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THE DEPENDENCE ON EXTERNAL CATIONS OF THE OXYGEN CONSUMPTION OF MAMMALIAN NON-MYELINATED FIBRES AT REST AND DURING ACTIVITY

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SUMMARY

1. A study has been made of the effect of potassium and other cations on the oxygen consumption of rabbit desheathed vagus nerves at rest and during activity.

2. In normal Locke solution (containing 5·6 mM-K) the resting oxygen consumption, Q_r, was 0·0903 μmole/g wet wt. min. The extra oxygen consumed as a result of stimulation, Q_s, at 3 stimuli/sec was about 600 p-mole/g.impulse; at 30 stimuli/sec it was less, being 240 p-mole/g.impulse.

3. Only a fraction of Q_r (18 % at 5·6 mM-K) was sensitive to ouabain (1 mM). The ouabain-sensitive component, however, increased as the external potassium concentration was increased, in the range 0–100 mM. Q_s was virtually abolished by ouabain.

4. Reduction of the external potassium concentration from 5·6 mM to zero reduced Q_r (by 10 %) and increased Q_s, but the changes were scarcely significant statistically.

5. The conclusions were drawn: that Q_s reflected the pumping of cations to restore the ionic imbalance following activity, particularly reflecting the extrusion of sodium ions from the fibre; that this pumping was normally absolutely dependent on the presence of potassium externally and that no pumping could occur in its absence; and that Q_s was not reduced to zero in ostensibly potassium-free solutions because enough potassium was released into the periaxonal space during activity to maintain pumping.

6. Thallium, rubidium, caesium and lithium could replace potassium and allowed pumping to occur.

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INTRODUCTION

When a nerve conducts impulses sodium ions accumulate in the axoplasm, and potassium ions are lost. During the subsequent recovery period the ionic balance is restored to its original resting state by the active extrusion of sodium ions from the axoplasm and by the associated uptake of potassium ions. Sodium extrusion takes place against a steep electrochemical gradient and so requires the expenditure of metabolic energy, which is reflected in an increased oxygen consumption (Meyerhof & Schultz, 1929; Fenn, 1927; Gerard, 1927, 1932; Brink, Bronk, Carlson & Connelly, 1952; Connelly, 1959, 1962; Baker, 1965; Baker & Connelly, 1966; Ritchie, 1967) and an increased heat production of the fibres (Gerard, 1927; Fenn, 1927; Hill, 1929, 1965; Bronk, 1931; Bugnard, 1936; Howarth, Keynes & Ritchie, 1968). The properties of the mechanism that is involved, the sodium pump, are now becoming well defined (see Baker, 1966): the pump is stimulated by an increase in the internal sodium concentration; it is associated with an ATP-splitting enzyme that needs both sodium and potassium for its activation; and, at least in crustacean and amphibian nerve, the evidence is strong that it depends on the presence of potassium ions in the external medium. It was therefore surprising to find in mammalian C fibres, which are particularly suitable for metabolic studies because of their small size (Ritchie & Straub, 1957; Greengard & Straub, 1962; Ritchie, 1967; Howarth *et al.* 1968), that both the recovery heat production (Howarth *et al.* 1968) and the amount of extra oxygen consumed as the result of electrical stimulation (Ritchie, 1967) were hardly, if at all, affected by removing the external potassium. The present experiments were designed to examine this apparently anomalous behaviour of mammalian C fibres.

METHODS

Both cervical vagi (each about 80 mm long) were rapidly removed from rabbits that had been killed by the injection of air into an ear vein. Each nerve was then desheathed under a dissectingmicroscope (× 10 to × 40) and mounted in a perfusion chamber in which its oxygen consumption was measured. The chamber, which has been described in detail before (Ritchie, 1967), consisted of a fine capillary glass tube whose internal diameter (0·8 mm) was just large enough to accommodate the nerve. A motor-driven syringe connected to the outlet of the chamber allowed Locke solution to flow at a constant rate, usually 0·09 ml./min, under its own hydrostatic pressure from one of a series of reservoirs that were connected to the inlet of the perfusion chamber. The reservoirs were bubbled slowly with air and were maintained at a constant temperature 2–3° C higher than that of the chamber; this prevented the formation of gas bubbles in the system. One end of the nerve was laid over indwelling platinum stimulating electrodes, and the fluid perfusing this region was drawn off by a guard-suck arrangement in order to prevent artifacts caused by the stimulating current (Ritchie, 1967). The effluent from the chamber passed via the platinum tubing to a small chamber

containing an oxygen cathode (Radiometer). The current through the electrode, which was proportional to the oxygen content of the fluid emerging from the chamber, was monitored by means of an electrometer (Keithley 610B) and a potentiometric pen recorder (Varian 2000). The temperature of the reservoir, the perfusion chamber, and the oxygen electrode was kept constant by water jackets. The temperature of the nerve chamber was 21–22° C.

The electrical stimuli, which were about 5–10 times the intensity required to stimulate the most excitable C fibres, were 0·5 msec in duration.

The composition of the Locke solution was (mM): NaCl, 154; KCl 5·6; $CaCl_2$ 2·2; tris-(hydroxymethyl) amino-methane (Tris) buffer at pH 7·2, 2·5; D-glucose, 5·0. Modifications of the Locke solutions will be described in the text.

