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Effect of chronic glutamate administration to pregnant rats during gestation on metabotropic glutamate receptors from mothers and full-term fetuses brain

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Summary. Chronic glutamate treatment during gestational period caused a significant decrease in total metabotropic glutamate receptors (mGluR) number. Similar results were observed on the steady-state level of $mGlu_1$ receptor detected by immunoblotting assays, suggesting that this is the main receptor subtype modulated by agonist exposure. Furthermore, no variations on mRNA coding $mGlu_1$ receptor were found, suggesting post-transcriptional modulation as a possible mechanism of the lost of receptor detected at the membrane surface. On the other hand, western-blotting to determine level of $G_{q/11}$ protein and phospholipase C β_1 revealed a significant decrease of both proteins in mothers brain. This decrease was associated with significant variation in glutamate and DHPG-stimulated phospholipase C activity. No significant differences on mGluR transduction pathway components were observed in fetuses brain. These results suggest that glutamate intake during pregnancy causes a down-regulation of different proteins involved in glutamate response mediated by mGluR only in mothers brain without significantly affecting fetuses brain.

Keywords: Gestation – Metabotropic glutamate receptors – Down-regulation – Desensitization

Introduction

Glutamate mediates the transmission of information at most excitatory synapses, being considered as the main excitatory neurotransmitter in the Central Nervous System (CNS). Therefore, in physiological conditions, glutamate participates in a wide variety of neuronal and glial processes such as learning and memory acquisition processes and formation of the neuronal network during development (for review see Riedel et al., 2003; Monaghan et al., 1989; Herrera-Marschitz et al., 1998). However, under pathological conditions, extracellular glutamate concentration can increase, acting as a neurotoxin and triggering neuronal death (Meldrum, 2000; Novelli and Tasker, 2000; Sattler and Tymianski, 2001). These functions are mediated by membrane receptors termed glutamate receptors (GluRs), which have been categorized into two main classes, ionotropic (iGluRs) and metabotropic glutamate receptors (mGluRs), based on pharmacological profiles, selectivity of coupling to second messenger pathways and molecular cloning. iGluRs are cation-selective receptor channels and their activation mediate ionic currents. These receptors include three families, named NMDA, AMPA and kainate. mGluRs belong to G-protein coupled receptor (GPCR) superfamily, sharing different structural properties such as: a N-terminal extracellular domain, an heptahelical domain and a C-terminal intracellular tail. mGluRs have been subclassified into three groups on the basis of their sequence similarity, pharmacological profiles and signal transduction mechanisms. Group I comprises $mGluR_1$ and $mGluR_5$, which are coupled to a stimulatory $G_{q/11}$ protein and activate the phospholipase C (PLC) activity. Group II ($mGluR_2$ and $mGluR_3$) and Group III ($mGluR_4$, $mGluR_6$, $mGluR_7$ and $mGluR_8$) are both coupled, in an inhibitory manner, to adenylyl cyclase (AC) through a $G_{i/o}$ protein (for review see Pin et al., 2003; Pin and Acher, 2002; Ozawa et al., 1998).

Chronic activation of GPCRs leads to an attenuation of receptor responsiveness (Ferguson, 2001; Bohm et al., 1997). This phenomenon, termed *desensitization*, has been observed in mGluR signaling in several systems, including C6 cells (Albasanz et al., 2002), primary neuronal cultures (Manzoni et al., 1990; Catania et al., 1991), hippocampal slices (Schoeep and Johnson, 1998; Alagarsamy

et al., 1999; Guerineau et al., 1997) and astrocytes (Balazs et al., 1997; Peavy and Conn, 1998) and it is the consequence of different mechanism that can involve the three components of the transduction pathway: receptor, heterotrimeric G-protein and the associated effectors. In this sense, it has been shown that chronic agonist exposure of GPCRs produces an internalization of cell surface receptors to intracellular compartments, where they can be recycled back to the cell surface or targeted to lysosomes for degradation (*down-regulation*) (for review see Ferguson, 2001; Claing et al., 2002; Krupnick and Benovic, 1998). We have previously reported the internalization of mGluR through coated vesicles in C6 cells after long-term L-Glu exposure (Albasanz et al., 2002). Apart from receptor protein, heterotrimeric G-protein and effector system can also be down-regulated in response to prolonged agonist exposure. Supporting this, we have shown the presence of mGluRs as well as PLC and G-protein in intracellular coated vesicles (Martín et al., 1991a, b, 1993).

