2 Fractional $\dot{V}_{max}$ after conditioning potential steps of 200 ms duration, relative to greatest $\dot{V}_{max}$ value (-90 mV for constant intensity stimulus curve, -100 mV for constant latency curves). Stimuli were 4 μs duration, intensity of either constant 80 mA/cm^2 for all potentials or varied to obtain constant latencies (2 or 5 ms) at all potentials.

This introduction of latency into the stimulation method eliminated the anomaly of lower $\dot{V}_{max}$ at the hyperpolarized conditioning potentials and produced fractional $\dot{V}_{max}$ curves that paralleled the h_∞ curves quite well, although the shifts of approximately +1 mV remained (5 ms latency curve being shifted slightly less). However, at the most depolarized conditioning potentials, the fractional $\dot{V}_{max}$ curve approached the h_∞ curve, dropping somewhat below it by a conditioning potential of −60 mV. The two different latencies produced very nearly the same curve, illustrating the great improvement over the constant strength method, where the results were significantly influenced by the stimulus strength chosen.

The next change in stimulation method was to use 10-ms stimulating pulses with constant latency. A 4-μs pulse is not experimentally practical. 10 ms was chosen because it is more representative of experimental protocols, and since it is probably at the other extreme of duration, could be expected to show up errors hidden by the extreme shortness of the 4-μs pulse. This method produced a fractional $\dot{V}_{max}$ curve that very closely fit the h_∞ curve, being shifted by only about +0.5 mV at $h_\infty = 0.5$ (Fig. 3).

ADJUSTMENT OF $\dot{V}_{max}$ FOR VARIATION OF V_m AND m-GATE VALUE AT $\dot{V}_{max}$ The membrane voltage at which $\dot{V}_{max}$ occurs ($\bar{V}$) is a parameter that can be experimentally determined, while the m-gate value cannot. The fractional $\dot{V}_{max}$ was adjusted for variation

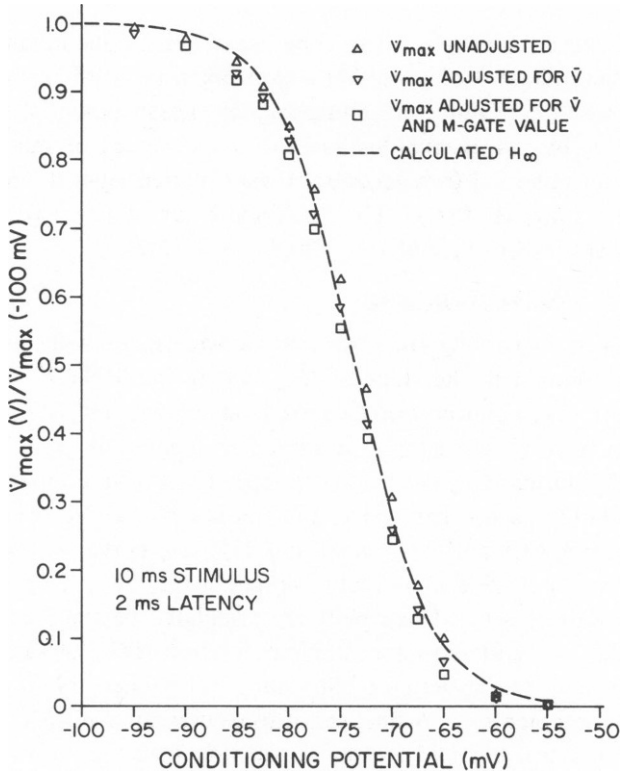

FIGURE 3 Fractional $\dot{V}_{max}$ after conditioning potential steps of 200 ms duration from resting potential, relative to value at −100 mV conditioning step. Values of $\dot{V}_{max}$ adjusted for $\bar{V}$, or for both $\bar{V}$ and m-value also shown. Dashed comparison curve of steady-state h values was calculated from equations in model.

