Maximal Upstroke Velocity as an Index of Available Sodium Conductance

Comparison of Maximal Upstroke Velocity and Voltage Clamp Measurements of Sodium Current in Rabbit Purkinje Fibers

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SUMMARY. We compared the maximal upstroke velocity of action potentials in short rabbit Purkinje fibers with sodium currents measured with a two-microelectrode voltage clamp. The number of sodium channels available to open during a sudden depolarization was varied either by blockade with tetrodotoxin or by inactivation with steady depolarizations. In both cases, the maximal upstroke velocity was found to be a very nonlinear measure of the number of available sodium channels. For example, 3 μM tetrodotoxin blocks 85% of the sodium channels, but reduces the maximal upstroke velocity by only 33%. Voltage clamp and upstroke velocity experiments were reconstructed with a computer model of the rabbit Purkinje fiber preparation that was closely based on experimental measurements of passive cable properties and sodium channel characteristics. The simulations indicate that our voltage clamp measurements of sodium current accurately report changes in channel availability, but they also show that the maximal upstroke velocity is a strongly nonlinear index of available sodium conductance. Most of the nonlinearity arises from the activation kinetics of the sodium channels: as the pool of available channels decreases, a greater percentage of those channels activate and contribute inward current at the time of the maximal upstroke velocity. Simulations predict that the maximal upstroke velocity-available sodium conductance relationship would still remain nonlinear at 37°C or under different stimulus conditions that give uniform or continuously propagated action potentials. The nonlinearity may invalidate inferences based on earlier maximal upstroke velocity experiments: the existence of two types of sodium channels with different tetrodotoxin sensitivity, steady state voltage dependence of tetrodotoxin block, voltage range over which sodium channels inactivate, and rapid, then slow recovery of sodium channel availability following a sudden repolarization. All of these conclusions need to be reevaluated. (Circ Res 54: 636–651, 1984)

IN 1951, Draper and Weidmann established that the rapid depolarizing phase of the cardiac action potential is generated by an increase in sodium conductance (gNa). Since their pioneering work, cardiac electrophysiologists have used the maximal upstroke velocity (V\text{max}) to characterize sodium channel availability and its dependence on membrane potential (Weidmann, 1955a), extracellular calcium (Weidmann, 1955b), and antiarrhythmic agents (Weidmann, 1955b; Johnson and McKinnon, 1957; Heistracher, 1971; see Hondeghem and Katzung, 1977, for a review). Interpretation of these studies requires knowledge of how V\text{max} and sodium channel availability are related. In the absence of direct evidence from voltage clamp experiments, most authors have assumed—either explicitly or implicitly—a linear relationship.

There are good reasons to question whether V\text{max} is proportional to the number of available sodium channels. The original rationale for using V\text{max} (Hodgkin and Katz, 1949; Weidmann, 1955a) involves several assumptions (see for example, Weld and Bigger, 1975; Tsien and Siegelbaum, 1978). These are as follows: (1) the impulse either propagates with constant conduction velocity in one dimension or not at all (uniform membrane action potential); (2) the membrane capacitance is independent of upstroke velocity; (3) total ionic current at the time of V\text{max} is dominated by sodium current (I\text{Na}); (4) the time of V\text{max}, I\text{Na} is strictly proportional to the available gNa just before the upstroke. Breakdown of one or more of these assumptions gives rise to a nonlinear relationship between V\text{max} and available gNa in nerve (Ulbricht and Wagner, 1975) and skeletal muscle (Colquhoun et al., 1974; Pappone, 1980), as predicted by models of nerve membranes (Frankenhaeuser and Huxley, 1964; Hunter et al., 1975; Khodorov and Timin, 1975; Cohen and Strichartz, 1977, Strichartz and Cohen, 1978; Cohen et al., 1981b).

In heart, the relationship between V\text{max} and available gNa remains a subject of considerable debate. The importance of this issue has been underscored by recent controversy over the inhibitory effect of
tetrodotoxin (TTX) on myocardial sodium channels. Baer et al. (1976) found that reduction of $V_{\text{max}}$ by TTX is markedly enhanced by steady membrane depolarization. They interpreted the results as genuine voltage-dependent TTX binding—a fundamental difference between sodium channels in heart and those in nerve or skeletal muscle. However, Cohen and Strichartz (1977) argued that $V_{\text{max}}$ could be a nonlinear measure of available $g_{\text{Na}}$ and that the nonlinearity might produce an apparent voltage dependence of TTX binding as an artifact. In response to this criticism, Hondeghem (1978) and Walton and Fozzard (1979) defended $V_{\text{max}}$ as a linear index of sodium channel availability, using calculations based on models of cardiac membranes (Beeler and Reuter, 1977; McAlistier et al., 1975). The controversy has not been resolved—in part, because the theoretical arguments hinge on the same assumptions as the original rationale for using $V_{\text{max}}$. Clearly, a direct comparison between $V_{\text{max}}$ and measurements of $I_{\text{Na}}$ under voltage clamp is needed.

Fortunately, such a comparison has become possible with recent improvements in methods for measuring $I_{\text{Na}}$ under voltage clamp (Colatsky and Tsien, 1979b; Lee et al., 1979; Ebihara et al., 1980). Our experiments were done with very short rabbit Purkinje fibers: favorable structural and electrical properties allow $I_{\text{Na}}$ to be measured with good voltage control (Colatsky and Tsien 1979a; Colatsky, 1980). We compared $V_{\text{max}}$ and $I_{\text{Na}}$, while the number of available sodium channels was varied with TTX or membrane potential. The results show that $V_{\text{max}}$ can be a seriously nonlinear measure of sodium channel availability, even under favorable conditions.

The voltage clamp experiments also allowed a realistic simulation of the electrical behavior of the rabbit Purkinje fiber preparation during $V_{\text{max}}$ or $I_{\text{Na}}$ experiments. These simulations predict a nonlinear relationship, in reasonable agreement with that found experimentally. Together, experiments and simulations suggest the need for reevaluation of earlier $V_{\text{max}}$ experiments. We find that $V_{\text{max}}$ is probably useful for some purposes, but that under other circumstances it can be very misleading. Preliminary reports of parts of this work have been published (Cohen et al., 1979, 1981a; Bean et al., 1980, 1982).

Methods

Measurements of $I_{\text{Na}}$ and $V_{\text{max}}$

Sodium currents were measured in short rabbit Purkinje fiber preparations using a two-microelectrode voltage clamp, as previously described (Colatsky and Tsien, 1979a; Colatsky, 1980; Cohen et al., 1981a). $V_{\text{max}}$ measurements were carried out in the same kind of preparation. To elicit action potentials, the preparation was released from voltage clamp by an electronic relay, and a depolarizing current was injected through the current-passing electrode. The stimulus strength was chosen so that $V_{\text{max}}$ occurred about 2.5 msec after the release from voltage clamp. The voltage signal was differentiated electronically, using a circuit similar to that of Diamantides (1962). The differentiator with a microelectrode was linear for velocities less than 500 V/sec, and had a time constant of 20 usec. Records of voltage and its time derivative were made with an oscilloscope camera, and values of $V_{\text{max}}$ were determined graphically.

