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PHOSPHATE EFFLUX AND OXYGEN CONSUMPTION IN SMALL NON-MYELINATED NERVE FIBRES AT REST AND DURING ACTIVITY

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SUMMARY

1. The oxygen consumption and the movements of labelled phosphate were measured in garfish olfactory nerve at rest and during activity.

2. In solutions with 2·5 mM-K and 0·2 mM-phosphate the resting oxygen consumption was 0·206 m-mole/kg.min; activity at 2 sec^{-1} produced an extra oxygen consumption of 2·46 μmole/kg.impulse. The extra oxygen consumption declined exponentially with a time constant of 2·62 min at 22–26 °C.

3. The phosphate efflux, measured simultaneously, had a resting efflux rate constant of $1{\cdot}24 \times 10^{-3}$ min^{-1}; activity at 2 sec^{-1} produced an extra fractional loss of $9{\cdot}38 \times 10^{-6}$ $impulse^{-1}$. The increase in phosphate efflux followed almost the same time course as the increase in oxygen consumption.

4. Increasing the frequency of stimulation from 2 sec^{-1} to 3 or 5 sec^{-1} decreased both the extra oxygen consumption and the extra fractional loss of phosphate. When the frequency was decreased to 0·5 or 1 sec^{-1} the extra oxygen consumption per impulse increased, while the extra phosphate liberation was lowered.

5. Changing the phosphate concentration did not much affect the extra oxygen consumption; on the other hand, lowering or increasing the phosphate from the standard 0.2 mM decreased both the resting and the stimulated phosphate efflux.

6. Lowering the K from the standard 2·5 mM did not affect the extra oxygen consumption, but increased both the resting and the extra loss of phosphate. At higher K concentrations the extra oxygen consumption and the extra fractional loss of phosphate decreased without much change in the resting phosphate efflux.

7. Application of 1–20 μM-strophanthidin produced a transient decrease in the resting phosphate efflux without much change in resting oxygen consumption. With 10 or 20 μM-strophanthidin the extra fractional loss of phosphate and the extra oxygen consumption were both lowered in approximately the same proportions.

8. The findings are consistent with the hypothesis that the increase in intracellular inorganic phosphate that results from increased break-down of ATP after activity, is the main cause for the increased phosphate efflux. A fraction of the increase in intracellular phosphate only appears to be liberated to the outside, the value of the fraction depending on the resting phosphate efflux before activity.

9. The initial increase in intracellular inorganic phosphate after an impulse, estimated from the oxygen consumption or the phosphate fluxes, appears to be about 12–19 μmole/kg nerve, remarkably close to the value known from chemical analysis.

INTRODUCTION

In several types of nerve fibres a small continuous efflux of inorganic phosphate has been observed, for example in frog sciatic nerve (Mullins, 1954), in squid giant axons (Tasaki, Teorell & Spyropoulos, 1961; Caldwell, Hodgkin, Keynes & Shaw, 1964), in crab nerve (Baker, 1965), in rabbit vagus nerve (Anner, Ferrero, Jirounek, Jones, Salamin & Straub, 1976), and in garfish olfactory nerve (Ritchie & Straub, 1978). Depending on the extracellular phosphate concentration, the efflux is balanced by an uptake of phosphate (Ferrero, Jirounek, Rouiller & Straub, 1978).

Recently it has been observed that during and immediately after activity the efflux of phosphate is increased (Ritchie & Straub, 1978). The increased efflux could be due to the increase in intracellular inorganic phosphate that results from increased break-down of ATP by the Na–K pump. Such an increase has actually been observed in garfish olfactory nerve (McDougal & Osborn, 1976) as well as in other types of nerve fibres (Baker, 1965; Chmouliovsky, Schorderet & Straub, 1969).

In the present experiments the increase in phosphate efflux during activity was further studied by simultaneously measuring the phosphate efflux and the oxygen consumption. Measurements of the oxygen consumption are likely to provide some insight into the phosphate turnover, since the increase in intracellular inorganic phosphate also stimulates the oxygen consumption. In addition the phosphate efflux and oxygen consumption were measured when the utilization of ATP was decreased by application of strophanthidin.

