J. Physiol. (1966), **182**, *pp.* 209–216
With 3 text-figures
Printed in Great Britain

CATION MOVEMENTS IN PERFUSED GIANT AXONS

By T. I. SHAW
From the Laboratory of the Marine Biological Association, Plymouth

(*Received* 21 *June* 1965)

SUMMARY

1. The net movements of Na and K into resting squid giant axons perfused with a K_2SO_4 artificial axoplasm have been determined. Na enters at a rate of 131 ± 25 p-mole/cm^2.sec. The K leakage from the fibre is 942 ± 566 p-mole/cm^2.sec; clearly this latter figure shows how large is the uncertainty in the exact value for the potassium leakage.

2. The net entry of sodium associated with activity in such fibres has been measured and is $5{\cdot}7 \pm 0{\cdot}7$ p-mole/cm^2.impulse.

INTRODUCTION

The sodium influx into the intact giant axons of squid has been measured by a number of workers (Shanes & Berman, 1955; Caldwell, Hodgkin, Keynes & Shaw, 1960); the flux is insensitive to cyanide and appears to be mainly a passive flux or leak. In the present work the influx of sodium into perfused fibres (Baker, Hodgkin & Shaw, 1962*a*) has been studied with the object of determining how much greater the leakage is into these fibres due to the manipulative treatment they have undergone. This information is important for deciding whether, in principle, it should be possible to reactivate the sodium pump in perfused fibres so as to induce a net outflow of sodium against both concentration and electrical gradients. Net movements of potassium have also been examined.

It has been known for several years that sodium enters rather rapidly into intact fibres whilst they are stimulated (Rothenberg, 1950; Keynes & Lewis, 1951) and indeed this evidence has been important in the development of the sodium hypothesis. Recently evidence in conflict with the sodium hypothesis has been put forward in connexion with perfused nerve fibres (Tasaki & Takenaka, 1964); it has therefore seemed worth while to examine whether there are large inward movements of sodium in these fibres during activity and this too has been examined in the present work.

METHODS

Materials

The fibres used were the hindmost giant axons from the stellate ganglion of *Loligo forbesi*; they were taken from large specimens whose refrigerated mantles had been brought in from the trawlers. The axons were dissected free from the mantle but were not separated from the nerve trunk or cleaned; at this stage their diameters were measured.

The technique of the initial extrusion and perfusion have been described elsewhere (Baker *et al.* 1962*a*). Only excitable axons were employed.

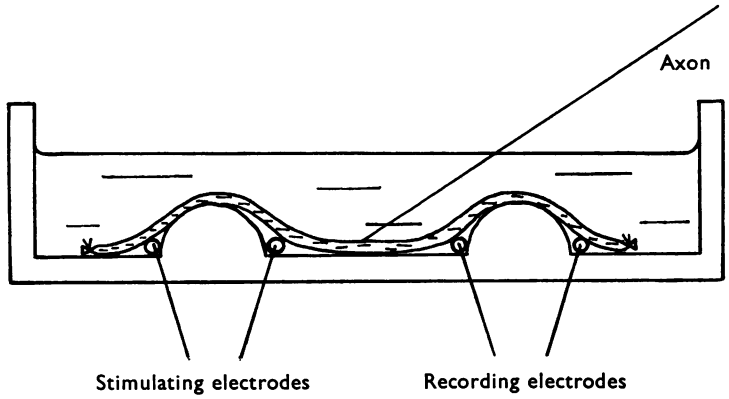

Fig. 1. Diagram of Perspex trough for perfused nerve fibres.

