LETTER TO THE EDITOR

Validity of $\dot{V}_{max}$ as a Measure of the Sodium Current in Cardiac and Nervous Tissues

Dear Sir:

In nerve the maximum rate-of-rise of the action potential ($\dot{V}_{max}$) is not a valid measure of the sodium current (I_{Na}), while in cardiac tissue there is a tight correlation between these two variables. The lack of correlation between $\dot{V}_{max}$ and I_{Na} in nerve is due to the existence of large background leakage currents, and the relatively fast development of the outward potassium current upon depolarization. Therefore, at the time of $\dot{V}_{max}$, the nonsodium currents contribute significantly to the total transmembrane ionic current in nerve. In contrast, in cardiac tissue the background current is much smaller, the instantaneous potassium current shows marked inward going rectification, and repolarizing currents turn on very slowly (several hundred millisecond time constant) so that at the time of $\dot{V}_{max}$, I_{Na} always comprises at least 98% of the total ionic current. When $\dot{V}_{max}$ is used to estimate the sodium conductance, drugs that decrease maximum sodium conductance will display an artifactual voltage dependence in nerve, but not in cardiac tissue.

The maximum upstroke velocity of the action potential ($\dot{V}_{max}$) has been used as an index of the inward sodium current (I_{Na}) by both nerve (1) and cardiac (2) electrophysiologists. Hodgkin and Katz (1) showed that at the time of $\dot{V}_{max}$, the *total* ionic current across the cell membrane is directly proportional to $\dot{V}_{max}$. Thus, the use of $\dot{V}_{max}$ to measure the sodium current accurately requires knowledge of the relative magnitudes of the nonsodium currents. Under normal conditions, the total ionic current in nerve at the time of $\dot{V}_{max}$ consists primarily of sodium current: therefore $\dot{V}_{max}$ will predominantly reflect this latter current. However, it was recently documented by Cohen and Strichartz (3) that $\dot{V}_{max}$ is not always a valid measure of the sodium current in nerve. They pointed out that when nonsodium currents contribute substantially to the total ionic current, the conditions that alter these nonsodium currents will also significantly affect $\dot{V}_{max}$. Moreover, using the Hodgkin-Huxley (4) equations, they showed that under such conditions, the use of $\dot{V}_{max}$ as a measure of I_{Na} could erroneously suggest a voltage-dependent action of tetrodotoxin on maximum sodium conductance ($\bar{G}_{Na}$). Cohen and Strichartz used these "nerve" results to offer an alternative explanation for the voltage-dependent action of tetrodotoxin on $\dot{V}_{max}$ of cardiac tissue reported by Baer et al (5). Their conclusion, that the use of $\dot{V}_{max}$ was also invalid in cardiac tissue, is extremely important because the sodium current cannot be adequately defined in heart with voltage clamp techniques (6,7). In fact, the use of $\dot{V}_{max}$ is the only readily available, generally accepted technique for studying the sodium current in cardiac tissue. I therefore decided to investigate the validity of $\dot{V}_{max}$ as a measure of the sodium current, using the technique described by Cohen and Strichartz, applied to *both* cardiac and axon models. I also investigated the possibility that the use of $\dot{V}_{max}$ could suggest a voltage-dependent effect of a drug that decreases $\bar{G}_{Na}$.

For simulation of nerve excitation I used the Hodgkin-Huxley equations (4) and the same conditions as Cohen and Strichartz (3). For the simulation of cardiac excitation I used the equations and constants given by Beeler and Reuter (8). For interactive convenience, programs

