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THE METABOLIC RESPONSE OF BRAIN SLICES TO AGENTS AFFECTING THE SODIUM PUMP

BY M. RUŠČÁK* AND R. WHITTAM†

From the Department of Biochemistry, University of Oxford, and the Laboratory of General Physiology, University of Leicester

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SUMMARY

1. Slices of brain cortex from rabbits were incubated in Ringer solution and in Ringer modified by the removal of calcium and sodium, and the addition of ouabain, oligomycin or extra potassium. The potassium content of the tissue, the oxygen consumption and the lactate production from glucose were measured and found to be interrelated.

2. Incubation in high-K Ringer caused an increase in oxygen consumption that was prevented by ouabain, oligomycin and deprivation of sodium. Lactate production was also raised, but this increase was unaffected by ouabain and raised further by oligomycin.

3. Calcium omission raised metabolism; the tissue K content was unaffected. Oligomycin always decreased oxygen consumption and raised lactate production further. The metabolic responses to calcium, potassium, ouabain and oligomycin depended on sodium.

4. After anaerobic incubation, the tissue potassium concentration was still 5 times higher than that in Ringer. It was unaffected by oligomycin but lowered markedly by ouabain.

5. The synergistic effects of sodium with potassium, oligomycin, calcium, and calcium plus ouabain suggest that the metabolic responses of brain cortex slices to a high-K Ringer depend on the operation of the sodium pump.

INTRODUCTION

Several features of brain metabolism suggest that cell membranes are involved in the process of metabolic regulation. First, there is a marked increase in the metabolism of brain cortex slices when they are incubated in Ringer solution to which extra potassium has been added. The effects are not found in brain homogenates nor in slices of non-excitable tissues, such

* Present address: Laboratory of Neurophysiology of Slovak Academy of Sciences, Bratislava, Czechoslovakia.

† Present address: Laboratory of General Physiology, University of Leicester.

as kidney cortex and liver. These discoveries of Dickens & Greville (1935), and Ashford & Dixon (1934) suggest that metabolism is stimulated when the excitable membranes of intact cells in the brain cortex are depolarized. Another special metabolic feature of brain cortex is the depression of oxygen consumption by calcium ions in the Ringer (Krebs, 1950). This observation also implicates neuronal membranes in metabolic regulation in view of the extensive electrophysiological evidence showing an action of calcium on membrane conductance. A deficiency of calcium leads to an increased permeability to sodium (Frankenhaeuser & Hodgkin, 1957). A third special feature of brain cortex is the dependence on calcium of the increase in oxygen consumption that follows inhibition of the sodium pump by ouabain (Schwartz, 1962). A decrease is found in brain homogenates and when slices are incubated in a Ca-free Ringer (Whittam, 1962*a*), suggesting that external calcium determines the kind of response.

These effects have a common feature in being associated with different rates of movement of sodium and potassium ions across cell membranes by means of the sodium pump. The sodium pump regulates the metabolic rate—whether of oxygen consumption or glycolysis—in simpler cells and non-excitable tissues, and there appears to be an interdependence between energy production and energy utilization (see Whittam, 1964). In order to ascertain whether the special features of brain cortex fit this general notion, which attributes a key role to sodium ions, slices have been incubated in Ringer solution under conditions that facilitate or inhibit the sodium pump. Oligomycin was used as an inhibitor, because it acts in red cells like ouabain (Whittam, Wheeler & Blake, 1964), and yet is also an inhibitor of oxidative phosphorylation in respiring cells (Lardy, Johnson & McMurray, 1958; Huijing & Slater, 1961). It was found to be unlike ouabain in its effects on brain cortex slices.

The results show that the metabolic responses to calcium, high external potassium, oligomycin and ouabain are all dependent on the presence of sodium. When the sodium pump was inactive, the special metabolic features of brain were abolished.

METHODS

Adult rabbits were stunned by a blow on the head, bled from the neck and decapitated. The brain was excised, and the medulla removed from the cortex. Slices of cortex were cut freehand with a razor blade moistened with the Ringer solution in which they were to be incubated (Deutsch, 1936). Only the outer and one inner slice were used so as to avoid contamination with white matter. The slices were placed in ice-cold Ringer in a Petri dish until they had all been cut. About 50 mg of tissue was then placed in 4·0 ml. Ringer in cups of Warburg manometers in which their oxygen consumption was measured during incubation for 1 hr at 37° C. The rate is expressed as μl. O_2/mg wet wt./hr. The time from the killing of the animal to the first reading of the manometers was between 30 and 35 min.

