 0014-4754/95/111052-03$1.50 + 0.20

High and low affinity transport of L-arginine in rat brain synaptosomes

C. H. Tan and F. H. Ng

Department of Biochemistry, Faculty of Medicine, National University of Singapore, 10 Kent Ridge Crescent, Singapore 0511 (Singapore), Fax + 65 7791453
Received 9 January 1995; received after revision 4 April 1995; accepted 17 May 1995

Abstract. The uptake of L-arginine into purified rat brain synaptosomes was investigated with respect to time and various concentrations of L-[^{3}H]arginine. Specific uptake was found to be linear with time for up to 5 min of incubation at 37 °C. Electrolytes, including sodium chloride, potassium chloride, magnesium chloride and calcium chloride, inhibited uptake of 3 μM L-arginine, and the inhibitory effect increased with increased electrolyte concentration under constant osmolarity. It was found that L-arginine was transported into synaptosomes by two uptake components – a high affinity component (3.5 μM) and a low affinity component (100 μM). These two components were similar to the Ly^+ system because of their extreme sensitivity to inhibition by L-lysine and L-ornithine but were distinguishable from each other by kinetic analysis of the uptake data and by their relative sensitivity to inhibition by several amino acids.
Key words. L-arginine; L-arginine transport; L-arginine uptake; rat brain; synaptosomes; nerve endings; amino acid uptake; transport systems.

The amino acid L-arginine is not only the precursor of urea, creatine and agmatine[1] but can lead via ornithine to the synthesis of proline and γ-aminobutyric acid[2]. Nitric oxide (NO), which is synthesized from L-arginine by nitric oxide synthase (NOS), plays an important role as a central neuronal messenger and is released in response to intracellular Ca^{++} signals that follow the stimulation of glutamate receptors[3].

The availability of L-arginine for NO synthesis by NOS would depend upon the presence of the amino acid at the nerve endings as well as on the interplay and delicate balance of enzymes metabolising L-arginine or contributing to its synthesis[2]. The existence of a complete metabolic machinery for transport and metabolism of L-arginine in mouse brain synaptosomes has been reported[2].

In this communication, we attempt to show that L-arginine is transported into rat brain synaptosomes by two sodium-independent processes (high and low affinity) which are similar to the Ly^+ system described previously in Ehrlich ascites tumour cells[4] and in mouse brain cells[5].

Materials and methods

Preparation of synaptosomes. Synaptosomes were isolated from dissected cerebral cortices of adult male Wistar rats (200–250 g) using the sucrose fractionation technique of Kurokawa et al.[6] with the additional step of purification through a discontinuous 3–13% Ficoll gradient[7] as described by Tan and Ng[8]. All procedures were carried out at 4 °C and the synaptosomal pellets were resuspended in a medium containing 10 mM Tris-HCl and 250 mM sucrose, pH 7.4. The protein content of the purified synaptosomes was 6–10 mg/g wet weight of brain tissue and was assayed by the method of Lowry et al.[9] using bovine serum albumin as standard.

Synaptosomal uptake assays. Uptake studies were immediately carried out after the preparation of synaptosomes. All incubations were carried out in triplicate in 1 ml of medium TS (10 mM Tris-HCl and 250 mM sucrose, pH 7.4) containing 0.25 mg of synaptosomal protein, 0.5 μCi of L-[^{3}H]arginine (60 Ci/mmol; Amersham International, UK) and unlabeled L-arginine in the absence or presence of other amino acids or analogs. Uptake was allowed to proceed at 37 °C and each incubation terminated with the addition of 3 × 5 ml of ice-cold medium TS containing 2 mM L-arginine, followed by gentle filtration on 25 mm glass fibre filters (Whatman GF/F). The filters were prepared for liquid scintillation counting as described previously[10]. Non-specific binding of the radiolabeled amino acid to the filter was determined in a parallel incubation at the end of which the filter was washed with distilled water containing 2 mM L-arginine instead of the TS washing medium. The difference in measured radioactivity (i.e. total uptake minus non-specific binding) represented net uptake into synaptosomes.

