

Effects of Changes in Ionic Strength on Inactivation and Threshold in Perfused Nerve Fibers of *Loligo*

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There is now general agreement that dilution of the internal potassium solution with a non-electrolyte shifts the threshold and the inactivation curve in the direction of a more positive internal potential (1-5). At first it was not clear whether the shift was due to dilution of potassium or to a change of ionic strength. Baker *et al.* (3) considered that the second alternative was more likely, and very clear evidence in support of this view was obtained last year by Chandler and Meves (5). They found that the change from 300 mM KCl to 50 mM KCl and 250 mM choline Cl (or NaCl) caused no detectable shift in the inactivation curve, whereas dilution with sucrose from 300 to 50 mM KCl gave a shift of 20 mv. A similar conclusion applies to the relation between sodium conductance and membrane potential, and presumably to the threshold.

Fig. 1 illustrates a possible explanation. It is assumed that there are phosphate or carboxyl groups on both sides of the membrane and that those on the outside are neutralized by Ca and Mg (the second assumption is not necessary, but it is simpler to consider only one set of charges). Since ions can pass through the membrane, the fixed charges do not make any direct contribution to the resting potential, but they do alter the distribution of potential. With a charge density of 1.4×10^{13} electronic charges/cm² and 300 mM KCl as the internal solution, the potential dip associated with the charges would be -17 mv; the space constant of the ion atmosphere in the internal solution is 5.5 A. These figures were calculated from the Debye-Hückel theory by the method of Verwey and Overbeek (6). On diluting the internal solution from 300 to 6 mM, the space constant of the ion atmosphere should increase from 5.5 to 39 A and the potential dip should change from -17 to -80 mv. Hence the p.d. across the membrane, as opposed to the total p.d. between internal and external solutions, would be the same in C (6 mM KCl, no resting potential) as it is in B (300 mM KCl, resting potential of -63 mv). Since changes in sodium permeability might depend on the p.d. across the

membrane, rather than on total p.d., the assumptions evidently provide a possible explanation of the effect of ionic strength on inactivation and threshold. Thus the shift predicted for the change from 300 to 6 mM is 63 mv and from 300 to 50 mM is 21 mv; both values are close to those observed experi-

