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SHORT COMMUNICATIONS

The effect of ouabain on potassium content, phosphoprotein metabolism and oxygen consumption of guinea pig cerebral tissue*

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OUABAIN or protoveratrine treatment lowers the potassium content of cerebral tissues.[1,2,3] To study the cause of this phenomenon, some concomitant changes have been measured. These are described briefly and then compared with earlier reports.

METHODS

Slices of cerebral cortex were prepared from guinea pigs, according to the method described by McIlwain,[4] and were incubated in a medium to which was added 134 mM NaCl, 5·2 mM KCl, 2·8 mM $CaCl_2$, 1·3 mM KH_2PO_4, 1·3 mM $MgSO_4$, 25 mM Tris (2-amino-2hydroxymethylpropane-1,3-diol), and 10 mM glucose at a pH of 7·4. The medium was gassed with oxygen prior to use. Experiments were also carried out utilizing the medium employed by Whittam,[3] which differed from the above essentially in that the Tris was replaced by a sodium phosphate buffer, and in addition was calcium free. The slices were incubated for 30 min at 37·5 °C in Warburg vessels, after which 12–15 μc of P^{32} and ouabain to a final concentration of 10^{-5}M were added from the side arms. Incubation was continued for 60 min. Control slices containing P^{32} without ouabain were incubated for the same period.

At the termination of the experiment, the slices were removed, drained and homogenized in 0·32M sucrose containing 0·5 mM EDTA (ethylenediaminetetracetic acid) at pH 7·0 Aliquots were removed for ion determination by flame photometry and the remainder utilized for phosphoprotein phosphorus assay by the procedure of Schmidt and Thannhauser.[5] The method employed by Heald[6] was utilized to estimate the rate of incorporation of P^{32} into phosphoproteins, both in the whole tissue homogenate and in subcellular fractions of the tissue isolated by the procedure of Aldridge and Johnson.[7]

RESULTS AND DISCUSSION

The effect of ouabain on the potassium content of cerebral slices incubated either in the Tris medium or the phosphate medium was the same, the values being 46·5 μmoles $\pm$ 2·4 (s.e) K^+/g wet weight for the control tissues and 19·5 μmoles $\pm$ 3·8 K^+/g wet weight for the ouabain treated slices. This represents about a 60 per cent depression in potassium concentration which is quite similar to the values reported by Whittam.[3] Concomitant with the potassium loss, a marked increase in oxygen consumption was observed. Fig. 1 represents the rate of oxygen uptake by the slices of cerebral cortex before and after addition of ouabain. It is of interest that even after one hour of exposure to the drug, the increased rate of respiration is maintained. In nine experiments, the QO_2 (μmoles/g wet weight/hr) of the control slices was 60 $\pm$ 1·9, while that of the slices which were treated with the drug was 81 $\pm$ 3·4. This increase in oxygen uptake is higher than that reported by Yoshida *et al.*[2] and may be due to the fact that these authors incubated their tissue in air rather than oxygen. While there was no decrease in oxygen consumption noted after ouabain treatment in any of the experiments, it is possible that with longer incubation periods a depression might have been observed. Wollenberger[8] found such a diphasic action with ouabain or protoveratrine treatment, that is, first an increase in respiration which lasted for about an hour, and then a marked decrease. In two experiments utilizing the calcium-free phosphate medium of Whittam, the oxygen consumption before addition of the drug was 90 μmoles/g wet weight/hr. Only a slight increase (5–10 per cent) in oxygen uptake was observed after addition of ouabain. The higher metabolic rate of respiring cerebral tissues in the absence of calcium may mask the stimulating effect of ouabain. Whittam, under the conditions described above, reported a 50 per cent depression in oxygen consumption.

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The effects of ouabain on oxygen consumption, reported in the present study, and on the maintenance of potassium tissue concentration are thus divergent and do not support the conclusions of Whittam.

Since phosphoproteins may be involved in ion movements,[6, 9] it was of interest to examine the effect of ouabain on phosphoprotein content and turnover in cerebral slices. Similar studies on phosphatidic acid have recently been reported [2, 10] The marked loss in potassium content of the cerebral slice induced by ouabain was accompanied by some changes in the phosphoprotein phosphorus distribution and content (Table 1). There appeared to be a slight but significant depression in