Determination of the resting and stimulated oxygen consumption. The oxygen consumption was determined in the manner that has been described in detail previously (Ritchie, 1967). Two methods were used to determine the resting consumption. In the first, the flow through the perfusion chamber was stopped for 1 (or 2) min, and then resumed. When the solution that had been stagnant over the nerve reached the oxygen electrode, a fall in the oxygen concentration was recorded; the area under the oxygen concentration–time curve gave the amount of oxygen consumed by the nerve in the period that the flow was stopped. In the second method the flow was increased from its normal flow, v_1, to a new flow, v_2. The resting oxygen consumption was then given by $\Delta C\, v_1 v_2/(v_2 - v_1)$ where ΔC is the measured change in the oxygen concentration of the effluent from the chamber. The stimulated oxygen consumption was measured by recording the fall in oxygen concentration that followed a period of activity. The area under the oxygen concentration–time curve gave the total extra oxygen used, and the shape of the curve gave information about the time course of the increased metabolism.

Various factors slowed the response of the recording system to changes in oxygen uptake by the tissue. These included the time taken for the fluid to travel the length of the nerve, the time taken for diffusion between the centre and the periphery of the bundle, the inherently slow response of the oxygen cathode, and the dead space between the nerve chamber and the oxygen cathode. The effect of these factors was checked by slowing the flow from its normal rate of 0·09 ml./min and waiting for a new steady state to be attained, and then returning the flow to the normal rate and following the time course with which the recorded oxygen tension returned to its original value. It was found that the dead space caused a delay of about 20 sec after which the return to the original level was 90% complete in about 40 sec. The effect of stimulation usually declined with a half-time of about 3 min, and therefore little distortion was introduced by the recording system.

The effects of the various procedures to which the nerves were subjected in these experiments were usually reversible. Therefore, to allow for any systematic drift in the condition of the preparation, the result of any procedure (such as removing the external potassium) was usually measured by expressing the test response as a fraction of the average of two control responses with the preparation in normal Locke solution obtained shortly before and shortly after the test.

RESULTS

Oxygen consumption at rest

When the external potassium is removed from the solution bathing crab nerve the resting oxygen consumption is reduced by about 40% (Baker & Connelly, 1966). In C fibres, as the top part of Table 1 shows, the same procedure had only a very slight effect on the resting oxygen consumption (reducing it by only $7{\cdot}9 \pm 3{\cdot}1$ %), a result in good agreement with the previous findings in this preparation (Ritchie, 1967).

In spite of the above negative finding with potassium-free solutions; it seemed worth while to investigate in more detail the effect of a range of higher potassium concentrations. For the improvement in the stability of the recording system and the reduction of artifacts accompanying solution changes made it possible to make far more measurements on a single nerve than was possible previously; the results are given in Table 2 and

TABLE 1. Effect of various cations on resting and stimulated oxygen consumption

			Q_r			Q_s	
Expt.	Temp. (° C)	Test solution	Control (μmole/ g.min)	Test / control	Stimulus	Control (p-mole/ g.imp.)	Test / control
7N6	21·7	K-free	0·0822	0·997	3/sec, 2′	—	0·917
10N6	20·6		0·0298	0·963	3/sec, 2′	—	1·061
22N6	20·9		0·0754	1·032	—	—	—
23N6	20·9		0·1002	0·926	—	—	—
28M7	21·0		0·1250	0·960	—	—	—
5A7A	21·9		0·0900	0·825	—	—	—
5A7B	21·9		0·0970	0·875	—	—	—
8D6	20·8		—	—	3/sec, 5′	684	0·967
26J7B	21·9		0·0925	0·785	3/sec, 5′	600	1·020
Mean ± S.E.	—	—	—	0·921 ± 0·031	—	—	0·991 ± 0·03
23N6	20·9	K-free 0·2 mM-Ca	0·1002	0·897	—	—	—
30N6	20·9		0·0909	0·988	3/sec, 2′	690	0·766
2D6	23·2		—	—	3/sec, 2′	667	0·915
6D6	20·5		—	—	3/sec, 5′	516	1·072
20D6A	20·9		—	—	3/sec, 5′	491	0·900
20D6B	20·9		—	—	3/sec, 5′	647	1·117
21D6	20·9		—	—	3/sec, 5′	687	1·033
Mean ± S.E.	—	—	—	0·945	—	—	0·967 ± 0·0
17N6A	21·9	K-free pH 9·2	0·0881*	1·119	3/sec, 2′	469*	0·936
17N6B	21·9		0·0878*	1·074	3/sec, 2′	437*	1·144
10F7B	21·9		—	—	30/sec, 30″	282*	0·870
10N6	21·9	56 mM-K pH 9·2	0·1128	1·260	3/sec, 2′	981	0·862
Mean ± S.E.	—	—	—	1·151 ± 0·056	—	—	0·953 ± 0·0

* Controls measured in K-free Locke solution pH 7·2. Standard errors are given only when three more determinations were made.

Fig. 1. Apart from short periods when the nerves were exposed to Locke solutions containing different concentrations of potassium, they were kept in normal Locke solution (5·6 mM-K), except for the three nerves at the bottom of Table 2; these latter nerves were kept in potassium-free Locke solution except for the brief testing periods in potassium-containing Locke solutions. Concentrations of potassium up to 20 mM were obtained by adding 1 M-KCl without altering any of the other constituents; higher concentrations of potassium were obtained by substituting potassium chloride for equimolar concentrations of sodium chloride. The type of solution in which the nerve had previously equilibrated made rather little

difference to the effect of potassium, which produced as large a burst of metabolism when applied to a nerve that had been kept in 5·6 mM potassium throughout most of the experiment as in a nerve that had been kept in potassium-free solution throughout. With potassium concentrations greater than 20 mM, the increase in respiration was usually not well maintained, and the values given in Table 1 refer to the peak effects; with lower potassium concentrations, however, the change in oxygen consumption was maintained indefinitely.

