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Comparison of the uptake of [^{3}H]-gabapentin with the uptake of L-[^{3}H]-leucine into rat brain synaptosomes

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1 Gabapentin is a novel anticonvulsant with an unknown mechanism of action. Homogenate binding studies described elsewhere have suggested that [^{3}H]-gabapentin binds to a site in brain similar to the large neutral amino acid (LNAA) uptake site, termed system-L.

2 This study describes an investigation into the uptake of [^{3}H]-gabapentin into a crude synaptosomal preparation from cerebral cortex of rat brain. Characterization studies showed that [^{3}H]-gabapentin is taken up into synaptosomes by a system that is similar to that responsible for the uptake of L-[^{3}H]-leucine. This system is sodium-independent, temperature-sensitive and requires ATP for function.

3 Kinetic studies of [^{3}H]-gabapentin uptake produced a Michaelis constant ($K_M = 160$ μM) similar to that observed for L-[^{3}H]-leucine ($K_M = 110.3$ μM). V_{max} values were 837.1 pmol mg^{-1} protein min^{-1} and 2.192 nmol mg^{-1} protein min^{-1} respectively.

4 Gabapentin and L-leucine mutually inhibit their uptake. Lineweaver-Burke plots of these data demonstrate that inhibition occurs by a competitive mechanism. Further to this the Dixon transformation of the data illustrates that these two substrates share a common uptake site by the similarity between their calculated K_i and K_M values (gabapentin inhibition of L-[^{3}H]-leucine uptake: $K_i = 160$ μM; L-leucine inhibition of [^{3}H]-gabapentin uptake: $K_i = 262$ μM).

5 Studies into the effect of gabapentin, the system-L-specific ligand 2-(−)-endoamino-bicycloheptane-2-carboxylic acid (BCH), and the system-A-specific ligand α-(methyl-amino)-isobutyric acid (MeAIB), on the initial rate of uptake of [^{3}H]-glycine, L-[^{3}H]-glutamate, L-[^{3}H]-glutamine, and L-[^{3}H]-leucine were performed. At 100 μM, gabapentin significantly inhibited initial rate of uptake of [^{3}H]-glycine (29%), L-[^{3}H]-glutamate (22%) and L-[^{3}H]-leucine (40%).

6 Gabapentin is taken up into synaptosomes by a system similar to system-L, responsible for the uptake of large neutral amino acids. Gabapentin will also inhibit the uptake of certain excitatory amino acids in this synaptosomal preparation. The implications of these findings for the mechanism of action for gabapentin are unclear. The data presented here may suggest an intracellular site for mechanism of action for this compound. Similarly changes in levels of amino acid pools may be involved in the mechanism of gabapentin's anticonvulsant action.

Keywords: Gabapentin; large neutral amino acid uptake; system-L; rat brain; synaptosomes

Introduction

Gabapentin is a novel anticonvulsant with an unknown mechanism of action. Despite being synthesized as a γ-aminobutyric acid (GABA)-mimetic, gabapentin has no activity at $GABA_A$ or $GABA_B$ receptors, no action on GABA uptake and no action on the GABA degradation enzyme GABA-transaminase transaminase (EC 2.6.1.19) (Chadwick, 1992). Gabapentin has been shown to be active orally in various animal models of epilepsy, including maximal electroshock in rats and pentylenetetrazol- or audiogenically-induced seizures in mice (Bartoszyk *et al.*, 1986; Dooley *et al.*, 1986; Chadwick, 1992). Suman-Chauhan and coworkers (1993) described a novel high affinity binding site for [^{3}H]-gabapentin in rat cerebral cortex synaptic plasma membranes. The same group described the autoradiographic localization of the gabapentin binding site in rat brain (Hill *et al.*, 1993). It was evident from this study that the gabapentin binding site appeared to be discretely localized in regions of the brain thought to be involved in the genesis of epileptiform activity. The gabapentin binding site has been shown to be present in mouse and pig cerebral cortex synaptic plasma membranes both demonstrating similar structure-activity relationships (Thurlow *et al.*, 1993).

Gabapentin has a small structure, with a molecular weight less than 200, is zwitterionic and resembles an amino acid except that it does not contain a chiral carbon and the amino group is not alpha to the carboxylate functionality. Investigations into the lack of proportionality between increasing dose and drug levels in plasma of gabapentin, using everted rat intestinal ring systems demonstrated that a saturable transport mechanism similar to what has been termed system-L for large neutral amino acid uptake (LNAA) was responsible for the uptake of gabapentin from the gastrointestinal tract (Stewart *et al.*, 1993).

More recently, it has demonstrated that the gabapentin binding site has a structure-activity relationship illustrating marked similarity to that of the system-L described in CHO cells by Shotwell *et al.* (1981) (Thurlow *et al.*, 1993). Recent work by Su and coworkers (1995) has demonstrated the uptake of [^{3}H]-gabapentin in glial cells and Chinese hamster ovary (CHO) cells.

Mammalian amino acid transport systems have been characterized over four decades following the pioneering work of Christensen *et al.* (1952). Original studies described the use of the Ehrlich cell for the characterization of uptake systems such as y^+, A, ASC, and L. These studies may well have facilitated the original description of the systems, but now this cell might be considered atypical in comparison to other mammalian cell types. This is highlighted by the fact that systems such as Gly, N, and those specific for imino or anionic amino acids, are not expressed by the Ehrlich cell.

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It has been shown that most mammalian cells express a common 'core' set of amino acid transport activities and that these vary between cell types in the total number, type, and activity of the transport systems. As a result each cell type is unique with regard to the process available for amino acid accumulation, and has undoubtedly adapted for its particular physiological role and metabolic needs (Kilberg *et al.*, 1993).

