Nonlinear Relationship Between V^+_{max} and h_∞ in Frog Skeletal Muscle

P. P. NÁNÁSI[1], M. DANKÓ[1], A. VARRÓ[2], and D. A. LATHROP[3]

1 *Department of Physiology, University Medical School of Debrecen, Debrecen, P.O. Box 22, H-4012 Hungary*
2 *Department of Pharmacology, Szent-Gyorgyi Albert Medical School, Szeged, H-6720 Hungary*
3 *Department of Physiology, Institute of Medical Biology, University of Tromso, P.O. Box 977, N-9001 Tromso, Norway*

Abstract. The relationship between the maximum velocity of action potential upstroke (V^+_{max}) and steady-state Na^+ channel inactivation (h_∞) was studied in frog skeletal muscle during repetitive discharges evoked in the presence of cevadine (1 μmol/l). Conventional microelectrodes and vaseline-gap voltage-clamp techniques were used. A severe degree of nonlinearity was found between (h_∞) and (V^+_{max}) especially when the Na^+ conductance (g_{Na}) was small. The observed nonlinearity could be explained as a property of the normal Na^+ channel gating in skeletal muscle rather than that of cevadine-modified channels. Part of this work has been published in abstract form in Biophys. J. **57**: 105A, 1990.

Key words: Sodium channels — Skeletal muscle — Repetitive discharges — Maximum upstroke velocity — Veratrum alkaloids

Introduction

The relation of maximal upstroke velocity of the action potential (V^+_{max}) to Na^+ conductance (g_{Na}), Na^+ current (I_{Na}), and steady-state sodium channel inactivation (h_∞) in excitable membranes has been a subject of interest for more than three decades (Weidmann, 1955; Hondeghem 1978; Strichartz and Cohen 1978). Two conventional methods have been applied in studies where these variables have been compared. Weidmann (1955) evoked cardiac action potentials from various levels of membrane potential by changing extracellular potassium. He concluded that V^+_{max} was a good measure of Na^+ channel availability. Another experimental approach has been to combine voltage and current clamp techniques (Cohen et al. 1984; Sheets et al. 1988). These studies have suggested that nonlinear relationships exists between V^+_{max} and g_{Na} as well as I_{Na} in isolated mammalian cardiac Pur-

kinje fibers; however, others described a linear correlation between g_{Na} and V^+_{max} in isolated ventricular myocytes (Yamaoka 1987). Since application of the space clamp technique did not allow investigation of propagative action potentials, which are most commonly applied to estimate drug effects, we used the cevadine-induced repetitive electrical activity (Macfarlane and Meares 1958) as a tool to study the relation between V^+_{max} and h_∞ in skeletal muscle membrane.

Materials and Methods

Isolated sartorius muscles obtained from frogs (*Rana esculenta*) were mounted in a plexiglass chamber and incubated at room temperature (20–22 °C) in Ringer solution containing (in mmol/l): Na^+ 120.15; K^+ 2.5; Ca^{2+} 1.8; Cl^- 121.1; HPO_4^{2-} 2.15 $H_2PO_4^{2-}$ 0.85 (pH 7.0±0.05). Following 60 min equilibration with 1 μmol/l cevadine (ICN Pharmaceuticals), superficial fibers were impaled with two glass microelectrodes. A single depolarizing current pulse (0.5 ms in duration and 1 μA in amplitude) was injected intracellularly through one of the microelectrodes to induce repetitive discharges in cevadine-treated muscles; the discharges were recorded by the other microelectrode. The membrane potential (V_m) and its first time derivative (dV/dt) obtained by analogue differentiation were sampled at 10 kHz, monitored on a dual beam digital storage oscilloscope (Gould OS-4000), and recorded on a chart recorder (Servogor 460).

In order to measure Na^+ current, short segments (1 to 1.5 mm) of frog semitendinosus muscle fibers were voltage clamped at 15 °C using the vaseline gap technique of Hille and Campbell (1976). Both cut ends of the fiber were bathed in "internal" solution consisting of (in mmol/l): K^+ 120.0; Mg^{2+} 2.0; Cl^- 4.0; glutamate$^-$ 120.0; EGTA 1.0; and TRIS-buffer 5. The 100 μm long test compartment contained "external" solution consisting of (in mmol/l): K^+ 2.5; Na^+ 115.0; Ca^{2+} 1.8; Cl^- 121.1; and TRIS-buffer 1.0. In each solution, pH was adjusted to 7.0±0.05 and osmolality was 250 mosmol. Potassium currents were suppressed by addition of 4-aminopyridine to the external solution or K-glutamate was replaced by CsF in the internal pools. To obtain voltage relations for Na^+ channel fast inactivation, test pulses to −20 mV were preceded by 20 ms long conditioning pulses (10 mV steps between −100 and −40 mV). The holding potential was −100 mV. The current was sampled at 77 kHz; capacitive and leakage currents were subtracted digitally using a hyperpolarizing step of 60 mV.