Resting cation movements

Approximately 80 μl. of artificial axoplasm were passed through the fibres to wash out residual axoplasmic sodium and to equilibrate the fibres. The perfusion was then halted and the fibres tied off at the end distal to the cannula. After lowering the hydrostatic pressure on the axon to about 1 cm water the cannula end was tied off, and the fibre removed from the cannula and placed in either sodium or choline artificial sea water. Those fibres that were to be kept for an hour in artificial sea water to permit sodium movements to proceed were placed in a special Perspex trough of the type shown in Fig. 1. The trough was designed to allow for the eventual testing of the axon for electrical excitability without permitting the solutions at either end of the fibre, where leakage may have been high, to mix with the main body of the contents. Recording and stimulating electrodes were mounted on the Perspex trough as shown. For the main duration of the influx experiments the fibre lay totally immersed in artificial sea water. About 2 min before the collection of the sample, the water level was lowered to expose the ridges in the trough, thereby separating it into three compartments. The axon was tested and two loose ligatures which, at the start of the experiment, had been placed round the fibre at the level of the ridges were drawn tight, thereby segregating the central part of the axon from the contaminated ends. Prior to extrusion of the artificial axoplasm for analysis the ends were cut off and the fibre washed for 5–10 min in choline artificial sea water. During this wash the fibre was cleaned, at one end, of much of the peripheral nerve trunk to ensure a minimum of contamination of extruded artificial axoplasm with extracellular fluid. After blotting with filter paper the axon was held over a small tared glass weighing vessel, its cleaned end down and just touching the glass. A cut was made into the axon and some of its contents drained into the vessel—the extrusion was completed with a roller.

Some fibres immediately after perfusion and tying off were washed directly in choline or sodium artificial sea water, cleaned, and their artificial axoplasm taken for immediate analysis as described above.

Sodium movements during activity

In these experiments the axon was retained in the perfusion apparatus shown by Baker *et al.* 1962*a*, Text-fig. 3, and the perfusion fluid was collected at 10 μl./min in the carefully washed cup. The samples were pipetted, with a teat, from this cup into tared weighing bottles before analysis. To permit stimulation and recording, the ends of the fibre had to be clear of the fluid but the minimum exposures were made. The excitability was carefully monitored during the periods of stimulation. For calculation of membrane area the lengths of the fibres between distal recording electrode and cannula tips were noted.

Analyses

The weighed samples of artificial axoplasm were diluted with distilled water and analysed for sodium and potassium by an EEL flame photometer.

Solutions

The composition of solutions is given in Table 1.

TABLE 1. Composition of solutions. Concentrations in mg ions/l. solution

	K^+	Na^+	$Choline^+$	Mg^{2+}	Ca^{2+}	Cl'	HCO_3'
Na artificial sea water	10	463	—	55	11	602	25
Choline artificial sea water	10	—	463	55	11	602	25

	K^+	SO_4^{2-}	$Glutamate^-$	Phosphate
K_2SO_4 artificial axoplasm*	1030	500	—	30
Glutamate artificial axoplasm*†	300	—	300	15

* The solution was made with KH_2PO_4 and KOH was added to bring the final pH to 7·7.

† Made isotonic with 0·5 molal sucrose.

RESULTS

Movements of cations in resting nerve

The sodium contents of artificial axoplasm collected immediately after perfusion and an hour later are shown in Table 2; both groups of fibres were washed in choline artificial sea water before the collection of samples. The mean initial sodium concentration was 24·6 m-mole/kg and this has been subtracted for each of the values for the sodium content of fibres maintained for an hour in sea water so as to calculate the net inward sodium movement in column 6. These movements were calculated from d, the fibre diameter in μ, t, the duration of the experiment in minutes, c, the difference in Na content of fibres due to the hour's immersion in sodium artificial sea water, and from the density (1·07) of the artificial axoplasm. The formula

$$F = \frac{cd}{2{\cdot}4t} \times 1{\cdot}07 \qquad (1)$$

gives F the cation movement stated in p-mole/cm^2.sec. Thus calculated, the mean inward movement of sodium is 115 p-mole/cm^2.sec.

