

Age and Brain Structural Related Effects of Glutaric and 3-Hydroxyglutaric Acids on Glutamate Binding to Plasma Membranes During Rat Brain Development

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Abstract (1) In the present study we determined the effects of glutaric (GA, 0.01–1 mM) and 3-hydroxyglutaric (3-OHGA, 1.0–100 μ M) acids, the major metabolites accumulating in glutaric acidemia type I (GA I), on Na^+ -independent and Na^+ -dependent [^3H]glutamate binding to synaptic plasma membranes from cerebral cortex and striatum of rats aged 7, 15 and 60 days. (2) GA selectively inhibited Na^+ -independent [^3H]glutamate binding (binding to receptors) in cerebral cortex and striatum of rats aged 7 and 15 days, but not aged 60 days. In contrast, GA did not alter Na^+ -dependent glutamate binding (binding to transporters) to synaptic membranes from brain structures of rats at all studied ages. Furthermore, experiments using the glutamatergic antagonist CNQX indicated that GA probably binds to non-NMDA receptors. In addition, GA markedly inhibited [^3H]kainate binding to synaptic plasma membranes in cerebral cortex of 15-day-old rats, indicating that this effect was probably directed towards kainate receptors. On the other hand, experiments performed with 3-OHGA revealed that this organic acid did not change Na^+ -independent [^3H]glutamate binding to synaptic membranes from cerebral cortex and striatum of rats from all ages, but inhibited Na^+ -dependent [^3H]glutamate binding to membranes in striatum of 7-day-old rats, but not in striatum of 15- and 60-day-old rats and in cerebral cortex of rats from all studied ages. We also provided some evidence that 3-OHGA competes with the glutamate transporter inhibitor *L-trans*-pyrrolidine-2,4-dicarboxylate, suggesting a possible interaction of 3-OHGA with glutamate transporters on synaptic membranes. (3) These results indicate that glutamate binding to receptors and transporters can be

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inhibited by GA and 3-OHGA in cerebral cortex and striatum in a developmentally regulated manner. It is postulated that a disturbance of glutamatergic neurotransmission caused by the major metabolites accumulating in GA I at early development may possibly explain, at least in part, the window of vulnerability of striatum and cerebral cortex to injury in patients affected by this disorder.

Keywords Glutaric acidemia type I · Glutaric acid · 3-Hydroxyglutaric acid · Glutamatergic system

Introduction

Glutaric acidemia type I (GA I, McKusick 23167; OMIM # 231670) is an autosomal recessive inherited neurometabolic disease caused by deficiency of the activity of the mitochondrial enzyme glutaryl-CoA dehydrogenase (GCDH, EC 1.3.99.7), which is involved in the catabolic pathway of lysine, hydroxylysine and tryptophan (Goodman et al. 1975). Increased concentrations of glutaric acid (GA, 500–5000 $\mu\text{mol/l}$), as well as of 3-hydroxyglutaric acid (3-OHGA) in lower amounts (40–200 $\mu\text{mol/l}$), are found in the body fluids and in the brain of GA I patients (Goodman and Frerman 2001; Strauss and Morton 2003; Strauss et al. 2003; Sauer et al. 2006). Clinical manifestations of GA I are predominantly neurological and appear especially after encephalopathic crises, which occur between 6 and 36 months of age and are accompanied by bilateral destruction of caudate and putamen (Hoffmann and Zschocke 1999; Morton et al. 1991). Frontotemporal atrophy, frequently detected at birth, is a distinctive radiological appearance that may be patho-gnomonic in GA I (Strauss et al. 2003). *Post mortem* examination of the basal ganglia and cerebral cortex of patients with GA I revealed postsynaptic vacuolization characteristic of glutamate-mediated brain injury (Goodman et al. 1977).

Glutamate is the main excitatory neurotransmitter in the mammalian brain and its interactions with specific membrane receptors are responsible for many CNS functions such as cognition, memory and movement (Ozawa et al. 1998; Danbolt 2001). The role of glutamate in mammalian brain is mediated by activation of glutamate-gated cation channels termed ionotropic receptors and of GTP-binding protein (G-protein)-linked receptors termed metabotropic receptors (Nakanishi 1992; Hollmann and Heinemann 1994; Ozawa et al. 1998). Ionotropic receptors can be divided into *N*-methyl-D-aspartate (NMDA: NR1 and NR2A-D) and non-NMDA, the latter including the α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA: GluR1-4) and kainate (GluR5-7 and KA1-2) receptors. Metabotropic glutamate receptors (mGluRs) have been divided into groups I, II and III (Conn and Pin 1997; Ozawa et al. 1998).