The modified Tyrode’s solution used in our experiments contained 155 mm NaCl, 4.0 mm KCl, 1.8 mm CaCl$_2$, 3.6 mm MnCl$_2$, 0.5 mm MgCl$_2$, 5.0 mm dextrose, and 10 mm HEPES, and was titrated to pH 7.4 with about 4.5 mm NaOH. Low sodium solutions were made by equimolar replacement of NaCl by choline chloride. TTX was obtained from Calbiochem. Temperature was maintained at 17.5 ± 1.0°C unless otherwise indicated. The Pattern-search algorithm (see Colquhoun, 1971) was used to obtain least squares fits to the dose-response and inactivation curves.

Mathematical Reconstruction of $V_{\text{max}}$ and $I_{\text{Na}}$: Formulation of Rabbit Purkinje Fiber Cable Model

Theoretical calculations were made in order to determine, first, what effect the cable properties of the preparation have on the $V_{\text{max}}$-$g_{\text{Na}}$ relation and, second, whether the cable properties compromise the ability of the preparation to be adequately voltage clamped. The calculations were made with a mathematical model that was based, as far as possible, on the experimentally determined membrane and cable characteristics of the preparations.

The cable equation, relating the voltage distribution along the cable to membrane current, is

$$\frac{\partial^2 V}{\partial x^2} = \frac{2R_i}{a} I_m$$

(1)

where $x$ is distance along the fiber, $a$ is the fiber radius, $R_i$ is the axial resistivity, and $I_m$ is the membrane current density. $I_m$ is composed of capacitative current ($I_c$) and ionic current ($I_I$).

$$I_m = I_c + I = C_m \frac{dV}{dt} + I_I$$

(2)

Under our conditions, $I_c$ has three components, background or leak current ($I_b$), sodium current ($I_{Na}$), and sodium channel gating current ($I_g$).

$$I = I_c + I_{Na} + I_g$$

(3)

The gating current results from the molecular rearrangement of charged groups within the sodium channels when the channels are activated by a depolarization [see Almers (1978) for a review]. It was included in the calculations because it affects the upstroke and conduction velocities (see Hodgkin, 1975; Adrian, 1975) but its presence or absence was found to have no significant effect on the $V_{\text{max}}$-$g_{\text{Na}}$ relation.

Sodium conductance or $g_{\text{Na}}$ is defined as $g_{\text{Na}} = I_{Na}/(V - V_{Na})$, where $V_{Na}$ is the reversal potential of the sodium channel. Following the experimental measurements of Colatsky and Tsien (1979b) and Colatsky (1980), the sodium current $I_{Na}$ was calculated by means of the constant-field equation

$$I_{Na} = P_{Na}N_{Na} \frac{V^2}{RT} \exp(V - V_{Na}) \frac{F/RT}{1 - \exp(VF/RT)}$$

(4)

where $P_{Na}$ is the sodium permeability, $N_{Na}$ is the external sodium concentration, and $R$, $T$, and $F$ have their usual meanings. $V_{Na}$ in 155 mm Na$_2$ was taken as +50 mV (see...
Colatsky, 1980); $V_{na}$ in solutions with lower $Na_0$ was calculated with the assumption that the effective internal cation concentration $[Na_0 + (P_0/Na_0) K_0]$ remains constant. The voltage- and time-dependence of $P_{na}$ were described by Hodgkin-Huxley-type equations,

$$P_{na} = P_{na 0} m^3 h$$

$$\frac{dm}{dt} = \alpha_m(1-m) - \beta_m m$$

$$\frac{dh}{dt} = \alpha_h(1-h) - \beta_h h$$

$$\alpha_m = 0.06(-32 - V)/exp((-32 - V)/10) - 1$$

$$\beta_m = 2.4 exp((-57 - V)/18)$$

$$\alpha_h = 0.001 exp((-76 - V)/8.6)$$

$$\beta_h = 0.33/\{exp((-40 - V)/9.4) + 1\}$$

where $N$ is the number of channels/cm$^2$, $z'$ is the effective valence/gating particle, $e$ is the unitary electronic charge, and $m$ and $h$ are the activation and inactivation variables given above. This expression was taken from Adrian (1975), but also includes the factor $h$, to take into account the recent finding that the gating charge immobilizes more strongly than $V^*$, is proportional to the number of available electronic charges. The channel density $N$ was related to the specific permeability of 4.7 x 10$^{-14}$ cm$^2$/sec (Sigworth and Neher, 1980),

$$N(cm^{-2}) = \frac{P_{na}(cm/sec)}{4.7 \times 10^{-14} cm^2/sec}$$

The leak current density ($I_L$) was made time independent, but voltage dependent, in accordance with experimental records. The voltage dependence was given by a third-order polynomial (see Jack et al., 1975, p. 393)

$$l_k = 3Nz'e \frac{dm}{dt} h$$

which, with $p$, $q$, and $r$ chosen appropriately, gave an excellent fit to the leak currents recorded in the regular fiber that was used for fitting the kinetics of sodium currents (see legend to Fig. 7). For the simulation of fibers with different dimensions, the expression for leak current density per cm$^2$ of cylindrical surface area was adjusted so that the leak current per $\mu F$ of total capacity was a constant.

The system of Equations 1-14 was solved numerically by the method of Cooley and Dodge (1966) to give membrane voltage and current at each point along the cable as a function of time. The cable was divided into 42 segments for the calculations. The ends of the cable were assumed to be disks of membrane with the same properties as the rest of the fiber. The integration step used in the calculation varied from 0.1 to 50 $\mu$sec and was selected in each simulation so that a further reduction made no significant difference in the result.

**Voltage Clamp Simulations**

Two sets of voltage clamp simulations were made. Each was designed to simulate the condition of a particular voltage clamp experiment (see Figs. 7 and 9). In each case, the cable geometry and electrical properties were chosen to be consistent with experimental data. In addition, the effective voltage clamp gain (determined by the actual voltage clamp gain and the resistance of the current electrode) was adjusted to match the experimental data. In all simulations, the current-passing electrode was placed at the middle of the cable, and the voltage electrode was located one-third of the distance from the middle to the end, in accordance with our experimental practice (see DiFrancesco and McNaughton, 1979). In the voltage clamp simulations, the current injected ($I_T$) by the current-passing electrode was determined by the difference between a command potential ($V_c$) and the membrane potential at the cable segment corresponding to the position of the voltage electrode ($V_v$),

$$I_T = G_{clamp} (V_c - V_v)$$

where $G_{clamp}$ is the gain of the voltage clamp in mhos. $G_{clamp}$ was frequency independent in the simulations because the bandwidth of the voltage clamp was limited by the cable properties of the Purkinje fibers. It was found experimentally that the current-passing electrodes passed outward current more effectively than inward current. Therefore, $G_{clamp}$ was given separate values in the simulations, depending on the sign of the current. In the simulation shown in Figure 7, $G_{clamp}$ is 0.051 for outward current and 0.0017 for inward current; for Figure 9, $G_{clamp}$ is 0.0021 for outward current and 0.007 for inward current.

In the simulations, as in the experiments, step changes in $V_c$ were lagged with an exponential time constant of 0.25 msec.