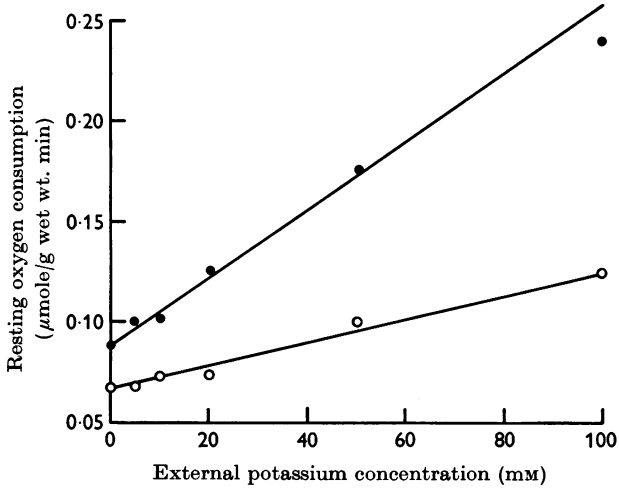

Fig. 1. The effect of potassium on the oxygen consumption of rabbit desheathed vagus nerves at rest. Filled circles show measurements made in normal Locke solution: open circles show measurements made in the presence of 1 mM ouabain. Results obtained in seven nerves have been combined. The standard error is, in most cases, no larger than the diameter of the point.

For convenience, the results of such experiments, which are summarized in Table 2, were always expressed as increases above the consumption in potassium-free solution regardless of whether the nerves had been equilibrated in 0 or 5·6 mM potassium Locke. The results shown in Table 2, which are plotted in Fig. 1, indicate that the increment in the resting oxygen consumption produced by potassium increased linearly with concentration over the whole of the range tested (0–100 mM).

The effect of ouabain on resting oxygen consumption

In the experiments of Table 2, after the dependence of the resting oxygen consumption on the external potassium concentration had been established, the preparations were exposed to ouabain. The resting oxygen consumption in potassium-free Locke solution, and its dependence on the external

TABLE 2. Effect of potassium concentration on the resting oxygen consumption

	Q_r in 0 mM-K (μmole/g.min)	ΔQ_r (μmole/g.min)				
		5 mM-K	10 mM-K	20 mM-K	50 mM -K	100 mM-K
28M7	0·1130 —	0·004 —	—	0·045 —	0·088 —	0·122 (0·047)
5A7A	0·0700 (0·0610)	0·011 (0·003)	—	0·015 (0·004)	—	—
5A7B	0·0860 (0·0660)	0·015 (−0·004)	—	0·038 (0·005)	—	—
17A7	0·0573 (0·0517)	—	—	0·022 —	0·061 (0·004)	—
19A7	0·0911 (0·0812)	—	0·020 (0·000)	—	0·072 (0·030)	—
20A7	0·1153 (0·0994)	—	0·005 (0·008)	—	0·084 (0·035)	—
20A7B	0·0991 (0·0852)	—	0·008 (0·005)	—	0·050 (0·034)	—
Means ± S.E.	0·0903 (0·0741) ±0·0081 ±0·0069	0·010 (0·000) ±0·003	0·011 (0·004) ±0·004 ±0·002	0·030 (0·005) ±0·007	0·071 (0·026) ±0·067 ±0·007	0·122 (0·047)

Values of ΔQ_r give the absolute increase in oxygen consumption when the solution was changed from potassium-free Locke to Locke solution containing potassium. Figures in brackets are measurements made in the presence of 1 mM ouabain.

potassium concentration, were then redetermined in the presence of the glycoside in a concentration of 1 mM, preliminary experiments having shown that the effect of 1 mM ouabain was no greater than that of 0·1 mM ouabain. The results of such experiments are indicated by the values within brackets in Table 2. There were two major findings in such experiments. First, at all potassium concentrations ouabain decreased the resting oxygen consumption. For example, in potassium-free solution the resting oxygen consumption fell from its original value of $0{\cdot}0903 \pm 0{\cdot}008$, μmole/g.min to $0{\cdot}0741 \pm 0{\cdot}0069$ μmole/g.min after exposure for at least 10 min to 1 mM ouabain, i.e. it fell by 18%; the corresponding fall in 50 mM potassium was much greater (38%). The second finding was that the sensitivity of the preparation to changes in the external potassium concentration was reduced by ouabain, but was by no means abolished. This is seen in Fig. 1 (open circles).

Baker & Connelly (1966) found that the oxygen consumption of crab nerve in potassium-free solution was substantially reduced in the presence of ouabain. They speculated that the low rate of flow resulted in an appreciable potassium concentration in the region of the nerve, and that this potassium could support activity of the sodium pump even in ostensibly potassium-free solution so permitting the residual sensitivity to ouabain. In the present experiments the flow rate was much greater and the effect of ouabain in potassium-free solution correspondingly less, in agreement with their speculation. Nevertheless, there was a remaining, though diminished, sensitivity to increased potassium concentration in the presence of a high concentration of ouabain, which clearly confuses the interpretation of resting oxygen consumption in terms of sodium pumping alone.

