

LETTERS TO THE EDITOR

Steady-State Membrane Hyperpolarization by Large Applied Currents in Nitella translucens

Dear Sir:

The hyperpolarizing region of the current-voltage characteristic curve for the plasma membrane of *Chara australis* has been examined in some detail by Coster (1). His results indicate a gradual decrease in the membrane differential resistance with increasing hyperpolarization until it becomes zero when the membrane is hyperpolarized by about 180 mv under an applied current density of about $50 \mu\text{amp}/\text{cm}^2$; with larger applied currents the differential resistance assumes current controllable negative values. The point on the characteristic at which the differential resistance is zero was referred to by Coster as "punch-through," in accord with solid state terminology. The punch-through effect is predicted by a fixed charge membrane model in which the membrane is envisaged as being made up of an anion-exchange resin joined in series with a cation-exchange resin, the junction between the resins then behaving in response to applied current in much the same way as does a semiconductor p-n junction. The model does not, however, predict a current controllable negative differential resistance region in the characteristic. We have recently carried out experiments to test for the existence of both punch-through and current controllable negative resistance in *Nitella translucens*.

In our experiments we were simply concerned with obtaining the steady membrane potential responses to various levels of applied current, and transients in these responses were largely ignored. Current was fed into a *Nitella* cell through a 10 megohm resistor with a low shunt capacitance, the internal current electrode being a pointed AgCl-coated silver wire inserted into the cell or cell section at its midpoint. The half-length of the cells used was always less than 1 cm, i.e. less than the space constant for these cells, which is between 2-4 cm (2, 3). Such a precaution ensures a high degree of uniformity of the applied current density over the whole of the membrane area being studied. The potential responses were picked up by a conventional 3.0 M KCl-filled glass microelectrode inserted into the vacuole of the cell and a calomel electrode in the external bathing medium. The current pulses were switched manually, there being no great difficulty in obtaining pulses with rise times of about 1 msec. For small applied current densities of less than $2 \mu\text{amp}/\text{cm}^2$, the membrane potential response was capacitative with a time constant between 10 and 100 msec. With larger applied currents, the membrane generated inductive responses (delayed rectification) with time constants of up to 15 sec. Accordingly, the duration of the applied current pulse was usually maintained for about 20 sec thus enabling the potential response to attain a steady level. With very large currents, however, it was found that pulses of such duration invariably caused irreversible damage to the cells. Consequently, pulses of shorter duration had to be used under these conditions and the potential responses were recorded as soon as they became steady.

The plot of a typical current-voltage characteristic curve for the hyperpolarizing region for *Nitella translucens* is shown in Fig. 1. The resting membrane resistance, i.e. at zero applied current, for this particular cell is 12 k ohm cm^2 . With increasing hyperpolarization, there is a gradual decrease in the differential resistance until it becomes practically zero when the

membrane is hyperpolarized by about 300 mv. This presumably means that a punch-through has been attained. In no case did we observe a current controllable negative resistance region in the characteristic, even with current densities in excess of $60 \mu\text{amp}/\text{cm}^2$. What we did observe with some of the cells, was a small voltage controllable negative differential resistance with small applied current densities of between 1 and $2 \mu\text{amp}/\text{cm}^2$. These observations are discussed elsewhere in the literature.

The results of our experiments with *Nitella translucens* are in good agreement with those of Coster for *Chara australis* in that we observed the gradual decrease in the membrane differential resistance with increasing hyperpolarization and also the punch-through effect, although punch-through apparently takes place at higher levels of hyperpolarization in

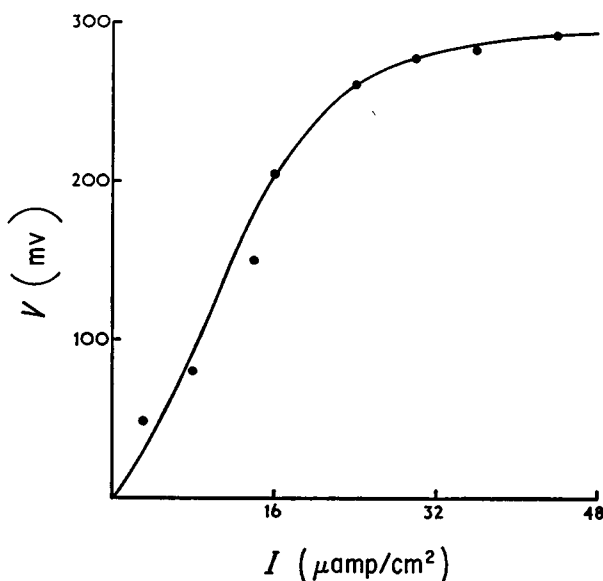


FIGURE 1 The steady-state current-voltage characteristic curve for *Nitella translucens* for hyperpolarizing current pulses. I is the membrane current density; V is the potential of the membrane (plasmalemma and tonoplast in series) below the normal resting value which is about -80 mv for cells bathed in 1.0 mM NaCl , 0.1 mM KCl , 1.0 mM CaCl_2 .

Nitella translucens than it does in *Chara australis*. What we have not obtained is a current controllable negative differential resistance region in the characteristic curve. It should, however, be pointed out that we used a different technique for current injection from that used by Coster. In our experiments we applied current pulses of long duration and recorded the steady potential responses of the membrane as far as possible. Coster's method was to inject continuously increasing current into the cell and to record the potential changes on a moving chart. The time taken for a complete scan of the characteristic is not given, but the continuous recording of the potential changes suggests that it was accomplished in a relatively short time, possibly half a minute. If such was the case, then the recorded membrane potentials would not have been the steady values but rather the transient values of these potentials. In view of these different methods of recording the potential responses, it is perhaps not unexpected that small differences should appear in the shapes of the characteristic curves obtained by the two methods.

It is relevant to observe that, as a result of differences in the transport numbers of different ionic species in the membrane, the application of a constant current can lead to ionic concentration changes in the environmental solutions on either side of the membrane. The ionic concentrations within the membrane itself can also change and it is on this assumption that Coster's analysis is based. These concentration changes, taking place either in the membrane or in the environmental solutions, are probably responsible for the initial transient behavior of the potential responses, and a current-voltage curve obtained under these transient conditions is thus not an accurate description of the steady state behavior of the membrane. However, it should be noted that the concentration changes also imply that the different points on a truly steady-state curve, as obtained in the present experiments, do not necessarily represent the membrane under identical ionic conditions.

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