The Uptake and Efflux of L-Phenylalanine and L-Tyrosine from Rat Brain Cerebral Cortex Slices

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The uptake of radioactive L-phenylalanine and L-tyrosine into the tissue water of rat brain cerebral cortex slices was shown to have saturation kinetics. The apparent K_m for the uptake of L-phenylalanine was 0.86 mM and for L-tyrosine was 1.64 mM; for phenylalanine the apparent V_{max} value was 0.64 $\mu\text{mol/min}$ per ml of tissue water, and for L-tyrosine it was 0.98 $\mu\text{mol/min}$ per ml of tissue water. The accumulation of the two amino acids by the tissue was depressed in the absence of O_2 , at 0°C, or in the presence of metabolic inhibitors. The influxes and effluxes of both L-isomers were more rapid than those of the D-isomers. Competition between these two amino acids and each with L-tryptophan in respect of uptake into tissue water was shown. Their rates of influx were faster, and rates of efflux were slower, in the presence than in the absence of Na^+ . It was concluded that these amino acids were taken up by active transport via a carrier mechanism.

The uptake of amino acids into mammalian cerebral slices is influenced by the availability of substrate (Abadom & Scholefield, 1962; Barbosa *et al.*, 1968), Na^+ and K^+ (Stern *et al.*, 1949; Tsukada *et al.*, 1963; Lahiri & Lajtha, 1964), the charge and site of entry of particular amino acids (Blasberg & Lajtha, 1965), the presence of other amino acids (Levi *et al.*, 1966; Joanny *et al.*, 1971) and the presence of inhibitors (Mase *et al.*, 1962; Cherayil *et al.*, 1967). There have been relatively few kinetic studies concerned with the mechanism of transport (Neame, 1964; Blasberg *et al.*, 1970; Joanny *et al.*, 1971). The present paper is an examination of the uptake by cerebral tissue of phenylalanine and tyrosine.

Methods

Wistar rats weighing 200–250 g were used. The methods have been previously described in detail (Joanny *et al.*, 1971). The tissue water was calculated as the difference between the fresh weight and the weight after dehydration for 12 h at 105°C. L-[2,3- ^{14}C]Phenylalanine (200–5000 mCi/mmol) and L-[2,3- ^3H]tyrosine (250–3000 mCi/mmol) were obtained from The Radiochemical Centre, Amersham, Bucks., U.K. They were dissolved to give 0.01 mCi/100 ml of incubation medium in the presence of 1 mM non-radioactive compound. Samples were mixed with 10 ml of the liquid scintillator of Bray (1960) and counted on a Packard 2003 TriCarb liquid-scintillation spectrometer, with external standards to correct for quenching.

Krebs–Ringer bicarbonate–glucose medium, bubbled with $\text{O}_2 + \text{CO}_2$ (95:5), was used as the ‘normal’ incubation medium in the experiments, except where a modification is stated; it had the following composition (mM): NaCl 124, KCl 5, KH_2PO_4 1.24, MgSO_4 1.3, CaCl_2 2.8, NaHCO_3 26 and glucose 10. When tissue was incubated in a Na^+ -free medium, the NaCl was replaced by Tris–HCl and the NaHCO_3 by KHCO_3 . When the effect of different concentrations of Na^+ or K^+ was being studied, the NaCl and KCl of the above medium were partially replaced by different concentrations of Tris or KCl, so that the concentrations of $\text{Na}^+ + \text{Tris}^+$ or of $\text{Na}^+ + \text{K}^+$ were always 150 mM, and all the other constituents were kept constant at the concentrations indicated here. All amino acids used were L-isomers unless otherwise stated. The ‘initial’ rates of uptake of amino acids were measured during the first 3 min after the addition of a particular amino acid.

Results

Uptake of amino acids

Accumulation in slices. The accumulation of amino acids in cerebral slices in Krebs–Ringer medium to which low concentrations of phenylalanine or tyrosine had been added was relatively much greater than when the amino acids were present in high concentrations in the medium, and seemed to fall exponentially (Fig. 1). The L-isomers were accumulated in the slices more rapidly and to significantly higher concentrations than the D-isomers; in a medium containing

Fig. 1. Accumulation of phenylalanine or tyrosine in the tissue water of rat cerebral cortex slices

The slices were incubated for 30 min in Krebs–Ringer bicarbonate–glucose saline containing different concentrations of (a) L-phenylalanine or (b) L-tyrosine. In this and all other figures, each value is the mean $\pm$ S.D. of at least six slices.

