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OXYGEN CONSUMPTION AND PHOSPHATE EFFLUX IN MAMMALIAN NON-MYELINATED NERVE FIBRES

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SUMMARY

1. A comparison has been made between the efflux of labelled phosphate from the non-myelinated fibres of the desheathed rabbit vagus nerve at 37 °C and the corresponding O_2 consumption at rest and during activity, and during a variety of experimental interventions.

2. The resting rate constant of phosphate efflux was $2{\cdot}61 \times 10^{-3}$ min^{-1}: electrical stimulation (10 sec^{-1}, 3 min) produced an extra fractional loss of $6{\cdot}75 \times 10^{-6}$ impulse^{-1}.

3. The corresponding resting O_2 consumption was 0·484 m-mole.kg^{-1} min^{-1} and the extra O_2 consumption with electrical activity was 0·467 μmole.kg^{-1} impulse^{-1}.

4. Ouabain (100 μM) produced a sustained depression (of about 40%) of the resting O_2 consumption, accompanied by a transient fall (of about 14%) in the rate constant of phosphate efflux.

5. Na salicylate (10 mM) or Na arsenate (1 mM) produced a much larger increase in phosphate efflux than in resting O_2 consumption.

6. Changing the external phosphate concentration (between 0·02 and 2 mM), addition of acetylcholine (1·7 mM), and addition of lanthanum (20 μM) – all of which are known to affect markedly the phosphate efflux in rabbit non-myelinated fibres – had little or no effect on the resting O_2 consumption or, where tested, on the extra O_2 consumption with electrical stimulation.

7. Changing the external Ca concentration (between 0·09 and 9 mM) had only minor effects on the O_2 consumption (resting and stimulated) and on the rate constant of resting phosphate efflux.

8. It is concluded that although changes in metabolism of the nerve produce changes in the phosphate efflux expected on the basis of the concomitant changes in the internal concentration of inorganic phosphate, the converse is not true; and increases and decreases in the rate constant of phosphate efflux do not necessarily signal the corresponding metabolic changes.

INTRODUCTION

A small continuous efflux of inorganic phosphate occurs in many different kinds of nerve fibre (for references see Ritchie & Straub, 1979), which is balanced by a corre-

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sponding uptake of phosphate (Ferrero, Jirounek, Rouiller & Straub, 1978). During, and immediately after, activity the efflux of phosphate increases, as does the O_2 consumption. The time courses of development, and subsequent decline, of these two responses are roughly parallel; and it has been proposed that both increases result from a common determining factor, namely, the increase in intracellular inorganic phosphate following the increased metabolic activity of the nerve as it extrudes the Na that had entered the nerve during the electrical activity (Ritchie & Straub, 1978, 1979).

The correspondence in the two types of response, however, is not exact. For example, the results of Ritchie & Straub (1979) suggest that, in the steady state, stimulation of the non-myelinated fibres of the garfish olfactory nerve at 2 sec^{-1}, would increase the inorganic phosphate efflux above the resting efflux for that period by about 90 % whereas the O_2 consumption would be increased by a larger amount, 140 %. In the non-myelinated fibres of the rabbit vagus nerve a similar discrepancy is found (Ritchie & Straub, 1978). The discrepancy in the two types of response might occur because the increase in O_2 consumption depends not just on the increase in the intracellular concentration of inorganic phosphate but also on other factors such as the [ADP]/[ATP] ratio (Ritchie & Straub, 1979), which is likely to rise at the same time as $[P_i]$ rises (Erecinska, Stubbs, Miyata, Ditre & Wilson, 1971; Ritchie & Straub, 1979).

Recently, Straub and his colleagues have found that various pharmacological interventions lead to dramatic changes in the phosphate efflux from mammalian non-myelinated fibres. For example, small concentrations of lanthanum ions in the bathing medium virtually abolish the phosphate efflux (P. Jirounek and M. Rouiller, unpublished observations). Since the La ion seems to inhibit the Ca pump in erythrocytes (see Rossi, Garrahan & Rega, 1978), the question arises whether these and other pharmacological effects are secondary to metabolic changes involving the phosphate turnover in the axon.

In the present experiments we have examined the question of the metabolic activity of the nerve cell, and the efflux of phosphate from it, by looking at the effect of these and other interventions on the resting and stimulated O_2 consumption and phosphate effluxes of the rabbit vagus nerve.