At present, there are not studies, to our knowledge, that analyse the regulation of mGluRs after chronic oral treatment with glutamate. This is specially important due to glutamate is highly consumed in the world through diet (where in some foods comprises 20% of the total amino acid content) and as a flavour enhancer (Geha et al., 2000). For this reason, the aim of the present work was to analyse the effect of *in vivo* L-glutamate exposure on metabotropic glutamate receptors from rat brain. The study was performed using pregnant rats that were daily treated in the drinking water with L-Glutamate during all gestational period. A group of female rats drinking tap water were used as control. Glutamate caused a down-regulation of mGlu receptors, $G_{q/11}$ and phospholipase C β_1 proteins in mothers brain, although no significant effect was observed in fetuses brain.

Materials and methods

Materials

L-[^{3}H]Glutamic acid (48.1 Ci/mmol) and phosphatidylinositol 4,5-bisphosphate, (myo-inositol-2-^{3}H(N)) ([^{3}H]PIP_2) (8 Ci/mmol) were obtained from NEN (Boston, MA, USA). L-glutamate, NMDA, AMPA, Kainate and DL-threo-β-hydroxyaspartic acid (TBHA) from Tocris (London, UK) and phosphatidylinositol 4,5-bisphosphate (tri-ammonium Salt) was purchased from Avanti Polar Lipids (Alabaster, AL). Anti-bovine PLC β_1 monoclonal antibody and anti-rat polyclonal $mGlu_1$ receptor antibody were from Upstate Biotechnology (New York, NY, USA). Anti $\alpha G_{q/11}$ protein was from Dupont NEN. All other reagents were of analytical grade.

Animals

Pregnant Wistar rats were kept on a 12 h light/12 h dark cycle (lights on at 7:00 am) and with free access to food and drinking water. The day when sperm was observed in the vaginal smear was designated day 1 of pregnancy. L-glutamate to a concentration of 1 g/l was administrated in the drinking water from the second to the last gestational day. At the end of pregnancy rats were sacrificed, fetuses were removed by caesarean and brain removed, frozen in N_2-liquid and stored at -70°C until experiments were performed. All experiments followed the European Community regulations about animal experimentation.

Plasma membranes isolation

Brain plasma membranes from pregnant and fetuses rat were isolated as described by Kessler et al. (1989) with some modifications. Brains were homogenized in 20 volumes of isolation buffer (50 mM Tris-HCl, pH 7.4 containing 10 mM $MgCl_2$ and protease inhibitors) in Dounce ($10 \times$ A, $10 \times$ B). After homogenization, brain preparations were centrifuged 5 min at $1.000 \times g$ in a Beckman JA 21 centrifuge. Supernatant was centrifuged 20 min at $27.000 \times g$ and pellet was finally resuspended in isolation buffer. Protein concentration was measured by the method of Lowry, using bovine serum albumin as standard.

Metabotropic glutamate receptor binding assays to plasma membranes

L-[^{3}H]Glutamate binding assays to rat brain membranes were performed as described previously (Martín et al., 1993; Albasanz et al., 2002). Briefly, membranes were treated with 0.04% Triton X-100 to facilitate the removal of endogenous glutamate (Compton et al., 1990). To determine mGluR binding, 50 μg of protein were incubated for 60 min at 25°C in the presence of 100 μM α-amino-3-hydroxy-5-methyl-isoxazole-4 propionic acid (AMPA), 100 μM kainate, and 100 μM N-methyl-D-aspartic acid (NMDA), in order to block ionotropic glutamate receptor binding, and different L-[^{3}H]Glutamate concentrations (100 nM–1200 nM) with 10 mM potassium phosphate pH 7.4. Non specific binding was obtained in the presence of unlabeled L-glutamate. All assays were performed in the presence of 1 mM DL-threo-β-hydroxyaspartic acid (THBA), a L-glutamate uptake inhibitor (Kimelberg et al., 1989).

