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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[9] R. ROSSITER, *Metabolism of the Nervous System* (ED. D. RICHTER, Pergamon Press, London 1957), p. 355.

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

[1] A. H. COONS, H. J. CREECH, and R. N. JONES, Proc. Soc. exp. Biol. Med. *47*, N. Y. 200 (1941).

valuable laboratory aid in the rapid identification of pathogenic and non-pathogenic microorganisms[2]. Major obstacles to a more widespread use of this procedure have been the laborious preparation of the labelling dye and its instability, the problem of protein denaturation due to organic solvents used in the labelling reaction and insufficient labelling of the protein. These difficulties have largely been overcome by the use of more stable fluorescent labelling agents such as fluoresceinisothiocyanate[3], the modified method of conjugation with the aid of filter paper[4] or the use of the solid labelling agents without organic solvents[5]. However, none of these techniques is entirely free of disadvantages. Thus, some fluorescent dyes are not readily prepared in solid form; others may be too insoluble in organic solvents for convenient preparation of filterpaper strips containing the desirable concentration of dye or they may be too reactive to remain stable on cellulosic materials for prolonged periods of time.

We believe to have found an alternative method which affords great convenience in preparing a variety of fluorescent labelling materials and incorporates all the advantages of the above mentioned modifications of COONS' original technique. The procedure used in our laboratories consists of adsorption of the labelling dye onto specially dried diatomaceous earth (Celite, hyflo super-cel, Johns-Manville Co.) or the like and is reported below in detail. Such 'fluorescent Celite powders' are more conveniently prepared and stored in quantity than impregnated filter paper and are easier to dispense than the latter or the minute quantities of solid dye required for fluorescent labelling. Their tremendously large reactive surface permits drastic reduction of the reaction time; 30 min or less have been found adequate for satisfactory labelling of protein solutions compared with 12 to 24 h used in the older techniques.

Materials and Methods. The following three fluorescent dyes were investigated: (1) 5-dimethylaminonaphthalenesulfonylchloride[6], (2) Lissamine Rhodamine B 200 (sulfonylchloride)[6], and (3) fluoresceinisothiocyanate[6]. The preparation of our labelling agents is illustrated in the following example. 2 g of Lissamine Rhodamine B 200 (red fluorescence) were suspended in a solution of 4 g phosphorus pentachloride in 40 ml ethyleneglycol dimethylether (dried over calcium hydride) and heated under reflux for 30 min. The solvent and phosphorus oxychloride were then removed in a rotary evaporator under reduced pressure, the excess phosphorus pentachloride extracted three times with carbontetrachloride and the Rhodamine B 200-chloride dissolved in 150 ml dry chloroform. After filtration from insoluble material the chloroform solution was added to 15 g of Celite (hyflo super-cel) which had been dried by heating at 300°C for 10 min. The chloroform was then distilled under reduced pressure in a rotary evaporator and traces of solvent removed from the residual powder with a high vacuum pump. Such preparations, when stored at room temperature in a tightly closed container retained satisfactory labelling activity for at least one year (longest period tested). The amount of active labelling dye on the Celite was assayed colorimetrically by extraction with specially dried chloroform and alkaline hydrolysis of the extract. The readings obtained with the hydrolysate were compared with a standard curve prepared with Lissamine Rhodamine B 200 (sodium salt). Similar fluorescent labelling powders were prepared in this way from 5-dimethylaminonapthtalenesulfonylchloride and fluoresceinisothiocyanate.

For the labelling tests 10–40 mg of 'fluorescent Celite powder' was added to 3 ml of rabbit serum and 3 ml of 0·05 *M* sodium carbonate-bicarbonate buffer (pH 9) in a centrifuge tube. The mixture was shaken mechanically for 30 min and centrifuged. The supernatant was dialysed for three days against running water and subjected to paper-strip electrophoresis. Satisfactory labelling of the proteins was observed in each case.

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H. RINDERKNECHT

California Corporation for Biochemical Research, Los Angeles (Calif.), February 1, 1960.

Zusammenfassung

Es wird eine neue Arbeitsweise zur Fluoreszenzmarkierung von Proteinen beschrieben unter Verwendung auf Celite adsorbierter Fluoreszenzfarbstoffe. Die neue Methode bietet gegenüber andern heute gebräuchlichen Verfahren bedeutende Vorteile.

[2] For review of the pertinent literature see H. UEHLEKE, Z. Naturforsch. *13b*, 722 (1958).

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PRO LABORATORIO

Eine einfache Apparatur zur serienweisen Redoxtitration

Für redoxpotentiometrische Titrationen von säulenchromatographisch aufgetrennten Stoffen (Polyphenole, Askorbinsäure, Redoxfarbstoffe) ist eine Schnellmethode vorteilhaft, da meist mit einer grösseren Anzahl von Fraktionen gerechnet werden muss. Die nachstehend beschriebene Apparatur erlaubt in kurzer Zeit eine grosse Anzahl von Fraktionen zu titrieren.

Im linken Tubus befindet sich das Vergleichshalbelement (zum Beispiel Chinhydron) mit einer Platinelektrode. Es steht über eine KCl-Agar-Brücke (KA) und eine Glasfritte (F) mit dem Titrationsgefäss in Verbindung, in das eine zweite Elektrode eintaucht. Diese wurden nach dem von BIRNSTIEL[1] angegebenen Verfahren hergestellt. Quecksilber (2) gewährleistet die Verbindung zwischen dem Kupferdraht (1) und dem ins Glasrohr eingeschmolzenen Platindraht (3) vom Durchmesser 1 mm. Diese Elektroden münden schräg von der Seite her ein, damit die obere Tubusöffnung zum Einbringen der zu titrierenden Fraktion und der Titerlösung frei bleibt. Zur Gewährleistung steter Mischung während der Titration wird durch den Stutzen A ständig reinster Stickstoff zugeführt. Nach erfolgter Titration kann die Zelle mittels Wasserstrahlvakuum (V) ebenfalls über den Stutzen A geleert und gespült werden, wonach sie zur Aufnahme der nächsten Fraktion bereit ist. Auf die gleiche Weise kann auch der linke Tubus gereinigt werden. Die Grösse des Titrationsgefässes ist für Fraktionen von 7–20 ml berechnet.

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