It is significant that the K content of the artificial axoplasm collected immediately after perfusion (lines 1–7, Table 2) is only 923 m-mole/kg and not the 1050 m-mole/kg obtained by analysis of the fresh solution. The discrepancy suggested that there was some dilution of the collected sample with extracellular fluid. To see if this was the case, and if so to correct for it, a number of fibres were briefly washed in Na artificial sea water rather than choline sea water and their Na contents were determined. In these analyses any dilution by extracellular fluid should be shown up by raised Na contents. The data are given in Table 3 where it is seen that the mean Na content is 75·9 m-mole/kg, an excess of 51·3 ± 13·7

TABLE 2. Na and K content of the artificial axoplasm of perfused fibres

Fibre reference	Diameter (μ)	Time in Na artificial sea water (min)	Na (m-mole/kg)	K (m-mole/kg)	Na* flux (p-mole/cm^2.sec)
iii 20F2	829	5	10·3	838	—
v 604	753	3	19·4	882	—
iv 704	922	3	20·3	854	—
iii 1404	753	5	66·5	859	—
v 20N4	852	4	11·8	1195	—
i 20N4	897	2	17·4	1017	—
iv 20F2	714	5	26·8	815	—
	Mean ± S.E. of mean		24·6 ± 7·3	923 ± 52	
ii 504	659	60	37·8	916	69·4
iii 604	873	60	38·3	401	95·4
i 704	741	61	55·6	831	180
ii 23N4	815	60	37·7	1059	85·0
ii 20F2	886	60	48·1	745	166
i 21F2	922	60	48·2	755	173
ii 21F2	903	60	30·0	815	38·9
		Mean ± S.E. of mean		789 ± 76	115·4 ± 21·4

* Na fluxes uncorrected for dilution with extracellular fluid—see text. In calculating the fluxes a mean time of 4 min has been taken for the duration of the experiments in lines 1–7; the density of artificial axoplasm has been taken as 1·07 g/c.c. Temperature 17–22° C.

TABLE 3. Na and K content of the artificial axoplasm of fibres washed in Na artificial sea water before sample collection

Fibre reference	Diameter (μ)	Na (m-mole/kg)	K (m-mole/kg)
vi 604	863	124	800
ii 704	721	76·4	918
iv 1504	674	77·2	603
iii 1504	654	51·0	939
i 15D4	716	76·7	957
ii 15D4	741	97·6	1003
iv 15D4	716	28·6	995
	Mean ± S.E. of mean	75·9 ± 11·6	

m-mole/kg over the sodium contents of fibres washed immediately in choline sea water (Table 2). This figure amounts to $12 \pm 3\%$ of the sodium content of artificial sea water and implies a dilution such that samples contained only $88 \pm 3\%$ of artificial axoplasm. Allowing for this dilution the mean K content of the axoplasm extruded immediately after perfusion becomes 1050 m-mole/kg while the mean K content of the axoplasm of fibres maintained for 1 hr in artificial sea water becomes 897 ± 92 m-mole/kg which is 153 ± 92 m-mole/kg less than the initial perfusate. From an equation like eqn. (1) the net outward K movement for a mean axon diameter of 828 μ is 942 ± 566 p-mole/cm^2.sec.

With allowance for the dilution of the artificial axoplasm by extracellular fluid, the inward Na movement becomes $131 \pm 24{\cdot}5$ p-mole/cm^2.sec. All the fibres in Tables 2 and 3 were perfused with K_2SO_4 artificial axoplasm.

Na movements in stimulated nerve

Figure 2 shows the results of one experiment on a fibre perfused with K_2SO_4 artificial axoplasm. The Na content was estimated in the perfusate collected at rest and during two periods of activity. The results clearly show the increased Na concentration in the fluid collected during activity. Another experiment in which a glutamate artificial axoplasm

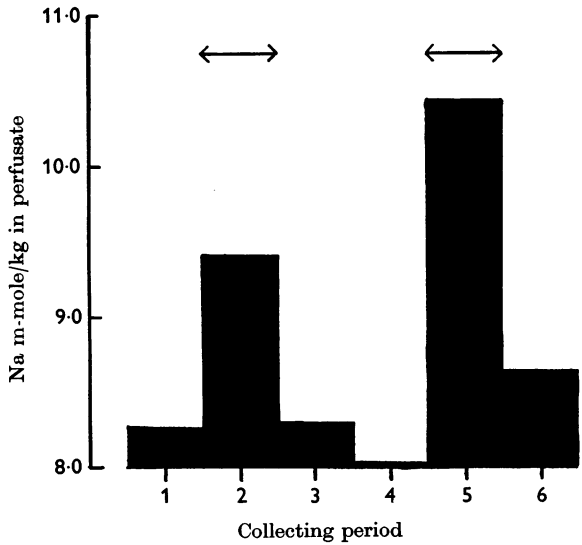

Fig. 2. Na content of perfusion fluid from a giant axon at rest and stimulated. The periods during which 110 c/s stimulation was applied are marked above; the first was applied for 5 min, the latter for 6 min. Perfusion of K_2SO_4 artificial axoplasm.

