PRELIMINARY NOTE

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Sodium transport and perfused axons

Three methods are available for changing the internal composition of squid axons: injection, dialysis and perfusion. While injection only allows substances to be added to those already present in the axon, both dialysis and perfusion provide means for setting the internal environment at any predetermined level, and these preparations should be very useful for studying membrane-dependent reactions such as the sodium pump. This has proved to be the case with dialysed axons where the addition of ATP as sole source of energy maintains a normal Na^+ efflux which can be reduced by the removal of external K^+ or by ouabain[1]; but experiments on continuously perfused axons, both in this laboratory and elsewhere, have been much less encouraging[2]. We now report a method whereby a K^+ and ouabain-sensitive sodium efflux can be obtained from perfused axons. The experiments suggest a possible cause of failure in the early experiments.

Giant axons of *Loligo forbesii* were extruded and perfused in the usual manner[3] with a solution containing 550 mM sucrose, 102 mM potassium aspartate, 102 mM glutamate, 5 mM borate buffer, 6 mM $MgSO_4$ and 2 mM ethylene glycol-bis-(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid (magnesium salt). Glutamate was added as both the sodium and potassium salts, so that the final Na^+ concentration was between 100 and 150 mM. 20 mM ATP with equimolar $MgSO_4$ was present in some samples: others contained 3 mM ATP, 27 mM creatine phosphate and 0.3 mg/ml creatine phosphokinase. The pH was adjusted to 7.3. These solutions do not sustain electrical activity as measured by the ability of axons to conduct action potentials; but they are not toxic, since perfused fibres can become excitable on either replacement of natural axoplasm or perfusion with buffered isotonic potassium fluoride. An important aspect of the technique was that the axon was tied off after perfusion of a limited volume of solution. ^{22}Na was then injected into the perfused axon, and the Na^+ efflux followed by counting samples of the external medium. In brief perfusion experiments, only 3–4 times the axon volume of perfusion fluid passed through the axon over a period of about 5 min, while in long perfusion experiments, 25–30 fibre volumes passed through over some 25 min.

It was important initially to establish that the mechanical actions of extrusion and perfusion do not damage the fibre. To demonstrate this an intact axon was injected with ^{22}Na towards one end, and the Na^+ efflux followed. Axoplasm from the uninjected half was then extruded and the radioactive axoplasm rolled from the intact zone into the flattened sheath, thereby perfusing the axon with its own axoplasm. The operation had little effect on the Na^+ efflux which remained sensitive to removal of external K^+ and to ouabain.

It was then important to establish that the perfusing medium was nontoxic. An axon was perfused and then refilled with natural axoplasm by first joining it

with a cannula to an intact fibre and then rolling axoplasm from the latter through the cannula into the perfused axon. The sodium efflux from this 'resurrected' axon was of normal magnitude and sensitive to both external K^+ and ouabain, showing that the perfusion solution was not irreversibly toxic.

The Na^+ efflux from perfused axons was studied under two kinds of conditions: after brief perfusion and after long perfusion. After brief perfusion, data of the type shown in Fig. 1a were obtained. The upper curve shows the Na^+ efflux from a fibre supplied with energy-rich phosphate compounds; the rate constant is similar to that of an intact fibre and is sensitive to both external K^+ and ouabain. In this particular experiment, ATP, creatine phosphate and creatine phosphokinase were present in the perfusion fluid; but similar results were obtained when ATP was provided as the only source of energy-rich phosphate. The lower curve in Fig. 1a shows the rate

