Glutamate uptake into cerebral cortex slices is reduced in the presence of a gamma-glutamyl transpeptidase inhibitor

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Summary. The uptake of L-glutamic acid into brain cortex slices of mice of different age was studied. The results suggest that GGT contributes to the transport of L-glutamic acid into the brain.

Gamma-glutamyl transpeptidase (GGT) occurs in many mammalian tissues, especially those actively engaged in transport and secretory phenomena (for review see Meister[1] and Meister et al.[2]). In mouse brain the highest activity of GGT is found in the choroid plexus and in capillary endothelium[3], but its presence in neurons and glial cells has also been demonstrated[4]. Because of the limited evidence for a direct involvement of GGT in transport in the nervous system[5], we have investigated the effects of the inhibition of GGT activity on L-glutamic acid uptake into mouse brain cortex slices in vitro.

Methods. 1 mm slices of cerebral cortex were prepared with an adapted rotating microtome (Reichert) from white male inbred mice of different ages. The slices were incubated in 'HEPES-buffered' medium[6], containing 13 mM serine + 13 mM borate or 13 mM serine and/or 13 mM borate alone. After 15 min incubation at 37 °C, ^{14}C-glutamic acid (0.1 mM) was added to the medium. After an additional 5 min the medium was filtered from the slices by suction and slices were washed 3 times with ice-cold NaCl (9 g · l^{-1}) (Sershen et al.[6]). To distinguish between active and passive uptake parallel slices were incubated at 4 °C. The slices were then transferred into perchloric acid (PCA; 50 g · l^{-1}) and homogenized in a glass homogenizer equipped with a motor driven Teflon pestle. After centrifugation (3500 rpm, 30 min) in the cold, a portion (0.5 ml) of the clear supernatant was added to 7.5 ml of scintillation fluid and counted for radioactivity. Pellets were dissolved in NaOH (1 mole · l^{-1}) and the protein content was estimated using the method of Lowry et al.[7]. The number of estimations corresponded to the number of animals (1 slice was prepared from each mouse brain) used for each age group (Table).

Results and discussion. The table shows that the uptake of L-glutamic acid into brain slices represents mainly active transport, as is evident from the different uptake rates at 37 and 4 °C. The apparent decrease in the rate of uptake between days 7 and 13 is undoubtedly due to the dilution of counts by the increase in protein content of slices from older brain. This increase in protein content in cortex slices from rodent brain of different ages was shown by Schousboe[8]. At all ages studied, glutamate uptake was inhibited in the presence of serine-borate by 25–35%. Neither L-serine nor borate alone had any statistically significant influence on glutamate uptake. The combination of L-serine with borate is one of the most potent inhibitors of GGT from a variety of mammalian tissues[2] but has no effect on Na^+, K^+-ATPase activity[9]. Thus the effect of this inhibitor on the uptake of L-glutamic acid in our experiments can be explained by the inhibition of GGT mediated membrane transport of this amino acid. The most significant inhibitory effect was observed in 7-day-old animals. At this age the Na^+-gradient across the cell membrane is not yet well established[10] and therefore a considerable proportion of transport is evidently sodium independent. Among the many transport systems for amino acids in the brain[6], GGT may represent one of those that is not dependent on the presence of sodium ions[5]. Thus it can be concluded from data presented here that GGT mediates membrane translocation of L-glutamate in brain tissues, and that the contributory role of this enzyme to transport decreases during early postnatal development, owing to the maturation of the sodium-dependent transport systems for amino acids.

The uptake of ^{14}C-glutamic acid (0.1 mM) into mouse brain slices incubated under various conditions (cpm/100 µg protein)

Age (days)	Control	Serine (13 mM) + borate (13 mM)	Serine (13 mM)	Borate (13 mM)	At 4°	Inhibition by serine + borate (%)
7	1881 ± 112 (8)	1232 ± 129 (8) $p < 0.01$	1748 ± 338 (12) n.s.	1588 ± 433 (10) n.s.	339 ± 43 (6)	35
13	714 ± 52 (6)	536 ± 13 (6) $p < 0.01$	793 ± 117 (8) n.s.	654 ± 93 (8) n.s.	128 ± 28 (6)	25
30	737 ± 112 (14)	541 ± 88 (16) $p < 0.01$	686 ± 70 (10) n.s.	681 ± 81 (8) n.s.	131 ± 18 (6)	27

Each value is the mean ± SD of number of estimations given in brackets. Statistical significance was calculated using Student's t-test. Non-significant values (n.s.) were over the limit of $p < 0.05$.

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