

LETTERS TO THE EDITOR

*Evidence for Membrane Surface Charge from Measurement of Potassium Kinetics as a Function of External Divalent Cation Concentration*

Dear Sir:

It is well known experimentally that a change in the external concentration of calcium ions causes a shift along the voltage axis of electric field-dependent axon parameters. Shifts of about 9 mV/*e*-fold change of calcium have been reported for squid giant axons (Frankenhaeuser and Hodgkin, 1957; Gilbert and Ehrenstein, 1969). Shifts have also been reported for myelinated nerve (Frankenhaeuser, 1957; Albrecht-Bühler, 1968; Hille, 1968) and for several other preparations (Narahashi, 1966; Frankenhaeuser and Lännergren, 1967; Hagiwara and Takahashi, 1967; Julian et al., 1962).

We have previously presented a surface charge model to explain these shifts (Gilbert and Ehrenstein, 1969). Briefly, the surface charge model explains the shift of an electric field-dependent parameter by postulating an additional component of electric field inside the membrane. This electric field is produced by a double charge layer consisting of a fixed negative charge on the external surface of the axon membrane and neutralizing cations in the external solution. When the external concentration of calcium cations is increased, the additional component of electric field is affected in two ways. First, more negative surface charge is neutralized, and secondly, the ionic strength is increased. Both effects tend to reduce the electric field and, therefore, shift the field-dependent parameters. Two recent experiments strengthen the case for the surface charge model. Mozhayeva and Naumov (1970) varied concentrations of several cations simultaneously in frog node, and found shifts of potassium conductance-voltage curves corresponding to a surface charge model. Also, McLaughlin et al. (1971) measured carrier flux through several different artificial membranes with different known concentrations of fixed charges, and obtained results in agreement with the surface charge model.

The chief weakness of the surface charge model is that there are plausible alternatives. A number of models have been proposed to explain the voltage dependence of sodium conductance on the basis of blocking of sodium current by calcium ions (Goldman, 1964; Stephens, 1969; Fishman et al., 1971; Moore and Jakobsson, 1971). In the model of Moore and Jakobsson, the adsorption of calcium ions at specific sites on the membrane is also invoked to explain the calcium-dependent shift of the sodium conductance-voltage curve along the voltage axis. The changes of conductance-voltage curve predicted by this model when external calcium concentration is varied are qualitatively similar to the changes predicted by the surface charge model. There is an apparent quantitative discrepancy between experimental observations and the model of specific blocking sites regarding the magnitude of the voltage shift of conductance parameters caused by changing calcium concentration. However, as Moore and Jakobsson (1971) have indicated, it is possible to account for this quantitative difference by an appropriate profile of electric field across the membrane.

The situation is rather similar with regard to transport of potassium ions, in that neither the surface charge model nor the model of specific blocking sites has been ruled out as the cause of shifts of voltage-dependent parameters along the voltage axis. We suggest that analysis of

time constant vs. voltage curves in the region where these curves have clear maxima should distinguish between the two mechanisms. The surface charge model, which predicts that changes in calcium concentration should cause shifts along the voltage axis, requires that the curves for different calcium concentrations cross each other; an increase in calcium concentration would increase the time constant at some voltages and decrease it at other voltages. According to the model of specific binding sites, however, an increase in external calcium concentration should speed up the process of calcium ion adsorption, and hence always decrease the repolarization time constant. For the condition of high external potassium concentration, the potassium conductance is large in the absence of an applied voltage. Thus, when the membrane is repolarized, there is a relatively large change in potassium current. This facilitates the measurement of potassium repolarization time constants on both sides of the peak and subsequent discrimination between the two models.

From our current vs. time data records made on squid axons in external solutions of high potassium concentrations with varying calcium concentrations, we have calculated time constants ( $\tau_n$ ) by a least-squares procedure using the following equation adapted from Hodgkin and Huxley (1952):

$$I = \left[ I_{\infty}^{1/4} - (I_{\infty}^{1/4} - I_0^{1/4}) \exp\left(-\frac{t}{\tau_n}\right) \right]^4,$$

where  $I$  is the current at time  $t$ ,  $I_{\infty}$  is the final current, and  $I_0$  is the initial current.