Media. Ringer solution contained (mM): NaCl, 146; KCl, 5; $CaCl_2$, 3; Na phosphate, 2·5, pH 7·4; glucose, 10. It was modified in composition either by omitting calcium, adding 0·1 M extra K, or replacing Na with choline. Additions of ouabain were made in aqueous solution. Oligomycin was added as a solution in ethanol and the same volume of ethanol was added to control slices incubated in normal Ringer.

Lactate determinations. Perchloric acid (0·8 ml. of 25 %) was added to the contents of the Warburg cups. Alkali (1 N-KOH) was then added until the solution was neutral towards an external indicator. Lactate was determined enzymically on a sample of the neutralized extract (Hohorst, Kreutz & Bücher, 1959); the error of the method was about 5 %. The production of lactate is expressed as μmoles/g wet wt./hr.

Potassium was determined by flame photometry. The slices after incubation were placed on a piece of muslin which was laid on a piece of hardened filter paper (Whatman No. 542) to absorb adherent fluid, and weighed on a torsion balance. Extracts of the tissue were made for at least 3 hr with 0·1 N-HNO_3. Significant differences were not found on more prolonged extraction.

RESULTS

Comparison of Ringer and Ca-free Ringer

Omission of calcium from Ringer does not affect the oxygen consumption of kidney cortex or liver slices but it increases that of brain slices (Krebs, 1950). Since brain cortex differs from the other tissues in having a high rate of lactate production from glucose under aerobic conditions, this was also measured after incubation in Ca-free Ringer. Table 1 shows

TABLE 1. Changes in metabolism due to calcium

		Oxygen consumption (μl./mg wet wt./hr)	Lactate production (μmoles/g wet wt./hr)	Tissue K content (μequiv/g wet wt.)
Ringer control		1·29 ± 0·05 (7)	24·0 ± 2·6 (13)	47·5 ± 1·2 (15)
Ca-free Ringer	control	1·68 ± 0·07 (7)	39·3 ± 5·3 (8)	47·9 ± 1·3 (11)
	+ ouabain	1·02 ± 0·06 (7)	41·1 ± 4·2 (4)	13·0 ± 1·0 (8)
	+ 0·1 M-KCl	1·84 ± 0·7 (12)	91·2 ± 5·8 (7)	—
	+ ouabain + 0·1 M-KCl	1·03 ± 0·07 (3)	91·7 ± 1·5 (3)	—
Change due to deprivation of Ca		−0·39 (−30 %)	+15·3 (+64 %)	0
Change on adding 0·1 M-KCl		+0·16 (+10 %)	+51·9 (+132 %)	—
Effect of ouabain on response to 0·1 M-KCl		−0·81 (−44 %)	0	—

S.E.M. are shown.

that the oxygen consumption was raised 30 % and lactate production 64 % on omission of calcium from Ringer. The effect of calcium would be half-maximal at about 0·5 mM (Fig. 1). The potassium content of the tissue was unaffected, potassium being maintained at a concentration some 10 times higher than in the external solution. The sodium content is also known to be unaffected (Lolley, 1963; Dawson & Bone, 1965), but

was not measured in the present experiments because of uncertainties about the amount of extracellular sodium in the slice. Since the sodium pump also transports potassium inwards and maintains the high internal potassium content, it was evidently not inhibited in slices by calcium in the medium.