Results and discussion

L-Arginine uptake into rat brain synaptosomes was linear with time up to 5 min at 37 °C (fig. 1). For subsequent kinetic studies, initial rates of uptake were measured at 3 min at this temperature. Unlike the poor uptake of L-arginine reported in mouse brain synapto-

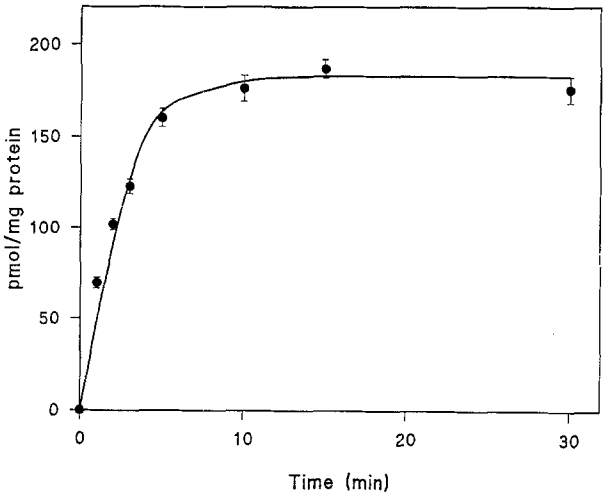

Figure 1. Time course of uptake of 3 μM L-arginine by rat brain synaptosomes.

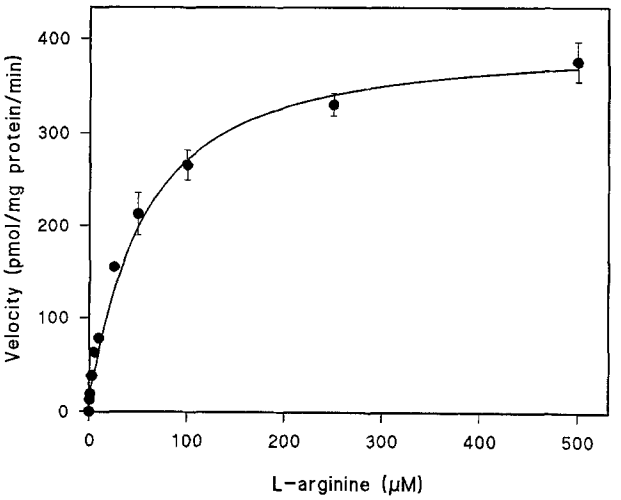

Figure 2. Effect of substrate concentration on synaptosomal uptake of L-arginine.

somes[2], purified rat brain synaptosomes took up as much as 2% of the ^{3}H-labeled L-arginine in 3 min at a substrate concentration of 1 μM (data not shown).

The accumulation of L-arginine by rat brain synaptosomal particles was previously reported to be independent of Na^+ at high substrate concentrations[11] or inhibited up to 30% by Na^+ at substrate concentrations of 10 μM or less[7,11]. Our results showed that the uptake of 3 μM L-[^{3}H]arginine was significantly reduced ($p < 0.05$) in the presence of various concentrations of Na^+ (table 1). In addition, we found that other electrolytes such as KCl, $MgCl_2$ and $CaCl_2$ were also inhibitory and the degree of inhibition increased with increasing electrolyte concentration.

The effect of substrate concentration on the uptake rates of L-arginine by rat cerebral cortical synaptosomes showed saturation at concentrations above 500 μM (fig. 2). An Eadie-Hofstee plot (fig. 3) of the uptake rates gave a biphasic curve which fits a non-linear equation[12] pointing to the existence of two or more components of uptake. By using a graphical and correction method[13,14], the biphasic curve was resolved into two linear components (lines 1 and 2 of fig. 3). As extrapolated from the plots, component 1 (the high affinity component) showed apparent transport K_m and V_{max} values of 3.5 ± 0.7 μM and 55 ± 5 pmol/mg protein/min, while component 2 (the low affinity component) gave apparent K_m and V_{max} values of 100 ± 15 μM and 328 ± 13 pmol/mg protein/min. The ratio of uptake rates due to the low affinity component to that due to simple diffusion (as measured by L-glucose uptake) was approximately 6:1 at a substrate concentration of 500 μM (data not shown). Thus simple diffusion or non-saturable uptake could only account for part of the uptake of L-arginine due to the low affinity component[15].