of these parameters to see their effects upon the fitting of both the 4-μs stimulus, 2 ms latency, and 10 ms stimulus, 2 ms latency curves to the h_∞ curve (Fig. 3). The $\overline{V}$ adjustment was performed by multiplying each fractional $\dot{V}_{max}$ value by the factor $(\overline{V}_{-100mV} - E_{Na})/(\overline{V}_V - E_{Na})$. The value of E_{Na} for these adjustments was taken as the V_{peak} of the upstroke from a conditioning potential of -100 mV (38.6 and 38.3 mV, while the actual programmed E_{Na} was 40 mV). For the 4-μs stimulus curve, $\overline{V}$ varied between -9.1 and -32 mV ($\overline{V} - E_{na}$ was -49 to -72 mV) and for the 10-ms stimulus curve $\overline{V}$ varied between -9.0 and -33.1 mV ($\overline{V} - E_{Na}$ was -49 to -73.1 mV). On the 4-μs curve, correction for $\overline{V}$ made the fractional $\dot{V}_{max}$ curve nearly identical to the h_∞ curve by lowering each point slightly, while on the 10-ms stimulus curve, correction made the midrange $\dot{V}_{max}$ nearly identical to h_∞, but accentuated the low values of fractional $\dot{V}_{max}$ for the extremes of conditioning potential, so that the net fit to h_∞ was not better.

The correction for variation in the m-gate value at the moment of $\dot{V}_{max}$ was made by multiplying each fractional $\dot{V}_{max}$ value (adjusted for $\overline{V}$) by $m^3_{-100\,mV}/m^3_{\overline{V}}$. The actual value of the m-gate varied only between 0.91 and 0.89 for both the 4 μs and 10 ms stimuli curves between -100 and -65 mV, but did drop to 0.81 and 0.80 for the -60 mV conditioning potential. As there was little actual variation, the corrections tended to drop the $\dot{V}_{max}$ curves slightly, but did not significantly alter the general fit of the $\dot{V}_{max}$ curves to h_∞ from that of the $\overline{V}$-corrected curves.

MEMBRANE RESPONSIVENESS This experimental technique involves stimulation of action potentials along phase 3 of a normal action potential, with plots of $\dot{V}_{max}$ (or fractional $\dot{V}_{max}$) vs. V resulting. For this simulation, an action potential was elicited from the resting membrane by a 4-μs stimulus and the actual values of the h-variable during phase 3 of the action potential (repolarization) were plotted against the V at which the h-values occurred (i.e.: $h(t)$ vs. $V(t)$). The fractional h curves (Fig. 4) show large differences, $h(t)$ being much steeper (V_{half}:$h(t)$ = -80, h_∞ = -73 mV).

Use of $\dot{V}_{max}$ *to Determine Relative* $\overline{G}_{Na}$

To simulate the effects of varying $\overline{G}_{Na}$, calculations were made with an altered $\overline{G}_{Na}$ and compared to calculations with the value of $\overline{G}_{Na}$ used in the MNT model (150 mS/cm^2). First were trials where $\overline{G}_{Na}$ was decreased to a fixed value (50%, 33%, 25% of normal). $\dot{V}_{max}$ curves resulted that were mostly simply decreased in magnitude, with the same voltage dependence as in the normal $\overline{G}_{Na}$ curve (Fig. 5, top). Plots of fractional $\dot{V}_{max}$ for each $\overline{G}_{Na}$ curve were all similar to each other and to the normal $\overline{G}_{Na}$ curve (Fig. 5, bottom), the greatest difference being between the normal and 25% $\overline{G}_{Na}$ curve, which was parallel and shifted by approximately -1.5 mV. A plot of apparent $\overline{G}_{Na}$ block (Fig. 6) was drawn for each of the decreased $\overline{G}_{Na}$ sets. These plots are calculated as the fractional decrease in $\dot{V}_{max}$ $(1-(\dot{V}_{max,test}/\dot{V}_{max,ctrl}))$ and show the apparent fraction of $\overline{G}_{Na}$ blocked. They showed a small voltage dependency (as suggested by Strichartz and Cohen, 1978), mostly at the depolarized conditioning potentials. A final set of trials used an explicitly voltage-dependent $\overline{G}_{Na}$. The dependency was sigmoid, with $\overline{G}_{Na}$ ranging from 75 mS/cm^2 ($\frac{1}{2}$ normal) at -100 mV to 0 at the least negative conditioning potentials (by -60 mV). The sigmoid dependency had a V_{half} of -77.2 mV and slope at V_{half} was 75 mS/cm^2 per 15 mV. Curves produced by this modification were distinctly different from all others. The $\dot{V}_{max}$ curve (Fig. 7, top) was decreased at -100 mV to the same degree as the $\overline{G}_{Na}$ = 50% normal curve

