PRELIMINARY NOTE

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Maintenance of sodium transport in perfused axons

Conditions under which a K^+- and ouabain-sensitive Na^+ efflux, characteristic of the Na^+ pump[1], can be obtained in perfused giant axons of Loligo were described in a previous note[2]. In contrast to observations on the intracellular dialysis of such axons[3,4], it was observed that a long and extensive flow of perfusate through the fibers caused the Na^+ transport system to fail. Thus after 25–30 axon volumes of perfusate had been passed through axons for about 25 min, the fibers often failed to show much K^+- or ouabain-sensitive Na^+ efflux. It was pointed out that toxic materials in the perfusate might progressively inhibit the Na^+ pump or that some component of high molecular weight might be eluted during the long perfusion. The present study was aimed at clarifying which of these factors was responsible for the failures and at giving a preparation which would maintain the Na^+ transport despite prolonged perfusion with large volumes of solution.

Axons were examined for Na^+ transport after perfusion with one of two perfusion media which, apart from the energy source (3 mM ATP, 27 mM creatine phosphate and 0.3 mg/ml creatine phosphokinase) and cofactor Mg^{2+}, contained different constituents. One medium (the glutamate medium) contained 600 mM potassium glutamate, 10 mM phosphate buffer, 6.8 mM $MgCl_2$ and 2 mM EDTA as its magnesium salt. The second perfusion medium (the sucrose medium) contained 810 mM sucrose, 5 mM borate buffer, 6.8 mM magnesium sulphate and 2 mM ethyleneglycol-bis-(aminoethyl ether)-*N*,*N*′-tetraacetic acid (magnesium salt). The concentration of Na^+ in the glutamate medium was 96 mM and in the sucrose medium 40 mM. Axons were perfused with these media, and the rate constants for Na^+ efflux into artificial sea water were measured in the manner previously described[2]; when the sucrose medium was used, 300 nM tetrodotoxin was added to the sea water bathing the nerve in order to abolish any spontaneous action potentials such as occur in fibers containing low ionic strength solutions.

Fibers which had been perfused with 3–4 axon volumes of either perfusion medium over a period of about 5 min showed a K^+- and ouabain-sensitive Na^+ efflux. However, after the passage of some 50 fiber volumes over a period of about 50 min (prolonged perfusion), the rate constant for the Na^+ efflux was both high and virtually insensitive to external K^+ or ouabain (Fig. 1a); none of the 9 fibers examined showed signs of Na^+ transport. Failure occurred on these very long perfusions whether sucrose or glutamate perfusion media were used. Clearly if toxic materials are progressively inhibiting the Na^+ pump, then the toxic materials are either ubiquitous, which seems improbable, or are associated with the energy supply which was common to both media. However, failure also occurred when 30 mM ATP was applied as the energy source. (Short perfusion of fibers with 30 mM ADP as an energy supply gave a Na^+ efflux which was insensitive to external K^+, reduced by replacing the external Na^+

with Li^+ and was sensitive to ouabain. This can be interpreted in the light of observations on intact axons and on red cells which indicate that ADP promotes Na^+–Na^+ exchange rather than coupled pumping[1,5].) The observations did not support the idea that toxic materials in the perfusate progressively poisoned the pump during long perfusions.

The possibility that a specific high molecular weight material might be eluted during the long perfusions then was tested by adding natural axoplasm to the perfusate. Previous observations[2] had shown that there was some reestablishment of Na^+ transport when intact natural axoplasm was replaced in long perfused fibers.