Fig. 2. Lineweaver–Burk plots of the uptake of phenylalanine and tyrosine into the tissue water of rat cerebral cortex slices

The slopes are derived from the rates of uptake for slices in different concentrations of (a) phenylalanine and (b) tyrosine during the initial 3 min after the addition of the amino acid (0.2 mM final concentration), after incubation for 30 min in its absence. ○, Normal Krebs–Ringer bicarbonate–glucose medium; ●, medium with Na^+ omitted.

0.2 mM L-phenylalanine, the concentration in the tissue water reached a mean of 0.6 mM in 15 min, whereas in medium with the same concentration of the D-isomer, it reached a mean of 0.48 mM; these concentrations remained constant for the 50 min of the experiment. However, after incubation for 40 min in 0.2 mM tyrosine, the L- and D-isomers reached maximum mean concentrations of 1.6 mM and 1.4 mM respectively, which fell to about two-thirds of these values during incubation for a further 80 min.

Effect of Na^+ concentration in the medium. The initial rates of the uptakes at different concentrations of the amino acids, in the presence and absence of Na^+ in the medium, were plotted on Lineweaver–Burk plots (Fig. 2). In the presence of Na^+ , the apparent K_m and apparent V_{max} values for the uptake of phenylalanine were 0.86 mM and $0.64 \mu\text{mol}/\text{min}$ per ml of tissue water respectively, whereas when Na^+ was omitted from the medium the apparent K_m rose to 1.97 mM, indicating an apparent inhibition constant, K_i , of 0.98 mM. In the presence of Na^+ , the apparent K_m and the apparent V_{max} values for the uptake of tyrosine were 1.64 mM and $0.98 \mu\text{mol}/\text{min}$ per ml of tissue water respectively, whereas when Na^+ was omitted from the medium the apparent K_m rose to 2.84 mM, indicating an apparent K_i of 1.04 mM (Fig. 2). In experiments in which Na^+ was omitted from the incubation medium, the Na^+ concentration in the tissue was found to be $14 \mu\text{equiv}/\text{g}$ and the K^+ was $26 \mu\text{equiv}/\text{g}$ of incubated weight.

Slices were incubated in Krebs–Ringer solutions modified so that the $[\text{Na}^+ + \text{K}^+]$ or $[\text{Na}^+ + \text{Tris}^+]$ always added up to 150 mM (see the Methods section). After preincubation for 30 min, tyrosine was added to give a final concentration in the medium of 0.2 mM. In the media in which Na^+ was substituted for K^+ or Tris^+ , the initial concentrations of tyrosine in the

tissue were different; the initial rates of uptake for 3 min, and the kinetics of the uptake, depended upon the particular combination of cations in the medium (Fig. 3).

Competition of amino acids. Tyrosine added to the incubation medium to give a final concentration of 5 mM altered the apparent K_m of the uptake of 0.2 mM-phenylalanine, giving an apparent K_i of 2.87 mM; phenylalanine (5 mM) had the same effect on the uptake of 0.2 mM-tyrosine, causing it to have an apparent K_m of 3.25 mM and an apparent K_i of 5.10 mM. The rate of uptake of tryptophan and its maximum concentration in the tissue, in the presence of 0.2 mM-tryptophan in the incubation medium, was diminished by the addition of 5 mM-tyrosine to the medium, and to a greater extent by 5 mM-tryptophan; likewise, the rate of uptake and the maximum concentration of tyrosine was diminished by 5 mM-tryptophan, and more so by phenylalanine (Fig. 4).

Absence of substrate or oxygenation, and the effect of inhibitors. The uptake of phenylalanine or tyrosine from a medium 0.2 mM in these amino acids was considerably decreased after 30 min incubation, in the absence of glucose or oxygen, if the slices were incubated at 0°C, or if various metabolic inhibitors were added (Table 1).

Efflux of amino acids

The cerebral slices were incubated in a medium containing the amino acid (0.2 mM) for 30 min, and then transferred to a similar medium without the amino acid. The rate of efflux of both L-phenylalanine and L-tyrosine was significantly greater than that of the two D-isomers for approximately the first 20 min, and

Fig. 3. Initial rates of influx of tyrosine into rat cerebral cortex slices incubated in media in which Na^+ was replaced by K^+ or Tris

Rat cerebral cortex slices were incubated in Krebs-Ringer bicarbonate-glucose medium for 30 min, and then transferred to iso-osmotic media containing 0.2 mM-tyrosine in which (a) Na^+ + K^+ or (b) Na^+ +Tris⁺ concentrations added up to 150 mM (see the Methods section); the initial rates of influx were measured for 3 min.