Glutamate receptors are involved in a variety of physiological processes during brain development, including synaptogenesis and synaptic plasticity, and present a unique profile of susceptibility to toxicity mediated by differential activation of the receptor subtypes (McDonald and Johnston 1990). Ionotropic receptor ontogeny is characterized by rapid maturational changes in various forebrain structures in the rat. NMDA receptor expression reaches the highest level in hippocampus and neocortex in the first postnatal week, whereas AMPA receptors density peaks occur in the second postnatal week (Insel et al. 1990; Petralia et al. 1999). On the other hand, NMDA receptor maturation occurs later than kainate and AMPA receptor expression in the neonatal rat

striatum (Colwell et al. 1998; Nansen et al. 2000). This variable receptor expression profile generates a regional- and age-specific window of susceptibility to many neurotoxins and diseases (Kölker et al. 2000a; Haberny et al. 2002; Jensen 2002).

The synaptic actions of glutamate are terminated by its removal from the synaptic cleft by a high-affinity sodium-dependent excitatory amino acid transporter (EAAT) system, mainly located in the astrocytic membranes (Danbolt 2001; Amara and Fontana 2002). The astroglial glutamate transporters GLAST (EAAT1) and GLT1 (EAAT2) are mainly responsible for the clearance of extracellular glutamate (Rothstein et al. 1996; Danbolt 2001). In the rat, GLAST is expressed at birth, whereas GLT1 is mainly expressed during the second to third postnatal week. Both transporters are fully expressed by postnatal week 5, but GLT1 is the predominant glutamate astroglial transporter in the adult brain (Ullensvang et al. 1997).

Glutamate is also a potent neurotoxin when present at high amounts in the synaptic cleft leading to excitotoxicity by over-stimulation of glutamate receptors, a process related to the neuropathology of acute and chronic brain disorders (Olney 1969; Lipton and Rosenberg 1994; Maragakis and Rothstein 2001).

A considerable body of evidence has emerged from recent studies indicating excitotoxic actions for GA and 3-OHGA, two organic acids with similar chemical structure to that of glutamate (Flott-Rahmel et al. 1997; Lima et al. 1998; Kölker et al. 1999, 2000a, b, 2002a, b; Ullrich et al. 1999; Porciúncula et al. 2000, 2004; Mello et al. 2001; Rosa et al. 2004). However, the exact underlying mechanisms by which GA and 3-OHGA lead to excitotoxicity are to date poorly known. Therefore, in the present work we studied the influence of GA and 3-OHGA on Na⁺-independent and Na⁺-dependent [³H]glutamate binding in synaptic plasma membranes from cerebral cortex and striatum of rats from different ages (7, 15 and 60 days) in order to better characterize the role of these organic acids on glutamate receptors and transporters during rat brain development. We choose these rat ages because children with GA I are more vulnerable to the effects of an acute metabolic stress during the first 3 years of age (Bjugstad et al. 2000), which correspond approximately to 7–15 days of life in rats, whereas 60-day-old rats match to young adult humans (Haberny et al. 2002).

Materials and Methods

Materials

Chemicals of analytical reagent grade were mainly purchased from Sigma (St Louis, MO, USA) and Tocris Cookson Inc. (Ellisville, MO, USA), whereas 3-OHGA was prepared with 99% purity by Dr Ernesto Brunet (Universidad Autonoma de Madrid, Spain). The labeled product L-[³H]glutamate (52 Ci/mmol) was purchased from PerkinElmer Life and Analytical Sciences (Boston, MA, USA), whereas [³H]kainic acid (58 Ci/mmol) was obtained from New England Nuclear (Germany).

Animals

Wistar rats of 7, 15 and 60 days of age were obtained from the Central Animal House of the Department of Biochemistry, ICBS, Federal University of Rio Grande do Sul, Porto Alegre, Brazil. They were maintained at 25°C, on a 12:12 h light/dark cycle, with free access to food and water. The “Principles of laboratory animal care” (NIH publications

No. 80-23, revised 1996) were followed in all experiments. All efforts were made to minimize the number of animals used and their suffering.