**Short Cable Action Potential Simulations**

In action potential simulations, the fiber was stimulated by a 2-msec pulse of current delivered through the current-passing electrode; as in the experiments, the size of the pulse was adjusted for each case so that the time from the beginning of the pulse to $V_{max}$ was 2.4–2.8 msec.

**Propagated Action Potential Simulations**

The propagated action potential simulations (Figs. 11 and 12) were performed by the wave-equation method of Hodgkin and Huxley (1952c), including the modifications of Adrian (1975) for incorporating gating current. In each simulation, the expression for leakage current was adjusted by adding or subtracting a constant in order to produce zero net membrane current at the desired resting potential. The propagation constant was obtained to seven significant digits in each case, a precision adequate for a stable solution well beyond the time of $V_{max}$. Euler integration was used, with a time step of 1 $\mu$sec.

**Results**

**Terminology and Rationale**

The core of the controversy over $V_{max}$ is whether or not $V_{max}$ is proportional to the number of available
sodium channels—those channels that are neither blocked by drug nor inactivated. Their conductance is called "available \( g_{Na} \)" in this paper; in the terminology of Hodgkin and Huxley (1952), available \( g_{Na} \) is \( g_{Na} h_0 \). \( g_{Na} \) is the aggregate conductance of all unblocked sodium channels; it would be measured if all unblocked channels could be opened at once. \( h_0 \) is the fraction of unblocked or blocked channels that are not inactivated just before a sudden depolarization. This formalism is useful here because we use two independent methods for varying available \( g_{Na} \). On one hand, TTX blocks sodium channels in rabbit Purkinje fibers without changing the voltage dependence of inactivation (Cohen et al., 1981a); its effect is expressed as a decrease in \( g_{Na} \). On the other hand, steady depolarizations simply promote inactivation, stated as a decrease in \( h_0 \). Voltage clamp measurements of \( I_{Na} \) provide a direct measure of changes in available \( g_{Na} \) during either intervention; the relative changes in available \( g_{Na} \) are then compared with relative changes in \( V_{max} \).

**V\(_{max}\) as a Measure of \( g_{Na}\): Experiments with TTX**

Figure 1 compares the effects of 3 \( \mu \)M TTX on \( V_{max} \) and \( I_{Na} \). All measurements were made in the same modified Tyrode’s solution at 17°C. For the \( V_{max} \) measurements shown in panels A and B, the preparation was held at a negative potential (−104 mV) under voltage clamp, then released from voltage clamp and stimulated intracellularly with a brief pulse of depolarizing current. The resulting action potential (top trace) was recorded on the same time scale as \( dV/dt \) (bottom trace). The peak value of \( dV/dt \) or \( V_{max} \) was 231 V/sec in the absence of drug (Fig. 1A); 3 \( \mu \)M TTX (Fig. 1B) reduced \( V_{max} \) to 194 V/sec, or 84% of control.

The lower panels of Figure 1 illustrate the effect of 3 \( \mu \)M TTX on \( I_{Na} \) in a different fiber. 3 \( \mu \)M TTX reduced \( I_{Na} \) to 13% of control, a much greater reduction than was seen with \( V_{max} \). In a total of five experiments of this type, 3 \( \mu \)M TTX reduced \( I_{Na} \) to 0.15 ± 0.02 (mean ± SEM) of control; in four upstroke velocity experiments, 3 \( \mu \)M TTX reduced \( V_{max} \) to 0.67 ± 0.03 of control. The difference in fractional reductions is statistically significant, as indicated by an unpaired t-test (\( P < 0.005 \)). Thus, \( V_{max} \) is much less sensitive to TTX than is \( I_{Na} \), or, to put it another way, \( V_{max} \) is a very nonlinear measure of \( g_{Na} \).

Figure 2 shows how \( V_{max} \) and \( g_{Na} \) respond to a wide range of TTX concentrations. Plotted are dose-response curves for the block of \( I_{Na} \) and \( V_{max} \) determined in two different fibers. Triangles show the
reduction of $V_{\text{max}}$ by various concentrations of TTX; the data points are fit by a dose-response curve corresponding to a $K_{0.5}$ of 8.4 $\mu M$. The filled circles in Figure 2 show the reduction of $I_{\text{Na}}$ by TTX; this test of the 1:1 binding curve ($K_D = 0.82$ $\mu M$) are taken from our earlier paper (Cohen et al., 1981a). The results show that the relative insensitivity of $V_{\text{max}}$ holds for a broad range of toxin concentrations. For example, it is reduced to about 30% by 24 $\mu M$ TTX, a concentration at which $I_{\text{Na}}$ would be only 3% of control. An interesting feature of Figure 3 is how well the $V_{\text{max}}$ data are fit by a 1:1 binding curve, despite the fact that the midpoint of the curve ($K_{0.5}$) is about 10 times higher than the true $K_D$ for the channel-toxin interaction. This feature of the $V_{\text{max}}$ dose-response curve, which was also seen by Baer et al. (1976), illustrates that fits by 1:1 binding curves cannot always be interpreted in terms of a simple molecular interaction. However, fortuitous as it may be, the fact that the $V_{\text{max}}$ curve is well-fit by this empirical 1:1 binding curve is convenient in using such dose-response curves to describe the dependence of $V_{\text{max}}$ on available $g_{\text{Na}}$.

The sodium current data in Figure 2 were fit to the equation:

$$g_{s\text{el}} = I_{\text{Na}}/I_{\text{Na}} = 1/(1 + [\text{TTX}]/K_D) \quad (16)$$

where $I_{\text{Na}}$ is the current amplitude in the absence of TTX. Empirically, we found that:

$$(V_{\text{max}})_{\text{rel}} = V_{\text{max}}/V_{\text{max}} = 1/(1 + [\text{TTX}]/10 K_D) \quad (17)$$

where $V_{\text{max}}$ is $V_{\text{max}}$ in the absence of TTX. By solving for $[\text{TTX}]/K_D$ in Equation 16, substituting this quantity into Equation 17 and rearranging, we obtain:

$$(V_{\text{max}})_{\text{rel}} = 10g_{s\text{el}}/(1 + 9 g_{s\text{el}}) \quad (18)$$

This relationship defines the solid curve seen in Figure 3, where relative $V_{\text{max}}$ is plotted as a function of relative $g_{s\text{el}}$. Equation 18 describes a rectangular hyperbola, whose slope decreases from 10 to 0.1 as $g_{s\text{el}}$ increases 0 to 1. In contrast, the line of slope equal to 1 shown in Figure 3 would pertain if $V_{\text{max}}$ were linearly proportional to $g_{s\text{el}}$. The solid curve in this figure falls within error limits of the crossed standard error bars, which represent collected results mentioned above for the effect of 3 $\mu M$ TTX on $V_{\text{max}}$ and $I_{\text{Na}}$.