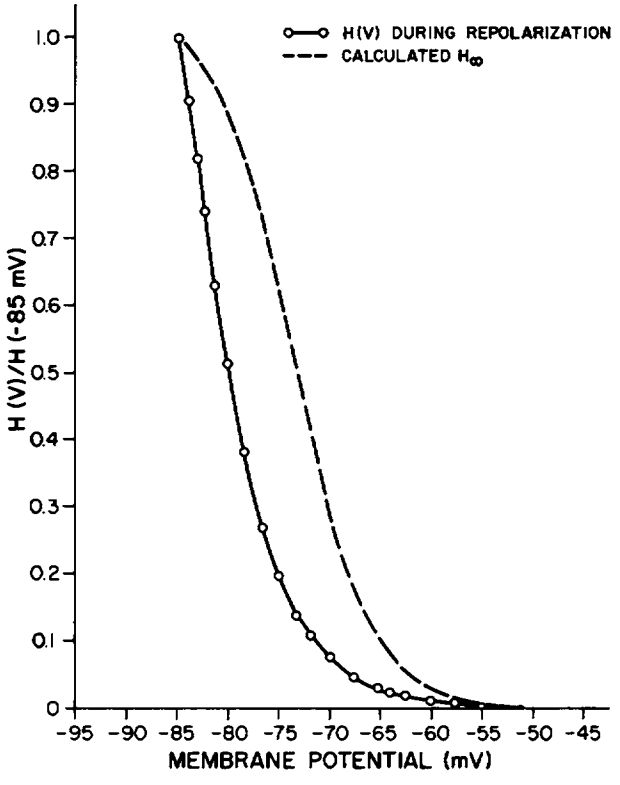

FIGURE 4

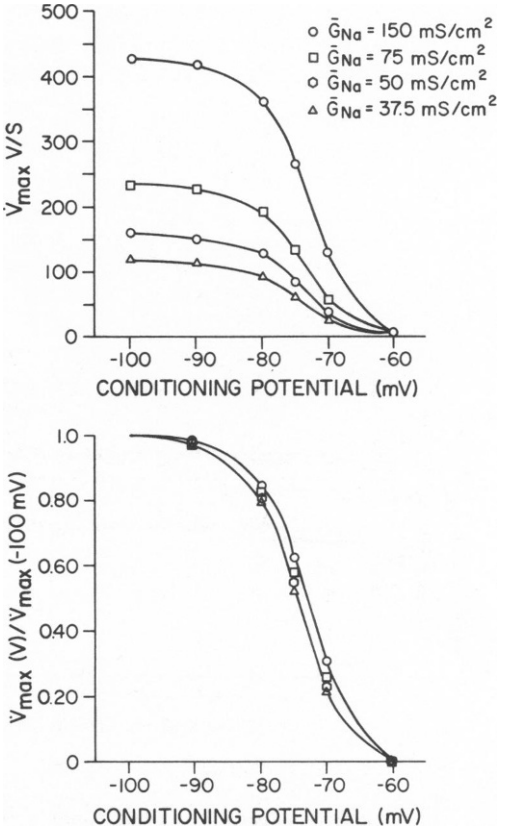

FIGURE 5

FIGURE 4 Fractional h value plotted against potential at which it occurred during repolarization process of an action potential, relative to value at −85 mV. Dashed comparison curve is from steady-state h values.

FIGURE 5 *Upper:* $\dot{V}_{max}$ after 200 ms conditioning potential steps from resting potential. The value of $\bar{G}_{Na}$ was reduced from the program's normal 150 mS/cm^2 to 75, 50, or 37.5 mS/cm^2. *Lower:* Same as above, but fractional $\dot{V}_{max}$ relative to value with −100 mV conditioning potential. Upper curve through 150 mS/cm^2 points, lower curve through 37.5 mS/cm^2 points.

was, but fell away to near 0 by −70 mV, a more negative potential than any of the other curves. The fractional V_{max} curve (Fig. 7, lower) did not match the normal $\bar{G}_{Na}$ curve, but fell at more negative potentials, and had a separation of 7.5 mV at half blockage levels. The apparent $\bar{G}_{Na}$ block curve was highly voltage dependent (Fig. 6). It showed the same difference from the actual $\bar{G}_{Na}$ block in the most negative potentials as did the $\bar{G}_{Na}$ = 50% normal curve, but matched the actual blockage curve closely in the middle range of its voltage dependency.