We used the total current instead of the potassium current corrected for the linear component. If it is assumed that the linear component is the leakage current and is not time dependent, then it does not make any difference for the determination of the time constant whether the total current or the corrected potassium current is used.

The raw data are the same as those obtained for conductance measurements (Gilbert and Ehrenstein, 1969). About 20 current-time pairs of values were used for each  $\tau_n$  determination. The standard deviation of the experimental current-time points from the calculated current-time points was less than 15% in every case. The average standard deviation was about 5%. Fig. 1 shows the time constant ( $\tau_n$ ) for the potassium conductance parameter  $n$  to reach equilibrium as a function of final membrane voltage and external calcium concentration.

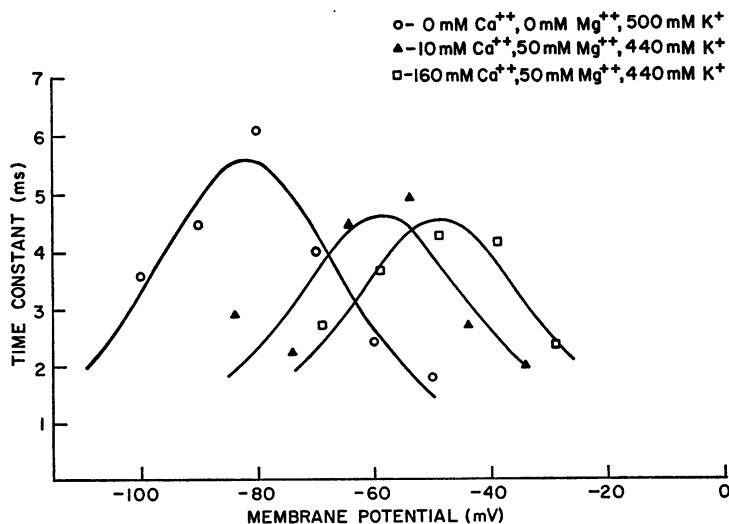


FIGURE 1  $\tau_n$  vs. membrane potential for various divalent cation concentrations.

The curve for the low divalent cation concentration is shifted to the left from the center control curve by about 25 mV whereas the curve for the high divalent cation concentration is shifted to the right from the control curve by about 10 mV. The initial membrane voltage was always the resting potential in the high potassium solution, which is approximately zero. Our experimental results were fit by least squares to the equation  $\tau_n = M \operatorname{sech}(W[V - A])$ , where  $V$  is the membrane voltage,  $M$  is the maximum value of  $\tau_n$ ,  $A$  is the value of  $V$  when  $\tau_n$  is a maximum, and  $W$  is the relative reciprocal width of the symmetrical curves around  $A$ . All curves have about the same relative width. The curve for low calcium has about 20% greater amplitude, possibly because of small differences in temperature among the illustrated experiments. In Fig. 1, the  $\tau_n(V)$  curve for normal calcium is centered at about  $-60$  mV, which is about the center of the  $\tau_n(V)$  curve in seawater. The similarity between the  $\tau_n(V)$  curves in seawater and in high potassium was previously suggested by Lecar et al. (1967), who used perfused squid axons in high external potassium solution.

In Fig. 1 the  $\tau_n(V)$  curves are shifted along the voltage axis as a function of calcium concentration. The magnitudes of the shifts are approximately the same as those found by analysis of conductance data (Gilbert and Ehrenstein, 1969), in agreement with the surface charge model. The model correlating a change of conductance with adsorption and desorption of calcium ions requires that an increase in external calcium concentration should decrease the time constant for all membrane voltages considered, in clear disagreement with Fig. 1. Thus, the data of Fig. 1 support the surface charge model for the potassium system in two ways: the voltage shifts are in agreement with those previously measured for conductance-voltage curves, and the qualitative form of Fig. 1 argues against a plausible alternative.

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GERALD EHRENSTEIN  
 DANIEL L. GILBERT  
 Laboratory of Biophysics  
 National Institute of Neurological  
 Diseases and Stroke  
 National Institutes of Health  
 Bethesda, Maryland 20014