This agreement is a reassuring check on the two methods we used to reduce $I_{\text{Na}}$ to manageable size. It would be ideal to study $I_{\text{Na}}$ under exactly the same conditions of high Na0 and negative holding potential used in $V_{\text{max}}$ experiments. This is not possible with our preparation; establishing good voltage control requires that $I_{\text{Na}}$ be kept small, either with low Na0 or with depolarized holding potentials, so that most sodium channels are inactivated. In the experiment shown in Figure 1C and 1D (and the collected results indicated by the crossed symbol in Figure 3), 3 $\mu M$ TTX was applied in the same high Na0 solution as was used in the $V_{\text{max}}$ experiment, and $I_{\text{Na}}$ was kept small by holding the membrane potential relatively depolarized. The rationale for this comes from experiments demonstrating that fractional TTX block is not detectably changed with depolarizations that inactivate most of the Na channels (Cohen et al., 1981a). The smooth curve in Figure 3 represents the other approach, of using the same holding potential (−104 mV), and relying on a reduction in Na0 as a means of attenuating $I_{\text{Na}}$ for the voltage clamp measurements. Here, the argument is based on other experiments which establish that Na0 has little if any influence on TTX affinity. The near coincidence of the crossed symbol and the solid curve is consistent with the view that neither reduced Na0 nor...
Cohen et al. Is $V_{\text{max}}$ Proportional to Available $g_{\text{Na}}$?

**FIGURE 3.** The relationship between normalized $V_{\text{max}}$ and normalized $g_{\text{Na}}$. The straight line at 45° represents a linear relationship between $V_{\text{max}}$ and available $g_{\text{Na}}$. The solid curve is based on the dose-response relationships for TTX shown in Figure 2. As given by Equation 18:

$$V_{\text{max}}/V_{\text{max}}^* = 10g_{\text{Na}}/(1 + g_{\text{Na}})$$

where

$$g_{\text{Na}} = (g_{\text{Na}} \text{ TTX})/(g_{\text{Na}} \text{ no TTX}).$$

For simplicity, the curve is drawn to correspond to an exactly 10-fold difference in the concentrations required for 50% block of $V_{\text{max}}$ and available $g_{\text{Na}}$. The bars show ±1 SEM. The dashed curve is the relationship between $V_{\text{max}}/V_{\text{max}}^*$ and $g_{\text{Na}}$ found in frog node of Ranvier by Ulbricht and Wagner (1975):

$$V_{\text{max}}/V_{\text{max}}^* = (1 + 0.267(V_{\text{H}} - V_{\text{h}}))^{-1}$$

This figure is reproduced with permission from Bean et al. (1982).

steady depolarization seriously alter the effectiveness of TTX.

The dashed curve in Figure 3 shows the empirical relationship between $V_{\text{max}}$ and available $g_{\text{Na}}$ in the node of Ranvier, as determined in TTX experiments by Ulbricht and Wagner (1975). Similar nonlinearity has been reported in skeletal muscle (Colquhoun et al., 1974; Pappone, 1980). Although theoretical arguments have been presented that $V_{\text{max}}$ is a more faithful measure of available $g_{\text{Na}}$ in heart (Hodgkin, 1978; Walton and Fozzard, 1979), our results show that, in rabbit Purkinje fibers at least, $V_{\text{max}}$ can be an even more nonlinear index than in other tissues.

$V_{\text{max}}$ as a Measure of $h_{\text{Na}}$

How is the interpretation of $V_{\text{max}}$ experiments affected by the finding that $V_{\text{max}}$ is not linearly proportional to available $g_{\text{Na}}$? Our results with TTX can be used to predict the effect on $V_{\text{max}}$ of other interventions which reduce available $g_{\text{Na}}$. For example, reducing the steady state fraction of noninactivated sodium channels ($h_{\text{Na}}$) should be equivalent to reducing $g_{\text{Na}}$ with TTX. The dependence of sodium-channel inactivation on holding potential is:

$$h_{\text{Na}} = g_{\text{Na}} = [1 + \exp((V_{\text{H}} - V_{\text{h}})/k)]^{-1}$$

where $V_{\text{H}}$ is the holding potential and $V_{\text{h}}$ and $k$ are constants. If we substitute Equation 19 for $g_{\text{Na}}$ into Equation 18, we obtain:

$$(V_{\text{max}})_{\text{est}} = [1 + \exp((V_{\text{H}} - (V_{\text{h}} + \Delta V_{\text{h}}))/k)]^{-1}$$

where $\Delta V_{\text{h}} = k \ln(10)$. In other words, the availability curve determined with $V_{\text{max}}$ should be similar in shape to the true $h_{\text{Na}}(V)$ curve, but its mid-point should be displaced to a less negative potential.

**FIGURE 4.** Steady state availability curves for $g_{\text{Na}}$ and $V_{\text{max}}$. Filled circles are the relative peak $I_{\text{Na}}$ measured at $-37$ mV plotted as a function of the holding potential (filled circles). The curve through these points is a least-squares best fit to Equation 19, with $k = 5.7$ and $V_{\text{h}} = -89.9$ mV. Equation 20 predicts that the mid-point of an availability curve determined with $V_{\text{max}}$ will be shifted by $k \ln(10) = 13.1$ mV, and that the slope factor will still be 5.7 mV. The open squares represent relative $V_{\text{max}}$ measurements made in 155 mm Na in the same fiber. The least squares best fit of Equation 19 to these points (smooth curve) gives a slope factor $k = 5.9$ and a shift of 10.3 mV, in fairly good agreement with the predictions. To control for possible drift, we returned to 6 mm Na and carried out another voltage clamp run (open circles). In this case, $k = 5.4$; the shift...
relative to the prior {\(V_{\text{max}}\) run is} \(\Delta V = 12.4\) mV. The separation between the \(V_{\text{max}}\) and \(g_{\text{Na}}\) curves in Figure 4 is representative of our other results, although the absolute position of these curves is somewhat more negative than usual. In 15 other voltage clamp experiments, \(V_{0} = -86.2 \pm 1.4\) mV, \(k = 4.70 \pm 0.22\), and in seven upstroke velocity experiments, \(V_{0} = -77.7 \pm 2.1\) mV, \(k = \pm 5.72 \pm 0.14\) mV. The difference in values for \(V_{0}\) is statistically significant (\(P < 0.005\)). In one experiment, the \(V_{\text{max}}\) availability curve was determined for latencies of \(1.9 \pm 0.2\) msec and \(3.5 \pm 0.1\) msec. The mid-point of these curves differed by \(<1\) mV, indicating that \(V_{\text{max}}\) is a non-linear index of \(g_{\text{Na}}\) for a wide range of stimuli.

The availability curve determined with \(V_{\text{max}}\) has a very similar shape to the true \(h_{\text{Na}}(V)\) curve. This was to be expected from the fit of \(V_{\text{max}}\) measurements to a 1:1 binding curve for TTX. Rewriting the dose-response relationship as

\[
g_{\text{rel}} = \left[1 + \exp(2.3 \log((\text{TTX})/K_{B}))\right]^{-1} \tag{21}
\]

we see that the \(h_{\text{Na}}(V)\) and TTX dose-response curves are of the same functional form. Hence, a displacement of the dose-response curve along the log concentration axis corresponds precisely to a translation of the \(h_{\text{Na}}(V)\) curve along the voltage axis.

