

Uptake of Radiolabeled Essential Amino Acids by Brain Following Arterial Injection (35270)

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The requirement of brain for amino acids is met either by synthesis from other amino acids or glucose *in situ* or from the blood amino acid pool. Uptake of amino acids from blood requires passage through the blood-brain barrier (BBB) which is highly impermeable to most molecules the size of amino acids. The observed selective free passage of some amino acids from blood to brain is attributed to carrier mediated BBB transport (1).

We have studied the uptake of 18 labeled amino acids by rat brain after carotid arterial injection and find a great variability in the percentage of amino acid extracted during the first few seconds after injection. The data indicate amino acids essential for brain are taken up from blood to a greater degree than those which can be synthesized within brain from other substrates.

Method. A mixture of approximately 0.5 μ Ci each of ^{14}C -labeled amino acid and ^3H water in 0.2 ml of a physiological salt solution (Elliott's "B" solution, Baxter Lab, Morton Grove, Illinois) is injected (approx. 0.25 sec) into the surgically exposed common carotid artery of 300-g Wistar rats immobilized with pentobarbital, with decapitation 15 sec later. Injection is through a 0.4-mm diameter needle and is sufficiently rapid that the artery clears of blood during injection thereby minimizing mixing of the injected solution with blood. The cerebral hemisphere ipsilateral to the injection is quickly dissected free and subjected to routine liquid scintillation analysis for ^{14}C and ^3H . Some of the injected isotope mixture is similarly analyzed and the ratio of $^{14}\text{C}/^3\text{H}$ in brain is compared with the same ratio in the injected solution.

The percentage extraction (E) relative to

water (100%) under these circumstances of injection is calculated by:

$$E = \frac{\text{brain tissue } ^{14}\text{C}/\text{brain tissue } ^3\text{H}}{\text{injected } ^{14}\text{C}/\text{injected } ^3\text{H}} \times 100.$$

Three rats were injected with each of 18 ^{14}C amino acids. ^{14}C mannitol was also studied (3 animals) as a metabolically inert substance with a molecular weight similar to the amino acids. The mannitol present in brain 15 sec after carotid injection probably represents both recirculation and residual tracer not yet washed out of the brain-blood compartment.

Results and Discussion. Approximately 8% of the injected solution distributes to brain, the remainder passing to the external carotid distribution (2). A large relatively fixed proportion of the tritiated water which enters brain is assumed still present at the time of decapitation, an equilibrium having been reached between brain exchangeable water and capillary water with virtually complete initial loss of labeled water from the capillaries during a single microcirculatory pass (3). A variable amount of the ^{14}C -labeled amino acid has left the capillary bed during the single microcirculatory pass and remained in the brain tissue. Approximately 40% of the initial brain uptake of ^3H water washes out during the first 15 sec (2). The labeled water remaining in the analyzed brain specimen serves as an internal standard against which to compare the amino acid extraction. By 15 sec, the amino acid not extracted is assumed to have been carried out of the brain circulation.

When arranged in descending order of extraction, Table I, there is no obvious correlation with molecular structure. However, 10 of the first 11 amino acids listed represent those considered nutritionally essential in the

TABLE 1. Percentage Uptake of ^{14}C L-Amino Acids and ^{14}C D-Mannitol Relative to $^3\text{H}_2\text{O}$ by Brain After Common Carotid Injection in Rat.

		(E)	
		% Taken up by brain	
Nutritional classification		Mean	SD ($n=3$)
H_2O		100	
Phenylalanine	Ess.	54.5	5.4
Leucine	Ess.	51.0	2.7
Tyrosine	Noness.	46.8	3.2
Isoleucine	Ess.	37.3	0.92
Methionine	Ess.	34.5	1.35
Tryptophan	Ess.	33.6	3.8
Histidine	Ess.	31.0	1.8
Arginine	Ess.	20.8	1.9
Valine	Ess.	19.8	2.2
Lysine	Ess.	13.9	2.5
Threonine	Ess.	10.7	0.23
Serine	Noness.	7.05	0.48
Alanine	Noness.	5.50	0.85
Citrulline	Noness.	4.71	1.19
Proline	Noness.	3.05	0.21
Glutamic	Noness.	2.81	0.15
Glycine	Noness.	2.47	0.25
Aspartic	Noness.	2.24	0.57
D-Mannitol	—	1.92	0.23

Relationship between classification of Rose *et al.* (4) into nutritionally essential or nonessential amino acids in rat and percentage taken up by brain after carotid injection. Amino acid concentration injected was different for each acid depending on the specific activity of the labeled material with a range of 0.003 to 0.05 mM. Radiochemicals were from New England Nuclear, Boston, or from Amersham/Searle, Arlington Heights, Illinois.

rat (4). The exception, tyrosine, considered nutritionally nonessential in the rat (4), is readily derived from phenylalanine by hepatic phenylalanine hydroxylase. The large influx of tyrosine noted here suggests tyrosine in rat brain is derived from blood and is compatible with the observed absence of phenylalanine hydroxylase in rat brain (5). These data suggest that although tyrosine is nonessential in rat extraneural tissues, it is essential in brain.

After intravenous injection of ^{14}C -labeled

glucose, ^{14}C rapidly appears in brain alanine, aspartic, and glutamic acids and, to a lesser extent, in serine and glycine (6). This suggests some, at least, of the brain requirement for these amino acids is met from a glucose source.

The amount of test substance extracted by brain is a function of its brain tissue distribution space and BBB permeability. Entrapment and utilization by brain cells increases the brain distribution space and would be expected to increase the amount of test substance extracted. A high BBB permeability should increase brain uptake. The relative importance of these factors in determining the amount of a given substance extracted by brain remains unsettled.

Summary. ^{14}C -labeled amino acids were injected into rat common carotid artery mixed with $^3\text{H}_2\text{O}$, with decapitation 15 sec later. The ratio of ^{14}C to ^3H in brain was compared with the same ratio in the injected solution allowing expression of brain uptake of amino acid as a percentage of $^3\text{H}_2\text{O}$ uptake. Eighteen amino acids showed a range of uptake between 2.24 and 54.5%. Those amino acids ordinarily considered nutritionally essential in the rat are taken up to a greater extent than nonessentials. The exception is tyrosine which is not nutritionally essential to the total organism because it is available from hydroxylation of phenylalanine. The large uptake of tyrosine by brain may be related to the absence in brain of phenylalanine hydroxylase thus necessitating an external source.

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