STUDIES ON PERMEABILITY IN RELATION TO NERVE FUNCTION*

IV. EFFECT OF GLUTAMATE AND ASPARTATE UPON THE RATE OF ENTRANCE OF POTASSIUM INTO BRAIN CORTICAL SLICES

by

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INTRODUCTION

The ionic concentration gradients between the inside of the nerve cells and its outside are generally considered as the energy source of the action potentials propagating nerve impulses. The maintenance of these gradients and the restoration following activity requires metabolic energy. It has been reported that both glucose and L-glutamate must be present in the medium, in aerobic conditions, to prevent loss of potassium from slices of rabbit and guinea pig brain cortex[1]. When present alone, neither glucose nor L-glutamate prevented the loss. Glutamate could be replaced by aspartate, but not by other amino acids.

In this paper observations are reported in which the rate of entry of potassium into slices of rabbit brain cortex has been measured. The effect of addition of L-glutamate and aspartate to the medium upon this rate has been tested.

METHODS

The preparation of rabbit cortical slices by means of the Stadie-Riggs slicers has been described in the preceding communication[2]. Slices weighing about 50 to 70 mg wet weight, were kept in oxygenated Krebs-Ringer solution for 25 min in order to adjust the cells to the medium. The solution had the following composition: 0.9% sodium chloride – 110 parts; 1.15% potassium chloride – 4 parts; 1.22% $CaCl_2$ – 3 parts; 2.11% KH_2PO_4 – 1 part; 3.82% $MgSO_4H_2O$ – 1 part; 1.3% $NaHO_3$ – 3 parts; 12% Na_2HPO_4 buffer – 3 parts; 5.4% glucose – 5 parts[3]. Temperature 23°.

When required the pH of the solution was adjusted by varying the ratio of mono- to di-basic sodium phosphate of the buffer. At pH 5.0 the solution was virtually unbuffered. The pH of the solutions used for the incubation was checked after the experimental run and was found not to have varied significantly.

After 25 min pre-exposure the tissue was transferred to a solution of the same composition, in which, however, part of the potassium was replaced by a radioactive species, ^{42}K. At various times tissue slices were removed, quickly washed by dipping 3–4 times into a series of Krebs-Ringer solution without isotopes or isotonic NaCl, blotted lightly and plated. The tissue was dried under an infrared lamp and then counted. Counts are reported in numbers per mg dry tissue per 100 seconds.

In the experiments designed to study the effects of pH the tissue slices were again pre-exposed for 25 min and then transferred into the test solution of identical pH but containing ^{42}K. After 20 min incubation the tissue slices were removed and treated as above.

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In the experiments investigating the effect of L-glutamate or aspartate in 0.03 *M* concentration on the rate of entry of ^{42}K, these amino acids were added to the media. Isotonicity was maintained by corresponding reduction of the concentration of NaCl. Again, pre-exposure time was 25 min and the incubation time 20 min at 23°.

Oxygenation of the solutions was continued throughout the experimental procedure.

RESULTS

The rate of entry of ^{42}K into cortical tissue slices is seen in Fig. 1. After 35 min equilibrium was attained between the concentration of ^{42}K in the cell and in the surrounding fluid. The slope of the curve at the 20 min interval exhibits a rate of change sufficiently rapid to manifest notable differences in the velocity of penetration of ^{42}K into the slice under varying conditions. For this reason experiments were run for 20 min and the amount of ^{42}K in the slice measured.

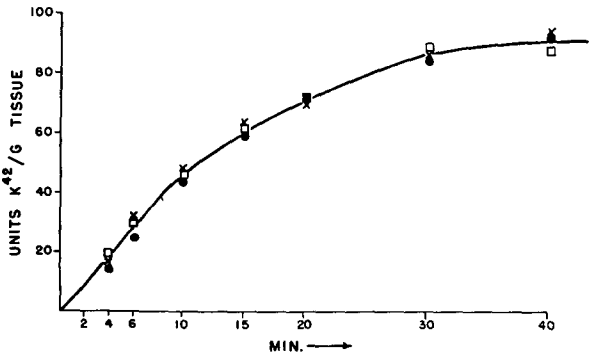

Fig. 1

Effect of pH. Cortical slices which were exposed to solution of pH 5.0, 5.7, 7.0 and 8.0 for 25 min prior to incubation as well as during the 20 min of incubation did not show differences in the rate of penetration of ^{42}K. Each figure in the Table represents the average of 3 slices:

TABLE I

pH	*Counts/mg/100 sec.*
8.0	3180
7.0	3433
5.7	3680
5.0	3406

TABLE II

Control	*Glutamate*	*Aspartate*
8966	9000	—
8250	—	8500

Effect of L-*glutamate and aspartate.* Slices bathed in L-glutamate or aspartate and then incubated in the presence of these amino acids did not reveal any effect upon the rate of entry of ^{42}K due to these dicarboxylic amino acids, as seen in Table II. Each figure represents again an average value of 3 determinations.

DISCUSSION

Changes of pH of the medium over a wide range did not affect the rate of entry of ^{42}K into the cortical slices of rabbit. Although the internal pH of the tissue slices

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was not measured, it seems fair to assume that the processes responsible for the inward diffusion of K ions are not particularly sensitive to variation of pH. Similar experimental results were obtained in studies of the isolated giant axon of Squid (WILSON unpublished experiments).

The absence of an effect on the rate of penetration of K by L-glutamate or aspartate is puzzling in view of the work of KREBS *et al.* indicating the importance of these amino acids in maintaining the cellular concentration of K in the brain[1]. One might, therefore, expect an accelerated rate of K entry in the presence of L-glutamate or aspartate. However, the total tissue K was not determined in the present experiments. It is therefore possible, and our experiments do not rule out the possibility, that in the presence of glutamate, the rate of inward diffusion may be unchanged, but the final equilibrium may be attained at a significantly higher concentration of intracellular K. Another possibility is the assumption that inward and outward diffusion of K ions are to a certain extent independent of each other and that glutamate causes the retention of intracellular K, but affects less markedly, if at all, the rate of entrance. This, however, does not seem kinetically a likely explanation.

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SUMMARY

Rabbit cortical slices maintained in oxygenated Krebs-Ringer solution were exposed to ^{42}K. The rate of penetration of this cation was determined and equilibrium between medium and the inside of the cell was noted to be attained in 35 minutes. Variations in pH of the external milieu between pH 5.0–8.0 did not affect the rate of ^{42}K entry into the cortical cells. The presence of L-glutamate and aspartate in the medium likewise did not modify the rate of ^{42}K penetration. The interpretation of the latter experiments offers some problems in view of certain results of previous workers, but several explanations are suggested.

RÉSUMÉ

Des tranches de cortex de lapin maintenues en solution Krebs-Ringer oxygénée ont été exposées à ^{42}K. Nous avons déterminé la vitesse de pénétration et avons constaté que l'équilibre entre le milieu ambiant et l'intérieur de la cellule est atteint en 35 minutes. Les variations du pH du milieu de 5.0 à 8.0 n'avaient pas d'effet sur la vitesse de pénétration de ^{42}K dans les cellules corticales. De même, la présence d'acide L-glutamique et d'acide aspartique dans le milieu n'affectait pas cette vitesse. L'interprétation de ces dernières expériences offre quelques difficultés en vue de certains résultats obtenus par d'autres chercheurs, mais des explications de ce désaccord sont présentées.

ZUSAMMENFASSUNG

Cortex-Scheiben (Kaninchen) in einer Krebs-Ringer Lösung wurden in ^{42}K-haltige Lösungen getaucht. Die Geschwindigkeit, mit welcher dieses Kation in die Zelle eindringt, wurde bestimmt und festgestellt, dass das Gleichgewicht zwischen dem Milieu und dem Zellinnern in 35 Minuten erreicht ist. pH-Variationen von 5.0 bis 8.0 in der Lösung hatten keinen Einfluss auf die gemessene Geschwindigkeit von ^{42}K. Ebenso waren L-Glutaminsäure und Asparaginsäure im Milieu ohne Einfluss. Die Interpretation dieser letzten Versuche ist etwas schwierig im Hinblick auf gewisse Resultate anderer Forscher; hierfür werden mehrere Erklärungen vorgeschlagen.

REFERENCES

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