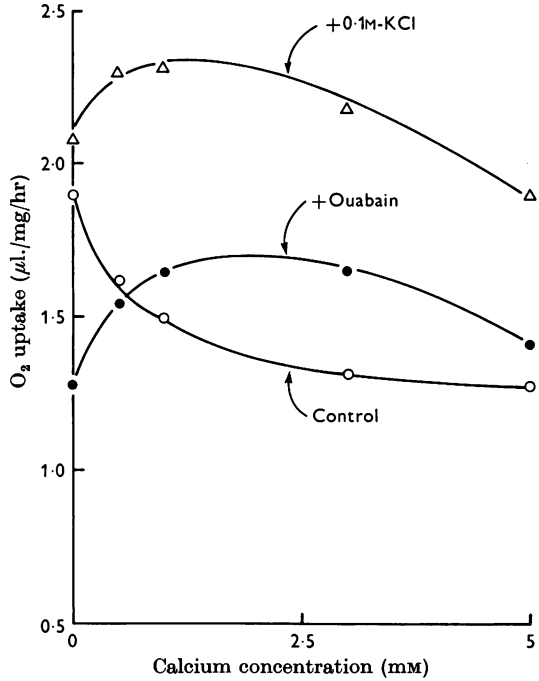

Fig. 1. Metabolic responses to 0·1 M-KCl, ouabain and calcium. (1 hr; 37° C; O_2).

Effect of ouabain. The metabolic changes with calcium suggest that it might also affect the metabolic response of brain to inhibition of the sodium pump by ouabain. The tissue potassium content at the start of incubation (µequiv/g wet wt.) was about 25, and was raised to 48 by a net uptake from the medium during incubation for 1 hr. When ouabain was added to Ringer or Ca-free Ringer, the potassium content fell to 12–13 showing inhibition of potassium uptake (Tables 1 and 2). Figure 1 shows that ouabain raised the oxygen consumption of slices in Ringer but decreased it in Ca-free Ringer. The values show the total change in 1 hr. When the time course is plotted, however, it is clear that the rate was constant only when slices were in Ringer and Ca-free Ringer. Figure 2 shows that the increased rate with ouabain in Ringer was found only during the first 30 min because, after 40 min, it fell to lower than in the control. In Ca-free Ringer containing ouabain there was a steady fall in

TABLE 2. Changes in metabolism with ouabain and an elevated K level in Ringer solution

Conditions		Oxygen consumption (μl./mg wet wt./hr)	Lactate production (μmoles/g wet wt./hr)	Tissue K content (μequiv/g wet wt.)
Ringer	control	1·29 ± 0·05 (7)	24·0 ± 2·6 (13)	47·5 ± 1·2 (15)
	+ ouabain	1·56 ± 0·08 (7)	34·5 ± 3·0 (9)	12·1 ± 0·1 (11)
	+ 0·1 M-KCl	2·12 ± 0·08 (12)	112·5 ± 3·5 (7)	—
	+ ouabain + 0·1 M-KCl	1·52 ± 0·03 (3)	114·3 ± 4·0 (3)	—
Na-free Ringer	control	0·82 ± 0·04 (8)	64·9 ± 6·7 (6)	7·0 ± 0·2 (4)
	+ ouabain	0·80 ± 0·05 (6)	65·3 ± 3·0 (4)	7·7 ± 0·2 (4)
	+ 0·1 M-KCl	0·82 ± 0·06 (4)	73·2 ± 8·9 (8)	—
	+ ouabain + 0·1 M-KCl	0·88 ± 0·09 (4)	77·8 ± 6·4 (5)	—
	Ca-free	0·93 ± 0·03 (4)	62·8 ± 0·03 (4)	—
Changes due to deprivation of Na		−0·47 (−36 %)	+40·9 (+170 %)	−40·5 (−85 %)
Change on adding 0·1 M-KCl				
With Na		+0·83 (+64 %)	+88·5 (+370 %)	—
Without Na		0	0	—
Effect of ouabain on response to 0·1 M−KCl				
With Na		−0·60 (−28 %)	0	—
Without Na		0	0	—

S.E.M. are shown.

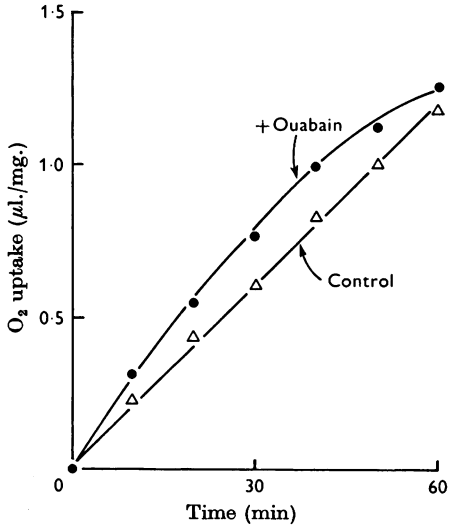

Fig. 2. Synergistic action of Ca and ouabain to raise O_2 consumption. (1 hr; 37° C; O_2).

rate with time in contrast to the constant rate in the control (Fig. 3). These results show that calcium and ouabain act together to cause a transient increase in oxygen consumption whereas singly each causes a decrease.