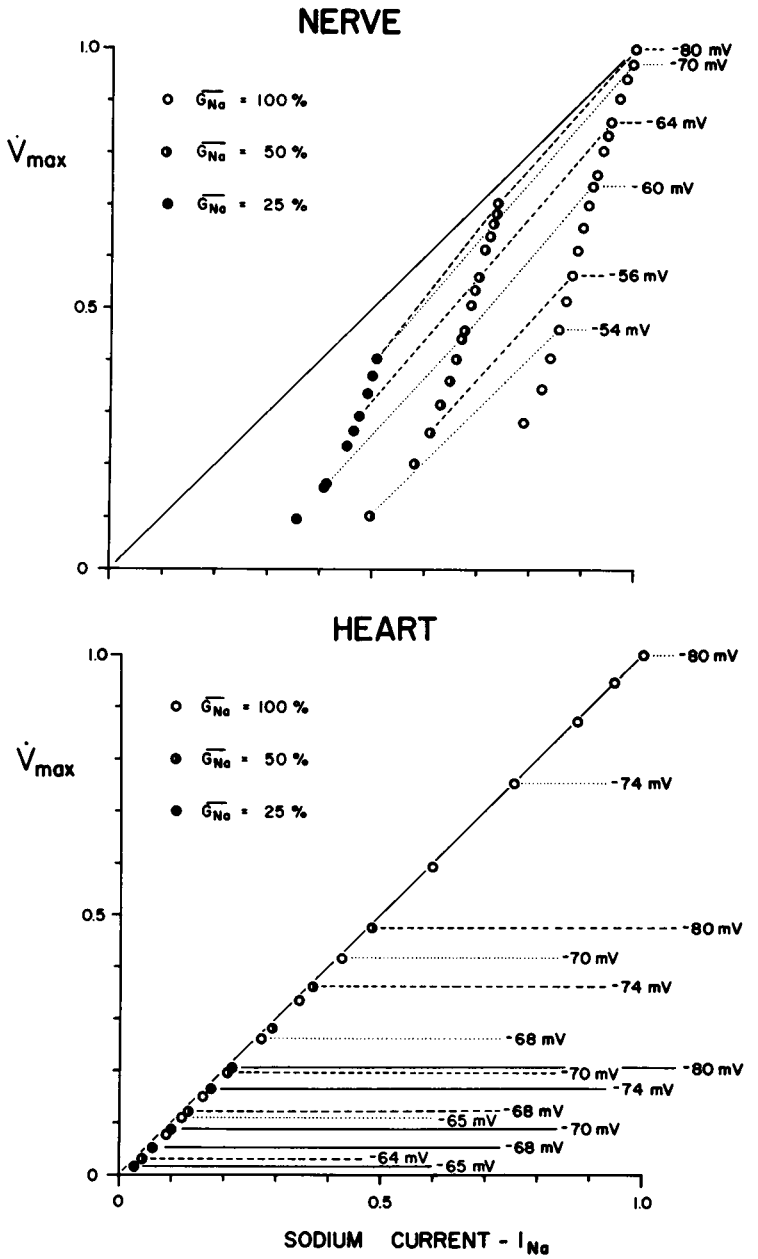

FIGURE 1 Relation between $\dot{V}_{max}$ and its corresponding sodium current in nerve (holding potential −90 mV to −50 mV range) and cardiac cells (−90 to −60 mV). The open circles represent a 100% sodium conductance ($\bar{G}_{Na}$ = 120 mmho/cm^2 in nerve, 4 mmho/cm^2 in heart), the half-filled and filled circles represent a 50% and 75% blockade of $\bar{G}_{Na}$, respectively. All values were normalized in relation to the values at −90 mV in the absence of blockade. The solid line with slope of one represents the expected relation between $\dot{V}_{max}$ and its associated sodium current if a linear and unequivocal relation were to exist. The dashed/dotted lines interconnect the data points at identical holding potentials.

were written in BASIC. The differential equations were integrated by the trapezoidal method and the step-size of integration was varied under program control so that the total integration error on any of the measured parameters remained negligibly small (i.e., less than 0.5%). Programs were run on a PDP 11/10 computer and graphic output was displayed on a VT11-VR17 oscilloscope package (Digital Equipment Corp., Marlboro, Mass.).

After confirming that the nerve simulations produced results identical to those published by Cohen and Strichartz (3) (their Fig. 1), I then proceeded to correlate $\dot{V}_{max}$ against the sodium current (I_{Na}) at the time of $\dot{V}_{max}$.

Fig. 1 shows the normalized $\dot{V}_{max}$ plotted against the corresponding normalized I_{Na} for 100%, 50%, and 25% values of $\bar{G}_{Na}$. From these results it is obvious that in nerve (top) the $\dot{V}_{max}$-I_{Na} ratio is not constant. At potentials more positive than −80 mV, the normalized $\dot{V}_{max}$ is significantly smaller than expected from the value of I_{Na}. This deviation becomes more marked, the more depolarized the cell. Furthermore, for any given potential, the deviation of $\dot{V}_{max}$ and I_{Na} from a 1:1 ratio increases as $\bar{G}_{Na}$ is decreased. Even more objectionable, Fig. 1 also indicates that for nerve the $\dot{V}_{max}$-I_{Na} relationship is ambiguous, i.e., a single value of $\dot{V}_{max}$ can represent many values of I_{Na}. These results support the conclusion of Cohen and Strichartz that such an ill-defined $\dot{V}_{max}$-I_{Na} relation cannot be used in any valid way. In contrast, the cardiac data (bottom of Fig. 1) indicate that at all potentials in the −90 to −60 mV range and for 100%, 50%, and 25% values of $\bar{G}_{Na}$, there exists a linear and unambiguous relation between $\dot{V}_{max}$ and the corresponding I_{Na}. Evaluation at even lower values of $\bar{G}_{Na}$ (not included in the figure) and studies over the full range of membrane potentials at which cardiac tissues can be excited never resulted in a deviation between these two parameters in excess of 2%. Thus, in cardiac tissue $\dot{V}_{max}$ can be considered an accurate estimate of the sodium current at the time of occurrence of $\dot{V}_{max}$ under all experimental conditions simulated in this study.