METHODS

The methods used were essentially similar to those described earlier (Ritchie, 1967; Rang & Ritchie, 1968; Ritchie & Straub, 1978, 1979). Rabbits weighing about 2·5–3 kg were shot and the cervical nerves rapidly removed and desheathed. A desheathed nerve (length 60–80 mm; dry mass/wet mass ratio, $0{\cdot}220 \pm 0{\cdot}004$, $n = 48$; $0{\cdot}202 \pm 0{\cdot}004$ mg/mm, $n = 55$) was then mounted in a glass or perspex capillary chamber (diameter 0·8 mm) that was continuously perfused with Locke solution by a peristaltic pump at a rate (v) of 0·07–0·20 ml./min. The effluent from the chamber then passed over an O_2 electrode (Orbisphere 2603) so that its O_2 concentration could be determined. An important new modification of the method of measuring the resting O_2 consumption was that by rotation of the switch S (Fig. 1) the nerve chamber could be bypassed and the perfusing fluid sent directly to the oxygen electrode so that its O_2 concentration before entering the nerve chamber could be determined. The resting O_2 consumption was then calculated as ΔCv, where ΔC is the change in concentration of O_2 in the solution at the electrode on switching. It should be noted that the flow past the O_2 electrode remained constant. This is

important because the O_2 electrode itself consumes oxygen, and small changes in flow lead to large changes in the recorded concentration in the effluent. In earlier experiments (Ritchie, 1967; Rang & Ritchie, 1968; Ritchie & Straub, 1978) this factor was less important because the O_2 electrode was much smaller; and when perfused by a solution at a given O_2 concentration, its output did not appreciably depend on flow. The extra O_2 consumption with stimulation was determined in experiments using a chamber similar to that described in Fig. 1, except that it had in addition at the left-hand side (in the diagram) a set of stimulating electrodes and a guard-suck arrangement (Keynes & Ritchie, 1965) to ensure that the solution bathing the stimulating electrodes themselves was drawn off separately and did not contaminate the solution flowing past the O_2 electrode.

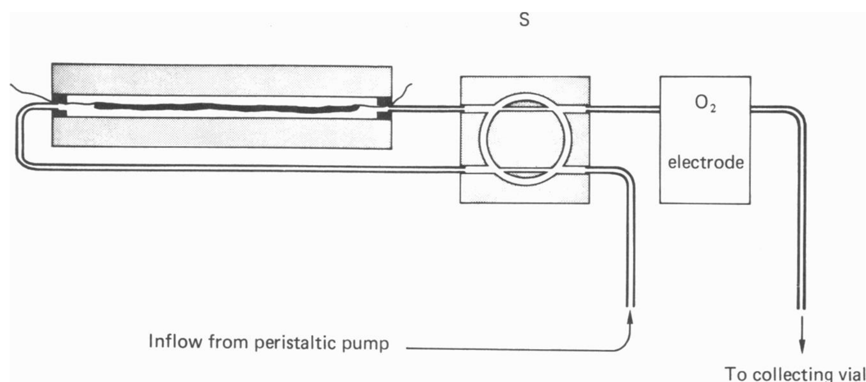

Fig. 1. The nerve chamber. The perfusion fluid can reach the O_2 electrode directly, or by way of the chamber, depending on the setting of the switch S.

The O_2 electrode was calibrated frequently throughout the series of experiments by noting the deflexion produced when a solution saturated with air at a given temperature which was flowing rapidly across the O_2 electrode was replaced by an N_2 equilibrated solution at the same temperature. Unfortunately, because the electrode itself consumed O_2 the sensitivity based on this deflexion with a high flow rate (about 1 ml.min^{-1}) was higher than that at the lower flow rates (0·07–0·2 ml.min^{-1}) used for perfusion of the nerve chamber. Empirically, it was found possible to allow for this since over the experimental range of flow rates the sensitivity at any flow rate v (ml.min^{-1}) was reduced from that obtained at the fast rate by a factor r given by the relation

$$r = 3{\cdot}700 \exp(-0{\cdot}1423 \ln 10{,}000\, v) \text{ at } 37\ ^{\circ}\text{C}$$

or

$$r = 1{\cdot}403 \exp(-0{\cdot}0398 \ln 10{,}000\, v) \text{ at } 20\ ^{\circ}\text{C}.$$

For the highest flow rates used the value of r was about 1·2: for the lower rates it was less than 1·4.

Measurement of the O_2 consumption

The method of determining the resting O_2 consumption is illustrated in Fig. 2. The O_2 concentration in the effluent from the nerve chamber was about 204 μM. However, the concentration in the solution flowing into the chamber, determined by by-passing the chamber and sending the solution directly to the O_2 electrode, was about 220 μM. Thus, with a flow rate of 0·156 ml.min^{-1} and a wet weight of 8·28 mg, one calculates a resting O_2 consumption of 0·301 m-mole.kg^{-1} min^{-1}. However, this value over-estimates the O_2 consumption. For when the nerve was removed and the same procedure was repeated switching from perfusing the now empty chamber to perfusing the O_2 electrode directly still led to an apparent increase in the oxygen concentration of the effluent (Fig. 2*b*). This 'blank' response, which was also present in previous experiments (Ritchie, 1967; Rang & Ritchie, 1968), could not be eliminated by a variety of procedures that included: perfusing the chamber for a long time with 10% acetic acid to sterilize it, removing all stainless steel connexions, frequently changing the tubing and chamber, and taking care that all

solutions were at temperature equilibrium (the latter was achieved by immersing the whole system, chamber and electrode, in a well stirred water-bath and passing the solutions through several feet of fine polyethylene tubing immersed in it). Fortunately, this artifact was relatively small (being about 20% in the experiment of Fig. 1*B* and less than that in nearly all other experiments) and relatively constant in size. It was therefore subtracted from the total response in the calculation of the true resting O_2 consumption. On this basis the resting O_2 consumption of the nerve in Fig. 2 was 0·236 m-mole.kg^{-1} min^{-1}; in forty-nine nerves the average resting consumption at 37 °C was 0·375 ± 0·015 m-mole.kg^{-1} min^{-1}.