The amino acid-uptake systems in the periphery have been reasonably well defined according to the substrate, and sodium-dependency (Christensen, 1990). As mentioned above, variations in the structure-activity relationship between cell types for the transport system studied can occur. In the main, however, attempts have been made to form a uniform classification of the systems (Christensen, 1990).

The large neutral amino acid transport system was one of the first to be described for mammalian cells (Oxender & Christensen, 1963). This system is Na^+-independent and until recently, most investigators have somewhat arbitrarily assigned all saturable, Na^+-independent uptake to this activity, especially in those cases where 2-(−)-endoamino-bicycloheptane-2-carboxylic acid (BCH) was used because of its system-L specificity in the Ehrlich cell (Christensen *et al.*, 1969). However, it is now clear that several distinct systems may contribute to the saturable Na^+-independent transport in any given cell type.

The kinetics of influx of large neutral amino acids seem largely to follow the simple Michaelis-Menten equation. Influx parameters have been shown to vary quite considerably, with K_M values ranging from 10^{-6} M in cellular preparations to 10^{-3} M in brain slices, and maximal velocities vary significantly between laboratories (Vahvelainen & Oja, 1972; Hannuniemi & Oja, 1981).

These differences are thought to be due to several factors. Firstly, more than one transport mechanism may exist for the transport of a certain amino acid and the differences in preparation and handling of tissue may selectively affect only one of the mechanisms. Secondly, the maximal velocity of influx may be influenced by the size and composition of the intracellular amino acid pools. Large neutral amino acids are known to participate in fast homo- and hetero-exchange across cell membranes (Grahame-Smith & Parfitt, 1970; Korpi & Oja, 1979). For instance, pre-loading of slices with L-histidine has markedly increased the maximal velocity of the initial influx of tryptophan (Korpi, 1983).

The qualities of transport of large neutral amino acids in the CNS appear in agreement with the transport system-L described for the periphery. L-Leucine influx has been shown to be reduced more efficiently by BCH and other LNAA than by α-(methyl-amino)-isobutyric acid (MeAIB), N-methyl-L-alanine, and small neutral amino acids (Blasberg & Lajtha, 1966; Picolli *et al.*, 1971; Sershen & Lajtha, 1979). In synaptosomes, leucine, serine and threonine significantly decrease each other's influx, but the attenuation of serine and threonine influx by leucine and the inhibition of leucine transport by serine and threonine were least pronounced (Picolli *et al.*, 1971). The uptake of tryptophan by cerebral cortex slices is inhibited most by other aromatic amino acids or by branched-chain aliphatic amino acids (Kiely & Sourkes, 1972). LNAA readily undergo either homo-exchange or hetero-exchange (Crnic *et al.*, 1973; Korpi & Oja, 1979). These studies have generally indicated a similar substrate specificity in the transport of all branch-chain and aromatic aminocarboxylic acids and amides of amino-dicarboxylic acids. The results do not, however, exclude the possibility that several transporting systems for LNAA exist with overlapping specificities. This has been shown for tryptophan in 5-hydroxytryptaminergic neurones and their synaptic terminals via a separate high-affinity transport system (Denizeau & Sourkes, 1977).

In this study the nature of the uptake of [^{3}H]-gabapentin into rat cerebral cortex synaptosomes has been determined and its characteristics compared to that of the uptake of L-[^{3}H]-leucine in the same tissue preparations.

Methods

Characterization and comparison of [^{3}H]-gabapentin uptake with L-[^{3}H]-leucine uptake into rat cerebral cortex synaptosomes

[^{3}H]-gabapentin was incubated with a crude synaptosomal preparation as previously described for early [^{3}H]-GABA uptake studies (Iversen & Johnston, 1971). Briefly, cerebral cortices were dissected out from rats, weighed and homogenized in 10 volumes of ice-cold 0.32 M sucrose in 10 mM HEPES buffer (pH 7.4 at 4°C), using a glass homogenizer with a Teflon pestle. The homogenate was centrifuged for 10 min at 1000 *g* at 4°C to remove unbroken tissue fragments and other debris.

The uptake of [^{3}H]-gabapentin was measured in the resulting supernatant such that 10 mg wet weight of original tissue was incubated with 2 ml of artificial cerebro-spinal fluid (aCSF composition, mM: NaCl 126, KCl 5, $MgCl_2$ 1.2, NaH_2PO_4 1.2, glucose 10, $NaHCO_3$ 25, $CaCl_2$ 2.4), gassed with 95% oxygen and 5% carbon dioxide. Unless otherwise stated pre-incubation was performed at 25°C for 15 min in a shaking water-bath (200 cycles min^{-1}) prior to the addition of labelled gabapentin.

Radioligands were added at reduced specific activity by addition of 5 nM labelled gabapentin to 0.995 μM unlabelled compound to give a final concentration of 1 μM. Incubation was continued for the desired time interval at 25°C before termination of uptake by filtration through Whatman GF/B filters followed by three, 10 ml washes with either ice-cold aCSF to define total uptake, or ice-cold 10 mM HEPES at pH 7.4 to define basal uptake (including the binding component). Filters were then transferred to scintillation vials and the amount of radioactivity present determined by liquid scintillation counting.

The above procedure was adopted for studies into the uptake characteristics of the system-L preferring ligand L-[^{3}H]-leucine and for [^{3}H]-GABA as control. Studies into the characteristics of each of the uptake systems were performed for time course of uptake and the effect of temperature on initial rate of uptake for each ligand. All initial rates were determined for 4 min periods.

