Effect of Ammonium and Other Ions on the Glucose-Dependent Active Transport of L-Histidine in Slices of Rat-Brain Cortex

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(Received 24 July 1964)

1. The influence of cations on the active transport into cells of rat-brain-cortex slices of L-histidine, an amino acid that is not metabolized by this tissue, has been studied. 2. Like other amino acids, L-histidine accumulated in the cells in the presence of glucose in concentrations up to over double that in the incubation medium. 3. The active transport of L-histidine was highest in a medium containing Ca^{2+} (3mM). The addition of K^+ (27mM) led to a marked decrease in the intracellular concentration of L-histidine, though the oxygen uptake of the slices was higher. 4. The active L-histidine transport was inhibited by NH_4^+ . The inhibitory effect increased with the NH_4^+ concentration, being about 25% at 8mM, 65% at 20mM, and 90% at 27 and 50mM. The oxygen uptake of the brain slices was depressed by only 25% by the highest NH_4^+ concentration used, and less by lower concentrations.

Brain tissue slices, in the presence of glucose, can accumulate amino acids to concentrations considerably above those present in the incubation medium (Stern, Eggleston, Hems & Krebs, 1949; Guroff, King & Udenfriend, 1961; Abadom & Scholefield, 1962a; Sellinger, Catanzaro, Chain & Pocchiari, 1962).

This phenomenon, referred to in the present paper for simplicity as 'active transport' of amino acids (without implying any particular mechanism but with the understanding that it is dependent on the intact hexose molecule) was shown by several workers to be influenced by inorganic ions (Takagaki, Hirano & Nagata, 1959; Guroff *et al.* 1961; Abadom & Scholefield, 1962a; Tsukada, Nagata, Hirano & Matsutani, 1963; Lahiri & Lajtha, 1964).

All investigators agreed that Na^+ was essential and that Ca^{2+} exerted a stimulating effect; K^+ has a stimulating effect on the transport of glycine (Abadom & Scholefield, 1962a), but, in low concentrations, does not influence the transport of L-tyrosine (Guroff *et al.* 1961), L-lysine and DL-aminoisobutyric acid (Lahiri & Lajtha, 1964), whereas in high concentrations it exerted an inhibitory effect on the latter.

The effect of NH_4^+ on active transport of amino acids in brain tissue does not seem to have been studied. The present authors considered the problem worth investigating because this ion was shown to be actively involved in the carbohydrate metabolism of brain tissue; it provides substantially the nitrogen for the main amino acids, glutamic acid

and aspartic acid, which in this tissue are formed from glucose (Chain, Chiozzotto, Pocchiari, Rossi & Sandman, 1960a).

The present paper describes the effect of NH_4^+ on the active transport of L-histidine. This amino acid was chosen because it was found not to be metabolized by brain tissue. Neame (1961) showed that L-histidine was actively transported in brain slices in the presence of glucose. The effects of Na^+ , Ca^{2+} and K^+ on active histidine transport were also studied and compared with those reported in the literature on other amino acids.

MATERIALS AND METHODS

L-[ring-2- ^{14}C]Histidine was obtained from The Radiochemical Centre, Amersham, Bucks. The radioactive material was diluted with non-radioactive substrate to give a specific activity of 0.13 $\mu\text{C}/\mu\text{mole}$.

Adult rats of the Wistar strain bred in this Institute were stunned and decapitated. The brains were removed and placed on crushed ice. Two slices were made from each hemisphere, weighed on a torsion balance and placed in the corresponding Warburg vessels kept on crushed ice containing 1.2ml. of incubation medium. Usually about 90–100mg. wet wt. of cerebral-cortex slices was used for each vessel. Incubation was carried out at 37.5° for 90 min., in an atmosphere of O_2 , by the standard manometric techniques.

Elliott & Henderson (1948) incubation medium (composition: NaCl, 98mM; KCl, 27mM; MgSO_4 , 1.2mM; KH_2PO_4 , 4mM; Na_2HPO_4 , 17.5mM) was used.

L-Histidine was used at a concentration of 6.45mM; glucose, when present, was at a concentration of 5.55mM.

Modifications of the ionic composition of the medium

included the substitution of K^+ by Na^+ , and (or) the addition of $CaCl_2$ (3mM), and the substitution of $NaCl$ by $LiCl$ or choline chloride. In experiments where NH_4Cl replaced KCl , the required amount of $NaCl$ was added to the medium to make up for its usual molarity. In every case the pH of the medium was kept at 7.4, and the solutions were always iso-osmolar.

Incubation was stopped by placing the Warburg vessels on crushed ice. The brain slices were removed, washed three times in the appropriate medium, extracted with 2ml. of 60% (v/v) ethanol in a Teflon-glass homogenizer and centrifuged, and the residue was washed twice with 9ml. of 60% ethanol and recentrifuged. The supernatants from the extraction and both washings were pooled and concentrated to 5.0ml., and a 1ml. sample was plated on aluminium planchets, dried, and counted under an end-window Geiger-Müller counter. Results are expressed as μ moles of L-histidine/100 mg. wet wt. of tissue.

