LETTERS TO THE EDITOR

Some Experimental Difficulties in the Use of $V_{\text{max}}$ as a Measure of $G_{\text{Na}}$

The report by Kishida, Surawicz, and Pu entitled "Effects of K" and K'-Induced Polarization on (dV/ dt)$_{\text{max}}$, Threshold Potential, and Membrane Input Resistance in Guinea Pig and Cat Ventricular Myocardium" (1979) is a careful study of the effects of changes in $[K^+]_o$ on ventricular muscle. Nevertheless, the separation of the effects of K' on $V_{\text{max}}$ into a membrane potential-dependent component and a component independent of the membrane potential (and presumably dependent on a specific action of K' on the Na+ channel) is premature. To achieve such a separation, all sources of possible error must be carefully quantified. I would like to discuss two of them: (1) variation in step potential, both across the sucrose gap node and between "identical" voltage steps in solutions of different values of $[K^+]_o$, and (2) the effects of differences in the instantaneous I-V relationships in solutions of different $[K^+]_o$.

Variation in Step Potential Alters $V_{\text{max}}$

When a membrane action potential is initiated, a patch of membrane is brought uniformly from an initial holding potential (which may be the resting potential) to a potential above the threshold for a regenerative response. In the present study, when the nodal region of the gap is brought to threshold, there is a small but finite difference in the potential at the opposite ends of the node (due to the finite cable length), and therefore, the takeoff potentials at opposite ends of the node are different. Figure 1a in the present study demonstrates this difficulty. Although the authors state that the response at opposite ends of the gap is "similar," the experimental record shows a 10–20% difference in the two values of $V_{\text{max}}$.

Similarly in Figure 6 of the same study, there is a substantial experimental difference in the value of $V_{\text{max}}$ obtained from action potentials initiated from a holding potential of -80 mV in 5.4 and 13 mM [K']o. However, the interpretation of this result is complex because the depolarizing current pulse has stepped the membrane to substantially different membrane potentials in the two conditions (this is probably due to the difference in input resistance).

To assess the possible effects of variations in step potential on $V_{\text{max}}$, I have computer-simulated the problem. The simulation contains three currents:

1. A Hodgkin-Huxley squid Na"+ current with a ratio of activation to inactivation rate constants of 3-5 over the rising phase of the action potential (-40 to 0 mV). The magnitude of the rate constants were increased by a factor of near four to account for the difference in temperature. Recent experiments (Lee et al., in press) suggest that these kinetics are far more appropriate for the ventricular Na+ current than those assumed in the model of Beeler and Reuter (1977) where the ratio of activation to inactivation rate constants was between 57 and 75 over the same voltage range.

2. An inwardly rectifying K" current with a magnitude about equal to that suggested by Beeler and Reuter (1977) for iK1. This is illustrated as the broadly dashed curve in Figure 2A.

3. A linear inward background current.

Other currents were not included because their effects should be minimal during the less than 2-msec rising phase of the ventricular action potential.

Figure 1 shows the effects of step potential on $V_{\text{max}}$. In a 20-mV range near threshold, $V_{\text{max}}$ changes by over 200%. This change is due largely to the difference in activation and inactivation rate constants at the various voltages corresponding to V step.

It is important to realize that this simulation only suggests what might be the case; the actual values of the voltage and time dependence of activation and inactivation are not yet available for any cardiac preparations. Nevertheless, the effect of step potential on $V_{\text{max}}$ may be large enough to be a significant concern in interpreting the present set of experiments.

Alterations in the Instantaneous I-V Relationship Change $V_{\text{max}}$

A second major concern in the present study is that alteration in [K'+]o might alter the instantaneous I-V relationship. This effect might be sufficient to produce the observed effects of [K'+]o without direct effects of K' on the Na+ channel. The authors doubt the seriousness of this concern, both because the K' channel inwardly rectifies, and because the experiments of McDonald and Trautwein (1978)

* The actual equations used in the simulation were:
   a. for $I_{Na}$—those described in Hodgkin and Huxley (1952) with $m$, $h$, $\theta$, $\xi$ multiplied by 3.84.
   b. $I_{K1} = 0.015 (V + 55)$
   c. $I_{K} = (V - V_d) A$, where $A = 5$, 12.5 25, for $V_d = -100, -80$, and -65, respectively.
suggest that for cat ventricular muscle there is little crossover in the background I-V relationships when extracellular $K^+$ is raised. There are several difficulties with these assumptions:

1. As the simulation below will demonstrate, the presence of inward rectification alone is not sufficient to eliminate the possible effects of crossovers in the instantaneous I-V relationships.