**\(V_{\text{max}}\) Studies of Voltage-Dependent Drug Binding**

Voltage-dependent drug binding is an important aspect of the interaction of many anti-arrhythmic agents with sodium channels (Hille, 1978). \(V_{\text{max}}\) measurements have often been used to identify and characterize voltage-dependent drug effects on cardiac sodium channels (Hondeghem and Katzung, 1977). Unfortunately, the nonlinear relationship between \(V_{\text{max}}\) and \(g_{\text{Na}}\) can give rise to the erroneous appearance of voltage dependence, since an intervention that reduces \(g_{\text{Na}}\) will appear to have a relatively greater effect at less negative holding potentials (Cohen and Strichartz, 1977). At a very negative potential (the condition used for Figs. 2 and 3), block of half of the sodium channels will reduce \(V_{\text{max}}\) by only about 9%. If the holding potential is changed so that \(V_{\text{max}}\) is reduced to one third in the absence of drug, then addition of the same half-blocking dose of drug will reduce \(V_{\text{max}}\) by 41%. In other words, drug binding will appear reversible, depending on the holding potential; if \(V_{\text{max}}\) is taken as a linear index of \(g_{\text{Na}}\)

In Figure 5A, the effect of TTX on \(V_{\text{max}}\) is studied at a broad range of voltages. An availability curve was first determined in the absence of drug (open triangles), and then after the addition of 5 \(\mu\)M TTX (filled squares). The effect of TTX was completely reversible (open diamonds). Although TTX binding is not voltage-dependent (Cohen et al., 1981a), the reduction in \(V_{\text{max}}\) by this drug clearly is voltage dependent. This is seen here as a shift of the availability curve to more negative voltages in the presence of TTX. The shift is quantitatively accounted for by Equation 18, even though this experiment was conducted at 30°C.

A reduction in external sodium also has a voltage-dependent effect on \(V_{\text{max}}\) (Fig. 5B). In the same fiber used for Figure 5A, availability curves were determined in 155 nM \(N_{a0}\) (open circles), 35 nM \(N_{a0}\) (filled squares), and then again in 155 nM \(N_{a0}\) (open triangles). Once again, the percentage reduction in \(V_{\text{max}}\) is greatest at less negative holding potentials. As Figure 5 illustrates, the response to TTX differs from the effect of lowering the external sodium concentration; reducing \(N_{a0}\) seems to be less effective at shifting the availability curve for a given reduction in the saturating value of \(V_{\text{max}}\). It is therefore unlikely that complete compensation for the nonlinearity of \(V_{\text{max}}\) can be attained by balancing drug block of Na channels against changes in \(N_{a0}\). Figure 5B also shows that the relationship between \(V_{\text{max}}\) and \(N_{a0}\) could in principle change from nonlinear to linear, depending on the holding potential; finding a linear relationship between \(V_{\text{max}}\) and \(N_{a0}\) (Déleze, 1959; Brady and Woodbury, 1960; Reuter et al., 1978; but see Kohlhardt, 1982) is by itself no
guarantee that $V_{\text{max}}$ is a linear measure of available $g_{Na}$.

$V_{\text{max}}$ Can Give a Distorted View of Sodium Channel Repriming Kinetics

Recovery of sodium channel availability occurs as a voltage- and time-dependent process following action potentials or depolarizing voltage clamp pulses; the time course of recovery can be dramatically slowed under the influence of local anesthetics, an effect which may contribute to their anti-arrhythmic action (Hondegem and Katzung, 1977; Hille, 1978). Recordings of $V_{\text{max}}$ are often used with the hope of determining the time course of recovery of sodium channel availability. Our evidence that $V_{\text{max}}$ is a nonlinear measure of available $g_{Na}$ leads to the prediction that recovery of $V_{\text{max}}$ will deviate from a single exponential time course, even if the true repriming of $I_{Na}$ is close to a single exponential (Colatsky, 1980; Ebihara et al., 1980; Cohen et al., 1981a) give or take a slight delay (Brown et al., 1981).

Consider an idealized experiment studying the recovery of available $g_{Na}$ and $V_{\text{max}}$ following a depolarizing pulse that completely inactivated sodium channels. Let us assume that removal of inactivation can be described as

$$ g_{\text{rel}} = h_e(1 - \exp[-t/r_{he}]) $$

Substituting this expression into Equation 18 gives

$$ \langle V_{\text{max}} \rangle_{\text{rel}} = h_e(1 - \exp[-t/r_{he}])/(0.1 + 0.9h_e(1 - \exp[-t/r_{he}])) $$

Figure 6 plots these two equations as simulations of $V_{\text{max}}$ and $I_{Na}$ experiments. The variables $h_e = \alpha_e/(\alpha_e + \beta_e)$ and $r_{he} = 1/|\alpha_e + \beta_e|$ were calculated from $\alpha_e$ and $\beta_e$ as described in Equations 10 and 11. Panel A shows the true time course of sodium channel repriming; the time courses are, in fact, typical for rabbit Purkinje fibers at 17°C. The solid curves in Figure 6B show how the recovery of $g_{rel}$ translates into a time course for $V_{\text{max}}$. At each potential, $(V_{\text{max}})_{\text{rel}}$ initially increases 10 times faster than $g_{rel}$; when $t \approx \tau/4$ the slopes are equal; thereafter $V_{\text{max}}$ changes more slowly than $g_{rel}$.

How would this be interpreted in a simulated experiment? Kinetic parameters determined from the simulated data would depend on the interval chosen for curve fitting. The dashed curves in Figure 6B are fits to the simulated data for $t > 10$ msec, as though information for $t < 10$ msec were not available. (This might be representative of most experiments. Very short repriming intervals are hard to study because the membrane potential takes some time to settle during action potential or voltage clamp repolarizations.) The dashed curves provide a very good fit, and one might be tempted to conclude that the recovery of $V_{\text{max}}$ was, in fact, a simple exponential. The main indication to the contrary is the large y-intercept, particularly at the more negative recov-
**FIGURE 6.** Recovery of sodium channel availability as seen in a simulated experiment using \( V_{\text{m}} \). Part A: true time course of sodium channel repriuning following a depolarizing pulse that inactivates all the channels. Time course is given by Equation 22 with \( h_{\text{m}} = 0.256 \), \( \tau_h = 161 \) msec at \(-80\) mV, and \( h_{\text{m}} = 0.967 \), \( \tau_h = 59.3 \) msec at \(-100\) mV. Part B: predicted time course of recovery of \( (V_{\text{max}})_{\text{rel}} \) as given by Equation 23. The dashed curves are single exponentials, fitted to the simulated "data" for recovery times >10 msec; at \(-80\) mV, \( (V_{\text{max}})_{\text{rel}} = 0.200 + 0.674(1 - \exp[-t/84.3]) \), and at \(-100\) mV, \( (V_{\text{max}})_{\text{rel}} = 0.541 + 0.456(1 - \exp[-t/26.5]) \).