METHODS

The technique used was essentially similar to that described by Ritchie & Straub (1975, 1978). Olfactory nerves of garfish (tip of snout – tail length, 65–80 cm; tip of snout – eyeball length, 16–19 cm) were isolated and soaked for 2 hr in [^{32}P]orthophosphate labelled garfish Ringer containing 0·2 mM total phosphate at room temperature (22–26 °C). The nerves (weighing $0{\cdot}349 \pm 0{\cdot}012$ mg.mm^{-1}, $n = 27$; dry wt./wet wt. $= 0{\cdot}172 \pm 0{\cdot}005$, $n = 15$) were then mounted in the apparatus described by Ritchie & Straub (1975) and perfused with label-free solutions at a constant rate (0·3 ml./min unless otherwise stated) by a peristaltic pump. The solutions were all equilibrated with air. As in the earlier experiments (Ritchie & Straub, 1978), the fluid bathing the portion of the nerve that lay underneath the stimulating electrodes (about 1/4) was collected by a guard-suck system and discarded. The remainder, which perfused the rest of the nerve, was collected and counted. For stimulation supramaximal shocks (10 × threshold) of 1 msec duration were used.

At the end of the experiment the nerve was removed, weighed and the water soluble phosphates were then extracted and counted as described (Ritchie & Straub, 1978). In some experiments part of the nerve was used for measuring the total water soluble phosphates and the proteins. This part of the nerve was first dried and re-weighed. One half of the dried preparation was then homogenized in 1 ml. solution containing 250 mM-sucrose, 0·1 mM-EDTA and 0·1 mM-dimercaptoethanol, and centrifuged for 30 min at 35,000 g. The inorganic phosphates of the supernatant were then measured by the method described by Anner & Moosmayer (1975). The other half was homogenized in 1 ml. TCA (5% w/v) and an aliquot was taken for measuring the proteins with the method of Lowry, Rosebrough, Farr & Randall (1951). Another aliquot was centrifuged for 20 min at 3000 g and the supernatant used for determination of the phosphates.

Both methods of preparing the extract of water soluble phosphates gave similar results suggesting that the TCA treatment did not liberate phosphates that were not also solubilized by the milder sucrose-EDTA procedure.

The oxygen consumption was determined as described in detail previously (Ritchie, 1967; Rang & Ritchie, 1968) by flowing the perfusion fluid of the nerve past an oxygen cathode. The resting consumption was measured by recording the change in steady-state oxygen concentration at the electrode when the flow through the nerve chamber was suddenly reduced from its normal value v_1 (0·3 ml./min) to a new value v_2 (0·1 ml./min). The resting oxygen consumption was then calculated as $\Delta C\ v_1 . v_2/(v_1 - v_2)$ where ΔC is the change in concentration of oxygen in the solutions at the electrode on switching (Rang & Ritchie, 1968). The stimulated oxygen consumption was determined by recording the fall in oxygen concentration of the effluent from the chamber resulting from activity. The area under the oxygen concentration–time course gave the total extra oxygen used; and the shape of the curve gave information about the time course of the increased metabolism. The oxygen electrode was calibrated at the end of each experiment by noting the deflexion when the perfusing fluid was changed from one that was well equilibrated with air to one equilibrated with nitrogen and from which the oxygen had been removed by vigorous bubbling (often after the solution had been boiled to remove any dissolved gases).

The action potential was recorded continuously as a check on the excitability of the preparation. All experiments were done at room temperature (22–26 °C). The Ringer solution had the following composition (mM): NaCl, 120; KCl, 2·5; $CaCl_2$, 0·9; $MgCl_2$, 0·5; sucrose, 60; dextrose, 24; Tris, 10. Na orthophosphate was present in the concentrations indicated in the text. In all experiments pH was adjusted to 7·2. The K concentration was changed by adding or omitting KCl. Na-free solution was prepared by replacing NaCl by Tris Cl.

The [^{32}P]phosphoric acid (carrier free) was obtained from New England Nuclear, Boston. It was used for 3 weeks at most.

Whenever possible mean values ± S.E. are given.

RESULTS

Oxygen consumption

Resting oxygen consumption

The resting oxygen consumption was difficult to determine reliably in these experiments because the high sugar content of the bathing solutions favoured growth of micro-organisms. As a result a relatively large fraction of the measured resting oxygen consumption was due to contamination and not to nervous metabolism. Allowance was made for this by determining the oxygen consumption without the nerve in the chamber. Switching from a flow of about 0·3 ml./min to 0·1 ml./min thus led to a decrease in oxygen concentration of effluent from the chamber. This bacterial oxygen consumption was then subtracted from the total oxygen consumption, determined similarly by decreasing the flow from 0·3 ml./min to 0·1 ml./min with the nerve in the chamber; the difference was taken to give the resting oxygen consumption of the nerve. Unfortunately, the background resting oxygen consumption was usually high (about 40 % of the total) and depended on the solution being used. For this reason routine measurements of the resting oxygen consumption in different solutions were not made. However, eight tests in five different nerves in normal Ringer solution with 0·2 mM-phosphate yielded an average value for the resting oxygen consumption of garfish nerve at about 25 °C of 0·206 ± 0·019 m-mole O_2/kg wet.min. Because of the uncertainty discussed above this must be regarded as giving only an approximate estimate of the true value.