The effect of the electron transport chain (ETC) inhibitors rotenone and antimycin A were investigated after a 30 min preincubation with synaptosomes at 37°C. Initial rates of uptake were determined for all three ligands.

The effects of depletion of sodium-ion concentration in the incubation buffer on uptake were investigated by replacing sodium ions in aCSF with choline chloride and potassium dihydrogen phosphate such that Na^+ ion concentration was no more than 25 mM due to the $NaHCO_3$ remaining for buffering. The effect of this buffer change on initial rate of uptake was compared with all three ligands.

Saturation studies of radioligand uptake were performed using 4 min incubations at varying concentrations of uptake substrate (10 nM to 1 mM). Increasing concentrations of each ligand were achieved by radioisotope dilution of 5 nM labelled ligand with various concentrations of unlabelled ligand and the results were transformed as follows:

$$\text{Corrected specific d.p.m.} = \text{specific d.p.m.} \times (1 + [\text{unlabelled}]/[\text{labelled}])$$

Inhibition of the initial rate of [^{3}H]-gabapentin uptake by amino acids and related compounds was investigated by 4 min incubations with 1 μM [^{3}H]-gabapentin in the presence of 100 μM of various amino acids and related compounds. Pre-incubation of synaptosomes was performed for 15 min at 25°C before the simultaneous addition of inhibitor and radioligand. Termination by filtration occurred as described above. All uptake initial rates were compared with those of control values by Tukey's multiple comparison test (InStat, GraphPad).

The type of inhibition of [^{3}H]-gabapentin uptake by large

neutral amino acids was investigated using the effects of various concentrations of L-leucine (50, 100 and 250 μM) on the initial rate of uptake of [^{3}H]-gabapentin at concentrations ranging from 10 nM to 300 μM. Inhibitor and substrate were added simultaneously to membranes pre-incubated for 15 min at 25°C, to facilitate direct competition between the two for the uptake site. Similar experiements were performed for the inhibition of L-[^{3}H]-leucine uptake by gabapentin.

The effect of gabapentin and amino acid uptake system-L and system-A specific ligands on the initial rate of uptake of [^{3}H]-glycine, L-[^{3}H]-glutamine, L-[^{3}H]-glutamate and L-[^{3}H]-leucine

Experiments were performed as described above for [^{3}H]-gabapentin and L-[^{3}H]-leucine uptake into synaptosomes. Gabapentin, BCH and MeAIB were included in the incubations at a concentration of 100 μM. Termination of the incuabation was by filtration in the methods described above. Initial uptake rate was calculated for each 4 min incubation and compared with control uptake with no additional compounds. Concentrations of radioligands used were as follows: [^{3}H]-glycine (1 μM); L-[^{3}H]-glutamate (1 μM) and L-[^{3}H]-glutamine (1 μM).

Materials

[^{3}H]-gabapentin was obtained by custom synthesis from Cambridge Research Biochemicals, ICI, at specific activity of 104 Ci mmol^{-1}. [^{3}H]-GABA and L-[^{3}H]-leucine were obtained from Amersham, specific activities 57 Ci mmol^{-1} and 140 Ci mmol^{-1} respectively. [^{3}H]-glycine, L[^{3}H]-glutamate, and L-[^{3}H]-glutamine were obtained from Amersham at specific activities of 48 Ci mmol^{-1}, 17 Ci mmol^{-1} and 30 Ci mmol^{-1}. All other compounds were purchased from Sigma Chemical Company.

Results

Characterization and comparison of [^{3}H]-gabapentin uptake with [^{3}H]-GABA and L-[^{3}H]-leucine uptake into rat cerebral cortex synaptosomes

Uptake of 1 μM [^{3}H]-gabapentin occurred into a lysable pool present within synaptosomes. The uptake assay was reproducible with a percentage specific uptake of 50–60%. Similar results were obtained for [^{3}H]-GABA and L-[^{3}H]-leucine.

Uptake of [^{3}H]-gabapentin occurred by a rapid mechanism with equilibrium being reached after approximately 20 min (Figure 1a). Similar results were obtained with L-[^{3}H]-leucine (Figure 2a) uptake and [^{3}H]-GABA (data not shown).

Figures 1b and 2b represent the temperature-sensitivity of uptake of the ligands studied. It can be seen that for [^{3}H]-gabapentin uptake, initial rate increases up to a temperature of 40°C and above this temperature, initial rate of uptake decreased rapidly (Figure 1b). Similar changes in initial rate of uptake were demonstrated with L-[^{3}H]-leucine (Figure 2b) except maximal initial rate occurred at 20°C.

The uptake of both ligands was significantly inhibited by the preincubation of synaptosomes in the presence of ETC inhibitors (Dunnett's multiple comparison test; InStat V2.02).

The uptake of [^{3}H]-gabapentin and L-[^{3}H]-leucine (Figure 1d and 2d) was unaffected by a decrease of sodium ions to a concentration of 25 mM in the incubation buffer. Control uptake studies with 10 nM [^{3}H]-GABA in the same preparation exhibited total inhibition of uptake by the decrease in sodium ion concentration in the incubation medium (data not shown).