Membrane Preparation

Animals were killed by decapitation without anesthesia, the brain rapidly removed and the cerebral cortex and striatum immediately dissected on a Petri dish on ice. Synaptic membranes were isolated from the cerebral cortex and striatum of rats, as described by Jones and Matus (1974) with slight modifications (Rosa et al. 2004), and stored frozen at -70°C for no more than 2 weeks. On the day of assay, membranes were thawed at 37°C for 30 min, and washed three times in hypotonic solution consisting of 5 mM Tris-acetate (glutamate binding) or Tris-HCl (kainate binding), pH 7.4, by centrifugation at 45,000g at 4°C for 15 min. We have previously observed that this procedure avoids membrane sealing, in agreement to the literature (Danbolt 1994).

[^3H]Glutamate Binding

Glutamate binding experiments were carried out in the absence of sodium, aiming glutamate binding to receptors, and in the presence of sodium, aiming glutamate binding to transporters, which depends on sodium for their activity. The incubations were carried out in triplicate in polycarbonated tubes (total volume 500 μl) containing 50 mM Tris-acetate (Na^+ -independent binding) or 50 mM Tris-acetate/120 mM NaCl (Na^+ -dependent glutamate binding), pH 7.4, 40 nM [^3H]glutamate (0.3 μCi) and GA ranging from 0.01 to 1 mM, or 3-OHGA ranging from 1 to 100 μM . Some experiments (Na^+ -independent glutamate binding) were performed in the presence of 100 μM of the ionotropic non-NMDA and NMDA receptors antagonists 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and DL-2-amino-phosphonovaleric acid (AP5), respectively. Na^+ -dependent [^3H]glutamate binding was also evaluated in the presence of 50 μM L-trans-pyrrolidine-2,4-dicarboxylate (PDC), which is a substrate inhibitor of glutamate transporters. Controls did not contain GA or 3-OHGA. Incubation was started by adding 50–100 μg of protein membrane, and run at 30°C for 30 min. The reaction was stopped by centrifugation at 16,800g for 15 min at 4°C . The pellet was carefully washed with ice-cold distilled water and resuspended with 0.1 M NaOH and 0.01% sodium dodecyl sulfate (w/v) overnight. Radioactivity was determined using a Wallac 1409 liquid scintillation counter. Non-specific binding (10–20% of the total binding) was determined by adding 40 μM non-radioactive glutamate to the medium in parallel assays. Specific binding was considered as the difference between total binding and non-specific binding. Results were calculated as pmol [^3H]glutamate/mg protein and expressed as percentage of control.

[^3H]Kainate Binding

[^3H]Kainate binding assays were carried out in small polycarbonate tubes (total volume 500 μl) using synaptic plasma membranes from cerebral cortex of 15-day-old rats. Incubations were started by the addition of membrane preparation (100–150 μg of protein) to a medium containing 50 mM Tris-HCl, pH 7.4, 10 nM [^3H]kainate (0.29 μCi), in the absence or presence of 400 μM non-radioactive kainate (non-specific binding). In some experiments GA (1.0 mM) was also added to the medium, whereas

controls did not contain this acid. Following 90 min of incubation at 0°C, bound and free [³H]kainate were separated by centrifugation at 16,800g for 15 min at 4°C. The pellet was washed with ice-cold distilled water and dissolved overnight with 0.1 M NaOH and 0.01% sodium dodecyl sulfate (w/v). Radioactivity was determined using a Wallac 1409 liquid scintillation counter. Specific binding was calculated as the difference between total binding and non-specific binding. Results were calculated as pmol [³H]kainate/mg protein and expressed as percentage of control.

Protein Determination

Protein concentration was measured by the method of Lowry et al. (1951) using bovine serum albumin as standard.

Statistical Analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by the Duncan's multiple range test when appropriate. The Student's *t*-test for paired samples was also used for comparison between two means. Only significant values are shown in the text. Analyses were performed using the SPSS (Statistical Package for the Social Sciences) software in a PC-compatible computer. A value of *P* < 0.05 was considered to be significant.