Stimulated oxygen consumption

Fig. 1 shows the effect of stimulation on the oxygen consumption of the garfish olfactory nerve at about 25 °C. About a minute after stimulation had started, the oxygen content of the solution leaving the nerve chamber fell; and it did not return to its pre-stimulation value until several minutes after the end of the stimulation. This delay in onset, which also appeared before recovery began at the end of the period of stimulation, presumably reflected the transit time of the relatively slow

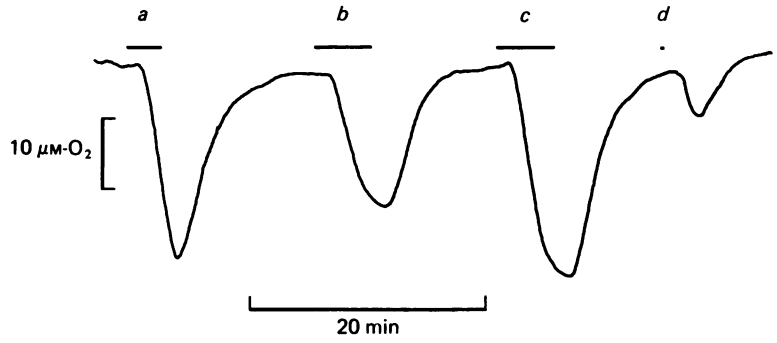

Fig. 1. Increase in oxygen consumption during activity in garfish olfactory nerve. The record shows the oxygen concentration of the effluent from the nerve chamber, a downward deflexion corresponding to a decrease in oxygen tension. During the periods indicated by the horizontal lines the nerve was stimulated at a frequency of: (*a*) 5 sec^{-1}, (*b*) 1 sec^{-1}, (*c*) 2 sec^{-1}, and (*d*) 5 sec^{-1}. The vertical bar represents a 10 μM change in oxygen concentration. Temperature 25 °C.

TABLE 1. Effect of frequency of stimulation on the extra oxygen and extra phosphate efflux per impulse (relative to response at 2 sec^{-1})

	Frequency of stimulation (sec^{-1})				
	0·5	1	2	3	5
Extra oxygen per impulse (relative)	1·25 ± 0·07 (11)	1·37 ± 0·07 (13)	1·00*	0·82 ± 0·06 (11)	0·61 ± 0·05 (8)
Extra P_i efflux per impulse (relative)	0·79 ± 0·08 (8)	0·84 ± 0·10 (9)	1·00†	0·84 ± 0·05 (5)	0·56 ± 0·07 (3)

Values in this and subsequent Tables are means ± S.E. of mean. The figures in parentheses represent number of nerves or experiments.

* The absolute value of the extra oxygen consumption was 2·46 ± 0·19 μmole/kg.impulse (n = 44).

† The absolute value of the extra fractional loss of phosphate was $9{\cdot}38 \pm 1{\cdot}20 \times 10^{-6}$ $impulse^{-1}$ (n = 22).

flowing solution from the nerve to the oxygen electrode. At the end of the period of stimulation, the response returned, more-or-less exponentially with a time constant of 2·62 ± 0·14 min (n = 20), to its pre-stimulation value. Records *a*, *b* and *c* show the response to stimulation for 3 or 5 min at 5, 1 and 2 sec^{-1}. Record *d* shows that even a brief period of stimulation (10 sec at 5 sec^{-1}) produced a distinct increase in the oxygen consumption of the preparation.

The effect of frequency. In twenty experiments, forty-four responses were obtained to 5 min periods of stimulation at 2 sec^{-1}. The oxygen uptake in these experiments was $2{\cdot}46 \pm 0{\cdot}19$ μmole O_2/kg.impulse. When the stimulation frequency was increased to 3 and 5 sec^{-1}, the extra oxygen per impulse fell (Table 1). On the other hand, when the frequency of stimulation was reduced, the extra oxygen per impulse rose to a value 32% greater than the value obtained at 2 sec^{-1} (average of responses at 0·5 sec^{-1} and 1 sec^{-1}). The extra oxygen consumed by relatively infrequent stimuli is thus of the order of 3·2 μmole/kg.impulse.