Results

In the presence of cevadine, the muscle membrane failed to repolarize completely after the initial rapid action potential upstroke. Instead, a volley of repetitive discharges propagated along the fibers (Fig. 1). Cevadine did not significantly affect the resting potential, amplitude, or V^+_{max} of the initial action potential evoked by stimulation. Increasing cevadine concentrations, however, decreased the "take-off potential" of the repetitive spikes (defined as the "maximal diastolic potential" preceding the respective spike) while the rate of discharge and rate of "diastolic depolarization" were increased. The amplitude and V^+_{max} of these spontaneous action potentials gradually decreased within one volley as the "take-off potential"

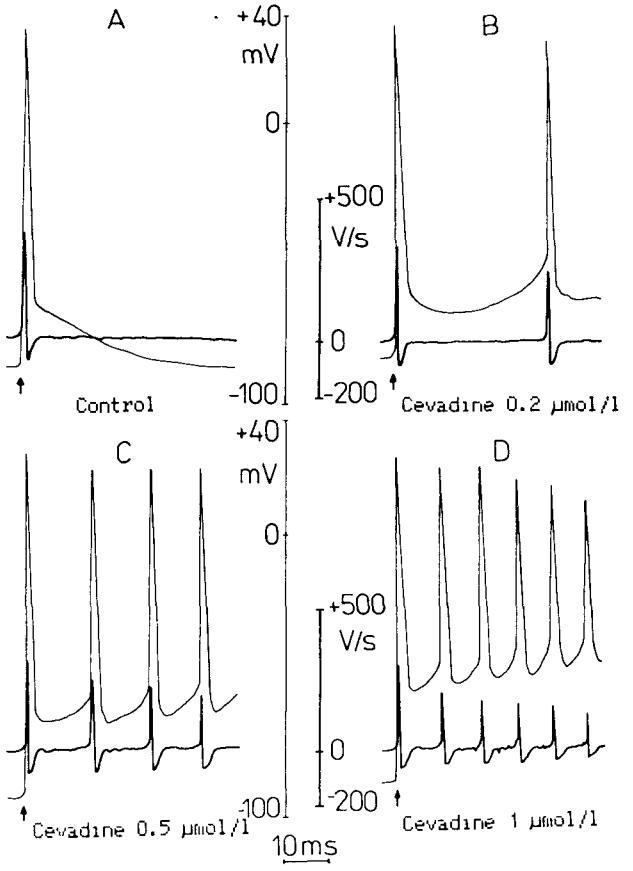

Figure 1. *A*: control action potential evoked in Ringer solution; *B*, *C*, *D*: repetitive electrical activity induced by a single electrical stimulus (arrow) after 60 min equilibration with 0.2, 0.5, and 1 μmol/l of cevadine, respectively. Upper curves and left scales (thin): membrane potential; lower curves and right scales (thick): first time derivative. Digitally reconstructed recordings from the initial 50 ms of the volley.

became progressively less negative. In the presence of 1 μmol/l cevadine (Fig. 1*D*), a 25–30 mV decrease was measured in "take-off potential" between the initial stimulated and first spontaneous action potentials, whereas this decrease in "take-off potential" was moderate (usually less than a few mV) for the subsequent repetitive spikes. Therefore, for the 4th repetitive action potential in the volley, the following approximation appears to be correct; i.e., the membrane potential during the last 20 ms preceding the spike was not more negative on average than the "take-off potential" of the 4th repetitive action potential. The instantaneous value of variable

h just prior to the 4th repetitive action potential should not have been consequently greater than the value of h at the end of a 20 ms long conditioning prepulse clamped to a potential equal to the "take-off potential" of the 4th repetitive spike.