RESULTS

Fate of L-[ring-2- ^{14}C]histidine in rat-brain-cortex slices. With the quantitative paper-radiochromatography technique described by Frank, Chain,

Pocchiari & Rossi (1959) it was found that histidine is practically not metabolized by rat-brain-cortex slices under the experimental conditions here employed.

Effects of K^+ and Ca^{2+} ions on the active transport of histidine. Table 1 summarizes the values obtained for the active transport of histidine in the presence of 27mM- K^+ and 3mM- Ca^{2+} in the incubation medium and in the absence of these ions. The maximal concentration of histidine in brain slices was obtained in a K^+ -free Ca^{2+} medium. The addition of K^+ to the medium decreased this value, whether Ca^{2+} was present or not. Thus the net transport value was decreased by K^+ from 1.81 to 1.13 in the absence of Ca^{2+} , and from 2.7 to 1.87 in the presence of 3mM- Ca^{2+} , in agreement with the findings of previous authors (Ashford & Dixon, 1935; Dickens & Greville, 1935; Kimura & Niwa, 1953; Kini & Quastel, 1959; Chain, Larsson & Pocchiari, 1960b; Quastel, 1962); the oxygen uptake by brain slices, with glucose as substrate, was stimulated by K^+ and decreased by Ca^{2+} (Table 1).

Table 1. *Effects of K^+ and Ca^{2+} on the oxygen uptake and active transport of L-histidine by rat-brain-cortex slices*

The results are expressed as μ moles of intracellular histidine and μ moles of oxygen consumed/100 mg. of tissue after 90 min. incubation in 1.2 ml. of phosphate medium, pH 7.4, at 37° in an atmosphere of O_2 with L-[ring-2- ^{14}C]histidine (6.45 mM), glucose (5.55 mM) and Ca^{2+} (3mM) or K^+ (27mM). Mean values $\pm$ S.E.M. are given, with the numbers of experiments in parentheses.

K ⁺ and Ca ²⁺ in incubation medium			Intracellular histidine (μ moles/100 mg. of tissue)			O ₂ uptake (μ moles/100 mg. of tissue/hr.)		
K ⁺	Ca ²⁺		No glucose	With glucose	Difference (transport)	No glucose	With glucose	Difference
+	—	(10)	0.70 $\pm$ 0.02	1.83 $\pm$ 0.09	1.13	6.9 $\pm$ 0.1	13.7 $\pm$ 0.5	6.8
—	—	(5)	0.67 $\pm$ 0.09	2.48 $\pm$ 0.18	1.81	5.6 $\pm$ 0.2	10.5 $\pm$ 0.2	4.9
+	+	(6)	0.69 $\pm$ 0.02	2.56 $\pm$ 0.10	1.87	5.8 $\pm$ 0.2	11.9 $\pm$ 0.6	6.1
—	+	(8)	0.88 $\pm$ 0.04	3.58 $\pm$ 0.09	2.70	5.5 $\pm$ 0.2	9.4 $\pm$ 0.2	3.9

Table 2. *Effect of replacement of Na^+ by Li^+ or choline on oxygen uptake and active transport of L-histidine by rat-brain-cortex slices*

Experimental conditions were as given in Table 1. The concentration of Na^+ in the complete medium was 98 mM. The concentration of Li^+ or choline replacing Na^+ was also 98 mM. Mean values $\pm$ S.E.M. are given, with the numbers of experiments in parentheses.

Replacement of Na ⁺ by Li ⁺ or choline in incubation medium			Intracellular histidine (μ moles/100 mg. of tissue)			O ₂ uptake (μ moles/100 mg. of tissue/hr.)		
Na ⁺	Li ⁺	Choline	No glucose	With glucose	Difference (transport)	No glucose	With glucose	Difference
+	—	—	0.70 $\pm$ 0.02 (10)	1.83 $\pm$ 0.09 (11)	1.13	6.9 $\pm$ 0.1	13.7 $\pm$ 0.5	6.8
—	+	—	0.44 $\pm$ 0.04 (6)	1.00 $\pm$ 0.04 (6)	0.56	4.4 $\pm$ 0.2	14.4 $\pm$ 0.5	10.0
—	—	+	0.58 $\pm$ 0.04 (3)	1.08 $\pm$ 0.03 (3)	0.50	6.3 $\pm$ 0.1	13.4 $\pm$ 0.6	7.1

Table 3. *Effect of NH_4^+ on the oxygen uptake and active transport of L-histidine in rat-brain-cortex slices*

Experimental conditions were as given in Table 1. The concentration of NH_4^+ replacing K^+ was 27 mM. Mean values $\pm$ S.E.M. of five experiments are given.