Results

Figure 1 shows the effect of GA on glutamate binding to synaptic plasma membranes from cerebral cortex and striatum of rats aged 7 (A), 15 (B) and 60 (C) days in the absence of sodium, which reflects glutamate binding to receptors. GA significantly decreased Na⁺-independent [³H]glutamate binding (up to 25%) at concentrations as low as 0.01 mM in cerebral cortex and at 1 mM concentration in striatum from rats of 7 (cerebral cortex: [*F*(3, 26) = 3.50; *P* < 0.05]; striatum: [*F*(3, 20) = 7.96; *P* < 0.01]) and 15 (cerebral cortex: [*F*(3, 26) = 8.73; *P* < 0.001]; striatum: [*F*(3, 19) = 4.88; *P* < 0.05]) days of life. In contrast, Na⁺-independent [³H]glutamate binding to synaptic membranes from rats aged 60 days was not changed by GA.

The next set of experiments tested the role of glutamate antagonists on GA-induced Na⁺-independent [³H]glutamate binding decrease in synaptic membranes from cerebral cortex and striatum of 15-day-old rats. GA (1 mM) and the ionotropic non-NMDA antagonist CNQX (100 μM) were incubated alone or combined. We observed that GA did not change the decrease provoked by CNQX on Na⁺-independent [³H]glutamate binding both in cerebral cortex [*F*(5, 36) = 14.09; *P* < 0.001] (Fig. 2A) and striatum [*F*(5, 36) = 5.56; *P* < 0.01] (Fig. 2B). Furthermore, when the NMDA antagonist AP5 (100 μM) was co-incubated with GA (1 mM), we observed a slight higher decrease of Na⁺-independent [³H]glutamate binding relatively to that of AP5 alone.

We then investigated the effect of GA on [³H]kainate binding to synaptic membranes from cerebral cortex of 15-day-old rats in order to clarify which non-NMDA receptors were involved in GA effect. We observed that [³H]kainate binding was markedly decreased (60% decrease) by 1 mM GA [*t*(4) = 4.13; *P* < 0.05] (Fig. 3).

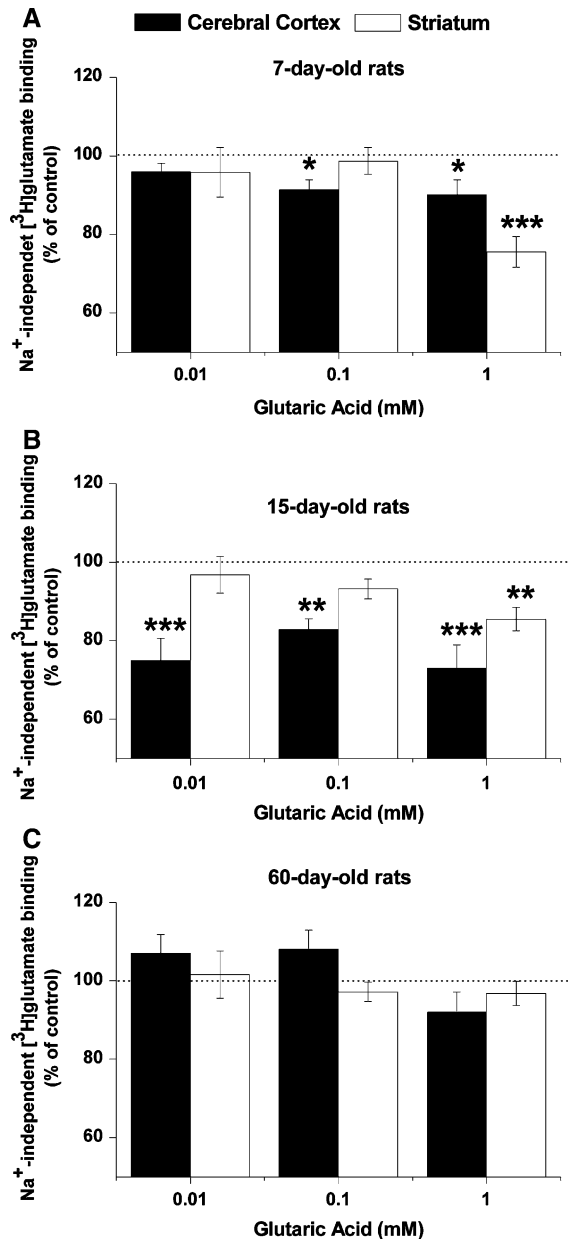


Fig. 1 Effect of glutaric acid (GA, 0.01–1 mM) exposure on Na⁺-independent [³H]glutamate binding (binding to receptors) to synaptic plasma membranes from cerebral cortex and striatum of rats aged 7 (A), 15 (B) and 60 (C) days. Results are presented as mean ± SEM from five to eight independent experiments performed in triplicate and are expressed as percentage of controls. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with controls (Duncan multiple range test)