During the by-passing period when the perfusion fluid was going directly to the O_2 electrode the flow through the nerve chamber stopped, resulting in a progressive decrease in the O_2 concentration in the remaining stagnant fluid in the chamber, due to the metabolic activity of the nerve. When perfusion was restored and the fluid thus depleted of its O_2 was flushed past the

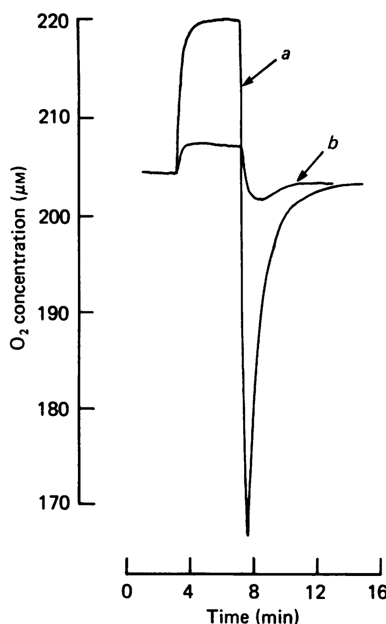

Fig. 2. The method for determining the resting O_2 consumption of nerve. Trace *a* shows change in O_2 concentration across chamber with nerve mounted in it, trace *b* without nerve. For further explanation, see text.

O_2 electrode, the recorded O_2 concentration fell rapidly to a low value and then gradually recovered. Taking the area under the O_2/time curve as a measure of the O_2 used during the by-passing period (usually 2–4 min) one can therefore calculate in an alternative way the resting O_2 consumption of the nerve; and in the experiment of Fig. 2 the resting O_2 consumption so calculated was about 0·190 m-mole.kg^{-1} min^{-1}. This value based on a 'stop-flow' technique, although close to, is slightly lower than the value based on the steady-state difference in O_2 concentration in the ingoing and outgoing solutions. Indeed, in all experiments the O_2 consumption determined by the stop–flow method was less than the value determined by the steady-state method first described. A likely explanation of the discrepancy is that during the stagnant period the O_2 concentration in the fluid surrounding the nerve falls considerably. Anaerobic glycolysis is therefore switched on with the production, presumably, of lactic acid which is subsequently washed away. Although this hypothesis was not tested directly, support for it comes from the observation that the discrepancy between the stop–flow and steady-state methods was greater the gerater was the fall in O_2 concentration during the period of stagnation (because large nerves or longer periods of by-passing were used). It was also much more pro-

nounced in experiments at 37 °C, where the O_2 consumption is high, than it was at 20° where the O_2 consumption is lower.

Because of these considerations all the resting O_2 consumptions reported in this paper depend on the steady-state difference in the recorded O_2 concentration between the in-flowing and outflowing fluid from the nerve chamber.

Phosphate efflux

In many experiments each nerve was soaked before mounting in a solution containing trace amounts of labelled orthophosphate (^{32}P, 1–10 μc/ml.) for 1–4 hr at about 20 °C. The nerves were then washed in label-free solution in the perfusion chamber and the efflux of labelled phosphate determined by scintillation spectroscopy. At the end of the experiment the nerve was weighed, cut into small pieces, and then extracted in 5 ml. distilled water. The water-soluble labelled phosphate was then determined and used to calculate the rate constant of phosphate efflux. The extraction time was about 30 min. No more labelled phosphate was extracted when this time was increased to 2 or 12 hr.

Solutions

The Locke solutions used, unless otherwise stated, had the following composition (mM): NaCl, 154; KCl, 1·0; $CaCl_2$, 0·9; $MgCl_2$, 0·5; Na orthophosphate, 0·2; morpholinopropionyl sulphonate buffer (pH 7·4), 10; D-glucose, 5. The experiments were usually carried out at 37 °C, although some experiments were carried out at room temperature (20–26 °C).

Calculations

Wherever possible, means of the values obtained in each individual nerve ± their standard errors are given. Except where stated, values are expressed on a wet weight basis.