circuit, and the resistance and rectification of the current-passing electrode (see Methods). The fitting proceeded as follows: first, we simulated a small depolarization from rest that elicited only capacitative current; the passive properties of the model cable (cable dimensions, specific resistance) were adjusted so that the simulated capacitative transient superimposed with the experimental transient for the same step (Fig. 7A). Next, the voltage dependence of the leakage current, determined experimentally as the current at the end of a 50-msec pulse, was fit using a polynomial approximation (see Methods). Finally, the time courses of the sodium currents at various potentials were fit as well as possible within the constraints of \( m^3h \) kinetics (Hodgkin and Huxley, 1952). The parameters to be fit at each potential were the peak sodium current, the time-to-peak sodium current, and the time constant for the inactivation of the current; the rate constants governing \( m \) and \( h \) were adjusted by trial and error to give the best possible match of these parameters. The time constant for inactivation could be fit very accurately at each potential, since it is determined in the model primarily by a single rate constant, \( \beta_h \). The peak sodium current and time-to-peak are complicated functions of all the rate constants, as well as passive cable properties, but after a number of iterations, a set of rate constants was obtained which simulated reasonably accurately the experimental results at all potentials (Fig. 7, C and D). At some potentials, the simulation gave a nearly perfect fit to the experimental currents (Fig. 7B). At other potentials, the simulated current did not precisely superimpose with the experimental current: even with the peak current and time-to-peak nearly perfectly matched, the experimental records showed more of a delay in the rising phase, as if \( m^3 \) kinetics did not give enough of a sigmoidal lag. This discrepancy is consistent with reports obtained with a number of nerve preparations (Keynes and Rojas, 1976; Neumcke et al., 1976; Bullock and Schauf, 1978).

**How Accurate are Measurements of Peak \( I_{\text{Na}} \) in Short Purkinje Fibers?**

The primary purpose of this voltage clamp simulation was to obtain a mathematical description of sodium current kinetics appropriate to the rabbit Purkinje fiber. However, the simulations are also useful for defining the conditions under which the two-microelectrode voltage clamp is relatively free of distortions due to longitudinal inhomogeneities. Arguments have been made previously (Colatsky and Tsien, 1979b; Colatsky, 1980) that when the currents are kept small, the cable properties of the preparation should have little effect on the measurement of peak sodium current at moderate depolarizations where the peak sodium current is well separated from the capacity transient. Figure 8 shows the result of simulations expressly designed to investigate this point. The filled circles show the peak sodium current vs. voltage (I-V) curve obtained in the same voltage clamp simulations shown in
Figure 7. The open circles show the peak sodium current vs. voltage curve which would be obtained for the same kinetics with an ideal voltage clamp, with perfect voltage control and spatial uniformity. The comparison shows that the I-V obtained by simulation of the experiment is quite similar to the ideal I-V. The main discrepancy from the ideal I-V is that the cable currents are about 20% smaller than the ideal currents near the peak of the I-V. This rather small error arises from the delay (due mainly to the axial resistance) in charging the cable to the desired voltage; this delay allows inactivation to proceed to a greater extent before peak current is reached than it would in the ideal case, and tends to make the currents smaller, especially at positive potentials where inactivation is fastest.

Simulated Measurements of Na Channel Availability

With appropriate expressions for sodium current in hand, the relation between $V_{\text{max}}$ and $h_n$, was modeled by simulating the type of experiment shown in Figure 4. First, voltage clamp steps for the measurement of an availability curve were simulated, with 6 mM Na, in the model as in the experiment of Figure 4. The total current in the simulated records was corrected for leak current, and peak sodium current was plotted as a function of holding potential (filled circles, Fig. 9). These points show the availability curve that would be determined in a short cable. The solid line is the true $h_n$ curve. From the close agreement, it is evident that the availability of Na channels is faithfully reported in short cables under our experimental conditions.
FIGURE 8. Effect of cable properties on measurement of peak \( I_{\text{Na}} \). Filled circles are from the same set of simulations described for Figure 7. Peak \( I_{\text{Na}} \) is plotted vs. membrane potential at the time of peak current. Triangles are from calculations of \( m^3h \) kinetics assuming perfect voltage control in time and space.

Next, the measurement of an availability curve by \( V_{\text{max}} \) was simulated for the same preparation, but with 155 mM \( \text{Na}_0 \). The passive cable properties and action potential latency were all adjusted to be consistent with the experiment in Figure 4. \( V_{\text{max}} \) is plotted as a function of holding potential (open squares, Fig. 9). As shown in Figure 4, the \( V_{\text{max}} \) availability curve has a similar shape to the \( I_{\text{Na}} \) curve (and true \( h_0 \) curve) but is shifted to more positive potentials. The simulated shift of 7 mV is not far from the experimental shift of 10 mV in Figure 4.

Figure 10 compares the simulated nonlinearity between \( V_{\text{max}} \) and \( I_{\text{Na}} \) (filled circles), constructed from the simulated availability curves in Figure 9, with the experimental relationship (solid line), derived from the fitted functions in Figure 4. Like the experimental curve, the simulated \( V_{\text{max}} \)-\( I_{\text{Na}} \) relationship is severely nonlinear, although somewhat less so than the experimental one. We believe that the difference probably arises from the fact that the \( m^3h \) kinetics used in the model did not precisely fit the time course of the experimental sodium currents; as mentioned previously, the experimental records frequently showed more of a lag in activation than \( m^3 \) kinetics could give. The \( V_{\text{max}} \)-\( I_{\text{Na}} \) relationship is very sensitive to the details of the activation kinetics of the channels, and since the relationship becomes more nonlinear with a greater delay in action (Cohen et al., 1981b), the difference between the real and theoretical activation kinetics could easily account for some or all of the small difference between the curves in Figure 10. The over-all conclusion is that the simulation successfully captures the fundamental features of the experimental situation.

Effect of Cable Geometry and Temperature on \( V_{\text{max}} \)

To what extent do the cable properties of our preparations contribute to the non-linearity between \( V_{\text{max}} \) and available \( g_{\text{Na}} \)? In Figure 11, the relationship between \( V_{\text{max}} \) and \( h_0 \) is shown for three different degrees of cable current flow. Using the same membrane properties, simulations were performed for spatially uniform action potentials (triangles); for action potentials in a 500-\( \mu \)m cable typical of the rabbit Purkinje fiber preparations that we used (filled circles); and for propagated action potentials in an infinitely long cable (open circles). In the simulations, available \( g_{\text{Na}} \) was altered by changing the holding potential, thus altering the degree of

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**FIGURE 9.** Simulation of availability curves using \( h_0 \) and \( V_{\text{max}} \). The parameters used in the simulation were based on those from the experiment of Figure 4. The cable dimensions (diameter = 120 \( \mu \)m, length = 420 \( \mu \)m) and capacitance (2.1 \( \mu \)F/cm\(^2\)) were chosen so that a simulated subthreshold capacity transient matched an experimental capacity transient. The simulation of the voltage clamp measurement of the availability curve in 6 \( \text{mM} \) \( \text{Na}_0 \) was obtained with \( P_{\text{Na}} = 92 \times 10^{-5} \text{ cm/sec} \), a value selected so that the current elicited from \(-120 \text{ mV} \) was 38 nA, the same value obtained in the experiment in Figure 4. Leak current taken as steady state current at 50 msec was subtracted. The theoretical \( h_0 \) curve is calculated as \( \alpha_h/\alpha_h + \beta_h \) with \( \alpha_h \) and \( \beta_h \) given in Equations 10 and 11 in Methods. The action potentials for the \( V_{\text{max}} \) availability curve were simulated in 155 \( \text{mM} \) \( \text{Na}_0 \) with \( P_{\text{Na}} = 107 \times 10^{-5} \text{ cm/sec} \), chosen so that \( V_{\text{max}} \) from \(-120 \text{ mV} \) was 270 V/sec, in agreement with the experiment in Figure 4.