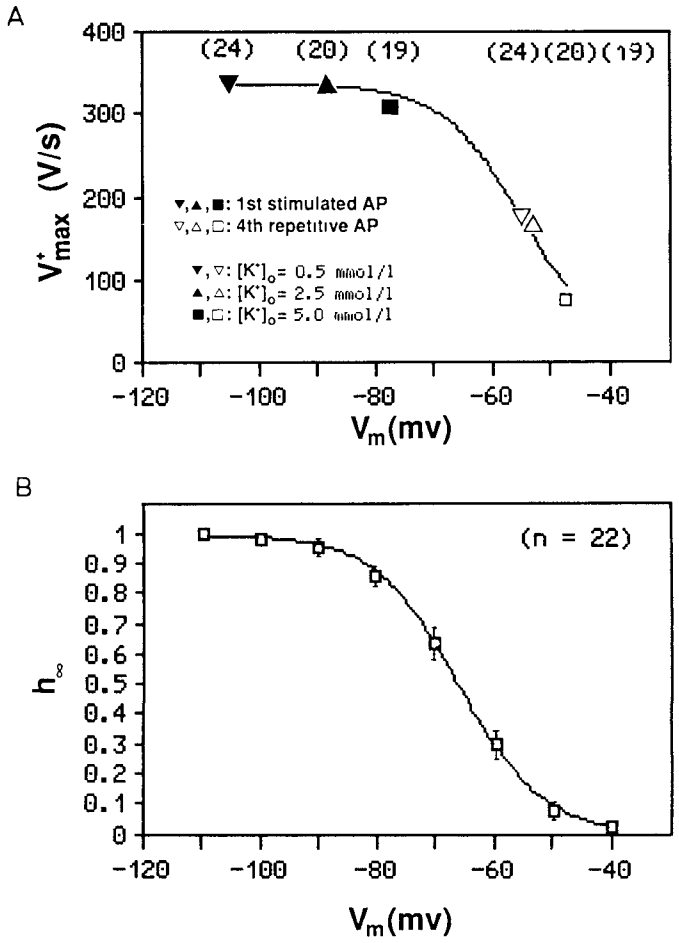

Figure 2. *A*: Measured V^{+}_{max} values of the initial action potential upstrokes (filled symbols) and those of the 4th repetitive action potentials (open symbols) as a function of their respective "take-off potentials". Volleys were evoked in the presence of 1 μmol/l cevadine at three different $[K^+]_o$ (0.5, 2.5, and 5 mmol/l). *B*: Steady-state $h_\infty - V_m$ relation measured in 22 single frog muscle fibers under voltage-clamp. Means ± S.E. are given, the figures in the parentheses indicate the number of muscle fibers. Where lacking, the error bars are covered by the symbols. Both continuous curves represent best fits to two-state Boltzmann function.

In Fig. 2*A*, the measured V^+_{max} values for the initial stimulated (filled symbols) and 4th repetitive action potentials (open symbols) are plotted as a function of their "take-off potential" measured in the presence of three different concentrations of external potassium: 0.5, 2.5 and 5 mmol/l. The continuous curve represents the best fit to a two-state Boltzmann function. In Fig. 2*B*, the $h_\infty - V_m$ relations obtained for 22 voltage-clamped muscle fibers are shown. In order to compare the V^+_{max} and h_∞ data directly, the experimentally observed V^+_{max} values were normalized to the V^+_{max} values of the initial action potential usptroke measured in 0.5 mmol/l $[K^+]_o$ where the mean resting potential was -105.4 ± 1.1 mV. This fractional $V^+_{max} - V_m$ relation and the $h_\infty - V_m$ curve were plotted in a common coordinate system (Fig. 3). Both curves fit well to the Boltzmann function ($R >$

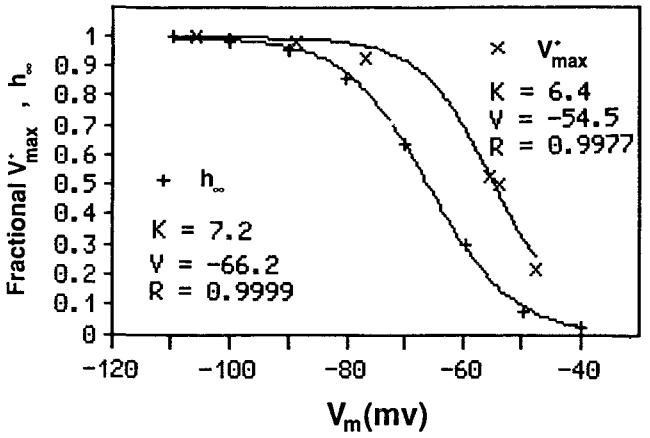

Figure 3. Comparison of voltage dependence of the normalized V^+_{max} curve (x) with that of the h_∞ curve (+) in a common coordinate system. The curves are best fits of the two-state Boltzmann function (V, midpoint potential; K, slope factor; R, regression coefficient). Data derived from Fig. 2.