RESULTS

The resting nerve and electrical stimulation

In the experiment of Fig. 3, the resting O_2 consumption was 0·563 m-mole.kg^{-1} min^{-1}. Stimulation led to an extra O_2 consumption of 0·585 μmole.kg^{-1} impulse^{-1}. If the nerve, therefore, had been stimulated for long enough for a steady state to be reached, and if there was no falling off in the response per impulse, the O_2 consumption would have increased by the fraction $(600 \times 0{\cdot}585 \times 10^{-3})/0{\cdot}563$ i.e. by 0·623. Similarly, the resting phosphate efflux, which was $2{\cdot}57 \times 10^{-3}$ min^{-1} during the first response and $2{\cdot}30 \times 10^{-3}$ min^{-1} during the second was increased on stimulation by an amount $8{\cdot}17 \times 10^{-6}$ impulse^{-1} during the first test and $10{\cdot}64 \times 10^{-3}$ impulse^{-1} in the second. In the steady state, therefore, stimulation would have increased the efflux by a fraction 1·91 and 2·78 above its resting value in the two tests. At 37 °C therefore the relative increase in the oxygen consumption is only about a quarter of the relative increase in phosphate efflux, in contrast to the finding at 22 °C where the relative increase in O_2 consumption is greater (see Introduction). Nine such tests on eight different preparations indicated that the relative increase in oxygen consumption with stimulation was only 0·436 ± 0·075 times the relative increase in the phosphate efflux.

The extra O_2 consumed per impulse with stimulation at 10 sec^{-1} was 0·467 ± 0·061 μmole.kg^{-1} impulse^{-1} at 37 °C (n = 9). At 22 °C this value fell to 0·346 ± 0·051 μmole.kg^{-1} impulse^{-1} (n = 7), presumably because the stimulation frequency was too high at the lower temperature. When the frequency was reduced to 3 sec^{-1}

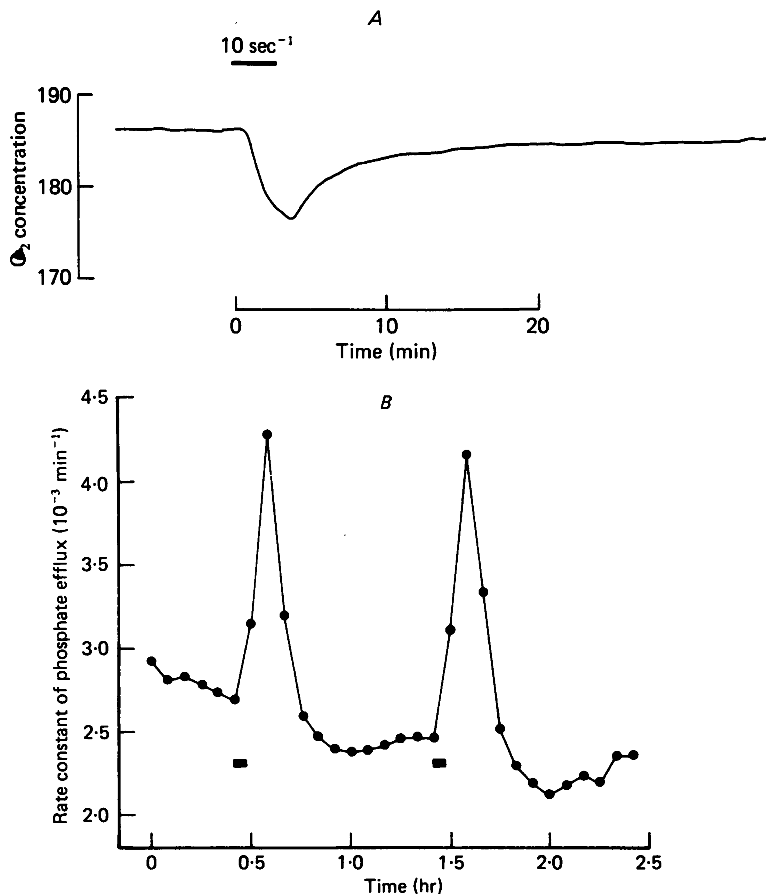

Fig. 3. The effect of electrical stimulation on the O_2 consumption (*a*) and the rate constant of phosphate efflux (*b*). At the bars the nerve was stimulated for 3 min at 10 sec^{-1}. Record *a* is the O_2 response to the first period of stimulation. Temperature, 37 °C.

at 22 °C, the extra O_2 consumption was $0{\cdot}631 \pm 0{\cdot}083$ μmole.kg^{-1} impulse^{-1}, i.e. close to the value obtained at the higher temperature with the higher frequency. With the dry mass/wet mass ratio in the present experiments of 0·220 this corresponds to an O_2 consumption of 2·87 μmole.kg dry^{-1} impulse^{-1} which agrees well with the value of 3·11 μmole.kg dry^{-1} impulse^{-1} obtained by Ritchie (1967) in experiments where the dry mass/wet mass ratio was 0·262. Similarly the resting O_2 consumption at 22 °C of $0{\cdot}113 \pm 0{\cdot}008$ m-mole.kg^{-1}.min^{-1} (n = 11) i.e. $0{\cdot}514 \pm 0{\cdot}036$ m-mole.kg dry^{-1} min^{-1} agrees quite well with the values of 0·092 m-mole. kg wet^{-1} min^{-1} i.e. 0·351 m-mole.kg dry^{-1} min^{-1} obtained by Ritchie (1967).