Cohen and Strichartz (3) showed that, for nerve, a voltage-dependent effect of tetrodotoxin (5) would result as an artifact if $\dot{V}_{max}$ were used as the measure of $\bar{G}_{Na}$. If $\dot{V}_{max}$ does not correctly reflect I_{Na} (as in nerve), then a priori any direct estimations of $\bar{G}_{Na}$ from $\dot{V}_{max}$ must be erroneous (see Eq. 6 of Appendix). In contrast, if $\dot{V}_{max}$ is proportional to I_{Na} (as in cardiac tissue) then, under certain conditions (see Appendix), one can directly derive valid information about the sodium conductance.

Fig. 2 shows the normalized $\dot{V}_{max}$ computed as a function of the normalized $\bar{G}_{Na}$ for two membrane potentials. It can be seen that this relation is nonlinear in nerve but relatively linear in cardiac tissue. As described by Cohen and Strichartz (3), the use of $\dot{V}_{max}$ to estimate $\bar{G}_{Na}$ will result in an artifactual voltage dependence of the potency of agents that decrease $\bar{G}_{Na}$. For example a drug dose that would decrease $\dot{V}_{max}$ by 50% at −80 mV decreases $\dot{V}_{max}$ by 65% at −60 mV, i.e., erroneously suggests a voltage-dependent action. In contrast, in cardiac tissue the dosage that decreases $\dot{V}_{max}$ by 50% at −90 mV also decreases $\dot{V}_{max}$ by 50% at more depolarized potentials. Thus drugs that simply decrease $\bar{G}_{Na}$ in cardiac tissue do not cause an artifactual voltage dependent decline of $\dot{V}_{max}$. The potentials for the present simulations were selected to be similar to those of the experimental conditions used by Baer et al. (5)

The reasons for the discrepancy between nerve and heart relate to the differences between the nonsodium currents in the two tissues. In nerve, as the tissue is progressively depolarized, the sodium current progressively declines, and becomes superimposed on a progressively increasing outward potassium current and a background leak current (4). Since $\dot{V}_{max}$ is linearly proportional to the total ionic current (1), it is then not surprising that when the sodium current becomes a small fraction of the total ionic current, $\dot{V}_{max}$ will give a highly erroneous estimate of I_{Na}.

In contrast, in cardiac tissue the outward currents are relatively small and show inward-going rectification (8). Actually, the only outward current fast enough to contribute to the $\dot{V}_{max}$ of the cardiac action potential shows marked instantaneous inward-going rectification and consequently constitutes only a minimal fraction of the total current at the time of $\dot{V}_{max}$.