Fig. 4. Uptake of (a) phenylalanine and (b) tyrosine in the presence of other amino acids

Slices were incubated for 30 min in Krebs-Ringer bicarbonate-glucose medium without amino acid. (a) After this, phenylalanine (0.2 mM) was then added alone (○), or 0.2 mM-phenylalanine was added with 5 mM-tyrosine (△) or 0.2 mM-phenylalanine with 5 mM-tryptophan (●). (b) Tyrosine (0.2 mM) was added alone (○) after the initial incubation, or 0.2 mM-tyrosine was added with 5 mM-tryptophan (●) or 0.2 mM-tyrosine with 5 mM-phenylalanine (■).

Table 1. *Effect of different incubation conditions on the concentrations of amino acids in cerebral slices*

The slices were incubated for 30 min at 37°C in Krebs–Ringer bicarbonate–glucose medium to which 0.2 mM-L-phenylalanine or 0.2 mM-L-tyrosine had been added, and with the amendments shown. Each value given is the mean $\pm$ s.d.; the number of slices is indicated in parentheses.

Modification of Krebs–Ringer medium	Concn. of amino acid in tissue water (mM)	
	L-Phenylalanine	L-Tyrosine
None	0.60 $\pm$ 0.04 (6)	1.50 $\pm$ 0.06 (6)
Glucose absent	0.36 $\pm$ 0.02 (6)	1.01 $\pm$ 0.04 (6)
Tris buffer; N ₂ +CO ₂ (95:5)	0.23 $\pm$ 0.06 (6)	0.36 $\pm$ 0.08 (8)
Incubation at 0.5°C	0.21 $\pm$ 0.07 (6)	0.32 $\pm$ 0.06 (6)
Addition of NaN ₃ (1 mM)	0.27 $\pm$ 0.10 (6)	0.61 $\pm$ 0.15 (8)
Addition of NaCN (2 mM)	0.26 $\pm$ 0.08 (6)	0.48 $\pm$ 0.09 (8)
Addition of 2,4-dinitrophenol (1 mM)	0.20 $\pm$ 0.07 (6)	0.25 $\pm$ 0.075 (8)
Na ⁺ replaced by Tris ⁺	—	0.31 $\pm$ 0.05 (6)

Fig. 5. *Efflux of D- and L-phenylalanine and -tyrosine*

Slices were incubated for 30 min in Krebs–Ringer bicarbonate–glucose medium containing 0.2 mM concentration of the D-(●) and L-(○) isomers of (a) phenylalanine or (b) tyrosine, and then transferred to similar medium without the amino acid.

then it continued at about the same rate for a further 40 min. The final concentration of D-phenylalanine in the tissue was about three times that of the L-isomer, although it had been 16% lower after the 30 min incubation period. The concentrations of the two isomers of tyrosine both fell to about the same value (Fig. 5).

The efflux of phenylalanine and tyrosine from the tissue after 30 min incubation in 0.2 mM-amino acid was markedly accelerated in the presence of other amino acids in the fresh medium to which the tissue had been transferred; the final concentrations in the tissue of the initial amino acids were also depressed. Tyrosine (5 mM) or tryptophan (5 mM) increased the initial rate of efflux of phenylalanine by 92% and 14% respectively (Fig. 6). After 30 min incubation in the normal Krebs–Ringer bicarbonate–glucose medium (see the Methods section), to which 0.2 mM-amino acid had been added, the tissue was then transferred either to a similar medium without amino acid, or to Krebs–Ringer medium without amino acid but modified by substitution of equimolar Tris⁺ for the Na⁺; the effluxes of both phenylalanine and tyrosine in these media were at a greater initial rate, and were more complete, in the absence of Na⁺ from the incubation medium (Fig. 7).

Discussion

Tissue accumulation

In the present experiments we have shown that the tissue-water concentration ratios for phenylalanine and tyrosine are higher with low than with high concentrations of the amino acids in the medium (Fig. 1), as has previously been shown for other amino acids (Neame, 1961, 1962; Lahiri & Lajtha, 1964;

Fig. 6. Efflux of phenylalanine or tyrosine in the presence of other amino acids

Slices were incubated for 30 min in Krebs–Ringer bicarbonate–glucose medium containing amino acids and then transferred to media without amino acids. (a) Efflux after incubation in media containing 0.2 mM-phenylalanine ($\circ$), or 0.2 mM-phenylalanine with 5 mM-tyrosine ($\bullet$) or 5 mM-tryptophan ($\blacktriangle$). (b) Efflux after incubation in media containing 0.2 mM-tyrosine ($\circ$), or 0.2 mM-tyrosine with 5 mM-phenylalanine ($\blacksquare$) or 5 mM-tryptophan ($\bullet$).

