

Ionic Selectivity in Perfused Giant Axons

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Information about the ionic selectivity of the sodium-carrying system of the active nerve membrane has been obtained by voltage clamp experiments on internally perfused giant axons of *Loligo forbesi*. These experiments were carried out in the Laboratory of the Marine Biological Association in Plymouth during the winter 1963–64.

Uncleaned axons were perfused by the method of Baker, Hodgkin, and Shaw (1962). The internal microelectrode consisted of a fluid-filled capillary for measuring internal potential and a 1 cm length of platinized platinum wire for sending current. Two Ag-AgCl wires each shaped as a C were used for measuring the membrane current by the voltage it produced in the external solution. All measurements were made at approximately 0°C. The axons lasted on the average 3 to 4 hours from the time of initial perfusion.

The membrane currents recorded from perfused axons with the voltage clamp technique had the same general characteristics as those recorded from intact axons (Cole, 1949; Hodgkin, Huxley, and Katz, 1952). With an isotonic solution of 305 mM KCl + sucrose inside and K-free artificial sea water outside, the membrane current associated with a small cathodal pulse consisted of a sodium inward current (Fig. 1A, record -10 mv) followed by a delayed outward current. The inward component disappeared for cathodal pulses which shifted the internal membrane potential to +60 or +70 mv (Fig. 1A, record +63 mv) and became outward for larger depolarizations (Fig. 1A, record +129 mv).

The appearance of an early outward current in the absence of internal Na strongly suggests that the internal K can partially substitute for Na in the early permeability change. The early outward current was also observed when the external Na was replaced by choline; *i.e.*, when the internal and external solutions were both Na-free. The amplitude of the early outward current was reversibly reduced when the internal KCl was replaced by either RbCl, CsCl, or sucrose. The time course of the early outward current corresponded to the time course of the sodium permeability measured by repolarizing the membrane to the resting potential at different times after the beginning of the cathodal pulse. The steady-state inactivation of the early outward current resembled the steady-state inactivation of the sodium inward

current. The conclusion is that potassium ions are able to cross the active membrane through the sodium-carrying system.

A quantitative estimate of the ionic selectivity of the sodium system was obtained by determining the potential at which the ionic current was flat initially; *i.e.*, the equilibrium potential V_e . With K-free sea water outside and 305 mM KCl inside, V_e is given by

$$V_e = \frac{RT}{F} \ln \frac{P_{Na}[Na]_o}{P_K[K]_i}.$$

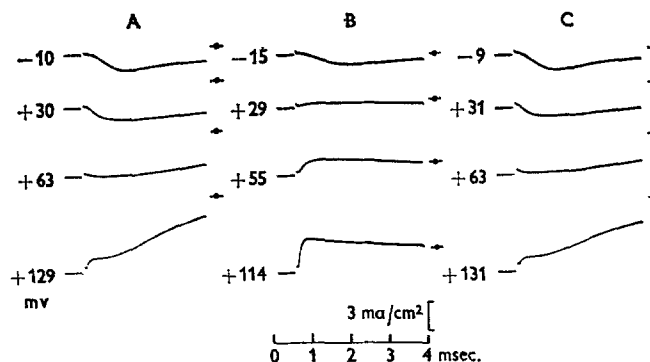


FIGURE 1. Voltage clamp records from a perfused giant axon (diameter 670μ). The records show the membrane currents associated with step depolarizations of different amplitude. Outward current upward, internal potential during the cathodal step given. Membrane hyperpolarized to about -104 mv for 18 msec. preceding each pulse. \bullet shows final level of delayed current at 17 msec. Internal solutions: A and C, 305 mM KCl; B, 153 mM KCl + 152 mM NaCl; isotonicity maintained by sucrose. External solution: K-free artificial sea water. Temperature 0°C . (Chandler and Meves, 1964)

Measurements on 23 axons perfused with 305 mM KCl + sucrose gave $V_e = +67.8$ mv at 0°C , corresponding to $P_{Na}/P_K = 12$. This permeability ratio was not significantly changed when the pH of the internal solution was lowered from 7.3 to 6.5, when the internal Cl was replaced with SO_4 , or when the fiber deteriorated. Furthermore, P_{Na}/P_K for the early permeability change remained constant as the permeability increased during a cathodal pulse; a flat ionic current corresponding to an apparent equilibrium potential would not be obtained if the permeability ratio P_{Na}/P_K changed with time. The only experimental situation which appeared to alter P_{Na}/P_K was when the internal ionic strength was reduced; P_{Na}/P_K decreased to 7.9 when the internal salt concentration was reduced from 305 to 24 mM KCl.

Partial replacement of internal K by Na decreased V_e and increased the early outward current seen with large depolarizations (Fig. 1B). In the experiment illustrated by Fig. 1, V_e was close to +63 mv with 305 mM KCl as the internal solution (Fig. 1A and C) while it was less than +29 mv with 153 mM KCl + 152 mM NaCl (Fig. 1B). Measurements of V_e with various amounts

of internal Na on a total of 12 fibers were consistent with the relation

$$V_e = \frac{RT}{F} \ln \frac{P_{\text{Na}}[\text{Na}]_o}{P_{\text{Na}}[\text{Na}]_i + P_{\text{K}}[\text{K}]_i}$$

with a $P_{\text{Na}}/P_{\text{K}}$ ratio of about 12. These results are in agreement with the idea that the active membrane is permeable primarily to Na and to a much smaller extent to K. No inward current was seen when the Na in the external sea water was replaced with choline; the early current was outward at -29 mv and all voltages to the positive side of this. Replacement of external Na with Li increased V_e slightly; by comparing $P_{\text{Na}}/P_{\text{K}}$ determined in Na sea water and $P_{\text{Li}}/P_{\text{K}}$ determined in Li sea water an average value of 0.88 for $P_{\text{Na}}/P_{\text{Li}}$ was obtained.

The early outward current decreased and the equilibrium potential V_e increased when the internal K was partially or totally replaced by Rb or Cs. From the change in V_e the permeabilities

$$P_{\text{Na}}:P_{\text{Rb}}:P_{\text{Cs}} = 1:1/40:1/61$$

for the sodium-carrying system were calculated.

The results can thus be summarized by saying that the sodium system of the active nerve membrane discriminates between the alkali metal ions in the ratio

$$\text{Li:Na:K:Rb:Cs} = 1.1:1:1/12:1/40:1/61.$$

This sequence is one of the possible sequences given by Eisenman (1962) for the cation-selective properties of glass electrodes.

Further experiments were concerned with the effect of internal Na, Rb, Cs, or choline on the magnitude of the delayed currents. Replacement of internal K by either of these ions decreased the magnitude of the delayed currents below the values expected from the independence principle. Replacement of half the K by Na, Rb, or Cs reduced the delayed outward current to 38, 27, or 2 per cent of the delayed current measured with full K. The ability of the alkali metal ions to reduce the magnitude of the delayed currents thus followed the sequence $\text{Na} < \text{Rb} < \text{Cs}$. Partial replacement of internal KCl with sucrose, on the other hand, decreased the delayed outward current in approximate proportion to the internal K activity.

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