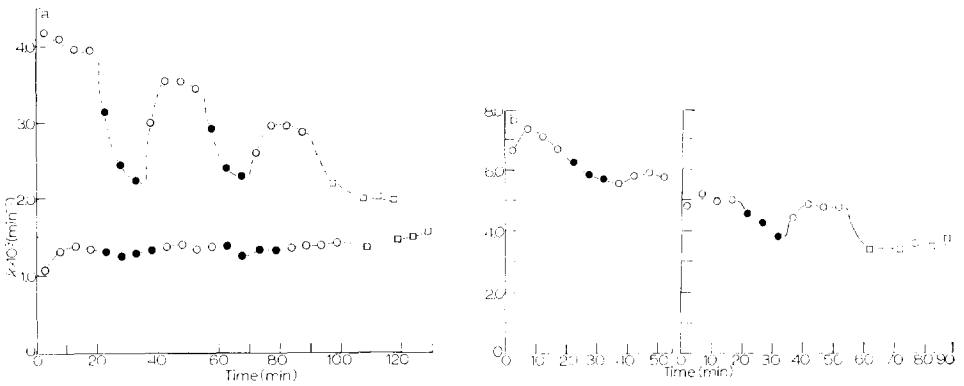

Fig. 1. a. The rate constant (k) for Na^+ efflux plotted against time: upper curve is for a fibre perfused with a solution containing ATP, creatine phosphate and creatine phosphokinase; lower curve is for a fibre perfused with a solution lacking energy-rich phosphate compounds. b. A similar plot of data for a long-perfused fibre which was resurrected after 55 min and injected with ^{22}Na. External solutions: ○, artificial sea water containing 10 mM K^+; ●, artificial sea water lacking K^+; □, artificial sea water with 10 mM K^+ and 10^{-5} M ouabain.

constant for Na^+ efflux from an axon perfused with solution lacking energy-rich phosphate. The rate constant is lower, relatively steady with time and independent of external K^+ or ouabain. Remembering that a rate constant of 10^{-3} min^{-1} represents an efflux of 39 pmoles/cm^2·sec for an axon 750 μ in diameter and containing 125 mM Na^+, it is clear that the basal effluxes in unfuelled fibres are higher than those found in poisoned intact axons. They are much higher than those found in unfuelled, dialysed axons where the efflux averaged 1.3 pmoles/cm^2·sec (ref. 4). The perfused axons may be leaky towards Na^+, although other experiments suggest that this leak may not be very profound[5]. Despite the large basal efflux, the K^+ and ouabain-sensitive fluxes are similar in magnitude to those seen in intact axons.

With long-perfused fibres the rate constant for Na^+ efflux in the presence of energy-rich phosphate compounds was often very large; but in about half of our 17 long-perfusion experiments, the efflux was little affected by either external K^+ or ouabain.

In view of the failure of many long-perfused axons to show signs of active transport, the possibility of resurrecting these fibres was investigated. Such a fibre

is shown in Fig. 1b. On perfusion it showed virtually no sensitivity to the removal of external K^+. After 55 min the axon was refilled with axoplasm and injected with ^{22}Na. Fig. 1b shows that the resurrected fibre was sensitive to potassium deprivation and to the application of ouabain. Evidently, some of the K^+ and ouabain-sensitive Na^+ efflux which is frequently lost after long perfusion can be recovered by replacement of intact axoplasm.

It may be that in long perfusions toxic material progressively inhibits the sodium pump but is removed when natural axoplasm is replaced. Alternatively, long perfusion may elute a component of the active transport mechanism. Presumably the material would be of high molecular weight, since prolonged dialysis does not interfere with sodium transport[1]. It would be of interest to know whether the $(Na^+ + K^+)$-activated ATPase[6], which seems to be part of the sodium pump mechanism and is present in perfused fibres[7], is still active in those fibres which lack evidence of active transport after long perfusion. If the enzyme is still active then it would seem to follow that prolonged perfusion interferes with a factor coupling the action of the enzyme to the movement of ions.

The present observations suggest a possible explanation for the failure of earlier experiments to demonstrate the sodium pump in perfused axons because in these cases a continuous flow method of perfusion was used. Moreover, the experiments demonstrate that a K^+ and ouabain-sensitive Na^+ efflux can take place after the bulk of the axoplasm has been removed.

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