The extra oxygen consumed following stimulation

The evidence relating the extra oxygen consumption following stimulation to sodium pump activity (Ritchie, 1967) is much less equivocal than it is for the resting oxygen consumption. We have therefore concentrated on the stimulated oxygen consumption in analysing further the potassium dependence of the sodium pump in this tissue.

Ritchie (1967) found that, in spite of clear evidence relating the stimulated oxygen consumption to sodium pump activity, the total extra oxygen used as a result of activity of the nerve was little, if at all, reduced in the absence of external potassium ions, a result seemingly at odds with the known dependence of the sodium pump on external potassium ions in nerve, red cells and muscle (Hodgkin & Keynes, 1955; Glynn, 1956; Horowicz & Gerber, 1965). But the lack of effect of removal of external potassium on the total stimulated oxygen consumption seems to be quite

TABLE 3. Effect of potassium concentration on stimulated oxygen consumption

Expt.	$[Cl]_o$	Stimulus	Q_s (p-mole/g.impulse) Potassium concentration (mM) 0	0·5	5·6	Maximum rate of stimulated oxygen consumption (arbitrary units) Potassium concentration (mM) 0	0·5	5·6	τ (min) Potassium concentration (mM) 0	0·5	5·6
26J7B	154	3/sec, 5′	611 (1·02)	—	600	66 (1·08)	—	61	12·6 (3·15)	—	4·0
30J7	154	3/sec, 5′	433 (0·090)	—	483	47 (0·98)	—	48	46 (1·64)	—	2·8
1F7	154	3/sec, 5′	—	621 (1·09)	572	—	63 (1·05)	60	—	7·3 (1·87)	3·9
1F7	154	30/sec, 30″	—	336 (1·52)	221	—	43 (0·96)	45	—	5·8 (1·87)	3·1
3F7	154	3/sec, 5′	—	785 (1·15)	680	—	51 (1·11)	46	—	9·2 (2·55)	3·6
6F7	154	30/sec, 30″	—	354 (1·30)	273	—	54 (1·20)	45	—	5·5 (2·20)	2·5
13F7	0	30/sec, 60″	324 (1·35)	—	226	58 (1·10)	—	51	19·2 (7·12)	—	2·7
Mean ± S.E.	—	—	(1·09)	(1·27) ±0·10	*	1·05	(1·08) ±0·05	—	12·1 (3·98)	7·0 (2·12) ±0·16	3·2 ±0·2

Figures in brackets are ratios of the values in reduced potassium and in normal Locke solution.
Values of τ are the time constants of the exponential phase of recovery after stimulation.
Standard errors are calculated only when more than 3 determinations were made.
* Value obtained with 3/sec is 584 ± 4·1; with 30/sec it is 240.

genuine and, as the results in Table 1 and Table 3 show, it was readily confirmed in the present experiments. Thus with stimulation either at 3 stimuli/sec or 30 stimuli/sec, where the extra oxygen consumption with stimulation (Q_s) in Locke solution containing 5·6 mM potassium was 580 and 240 p-mole/g wet wt. impulse respectively (Table 3), a reduction of the external potassium concentration to zero or to 0·5 mM had little or no effect on Q_s. However, as Baker & Connelly (1966) pointed out, partial inhibition of the sodium pump might be expected to alter the time course, rather than the total magnitude, of the stimulated oxygen consumption; so we have re-examined the problem with this in mind. Before doing so, however, we considered one possible explanation for the lack of effect of removing the external potassium ions, namely that external cations other than potassium can support the operation of the sodium pump in C fibres. It seemed unlikely that any of the other cations present in potassium-free Locke solution (calcium, hydrogen, and buffer) could function in this way in the low concentrations present, but the possibility was tested experimentally. Table 1 shows that there was little effect either on the resting or on the stimulated oxygen consumption when the external calcium concentration was reduced from 2·2 to 0·2 mM in potassium-free Locke solution; thus the stimulated oxygen consumption in calcium-deficient potassium-free Locke solution was $96{\cdot}7 \pm 5{\cdot}0\,\%$ of the corresponding value in normal Locke solution, an effect that is not statistically significant. Similarly, when the pH of the bathing solution was increased from 7·2 to 9·2, which would considerably decrease both the hydrogen ion concentration and the concentration of the cationic form of the buffer, there was little change in the resting oxygen consumption (an increase of $15{\cdot}1 \pm 5{\cdot}6\,\%$) and no change in the stimulated oxygen consumption (decrease of $4{\cdot}7 \pm 5{\cdot}1\,\%$). Reducing the calcium concentration or altering the pH of the Locke had no appreciable effect on the action potential, so it is unlikely that these findings were confused by large differences in the sodium load incurred during stimulation in the different solutions.

The time course of stimulated oxygen consumption

Though removal of external potassium consistently had no effect on the total oxygen consumed following stimulation, the time course of the increased metabolism *was* sensitive to potassium, as is shown in Fig. 2 and Table 3. In these experiments the stimulated oxygen consumption was recorded in the presence of 5·6 mM potassium, and in a reduced potassium concentration of either 0·5 mM or zero. Three effects of reduced potassium were seen, and these are evident in the records of Fig. 2: first, a slight increase in the maximum amplitude of the response; second, an increase

in the initial rate of fall following the peak (this was seen in most, but not all, preparations); and, third, a marked slowing of the later stages of recovery. These effects are seen best, as in Fig. 2*b*, when the tracings are plotted semilogarithmically.