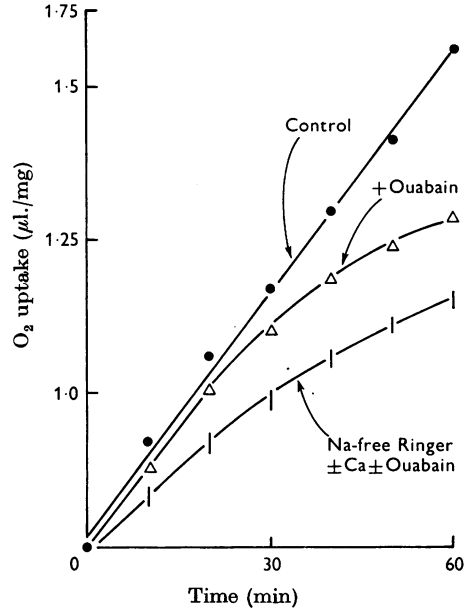

Fig. 3. Changes in O_2 consumption with Na and ouabain in Ca-free Ringer. (1 hr; 37° C; O_2).

Response to 0·1 M-*KCl*

Oxygen consumption. Addition of an extra 0·1 M-KCl to Ringer or Ca-free Ringer increased both oxygen consumption and lactate production. Controls with an extra 0·1 M-NaCl did not produce an increase. The increased oxygen consumption was dependent on the calcium concentration, the increase (in μl./mg wet wt./hr) being 0·16 in the absence of calcium, but 0·83 in Ringer (Tables 1 and 2). Calcium thus enhances the respiratory response to potassium, although alone it decreases metabolism.

When oxygen consumption was raised in high-K Ringer, addition of ouabain caused a fall, and not an increase as it did in slices in Ringer (containing 5 mM-K) (Fig. 4). In Ca-free Ringer containing an extra 0·1 M-KCl, there was also a fall with ouabain very similar to the effect in medium containing calcium. Addition of 0·1 M-KCl or omission of calcium are two changes in Ringer that abolish the increased oxygen consumption that is otherwise found with ouabain. Figure 4 shows that a concentration of about 10 μM is required to elicit a half-maximal inhibition. This is the

same as that needed to produce half-maximal stimulation in Ringer without extra KCl.

The effect of ouabain suggests that operation of the sodium pump is necessary to elicit the metabolic response to 0·1 M-KCl. Incubations were therefore made in Na-free Ringer. The slices lost sodium during preliminary incubation and active transport of sodium and potassium no

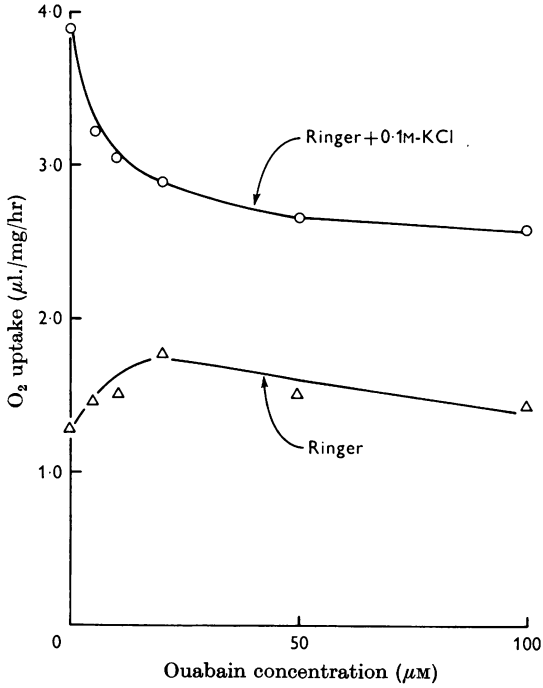

Fig. 4. Change in O_2 consumption with ouabain (37° C; 1 hr).

longer occurred. In Na-free conditions the rate of oxygen consumption was at the same level irrespective of the presence of extra potassium, ouabain or calcium (Table 2). It appears that the sodium pump is necessary to produce with these materials the effects that are characteristic of brain cortex.