DISCUSSION

Sources of Error in Relating $\dot{V}_{max}$ *to* h_∞

These calculations depend on the assumption that the model of excitation used in the MNT equations does reflect the behavior of the Purkinje fiber for a uniform membrane action

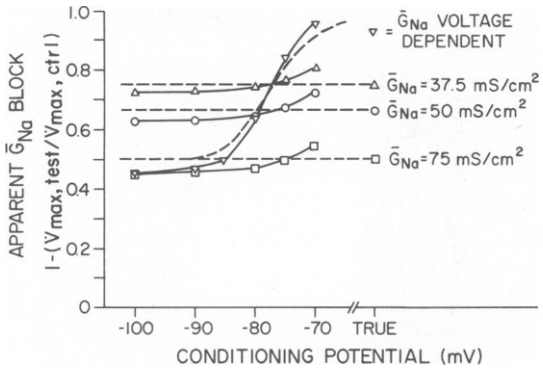

FIGURE 6

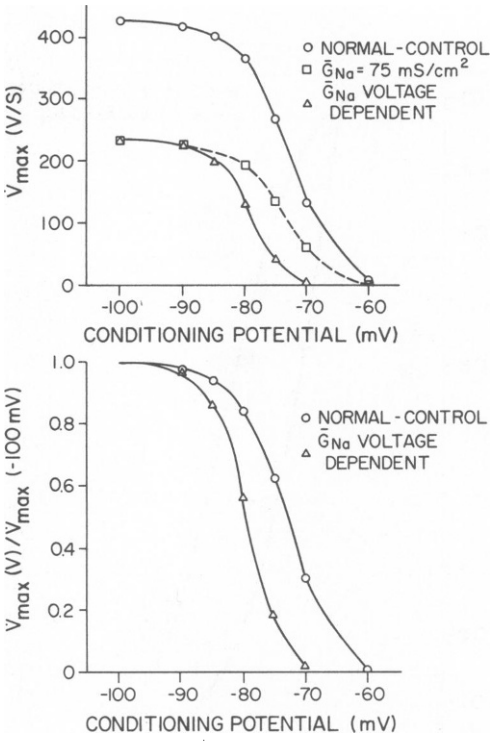

FIGURE 7

FIGURE 6 Apparent block (reduction) of $\bar{G}_{Na}$ after 200 ms conditioning potential steps. Points are apparent fraction of $\bar{G}_{Na}$ blocked with $\bar{G}_{Na}$ of 75, 50, or 37.5 mS/cm^2 or sigmoid voltage dependency (see text) relative to results with $\bar{G}_{Na}$ of 150 mS/cm^2. Dashed lines and curve are of corresponding actual programmed blockage of $\bar{G}_{Na}$.

FIGURE 7 *Upper:* $\dot{V}_{max}$ after 200 ms conditioning potential steps from resting potential. Curves shown are through points for $\bar{G}_{Na}$ of normal (150 mS/cm^2), 75 mS/cm^2, and voltage-dependent, as described in text. *Lower:* Same as upper, but fractional $\dot{V}_{max}$ relative to value after −100 mV conditioning potential.

potential. If this assumption is accepted, then it can be concluded that the experimental method of using $\dot{V}_{max}$ to provide a measure of I_{Na}, and thereby of h_∞ and $\bar{G}_{Na}$ is valid. If this were not true, the $\dot{V}_{max}$ curves could not have matched the h_∞ curves as well as they did. This implies that I_i in Purkinje fibers, the actual current reflected in the $\dot{V}_{max}$, is not significantly different from I_{Na} at the time of $\dot{V}_{max}$. There are, however, several factors that must be considered because they may introduce error into the result if their effects are not minimized.

