LETTER TO THE EDITOR

$\dot{V}_{max}$ *as a Measure of* $\bar{G}_{Na}$ *in Nerve and Cardiac Membranes*

Dear Sir:

Hondeghem's present study compares the validity of the maximum rate-of-rise of the action potential ($\dot{V}_{max}$) as a measure of sodium current (I_{Na}) and sodium conductance ($\bar{G}_{Na}$) in both the squid axon and in ventricular muscle (Hondeghem, 1978). From the application of computer models for each of these systems, he concludes that $\dot{V}_{max}$ is a valid, linear measure of these parameters in ventricle, but not in the axon. This difference supposedly arises from the presence of significant nonsodium currents in nerve, which flow during the rising phase of the action potential. According to Hondeghem, such nonsodium currents are insignificant in ventricular muscle, thus the linear relation between $\dot{V}_{max}$ and I_{Na} or $\bar{G}_{Na}$ in this tissue.

If all other ionic currents are small during the upstroke, then we agree with Hondeghem's conclusion that the sodium current will be proportional to $\dot{V}_{max}$. The main issue we are concerned with is the relation between $\dot{V}_{max}$ and $\bar{G}_{Na}$. On this issue Hondeghem's simulation and derivation are not in error, but they depend critically on the applicability of the Beeler and Reuter (1977) model and the sodium kinetics assumed by that particular simulation. We disagree with Hondeghem's conclusions concerning the relationship between $\dot{V}_{max}$ and $\bar{G}_{Na}$. The nonlinear relationship between $\dot{V}_{max}$ and $\bar{G}_{Na}$ in nerve does not arise uniquely from the presence of nonsodium currents, but rather from the inherent kinetics of the sodium conductance system. Even in the absence of nonsodium currents in ventricular membranes, it cannot be proven that $\dot{V}_{max}$ is proportional to $\bar{G}_{Na}$.

Before proceeding into our specific disagreements with Hondeghem's conclusions, we think it is important to emphasize one general point. Computer modeling of the squid action potential is soundly based on a voltage-clamp analysis of all relevant membrane currents (Hodgkin and Huxley, 1952). The computer reconstruction of the action potential is a necessary outcome of the voltage-clamp results. This is not the case for cardiac muscle simulations; the properties of only a few of the relevant membrane currents are known, and in particular the characteristics of the sodium current have not been experimentally obtained. In reconstructing the cardiac action potential, it is thus necessary to assume the kinetics of the sodium current. These kinetics are then appropriately modified to obtain the desired voltage response. There is no direct experimental verification of these kinetics, but there is some justification for this process, because it allows a synthesis of presently available experimental fact with reasonable supposition. However, the validity of the relationship between $\dot{V}_{max}$ and $\bar{G}_{Na}$ cannot be tested in a framework where the sodium kinetics are based on conjecture.

There are also specific difficulties with the models on which the author's conclusions are based. First, we consider the contribution of the nonsodium ionic currents during a membrane action potential in the Hodgkin-Huxley (1952) axon. We have performed a computer simulation as before (Cohen and Strichartz, 1977), measuring $\dot{V}_{max}$ for uniform action potentials in squid axons with different $\bar{G}_{Na}$ values; but in this case outward potassium and leak currents were kept at zero (Fig. 1). Sodium inactivation (h_∞) was set at one of three steady-state levels by different holding potentials, and the axon was then depolarized to a membrane potential above threshold. As Fig. 1 shows, even in the absence of potassium and leak currents $\dot{V}_{max}$ is not proportional to the normalized $\bar{G}_{Na}$; the nonlinearity is not improved by removing resting in-

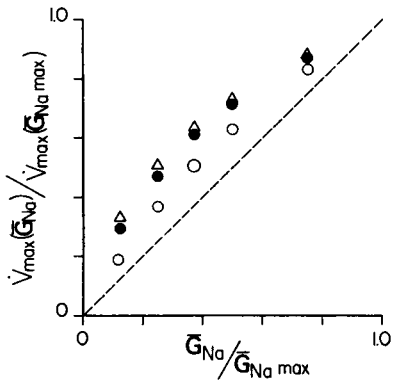

FIGURE 1 The relationship between normalized $\dot{V}_{max}$ and normalized $\bar{G}_{Na}$ for a simulated uniform action potential in a Hodgkin-Huxley squid axon with no potassium and leak currents. Different $\dot{V}_{max}$ normalization values were used for $E_{hold} = -60$ mV($h_\infty = 0.60$) (filled circle), -45 mV($h_\infty = 0.15$) (open circle), and -80 mV($h_\infty = 0.97$) (triangle), $\bar{G}_{Na}$ max = 120 mScm^{-2}. T = 6°C.

activation ($E_{hold} = -80$ mV). Obviously, nonsodium currents are not the only cause of nonlinear relationships between $\dot{V}_{max}$ and $\bar{G}_{Na}$. Fig. 1 also demonstrates that the $\dot{V}_{max}$ is relatively smaller for a particular reduction of $\bar{G}_{Na}$ in an inactivated axon ($E_{hold} = -45$ mV) than in one with inactivation removed ($E_{hold} = -80$ mV), even in the absence of potassium and leak currents. Therefore, an apparently voltage-dependent drug (e.g. tetrodotoxin) action could appear from measurements of $\dot{V}_{max}$ in membranes with only a Hodgkin-Huxley sodium conductance (Cohen and Strichartz, 1977).