Lactate production. Lactate production was raised in Na-free Ringer, and again further addition (extra potassium, or ouabain) or deletion (calcium) was without effect (Table 2). Sodium thus appears to be needed for the responses to high-K Ringer, ouabain and calcium.

Comparison of oligomycin and ouabain

Oligomycin is like ouabain in inhibiting the ATPase of cell membranes associated with the sodium pump, but different in inhibiting oxidative

phosphorylation. For both reasons it would be expected to decrease active transport. Since oligomycin has been claimed to produce effects that indicate an energy supply other than ATP for the sodium pump (Van Rossum, 1964; Hempling, 1966), it was crucial to compare it with ouabain, which is known to act directly on the membrane transport ATPase. The

TABLE 3. The different effects of ouabain and oligomycin on the tissue K content and metabolism

	Oxygen consumption (μl./mg wet wt./hr)	Lactate production (μmoles/g wet wt./hr)	Tissue K content (μequiv/g wet wt.)
Control	1·28	33·3	55
+ ouabain	1·35	49·7	12
+ oligomycin 0·5 μg/ml.	0·95	85·1	36
1·0 μg/ml.	0·94	91·9	28
10·0 μg/ml.	0·91	89·2	28
+ ouabain + oligomycin (10 μg/ml.)	0·99	78·5	13

O_2; 37° C; concentration of ouabain 20 μM. Normal Ringer solution; means of three incubations.

key question is whether the effects of oligomycin can be explained by inhibition of either ATP production or utilization, or both, without postulating an alternative energy supply for active transport. Three differences were immediately apparent. First, oxygen consumption was always partially inhibited by oligomycin, even in Ringer where ouabain caused an increase (Table 3). Secondly, the increase in lactate production was much greater with oligomycin (from 33·3 to about 90 μmoles/g wet wt./hr) than with ouabain (from 33·3 to 49·7 μmoles/g wet wt./hr). In both these metabolic changes the effects of oligomycin were found to predominate over ouabain when the two inhibitors were added together. The presence or absence of calcium made no difference to the increase in glycolysis found with oligomycin. The rate (in μmoles/g wet wt./hr) increased from 41 to 89 in Ringer, and from 52 to 91 in Ca-free Ringer. Thirdly, the tissue potassium content fell only to about 28 μequiv/g with oligomycin but to 13 μequiv/g with ouabain (Table 4). When oligomycin and ouabain were present together the tissue potassium content was the same as with ouabain alone.

Response to 0·1 M-*KCl.* Oligomycin was like ouabain in inhibiting the rise in oxygen consumption due to 0·1 M-KCl (Table 4). On the other hand, it differed in causing an increase in lactate production under this condition. Ouabain did not cause a further increase over that found with 0·1 M-KCl alone. The results show that oligomycin has a stimulating action on glycolysis both in the presence and absence of calcium and of an extra 0·1 M-KCl.

A critical result localizing the effect of ouabain is that the inhibitor is without effect in Na-free conditions. Table 5 shows that, in this respect, oligomycin is like ouabain. The oxygen consumption and lactate production in Na-free Ringer were unaffected by oligomycin, and the values are

TABLE 4. Stimulation of lactate production and inhibition of oxygen consumption by oligomycin

		Oxygen consumption (μl./mg wet wt./hr)	Lactate production (μmoles/g wet wt./hr)	Tissue K content (μequiv/g wet wt.)
Ringer	control	1·19	40·9	38
	+ oligomycin	0·72	89·3	19
Ringer + 0·1 M-KCl	control	1·97	99·1	139
	+ oligomycin	1·06	138·8	133
Ca-free Ringer	control	1·35	51·8	41
	+ oligomycin	0·75	90·8	21
Ca-free Ringer + 0·1 M-KCl	control	1·81	71·8	149
	+ oligomycin	1·03	138·6	132

O_2; 37° C; concentration of oligomycin 10 μg/ml. Values are means of three separate incubations.

