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Interaction of Kainic Acid with Na^+-Dependent Glutamate Binding and Uptake in the Cerebral Cortex of the Developing Mouse

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Summary

The effect of kainic acid, a structural analogue and specific agonist of glutamate, was studied on the Na^+-dependent binding and uptake of this amino acid in cerebral cortex preparations from 7-day-old and 30-day-old mice. The specific binding of glutamate to a crude synaptic membrane fraction and uptake into cortical slices increased several fold during this period. Kainic acid (0.5 mM or 5 mM) significantly reduced glutamate binding and this effect was more pronounced in membrane fractions from older animals. In contrast to this, the inhibitory action of kainic acid on glutamate uptake was twofold more potent in 7-day-old mice. The results are discussed from the viewpoint of the relationship between the Na^+-dependent binding of glutamate and its uptake.

Key words
Glutamate binding – Glutamate uptake – Kainic acid – Cerebral cortex – Developing mouse

Introduction

Glutamate, the most prevalent amino acid in the mammalian cerebral cortex, has been shown to be a powerful excitatory neurotransmitter in the central nervous system (Emson and Lindvall 1979, Watkins and Evans 1981, Fonnum 1984, Engelsen 1986). Besides the specific metabolic pathways for glutamate in the brain, transport systems are present which ensure the replenishment of this synaptically released amino acid by specific reuptake mechanisms and also its uptake into glial cells (Watkins and Evans 1981, Nicklas 1983, Fonnum 1984). The uptake of glutamate into glia and neurones consists of both low- and high-affinity components which differ in sensitivity to the presence of Na^+. In the case of low concentrations of glutamate the uptake seems to be more than 90 % Na^+-dependent (Bennet *et al.* 1974, Hertz *et al.* 1983). The precise molecular and cellular processes that underlie glutamate uptake are not known, but it was proposed that the uptake sites may comprise a receptor subtype exhibiting Na^+-dependence (Watkins 1978, Baudry and Lynch 1981, LaBella 1985, Ogita and Yoneda 1986).

Kainic acid (KA), a cyclic analogue of glutamate, can reduce its uptake in the cerebral cortex (McGeer *et al.* 1978, Johnston *et al.* 1979) and also displace

glutamate binding to receptors (Watkins 1978, Vincent and McGeer 1980). In addition, controversial data exist on the inhibitory effect of kainic acid on gamma-glutamyl transpeptidase (GGT; EC 2.3.2.2), the enzyme which might be implicated in membrane translocation of glutamate in the brain (Minn and Besagni 1983, Lisý *et al.* 1983a, Lisý and Murphy 1984, Rothe and Wolf 1985).

The aim of this study is to correlate the developmental differences in Na^+-dependent glutamate binding and uptake in cerebral cortex preparations *in vitro* and to specify the influence of kainic acid on these processes in media of the same ionic composition.

Material and Methods

L-(U-^{14}C)glutamic acid (specific activity 185 mCi/mmol) was obtained from the Institute for Research, Production and Applications of Radioisotopes (Prague, CSFR). Kainic acid and L-glutamic acid were purchased from Sigma Chemical Co. All other chemicals were of the analytical grade.

The preparation of a crude synaptic fraction (P_2) from brain cortices of 7- and 30-day-old mice was based on the method of Cotman and Matthews (1971). The P_2 fraction was subjected to lysis in a hypotonic buffer (5 mM Tris-HCl, pH 7.4) as described by Sanderson and Murphy (1982). Membranes sedimented by centrifugation (18 000xg; 30 min) were resuspended in a "HEPES-buffered" medium (pH 7.2) containing 110 mM NaCl, 5.0 mM KCl, 0.75 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.0 mM NaH_2PO_4, 1.0 mM $NaHCO_3$, 25 mM HEPES (Sigma Chemical Co.) and 12 mM NaOH (Sershen and Lajtha 1979, Sanderson and Murphy 1982). The membrane suspension was diluted to 10 mg of protein per 1 ml.

Binding assays were run in plastic tubes containing 0.8 ml of "HEPES-buffered" medium (without or with kainic acid in a final concentration of 0.5 or 5 mM), 0.1 ml of the membrane suspension and 0.1 ml of 5 μM ^{14}C-glutamate. Specific binding was defined as that displaced by 95 μM unlabelled glutamate. Samples were incubated in an ice water bath for various intervals. The incubation was terminated by rapid centrifugation at 18 000xg in a refrigerated rotor. The supernatant was carefully decanted and the pellet was superficially rinsed twice with 5 ml of ice-cold distilled water, drained overnight at 4 °C and solubilized in 1 ml of 0.1 M NaOH. Scintillation fluid (5 ml) containing toluene-Triton mixture (v/v 2:1) with PPO (Sigma Chemical Co.) was added to a 0.5 ml aliquot of this solution and radioactivity was assayed in a Beckman 980 Scintillation Counter.