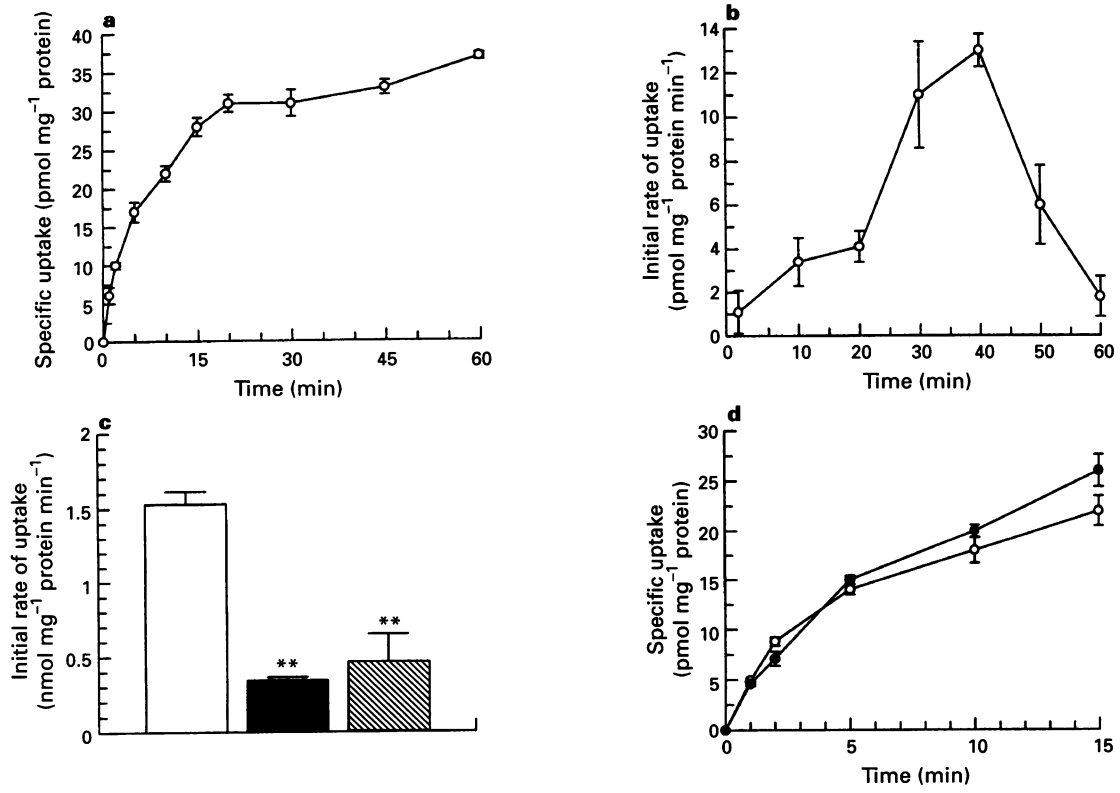

Figure 1 Characterization of the uptake of 1 μM [^{3}H]-gabapentin in rat brain synaptosomes prepared from cerebral cortex. (a) Time course of uptake at 21°C. (b) Effect of temperature on initial rate of uptake. (c) Effect of electron transport chain inhibitors 25 μM rotenone (solid column) and 25 μM antimycin A (hatched column) on control (open column) initial rate of uptake at 37°C. (d) Time course of uptake in the presence of high sodium ion concentration (○) and low sodium ion concentration (●) at 21°C. Data represents the mean of three separate experiments (**$P<0.01$ Dunnett's multiple comparison test; InStat V 2.02).