TABLE 5. The dependence on Na of the effects of oligomycin

		No. of values	Oxygen consumption (μl./mg wet wt./hr)	Lactate production (μmoles/g wet wt./hr)	Tissue K content (μequiv/g wet wt.)
Ringer	control	4	1·35 ± 0·07	36·0 ± 3·7	45·7 ± 0·6
	+ oligomycin	4	0·94 ± 0·09	91·9 ± 4·1	27·6 ± 2·9
Na-free Ringer	control	6	0·67 ± 0·04	64·4 ± 6·2	12·3 ± 0·6
	+ oligomycin	6	0·61 ± 0·04	69·1 ± 2·8	10·9 ± 0·5

O_2; 37 °C; 1 hr incubation. Standard errors of mean values are shown.

comparable to those found in the same experiment with ouabain (Table 2). Sodium deprivation decreased the tissue potassium content to a low level (11–12 μequiv/g wet wt.). These results suggest that, whatever the site of action of oligomycin there is a requirement for sodium in intact cells of the slices in order for the inhibition of oxygen consumption and stimulation of glycolysis to be elicited.

Effects of oligomycin under anaerobic conditions. A way of clarifying the mode of action of oligomycin is to make use of the fact that brain cortex, like red blood cells, can utilize energy from glycolysis for active transport under conditions when oxidative phosphorylation is inhibited. Oligomycin inhibits both lactate production and the sodium pump in red cells in a way that can be attributed to direct inhibition of the pump rather than impairment of the supply of ATP to the pump from metabolism (Whittam *et al.* 1964). If oligomycin in brain slices also acted directly on the sodium pump, then those concentrations that inhibited active transport under aerobic conditions (1 and 10 μg/ml.) should also inhibit under anaerobic conditions.

The tissue potassium content under anaerobic conditions was unaffected by oligomycin, as was the rate of lactate production. The latter was 2 or 3 times greater anaerobically than aerobically. The failure of oligomycin to inhibit the maintenance of a tissue potassium content some 5 times higher than the medium is in contrast to the decrease produced by ouabain. There was no decrease in the rate of lactate production when oligomycin

TABLE 6. Different effects of ouabain and oligomycin under anaerobic conditions

		Lactate production (μmoles/g wet wt./hr)	Tissue K content (μequiv/g wet wt.)
Expt. 1	control	90·1	27
	oligomycin (1 μg/ml.)	88·1	25
	oligomycin (10 μg/ml.)	90·6	27
Expt. 2	control	131	24
	oligomycin (1 μg/ml.)	136	23
	ouabain (20 μM)	104	10

N_2/CO_2; 37° C Ringer solution; 1 hr incubation.

was present. Table 6 (Expt. 2) shows that the tissue potassium content was decreased to a low level (10 μequiv/g wet wt.) in the presence of ouabain whether or not oligomycin was present. These results under anaerobic conditions add to those under aerobic conditions in showing that, so far as active transport is concerned, oligomycin acts differently from ouabain and that, metabolically, the effect of oligomycin is dominant over that of ouabain. It thus appears that brain slices respond in a more complex way to oligomycin than do red cells, and that in this tissue the inhibitor exerts its effect at a different site of action than ouabain.

DISCUSSION

The first question to consider is the use of slices of tissue in experiments designed to elucidate the behaviour of cells. There are clear drawbacks with tissue slices, such as the presence of damaged cells, the lack of a circulating fluid and the heterogeneity of the cells in the slice. It is clearly not valid to extrapolate from results with tissue slices, such as those of the brain and kidney cortex, to the physiological function of the cells in the intact organ. There are, however, some aspects of cell metabolism such as gluconeogenesis (e.g. see Krebs, Bennett, de Gasquet, Gascoyne & Yoshida, 1963), and the influence of active transport on metabolism (see Whittam & Willis, 1963), that can profitably be investigated with tissue slices. The main results of this paper direct attention to the role of the sodium pump in the metabolic responses of the brain cortex towards various ions and agents that do not elicit similar responses in the liver and kidney cortex.