TABLE 2. Effect of external phosphate concentration on the extra oxygen consumption with activity and on the resting and stimulated phosphate effluxes

	Phosphate concentration (mM)			
	0·002	0·02	0·2	2
Extra oxygen per impulse (relative)	$1{\cdot}31 \pm 0{\cdot}04$ (2)	$1{\cdot}01 \pm 0{\cdot}08$ (3)	1·00	$1{\cdot}17 \pm 0{\cdot}17$ (4)
Extra P_i efflux per impulse (relative)	$0{\cdot}90 \pm 0{\cdot}03$ (3)	$0{\cdot}86 \pm 0{\cdot}06$ (3)	1·00	$0{\cdot}66 \pm 0{\cdot}13$ (5)
Resting P_i efflux (relative)	$0{\cdot}84 \pm 0{\cdot}06$ (3)	$0{\cdot}91 \pm 0{\cdot}04$ (3)	1·00*	$0{\cdot}86 \pm 0{\cdot}05$ (5)

* The absolute value of the rate constant of resting phosphate efflux was $1{\cdot}24 \pm 0{\cdot}07 \times 10^{-3}$ min^{-1} ($n = 22$).

TABLE 3. Effect of external K concentration on the extra oxygen consumption with activity and on the resting and stimulated phosphate effluxes

	K concentration (mM)		
	0	2·5	5
Extra oxygen per impulse (relative)	$1{\cdot}02 \pm 0{\cdot}08$ (5)	1·00	$0{\cdot}87 \pm 0{\cdot}08$ (4)
Extra P_i efflux per impulse (relative)	$1{\cdot}65 \pm 0{\cdot}10$ (4)	1·00	$0{\cdot}62 \pm 0{\cdot}05$ (4)
Resting P_i efflux (relative)	$2{\cdot}04 \pm 0{\cdot}17$ (4)	1·00	$0{\cdot}96 \pm 0{\cdot}06$ (4)

The effect of potassium and of phosphate. The oxygen consumption during activity was measured in solutions with different potassium and phosphate concentrations (Tables 2 and 3). Changing the ionic content of the bathing medium by doubling or removing the potassium, or by varying the external phosphate concentration from 0·002 mM to 2 mM, had only a slight effect on the extra oxygen consumption per impulse.

Recovery of the increased oxygen consumption

As mentioned above the recovery showed an approximately exponential time course. After stimulation for 5 min at 2 sec^{-1} the mean rate constant of recovery was $0{\cdot}38 \pm 0{\cdot}01$ min^{-1} ($n = 12$) in the presence of 2·5 mM-K and 0·2 mM-phosphate. The rate of recovery usually showed a small tendency to decrease in the course of an experiment.

The rate of recovery did not appear to be much affected by the frequency of stimulation. In three nerves where the effect of frequency was studied the normalized values after 5 min stimulation were: at 0·5 sec^{-1}, 0·81 ($n = 2$); at 1 sec^{-1}, 1·05 ± 0·07 ($n = 4$); at 2 sec^{-1}, 1·00 ($n = 5$); at 3 sec^{-1}, 1·19 ± 0·03 ($n = 3$); at 5 sec^{-1}, 1·07 ± 0·12 ($n = 3$).

The rate of recovery increased with increasing potassium concentrations as in the experiments of Baker & Connelly (1966), and Rang & Ritchie (1968). The normalized values for 5 min periods of activity at 2 sec^{-1} at the various K concentrations were: 0 mM-K, 1·5 ± 0·18 ($n = 3$); 1 mM-K, 1·10; 2·5 mM-K, 1·00 ($n = 7$); 4 mM-K, 0·94; 5 mM-K, 0·73 ± 0·07 ($n = 3$).

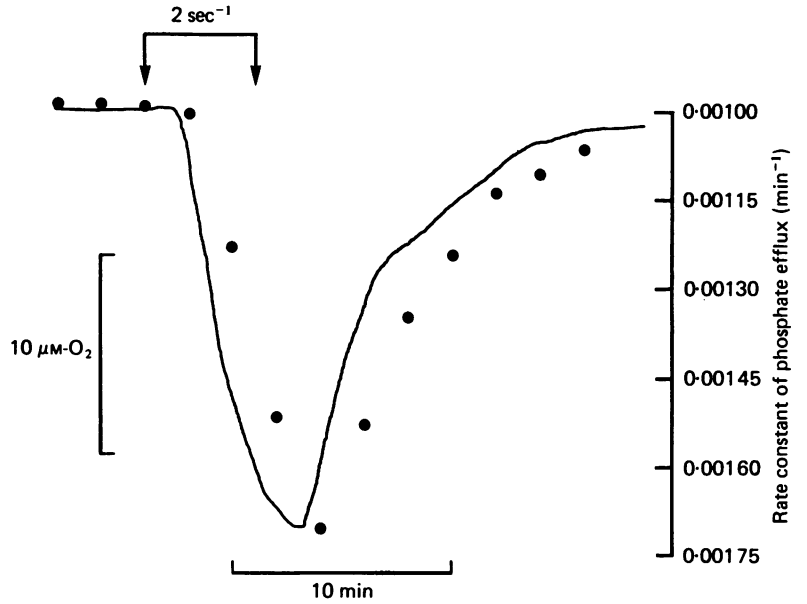

Fig. 2. Increase in oxygen consumption and phosphate efflux during activity in garfish olfactory nerve. The continuous record shows the oxygen concentration of the effluent of the nerve chamber, a downward deflexion representing a decrease in oxygen tension. The points are rate constants of phosphate efflux, an *increase* in efflux corresponding to a downward deflexion. The nerve was stimulated during the period shown by the horizontal line. Temperature 26·2 °C.