was used is illustrated in Fig. 3. Again during the two periods of stimulation the Na content is increased in the perfusate. The Na concentration also appears to be raised in the collecting periods immediately after stimulation. This experiment, the only one using a glutamate perfusion fluid, was unique in showing this carry-over. Tentatively the effect may be ascribed to the high viscosity of the perfusion fluid delaying mixing so that some of the excess Na brought into the fibre during the period of stimulation is only swept out after the electrical activity has been completed. In calculating the Na entry per impulse the residual Na in the hang-over periods has been included with the extra Na during the period of stimulation.

The net movements of Na during activity was determined in a number of experiments and the results are summarized in Table 4 which shows that Na entry amounted to 5·7 p-mole/cm^2.impulse for fibres perfused with K_2SO_4 artificial axoplasm.

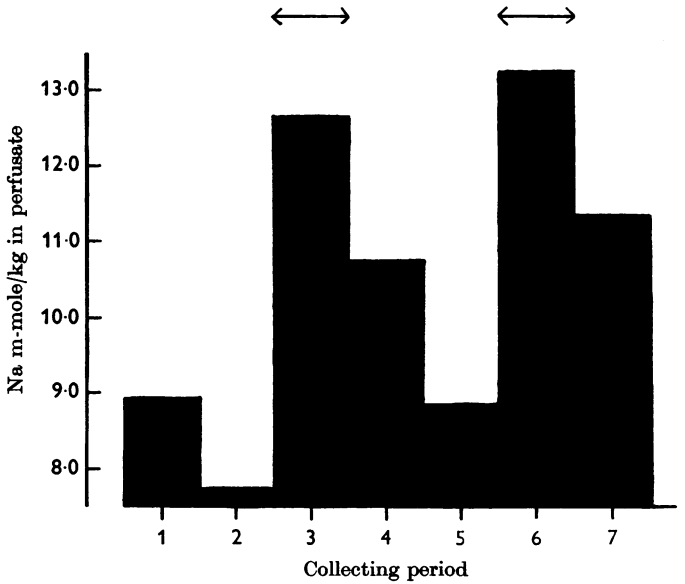

Fig. 3. Na content of perfusion fluid from a giant axon at rest and stimulated. The periods during which 110 c/s stimulation was applied are marked above; stimulation was for 5 min. Perfusion of glutamate artificial axoplasm.

DISCUSSION

The net movements of Na measured in this work may be compared with the influx as ordinarily measured, for the present fibres were virtually free of Na at the start of the experiment. Shanes & Berman (1955) found that the Na influx averaged 63 p-mole/cm^2.sec in *L. pealii*; Caldwell *et al.*

(1960) quote a figure of 42 p-mole/cm².sec for *L. forbesi*. In comparison with these figures the value of 131 p-mole/cm².sec found in the current study is considerably higher, and probably indicates that a fair degree of damage is done during the manipulation of extrusion or perfusion.

TABLE 4. Na entry per impulse into perfused fibres

Fibre reference	Perfusate	Na entry* p-mole/cm².impulse	T (° C)
i 10N4	K_2SO_4 artificial axoplasm	8	21
i 23N4		4	21
i 27N4		8	22
i 30N4		5	21
		6	21
i 1D4		3	17
		6	—
	Mean	5·7 ± 0·7	
ii 10D4	Glutamate artificial axoplasm	12	18
		9	20
	Mean	10·5	

* Stimulation periods were for 5–6 min and were at a frequency of 110 c/s except for i 10N4 which was at 43 c/s.