CONDITIONING PULSE The first of these potential errors is the effect of variation in the conditioning pulse. The criterion for validity here is that it must be long enough for all factors affecting I_{Na} to come to their equilibrium position, especially the h-gating variable, because it is the slower of the two voltage and time-dependent properties. The h-gate has a τ vs. V curve that is bell-shaped, with a maximum value of about 40 ms at approximately −70 mV. Very short conditioning durations could be used at the most negative potentials. Longer durations are required at potentials with longer τ_h. The interaction of several fac-

tors results in an optimal holding potential of −70 mV for lessening the effects of conditioning duration. This is because in the potential range of the longest τ_h, there is the least difference between starting and equilibrium values. In this way, with a conditioning step of less than 200 ms, the approach to equilibrium might be only partially complete, but the actual difference from equilibrium might be no longer experimentally significant. The MNT model (and most other models, as well) bases its h-variable kinetics and steady-state values on the experimental measurements of Weidmann (1955). He used 50-ms clamp steps as conditioning steps. This is by no means sufficient to allow equilibrium at the range of longer time constants. However, he appears to have started the conditioning step during phase 4, when the membrane potential was at about −75 mV. In this way it appears that his fractional $\dot{V}_{max}$ could have resulted in a curve sufficiently like the "true" h_∞ curve to be within experimental error. In addition to this method, Weidmann also tried starting his 50-ms conditioning steps during the action potential plateau period. The $\dot{V}_{max}$ obtained were significantly less than for the previous trials for the conditioning potentials of −65 to −75 mV, while results for the trials with hyperpolarizing conditioning potentials were the same as previously. Weidmann attributed this variance to a deficiency in the experimental control of the tissue, but these simulations suggest a different explanation. The low $\dot{V}_{max}$ would be expected with that method in the range of conditioning potentials where it occurred. A step from the plateau ($h_\infty = 0$) to potentials with very short τ_h could easily produce normal $\dot{V}_{max}$, as 50 ms could be a sufficient number of time constants to allow close approach to the equilibrium value of h. Conditioning potentials in the range of −65 to −75 mV would be expected to poduce low $\dot{V}_{max}$, because these potentials have the longest τ_h values. Hence, even though Weidmann's protocol was not the safest method, it should have produced valid results, and the MNT model's h-variable may be considered as having a valid experimental basis. It should be noted that study of an intervention that alters the gating time constants may be misleading, unless this problem is handled carefully.

STIMULATION PULSE The second factor that can introduce error in the $\dot{V}_{max}$-h relationship is the simulation pulse itself. By varying the extent to which it is suprathreshold, a stimulus can vary the rate of activation of the Na channel and in that way affect the $\dot{V}_{max}$ rates. Examination of the results in the 4 μs constant size stimulus trials showed two opposing trends in changes in $\dot{V}_{max}$. Both are related to the activation rate of the Na channel, but produce opposite effects due to the way in which activation rate acts as a limiting process. With the most negative potential, a successful, constant-size stimulus will raise the membrane the least amount above threshold. The potential will rise only slowly at first, so that the latency will be the longest. This allows additional time for the h-gate to shift (decrease) from its value at the end of the conditioning pulse. This shift of the h-values will be greatest for the more negative conditioning potentials, because they will have the longest latencies. As the size of the stimulus is increased, the potential will be more suprathreshold than previously, will have shorter latencies than previously, and will have less time for the h-variable to decrease. In this way increased stimulus size produces higher $\dot{V}_{max}$ by enabling the Na channel to activate faster than the h-variable can inactivate. Also, for any single stimulus strength the fractional $\dot{V}_{max}$ values near the most hyperpolarized potential will be higher than from the most hyperpolarized potential, because these upstrokes will have shorter latencies, and therefore less decrease in h value than the most hyperpolarized trial.

The other trend shown by increasing the stimulus size is to decrease the $\dot{V}_{max}$ from the least negative potentials. This is again an activation time effect, but through a different process. The membrane is pushed well above threshold (by a stimulus large enough to be at least threshold for the more negative potentials) and hence long latency cannot occur. However, as potential rises, τ_m levels off, so that progressively higher end-stimulus potentials will decrease activation time by ever-lessening amounts. Because the end stimulus potential was fairly high for these conditioning potentials, each 1-mV rise in V can cause a significant decrease of Na driving potential ($V - E_{Na}$). In this way two opposing processes occur during the latent period, m increasing and $V - E_{Na}$ decreasing. The net result is a decreased $\dot{V}_{max}$, as compared to the case of a slightly lower end stimulus potential, due to a smaller driving potential at $\dot{V}_{max}$. This effect was not seen in the most negative conditioning potentials, because the end-stimulus potential was much more negative than with the high conditioning potentials, so that each 1-mV rise in V is a small fraction of the total driving potential, and could be hidden by the decreased latency effect. The middle range of conditioning potentials did seem to show both effects, with $\dot{V}_{max}$ first increasing, then decreasing as stimulus size increased.