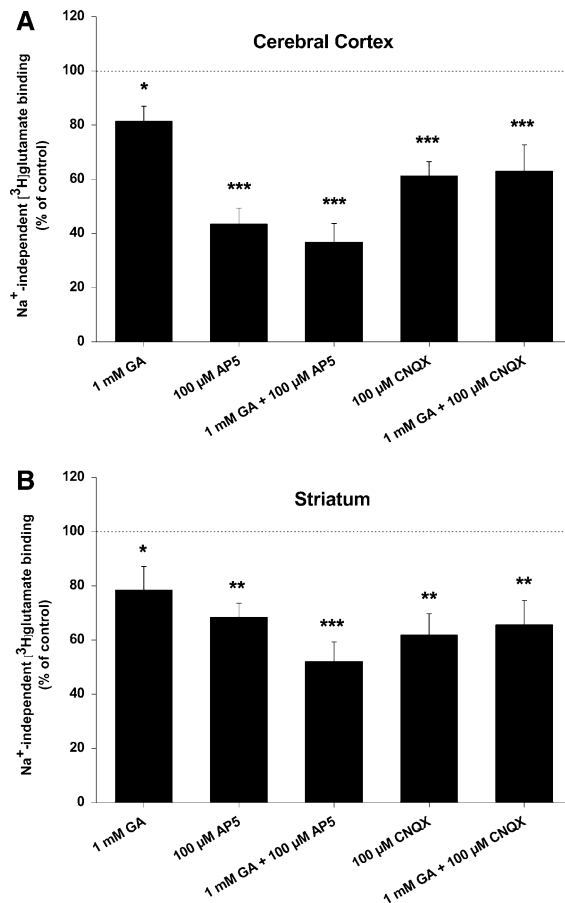


Fig. 2 Effect of glutaric acid (GA, 1 mM), AP5 (100 μM) and CNQX (100 μM) on Na⁺-independent [³H]glutamate binding to synaptic plasma membranes from cerebral cortex (**A**) and striatum (**B**) of 15-day-old rats. Results are presented as mean ± SEM from seven independent experiments performed in triplicate and are expressed as percentages of controls. **P* < 0.05, ***P* < 0.01, ****P* < 0.001, compared with controls (Duncan multiple range test)

We also verified that GA (0.01–1 mM) did not modify Na⁺-dependent [³H]glutamate binding to synaptic membranes in cerebral cortex and striatum from rats of 7, 15 and 60 days of life (data not shown).

The next set of experiments were carried out in order to test the influence of 3-OHGA on Na⁺-independent [³H]glutamate binding. We observed that 3-OHGA did not change Na⁺-independent [³H]glutamate binding in cerebral cortex and striatum from rats of 7, 15 and 60 days of life (data not shown). In contrast, 3-OHGA (at 100 μM concentration) significantly inhibited (20%) Na⁺-dependent [³H]glutamate binding to membranes in striatum of 7-day-old rats [*F*(3, 16) = 3.58; *P* < 0.05] (Fig. 4A), but not in striatum of 15- (Fig. 4B) and 60-day-old rats (Fig. 4C) and in cerebral cortex from all rat ages. Next we tested the action of 3-OHGA on Na⁺-dependent [³H]glutamate binding in synaptic membranes from striatum of 7-day-old rats in the presence of 50 μM of the competitive inhibitor (PDC) of glutamate transporters. We observed that both

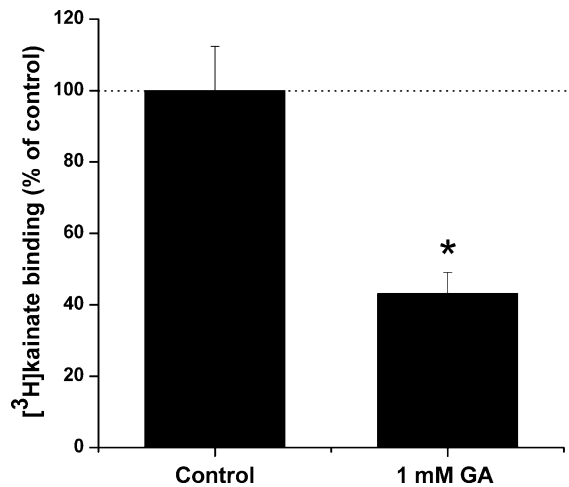


Fig. 3 Effect of glutaric acid (GA, 1 mM) on [³H]kainate binding to synaptic plasma membranes from cerebral cortex of 15-day-old rats. Results are means \pm SEM of five independent experiments performed in triplicate and are expressed as percentage of control. * $P < 0.05$, compared with control (Student's *t*-test for paired samples)