Under these conditions no difference was observed between the total and the non-specific binding of radioactive glutamate if 110 mM NaCl was omitted during the incubation. Thus, all the specific binding estimated in the presence of NaCl was considered as Na^+-specific.

For the uptake experiments, cerebral cortex slices were prepared and incubated in "HEPES-buffered" medium (pH 7.2) containing 10 mM glucose. The final concentration of added glutamate was 0.1 mM including 5 μM ^{14}C-labelled glutamate. The radioactivity of the acid soluble fraction was measured as described previously (Lisý *et al.* 1983b).

The extracellular and intracellular compartments in brain slices, the determination of which is a prerequisite for the calculation of intracellular glutamate accumulation, were estimated by measuring the inulin space (Heyrovský 1956, Šťastný 1974).

The protein content was estimated by the method of Lowry *et al.* (1951).

The statistical significance of the results was evaluated by Student's t-test.

Results

The time course of total and non-specific Na^+-dependent binding of ^{14}C-glutamate to cortical membranes from mice of both age groups shows an initial rapid elevation during the first 5 min followed by a slower increase up to 25 min of incubation (Fig. 1). Specific binding of labelled glutamate was obtained by subtracting the amount that was not displaced by an excess of unlabelled glutamate,

from the totally bound radioactivity. Routinely, specific binding represented about 50 and 70% of the total binding in 7-day-old or 30-day-old mice, respectively. During this developmental period, there was a twofold increase of non-specific binding and a fivefold elevation of Na^+-dependent specific binding (as determined after 25 min of incubation).

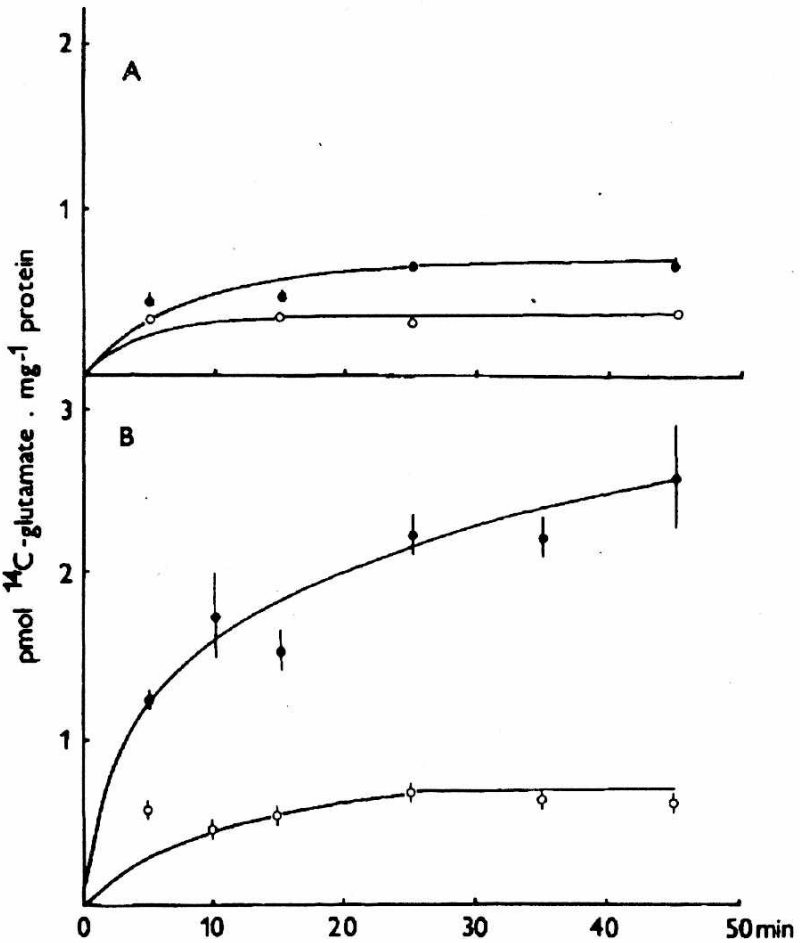

Fig. 1
Time-course of ^{14}C-glutamate binding to membranes of the P_2 fraction prepared from 7-day-old (A) or 30-day-old mice (B). Full dots – total binding; open circles – non-specific binding. Each point represents the mean ± S.E.M. of 4–9 estimations.