0.99) indicating that this treatment of data is reasonable. The midpoint of the $V^+_{max} - V_m$ relation was less negative (by 11.7 mV) than that of the $h_\infty - V_m$ curve, with only a moderate difference in the slope factor ($K = 6.4$ and $K = 7.2$, respectively). When the slope factor of the V^+_{max} curve was set to 7.2 (equal to the slope of the h_∞ curve), and held constant, the change in the calculated midpoint potential was only 0.2 mV. In other words, the V^+_{max} curve could be generated from the h_∞ curve by shifting it 11.7 mV to the right. According to Fig. 3, the skeletal muscle membrane was capable of generating action potentials with fast upstrokes arising from take-off potentials where the Na^+ channel availability is expected to be very low during repetitive activity; i.e. V^+_{max} appeared as a strongly nonlinear function of h_∞.

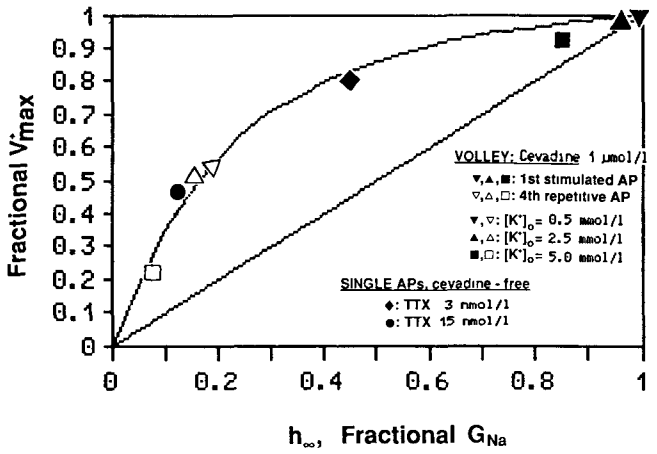

Figure 4. Nonlinear relationship between h_∞ and V^+_{max}. Fractional V^+_{max} from Fig. 2*A* (triangles and squares) are plotted against h_∞. The closed diamond and circle represent normalized V^+_{max} values measured in the presence of TTX (3 nmol/l, n = 24 and 15 nmol/l, n = 34, respectively). For these two points the abscissa represents normalized g_{Na}. The solid line was drawn with a slope of 1.0 to indicate linear correlation.

In Fig. 4, each normalized V^+_{max} value was plotted against the corresponding h_∞ to show the magnitude of nonlinearity. The "nonlinearity" curve was obtained by plotting the two Boltzmann functions against each other, with the symbols representing discrete values. The triangles and squares (both open and filled ones) represent V^+_{max} data derived from the experiments discussed above. The filled diamond and circle represent values obtained for sartorius muscles exposed to tetrodotoxin (TTX, Calbiochem). To check that the $V^+_{max} - h_\infty$ relationship was not distorted by the presence of cevadine, V^+_{max} of single action potentials were measured in the presence of TTX (3 nmol/l and 15 nmol/l, respectively), but in absence of cevadine. These V^+_{max} data were normalized to control V^+_{max} and plotted against the fractional g_{Na} taken from Jaimovich et al. (1983). The abscissa corresponding to these two points was scaled in normalized g_{Na}. The good accordance of the results obtained with TTX (V^+_{max} against g_{Na}) and those obtained with cevadine (V^+_{max} against h_∞) indicates that V^+_{max} is a nonlinear function of both g_{Na} and h_∞. This good accordance also suggests that the nonlinear correlation between h_∞ and V^+_{max} is likely to be the property of the normal skeletal muscle Na^+ channels rather than that of the cevadine-modified ones.