3-OHGA and PDC inhibited Na⁺-dependent [³H]glutamate binding to synaptic membranes from striatum. Furthermore, 3-OHGA did not change the inhibitory effect of PDC [$F(3, 12) = 209.12$; $P < 0.001$] (Fig. 5).

Discussion

The cause of the frontal and temporal cortex vulnerability during gestation and at early development and of the striatum degeneration during the first three years of life in GA I is still obscure despite intense investigation performed in cultivated neurons from chick and rats, in fresh rat neural tissue and also in tissues from mice deficient in GCDH. However, in the last decade alterations of energy metabolism (Ullrich et al. 1999; Silva et al. 2000; Kölker et al. 2002b, 2004; Das et al. 2003; Latini et al. 2005a; da Costa Ferreira et al. 2005a, b; Sauer et al. 2005), oxidative stress (de Oliveira Marques et al. 2003; Latini et al. 2002, 2005b) and particularly disturbance of the glutamatergic system due to the structural similarity between glutamate, GA and 3-OHGA (Flott-Rahmel et al. 1997; Lima et al. 1998; Kölker et al. 1999, 2000a, b, 2002a, b; Ullrich et al. 1999; Porciúncula et al. 2000, 2004; Mello et al. 2001; Rosa et al. 2004; Frizzo et al. 2004) have been considered important pathomechanisms underlying neural damage in GA I. On the other hand, recent works performed on neurons in culture did not confirm excitotoxic actions for 3-OHGA (Lund et al. 2004; Freudenberg et al. 2004), suggesting that more work is necessary to clarify this matter.

The results of the present investigation demonstrate that glutamate binding to receptors is decreased by GA in cerebral cortex and striatum from rats aged 7 and 15 days and that 3-OHGA decreased glutamate binding to transporters in striatum of 7-day-old rats, indicating developmentally regulated and tissue-specific effects of these metabolites.