A consistent feature of the stimulation experiments was that during recovery the rate constant of phosphate efflux seemed to dip slightly below the value expected from the general drift of the wash-out curve (see also Figs. 4 and 6). The effect, though small, seems to indicate a slight slowing in phosphate loss immediately after a period of repetitive electrical activity.

The values for the resting and stimulated O_2 consumptions, and those for the resting and stimulated rate constants of phosphate efflux, are summarized in Table 1.

Temperature

Since much of the earlier work on the O_2 consumption (Ritchie, 1967; Rang & Ritchie, 1968) was done at room temperature (20–22 °C) in contrast to the present experiments which were usually done at 37 °C, several experiments were done to determine the temperature dependence of the O_2 consumption. The resting value was determined first at room temperature (22 °C) and then at 37 °C on the same nerve.

Six such experiments indicated an increase in the resting O_2 consumption of $3{\cdot}56 \pm 0{\cdot}52$. On the assumption that the temperature coefficient of dependence did not change over this range, this corresponds to a Q_{10} of $2{\cdot}17 \pm 0{\cdot}20$.

The rate constant of phosphate efflux at 37 °C was found to be $2{\cdot}42 \pm 0{\cdot}10 \times 10^{-3}\ \text{min}^{-1}$ (n = 17), compared with the value found previously at 22 °C, $0{\cdot}49 \times 10^{-3}\ \text{min}^{-1}$ (Ritchie & Straub, 1978). These values correspond to a Q_{10} for phosphate efflux of about 2·90, which is close to that determined in the present experiments for the temperature dependence of the oxygen O_2 consumption. This value agrees well with the value calculated by Maire & Straub (1980) for the restng efflux, although it should be noted that the absolute values of the stimulated fluxes in their experiments at 37 °C and 22 °C ($1{\cdot}81 \times 10^{-6}$) impulse and $0{\cdot}12 \times 10^{-6}$ impulse^{-1} respectively) are much smaller than those reported here and by Ritchie & Straub (1978).

TABLE 1. The resting (Q_r) and stimulated (Q_s) O_2 consumptions and the resting (k_r) and stimulated (k_s) rate constants of phosphate efflux. (No. of experiments in parentheses)

Temperature	Q_r (m-mole.kg^{-1} min^{-1})	Q_s* (μmole.kg^{-1}.impulse^{-1})	k_r ($\times 10^{-3}$ min^{-1})	k_s ($\times 10^{-6}$ impulse^{-1})
37†	$0{\cdot}484 \pm 0{\cdot}036$	$0{\cdot}467 \pm 0{\cdot}061$	$2{\cdot}61 \pm 0{\cdot}11$	$6{\cdot}75 \pm 0{\cdot}96$
22	$0{\cdot}113 \pm 0{\cdot}008$	$0{\cdot}631 \pm 0{\cdot}083$	—	—

* From experiments at 10 sec^{-1} at 37 °C and 3 sec^{-1} at 22 °C.

† Average of nine tests in eight experiments in which all parameters were measured. For all experiments average value of Q_r was $0{\cdot}375 \pm 0{\cdot}015$ m-mole.kg^{-1} min^{-1} (n = 49) and of k_r was $2{\cdot}42 \pm 0{\cdot}10 \times 10^{-3}$ min^{-1} (n = 17)..

Pharmacological experiments

A number of experiments were done to examine the effect of various drugs on the O_2 consumption (usually only the resting value, Table 2) and the rate constant of phosphate efflux.

Ouabain

Fig. 4 shows the effect of adding a relatively large concentration of ouabain (100 μM) to the bathing medium. Both the phosphate efflux (Maire & Straub, 1980) and the O_2 consumption fell abruptly: the O_2 consumption had fallen by 31 ± 3 % (n = 9) after 30 min and by 40 ± 4 % (n = 5) after 60 min. The phosphate efflux fell, by a smaller amount. Furthermore, the reduction was not maintained. In all experiments, after reaching a maximum fall of about 14 ± 3 % (n = 4) in about 10 min, the

rate constant of phosphate efflux began to rise. By 30 min after the initial exposure to ouabain the efflux had returned to within 0 ± 4 % of its initial value; and in some cases the efflux continued to rise above the pre-ouabain value (Fig. 4).

Clearly, there is no direct correspondence between either the time courses, or the magnitudes, of the O_2 consumption and phosphate efflux responses to ouabain.