Changing the phosphate concentration also affected the rate of recovery, which was highest in 0·2 mM-phosphate (1·00) and decreased to 0·89 in 0·02 mM and to 0·63 in 0·002 mM and to 0·67 in 2 mM-phosphate.

Phosphate efflux and oxygen consumption

In a number of experiments the oxygen consumption and the phosphate efflux were measured simultaneously.

Time course

Fig. 2 shows a typical experiment for which both the rate constant of the phosphate efflux and the oxygen consumption were plotted on the same time scale. It can be seen that upon stimulation the increase in phosphate efflux (see Ritchie & Straub,

1978) roughly paralleled the increase in oxygen consumption (note that increasing phosphate efflux is plotted downwards in this Figure to make the comparison with the oxygen record easier).

The changes in phosphate fluxes were slightly slower in onset than the increase in oxygen consumption and delayed in recovery. In order to compare the two measurements it must be kept in mind that the efflux was collected over a fixed period (2 or 5 min) so that the values in Fig. 2 correspond to the mean efflux during this period. On the other hand, the recorded oxygen consumption reflects more closely the instantaneous events since the time constant of the recording equipment was small, being less than 1 min. Furthermore, any re-uptake mechanism for phosphate released during activity would slow its appearance in the perfusate.

The conclusion from these experiments is that phosphate efflux and oxygen consumption are closely linked. This applies to the rate of onset as well as to the rate of decline.

Relation between amount of phosphate released and amount of oxygen consumption

Phosphate efflux and oxygen consumption were measured at different frequencies of stimulation and at different phosphate and K concentrations.

Frequency. In Ringer with 0·2 mM-phosphate and 2·5 mM-K the mean rate constant of resting phosphate efflux was $1{\cdot}24 \pm 0{\cdot}07 \times 10^{-3}$ min^{-1} ($n = 22$); with stimulation at 2 sec^{-1} there was a mean extra fractional loss of $9{\cdot}38 \pm 1{\cdot}20 \times 10^{-6}$ imp^{-1} ($n = 22$). This is somewhat smaller than the corresponding values of $10\text{–}23 \times 10^{-6}$ impulse^{-1} at stimulation frequencies of 1–5 sec^{-1} obtained previously (Ritchie & Straub, 1978). Table 1 shows that the extra oxygen consumption was largest when low frequencies of stimulation were used. The extra fractional loss of phosphate did not show the same dependency on the frequency of stimulation. The phosphate efflux per stimulus increased when the frequency was lowered from 5 sec^{-1} to 2 sec^{-1}, but further lowering of the frequency of stimulation *lowered* the extra fractional loss per impulse. The decrease of both oxygen consumption and phosphate efflux per impulse at high frequencies of stimulation may reflect a decrease in metabolic load per impulse, for phosphate efflux seems to decrease *pari passu* with the extra oxygen consumption. The decrease in extra phosphate per impulse at low frequency is more difficult to understand: it might reflect the working of the phosphate uptake mechanism which may recapture some of the radiophosphate lost during activity.

Phosphate. The extra oxygen consumption does not seem to depend much on the phosphate concentration (Table 2). Changing the phosphate concentration from the standard value of 0·2 mM did not affect it significantly except for the value in 0·002 mM-phosphate which is based on two measurements only. On the other hand both the extra fractional loss of phosphate and the resting phosphate efflux were consistently decreased by a change in the phosphate concentration of the bathing fluid: the largest fluxes were found in 0·2 mM-phosphate and a decrease of both resting and extra efflux occurred when the phosphate concentration of the bath was either lowered or increased. In these experiments there was thus a certain parallelism between resting and extra efflux of phosphate.

Potassium. Table 3 shows that increasing the potassium concentration from 0 to 2·5 mM has no effect on the extra oxygen consumption, a further increase led

to a lowered consumption per impulse. The extra fractional loss of phosphate was not affected in the same way: in K-free solution the extra fractional loss was large, it was lowered in 2·5 mM-K and still further lowered in 5 mM-K. Comparison with the resting efflux shows that the resting efflux of phosphate had a similar dependency on potassium, so that the extra fractional loss of phosphate appeared to depend on the resting efflux, and the oxygen consumption per impulse.