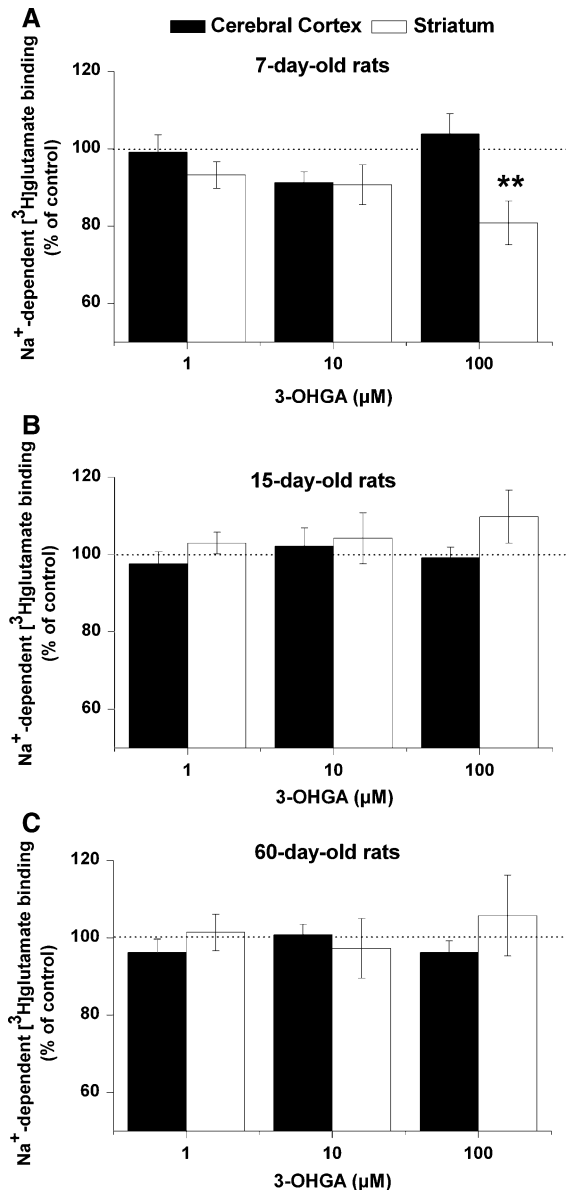


Fig. 4 Effect of 3-hydroxyglutaric acid (3-OHGA, 1–100 μM) exposure on Na⁺-dependent [³H]glutamate binding (binding to transporters) to synaptic plasma membranes from cerebral cortex and striatum of rats aged 7 (**A**), 15 (**B**) and 60 (**C**) days. Results are presented as mean ± SEM from five to six independent experiments performed in triplicate and are expressed as percentage of controls. ***P* < 0.01, compared with controls (Duncan multiple range test)

We first observed that GA (0.01–1 mM) induced a significant decrease of Na⁺-independent glutamate binding to synaptic membranes at the concentrations found in *post mortem* brain examination of GA I patients (Kölker et al. 2003; Sauer et al. 2006). Furthermore, this effect was mainly directed to glutamate receptors given that the

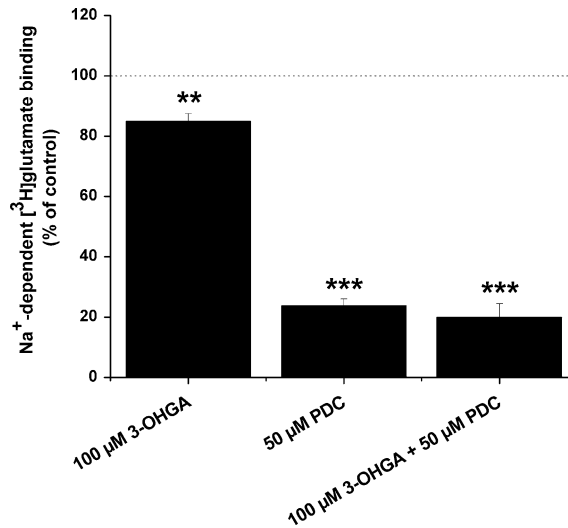


Fig. 5 Effect of 3-hydroxyglutaric acid (3-OHGA, 100 μM) and *L-trans*-pyrrolidine-2,4-dicarboxylate (PDC, 50 μM) exposure on Na⁺-dependent [³H]glutamate binding (binding to transporters) to synaptic plasma membranes from striatum of 7-day-old rats. Results are presented as mean ± SEM from four independent experiments performed in triplicate and are expressed as percentage of controls. ***P* < 0.01, ****P* < 0.001 compared with controls (Duncan multiple range test)

incubation medium did not contain sodium, which is necessary for glutamate to bind to its transporters. The inhibitory effect provoked by GA was probably directed to non-NMDA glutamate receptors and possibly to kainate receptors since co-incubation of GA with CNQX (a non-NMDA receptor antagonist) resulted in an identical reduction of glutamate binding as that found for CNQX alone, and GA markedly displaced kainate (60% decrease) from its receptors. Our present findings may possibly explain previous *in vivo* findings showing that the behavioral alterations and clonic convulsions provoked by intrastriatal administration of GA to adult rats were prevented by the non-NMDA antagonist DNQX, but not by the NMDA antagonist MK-801 (Lima et al. 1998). A recent report also showed that GA binds to non-NMDA receptors in brain from 30-day-old rats (Porciúncula et al. 2004). Furthermore, patch clamp studies failed to find any effect of GA on AMPA receptors (Ullrich et al. 1999; Kölker et al. 2002b; Lund et al. 2004), which is in agreement with our present findings indicating that GA binds preferentially to kainate receptors. In contrast, previous *in vitro* studies demonstrated the inefficacy of CNQX to prevent death of neonatal cultured neurons induced by GA and 3-OHGA (Kölker et al. 2000a). It should be stressed that our present findings and those of Lima and collaborators (1998) were carried out with postnatal striatum and cerebral cortex of rats aged 7–60 days rats, whereas Kölker and colleagues (2000a) used chick embryonic telencephalons and mixed neuronal and glial cell cultures from neonatal rat hippocampus in their experiments. Therefore, it is feasible that the effects provoked by GA are age-, species- and regional-dependent, being probably related to the differential ontogenetic expression and properties of glutamate receptor subtypes (Luo et al. 1996; Anderson et al. 1999; Ritter et al. 2002). This agrees with the fact that the variable subunits pattern of these proteins expressed ontogenetically usually confer different pharmacological and physiological responses (McDonald and Johnston 1990; Ozawa et al. 1998). One fact that may contribute to the