In the presence of 5·6 mM potassium, within about 2 min of the end of the period of stimulation, recovery became exponential, as is evident from the linearity of the semilogarithmic transformations in Fig. 2*b*. In

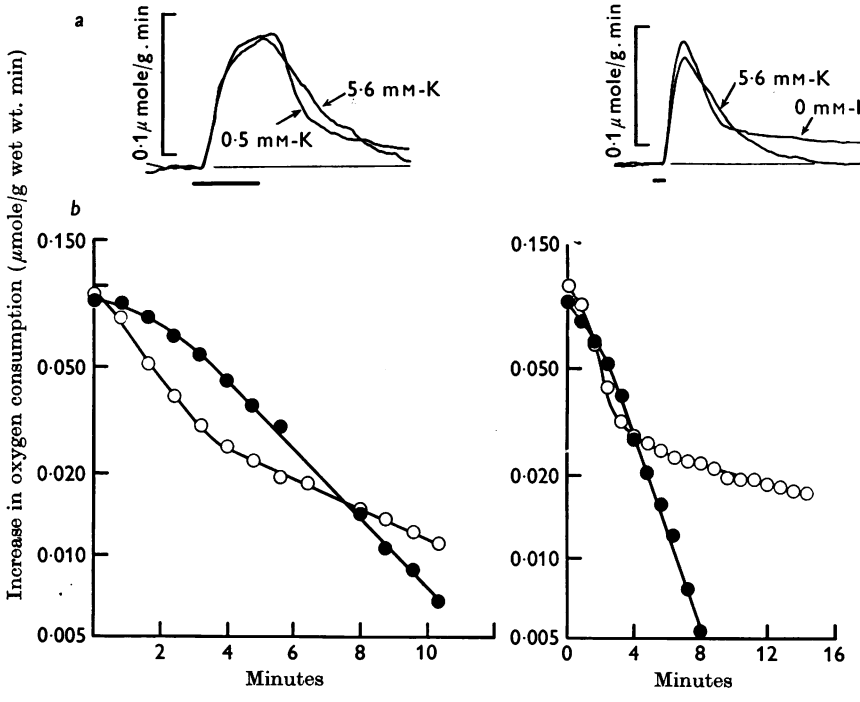

Fig. 2. The effect of potassium concentration on the time course of the increase in oxygen consumption of rabbit desheathed vagus nerves following stimulation. The upper panels (*a*) show superimposed responses to stimulation in the presence of 5·6 mM potassium and in the presence of zero or 0·5 mM potassium. The lower panels (*b*) show the height of the trace plotted logarithmically against time (measured from the peak of the response). Open circles: zero or 0·5 mM potassium; filled circles: 5 mM potassium. The frequency of stimulation was 3/sec for 5 min in the left-hand records and 30/sec for 1 min in the right-hand records, the period of stimulation being marked by the horizontal bars below each record.

the reduced potassium solutions recovery usually also became exponential following the early, rapid fall. The time constant for the later stage of recovery is given in Table 3. There was a good deal of scatter in these measurements, but an inverse relationship between potassium concentration and the recovery time constant was quite clear, the mean values

being 12·1, 7·0 and 3·3 min respectively for 0, 0·5 and 5·6 mM potassium. This pronounced dependence of recovery time course on external potassium is contrasted in Table 3 with the small effect of potassium concentration on the peak rate of oxygen consumption and on the total extra oxygen used.

Activation by potassium and other external cations

Potassium. One explanation for this apparent anomaly is that the sodium pump is indeed markedly dependent on the external potassium concentration; but that in potassium-deficient solution this dependence is not obvious because enough potassium ions are released into the periaxonal space during stimulation to support the pump for some time. However, after the released potassium diffuses away, the rate of sodium extrusion is determined by the steady-state value of the potassium concentration in the periaxonal space, which must be near that of the bulk of the solution. Thus, in low-potassium Locke solution, the rate of oxygen consumption should decline after a period of stimulation to a low level although the sodium debt is not yet paid off; and in this condition it should be possible to stimulate oxygen consumption by then increasing the potassium concentration. That this is indeed the case is shown by the following experiment in which a nerve was stimulated in potassium-free solution (usually 30 stimuli/sec, for 30 sec) and the time course of the extra oxygen consumption followed. When the response had returned almost to the base line the perfusion fluid was switched to a Locke solution that did contain potassium. As can be seen in Fig. 3*a*, which shows the result of a typical experiment, this change from potassium-free Locke solution to one containing 10 mM potassium produced an immediate and prolonged increase in the extra oxygen consumption.

In many experiments the procedure described above was then repeated exactly as first described, except that ouabain was added to the perfusing Locke solution between the period of stimulation and the introduction of the high potassium solution (Fig. 3*b*). As can be seen the subsequent introduction of potassium no longer increased the stimulated oxygen consumption. Furthermore, when a third period of stimulation was applied with the nerve now in ouabain, the phase of stimulated oxygen consumption was found to be virtually absent (Fig. 3*c*).