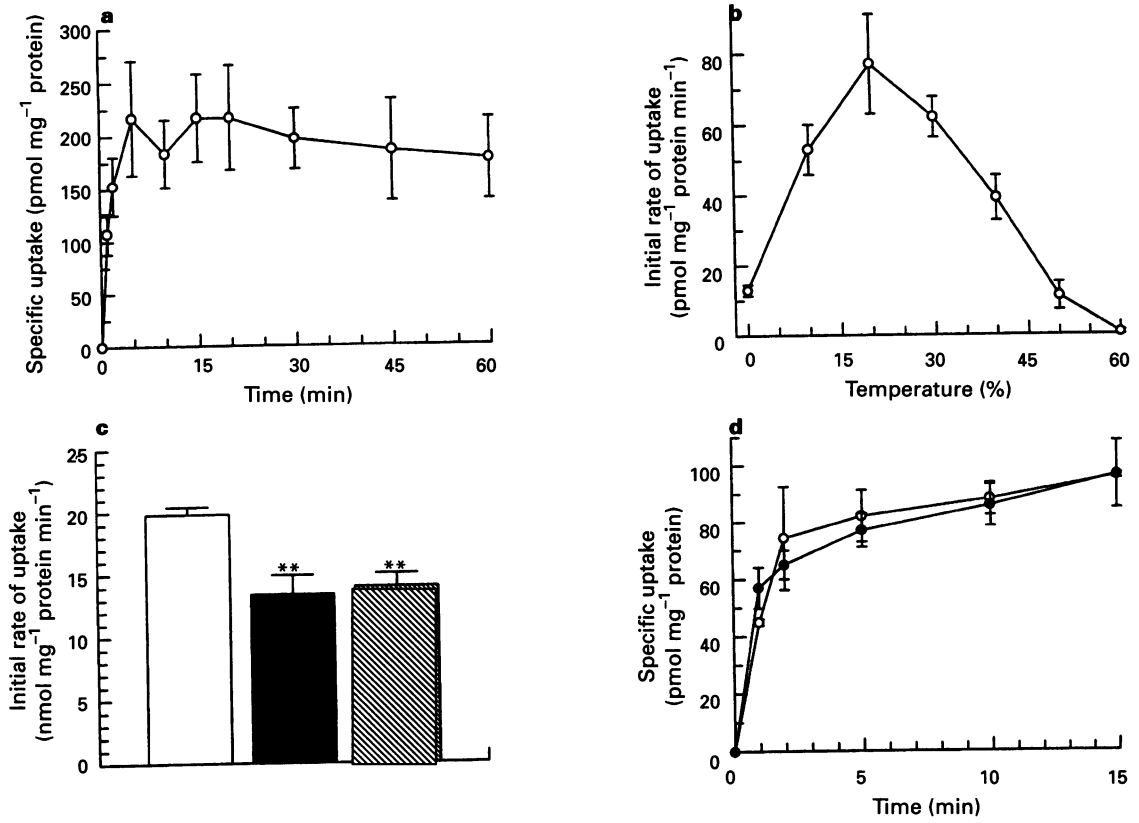

Figure 2 Characterization of the uptake of 1 μM L-[^{3}H]-leucine in rat brain synaptosomes prepared from cerebral cortex. (a) Time course of uptake at 21°C. (b) Effect of temperature on initial rate of uptake. (c) Effect of electron transport chain inhibitors 25 μM rotenone (solid column) and 25 μM antimycin (hatched column) on control (open column) initial rate of uptake at 37°C. (d) Time course of uptake in the presence of high sodium ion concentration (○) and low sodium ion concentration (●) at 21°C. Data represents the mean of three separate experiments (**$P<0.01$ Dunnett's multiple comparison test; InStat V 2.02).

[^{3}H]-gabapentin uptake into synaptosomes occurs by a saturable process (Figure 3). It can be seen from the direct plot of v against [S] with the curve fitted by least-squares regression (Grafit V3.0) that the uptake is of high affinity and is concentrative ($K_M = 160.2 \pm 19.1$ μM; $V_{max} = 837.1 \pm 43.6$ pmol mg^{-1} protein min^{-1}). Uptake appears monophasic as is illustrated by the linear nature of the Eadie-Hofstee transformation (Figure 3b) of the data shown in Figure 3a. The Michaelis constant derived from the Eadie-Hofstee plot is of the same order as that derived from the direct plot of the uptake data ($K_M = 150.6 \pm 10.6$ μM; $V_{max} = 813.1 \pm 39.6$ pmol mg^{-1} protein min^{-1}).

Figure 4 demonstrates a structure-activity relationship for inhibition similar to that observed in [^{3}H]-gabapentin homogenate binding studies (Thurlow *et al.*, 1993). It can be observed that large neutral amino acids, such as leucine, isoleucine, phenylalanine and methionine, inhibit [^{3}H]-gabapentin uptake significantly and stereospecifically. The 3-isobutyl GABA derivatives do not inhibit [^{3}H]-gabapentin uptake significantly, suggesting a difference in the structure-activity relationship between uptake and homogenate binding studies. The amino acid uptake system-L specific ligand, BCH, inhibits the uptake significantly while the system-A specific ligand, MeAIB, does not. Although L-glutamate inhibited uptake slightly, this was not significantly different from control uptake initial rate. The other compounds tested did not inhibit uptake.

The inclusion of increasing concentrations of L-leucine in the incubation caused increasing amounts of inhibition in the initial rate of [^{3}H]-gabapentin uptake. When the double reciprocal of these inhibition data is plotted (Figure 5a) no change in the y-axis intercept is observed. This indicates that no change in V_{max} occurs (control $V_{max} = 765.5$ pmol mg^{-1} protein min^{-1}; +250 μM L-leucine $V_{max} = 791.77$ pmol mg^{-1} protein min^{-1}). The x-axis intercept, however, changes significantly and illustrates a change in the Michaelis constant for the uptake site (control $K_M = 137.2$ μM; +250 μM L-leucine $K_M = 410.5$ μM). These changes are indicative of a competitive interaction for L-leucine at the [^{3}H]-gabapentin uptake site. When the inhibition data in the presence of all the concentrations of L-leucine tested (50, 100 and 250 μM) are transformed and plotted in the form of the Dixon plot (Figure 5b), the resulting linear regressions of each set of data points intercept, yielding a K_i of 160 μM.

The double reciprocal plot of the initial rate data for gabapentin inhibition of L-[^{3}H]-leucine uptake illustrates results similar to those observed for inhibition of [^{3}H]-gabapentin uptake by L-leucine (Figure 6). It can be seen that a competitive interaction appears to take place, as only a change in the K_M is observed (control $V_{max} = 2.192$ nmol mg^{-1} protein min^{-1}; $K_M = 110.3$ μM; +250 μM gabapentin $V_{max} = 2.656$ nmol mg^{-1} protein min^{-1}; $K_M = 259.3$ μM). The Dixon transformation of the data yields a K_i value of 261.9 μM.

The effect of gabapentin and amino acid uptake system-L and system-A specific ligands on the uptake initial rate of [^{3}H]-glycine, L-[^{3}H]-glutamine, L-[^{3}H]-glutamate and L-[^{3}H]-leucine

Table 1 depicts the results of these studies. Gabapentin at 100 μM can be seen to inhibit uptake of glycine (29% inhibition), glutamate (22% inhibition) and leucine (40% inhibition). BCH, the system-L-specific ligand has no effect on glycine uptake but does inhibit glutamate (21% inhibition) and leucine (24% inhibition) uptake significantly. MeAIB inhibits uptake of glycine (23% inhibition) and glutamate (33% inhibition) but stimulates the uptake of leucine. These compounds have no significant effect on glutamine uptake.