The effect of strophanthidin

Effect on resting nerve

Strophanthidin in concentrations of 1, 10 and 20 μM had a pronounced effect on the phosphate efflux of resting nerve. Immediately after the application of the glycoside the phosphate efflux fell: with 1 μM the efflux during the first 5 min was 0·817 ($n = 2$) of the control, with 10 μM the efflux was $0{\cdot}935 \pm 0{\cdot}014$ ($n = 3$), and with 20 μM $0{\cdot}944 \pm 0{\cdot}021$ ($n = 4$). The efflux was also lowered in the following samples: for the first 15 min the values, for the same concentrations of glycoside, were 0·813, $0{\cdot}847 \pm 0{\cdot}013$, and $0{\cdot}850 \pm 0{\cdot}027$. The initial fall was followed by a slow rise and after 30–45 min the efflux approached the control value. At a concentration of 20 μM, strophanthidin produced a small transient increase in the resting oxygen consumption, at the other concentrations there was no measurable effect.

In one experiment strophanthidin was applied to a nerve that had been exposed for 20 min to Na-free solution. In this case the initial decrease in phosphate efflux on addition of the glycoside was absent. Rather the efflux increased progressively with time, being 10% higher than the control value at 15 min and 14% higher at 25 min. This observation has since been confirmed on rabbit vagus nerve (Maire & Straub, 1978).

Stimulated nerve

Nerves exposed for 20 min to 0·01 μM or 1 μM-strophanthidin showed an increased extra fractional loss of phosphate during stimulation. The mean increase was 40%, the oxygen consumption per impulse was either slightly increased or lowered. At 10 or 20 μM-strophanthidin the extra fractional loss of phosphate was lowered by about 80% and the oxygen consumption showed a comparable decrease.

Phosphate content

In a number of nerves the total amount of water soluble phosphate and the protein content were measured at the end of the experiment. The phosphate content in sixteen nerves was $10{\cdot}28 \pm 0{\cdot}52$ m-mole/kg wet. There seemed to be a small difference between the nerves that had been used solely for measurements of oxygen consumption, where the mean was $10{\cdot}97 \pm 0{\cdot}46$ ($n = 12$), and those exposed to the radiophosphate where $8{\cdot}22 \pm 1{\cdot}16$ m-mole/kg ($n = 4$) was found. The difference may be due to a slow loss of phosphate during the perfusion: the experiments where the oxygen consumption only was measured lasted about 6 hr, while those with radiophosphate had a duration of 12–14 hr.

The mean phosphate content in the present experiments is smaller than the content reported in the previous paper (17·4 m-mole/kg wet) or the value of McDougal & Osborn (1976) which corresponds to 13·5 m-mole/kg. The difference

may come from the fact that in the previous experiments the fish were used within 1–2 weeks after capture, while in the present experiments the animals were often kept for longer periods.

The mean protein content was $0{\cdot}57 \pm 0{\cdot}04$ mg/mg dry nerve ($n = 15$) and the mean phosphate was $0{\cdot}11 \pm 0{\cdot}01$ μmole/mg protein ($n = 15$).

DISCUSSION

The present experiments demonstrate that in garfish olfactory nerve at 22–26 °C a single impulse (at low frequency) increases the oxygen consumption by 3·2 μmole/kg wet nerve. As has been shown previously an impulse gives rise to an extra efflux of phosphate of 206 n-mole/kg wet nerve (Ritchie & Straub, 1978) and an extra loss of K of 15 μmole/kg (Ritchie & Straub, 1975).