Other cations. The results described above with potassium are fully consistent with the hypothesis that the extra oxygen consumption is the result of the activity of a sodium pump that depends on external potassium ions for its activation and that is inhibited by ouabain. In similar experiments Baker & Connelly (1966) have shown that the sodium pump in crab nerve, which is also activated by potassium ions, can be activated

by a variety of other cations. Tests were therefore carried out in which various concentrations of the cations tested by Baker & Connelly (1966) were compared with 5·6 mM potassium for their ability to increase the oxygen consumption of C fibres that had been stimulated in potassium-free solution. Experimental records are shown in Fig. 4. Precise comparison

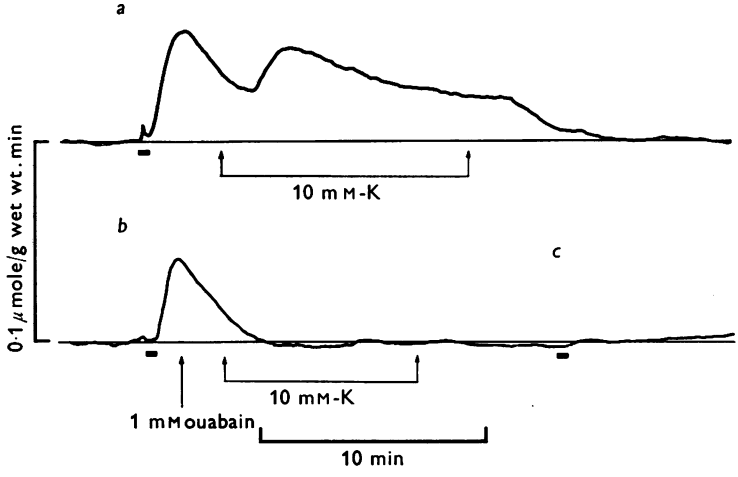

Fig. 3. The oxygen consumption of rabbit desheathed vagus nerves following stimulation for 30 sec at a frequency of 30/sec. *a*, The nerve was stimulated in potassium-free Locke solution. The solution was changed 2½ min after the end of the period of stimulation to one containing 10 mM potassium (indicated by the horizontal bar); *b*, as in *a*, except that 1 mM ouabain (at vertical arrow) was applied 1 min after the end of stimulation; *c*, the nerve was stimulated in potassium-free solution in the continued presence of ouabain.

of the activity of different ions was not attempted, because no more than three or four tests could usually be made on each nerve, but rough comparisons indicated that thallium (2 experiments) and rubidium (2 experiments) were about as effective as potassium, whereas lithium (4 experiments) and caesium (4 experiments) were about 1/4 as effective. In the experiments with lithium, concentrations up to 50 mM were tested, and controls were necessary to establish that the resulting hypertonicity was without effect. Thus, addition of equiosmotic amounts of sucrose or sodium chloride had no effect at any of the concentrations tested; but 50 mM choline chloride regularly caused an increase in oxygen consumption almost as big as that seen with lithium. It therefore appeared possible that choline could increase sodium extrusion. But whether this was an action comparable to that of the other cations tested, or an indirect effect following the changes in membrane properties that are known to be produced by acetylcholine and by choline (Armett & Ritchie, 1961), has not been

established; certainly choline does not seem to be able to activate the electrogenic component of the sodium pump (Rang & Ritchie, 1968*a*).

The effect of chloride ions

Replacement of chloride ions by impermeant anions such as isethionate has a dramatic effect on the after-potentials associated with activity of the sodium pump in C fibres (Rang & Ritchie, 1968*a*). In a few experiments in which chloride was replaced by isethionate the oxygen consumption

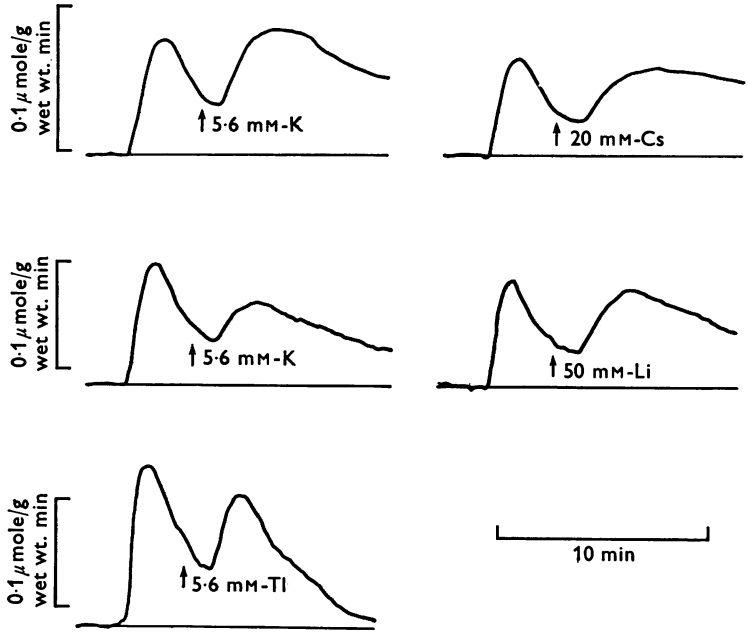

Fig. 4. The effect of adding various cations on the oxygen consumption of rabbit desheathed vagus nerves following a period of stimulation (30/sec for 30 sec) in potassium-free Locke solution. The cations were added at the arrows in the concentrations shown. The upper pair of responses was obtained from one nerve and the middle pair from another.

associated with stimulation was not altered in amount or in time course; nor did it appear that the effect of removal of potassium on resting or stimulated oxygen consumption was appreciably altered by the absence of chloride. This observation rules out the unlikely possibility that sodium extrusion in potassium-free Locke solution might become coupled to chloride extrusion instead of potassium entry. Thus neither chloride ions inside the fibres, nor calcium, hydrogen, or buffer cations outside, appear able to act as substitutes for external potassium in C fibres.