Our second objection concerns the conclusion that $\dot{V}_{max}$ is proportional to $\bar{G}_{Na}$ in cardiac tissue. This is a surprising result. Under fortuitous circumstances $\dot{V}_{max}$ might be proportional to $\bar{G}_{Na}$ for certain discrete membrane potentials, but these are exceptional situations. The conditions directly relevant to the system being examined here are derived from the Hodgkin-Huxley formalism. As Hondeghem has noted, when

$$I_{Na} = I_m = C_m \dot{V}, \tag{1}$$

then

$$\dot{V} \cdot C_m = \bar{G}_{Na}[m^3(V,t) \cdot h(V,t) \cdot (V - V_{Na})], \tag{2}$$

and

$$\dot{V}_{max} = \bar{G}_{Na}/C_m[m^3(V',t') \cdot h(V',t') \cdot (V' - V_{Na})], \tag{3}$$

where the primes denote the membrane potential and time at which $\dot{V}_{max}$ occurs. A strictly linear relationship between $\dot{V}_{max}$ and $\bar{G}_{Na}$ results when the bracketed factor remains constant, independent of $\bar{G}_{Na}$.

The specific conditions for this are: (*a*) the kinetics of sodium activation must be fast compared to the discharge time for the membrane, in order for $m(V',t')$ to reach its steady-state value, $m_\infty(V')$. (*b*) the kinetics of inactivation must be slow compared to the discharge time, so that $h(V',t')$ changes little from its resting value, $h_\infty(V_{rest})$, during the action potential rise. This usually means that the inactivation rate is at least 10 times slower than the rate of activation. (*c*) the voltage at which $\dot{V}_{max}$ occurs must be independent of $\bar{G}_{Na}$. The membrane dis-

charge time can be slowed, relative to the kinetics of sodium activation, by two factors; a decrease of the depolarizing sodium current accomplished by reducing $\overline{G}_{Na}$, or an increase of the specific membrane capacitance, C_m. The specified conditions will probably vary in different tissues, and one can only test them directly by the application of voltage-clamp methods.

The model which Hondeghem has adopted for his simulations (Beeler and Reuter, 1977) incorporates the conditions for linearity, described above, but the parameters of the model were not experimentally determined and we have reason to question their validity. Specifically, Beeler and Reuter (1977) state that no evidence is available on the time-course of activation of the sodium conductance in cardiac muscle, and that little evidence is available on the time-course of inactivation, yet their model assumes at least a 50-fold ratio of inactivation to activation time constants over the rising phase of the action potential (−40 to 0 mV). The one available voltage clamp study in cardiac muscle by Dudel and Rüdel (1970) does not support this large difference. When the Dudel and Rüdel results are scaled to the appropriate temperature (assuming $Q_{10} = 3$ for τ_h) it is probable that inactivation is complete within 1 ms (see McAllister et al., 1975) or, assuming three time constants to "completion," that $\tau_h = 0.3$ ms, 10 times faster than Beeler and Reuter's value.

The Beeler and Reuter ventricular model is patterned after the model derived for the cardiac Purkinje fiber by McAllister et al. (1975) from voltage-clamp and $\dot{V}_{max}$ studies. Both models admit an ignorance of $\overline{G}_{Na}$ kinetics in the heart and therefore rely on the assumption that these kinetics are very similar to those in squid. However, during the manipulations to reconstruct the cardiac action potential, the sodium conductance becomes greatly modified. The ratio $\tau_h{:}\tau_m$ (from −40 to 0 mV) is 3–5 in the Hodgkin-Huxley squid axon, as it is in frog node of Ranvier (Dodge, 1961), whereas in the heart models $\tau_h{:}\tau_m$ is 57–75 (Beeler and Reuter, 1977) and 28–104 (McAllister et al., 1975).

Furthermore, the squid axon has a maximum sodium conductance of 120 $mScm^{-2}$, while $\overline{G}_{Na}$ is reduced to 4 $mScm^{-2}$ in the ventricular model; $\overline{G}_{Na}$ is set at the large value of 150 $mScm^{-2}$ for Purkinje fibers, but that same model assumes a C_m of 10 μFcm^{-2}, which makes the effective $\overline{G}_{Na}$ during the upstroke equal to only 15 $mScm^{-2}$ (see Eq. 3, above). These modified conductance or membrane parameters result in a linear relationship between $\dot{V}_{max}$ and $\overline{G}_{Na}$, but ironically they no longer resemble the Hodgkin-Huxley membrane parameters. Hondeghem's simulation and derivation is not in error, but the confident application of the model requires more experimental confirmation than is now available. Until the actual kinetics of sodium conductance in the heart have been measured, the application of model simulations remains an academic exercise, valuable for the insights it may provide, but insufficient for answering experimental questions.

Incidentally, it was for this reason we chose to simulate our initial results on the squid axon. Although the results of such a simulation may not be strictly applicable to cardiac muscle, at least they are based on firmer experimental evidence.

This work was supported by U.S. Public Health Service grants HL 20558 and NS 12828.

Received for publication 6 February 1978 and in revised form 20 March 1978.

REFERENCES

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

COHEN, I. S., and G. R. STRICHARTZ. 1977. On the voltage-dependent action of tetrodotoxin. *Biophys. J.* **17**:275–9.

DODGE, F. A. 1961. Ionic permeability changes underlying nerve excitation. *In* Biophysics of Physiological and Pharmacological Actions, A.A.A.S., Washington, D.C.

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

HODGKIN, A. L., and A. 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 fibres. *J. Physiol. (Lond.).* **251**:1–59.

GARY STRICHARTZ
IRA COHEN
Department of Physiology and Biophysics
Health Science Center
State University of New York at Stony Brook
Stony Brook, New York 11794 U.S.A.