interaction of GA with glutamate receptors is that glutamate and GA are structurally chemical similar molecules so that a competition for the same receptor sites may have occurred. Furthermore, our present *in vitro* results showing that GA inhibits glutamate binding to cortical and striatal receptors at an early postnatal age (7- and 15-day-old rats), but not in older animals (60-day-old rats), may be possibly related to the amount of glutamate receptors that are expressed at higher levels during early postnatal development, a fact that may explain the higher vulnerability of neonatal brain to excitotoxicity (Ritter et al. 2002). This is in line with the observations that AMPA and kainate receptors in rat brain reach the density peaks around the second to third postnatal weeks, decreasing thereafter (Insel et al. 1990; Miller et al. 1990; Kossut et al. 1993; Brennan et al. 1997).

On the other hand, we also observed that GA (0.01–1 mM) did not inhibit Na^+ -dependent glutamate binding to synaptic membranes obtained from cerebral cortex and striatum of rats from all ages, implying that GA, at concentrations found in the brain of glutaric acidemic patients, does not alter glutamate binding to these transporters.

With respect to 3-OHGA, we found that this organic acid was unable to alter glutamate binding to receptors (Na^+ -independent glutamate binding) in cerebral cortex and striatum of rats from all ages. In contrast, 3-OHGA significantly decreased Na^+ -dependent glutamate binding (binding to transporters) only in striatum from 7-day-old rats, but not in striatum from older animals and in cerebral cortex from rats aged 7–60 days. We further observed that 3-OHGA did not alter the inhibition provoked by the competitive glutamate transporter inhibitor PDC on Na^+ -dependent glutamate binding to synaptic membranes from 7-day-old rat striatum, suggesting that 3-OHGA in fact binds to glutamate transporters rather than to unspecific sites on the synaptic membranes. Considering that our synaptic membrane preparations contain glial cell membranes (astrocytic glutamate transporters) and that at this age GLAST transporters are more prevalent, it could be presumed that 3-OHGA interfered with glutamate binding to these Na^+ -dependent high affinity transporters. Although the mechanism by which 3-OHGA, but not GA, inhibited glutamate binding to membrane transporters is unknown, it has been suggested that some substances with the hydroxyl group attached to carbon 3 have more affinity to glutamate transporters and can competitively inhibit glutamate binding (Balcar et al. 1977).

As regards to the physiological significance of our findings, although we cannot establish with certainty whether our *in vitro* data is related to the neurotoxicity observed in GA I *in vivo*, it should be emphasized that the effects provoked by GA and 3-OHGA were observed with concentrations similar to those encountered in brain of glutaric acidemic patients (Goodman et al. 1977; Kölker et al. 2003; Kulkens et al. 2005; Sauer et al. 2006). In this context, untreated GA I patients present brain GA concentrations of 500–5000 $\mu\text{mol/l}$ and 3-OHGA concentrations of 40–200 $\mu\text{mol/l}$ (Sauer et al. 2006). Furthermore, the degree of alterations of the glutamatergic system detected in our study is widely accepted to cause excitotoxicity in systems testing the effect of potential excitotoxins (Ozawa et al. 1998; Danbolt 2001; Meldrum 2002). Taken together these observations and previous reports demonstrating that GA and 3-OHGA markedly reduce viability of neurons in culture via glutamate receptors (Kölker et al. 2000a, b), it is conceivable that our findings may be related to these findings.

In summary, it is likely that the effects demonstrated here for GA and 3-OHGA at specific ages of rat development and also in distinct cerebral structures are probably associated to the differential ontogenetic and regional-specific expression of these proteins in rat brain synaptic membranes. The present findings suggest a disturbance of

glutamatergic neurotransmission caused by the major metabolites accumulating in GA I at early development. They may be possibly related to the regional- and age-specific window of susceptibility responsible for the neuropathology of GA I characterized by frontotemporal subcortical atrophy and striatum degeneration that occur during the first three years of life in the affected patients.

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