DISCUSSION

The observation that prompted this study was that neither the resting nor the stimulated oxygen consumption in mammalian C fibres appeared to be altered by the absence of external potassium. The present results suggest that the reason for the lack of effect on the *resting* oxygen consumption is that only a small fraction of this resting consumption is concerned with the active transport of sodium. Thus there is only a small effect of ouabain even at an apparently supramaximal concentration (1 mM) on the resting oxygen consumption in normal Locke solution, although this concentration of ouabain quickly and completely abolishes the increase in oxygen consumption with stimulation (Fig. 3); so, unless there are two separate sodium pumping mechanisms differing widely in their sensitivity to ouabain, the conclusion seems inescapable that not more than about 10 % of the resting oxygen consumption is concerned with sodium pumping. Our finding that the response of the resting oxygen consumption to added potassium was much the same whether the nerve had been previously soaked in potassium-free solution or in normal Locke solution, and that the metabolic response to potassium was maintained and not transient (at least at low potassium concentrations), contrasts with the results of Baker & Connelly (1966); it also argues against a close relation between resting oxygen uptake and sodium transport in the tissue since the internal sodium concentration, which is presumed to determine the rate of extrusion of sodium ions, must have been considerably higher in nerves which had been exposed to potassium-free solution (Rang & Ritchie, 1968*b*).

Increasing the external potassium concentration increased both the ouabain-sensitive and the ouabain-insensitive components of the resting oxygen consumption. Thus when the potassium concentration was increased from zero to 100 mM the ouabain-insensitive component increased from 0·074 to 0·21 μmole/g.min, while the ouabain-sensitive component increased from 0·016 to 0·091 μmole/g.min. This means that the relative contribution of the ouabain-sensitive component increased, from 18 to 43 % of the total resting oxygen consumption. If the action of ouabain is to interfere specifically with sodium transport, the relatively greater effect of ouabain on oxygen consumption at high potassium concentrations indicates that at these high potassium concentrations sodium transport accounts for a relatively large fraction of the total oxygen uptake. Whether the ouabain-insensitive increase in resting oxygen consumption results from a potassium-sensitive metabolic process other than sodium transport, or from surmountability of the ouabain inhibition in the presence of raised potassium concentration, is not clear. Glynn (1957) found that

inhibition of potassium transport in red cells with the low concentration of cardiac glycoside that he was then using could be completely overcome by potassium; but in red cell ATPase when higher concentrations of glycoside were used (Dunham & Glynn, 1961), and in crab nerve (Baker & Connelly, 1966), only partial surmountability was found.

The effect of potassium in increasing the resting respiration of mammalian C fibres seems to be a general property of nerve for it is also found in frog myelinated (Oberholtzer, 1951) and crab non-myelinated (Shanes & Hopkins, 1948; Baker & Connelly, 1966) nerves. In mammalian C fibres about two-thirds of this potassium sensitivity is removed by ouabain (Fig. 1; Table 2). A plausible interpretation of the stimulating effect of potassium on respiration, therefore, is that part, but not all, of the resting metabolism of nerve depends on the activity of the sodium pump; increasing the external potassium concentration enhances the activity of this pump so that the resting oxygen consumption is increased and there is a lower steady-state internal sodium, and a higher internal potassium concentration. A similar effect of potassium (and also of rubidium and caesium) is also found in muscle, and is called the Solandt effect (Fenn, 1931; Solandt, 1936; Van der Kloot, 1967). However, the effect on muscle may not be caused in the same way as just proposed for nerve, for the Solandt effect is much larger in muscle than in nerve; thus increasing the external potassium concentration from zero to 20 mM causes a 20-fold increase in the heat production of frog muscle (Solandt, 1936) but an increase of only 0·35 in the resting oxygen consumption of mammalian C fibres (Table 2). Indeed, Van der Kloot (1967) has proposed that the Solandt effect in muscle is a result of a rise in the sarcoplasmic calcium concentration secondary to a membrane depolarization produced by potassium rather than by a direct effect of the potassium on the sodium pump.

It had previously been suggested (Ritchie, 1967) that the resting metabolism of C fibres was of the right order of size for most of it to be involved in sodium pumping. But the argument used was somewhat indirect; and the present, contrary conclusion that relatively little of the resting oxygen consumption is related to sodium extrusion seems to be better founded. For example, the earlier suggestion would imply that a substantial part of the sodium extrusion process was insensitive to ouabain (ouabain causing only a 26 % decrease in the resting oxygen consumption in normal Locke solution, as Table 2 shows). But this implication is clearly false since, as Fig. 3 shows, the extra pumping following stimulation is *completely* abolished by ouabain. Unfortunately, the critical experiment to decide between the two possibilities cannot readily be done; for transmembrane fluxes of sodium cannot be measured reliably in C fibres (Keynes

& Ritchie, 1965). The relation between sodium transport and oxygen consumption in resting nerves thus remains for the moment unclear.

The effect of reduced potassium on the stimulated oxygen consumption (Table 3, Fig. 2), together with the effect of introducing potassium following stimulation in potassium-free solution (Figs. 3 and 4), can be explained in the following way. The sodium pump in C fibres is potassium-dependent, but sufficient potassium is released into the periaxonal space during activity to support the activity of the sodium pump during the period of activity in potassium-free solution and for a short time afterwards until the released potassium diffuses away (or is recaptured by the fibres). The pump then stops working (as far as can be determined from oxygen consumption) before the sodium debt is fully paid off. This accounts for the abrupt decline in oxygen consumption at the end of stimulation in potassium-free solution compared with the time course in normal Locke solution (Fig. 2). That a debt remains is shown by the burst of oxygen uptake that occurs when potassium is readmitted after the initial response has returned, or nearly returned, to the base line after stimulation in potassium-free solution.