Cause of increased efflux of phosphate during and after activity

It has previously been suggested (Ritchie & Straub, 1978) that the increased efflux of phosphate during and after activity may result from the increase in intracellular inorganic phosphate that has been found after activity in garfish olfactory nerve (McDougal & Osborn, 1976), as well as in other types of nerve fibres. The present results are in line with this hypothesis. The increase in oxygen consumption which probably results to a large extent from the decrease in ATP and the concomittant increase in inorganic phosphate and ADP, has a time course that closely parallels the increase in phosphate efflux. Further, in a number of conditions, when the extra oxygen consumption is lowered, there is also a smaller extra fractional loss of phosphate. This parallelism is found particularly when the frequency of stimulation is increased above 2 sec^{-1} or when the working of the Na–K pump is partially inhibited by strophanthidin. There are, however, a number of conditions where the extra oxygen consumption and the extra fractional loss of phosphate are not affected in the same way. For instance, when the phosphate is either increased above 0·2 mM or lowered below this value the extra fractional loss of phosphate is decreased without much change in the extra oxygen consumption. On the other hand in K-free solution, the extra fractional loss of phosphate is increased without much effect on the extra oxygen consumption. In these conditions, however, a parallelism is found between the *resting* phosphate efflux *before* stimulation and the extra fractional loss of phosphate. In high or low phosphate solutions the resting phosphate efflux is lowered, as is the extra efflux, in K-free solution the resting efflux is raised by the same amount as is the extra fractional loss of phosphate. The conclusion from these observations then is that the increase in internal inorganic phosphate *per se* does not determine the amount of extra release; rather the amount that is actually released also depends on the ability of the phosphate to escape to the outside. This is also in line with the conclusion (see below) that only a small fraction of the phosphate liberated inside escapes to the outside. In rabbit vagus, where the resting fluxes of phosphate have been studied in more detail than in the garfish, a Na-dependent transport system has been described which mediates a large proportion of influx and efflux of inorganic phosphate (see Ferrero *et al.* 1978). The experiments in garfish nerve suggest that a similar system may also be at work

in this tissue. If so, the efflux through this system would be the factor that determines the proportion of the increase in intracellular inorganic phosphate that is released to the outside.

Increase in intracellular phosphate and phosphate turnover after activity

The present experiments show that activity at 2 sec^{-1} increases the oxygen consumption by 2·5 μmole/kg.impulse (Q) and that the increase in the oxygen consumption declines exponentially with a time constant (τ) of 2·62 min. Taking into account that the increase in oxygen consumption was measured over a period of 10–12 min, the initial increase in the rate of oxygen consumption (Y_0) is given by $Y_0 = Q\ (1-e^{-4})/\tau$ (see Ritchie & Straub, 1978) and becomes after an impulse 0·97 μmole/kg.min. Assuming then that the increase in the rate of oxygen consumption is proportional to the increase in intracellular inorganic phosphate, and taking the latter value as 4·11 m-mole/kg wet nerve (McDougal & Osborn, 1976) and taking the resting oxygen consumption as 210 μmole/kg.min, one calculates that the initial increase in internal inorganic phosphate would be 19·0 μmole/kg per impulse. The increase in internal phosphate can be calculated also, by a similar argument, from the phosphate fluxes. At 2 sec^{-1} the total extra fractional loss per impulse is $9{\cdot}38 \times 10^{-6}$ $impulse^{-1}$; and the response declines at about the same rate as the oxygen consumption. From the resting phosphate efflux rate constant of 1240×10^{-6} min^{-1} (Table 2) and the internal phosphate of 4·11 m-mole/kg (McDougal & Osborn, 1976) the initial increase in the phosphate concentration becomes 11·6 μmole/kg per impulse. Compared to this value the change in internal phosphate measured by McDougal & Osborn after 180 impulses at 1 sec^{-1} is 6 μmole/kg per impulse, close to the values calculated above.

The finding that the increase in internal phosphate calculated from the oxygen consumption is larger than the corresponding value estimated from the phosphate fluxes may be due to the simplifying assumptions used. Indeed oxygen consumption is probably not stimulated only by the increase in P_i, but appears to depend on the $ADP \times P_i/ATP$ ratio (cf. Erecinska, Stubbs, Miyata, Ditre & Wilson, 1977). If this ratio is used, and the concentrations for ADP, P_i, and ATP in resting garfish nerve given by McDougal & Osborn (1976), the initial increase in internal phosphate calculated from the oxygen consumption becomes 11 μmole/kg.impulse, almost identical with the value estimated from the phosphate fluxes.

However, both the estimate from the oxygen consumption and the estimate from the phosphate fluxes are larger than the result of direct measurements by McDougal & Osborn (1976). This may be due to the fact that the true internal inorganic phosphate is smaller than the 4·11 m-mole/kg measured by chemical analysis, as suggested by comparison of values of P_i determined by nuclear magnetic resonance and by chemical methods in muscle (see Dawson, Gadian & Wilkie, 1977).

Phosphate turnover. With a time constant of recovery (τ) of 2·6 min, the calculated peak increases in internal phosphate (P_p) of 19 and 12 μmole/kg.impulse (based on the oxygen consumption and phosphate efflux measurements, respectively) correspond to increases in phosphate turnover rates (P_p/τ) of 7·3 and 4·6 μmole/kg.min, respectively. Similarly, the resting phosphate efflux corresponds to a phosphate turnover rate of 1·58 m-mole/kg.min ($4{\cdot}11 \times 10^{-3}$ mole/kg.2·6 min).