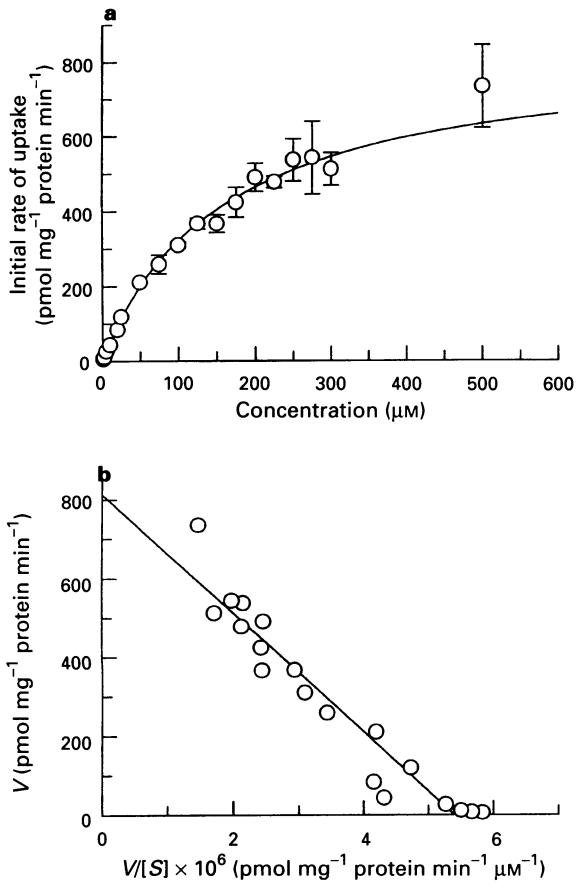

Figure 3 The effect of variation in concentration of uptake substrate on the initial rate of uptake. Graphs represent the data of 3 separate experiments. (a) Direct plot: curve fitted by least squares regression as described in results (K_M = 160.2 ± 19.1 μM; V_{max} = 837.1 ± 43.6 pmol mg^{-1} protein min^{-1}). (b) Eadie-Hofstee transformation of data shown in (a). Linear fit by least squares linear regression (K_M = 150.6 ± 10.6 μM; V_{max} = 813.1 ± 39.6 pmol mg^{-1} protein min^{-1}).

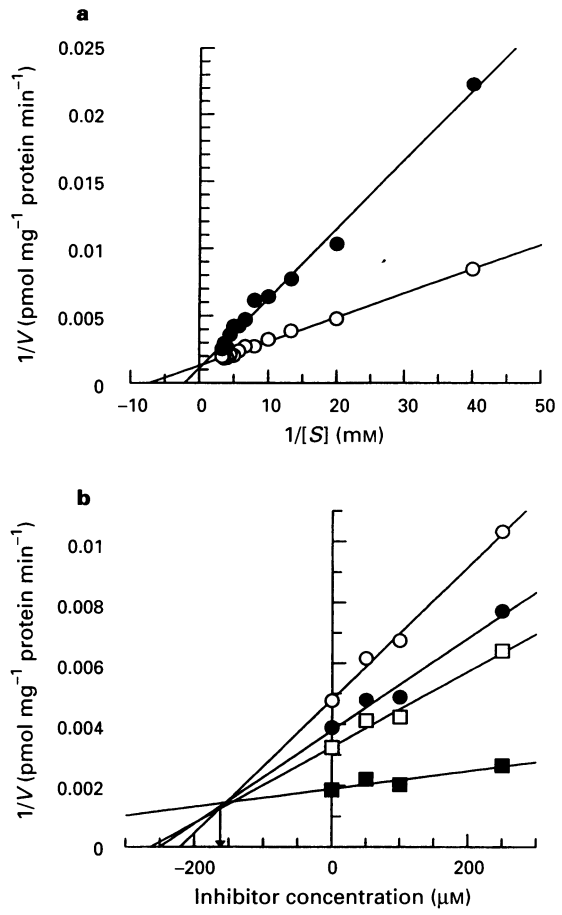

Figure 5 (a) The double reciprocal plot of the initial uptake rates of various concentrations of [^{3}H]-gabapentin in the absence (○) and presence (●) of 250 μM L-leucine (control: K_M = 137.2 μM; V_{max} = 765.5 pmol mg^{-1} protein min^{-1}; + 250 μM L-leucine: K_M = 410.5 μM; V_{max} = 791.77 pmol mg^{-1} protein min^{-1}). (b) Represents the Dixon transformation of the 50 (○), 75 (●), 100 (□) and 250 (■) μM [^{3}H]-gabapentin data from (a) yielding K_i = 160 μM. Graphs represent the mean of data from 3 separate experiments.

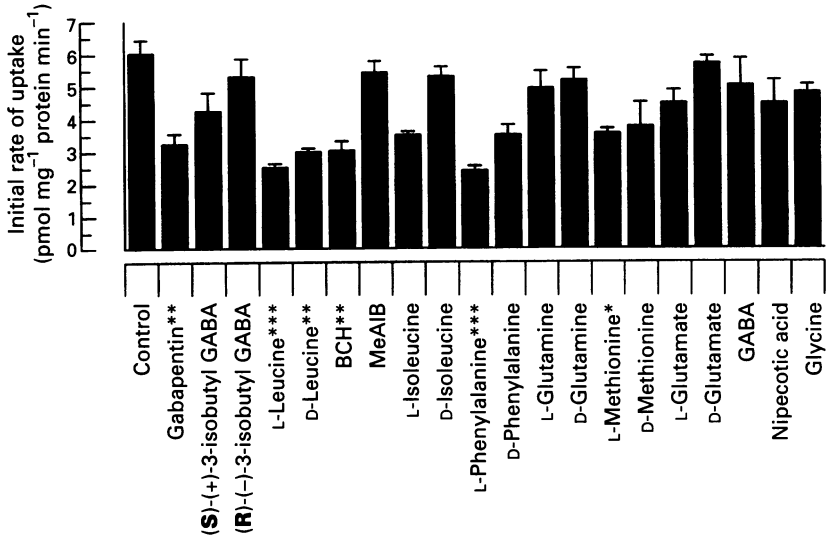

Figure 4 The effect of 100 μM of various compounds on the initial rate of uptake of 1 μM [^{3}H]-gabapentin in rat cerebral cortex synaptosomes. Graph represents the mean of 3 separate experiments. Statistical analysis by Tukey's multiple comparison test (InStat; ***P<0.001, **P<0.01; *P<0.05).