If the nonlinearity can be properly derived from a virtual shift between the h_∞ and V^+_{max} curves, then the magnitude of the observed nonlinearity will critically depend on the accuracy of determination of the "take-off potentials". In Fig. 5,

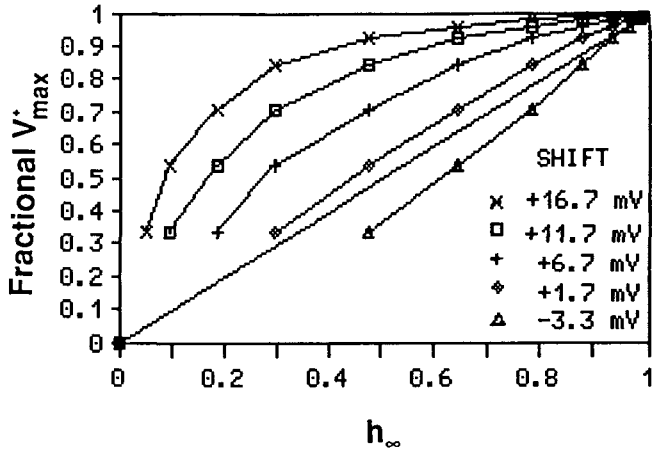

Figure 5. Simulated effect of a shift (graded in 5 mV steps) between the $V^+_{max} - V_m$ relation and the $h_\infty - V_m$ curve on the degree of nonlinearity. The +11.7 mV shift (squares) corresponds to the experimentally observed value. The curves were plotted only for relative V^+_{max} values > 0.3, since at very low values the comparison might be less reliable.

therefore, we simulated the effect of a shift (graded in 5 mV steps) between the $V^+_{max} - V_m$ relation and the $h_\infty - V_m$ curve on the degree of nonlinearity. The +11.7 mV shift (squares) corresponds to the experimentally observed value. In this respect, we might rather underestimate than overestimate the actual nonlinearity due to using the "maximal diastolic potential" in absence of the true membrane potential value just prior to the 4th repetitive spike. These calculations suggest that the observed nonlinearity cannot be attributed to inappropriate determination of the "take-off potential". It is also important to note that the 5–7 °C difference in temperature between the two types of measurements (i.e.; measurement of V^+_{max} and determination of the $h_\infty - V^+_{max}$ relation) cannot introduce nonlinearity. Changing the temperature by 10 °C (between 13 and 23 °C) had no significant effect on the midpoint potential of the $h_\infty - V_m$ curve.

Discussion

The major finding of the present study (i.e., the severe nonlinearity observed between V^+_{max} and h_∞ in amphibian skeletal muscle) may not be surprising considering the results of earlier experiments performed in mammalian cardiac Purkinje fibers (Cohen et al. 1984; Sheets et al. 1988). However, this is the first analysis of the $V^+_{max} - h_\infty$ relationship using propagating action potentials in muscle. Since those

action potentials which are commonly studied in the presence of various drugs are also propagating ones, our repetitive activity model may be a more realistic approach to this problem than previous work using membrane action potentials. Three major sources for the nonlinearity have been predicted in cardiac tissue: (1) Na^+ channel activation is not fast enough to precede filling of membrane capacitance (τ_m not $\ll \tau_{memb}$) (2) Na^+ channel inactivation is not slow enough to allow the membrane capacitance to be completely filled (τ_h not $\gg \tau_{memb}$) and (3) I_{Na} is not the only current flowing across the membrane at the time when V^+_{max} occurs ($g_L > 0$). The upward deviation from the theoretical solid line in Fig. 4 can only be attributed to the first of these factors (i.e., τ_m not $\ll \tau_{memb}$), since the other possibilities would be expected to introduce deviations in the opposite direction (Cohen et al. 1981). Both τ_m and τ_{memb} in frog skeletal muscle have been estimated to be in the range of 1 ms at 1 °C (Adrian et. al. 1970), whereas at room temperature (20 to 24 °C), τ_m was determined to be 0.2 ms at −50 mV, and τ_{memb} was found to have two components equal to 0.015 ms and 0.37 ms, respectively (Ildefonse and Rougier 1972). The required condition of $\tau_m \ll \tau_{memb}$ is therefore not met in skeletal muscle, leading to the conclusion that the nonlinearity between V^+_{max} and h_∞ in this tissue is a result of the Hodgkin-Huxley kinetics of Na^+ channel gating. The nonlinearity between h_∞ and V^+_{max} (or between g_{Na} and V^+_{max} was the largest when the magnitudes of these variables were small. The practical implication of such a behavior is that measurement of V^+_{max} block induced by local anesthetics and antiarrhytmic drugs, or by any agent which decrease g_{Na} by shifting the $h_\infty - V_m$ relation to the left, will evidently underestimate the fraction of Na^+ channels being actually blocked.