Fig. 2 shows that ^{14}C-glutamate specific binding is affected by the presence of kainic acid in concentrations of 0.5 and 5 mM. The lower concentration of kainic acid effectively reduces the specific binding of this amino acid in cortical membranes of both age groups. The elevation of kainic acid concentration leads to a significant increase in the inhibition of glutamate binding only in the older animals.

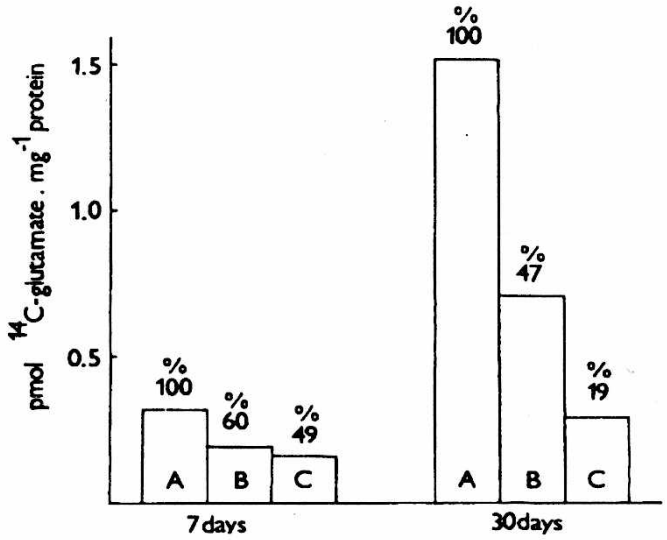

Fig. 2
Effect of kainic acid (KA) on the specific binding of ^{14}C-glutamate (25 min) to membranes of P_2 fraction prepared from 7-day-old and 30-day-old mice. A, controls; B, 0.5 mM KA; C, 5 mM KA. The residual binding after kainic acid inhibition is expressed as percentage of control values.

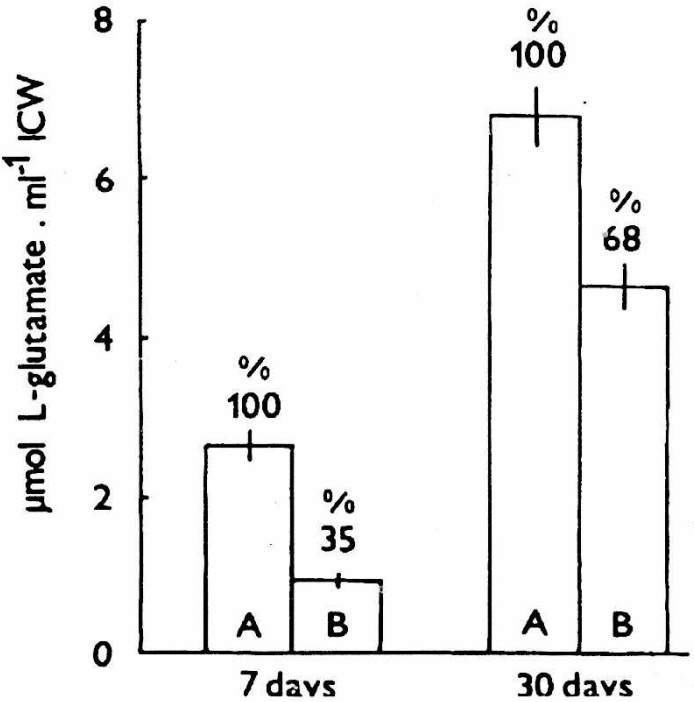

Fig. 3
Effect of kainic acid (KA) on the uptake of L-glutamate in cerebral cortex slices prepared from 7-day-old and 30-day-old mice. A, controls; B, 5 mM KA. Each column represents the mean ± S.E.M. of 11–44 estimations. The residual uptake after kainic acid inhibition is expressed as percentage of control values. ICW – intracellular water content.

The effect of 5 mM KA on the uptake of ^{14}C-glutamate into cortical slices is shown in Fig. 3. The slices were incubated for 5 min to measure the initial rates of uptake. The inhibitory effect of kainic acid was more pronounced in cortical slices from younger mice.