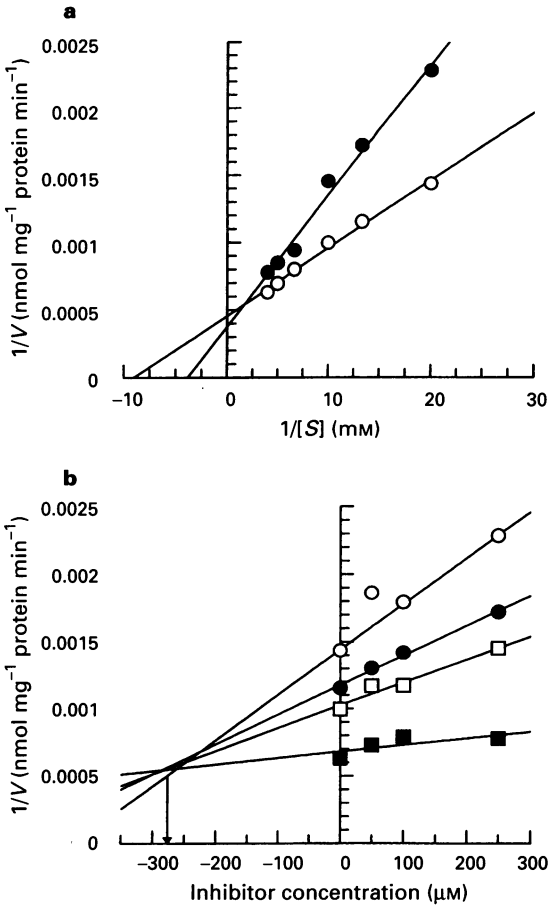

Figure 6 (a) The double reciprocal plot of the uptake initial rates of various concentrations of L-[^{3}H]-leucine in the absence of (○) and presence (●) of 250 μM gabapentin (control: $K_M = 110.3\,\mu$M; $V_{max} = 2.192$ nmol mg^{-1} protein min^{-1} + 250 μM gabapentin: $K_M = 259.3\,\mu$M; $V_{max} = 2.656$ nmol mg^{-1} protein min^{-1}). (b) Represents the Dixon transformation of the 50 (○), 75 (●), 100 (□) and 250 (●) μM L-[^{3}H]-leucine data from (a) yielding $K_i = 261.9\,\mu$M. Graphs represent the mean of data from 3 separate experiments.

Table 1 The effect of gabapentin, BCH and MeAIB on the uptake of (**A**) 1 μM [^{3}H]-glycine, (**B**) 1 μM L-[^{3}H]-glutamine (**C**) 1 μM L-[^{3}H]-glutamate and (**D**) 1 μM L-[^{3}H]-leucine into rat cerebral cortex synaptosomes prepared as described in the methods

A		
[^{3}H]-glycine uptake	*Mean initial rate of uptake (pmol mg^{-1} protein^{-1} min^{-1})*	P
Control	32.62 ± 0.77	—
+ 100 μM gabapentin	23.23 ± 0.47	**
+ 100 μM BCH	31.32 ± 0.74	—
+ 100 μM MeAIB	24.98 ± 0.68	**
B		
L-[^{3}H]-glutamine uptake	*Mean initial rate of uptake (pmol mg^{-1} protein^{-1} min^{-1})*	P
Control	24.54 + 1.66	—
+ 100 μM gabapentin	22.87 ± 1.98	—
+ 100 μM BCH	24.05 ± 1.74	—
+ 100 μM MeAIB	22.36 ± 1.44	—
C		
L-[^{3}H]-glutamate uptake	*Mean initial rate of uptake (pmol mg^{-1} protein^{-1} min^{-1})*	P
Control	435.67 + 28.08	—
+ 100 μM gabapentin	340.62 ± 17.09	**
+ 100 μM BCH	343.85 ± 4.47	*
+ 100 μM MeAIB	295.56 ± 0.90	**
D		
L-[^{3}H]-leucine uptake	*Mean initial rate of uptake (pmol mg^{-1} protein^{-1} min^{-1})*	P
Control	48.74 + 1.31	—
+ 100 μM gabapentin	29.37 ± 0.99	**
+ 100 μM BCH	37.21 ± 0.69	**
+ 100 μM MeAIB	56.23 ± 1.65	**

Results represent the mean and s.e. mean of 3 separate experiments. Statistical tests performed using InStat V2.02 (Dunnett's multiple comparison test).*$P < 0.05$; **$P < 0.01$.

Discussion

Recently, the existence of a structure-activity relationship for the [^{3}H]-gabapentin binding site similar to that observed for the amino acid transport system-L has been discussed (Thurlow *et al.*, 1993). In order to determine whether the binding site for gabapentin is indeed an amino acid uptake site, the above studies were performed.