Fig. 7. Efflux of phenylalanine and tyrosine in the absence of Na^+ in the incubation medium

Slices were incubated for 30 min in Krebs–Ringer bicarbonate–glucose medium containing (a) 0.2 mM-phenylalanine or (b) 0.2 mM-tyrosine, and then transferred to normal medium without amino acid ($\circ$) or to Na^+ -deficient medium ($\bullet$). For further details see the Methods section.

Joanny *et al.*, 1971). The different rates of uptake and final concentrations of the L- and D-isomers of phenylalanine and tyrosine found here parallel similar differences with isomers of alanine, methionine, leucine and lysine, found by Blasberg & Lajtha (1965), although these authors incubated their mouse brain slices in the presence of 2 mM-amino acids, ten times the concentrations used in the present experiments.

Reaction of presumed carrier with Na^+

Lineweaver–Burk plots showed different affinities of the tissue for the two amino acids studied here, and indicated saturation kinetics (Fig. 2). However, in the absence of Na^+ in the medium, although the initial velocities were similar, the plot extrapolated back to a point nearer the origin to give a result similar to a plot for diffusion kinetics. Thus the uptake mechanism, which is concluded to be a carrier (see below), probably requires the presence of Na^+ for the reaction of the amino acid to occur with the carrier. This locates the action of the cation more precisely than previous experiments in which the maximum concentration of amino acid in the tissue at the end of incubation was shown to be diminished when Na^+ was absent from the medium (Tsukada *et al.*, 1963; Margolis & Lajtha, 1968). The initial rate of influx of tyrosine was apparently linear with the concentration of Na^+ in the medium, when the tissue was kept in iso-osmotic conditions by replacing the Na^+ with K^+ in the medium (Fig. 3). This could be interpreted either as a 'specific' effect of Tris on the tyrosine carrier, or a requirement of the carrier for K^+ , as suggested by Margolis & Lajtha (1968).

Competition of amino acids

The initial rates of uptake of either amino acid were decreased by the presence in the incubation medium of another amino acid (Fig. 4); this is most easily explained as competition for a carrier. The further diminution of the final tissue concentrations by each other as well as by tryptophan in respect of both of them indicates either that they reach different equilibria within the tissue, or that the rates of incorporation of each of them into proteins are different. The present experiments do not distinguish between these two possibilities, although they are not mutually exclusive. The rate of efflux showed stereospecificity, and there was also competition between the amino acids in their efflux.

'Counter-acceleration'

The rate of efflux of phenylalanine from the tissue was accelerated by the presence of tyrosine or tryptophan in the medium, as was the efflux of tyrosine by phenylalanine or tryptophan (Fig. 6). This shows 'counter-acceleration', in which the presumed increased influx of one amino acid accelerated the rate of efflux of another. The influence of one amino acid on the fluxes of another (Fig. 4 and Fig. 6). could indicate several sites on the same carrier, or different carriers. The two possibilities must remain equally likely until the carriers have been identified chemically.

Dependence on metabolism

The dependence of the uptake of phenylalanine and tyrosine on metabolism (Table 1) has been shown previously in respect of glycine (Joanny *et al.*, 1971). The accumulation of the amino acids is correlated with the concentration of ATP in the tissue (Abadom & Scholefield, 1962; Barbosa *et al.*, 1968). In the present experiments the amino acids accumulated in the tissue in a ratio three to twenty times that in the incubation medium, as has previously been shown by Blasberg & Lajtha (1965), when the incubation media contained low concentrations of amino acids

similar to those found in plasma (Harper *et al.*, 1952).

In the present study it has been shown that the influx and efflux of phenylalanine and tyrosine are stereospecific, and are affected by the presence of Na⁺; they compete during the influx into, and the efflux from, the tissue. The tissue exhibits counter-transport in respect of them. The concentrations of these amino acids depend upon the availability of oxidizable substrate. All these findings are compatible with carrier-mediated active transport into cerebral slices of these amino acids (Wilbrandt & Rosenberg, 1961).

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