ROLE OF τ_h Given the above analysis, a solution would seem to be to redefine the stimulus to include some control over activation time. This was done by adjusting the stimulus in each trial to achieve a constant latency. This does not completely eliminate the error, but rather tries to give each effect equal chance over all conditioning potentials to produce alterations. In this way, it is hoped that each potential will be affected by the same amount, so that when fractional $\dot{V}_{max}$ is measured, the alteration effects will all cancel out. This would seem to be a reasonably good assumption, as shown by the results of all curves obtained with controlled latencies. The claim could be made that this close a match with such widely different stimulating pulses (4 μs and 10 ms) is purely fortuitious, for the following reason. While a constant latency maintains greater uniformity in the activation process throughout the trials, it does not really force uniformity on the inactivation process (decrease in h-value) that occurs during excitation, so that the good results obtained from the model are due purely to the h-variable's kinetics having such long time constants in relation to the activation process.

A straightforward way to evaluate this argument is by decreasing the τ_h to half their values in the MNT model. With this modification, they are all shorter than the time constants obtained by Weidmann. They are also shorter than the time constant of inactivation for large depolarizations estimated by Dudel et al. (1966). Sets of trials (2-ms latency) with this modification showed that 4 μs and 5 ms stimuli fit h_∞ well. Since this modification in time constant can be expected to encompass any differences between the h-variable kinetics in the MNT model and an actual Purkinje fiber, the argument of latency providing an erroneously fortuitous close fit may be rejected at least for stimuli 5 ms or less.

CORRECTION OF $\dot{V}_{max}$ CURVES Of the two types of adjustments considered here, variation in m-gate value is small, and adjusting for m-value at $\dot{V}_{max}$ is of small benefit. Hence, in an experimental situation it seems that assuming the m-gate value to be the same for all trials would be sufficiently close to the actual case to maintain the validity of the results. The other adjustment, for actual V at $\dot{V}_{max}$ ($\overline{V}$), produces similar results. For the 4-μs stimuli cases, this adjustment was of some benefit, although the un-

adjusted curve was not in gross error. The 10-ms stimuli case gave similar results. The general conclusion here is that these two corrections are not necessary, since variations in the factors are small and would produce corrections of the same order as experimental error. This agrees well with the findings of Weidmann (1955) and Baer et al. (personal communication) that $\bar{V}$ varies little.

10-ms stimuli are longer than necessary in an experimental situation. Voltage uniformity along with a short segment of a Purkinje fiber can be expected with significantly shorter stimulus durations. Considering the results discussed above, the best course in an experimental situation might be to use a stimulus duration as short as is feasible, given the limitations of a particular experimental arrangement. The expectation then would be that, while some small difference might be detected if significantly different stimuli durations are used, the differences between sets of trials and from the theoretical ideal result would be within the limits of experimental error.

Membrane Responsiveness

An important aspect of the commonly used technique known as membrane responsiveness is demonstrated here. The h-gating variable's kinetics at most potentials involved are slower than $\dot{V}$ during phase 3, so that the h-value is not at its equilibrium value during phase 3. This is well demonstrated by the plot of $h_\infty(V)$ and $h(t)$ in Fig. 4. Since there are no basic differences in the processes involved, it is to be expected that the membrane responsiveness $\dot{V}_{max}$ curve would closely follow the $h(t)$ curves. This has been shown by Chen et al. (1975). In this way, factors altering $h(V)$ during phase 3 alter the membrane responsiveness. If, for example, an intervention decreases the repolarizing currents (I_{x1} and I_{x2} in the MNT model) then the dV/dt during phase 3 would decrease, and a slower repolarization rate is analogous to a longer conditioning step duration. This enables the h-variable to increase more towards its equilibrium value during repolarization. In this manner an intervention decreasing repolarization currents has produced a higher membrane responsiveness curve, even though the sodium system's basic parameters have not been affected in any manner. If the sodium system is not altered, then faster phase 3 lowers "responsiveness," while a slower phase 3 increases it. Of course, it is also true that if an intervention altered the sodium system, then this would certainly produce altered membrane responsiveness.