Immunodetection of $mGluR_1$, $\alpha G_{q/11}$ and the phospholipase C β_1 isoform

Fifty micrograms of protein were subjected to 7.5% polyacrilamide gel electrophoresis in the presence of SDS. Western blotting was performed as described earlier (Martín et al., 1998). Immunodetection was carried out by incubating the nitrocellulose membranes with isoenzyme-specific polyclonal antibody (anti-$mGlu_1$ and anti-αGq/11) diluted 1:1000 and isoenzyme-specific monoclonal antibody (anti-PLCβ_1) diluted 1:400. After washing, blots were incubated with horseradish peroxidase-coupled goat anti-rabbit or -mouse IgG respectively, diluted 1:3000. Antigen was visualized using the ECL chemiluminiscent detection kit from Amersham and specific bands were quantified by densitometry in a BioRad GS-690 densitometer (BioRad laboratories, Hercules, CA, USA).

RT-PCR analysis

Total RNA was isolated by guanidium thiocyanate/phenol/cloroform extraction following the method of Chomczynski and Sacchi (1987). RT-PCR assays of different αG subunits were performed as described by Vendite et al. (1998) using the primers 5′-AAA TCT ACA GCA ATG CTG GCG A-3′ and 5′-CTT CGA TGA CTT CAT CTC TGT-3′ for $mGluR_1$, 5′-GAG AAC CGA ATG GAG GAG AGC AA-3′ and 5′-GTC CAC GAA CAT CTT CAG GAT GAA-3′ for $\alpha G_{q/11}$, 5′-TTT TCG GCA GAC CGG AAG CGA-3′ and 5′-TGC TGT TGG GCT CGT ACT TCT-3′ for PLCβ_1. PCR products were analyzed by electrophoresis in 2% agarose gels and stained with ethidium bromide. The PCR product size expected for $mGluR_1$, $\alpha G_{q/11}$ and PLCβ_1 were 206, 212 and 315 bp

respectively. In all cases, amplification of a fragment corresponding to the β-actin sequence was carried out in parallel using the same cDNA samples in order to correct possible variations in the amount of cDNA used for the process. The primers used for β-actin were 5′-GGT ATG GAA TCC TGT CGC ATC CAT GAA A-3′ and 5′-GTG TAA AAC GCA GCT CAG TAA CAG TCC G-3′. The size of the PCR product for the β-actin was 320 bp. Bands corresponding to PCR products were quantified by densitometry in a BioRad GS-690 densitometer.

Phospholipase C assay

Phospholipase C in plasma membranes was assayed in the presence of exogenous [^{3}H]PIP_2 as described by Tiger et al. (1990). [^{3}H]PIP_2 was dried under a N_2 stream, dissolved in 2 mM sodium deoxicholate, 50 mM Tris-HCl pH 6.5 and sonicated using an Ultrasonic Processor UP 200 S. Phospholipase C assay was carried out for 10 min at 37°C, incubating [^{3}H]PIP_2 (17000 dpm) with or without 20 μg of plasma membrane protein in 100 μl of buffer (100 mM NaCl, 1 mM sodium deoxicholate, 1 mM EGTA, 250 nM Cl_2Ca, 40 mM ClLi and 50 mM Tris-HCl pH 6.8). The incubation was terminated by the addition of 360 μl of chloroform/methanol/HCl (1:2:0.2 v/v) and putting the tubes on ice. After addition of 120 μl 2 M KCl and 160 μl of chloroform, the tubes were centrifuged 5 min at 3500 × g. 250 μl of the upper aqueous phase containing [^{3}H]inositol phosphates were mixed with 3.5 ml of Optiphase-Hi-Safe® for scintillation counting.

Spatial learning in the Morris water maze

Spatial learning was tested in a Morris water maze, similar to that developed by Morris (1984). Our version of the maze consists of a large round tank (190 cm diameter, 50 cm high) filled up with water to a depth of 40 cm (water temperature 22 ± 1°C). The water was occluded with a floating monolayer of white polypropylene beads (Czech et al., 2000). The escape platform was a cylinder with a white wooden top (15 cm diameter) submerged 2 cm below the water surface in the hidden-platform water maze version. In the visible-platform water maze version, the platform was placed to be visible 2 cm above the water surface and additionally signaled by a tennis ball. In the experimental room, there were various extramaze cues surrounding the maze at least of 1 m. The time taken to find the platform (escape latency) were recorded using a digital Sony video camera mounted on the ceiling above the pool.