Salicylate

Ritchie (1967) showed that the uncoupling agent, Na salicylate, produced a 24 % increase in the resting O_2 consumption of the rabbit vagus nerve. Fig. 5 shows that this effect is accompanied by a large increase in phosphate efflux. The two responses

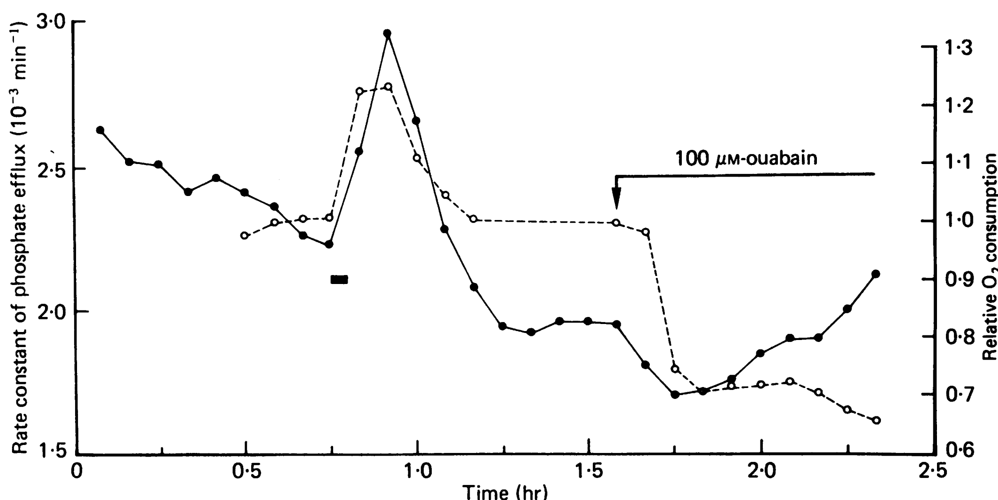

Fig. 4. The effect of electrical stimulation and of ouabain on the resting O_2 consumption (○, interrupted line) and rate constant of phosphate efflux (●). In this experiment, and that of Figs. 5 and 6, the O_2 consumption was followed by the modified steady-state method described in detail in Ritchie & Straub (1980). Ouabain (100 μM) was present in the bathing solution at the end of the experiment where indicated (by the arrow and horizontal line). The record also shows the response to stimulation (10 sec^{-1}, 3 min) at the bar. Temperature, 37 °C.

are not, however, strictly parallel. First, the phosphate efflux response was found to be more transient than the O_2 consumption. Secondly, the peak effect on the phosphate efflux is much greater than that on the O_2 consumption. Thus, in four experiments exposure to 10 mM Na salicylate increased the rate constant for phosphate efflux by 233 ± 46 % after about 10 min. The corresponding maximum increase in O_2 consumption, which occurred somewhat later, was only 34 ± 7 %, i.e. about 7 times smaller (Table 2).

Arsenate

Ferrero *et al.* (1978) have already shown that exposure to sodium arsenate produces a maintained increase in the phosphate efflux. We have confirmed this in two experiments (Fig. 6) and have, in addition, shown that this effect is accompanied by a roughly parallel increase in O_2 consumption. The increase in the rate constant of

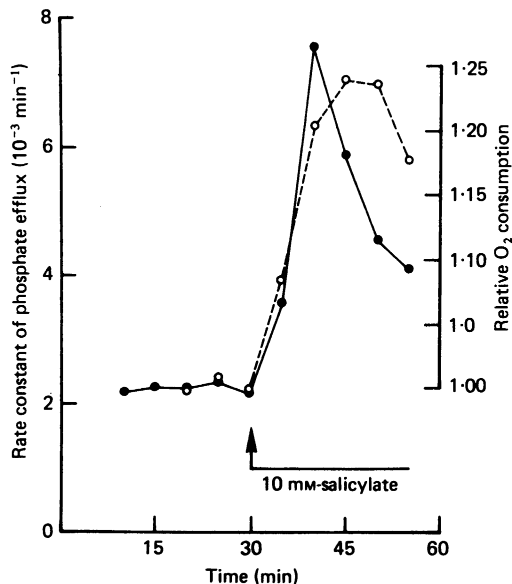

Fig. 5. The effect of salicylate on the resting O_2 consumption (○, interrupted line) and rate constant of phosphate efflux (●). Na salicylate (10 mM) was present in the bathing solution at the end of the experiment where indicated (by the arrow and horizontal line). Temperature, 37 °C.