Relative $\bar{G}_{Na}$ from $\dot{V}_{max}$

These simulations have demonstrated that in a system modeled by the MNT equations, the technique of using $\dot{V}_{max}$ to provide information on the h-gate component is valid, with the limitations discussed above, and with the assumptions and approximations outlined in the introduction. Among those assumptions was that $\bar{G}_{Na}$ was a constant, an unchanged value common to all trials. Use of TTX or other similar substances is an occasion where $\bar{G}_{Na}$ would change. The basic assumption is that two processes occur, alteration of h-value within each trial set, and alteration of $\bar{G}_{Na}$ between the trial sets. The h-value variation within a set of trials is eliminated by comparison ($\dot{V}_{max\text{-}TTX}/\dot{V}_{max\text{-}control}$) of the $\dot{V}_{max}$ values obtained at the same conditioning potential. Again assuming that m-value and driving potential remain constant between sets, approximations previously seen to be acceptable, this should yield a value for the apparent fraction of $\bar{G}_{Na}$ still available at each potential. Since TTX acts to reduce $\bar{G}_{Na}$, (1 – apparent $\bar{G}_{Na}$) is more appropriate to this

analysis, and can be called apparent $\bar{G}_{Na}$ block. These simulations showed that if $\bar{G}_{Na}$ is altered to some smaller constant value, the apparent blockage curve does fairly well indicate what has happened. The curve is relatively flat, and near, although not at the actual level. The rise at the depolarized conditioning potentials is due to the same process that produced the errors in the fractional $\dot{V}_{max}$ curves previously at the depolarized potentials. With that error-inducing factor, the apparent blockage curves do represent the actual alteration to a reasonable degree. This is also indicated by the near match of all the fractional $\dot{V}_{max}$ curves of constant $\bar{G}_{Na}$ to each other and to the curve with normal $\bar{G}_{Na}$. Since the fractional display method eliminates $\bar{G}_{Na}$ if it is constant throughout the set of trials, the curves would be expected to be the same, which, as before, should also match the h_∞ curve.

TTX is believed to work in this manner in many tissues, but mammalian cardiac ventricle may have an additional process involved, in that the TTX effectiveness seems to be voltage-dependent (Baer et al., 1976). Simulations utilizing sigmoid voltage dependency of $\bar{G}_N$ were designed to test this situation. Like the demonstrated TTX effect (P. Best, personal communication) $\bar{G}_{Na}$ was decreased to some base level, and over the range of approximately -85 to -70 mV it is further dropped to near zero. This set of upstroke trials showed an apparent blockage that accurately reflected the actual programmed blockage process. In addition, fractional $\dot{V}_{max}$ did not match the previous curves, as would be expected with the value of $\bar{G}_{Na}$ varying during the set of trials, so that the $\dot{V}_{max}$ at -100 mV could not be used to normalize for $\bar{G}_{Na}$ throughout the trials. This supports the conclusion of Baer et al. (1976) of TTX's voltage-dependent effect, and is contrary to the extrapolated conclusion reached by Cohen and Strichartz (1977) in their report of results of simulations. The causes of this difference can be understood by consideration of the methods that led to their conclusion.

Cohen and Strichartz performed their simulations using the Hodgkin-Huxley model for squid axon, and applied their results to cardiac tissue. The squid results seemed to show a false voltage-dependent effect because of the presence of a significant amount of outward current at the time of $\dot{V}_{max}$, so that I_i did not equal I_{Na}. In cardiac membranes the action potential is much longer, due both to rectifying K channels that decrease K conductance at depolarized potentials, and to the much slower activation of the repolarization currents. Because of these factors, I_i is much closer to I_{Na} in cardiac membranes, and shows noticeable deviation only at the most depolarized conditioning potentials (Hondeghem, 1978). Therefore, due to differences in the ionic current systems, as reflected in the two models, the false voltage dependency proposed by Cohen and Strichartz (1977) may be of little importance. Because $\dot{V}_{max}$ seems to follow theoretically varying components of I_{Na} so well, a strongly voltage-dependent apparent blockage curve could arise only from a voltage-dependent $\bar{G}_{Na}$ process. In addition, Cohen and Strichartz's (1977) results for squid axon simulation may be subject to errors introduced by their method of stimulation. They used a constant magnitude (26 mV) instantaneous depolarization, quite similar to a 4 μs constant current intensity stimulus. This type of stimulation was found to provide erratic results in these calculations.