In the hidden-platform water maze task, during three gestational days (GD 13, 14 and 15) in sessions consisting of four trials per day, 8 pregnant rats (control n = 4, L-Glu treated n = 4) were gently placed in the water from one of the four starting points located on the pool (N, S, E or W) with their heads facing the wall of the pool, and were allowed 60 sec to locate the hidden platform. The submerged platform was located in the same position on every trial. When a rat could not reach the platform after this time, it was placed on it by the experimenter, and it is assigned a latency of 60 sec. Rats were always allowed a 20 sec rest on the platform. The intertrial interval was approximately 1 min. During this time, the animal was placed in a holding cage.

The visible-platform water maze task was performed following the hidden-platform version, during the gestational days (GD 16, 17 and 18) in sessions consisting of four trials per day. The visible platform was moved to different points of the maze between trials such that the four designated start points (N, S, E, W) was used once within the four trials. This version of water maze provides an assessment of sensorimotor and motivational factors that might influence performance in the spatial learning task.

Statistical and data analysis

Data statistical analysis was performed using the Student *t*-test. Differences between mean values were considered statistically significant at $p < 0.05$. The binding data were analyzed with the GraphPad Prism 3.03 program (GraphPad Software, San Diego, CA, USA). In behavioural studies, escape latency data were analyzed with two way ANOVA with repeated measures.

Results

Pregnant rats were chronically treated with L-Glutamate and the status of different components of mGluR transduction pathway was studied by different approaches, including radioligand binding, Western-blotting and RT-PCR assays and by determining phospholipase C activity. Treatment did not cause significant variation in both the food intake and weight gain of animals. However, water intake was slightly but significantly decreased in pregnant rats treated with glutamate (Fig. 1).

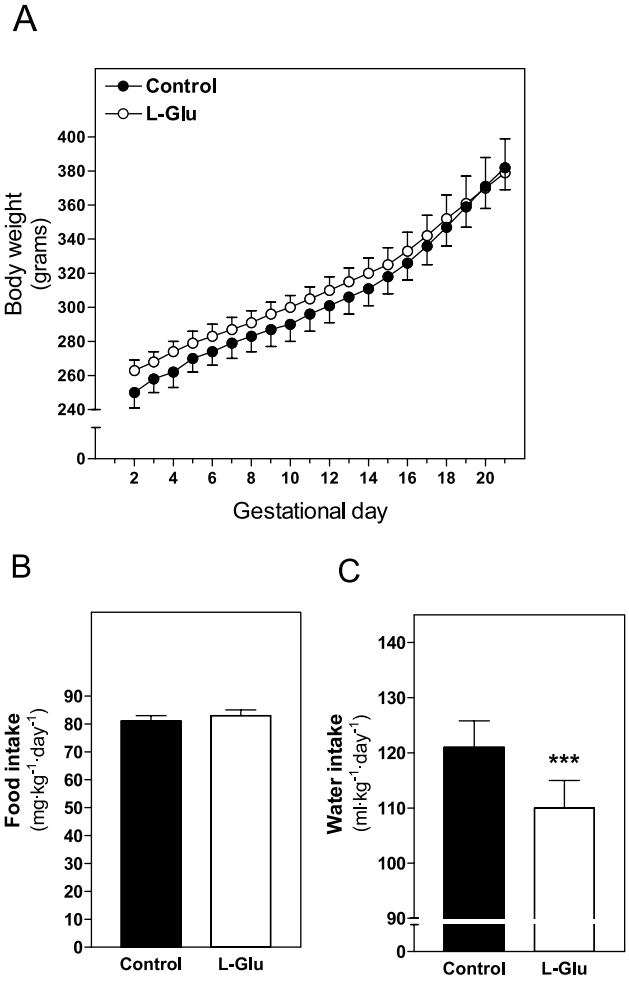

Fig. 1. Effect of chronic glutamate treatment on the body weight gain, water and food intake. The gain of body weight (**A**), food (**B**) and water intake (**C**) were monitored during gestational period in control (n = 7) and L-Glu treated (n = 11) rats. Values are expressed as the mean ± SEM. ***$p < 0.001$ significantly different from control