In addition to kinetic analysis of the uptake data, inhibition of L-arginine uptake with a variety of amino acids and non-metabolisable amino acid analogs were carried out to examine the nature of the transport

Table 1. Effect of electrolytes on L-[^{3}H] arginine uptake.

Additions	L-[^{3}H]arginine uptake (% control)
Control (medium TS)	100 ± 1
1 mM NaCl	81 ± 2
10 mM NaCl	65 ± 3
100 mM NaCl	38 ± 1
1 mM $MgCl_2$	78 ± 5
10 mM $MgCl_2$	42 ± 1
1 mM KCl	79 ± 5
10 mM KCl	36 ± 2
1 mM $CaCl_2$	59 ± 2
10 mM $CaCl_2$	24 ± 3

Synaptosomes were incubated with 3 μM L-arginine at 37 °C for 3 min. Values shown were the means ± SEMs of 3 determinations.

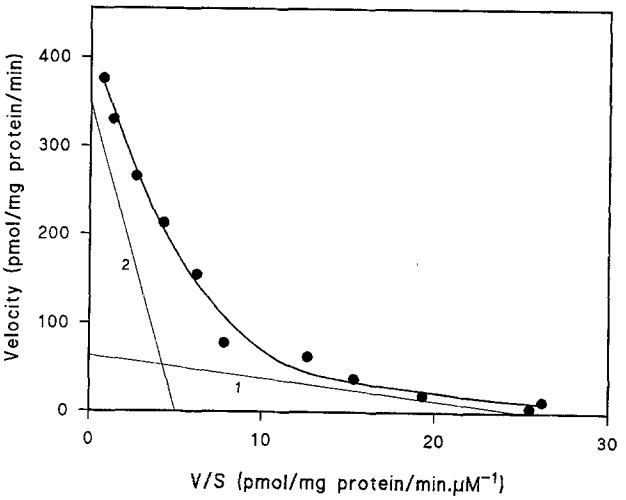

Figure 3. Eadie-Hofstee plot of L-arginine uptake.

Table 2. Effect of amino acids and non-metabolizable amino acid analogs on synaptosomal uptake of L-arginine.

Inhibitor	Potency index for inhibition of L-arginine uptake	
	low substrate (1 μM)	high substrate (50 μM)
L-lysine	0.76 ± 0.02	0.93 ± 0.05
L-ornithine	0.70 ± 0.03	0.91 ± 0.07
D-arginine	0.24 ± 0.03	0.61 ± 0.04
L-glutamic acid	0.12 ± 0.02	0
L-glutamine	0.09 ± 0.03	0
L-leucine	0.05 ± 0.03	0.13 ± 0.02
L-alanine	0	0
L-glycine	0	0
L-cysteine	0	0
L-proline	0	0
L-asparagine	0	0
L-taurine	0	0
γ-aminobutyric acid (GABA)	0	0
N-methyl-L-alanine (L-NMA)	0	0
N-methylaminoisobutyric acid (MeAIB)	0	0
2-aminobicyclo-(2,2,1)-heptane-2-carboxylic acid (BCH)	0	0

Incubation conditions were as described in the text. The ratio of inhibitor:substrate was 20:1. Inhibition of uptake by added L-arginine was 91% and 94% for low and high substrate concentrations respectively. Values shown were the means ± SEMs (n = 6).

components. Inhibition was carried out at two substrate concentrations (1 μM and 50 μM) using an inhibitor:substrate ratio of 20:1. The degree of inhibition of L-arginine uptake by an inhibitor was compared using the potency index (PI) which is defined as the ratio of % inhibition of uptake by an inhibitor to the % inhibition of uptake by the same concentration of added L-arginine. The results (table 2) showed that L-alanine, L-glycine, L-cysteine, L-asparagine, L-proline, L-taurine, GABA, MeAIB, L-NMA and BCH had no inhibitory effect (PI = 0) on L-arginine uptake at either low and high substrate concentrations, showing that L-arginine uptake in rat brain synaptosomes is unlike the Na^+-dependent A-type or the ASC-type transport systems found in the Ehrlich ascites cell[16, 17]. Sershen and Lajtha[5] observed in mouse brain slices that there were as many as ten or more amino acid transport systems with overlapping specificities including a specific system (Ly^+ system) for L-lysine uptake which was also shared by L-arginine. Our investigation with purified rat brain synaptosomes showed that L-lysine and L-ornithine were strongly inhibitory towards L-arginine uptake (PI values ≥ 0.7, table 2) thereby indicating that they share common transport modes. The results are supportive of a transport system for L-arginine similar to the Ly^+ system. However, the PI values of these inhibitors differed significantly ($p < 0.05$) at low and high substrate concentrations and are consistent with the biphasic nature of the Eadie-Hofstee plot which demonstrated the presence of two uptake components for L-arginine. Further evidence could be seen from the inhibition of L-arginine uptake by D-arginine, L-glutamic acid, L-glutamine and L-leucine where the PI values also differed significantly ($p < 0.05$) at low and high substrate concentrations. The existence of two or more uptake components for other amino acids such as L-leucine[18, 19], L-proline[20] and L-taurine[21] have also been described previously.