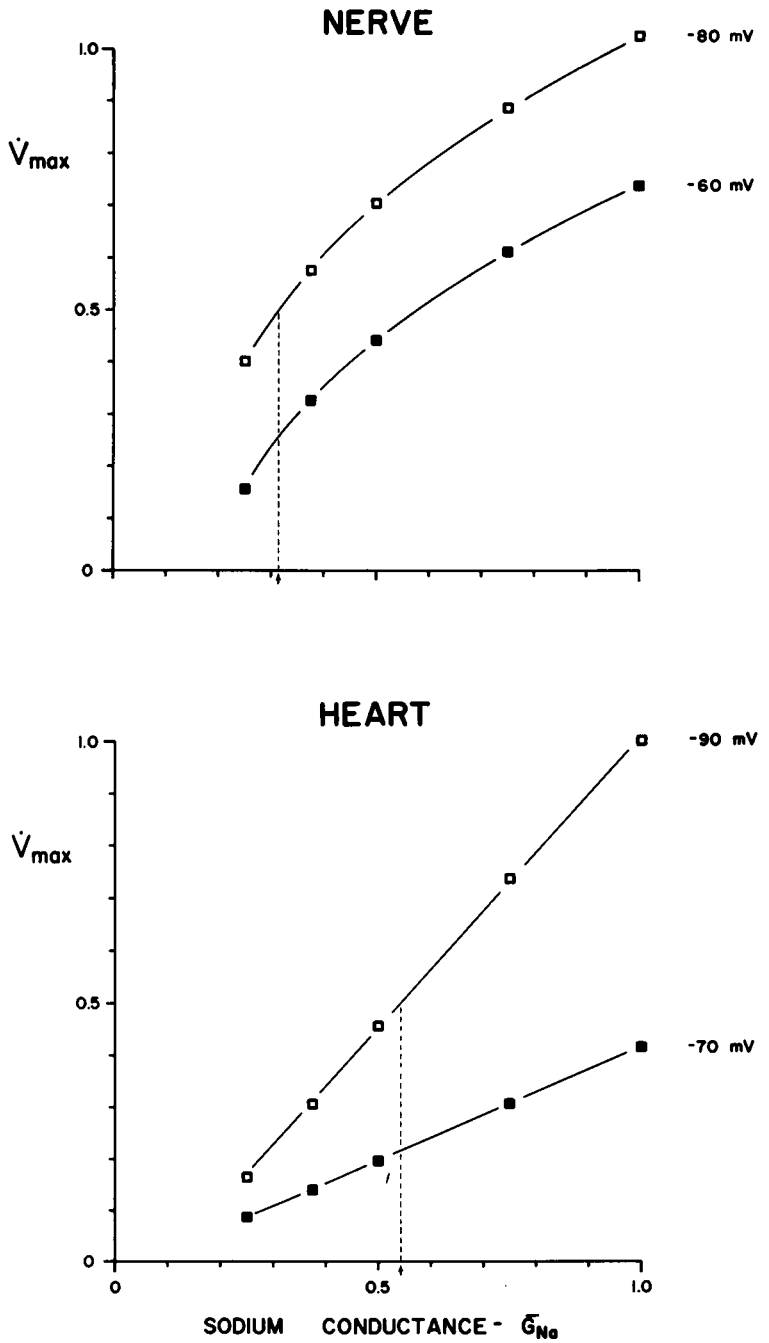

FIGURE 2 $\dot{V}_{max}$ as a function of $\bar{G}_{Na}$ for two holding potentials. All values were normalized in relation to the values at −90 mV in the absence of blockade. The vertical dashed line indicates the level of decrease in $\bar{G}_{Na}$ that results in a 50% decrease of $\dot{V}_{max}$ at the more negative holding potential.

The other outward currents turn on very slowly and very little of them is activated at holding potentials more negative than −50 mV. As a consequence, during the upstroke of the cardiac action potential, the only significant current flowing is the sodium current. The nonsodium currents account for less than 2% of total current at the time of $\dot{V}_{max}$ and this explains the tight relation between $\dot{V}_{max}$ and I_{Na} in cardiac tissue. Since this small error (less than 2%) is below the experimental error involved in the measurement of $\dot{V}_{max}$, it can be concluded that $\dot{V}_{max}$ is a valid index of the sodium current in cardiac tissue.

In summary, the present computations support the conclusions of Cohen and Strichartz for nerve, but not for cardiac muscle.

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APPENDIX

According to the standard equations (ref. 1):

$$I_{ionic} = -C_m dV/dt, \tag{1}$$

or:

$$I_{Na} = -C_m dV/dt - I_0 \tag{2}$$

where I_0 represents the sum of the other (nonsodium) currents.

Since: $$I_{Na} = \bar{G}_{Na} m^3 h (E - E_{Na}), \tag{3}$$

Eq. 2 can be rewritten as:

$$\bar{G}_{Na} m^3 h (E - E_{Na}) = -C_m dV/dt - I_0. \tag{4}$$

At $\dot{V}_{max}$:

$$\bar{G}_{Na} m'^3 h' (E' - E_{Na}) = -C_m \dot{V}_{max} - I'_0, \tag{5}$$

where the primes indicate the values at $\dot{V}_{max}$.

Or: $$\dot{V}_{max} = -[m'^3 h' (E' - E_{Na})/C_m] \bar{G}_{Na} + I_0/C_m. \tag{6}$$

Eq. 6 shows that if I_0 is significant, changes in $\dot{V}_{max}$ will not necessarily reflect $\bar{G}_{Na}$ directly. However, in cardiac tissue I_o/C_m is minimal at the time of $\dot{V}_{max}$ (see text);

therefore: $$\dot{V}_{max} \simeq -[m'^3 h' (E' - E_{Na})/C_m] \bar{G}_{Na}. \tag{7}$$

In cardiac tissue the slope of Eq. 7 happens to be quasi-constant (see Fig. 2) for any given potential. The latter results from the fact that m^3h, E', E_{Na}, and C_m all are relatively fixed for any given resting potential, according to our computations.

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