TABLE 2. Effect of drugs on the relative resting (Q_r) and stimulated (Q_s) O_2 consumptions (fraction of control value). (No. of experiments in parentheses)

Drug	Temperature (°C)	Relative O_2 consumption Q_r	Q_s
Ouabain, 100 μM	37	0·69 ± 0·03 (9)	—
Salicylate, 10 mM	37	1·34 ± 0·07 (4)	—
Arsenate, 1 mM	37	1·15 ± 0·01 (2)	—
10 × P_i	21·8	0·88 ± 0·05 (10)	0·91 ± 0·07 (5)
10 × P_i	37·1	0·92 ± 0·06 (7)	—
ACh, 1·7 mM	22	1·12 ± 0·01 (4)	—
ACh, 1·7 mM	37	1·01 ± 0·03 (9)	—
La^{3+}, 20 μM	37	1·00 ± 0·04 (9)	—
10 × Ca	20·7	0·85 ± 0·11 (3)	1·13 ± 0·04 (3)
10 × Ca	37	0·94 ± 0·01 (3)	—
0·1 × Ca	20·7	1·04 ± 0·02 (3)	0·97 ± 0·05 (3)
0·1 × Ca	37	1·11 ± 0·02 (3)	—

phosphate efflux (79 %) was, however, substantially greater than that in the O_2 consumption, which was 15 % (Table 2). In some respects therefore the response to the arsenate anion is similar to that of the salicylate anion; and it is tempting to suggest that both are due to a similar uncoupling action on mitochondrial phosphorylation.

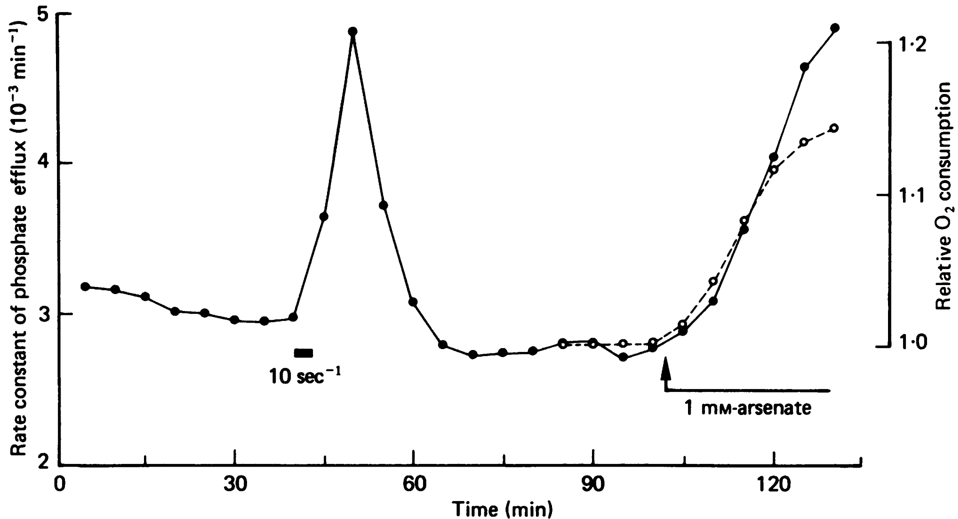

Fig. 6. The effect of arsenate on the resting O_2 consumption (○, interrupted line) and rate constant of phosphate efflux (●) Na arsenate (1 mM) was present in the bathing medium at the end of the experiment where indicated (by the arrow and horizontal line). The record also shows the response to stimulation (10 sec^{-1}, 3 min) at the bar. Temperature, 37 °C.

2-Deoxyglucose

It has been shown (P. Jirounek, M. Rouiller & R. W. Straub, unpublished observations) that exposure of the rabbit vagus to glucose-free Locke solution containing the antimetabolite 2-deoxyglucose (20 mM) causes a prolonged decrease in the phosphate efflux. There is also a prolonged progressive decrease in O_2 consumption (Fig. 7).

Phosphate

In garfish olfactory nerve (Easton, 1971) increasing the phosphate concentration from 0·2 to 2 mM leads to a slight decrease (about 14 %) in the resting P_i efflux. In the rabbit vagus nerve at 37 °C, this effect is in the opposite direction and more pronounced, especially in a Locke solution containing 1 mM-K. Ferrero *et al.* (1978) and Maire & Straub (1980) found that increasing the phosphate in the bathing medium from 0·2 to 2 mM *increases* the resting phosphate efflux, nearly doubling it. Parallel experiments on the O_2 consumption showed (Table 2) that increasing the external phosphate for periods of 30–90 min produces a small *fall* in the resting O_2 consumption both at room temperature (12 %) and at 37 °C (8 %). There was also a small decrease (9 %) in the extra O_2 consumption with activity (at room temperature).

Acetylcholine

Straub, Ferrero, Jirounek, Rouillet & Salamin (1978) have shown that acetylcholine produces a dramatic transient increase in the phosphate efflux of the rabbit vagus nerve. However, as Table 2 shows, acetylcholine 1·7 mM had relatively little effect, if any, on the resting O_2 consumption. Ritchie (1967) reported that this concentration of acetylcholine did indeed increase the resting O_2 consumption. However, this only occurred in experiments at room temperature; in a single experiment done then at 37 °C, acetylcholine had no effect. In agreement with the earlier findings four tests at 22° showed that the response was in fact present at the lower temperature, acetylcholine producing an increase, of 12 ± 1 %, in the resting O_2 consumption.