From the results described it is evident that gabapentin uptake occurs in crude synaptosomes prepared from rat cerebral cortex. The uptake of 1 μM gabapentin is concentrative but to an extent much less than that of 10 nM [^{3}H]-GABA (unpublished personal observations). A similar time course for uptake is illustrated in the study using L-[^{3}H]-leucine. The reason for the difference in temperature maximum between the initial uptake rate for [^{3}H]-gabapentin and L-[^{3}H]-leucine is unclear but may be due to the stability of the radioligands studied. Gabapentin has been shown to be excreted in man unmetabolized (Stewart *et al.*, 1993) whilst L-leucine can undergo metabolism. For this reason it might be assumed that whilst some metabolism of L-leucine occurs, there is no metabolism of gabapentin. Hence, the maximum for L-leucine uptake is at 20°C, as at higher temperatures metabolism will have significant effects on the intracellular L-[^{3}H]-leucine pool. For [^{3}H]-gabapentin, however, the maximum is at 40°C as no metabolism of this radioligand occurs.

The characteristics for uptake of gabapentin appear to mirror those for the uptake of L-leucine and the uptake system-L observed in the periphery (Shotwell *et al.*, 1981) but not those of GABA. The system in synaptosomes has temperature-sensitivity, is Na^+-independent, and requires a supply of energy in the form of ATP. These findings agree well with those described for system-L by Banay-Schwartz *et al.* (1974).

Uptake of gabapentin occurs by a system with single affinity state similar to that observed for L-[^{3}H]-leucine uptake in the same membranes. The initial rate of uptake is inhibited by amino acids that have been shown to utilize system-L (L-leucine, L-isoleucine and L-phenylalanine) in CHO cells (Shotwell *et al.*, 1981) but not those that inhibit system-A (MeAIB) or excitatory (glutamate) and inhibitory (GABA; nipecotic acid) amino acid uptake (Kilberg *et al.*, 1993).

Further evidence that gabapentin uptake occurs by system-L-like activity is illustrated by the inhibition by 250 μM L-leucine of the initial rate of uptake of gabapentin at various gabapentin concentrations when the data are transformed to the double-reciprocal plot or Lineweaver-Burke plot. The resulting change in K_M, with no change in V_{max} is indicative of a competitive interaction at the uptake site. This suggests that L-leucine can compete directly for this gabapentin uptake system. The converse is true for the system when utilising L-leucine as substrate for uptake and gabapentin as inhibitor.

The K_i value for inhibition by leucine of gabapentin uptake was virtually the same as the K_M value for gabapentin uptake. This has been proposed by some authors as evidence that this

uptake system is the major uptake system shared by the two substrates (Christensen, 1984; McGivan & Pastor-Anglada, 1994). For the inhibition of L-leucine uptake by gabapentin, however, these values are not as close but with only a two fold increase. L-Leucine has been shown to utilize system-A for uptake when system-L has been completely inhibited by BCH (Shotwell *et al.*, 1981) in CHO cells and this may well be the reason for this difference. From these results it is possible to conclude that gabapentin utilizes only system-L for transport across membranes in synaptosomes.

The inhibition of other amino acid uptake systems by LNAA has been shown to occur in a variety of systems, but of the greatest interest are those inhibitions that have been shown in CNS tissue (Picolli *et al.*, 1971). In this study the system-L-specific substrate, BCH, and LNAA, inhibited the 15 min uptake of glycine more than the system-A-specific substrates, MeAIB or L-alanine. This is particularly important in the fact that glycine plays such an important role in excitatory events, and together with the possible glycine site partial agonist properties of gabapentin described in various animal and electrophysiological studies (Sprosen, 1990; Oles *et al.*, 1990), may demonstrate a possible mechanism of action.

The preliminary experiments described here demonstrate that gabapentin inhibits glycine uptake by almost 30% at 100 μM, while MeAIB inhibits the uptake by only 23% and BCH did not demonstrate inhibition at all. It is not surprising that BCH in this experiment did not inhibit to the same extent as in the study by Picolli *et al.* (1971) as they used a concentration of 10 mM while we used a concentration of 100 μM. The level of inhibition has been shown to be higher than this in a purer synaptosomal preparation at the more physiologically relevant cerebrospinal fluid concentration, observed after lumbar puncture, of 1 μM (Hill, unpublished observations). Whether this decrease in glycine uptake could elicit anti convulsant effects *in vivo* is still unclear.

From these results it would appear that the structure-activity at the gabapentin binding site (Thurlow *et al.*, 1993) and the uptake characteristics illustrated here, that gabapentin binding and uptake occur at the same molecular site. If this is indeed the case, comparative autoradiography studies into the distribution of gabapentin and LNAA binding sites should elicit identical distributions. Preliminary studies of this type have been performed and comparison of the two distributions yields a correlation coefficient of 0.90 (Thurlow *et al.*, 1996).

In conclusion, gabapentin is taken up into a crude synaptosomal preparation by a process with characteristics virtually identical to those of the LNAA uptake system, termed system-L (Su *et al.*, 1995). Although gabapentin at 100 μM inhibits glycine uptake significantly it is unclear at present how this could elicit an anticonvulsant effect and further work in this area is required. It would appear from these studies that [^{3}H]-gabapentin is a substrate of the LNAA uptake site, system-L.

Gee and co-workers in a personal communication have described the purified, solubilized, [^{3}H]-gabapentin binding site to be the $\alpha_2\delta$-subunit of a calcium channel. The pharmacological site of action therefore does not appear to involve the LNAA uptake site, system-L. The autoradiographical studies described by us (Thurlow *et al.*, 1996) show that the L-[^{3}H]-leucine binding site has a similar distribution to that of [^{3}H]-gabapentin and for this reason suggest L-leucine may be the endogenous ligand for the gabapentin binding site. If this is the case, any alteration in the uptake of L-leucine may affect the levels of this amino acid and hence have a pro- or anti-convulsant action. Further work in this area is required.

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