Synergistic effects of ions. Earlier work on the influence of a high external potassium concentration has been confirmed (Ashford & Dixon, 1934; Dickens & Greville, 1935; Dixon, 1949). The salient features are an increase in oxygen consumption and in the aerobic utilization of glucose to lactic acid. These responses of brain slices to potassium depended upon the concomitant presence of sodium ions, and they were not found when the sodium pump was poisoned with the glycoside, ouabain (Hertz & Clausen, 1963; Gonda & Quastel, 1962; Elliott & Bilodeau, 1962). A further finding is that calcium ions affected the metabolism of brain slices in a way that depended on the sodium and potassium concentrations. When slices were incubated in Ringer with physiological potassium concentration (5 mM), the effect of calcium was to depress the oxygen consumption and the lactate production. On the other hand, when the Ringer contained an extra 0·1 M-KCl, the increase in oxygen consumption was enhanced by low concentrations of calcium. Calcium ions thus have a dual effect depending on the concentration of potassium. A recent study by Rose (1965) has also described the effect on oxygen consumption of the ratio of calcium/potassium in the medium, thus recalling the suggestion of Quastel & Quastel (1961) that the calcium/potassium ratio in the medium determines the magnitude of the stimulation by potassium. Although this ratio is important, it is nevertheless true that extra potassium in the Ringer stimulates oxygen consumption irrespective of the presence of calcium (Table 2; Elliott & Bilodeau, 1962).

Role of calcium. There is much evidence that calcium is involved in physical changes at cell surfaces, for in excitable tissues it affects the membrane resistance, resting and action potentials, and the sodium and potassium conductances. Much is also known of the effect of calcium on isolated mitochondria. In contrast, the question of regulation of metabolism by an action of calcium on the cell membrane has been neglected. In order to clarify the possible mechanism of the action of calcium, tissue slices were incubated under conditions when the sodium pump was not functioning either because of the absence of sodium, or because it was inhibited by ouabain. The results indicated that the metabolic effects of calcium ions were abolished when the sodium pump was inactive. In keeping with this interpretation is the increased metabolism of slices in Ca-free Ringer. Under this condition, there is a rise in permeability to sodium in other tissues (Curran, Herrera & Flanigan, 1963; Bolingbroke & Maizels, 1959; Solomon, 1960). In the present experiments the tissue potassium content was unaffected by calcium lack. If, however, the exchanges of ions were then increased, it follows that the extra energy needed to maintain the ionic gradients would be met by an increased rate of metabolism.

Role of potassium. The main conclusion in this work is that the metabolic effects of a high external potassium concentration and of calcium were elicited only when the sodium pump functioned. It is noteworthy that similar experiments with red blood cells or with slices of kidney cortex and liver, or with brain homogenates have not produced the characteristic effects that have been described above with brain cortex slices. It may be tentatively concluded that the effects depended upon the excitable membranes of intact cells in the brain slice. It seems reasonable to suppose that the raised metabolism followed membrane depolarization, which has been demonstrated in brain cortex slices (Hillman & McIlwain, 1961), and was a consequence of an accompanying increased permeability to, and active transport of, sodium and potassium ions. Attempts to show an increased turnover of ions in brain slices failed because of the fragile, ill-defined nature of the preparation. However, in crab nerve and *Sepia* nerve axons the efflux of potassium is markedly increased on raising external potassium (Keynes & Lewis, 1951; Hodgkin & Keynes, 1955). Since these permeability and metabolic effects are not found in non-excitable cells, they may indicate a fundamental difference between excitable and non-excitable cells.

Site of action of ions

The question arises as to the site of action of the ions (sodium, potassium and calcium). By analogy with red blood cells and nerve axons, it seems likely that the stimulation of metabolism by potassium is due to activation of the sodium pump from the external surface of the cells in a way that depends upon internal sodium (Glynn, 1962; Whittam, 1962*b*; Baker, 1965; Baker & Connelly, 1966). Sodium and potassium ions therefore exert their effect via the sodium pump. The phosphates involved in the activity (ATP, ADP and inorganic phosphate) are known to regulate the rates of oxygen consumption and glycolysis.