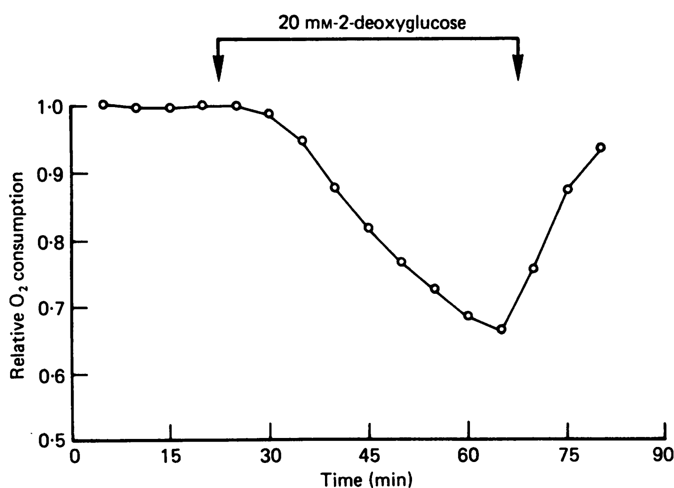

Fig. 7. The effect of 2-deoxyglucose on the resting O_2 consumption. Glucose-free Locke solution containing 2-deoxyglucose (20 mM) was present where indicated (by the arrow and horizontal line). Temperature, 37 °C.

La

P. Jirounek & M. Rouiller (unpublished observations) have found that La in small concentrations (20 μM) produces a rapid almost complete inhibition of the phosphate efflux from the rabbit vagus nerve. However, in nine tests exposure of 10–20 min to 20 μM-La had no effect on the resting O_2 consumption (Table 2).

Ca

As can be seen in Table 2, a tenfold increase in the Ca concentration produced a slight decrease in the resting O_2 consumption and a slight increase in the extra O_2 consumption with activity. Bathing the nerve in Ca-poor solutions had the reverse effect, namely, the resting O_2 consumption was slightly increased and the stimulated O_2 consumption might have been decreased. There were also slight changes in the phosphate efflux, but in the opposite direction. The small effect of Ca was somewhat unexpected in view of the known involvement of Ca in the metabolic response of the nerve cell to electrical activity (Landowne & Ritchie, 1971).

DISCUSSION

The energy for the recovery process following a nerve impulse is derived from ATP which, after electrical activity, is broken down at an increased rate. As a result the intracellular concentration of ATP falls and those of ADP and P_i rise, as has been shown in frog muscle by Dawson, Gadian & Wilkie (1977), in crab nerve by Baker (1965), and in the rabbit vagus nerve by Chmouliovsky, Schorderet & Straub (1969). The latter leads, at least in part, to the increased efflux of phosphate after activity. One major objective in this paper was to examine how well the increase in phosphate efflux serves as a measure or index of the increased metabolic activity.

If the O_2 consumption is taken as a direct measure of the underlying metabolic activity it is clear that the phosphate efflux is less well related directly, for several reasons. First, although electrical stimulation increases both the O_2 consumption and the phosphate efflux it does so unequally. As mentioned in the Introduction, in previous studies at about 22 °C it was found that the O_2 consumption is relatively much more increased than the phosphate efflux. The present experiments at 37 °C similarly show a discrepancy, but in the opposite direction: the relative increase in O_2 consumption is less than half that of the rate constant of phosphate efflux. Secondly, although salicylate (or arsenate) increases both the O_2 consumption and the phosphate efflux, the latter is much more affected than the O_2 consumption; and furthermore the effect on the phosphate efflux is more transient. Thirdly, a diverse set of agents that increase (acetylcholine and increased P_i), or decrease (La^{3+}, decreased P_i) the rate constant of phosphate efflux have relatively little effect on the O_2 consumption. Finally, there is the divergence in the two responses in Li-Locke solution (see Ritchie & Straub, 1980), namely that on first switching to Li-Locke from Na-Locke solution the fall in O_2 consumption is small and transient whereas that in the phosphate efflux is profound and maintained; and on subsequent exposure to ouabain the O_2 consumption falls substantially, whereas the rate constant of phosphate efflux does not (and may even rise).

All in all therefore it is difficult to avoid concluding that changes in the phosphate efflux are not simply determined by changes in the intracellular concentration. Some other factor must be involved. Possibly there is more than one pool from which the phosphate is escaping; for example Straub *et al.* (1978) have suggested that acetylcholine may release phosphate from a superficial pool rather than from the axoplasm. Alternatively, the various procedures used (electrical stimulation, application of drugs, etc.) may alter the permeability of the membrane to the phosphate anion. We see no clear way to distinguish between these two possibilities at the moment; and further studies here should be rewarding. In summary, it seems that when the metbolism of the nerve cell is affected, expected changes in phosphate efflux are indeed obtained: increased metabolic activity leads to an increased efflux, and decreased activity to a decreased efflux. However, the converse is not true; and increases and decreases in the rate constant of phosphate efflux do not necessarily signal corresponding metabolic changes.

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