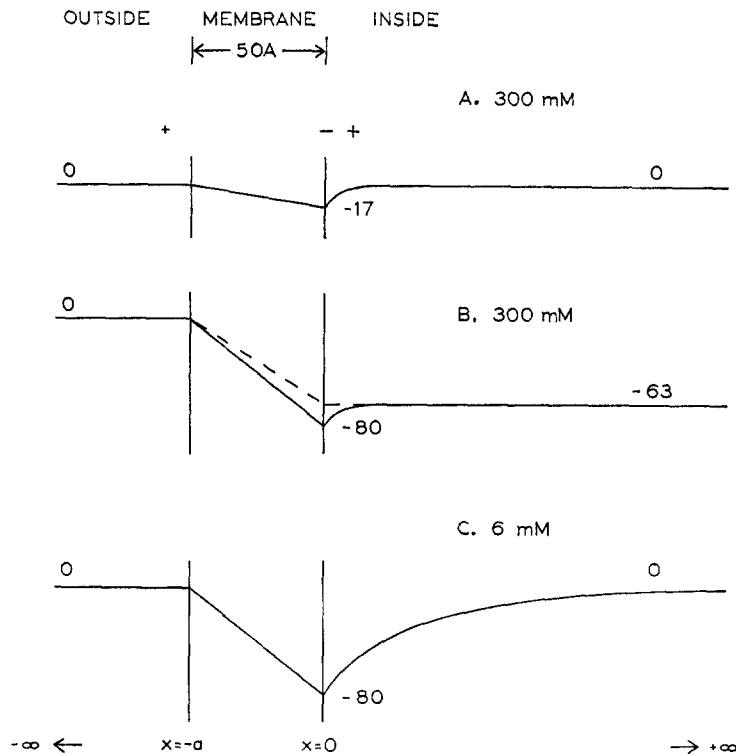


FIGURE 1 Theoretical effect of fixed charges at inside of membrane on potential distribution, calculated from the Debye-Hückel theory by methods similar to those of Verwey and Overbeek (6): *A*, no resting potential; fixed charge density of -2.23μ coulomb/cm²; internal concentration 300 mM. *B*, broken curve: resting potential, -63 mv; no fixed charges, 300 mM inside; full curve: resting potential, -63 mv; same fixed charge density as in *A*, 300 mM inside. *C*, no resting potential; same fixed charge density as in *A*, 6 mM inside.

The numbers give the potential in mv with reference to the external solution; the external solution is 600 mM in all cases; a membrane capacity of 1μ f/cm² is assumed. From Chandler, Hodgkin, and Meves (7).

mentally. The charge density chosen for this calculation does not seem unreasonably high since it corresponds to a linear separation of 27 Å between charges.

This type of hypothesis also provides a reasonable explanation of another puzzling result. From the effect of diluting internal KCl on the resting potential, Baker *et al.* (3) concluded that the permeability ratios changed from

$P_K:P_{Na}:P_{Cl} = 1:0.05:0.1$ at concentrations of 100 to 600 mM, to $1:0.035:0.02$ at concentrations below 50 mM. A change of this kind is expected from a potential dip such as that shown in Fig. 1, since this will tend to dilute anions and concentrate cations near the inside of the membrane. On the constant field theory, the over-all effect is formally equivalent to increasing the permeability to cations relative to that of the anions. However, there is no direct evidence to show that such permeability changes do in fact occur.

In addition to describing these results, an account was given of experiments carried out during the latter part of 1964. The aim of this work was to investigate an apparent objection to the sodium hypothesis. If the theory is correct the membrane potential at the crest of the spike should not exceed the sodium equilibrium potential. Baker *et al.* (1) tested this point using sulfate as the internal anion, and found that the overshoot was decreased by raising the internal sodium and that it did not exceed E_{Na} . More recently Tasaki and Takenaka (8, 9) and Tasaki and Luxoro (10) have described a very different result. They used glutamate, aspartate, or fluoride as the internal anion and found that the overshoot exceeded the sodium equilibrium potential by a large amount. With about 300 mM Na on either side of the membrane and glutamate inside, Tasaki and Takenaka (8) recorded an overshoot of 40 mv in perfused axons from *Loligo pealii*. We carried out similar experiments on axons from *Loligo forbesi*. Glutamate and fluoride were used as internal anions, but we failed to confirm the discrepancy with the sodium theory. The overshoot varied with internal sodium in the expected manner and the potential did not exceed E_{Na} , although it was close to the equilibrium value when the internal sodium concentration was high.

The internal electrode used in these experiments consisted of a 100 μ capillary filled with 0.6 M KCl. It also contained a 20 μ platinum wire, Baker *et al.* (3), which reduced the high frequency impedance and enabled the spike to be recorded without distortion. Use of the wire also has the advantage that it eliminates an artifact which can give rise to large errors. When an action potential reaches the cannula the potential of the fluid in the cannula increases by 100 mv or more; this rapid change gets through the capacity of the glass and adds a differentiated component to the recorded action potential. With a low impedance electrode containing a wire, the error was less than 1 mv, but it exceeded 50 mv when a high resistance electrode without a wire was employed. If the artifact coincided with the crest of the action potential the result was a peaked wave which went well beyond the sodium equilibrium potential. These large potentials were obtained by using a 7 M Ω electrode (without a wire) and a column of fluid about 35 mm in length; the experimental conditions were therefore similar to those described by Tasaki and Takenaka (8) who used 5 to 15 M Ω electrodes and a column of fluid about 30 mm in length (11). It therefore seems that a large cannula artifact may

be a possible explanation of the two very divergent sets of results. An account of this work is in preparation for the *Journal of Physiology*.

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