2. McDonald and Trautwein (1978) estimated the background I-V relationship, not the instantaneous I-V relationship. These are not equivalent, since the background I-V relationship includes steady state components of both tetrodotoxin (TTX)- and D600-sensitive channels (Attwell et al., in press; Kass, Siegelbaum and Tsien, 1976).

3. The estimate of McDonald and Trautwein (1978) involves extrapolation of a time-dependent $K^+$ current back to the origin. This method is theoretically invalid in preparations where $K^+$ accumulation exists (D. Eisner, I. Cohen, D. Attwell. Voltage clamp and tracer flux data: Effects of a restricted extracellular space, submitted to Quarterly Reviews of Biophysics).

4. McGuigan (1974) also estimated the background I-V relationship in ventricular muscle. These experiments were performed in the presence

![Figure 1](image1)

**Figure 1** The effects of step potential on the maximum upstroke velocity ($V_{\text{max}}$). The maximum upstroke velocity is plotted on the ordinate in volts/sec, and the step potential is plotted on the abscissa in mV. The initial holding potential for all action potentials generated in this simulation was $-60 \text{ mV}$.

![Figure 2](image2)

**Figure 2** The effects of a crossover in the instantaneous I-V relationship. A: The actual I-V relationships for the several values of $E_s$ used in the simulation ($-100, -80, -65 \text{ mV}$). B: A plot of $V$ against time demonstrating the differences in the trajectories of the rate of rise. C: A plot of the upstroke of the action potential against time demonstrating the differences in the upstroke in the three different conditions.
of TTX and involve extrapolation of less significant magnitude (since delayed rectification was small.) In these experiments there was significant crossover in the background I-V relationships when the extracellular K+ was elevated.

Since the majority of experiments in this report were performed on guinea pig ventricular muscle, where the effects of K+ on the instantaneous I-V relationship have yet to be assessed, it seems worthwhile to consider the influence of a K+ current exhibiting inward rectification with crossover on the results of the present study.

Figure 2A shows the results of a simulation on the effects of a crossover of the instantaneous I-V relationships on Vmax. Figure 2A shows the instantaneous I-V relationships used for Ik, in the simulation. The magnitude of the crossover is consistent with the experimental results of McGuigan (1974). The effects of these changes on the upstroke of the action potential and on the trajectory of V are shown in Figure 2, B and C. The difference in background current at V(Vmax) for the most depolarized and hyperpolarized values of Ek is sufficient to reduce Vmax by 13 V/sec. The actual reduction is 36 V/sec. Thus, the magnitude of the reduction in Vmax cannot be estimated readily from the difference in background current but is much larger due to alterations in the trajectory of the upstroke. If Ek is shifted to even more depolarized potentials, the effects of the change in trajectory on Vmax are even more dramatic.

It is worth noting that the present simulations attempt to evaluate quantitatively only two of the difficulties in the present study. The complete effects of both longitudinal and radial nonuniformity are highly nonlinear and cannot be estimated without a three-dimensional cable simulation. The present study is more carefully conceived and executed than many using Vm as a measure of the sodium conductance. Nevertheless, there are substantial sources of error, and because of these difficulties, the conclusions, though cautiously stated, are still premature.

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Reply to the Preceding Letter

Dr. Kishida, Dr. Fu, and I appreciate Dr. Cohen's interest in our study, and we acknowledge the appropriateness of his concerns. We concur with Dr. Cohen that the absolute value of Vmax may not be a valid index of rapid inward sodium current. For nothing to add to the discussion of this important problem.