The site of action of calcium is less easily inferred but, like the effect of potassium, it would appear to be on the external surface of the membranes of intact cells. Thus, when the cells have been disrupted by homogenization, which allows access of calcium to previously intracellular structures, the co-operative effects of calcium and potassium described above for slices are not then found (Whittam & Blond, 1964). In the homogenate, addition of calcium leads always to a decrease in metabolism—never an increase, such as was found when calcium ions enhanced the effect of high external potassium. It is, moreover, known in red cells that calcium inhibits the sodium pump from inside but not outside (Hoffman, 1961; Rummel, Seifen & Baldauf, 1963). These facts taken together suggest that the site of action of calcium may be both on the internal and external surfaces of the membranes of intact excitable cells.

Attention must be drawn to an anomalous finding, namely that the synergistic effects of calcium and ouabain in stimulating the oxygen consumption of brain slices when either alone otherwise caused a fall (Whittam, 1962*a*; Schwartz, 1962). The results indicate that the sodium pump was inhibited, as shown by the loss of tissue potassium, but nevertheless the oxygen consumption was slightly raised. The effect of calcium and ouabain together was not found in the absence of sodium ions or in the presence of high external potassium. It is not easy to explain this result in terms of the simple hypothesis described above, inasmuch as a decrease in the rate of metabolism would be expected when the sodium pump is inhibited. As the effect is not found with liver and kidney (Whittam & Willis, 1963; Elshove & Van Rossum, 1963), it may be connected with the excitable nature of neuronal membranes. Further study is needed with a system more amenable to precise work than is possible with tissue slices.

The action of oligomycin

Oligomycin is an antibiotic that inhibits oxidative phosphorylation in mitochondria (Lardy *et al.* 1958; Huijing & Slater, 1961). It also inhibits the sodium pump in red blood cells in which there are no mitochondria, thus raising the possibility that it may also be an inhibitor of the sodium pump in cells that do contain mitochondria (Glynn, 1963; Whittam *et al.* 1964). Brain slices are particularly suitable for investigating the effect of oligomycin on active transport, because, although they depend on respiration for most of their energy supply, they can still maintain some 5 or 6 times more potassium than the medium in the absence of oxygen. Brain slices under anaerobic conditions, therefore, would resemble red blood cells in so far as the energy supply for the sodium pump is concerned.

Comparison with ouabain. The results indicate that oligomycin is different from ouabain in four respects, and that its effects in brain slices are much more complex than in red cells. First, in Ringer solution, oligomycin inhibited oxygen consumption, and did not stimulate in the way that ouabain did in the presence of calcium. Secondly, oligomycin stimulated lactate production from glucose some 2–3 times, unlike ouabain which stimulated only some 25–50 %. Thirdly, oligomycin, in concentrations that gave a maximum aerobic metabolic response, did not fully inhibit the sodium pump. Fourthly, under anaerobic conditions there was no difference with or without oligomycin in the tissue potassium content (about 5 times higher than the medium). The feature in which oligomycin resembled ouabain was its apparent dependence on sodium ions for its effects.

It is evident that oligomycin affected brain slices in a way that can be attributed to inhibition of mitochondrial oxidative phosphorylation, without the need to postulate a direct inhibition of the sodium pump. Again,

it hardly seems necessary to postulate that a hypothetical intermediate of oxidative phosphorylation plays a role in active transport at the cell membrane (Van Rossum, 1964; Hempling, 1966). With isolated mitochondria there is good evidence that ion transport is sensitive to oligomycin through an effect on a high-energy compound—other than ATP—which is a component of the oxidative phosphorylation system (Brierley, 1963; Chappell, Cohn & Greville, 1963). On the other hand, the sodium pump is not found in mitochondria but at the cell membrane, and further evidence is needed before discarding the hypothesis that ATP is its source of energy. The present results can be explained according to this view. Thus, if oxygen consumption and the ATP supply from mitochondria is inhibited, then an increase in lactate production would be expected both because of the Pasteur effect and in order to supply ATP for active transport. This was found. The potassium content of the tissue was maintained at a level higher than the medium in the presence of oligomycin, both aerobically and anaerobically, presumably through utilization of ATP derived from lactate production. Tobin & Slater (1965) have also noted an increased lactate production with oligomycin. The present results show that the effects of oligomycin on active transport and metabolism in brain slices are different from expectations derived from work with red cells. The effects of oligomycin in brain slices are most easily explained by inhibition of oxidative phosphorylation, whereas in red blood cells it is necessary to suppose that oligomycin directly inhibits the sodium pump.

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