Discussion

In the synaptic cleft, the neurotransmitter amino acids (glutamate and aspartate) are removed from the vicinity of their membrane receptors by uptake systems present in neurones, synaptic endings and adjacent glia (Schousboe *et al.* 1988, Storm-Mathisen and Ottersen 1988). The concept of the reuptake of neurotransmitter amino acids is correlated with the existence of receptor uptake sites involved in translocation of the ligand-receptor complex into a cell compartment (LaBella 1985). Glutamate binding to the physiological uptake sites requires the presence of Na^+, similarly as the Na^+-dependent glutamate uptake mechanism (Vincent and McGeer 1980).

Our findings suggest that there is some relationship between the developmental increase in glutamate binding and uptake estimated in the medium containing Na^+. For this comparison, slices were incubated in the optimal uptake medium described by Sershen and Lajtha (1979) and binding experiments were run in the same medium at 4 °C in order to attain the optimum in maximum binding (Sanderson and Murphy 1982, Koshiya 1985, Ogita and Yoneda 1986).

The partial discrepancy between the developmental increase of Na^+-dependent glutamate uptake (twofold) and its binding (fivefold) may be explained, at least in part, by the fact that uptake increase seen in cortical slices does not fully reflect the situation of glutamate binding in isolated cortical cell membranes (Lakshmanan and Padmanaban 1974). Isolation procedure can induce changes in the total number of receptors or in their binding affinity (Perlmutter and Raichle 1986).

A structurally rigid analogue of glutamate, kainic acid (KA), is a moderately potent inhibitor of glutamate uptake into cortical slices (Johnston *et al.* 1979, Krespan *et al.* 1982, Yoneda *et al.* 1989). The degree of the inhibitory action of kainic acid on glutamate uptake *in vitro* strictly depends on the concentration used (Nicklas 1983, Anand *et al.* 1986). A relatively high concentration of kainic acid is necessary for a significant inhibition of glutamate uptake (Cox and Bradford 1978). A recommended concentration of 1 mM KA that was used in our experiments (data not shown) reduced the Na^+-dependent uptake of L-glutamate into slices of 7-day-old mice by 18 %, but in the case of cortical slices from older animals this concentration of kainic acid was ineffective. The concentration of 5 mM KA was significantly effective in both age groups, but its effect on uptake was much less in older than in young mice. This could be due to the fact that larger extracellular compartment in the cerebral cortex of younger animals enables easier penetration of kainic acid to the vicinity of glutamate binding/translocation sites. Under such circumstances a higher proportion of KA molecules to a low number of glutamate translocation sites (in contrast to a developmentally increased number of these sites in the cerebral cortex of older animals) contributes to a more effective reduction of glutamate uptake in cortical slices of younger mice.

Na^+-dependent glutamate binding probably represents binding to sites involved in amino acid transport (Baudry and Lynch 1981, LaBella 1985, Ogita and Yoneda 1986). Kainic acid may displace glutamate from the receptor site(s) and thus inhibit Na^+-dependent binding of this amino acid (Watkins 1978, Vincent and McGeer 1980). However, there are some data demonstrating the inability of kainic acid to inhibit glutamate binding to membrane preparations (Watkins and Evans 1981, Sanderson and Murphy 1982, Yoneda *et al.* 1986). Using the same incubation medium as for the uptake experiments, we found a significant reduction (40 % and 53 %) of Na^+-dependent binding by 0.5 mM KA in 7-day-old and 30-day-old mice, respectively. A tenfold increase in KA concentration led to further inhibition (81 %) of specific glutamate binding, but only in cortical membranes of 30-day-old animals. This increase in the inhibitory action of kainic acid could be explained by the developmental enrichment of nerve cell membranes with KA-preferring glutamate binding sites. It is worthwhile to note in this context that some data exist on the dependence of glutamate or kainic acid-related phenomena, including binding capacity, on the stage of postnatal maturation of glutamatergic systems (Wolf and Keilhoff 1984).

We conclude that kainic acid plays a role in alterations of the Na^+-dependent binding and uptake of glutamate in the cerebral cortex of the developing mouse and that both membrane functions are closely related. Membrane receptors for Na^+-dependent binding of glutamate seem to overlap, at least in part, with glutamate uptake sites.

It can be speculated that kainic acid-induced decrease of glutamate uptake in brain slices is a consequence of the inhibitory action of the neurotoxin on membrane-bound gamma-glutamyl transpeptidase (GGT) (Lisý and Murphy 1984). As both, GGT and receptor sites for glutamate, are of a glycoprotein nature (Meister *et al.* 1976, Michaelis 1979), one could suspect that the enzyme is a part of the receptor-carrier complex and that its inactivation with kainic acid blocks the binding and/or uptake function of this system.

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