However, Dr. Cohen also suggests that the decrease in Vmax, attributed by us directly to the increase in [K+]o, could have been produced by changes in the step potential or by changes in the instantaneous current-voltage relationships. To study the possible effects of these two variables, he used the technique of computer simulation. The computer-simulated relation between the step potential and the Vmax has resulted in a very steep curve (Fig. 1, in the letter preceding). However, even this curve does not seem to be sufficiently steep to account for the marked differences in Vmax observed by us in the experiments in which the step potential differences were in the order of only a few mV (Kishida et al., 1979). Perhaps, more important is the question of how valid is the model of the action potential upstroke stimulated by Dr. Cohen? Dr. Cohen refers to the model of ventricular action potential computed by Beeler and Reuter (1977) but challenges the kinetics of sodium current used in that particular model. He substitutes for it a squid axon sodium current with a much smaller ratio of activation to inactivation rate constants. The precise kinetics of rapid inward sodium current in the ventricular myocardium obviously are not available, but Beeler and Reuter (1977) stated that in their model "rate constants for h were chosen by considering the effect of the time constant of h on the excitability of the model, and the shape of the spike of the computed action potential." In other words, Beeler and Reuter (1977) chose the values needed to compute the whole action potential, whereas Dr. Cohen computed only an upstroke of a ventricular action potential using a squid axon current. This model remains untested. Extrapolating from the rate constants of recovery from inac-
tivation (Gettes and Reuter, 1974), we should expect marked differences between the kinetics of the rapid inward sodium current in the ventricular myocardium and in the squid axon. In short, Dr. Cohen has not convinced us that his model of the action potential upstroke is appropriate for calculating errors in our study.

Similarly, Dr. Cohen's simulations of the instantaneous current-voltage relationships at high [K+]o are not free of bias in relation to our results which were obtained in the guinea pig and cat myocardial fibers. Therefore, it would have been more appropriate for Dr. Cohen to use the currents measured by McDonald and Trautwein (McDonald and Trautwein, 1978; Trautwein and McDonald, 1978) in the cat than those measured by McGuigan (1974) in the cow and sheep ventricular fibers. As pointed out by McDonald and Trautwein (1978), the outward current-voltage relations in cat ventricular fibers crossed each other not only once ("cross-over"), but in most cases twice ("cross-back") when [K+]o was raised from 3 to 10, 20, and 30 mM. The current-voltage relations in bovine ventricular fibers were different from those in the cat fibers, but the 10 mM [K+]o relation also "crossed-over" and "crossed-back" the 3 mM [K+]o relation (Trautwein and McDonald, 1978). It is true that in the experiments of McGuigan, the increases in outward current in the instantaneous current-voltage relations that occurred when [K+]o was raised were much greater than the current increases in the steady state current-voltage relations of McDonald and Trautwein under similar circumstances. Dr. Cohen suggests that this may be due to the differences in the background current. However, this is unlikely, because the tetradotoxin- and D600-sensitive components of the steady state currents are practically not detectable in the ventricular myocardium (Professor W. Trautwein, personal communication). McDonald and Trautwein (1978) suggested that the difference between their results and those of McGuigan could have been due, in part, to the species difference and, in part, to methodology, because they studied their muscle in a single sucrose gap and McGuigan (1974) in a double sucrose gap. Dr. Cohen criticized the study of McDonald and Trautwein for not taking into consideration the effect of K+ accumulation. However, McDonald and Trautwein (1978) considered in great detail the possible effects of K+ accumulation. Their data suggest that a measurable shift in the reversal potential for the outward current would occur only in response to prolonged membrane depolarization. In short, we question again the validity of Dr. Cohen's model for checking the veracity of our data.

We cannot accept the described computer-simulated changes in Vmax as pertinent to the experimental conditions in our study, and therefore, we disagree with Dr. Cohen's statement that our conclusions are "premature." The results obtained by us probably are not unique to the mammalian ventricular myocardium, because an almost identical effect of [K+]o of 13.5 mM on Vmax was found recently in frog atrial fibers mounted in a double sucrose gap (R. Kern et al., 1978, and unpublished observations).

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