A very large uncertainty amounting to 60 % is associated with the figure of 942 p-mole/cm².sec for the rate of net loss of K from perfused fibres. The efflux from *Sepia* fibres, measured with tracers, amounts to only 28 p-mole/cm².sec (Hodgkin & Keynes, 1955). According to the constant field theory (Hodgkin & Katz, 1949), I_{Na}, the inward sodium current, and I_K, the outward potassium current, are related to P_{Na} and P_K, the permeabilities towards Na and K, by the equation

$$-\frac{I_{Na}}{I_K} = \frac{P_{Na}\,(Na_o - Na_i e^{-VF/RT})}{P_K\,(K_o - K_i e^{-VF/RT})} \qquad (2)$$

Here Na_o, Na_i, K_o and K_i are the activities of these ions inside and outside the cell, respectively, V is the potential of the outside solution minus that of the inside solution, RT and F have their usual significance. Taking the activity coefficients from the data given by Baker *et al.* (1962*a*) and taking the mean value of V for K_2SO_4 fibres as 60·5 mV with the value of P_{Na}/P_K as 0·045 given by Baker, Hodgkin & Shaw (1962*b*), also taking I_{Na} from the present data, the expected value for I_K becomes approximately 300 p-mole/cm².sec.

During activity, Rothenberg (1950) found a net entry into *L. pealii* axons of 4·5 p-mole/cm².impulse and in the same species but using a different method Moore & Adelman (1961) obtained a value of 1·5 p-mole/cm².impulse. In giant axons of *L. forbesi*, Keynes & Lewis (1951) found a net entry of 3·8 p-moles/cm².impulse. The present value of

$5{\cdot}7 \pm 0{\cdot}7$ p-mole/cm^2.impulse seems moderately close to these values and a good deal less than the increased influx of Na due to activity and measured by tracers. According to Keynes (1951) this increased influx amounts in *Sepia* to 10·3 p-moles/cm^2.impulse.

I am indebted to Mr B. J. Morgan for assistance with the flame photometry.

REFERENCES

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1962*a*). Replacement of the axoplasm of giant nerve fibres with artificial solutions. *J. Physiol.* **164**, 330–354.

BAKER, P. F., HODGKIN, A. L. & SHAW, T. I. (1962*b*). The effects of changes in internal concentrations on the electrical properties of perfused giant axons. *J. Physiol.* **164**, 355–374.

CALDWELL, P. C., HODGKIN, A. L., KEYNES, R. D. & SHAW, T. I. (1960). The effects of injecting 'energy rich' phosphate compounds on the active transport of ions in the giant axons of *Loligo*. *J. Physiol.* **152**, 561–590.

HODGKIN, A. L. & KATZ, B. (1949). The effect of sodium ions on the electrical activity of the giant axon of the squid. *J. Physiol.* **108**, 37–77.

HODGKIN, A. L. & KEYNES, R. D. (1955). Active transport of cations in giant axons from *Sepia* and *Loligo*. *J. Physiol.* **128**, 28–60.

KEYNES, R. D. (1951). The ionic movements during nervous activity. *J. Physiol.* **114**, 119–150.

KEYNES, R. D. & LEWIS, P. R. (1951). The sodium and potassium content of cephalopod nerve fibres. *J. Physiol.* **114**, 151–182.

MOORE, J. W. & ADELMAN, W. J. JR. (1961). Electronic measurements of the intracellular concentration and net fluxes of sodium in the squid axon. *J. gen. Physiol.* **45**, 77–92.

ROTHENBERG, M. A. (1950). Studies on permeability in relation to nerve function. II. Ionic movements across axonal membranes. *Biochim. biophys. Acta*, **4**, 96–102.

SHANES, A. M. & BERMAN, M. D. (1955). Kinetics of ion movement in the squid giant axon. *J. gen. Physiol.* **39**, 279–300.

TASAKI, I. & TAKENAKA, T. (1964). Effects of various potassium salts and proteases upon excitability of intracellularly perfused squid giant axons. *Proc. natn. Acad. Sci., U.S.A.*, **52**, 804–810.