These simulations utilized the MNT model, based upon Purkinje fiber data, not ventricle. While there are differences between Purkinje and ventricle, they are much less than between squid axon and ventricle. However, Hondeghem (1978) used the ventricular muscle model

of Beeler and Reuter (1977) for simulation of upstrokes at various conditioning potentials and fixed values of $\bar{G}_{Na}$. He reports that in all cases I_i is within 2% of I_{Na}. He also used an instantaneous depolarization method of stimulation, but that is not significant for his work, since he did not make any comment upon the relation of fractional $\dot{V}_{max}$ curves or apparent block curves obtained to the ideal results. However, it is to be expected that, as in the simulations here, the fractional $\dot{V}_{max}$ or apparent blockage curves would not show a false voltage dependency.

In a reply to Hondeghem's study, Strichartz and Cohen (1978), using the squid axon model modified for zero potassium and leak currents, reported that there was a false voltage-dependent effect due to change in the h-value between the start of the stimulus and the moment of $\dot{V}_{max}$. They suggest that the good results obtained with the MNT model are because the h kinetics are so slow relative to the m kinetics, and question the validity of the MNT model's h kinetics.

Their false voltage-dependent results, however, may be due to their method of stimulation (instantaneous constant size depolarization), without control over latency. That method of stimulation could produce erroneous results, in large part by uncontrolled changes in the h-value during the variable latent period. The use of constant latency largely eliminates this problem, not by preventing any change in the h-variable but by giving the same opportunity for change in the h-value to occur from all conditioning potentials. While not indicating the exact level of $\bar{G}_{Na}$ block with a decreased constant $\bar{G}_{Na}$, the apparent block plot was reasonably flat until the most depolarized levels, and could unmistakably show a voltage-dependent $\bar{G}_{Na}$ occurring over the midrange of h_∞ potentials.

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REFERENCES

BAER, M., P. M. BEST, and H. REUTER. 1976. Voltage-dependent action of Tetrodotoxin in mammalian cardiac muscle. *Nature (Lond.).* **253**:344–345.

BEELER, G. W., and H. REUTER. 1977. Reconstruction of the action potential of ventricular myocardial fibers. *J. Physiol. (Lond.).* **268**:177–210.

CHEN, C. M., L. S. GETTES, and B. G. KATZUNG. 1975. Effect of lidocaine and quinidine on steady-state characteristics and recovery kinetics of $(dV/dt)_{max}$ in guinea pig ventricular myocardium. *Circ. Res.* **37**:20–29.

COHEN, I. S., and G. R. STRICHARTZ. 1977. On the voltage-dependent action of tetrodotoxin. *Biophys. J.* **17**: 275–279.

DUDEL, J., K. PEPER, R. RÜDEL, and W. TRAUTWEIN. 1966. Excitatory membrane current in heart muscle (Purkinje fibers). *Pflügers Arch. Gesamte Physiol. Menschen Tiere.* **292**:255–273.

DUDEL, J., and R. RÜDEL. 1970. Voltage and time dependence of excitatory sodium current in cooled sheep Purkinje fibres. *Pflügers Arch. Eur. J. Physiol.* **315**:136–158.

GETTES, L. S., and H. REUTER. 1974. Slow recovery from inactivation of inward currents in mammalian myocardial fibres. *J. Physiol. (Lond.).* **240**:703–724.

HODGKIN, A. L., and A. F. HUXLEY. 1952. A quantitative description of membrane current and its application to conduction and excitation in nerve. *J. Physiol. (Lond.).* **117**:500–544.

HONDEGHEM, L. M. 1978. Validity of $\dot{V}_{max}$ as a measure of the sodium current in cardiac and nervous tissues. *Biophys. J.* **23**:147–152.

McALLISTER, R. E., D. NOBLE, and R. W. TSIEN. 1975. Reconstruction of the electrical activity of cardiac Purkinje fibers. *J. Physiol. (Lond.).* **251**:1–59.

SCHOENBERG, M., and H. A. FOZZARD. 1979. The influence of intercellular clefts on the electrical properties of sheep cardiac Purkinje fibers. *Biophys. J.* **25**:217–234.

STRICHARTZ, G., and I. COHEN. 1978. $\dot{V}_{max}$ as a measure of $\bar{G}_{Na}$ in nerve and cardiac membranes. *Biophys. J.* **23**: 153–156.

WEIDMANN, S. 1955. The effect of the cardiac membrane potential on the rapid availability of the sodium carrying system. *J. Physiol. (Lond.).* **127**:213–224.