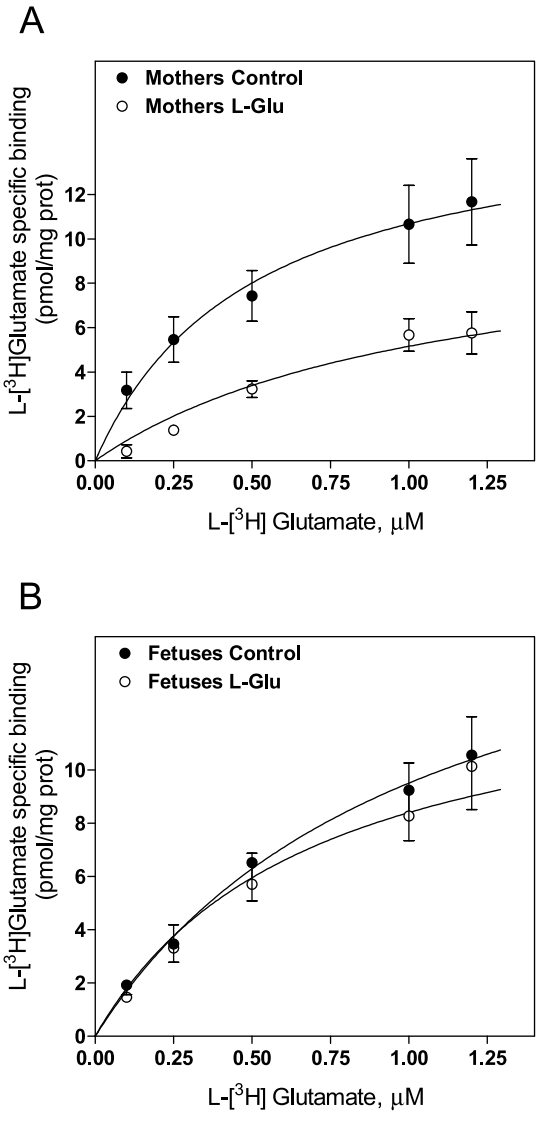

Fig. 2. Saturation curves of L-[^{3}H] Glutamate binding to plasma membranes from maternal and fetal brain. Fifty micrograms of plasma membranes from control and L-Glu-treated maternal (**A**) and fetal (**B**) rat brain were incubated with different concentrations of L-[^{3}H] Glutamate, as described in Materials and methods, after pre-treatment with 0.04% Triton X-100, in order to remove endogenous glutamate from the samples. Total receptor number (B_{max}) and receptor affinity (K_d) were determined by Scatchard analysis of saturation curves and these values are shown in Table 1. Data are mean ± SEM of 5–7 experiments performed using different plasma membranes isolations

L-Glu intake effect on metabotropic glutamate receptors from maternal and fetal brain

L-glutamate administration to pregnant rats caused a significant decrease in mGluR number (B_{max}) determined by saturation binding assays, associated with a significant decrease in receptor affinity as it can be seen in Fig. 2, panel A, and Table 1. No significant differences in receptor number or affinity were obtained when the assays were performed in membranes from fetuses brain (Fig. 2, panel B). These results suggest that L-glutamate causes a down-regulation of metabotropic glutamate receptors in maternal brain without significant effect on fetal brain. The decrease in total receptor number observed in maternal brain was in agreement with the lower level of $mGlu_1$ receptor obtained by immunoblotting assay (Fig. 3), suggesting this receptor type as the main modulated by agonist exposure. No significant variations on $mGluR_1$ level from fetuses brain was observed (Fig. 3).

To determine whether the decrease in $mGluR_1$ density was due to a decrease in $mGluR_1$ transcript levels, we carried out RT-PCR assays following control and treated brain RNA total isolation, using primers corresponding to this receptor subtype. As it can be observed in Fig. 4, chronic glutamate treatment during all gestacional period did not produce a significant change in $mGluR_1$ mRNA level present in both maternal and fetal brain. These results points to a post-transcriptional mechanism as responsible for the down-regulation of $mGluR_1$.