The significance of having two or more Na^+-independent transport processes for L-arginine is at present unknown. The high affinity component has a K_m value similar to other high affinity uptake systems for neurotransmitter amino acids[22]. The accumulation of L-arginine by synaptosomes could result in increased synthesis of important neurotransmitters such as GABA, L-glutamic acid and L-proline via ornithine[2]. However, L-arginine is also the precursor of neuronal NO through the activity of NOS and it is noteworthy that the rat brain expresses high NOS activity[23]. Hence L-arginine uptake into synaptosomes may be important in regulating the synthesis of NO as was suggested by Westergaard et al.[24] in their studies with cultured neurons.

Acknowledgement. This project was funded by a grant (RP87347) from the National University of Singapore.

1 Reyes, A. A., Karl, I. E., and Klahr, S., Am. J. Physiol. *267* (1994) 331.
2 Johnson, J. L., and Roberts, E., J. Neurochem. *42* (1984) 1123.
3 Garthwaite, J., Charles, S. L., and Chess-Williams, R., Nature *336* (1988) 385.
4 Christensen, H. N., Adv. Enzym. *32* (1969) 1.
5 Sershen, H., and Lajtha, A., J. Neurochem. *32* (1979) 719.
6 Kurokawa, M., Sakamoto, T., and Kato, M., Biochem. J. *97* (1965) 833.
7 Peterson, N. A., and Raghupathy, E., J. Neurochem. *19* (1972) 1423.
8 Tan, C. H., and Ng, F. H., Biochem. Pharmac. *39* (1990) 955.
9 Lowry, O. H., Rosebrough, N. J., Farr, A.L., and Randall, R. J., J. biol. Chem. *193* (1951) 265.
10 Tan, C. H., Leong, M. K., and Ng, F. H., Neurochem. Int. *12* (1988) 91.
11 Snyder, S. H., Logan, W. J., Bennett, J. P., and Arregui, A., Neurosci. Res. *5* (1973) 131.
12 Handlogten, M. E., Weissbach, L., and Kilberg, M. S., Biochim. biophys. Res. Commun. *104* (1982) 307.
13 Rosenthal, H. E., Analyt. Biochem. *20* (1967) 525.
14 Chamness, G. C., and McGuire, W. L., Steroids *26* (1975) 538.
15 Morre, M. C., and Wurtman, R. J., Life Sci. *28* (1981) 65.
16 Oxender, D. L., and Christensen, H. N., J. biol. Chem. *238* (1963) 3686.
17 Christensen, H. N., Adv. Enzym. *49* (1979) 41.
18 Peterson, N. A., Analyt. Biochem. *114* (1980) 322.
19 Tan, C. H., Leong, M. K., Ng, F. H., and Thiyagarajah, P., Biochem. Int. *14* (1987) 161.
20 Hauptmann, M., Wilson, D. F., and Erecinska, M., FEBS Lett. *161* (1983) 301.
21 Schmidt, R., Sieghart, W., and Karobath, M., J. Neurochem. *25* (1975) 5.
22 Usherwood, P. N. R., Adv. comp. Physiol. Biochem. *7* (1978) 227.
23 Salter, M., Knowles, R. G., and Moncada, S., FEBS Lett. *291* (1991) 145.
24 Westergaard, N., Beart, P. M., and Schousboe, A., J. Neurochem. *61* (1993) 364.