P/O ratio. From the total amount of phosphate released intracellularly after an impulse of 12–19 μmole/kg (see above) and the extra oxygen consumption of 2·5 μmole/kg.impulse (at 2 sec^{-1}), the value for the P/O ratio can be calculated to be 2·4–3·8. Similarly, from the resting phosphate turnover of 1·58 m-mole/kg.min and the observed resting oxygen consumption of 0·21 m-mole/kg.min, the calculated P/O ratio is 3·7. Considering the uncertainties of the various experimental measurements, we feel that these three estimates are in reasonably good agreement with the commonly assumed ratio of 3·0 (see Baker, 1965).

Effect of strophanthidin on resting phosphate efflux

After the application of strophanthidin the resting efflux of phosphate fell initially, and later slowly returned to near its original value. A plausible explanation for the

TABLE 4. Comparison of ionic fluxes and oxygen consumption in rabbit vagal and garfish olfactory nerve fibre (at room temperature)

	Rabbit vagus	Garfish olfactory nerve	References
Axonal area (m^2/kg wet)	600	6500	Keynes & Ritchie (1965), Ritchie & Straub (1975)
Resting oxygen consumption (m-mole/kg wet.min)	0·09	0·21	Ritchie (1967) (This paper)
Stimulated oxygen consumption (μmole/kg wet)	1·20	3·2	Ritchie (1967) (This paper)
Time constant of recovery of oxygen consumption (min)	3·3	2·6	Rang & Ritchie (1968) (This paper)
Resting K efflux (m-mole/kg wet.min)	0·69	0·81	Rang & Ritchie (1968), Ritchie & Straub (1975)
Stimulated K efflux (μmole/kg wet)	7·35	15·1	Rang & Ritchie (1968), Ritchie & Straub (1975)
Resting phosphate efflux (m-mole/kg wet.min)	0·44	1·71	Ritchie & Straub (1978)
Stimulated phosphate efflux (n-mole/kg wet.impulse)	7·7	206 79	Ritchie & Straub (1978) (This paper)
Calculated increase in intracellular phosphate (μmole/kg wet.impulse)	—	12–19	(This paper)

initial fall is that it simply reflects the decrease in the intracellular inorganic phosphate expected from the inhibition of the Na–K–ATPase by the glycoside. The subsequent slow recovery is less readily accounted for, but it may well be due to the sodium-dependence of the phosphate efflux, as shown by the increased efflux when the extracellular sodium concentration is increased. If the intracellular sodium similarly affected the phosphate efflux, the progressive accumulation of the intra-

cellular sodium by the poisoned axons would lead to the observed recovery of the phosphate efflux towards the original value.

Oxygen consumption and K fluxes; comparison with rabbit vagus

When it was found (Ritchie & Straub, 1975) that both the resting and stimulated potassium loss in garfish olfactory nerve fibres were a good deal smaller than in rabbit vagal nerve fibres, it was suggested that this might represent an evolutionary adaptation making conduction in extremely small fibres more economical. This conclusion is confirmed in the present experiments which show that in spite of the fact that the axonal area in garfish olfactory nerve fibres is more than 10 times that in rabbit vagal fibres (see Ritchie & Straub, 1975) and that the garfish nerve has many times more fibres, both the resting and stimulated oxygen consumptions are only double that in the rabbit vagus nerve (Table 4).

The extra efflux of K with stimulation at 0·5 sec^{-1}, 15 μmole/kg.impulse (Ritchie & Straub, 1975) is according to the present experiments accompanied by an extra oxygen consumption of 3·2 μmole/kg.impulse. The K efflux per mole of oxygen used, 4·6, is thus reasonably close to that in rabbit vagus fibres where it is 6·1 (Keynes & Ritchie, 1965; Ritchie, 1967). A similar value has been found in desheathed frog sciatic (Connelly, 1959). The corresponding values for garfish and rabbit non-myelinated fibres based on the resting values (Table 4) are 3·9 and 7·7, respectively. The ratio is thus far from the theoretical value of 18 obtained with the commonly accepted K:P ratio of about 3 and P:O ratio of 3. Values for the $K:O_2$ ratio around 18 have in fact been observed (see Whittam & Willis, 1963). Several reasons could account for the low $K:O_2$ ratio. First, the increased oxygen consumption may not be used only for restoring the ion gradients, and secondly, perhaps only a fraction of the increased potassium efflux during activity appears in the perfusate because of rapid re-uptake of the released K: indeed, in garfish olfactory nerve the measured potassium released during activity seems marginally large enough to account for the observed action potential (see Ritchie & Straub, 1975). Further, the amount of potassium lost per unit membrane area during the repolarization may indeed be smaller in these fibres. Only this latter factor would represent a true economy for the conduction process.

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