Intracellular histidine ($\mu\text{mole}/100\text{ mg. of tissue}$)			O_2 uptake ($\mu\text{moles}/100\text{ mg. of tissue/hr.}$)		
No glucose	With glucose	Difference (transport)	No glucose	With glucose	Difference
0.59 ± 0.03	0.61 ± 0.05	0.02	5.0 ± 0.4	9.5 ± 0.7	4.5

Fig. 1. Effect of concentration of NH_4^+ on the active transport of L-histidine (O) and O_2 uptake (●) in rat-brain-cortex slices. Experimental details are given in the text.

Effect of Na^+ ions on the active transport of histidine. Substitution in the incubation medium of Na^+ by choline or Li^+ resulted in a decrease of the active transport of histidine (Table 2).

Effect of NH_4^+ ions on the active transport of histidine. Substituting 27 mM- NH_4^+ for K^+ or Ca^{2+} in the incubation medium caused a strong inhibition of the active transport of histidine (Table 3). The inhibitory effect of NH_4^+ also manifested itself in the presence of 27 mM- K^+ . It increased with increasing NH_4^+ concentration: there was 25% inhibition of active transport with 8 mM- NH_4^+ , 65% with 20 mM, and 90% with 27 and 50 mM (Fig. 1).

The rate of oxygen uptake was only slightly affected at the highest NH_4^+ concentration (27 mM) at which transport inhibition was complete, though at the highest concentration (50 mM) tested there was an inhibition of about 25% (Fig. 1 and Table 3.)

DISCUSSION

The influence of cations on the active transport of amino acids into brain tissue slices, found by several groups of workers, has been confirmed by the present authors, using histidine.

It has been found that Na^+ is essential for the transport of glutamate (Takagaki *et al.* 1963), glycine (Abadom & Scholefield, 1962a), D- and L-leucine, L-cycloleucine, D- and L-lysine and α -aminoisobutyrate (Lahiri & Lajtha, 1964). In agreement with these results it has now also been found essential for the active transport of L-histidine. In this process Li^+ only partially substituted for Na^+ , as it was reported to do for the active transport of L-lysine, L-leucine, L-cycloleucine and α -aminoisobutyrate (Lahiri & Lajtha, 1964). The same applies to the choline ion, which had previously been shown to substitute only partially for Na^+ in the active transport of glycine (Abadom & Scholefield, 1962b), L-lysine and α -aminoisobutyrate (Lahiri & Lajtha, 1964).

Also, Ca^{2+} , again in agreement with what was found by other authors for the active transport of glycine (Abadom & Scholefield, 1962b), L-tyrosine (Guroff *et al.* 1961) and α -aminoisobutyrate (Lahiri & Lajtha, 1964), stimulated the active transport of histidine, and the highest intracellular concentrations of histidine in brain tissue were obtained in a medium containing only Ca^{2+} (3 mM) in addition to Na^+ .

At 27 mM K^+ exerted a strong inhibitory effect on histidine transport, in the presence as well as in the absence of Ca^{2+} . In this respect the behaviour of this ion in the active transport of L-histidine differed from its effect in the active transport of glycine, which has been reported to be stimulated by K^+ (Abadom & Scholefield, 1962a). At 5 mM K^+ was stated to have no effect on the active transport of L-tyrosine (Guroff *et al.* 1961), L-lysine, L-leucine and α -aminoisobutyric acid (Lahiri & Lajtha, 1964), but at higher K^+ concentrations (59 and 105 mM) an inhibitory effect was observed.

As is well known, K^+ stimulates the oxygen

uptake of brain slices in the presence of glucose, and Ca^{2+} inhibits it (Quastel, 1962); these observations were confirmed (Table 1). Thus, under the experimental conditions employed in the present study, K^+ and Ca^{2+} appear to influence oxygen uptake and the active transport of L-histidine in opposite directions.

The presence of NH_4^+ in the incubation medium caused a strong inhibition of the active transport of L-histidine, both in the absence and the presence of 27 mM-K^+ , the inhibitory effect of NH_4^+ increasing with its concentration. As the oxygen uptake of the brain slices was only slightly decreased in the presence of the highest NH_4^+ concentration bringing about complete inhibition of amino acid transport it is clear that the presence of glucose in incubation media of brain slices and the resulting high aeration rate with presumably concomitant high ATP production are not sufficient to maintain active transport of L-histidine, but this process can be interfered with or even completely abolished by factors such as the simple NH_4^+ . The values for active transport of amino acids in brain slices do not, in fact, seem to be correlated in any way with those of oxygen uptake.

This work was supported by U.S. Public Health Service Grant no. NB04266-02 to E.B.C. from the National Institutes of Health, Bethesda, Md., U.S.A.

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