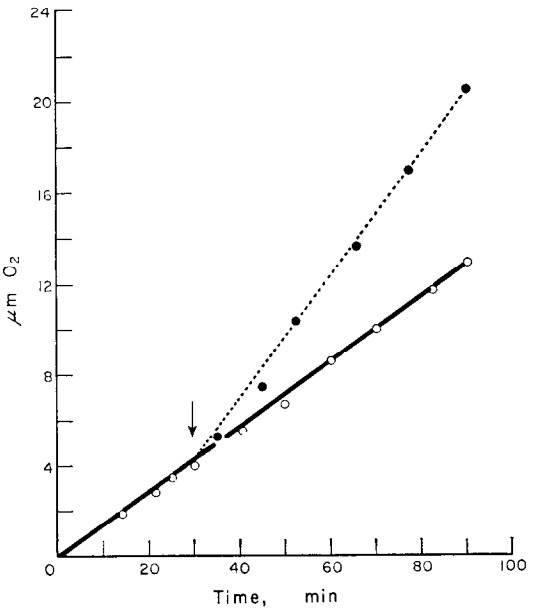

Fig. 1. The effect of ouabain on the rate of respiration of G.P. cerebral cortical slices
● — — — ● ouabain treated ○————○ control
The slices were incubated at 37·5 °C in Tris-saline medium, as described in the text. Ouabain was added after 30 min incubation (indicated by the arrow).

TABLE 1. THE EFFECT OF OUABAIN ON PHOSPHOPROTEIN OF GUINEA PIG CEREBRAL CORTICAL SLICES

Fraction	No. of expt.	Before ouabain: μmoles Pi‡/g fresh weight	Before ouabain: Specific radioactivity*	After ouabain: μmoles Pi‡/g fresh weight	After ouabain: Specific radioactivity*
Whole slice (homogenate)	5	0·670 ± 0·06 (S.E.)	533 ± 23	0·58 ± 0·06	530 ± 30
Nuclear	5	0·373 ± 0·04	441 ± 50	0·465 ± 0·02	501 ± 45
Mitochondrial	5	0·167 ± 0·05	414 ± 38	0·118 ± 0·02	442 ± 30
Supernatant-microsomal†	5	0·134 ± 0·01	319 ± 21	0·090 ± 0·03	264 ± 48

* counts per min μg Pi
† the post-mitochondrial supernatant
‡ Phosphoprotein phosphorus

After incubation, the brain slices were homogenized in ice cold 0·32 M sucrose containing 0·5 mM EDTA at pH 7·0. The various sub-fractions were obtained by centrifugation according to the procedure of Aldridge and Johnson.[7] The phosphoprotein content and P^{32} incorporation was determined as described in the text.

the level of phosphoprotein phosphorus in the whole tissue homogenate. This loss was reflected also in diminished radioactivity in the whole slice. However, the specific radioactivities (counts per min/μg Pi) of the phosphoproteins of both the homogenate and the subcellular fractions were not different from the control values (Table 1). This would suggest that the turnover of phosphoprotein phosphorus in the cerebral tissue was not altered by the treatment with ouabain. It is worth emphasizing that the phosphoprotein content and turnover were determined by measuring the release of inorganic phosphate after prolonged alkaline digestion of the protein. This procedure affords an estimate of the entire phosphoprotein fraction and does not preclude the possibility that some small fraction of the total phosphoprotein molecule may be involved in ionic movements which may, therefore, be susceptible to the action of drugs which affect ionic movements.

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Reversal of mitochondrial inhibition of glycolysis by styrylquinolines

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CERTAIN styrylquinolines have caused a marked regression in the growth of lymphoma 8 tumors in rats, but their successful therapeutic use has been hindered by their toxicity and the limited range of tumors affected.[1–3] The styrylquinolines have little resemblance in either structure or action to any of the known antitumor agents, and the only reports of their effects *in vitro* are those of Dickens[4] and Emmelot and co-workers,[5–7] who found that styrylquinolines inhibit cellular respiration and promote the breakdown of esterified phosphate.

One model system for investigating effects of drugs on energy metabolism is that of Aisenberg and colleagues,[8] in which some of the interactions of oxidative and glycolytic systems can be investigated. The glycolytic system is the supernatant fraction remaining after high speed centrifugation of a rat brain homogenate, and it readily utilizes glucose and produces lactic acid. The oxidative system is supplied by rat liver mitochondria. These mitochondria are not capable of coupling electron transport with oxidative phosphorylation, because they were isolated and incubated in KCl buffer systems. In this type of model system the addition of liver mitochondria to the supernatant fraction of brain depresses glycolysis. Presumably the effects of the mitochondria are related to their ability to carry out electron transport, since inhibitory effects are seen only under aerobic conditions and are reversed by the addition of electron transport inhibitors such as cyanide or antimycin A.[8]

The glycolytic system was prepared by centrifugation of a 30% homogenate of rat brain in 0·154 M KCl containing 0·02 M nicotinamide for 40 min at 76,000 g; 0·67 ml of the clear supernatant fraction was added to each flask. Mitochondria were prepared by differential centrifugation of a