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**FIGURE 10.** Simulated \( V_{\text{max}} \)-\( h_0 \) relation compared with experimental data. Note that the solid curve is the experimental relationship, constructed from the fitted curves in Figure 4, while the filled circles are plotted based on the simulated points in Figure 9.
steady state inactivation; relative $V_{\text{max}}$ has been normalized and plotted against the parameter $h_0$, the fraction of available channels. It is striking how similar the three relationships are; clearly, under our conditions, cable properties have little effect on the nonlinear relation between $V_{\text{max}}$ and Na channel availability. The points from the 500-μm cable simulation are nearly superimposable with those from the spatially uniform action potential simulation.

An important question is how sensitive to temperature the $V_{\text{max}}$-$h_0$ relationship is likely to be. Our experimental relationship was obtained at 17°C. A reduced temperature is necessary in the voltage clamp experiments so that the sodium currents are slow enough to be well-separated from the capacity transient. What amount of nonlinearity is expected at 37°C, where the $V_{\text{max}}$ experiments of most workers are performed? The principle effect of temperature is expected to be an increase in the rate constants governing sodium channel kinetics. The temperature dependence of the inactivation process between 16 and 26°C in rabbit Purkinje fibers has been determined by Colatsky (1980), who found a $Q_{10}$ of about 2.5. The temperature dependence of the activation process has not been determined for the temperature range of 17–37°C but from 0–20°C $Q_{10}$'s range from 1.6 to 2.6 in nerve and skeletal muscle (Frankenhaeuser and Moore, 1963; Schauf, 1973; Kimura and Meyers, 1979; Schwarz, 1979). It seems reasonable, therefore, to assume a $Q_{10}$ of 2.5 for activation as well as inactivation rate constants. Figure 12 shows the relationship expected between $V_{\text{max}}$ and $h_0$ at 37°C with the rate constants scaled in this way (filled circles). Although the relation is less severely nonlinear than at 17°C, it is still strikingly nonlinear; a reduction of $h_0$ to 0.25 results in a reduction of $V_{\text{max}}$ to 0.48.

It is, of course, possible that the rate constants in other cardiac preparations at 37°C will differ from those used in the simulation of Figure 12. The only available information about the kinetics of cardiac Na channels at 37°C comes from the experiments in aggregates of chick embryonic cells (Ebihara et al., 1979; Ebihara and Johnson, 1980). Figure 12 shows results of simulations with their kinetic rate constants, for a 500-μm cable (open triangles), and for uniformly propagated action potentials in an infinitely long cable (solid squares). There is very little difference between the results: in both cases, the relationship between $V_{\text{max}}$ and $h_0$ is somewhat more nonlinear than for rabbit Purkinje fiber parameters scaled to 37°C.

**Basis for Nonlinear $V_{\text{max}}$-$h_0$ Relationship**

Figure 13 shows the three major factors that determine the inward sodium current at the moment

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**Figure 11.** Effect of cable properties on the $V_{\text{max}}$-$h_0$ relationship. The $V_{\text{max}}$-$h_0$ relationship for a short cable (filled circles) was obtained exactly as in Figures 9 and 10, but for different cable parameters: diameter = 80 μm, length = 500 μm, $C_m$ = 5.4 μF/cm², $P_{\text{leak}}$ = 341 × 10⁻⁷ cm/sec. $P_{\text{leak}}$ was adjusted so that at negative holding potentials $V_{\text{max}}$ was about 270 V/sec, close to the experimental mean of 268 ± 30 V/sec (seven experiments). The $V_{\text{max}}$-$h_0$ relationship for spatially uniform action potentials (triangles) and propagated action potentials (open circles) were calculated using the same membrane properties. In each case, action potentials were simulated for holding potentials from −120 to −65 mV, and $V_{\text{max}}$ was normalized relative to the largest value of $V_{\text{max}}$ obtained, 273 V/sec from −110 mV for the short cable, 294 V/sec from −120 mV for the spatially uniform action potentials, and 171 V/sec from −95 mV for the propagated action potentials.

**Figure 12.** $V_{\text{max}}$-$h_0$ relation expected at 37°C. Filled circles: same cable properties and parameters as in Figure 11, but with the following changes: $\alpha_m$, $\beta_m$, $\alpha_h$, and $\beta_h$ multiplied by 6.25, corresponding to a $Q_{10}$ of 2.5; leak current and $P_{\text{leak}}$ multiplied by 1.44, corresponding to a $Q_{10}$ of 1.2. $V_{\text{max}}$ = 870 V/sec when $h_0 = 1$. Triangles: same properties except with $\alpha_m$, $\beta_m$, $\alpha_h$, and $\beta_h$ given by Equations 9–14 in Ebihara and Johnson (1980). Note that the denominator in their Equation 9 should be $1 - \exp(-V_{\text{th}} - 47.13)$. $V_{\text{th}} = 870$ V/sec when $h_0 = 1$. Filled squares: relation for propagating action potentials in a long cable with the same properties, using the Ebihara-Johnson rate constants. $V_{\text{max}}$ = 650 V/sec when $h_0 = 1$. 
FIGURE 13. Basis for simulated nonlinear relationship between $V_{\text{max}}$ and available $g_{Na}$. Each panel gives values at the point of maximal upstroke velocity for the simulation in Figure 10. For more details, see text.

Discussion

The experiments and simulations represent two largely independent ways of examining the relationship between $V_{\text{max}}$ and sodium channel availability. Both approaches point to the same overall conclusion: the relationship between $V_{\text{max}}$ and the number of available sodium channels can be strongly nonlinear in cardiac tissue, even under relatively favorable circumstances.

Earlier Arguments Concerning Necessary but not Sufficient Conditions for the Validity of $V_{\text{max}}$

Our findings disagree with earlier work that defends $V_{\text{max}}$ as a linear measure of Na channel properties. The controversy can be broken down into two questions: first, whether $V_{\text{max}}$ is proportional to $I_{Na}$, and second, whether $I_{Na}$ is proportional to the available sodium conductance.

Initially, Cohen and Strichartz (1977) directed attention to the first question and the issue of whether sodium current was seriously overlapped by outward K channel currents. Defenders of $V_{\text{max}}$ argued that outward K currents are small (e.g., Hondeghem, 1978; Reuter, 1979) or tried to eliminate K currents with Cs (Gintant and Hoffman, 1981). The relationship between $I_{Na}$ and available $g_{Na}$ has also been controversial (Strichartz and Cohen, 1978); some investigators have sought to eliminate nonlinearity between $I_{Na}$ and available $g_{Na}$ by minimizing variations in the driving force for sodium influx (Coromilas et al., 1981) or by fixing the latency between stimulus and the attainment of $V_{\text{max}}$ (Kollhardt, 1982; see Walton and Fozzard, 1979).