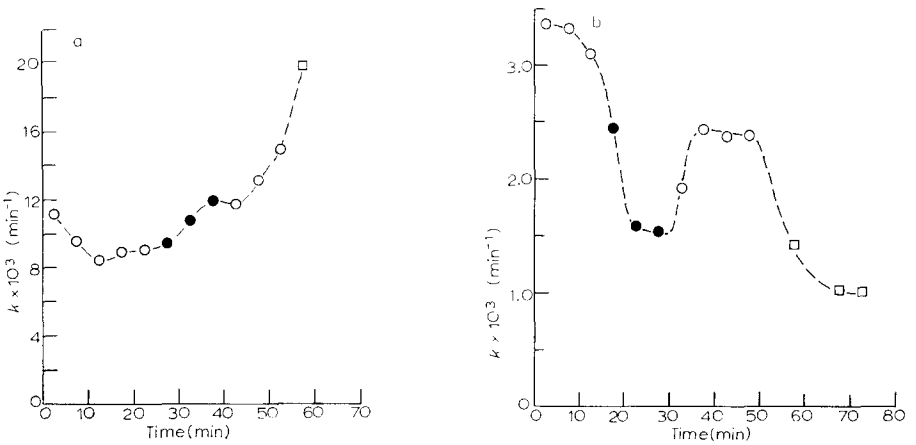

Fig. 1. (a) The rate constant (k) for Na^+ efflux plotted against time. The curve is for a fiber long perfused with 51 times its volume of glutamate medium containing 3 mM ATP, 27 mM creatine phosphate and 0.3 mg/ml creatine phosphokinase. (b) A similar plot of data for a fiber long perfused with 50 times its volume of a similar medium containing 35 mg/ml dextran. External solutions: ○, artificial sea water containing 10 mM K^+; ●, artificial sea water lacking K^+; □, artificial sea water with 10 mM K^+ and 10 μM ouabain.

In the present studies natural axoplasm was dispersed by sonication or by freezing and thawing. The axoplasm, dispersed in 4–6 times its volume of perfusion medium, was introduced into axons which had already been long perfused with either the sucrose medium or the medium described in the previous note[2]. In contrast to the findings on replacement of intact natural axoplasm, the K^+- and ouabain-sensitive Na^+ efflux was not restored after the dispersed axoplasm had been put into the fibers. The Na^+ efflux from such fibers became large and they appeared to become leaky. Even a briefly perfused fiber containing dispersed axoplasm failed to show appreciable K^+- and ouabain-sensitivity. Slight pressure was required to introduce the dispersed axoplasm into the perfused fibers, but nevertheless the observations suggested that dispersed axoplasm far from restoring Na^+ transport caused a deterioration in the condition of the fibers and that damaged axoplasm could be harmful to a fiber. Now dispersion of axoplasm by sonication or by freezing and thawing would seem unlikely to affect directly its chemical composition although structural components, such as vesicles, are likely to be damaged. Objects resembling distended vesicles have already been noted in the residual axoplasm[6], and the present results are consistent with the view that the integrity of such a structural component is essential for the maintenance

of the Na^+ transport. This integrity could be lost during prolonged perfusions with the elution of high molecular weight solutes which provide colloid osmotic pressure.

To examine this possibility, dextran (35 mg/ml; mol. wt., 60000–90000) was added to the glutamate media, and fibers were perfused with about 50 times their own volume over a period of about 50 min. Provided the total osmotic pressure of the medium was adjusted to be 2 % less than that of the sea water surrounding the fiber, it was found that fibers long perfused with this medium and distended with about 1 cm of water hydrostatic pressure showed both electrical excitability and sodium transport (Fig. 1b). When the osmotic pressure of the medium exceeded that of sea water, so that water would be drawn inwards through the fiber membrane during perfusion, thereby opposing the diffusion of dextran into the residual axoplasm, the fibers failed to show Na^+ transport. A fiber for which the total osmotic pressure of the perfusion medium was adjusted but to which no dextran added, failed to show a normal Na^+ transport.

Thus it has been found that the active transport of Na^+ occurs in squid giant axons briefly perfused with two different media. There are no indications from such experiments of specific intracellular requirements apart from ATP and Mg^{2+}. Indeed with the sucrose medium the resting potential is greatly reduced so that a substantial resting potential is not a prerequisite for pump activity. With either medium the transport mechanism was lost on prolonged perfusion. The addition of dextran combined with the adjustment of the total osmotic pressure of the perfusate, however, can preserve Na^+ transport in long perfused fibers. It seems possible that by providing colloid osmotic pressure, the dextran maintains the structural integrity of some component of the nerve sheath, possibly the vesicles detectable in residual axoplasm. If this is so, it is not yet clear whether the structural components are essential parts of the Na^+ transport mechanism or whether on injury they liberate enzymes which damage the Na^+ pump and perhaps the fiber membrane.

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