The near equality of the extra oxygen consumption in nerves that have been stimulated and kept wholly either in normal Locke solution or in potassium-free Locke solution is therefore fortuitous; for in the potassium-free solution the debt acquired during the period of stimulation has not been fully paid off. Indeed, the stimulated oxygen consumption in 0·5 mM potassium solution exceeded that in 5·6 mM potassium by 27 % (Table 3), although no great statistical significance is attached to this increase ($0{\cdot}3 > P > 0{\cdot}2$). Furthermore, the value obtained by adding the extra oxygen consumed on readmitting potassium to that consumed while the nerve was still in potassium-free medium is considerably above the value for a nerve kept in normal Locke solution for the whole time. No experiments were done to measure this carefully, but rough evaluation suggested that the peak produced by adding 5·6 mM potassium after stimulation in potassium-free solution amounted to 30–40 % (Fig. 4*a*, *c*) of the size of the initial peak, which was normally the same size (Table 1) as that obtained after stimulation in 5·6 mM potassium.

Adding the potassium after a delay, rather than having it present throughout, thus appeared to increase the total oxygen used during recovery by 30–40 %. This may merely be the result of greater sodium entry during each impulse in potassium-free solution owing to the resultant hyperpolarization and greater amplitude of the action potential. But the effect on the size of the compound action potential is quite small (see fig. 7, Ritchie, 1961) and seems hardly enough to account for the greater oxygen consumption. An interesting possibility is that the greater chemical work

done in recapturing potassium from a low external concentration accounts for the difference. The free energy change involved in the operation of a 1:1 coupled sodium:potassium pump is given by

$$RT\left(\ln\frac{[\mathrm{Na}]_o}{[\mathrm{Na}]_i}+\ln\frac{[\mathrm{K}]_i}{[\mathrm{K}]_o}\right)\text{cal/mole},$$

where $[\ ]_o$ and $[\ ]_i$ denote the external and internal concentrations respectively, R is the gas constant and T is the absolute temperature. Rang & Ritchie (1968*b*) found that for C fibres in 5·6 mM potassium Locke solution $[K]_i$ = 165 mM and $[Na]_i$ = 76·6 mM. Decreasing $[K]_o$ from 5·6 to 0·5 mM, assuming $[K]_i$ does not change quickly, increases the free energy change from 2390 to 3800 cal/mole at 21° C, i.e. by about 59%. If the pump worked at more or less constant efficiency, this increase could account for the greater oxygen uptake in low potassium solutions. However, the evidence from red cells (Sen & Post, 1964; Whittam & Ager, 1965; Garrahan & Glynn, 1967), frog skin (Zerahn, 1956), and invertebrate nerve (Baker, 1966) suggests that this stoicheiometry rather than the efficiency remains constant so the above explanation is not entirely satisfactory.

The activity of different cations in stimulating the sodium pump agrees quite well with the findings of Baker & Connelly (1966) on crab nerve. It is interesting that lithium ions, which have been shown to activate the sodium- and potassium-dependent ATP-ase of red cell membranes (Whittam & Ager, 1964) and crab nerve (Skou, 1965), also activate the sodium pump in C fibres, shown for oxygen consumption in the present work and for the electrogenic sodium pump in the accompanying paper (Rang & Ritchie, 1968*a*). It must be emphasized, however, that this effect is exerted at the external activation site that is normally potassium sensitive. There is no evidence that the internal sodium site is affected and the earlier conclusion remains valid, namely that the sodium pump does not extrude lithium ions at any appreciable rate (Keynes & Swan, 1959; Ritchie & Straub, 1957).

One interesting difference between mammalian and crustacean non-myelinated fibres has been revealed by the present experiments. The time recovery of C fibres in 5·6 mM potassium is 3·3 ± 0·2 min (Table 3), which contrasts with the value of 24·8 ± 4 min found by Baker & Connelly (1966) for crab nerve recovering in 5 mM potassium. The total extra oxygen used per impulse is roughly the same in both preparations, so C fibres show short, intense bursts of pumping after activity by comparison with the prolonged responses of crab nerve. Our experiments were done at a higher temperature (21° C) than was used by Baker & Connelly (16° C), but this seems insufficient to account for a sevenfold difference in recovery rate. More

probably the difference is a result of the smaller size of C fibres. There are no readily available details on the fibre diameters of the crab preparation used by Baker & Connelly (1966), but the average diameter of the corresponding fibres in a related species, *Maia*, can be calculated from the data of Abbott, Hill & Howarth (1958) to be about 2 μ, which is much larger than the corresponding value of 0·75 μ in rabbit C fibres (Keynes & Ritchie, 1965). If the rate of sodium extrusion is determined by the internal sodium concentration, a simple analysis shows that the time constant of recovery should be directly proportional to the fibre diameter. There is thus a *qualitative* fit between the relative sizes of the experimentally obtained time constants of recovery and those predicted. The remaining discrepancy—the experimental value for time constant being about 7 times greater in crustacean than in mammalian nerve whereas the fibre diameter is only about 3 times greater—could be accounted for if the relationship between rate of pumping and internal sodium concentration were rather steeper in C fibres than in crab nerve.

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