Our results show that, even with stringent precautions, $V_{\text{max}}$ can be a seriously nonlinear measure of available $g_{Na}$. In our experiments: (1) interference from ionic currents other than $I_{Na}$ was ruled out—Ca channels and Ca-activated K channels were blocked with Mn, and leak currents were demonstrably smaller than $I_{Na}$ at the time of $V_{\text{max}}$; (2) variations in the driving force for sodium influx or variations in latency between the stimulus and the time of $V_{\text{max}}$ were minimal—$V_{\text{max}}$ occurred at nearly a constant potential, and latency was carefully kept constant; (3) longitudinal cable current flow, a factor of possible importance in ventricular preparations where the action potential is neither spatially uniform nor uniformly propagated, was probably of little consequence in rabbit Purkinje fibers (see Fig. 11); (4) the membrane capacitance changed little
with reductions in $V_{\text{max}}$, in contrast to sheep Purkinje fibers (Fozzard, 1966; Carmeliet and Willems, 1971). Our experimental findings are strongly reinforced by the computer simulations, in which theoretically ideal conditions for $V_{\text{max}}$ measurements were chosen.

Unfortunately, all of these efforts are insufficient to make $V_{\text{max}}$ a linear index of available $g_{\text{Na}}$. Most of the nonlinearity is due to the kinetics of Na channel activation (Fig. 13). As the pool of available sodium channels decreases, a stronger stimulus must be applied to keep latency constant. Since more time is spent at stronger depolarizations where channel activation is faster, a much larger proportion of the available pool activates and contributes to $V_{\text{max}}$. In effect, the action potential upstroke is strongly buffered against interventions that reduce available $g_{\text{Na}}$.

This type of compensation was stressed by Strichartz and Cohen (1978) and Cohen et al. (1981b), who suggested that the relationship between $V_{\text{max}}$ and available $g_{\text{Na}}$ could be nonlinear. Their simulations used nerve axon parameters, and thus left room for optimism that the kinetics of cardiac sodium channels might be different enough to avoid compensation for decreased availability by increased activation (Hondeghem, 1978; Walton and Fozzard, 1979). It was clear that experimental information from cardiac tissue was needed because calculations of action potential upstroke were done without the benefit of reliable $I_{\text{Na}}$ measurements in cardiac tissue.* Now, our experiments and simulations focus the argument squarely on cardiac preparations and suggest that earlier optimism about $V_{\text{max}}$ was probably unfounded. In both rabbit Purkinje fiber and chick myocardium, the relationship between $V_{\text{max}}$ and available $g_{\text{Na}}$ appears even more nonlinear than in nerve. This is because the activation kinetics of $I_{\text{Na}}$ in myocardial cells are slower than those of nerve [compare Equations 8 and 9 with the values for $\alpha_{\text{m}}$ and $\beta_{\text{m}}$ given by Hille (1971) adjusted for a $Q_{10}$ of 1.8 (Frankenhaeuser and Moore, 1963)].

Can a Reliably Linear Relationship between $V_{\text{max}}$ and Available $g_{\text{Na}}$ be Obtained under Other Experimental Conditions?

This seems unlikely. Sodium channel kinetics qualitatively similar to those found for rabbit Purkinje fiber or chick embryonic myocardium can be seen in recently published recordings from single cells (Brown et al., 1981; Bodewei et al., 1982; Bus-tamante and McDonald, 1983; Cachelin et al., 1983). Furthermore, our experimentally based calculations suggest that varying the temperature or altering the cable geometry will not make a crucial difference (Figs. 11 and 12). Of course, the ultimate answer to this question must await experimental comparisons between $V_{\text{max}}$ and $I_{\text{Na}}$ measurements in individual preparations. Meanwhile, we feel that the burden of proof has now shifted to those who would take $V_{\text{max}}$ as a linear index of available $g_{\text{Na}}$. It will be particularly important to study guinea pig papillary muscles at 37°C, since so much work has already been done with this system. A number of puzzling results from papillary muscle would be explained if the relationship between $V_{\text{max}}$ and available $g_{\text{Na}}$ is quite nonlinear (see below).

Reevaluation of Earlier Conclusions Based on $V_{\text{max}}$ Experiments

A nonlinear relationship between $V_{\text{max}}$ and available $g_{\text{Na}}$ may explain some major discrepancies between studies using $V_{\text{max}}$ and voltage clamp measurements of $I_{\text{Na}}$.

1. $V_{\text{max}}$ experiments have been used as evidence that Purkinje fibers show two types of sodium conductance: a large rapidly changing conductance during the action potential upstroke that is relatively resistant to TTX, and a small, maintained conductance during the plateau that is relatively sensitive to TTX (Corazone et al., 1979). This view is in conflict with voltage-clamp data indicating that transient and steady sodium currents are about equally sensitive to TTX (Cohen et al., 1981a; Colatsky and Gadsby, 1980).

2. When studied with $V_{\text{max}}$, cardiac sodium channels seem to show steady state voltage dependence of TTX block. The voltage dependence is very dramatic in guinea pig papillary muscles (Baer et al., 1976; Reuter et al., 1978), and less so in other cardiac preparations (Fig. 5, this paper; Inoue and Pappano, unpublished data). On the other hand, voltage clamp experiments show no detectable steady state voltage dependence (Cohen et al., 1981a).

3. According to $V_{\text{max}}$, experiments in guinea pig papillary muscles, the midpoint of the sodium channel inactivation curve is −65 mV (Gettes and Reuter, 1974). This value is 15–20 mV less negative than the midpoint of the steady state inactivation curve determined by voltage-clamp experiments in single ventricular cells (e.g., Lee et al., 1981). The difference is even larger in ventricular tissue than in rabbit Purkinje fibers (Fig. 4, this paper). Some, if not all, of the discrepancy might be explained if the relationship between $V_{\text{max}}$ and available $g_{\text{Na}}$ were severely nonlinear in ventricular muscle.

4. Judged by $V_{\text{max}}$, the time course of sodium channel repriming shows a very rapid initial phase before a slower exponential recovery, but no such rapid phase is seen in voltage clamp experiments (see p. 20).

Although $V_{\text{max}}$ measurements probably give distorted information about the absolute location of the Na channel availability curve, they may accurately report relative displacements of the curve for interventions where this is the only effect, as in the case of extracellular Ca and local anesthetics in moderate

* McAllister et al. gave explicit warnings about the limitations of their model on pp. 2, 3, 4, and 54, of their 1975 paper.
doses (Weidmann, 1955b; Chen et al., 1975). In particular, there seems to be good agreement between studies using $V_{\text{max}}$ or $I_N$ to measure lidocaine-induced shifts in the sodium channel availability curve (see Bean et al., 1983, for references).

With these exceptions, the overall conclusion is that $V_{\text{max}}$ can be a very misleading index of availability of Na channels. Measurements of $V_{\text{max}}$ may be useful in preliminary qualitative analysis but are best avoided for quantitative analysis of Na channel properties.

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Circulation Research/ Vol. 54, No. 6, June 1984

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