The possibility arose that the small but persistently open population of the cevadine-modified Na^+ channels (Sutro 1986; Leibowitz et al. 1986; 1987) might contribute to the nonlinearity between V^+_{max} and h_∞ due to a shift in the voltage dependence of the Na^+ channel availability curve. This assumption, however, would require that the voltage dependence of Na^+ channel availability is shifted by cevadine toward *positive* potentials. In contrast, the $h_\infty - V_m$ relation was shifted toward *negative* potentials by aconitine (Grishchenko et al. 1983) and grayanotoxin (Seyama and Narahashi 1981), drugs shown to share a common mechanism of action with veratrum alkaloids (Catterall 1980). Therefore, the significant contribution of the cevadine-modified Na^+ channels to the observed nonlinearity is unlikely. In addition, the effect of TTX on the $g_{Na} - V^+_{max}$ relation was similar to the action of the cevadine-induced depolarization on the $h_\infty - V^+_{max}$ relation. These arguments strongly support the view that the severe nonlinearity (which might even be underestimated due to our definition of the "take-off potential") is an intrinsic feature of the skeletal muscle membrane similarly to neural and cardiac preparations.

Acknowledgements. We are grateful to Professor E. Varga for continuous support, and to Mrs. E.Deák, Mrs. A. Molnár and Miss E. Sági for excellent technical assistance. This work was supported by OTKA (No: 1453).

References

Adrian R. H., Chandler W. K., Hodgkin A. L. (1970): Voltage clamp experiments in striated muscle fibres. J. Physiol. (London) **208,** 607—644

Catterall W. A. (1980): Neurotoxins that act on voltage-sensitive sodium channels in excitable membranes. Annu. Rev. Pharmacol. Toxicol. **20,** 15—43

Cohen I., Attwell D., Strichartz G. (1981): The dependence of the maximum rate of rise of the action potential upstroke on membrane properties. Proc. Roy. Soc. London Ser. B. **214,** 85—98

Cohen C. J., Bean B. P., Tsien R. W. (1984): Maximal upstroke velocity as an index of available sodium conductance. Circ. Res. **54,** 636—651

Grishchenko. I. I., Naumov A. P., Zubov A. N. (1983): Gating and selectivity of aconitine-modified sodium channels in neuroblastoma cells. Neuroscience **9,** 549—554

Hille B., Cambell D. T. (1976): An improved vaseline gap voltage clamp for skeletal muscle fibers. J. Gen. Physiol. **67,** 265—293

Hondeghem L. M. (1978): Validity of V_{max}^{+} as a measure of the sodium current in cardiac and nervous tissues. Biophys. J. **23,** 147—152

Ildefonse M., Rougier O. (1972): Voltage-clamp analysis of the early current in frog skeletal muscle fiber using the double sucrose-gap method. J. Physiol. (London) **222,** 373—395

Jaimovich E., Chicheportiche R., Lombet A., Lazdunski M., Ildefonse M., Rougier O. (1983): Differences in the properties of Na^{+} channels in muscle surface and T-tubular membranes revealed by tetrodotoxin derivatives. Pflügers. Arch. **397,** 1—5

Leibowitz M. D., Sutro J. B., Hille B. (1986): Voltage-dependent gating of veratridine-modified Na channels. J. Gen. Physiol. **87,** 25—46

Leibowitz M. D., Schwarz J. R., Holan G., Hille B. (1987): Electrophysiological comparison of insecticide and alkaloid agonists of Na channels. J. Gen. Physiol. **90,** 75—93

Macfarlane W. V., Meares J. D. (1958): Chemical modification of intracellularly recorded after potentials of frog skeletal muscle. J. Physiol. (London) **142,** 78—96

Seyama I., Narahashi T. (1981): Modulation of sodium channels of squid nerve membranes by grayanotoxin I. J. Pharmacol. Exp. Ther. **219,** 614—624

Sheets M. F., Hanck D. A., Fozzard H. A. (1988): Nonlinear relation between V_{max} and I_{Na} in canine cardiac Purkinje cells. Circ. Res. **63,** 386—398

Strichartz G., Cohen I. (1978): V_{max} as a measure of $\bar{G}_{Na}$ in nerve and cardiac membranes. Biophys. J. **23,** 153—156

Sutro J. B. (1986): Kinetics of veratridine action on Na channels of skeletal muscle. J. Gen. Physiol. **87,** 1—24

Weidmann S. (1955): The effect of the cardiac membrane potential on the rapid availability of the sodium-carrying system. J. Physiol. (London) **127,** 213—224

Yamaoka K. (1987): Does the maximum upstroke velocity of the action potential (V_{max}) represent available sodium conductance in frog ventricular cells? Jpn. J. Physiol. **37,** 585—599

Final version accepted December 18, 1991