Effects of Potassium Ions on Brain Respiration and Aminoacid Incorporation into Brain Proteins *in vitro*

The stimulating effects of potassium ions on brain respiration were shown many years ago by ASHFORD and DIXON[1] and DICKENS and GREVILLE[2]. This stimulation, which seems greatly to resemble that brought about by oscillating electric pulses, is highly sensitive to drugs that have no demonstrable effect on the unstimulated respiration in the presence of glucose[3].

In the course of researches on the metabolism of brain cortex slices from hypoxic rats, preliminary experiments have been carried out, in an attempt to gain a better understanding of the relationship between stimulation of brain respiration by potassium ions and incorporation of 1-^{14}C-glycine into the proteins of brain cortex slices incubated *in vitro*.

All the experiments have been carried out on brain cortex slices from albino rats of either sex, weighing about 200 g. The slices were incubated in a conventional Warburg apparatus, at 37°C, the gas phase being air in some experiments and oxygen in others. Different phosphate salines, having respectively the following compositions, were used: (A) $1{\cdot}28\times10^{-1}$ *M* NaCl; $1{\cdot}35\times10^{-2}$ *M* KCl; $1{\cdot}94\times10^{-3}$ *M* $CaCl_2$; $6{\cdot}8\times10^{-4}$ *M* $MgSO_4$; 1×10^{-2} *M* sodium phosphate buffer, pH 7·2; 2×10^{-2} *M* glucose. (B) $2{\cdot}0\times10^{-2}$ *M* NaCl; $1{\cdot}2\times10^{-1}$ *M* KCl; the other components as in (A). (C) $1{\cdot}28\times10^{-1}$ *M* NaCl; $1{\cdot}2\times10^{-1}$ *M* KCl; the other components as in (A). Phosphate salines A and B had a final concentration of 0·319 os*M*/l; phosphate saline C had a final concentration of 0·531 os*M*/l.

The incorporation of 1-^{14}C-glycine into the proteins of brain cortex slices was studied as described in previous papers[4,5], using phosphate salines A and B as incubation mediums.

From the data given in Table I, it can be seen that the stimulation of brain respiration induced by potassium ions in the presence of glucose takes place only if the slices are incubated in oxygen ($P < 0{\cdot}01$), and not if they are incubated in air ($P > 0{\cdot}05$). When the slices are incubated in oxygen, the respiration is significantly increased if the slices are incubated in the presence of large quantities of potassium, even if large amounts of sodium are present in the medium, which is therefore hypertonic ($P < 0{\cdot}01$). If large quantities of potassium are present in the medium, it is not possible to see any difference due to a different sodium concentration in the phosphate saline ($P > 0{\cdot}05$).

The results of the experiments on the incorporation of 1-^{14}C-glycine into brain proteins are given in Table II. It can be seen that the incubation of brain cortex slices in a potassium-rich phosphate saline, i. e. in a medium which induces an increase in the respiration of the slices, does not induce a similar increase in amino acid incorporation. On the contrary, this is markedly inhibited.

Our observations are in keeping with the results obtained by several workers[6-8] with different biological materials, which however are not responsive to the respiratory stimulation by potassium ions. It had been reported by ROSSITER[9] that an increased concentration of potassium ions provokes an increase of oxygen uptake by cat brain sections, and a diminution of the incorporation of ^{32}P into organic phosphorus components, as represented by lipid phosphorus, ribonucleic acid, and phosphoprotein. It must therefore be kept in mind that the stimulation of the respiration of brain cortex slices induced by potassium ions can be uncoupled from energy-requiring reactions, such as phosphorylation in the experiments of ROSSITER, or glycine incorporation into proteins, as demonstrated in the present paper.

Tab. I. Oxygen consumption by rat brain cortex slices, suspended in different phosphate salines in the presence of 2×10^{-2} *M* glucose. Results expressed as Q_{O_2}. Means of 6 experiments ± s. e. m.

Phosphate saline	A	B	C
Gas phase: air	8·2 ± 0·31	8·1 ± 0·47	8·3 ± 0·38
Gas phase: oxygen	13·8 ± 0·60	16·4 ± 0·32	16·8 ± 0·36

Tab. II. Incorporation of 1-^{14}C-glycine into the proteins of rat brain cortex slices, incubated in two different phosphate salines, for 1 h, in the presence of 2×10^{-2} *M* glucose. Gas phase, oxygen. Means of 6 experiments ± s. e. m.

Phosphate saline	A	B
c. p. m./mg protein	65·4 ± 2·5	20·8 ± 2·1

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Riassunto

È stata studiata l'azione degli ioni potassio sulla respirazione di sezioni di corteccia cerebrale di ratto e sull'incorporazione di glicina-1-C^{14} nelle proteine di tali sezioni. Il potassio, che stimola la respirazione delle sezioni se queste sono incubate in ossigeno in presenza di glucosio, inibisce invece l'incorporazione di glicina-1-C^{14} nelle proteine delle sezioni stesse.

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PRO EXPERIMENTIS

A New Technique for the Fluorescent Labelling of Proteins

COONS'[1] technique of labelling antibody with fluoresceine has proved to be a useful research tool in immunochemistry, virology, and bacteriology and has become a

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