L-Glu intake effect on $\alpha G_{q/11}$ from maternal and fetal brain

Group I mGluRs are positively coupled to PLC through a $G_{q/11}$ protein. Since chronic agonist stimulation can produce a down-regulation of heterotrimeric G-protein, we assessed the effect of chronic glutamate intake on $\alpha G_{q/11}$ protein in control and treated rat brains. Membranes from both groups were analysed by Western-blotting using a specific $\alpha G_{q/11}$ antibody. Two distinct

Table 1. Kinetic parameters of L-[^{3}H] Glutamate binding to plasma membranes from mothers and fetuses rat brain. Data represent B_{max} and K_d values from mothers and fetuses rat brain membranes determined by Scatchard analysis of binding data in Fig. 2. Data are mean ± SEM of 5–7 experiments performed using different plasma membranes isolations. * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$ significantly different from control

	Mothers		Fetuses	
	B_{max} (pmol/mg prot)	K_D (nM)	B_{max} (pmol/mg prot)	K_D (nM)
Control	14.6 ± 2.4	534.3 ± 89.7	12.8 ± 2.7	599.0 ± 89.7
Glutamate	7.2 ± 0.7***	1060.3 ± 251.8***	12.6 ± 2.3	667.8 ± 137.8

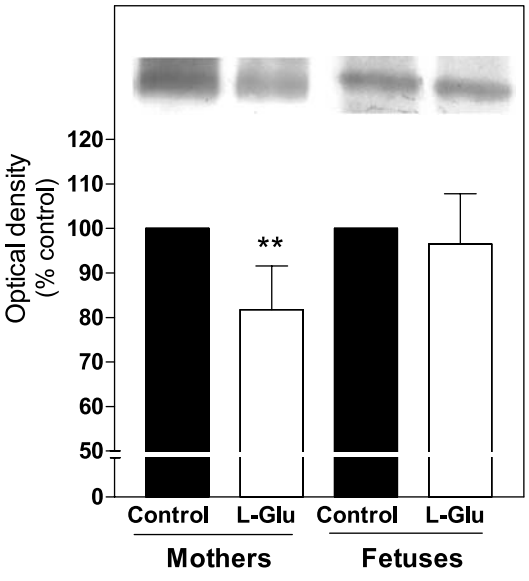

Fig. 3. Immunoblotting analysis of $mGluR_1$ in plasma membranes from maternal and fetal brain. Fifty micrograms of plasma membranes from control and L-Glu-treated maternal and fetal rat brain were subjected to SDS/PAGE, transferred electrophoretically to nitrocellulose membrane and incubated with an isoenzyme-specific polyclonal antibody (anti-$mGlu_1$), as described in Materials and methods. Data, expressed as percentage of control values, are mean ± SEM of 5–9 experiments performed using different plasma membranes isolations. $^{**}p < 0.01$ significantly different from control

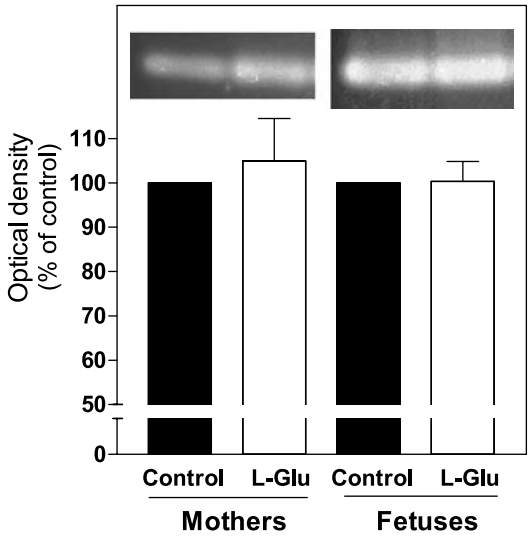

Fig. 4. Effect of chronic L-Glu treatment on $mGluR_1$ expression detected by RT-PCR. After RNA isolation from control and L-Glu treated maternal and fetal brain, RT-PCR assays were performed using specific oligonucleotides to $mGluR_1$ as described in Materials and methods. The histograms show the mean ± SEM of 5–9 experiments performed using different RNA isolations

bands migrating at expected molecular masses and corresponding to αGq (42 kDa) and αG_{11} (43 kDa) are shown in Fig. 5. Analysis of bands revealed that there

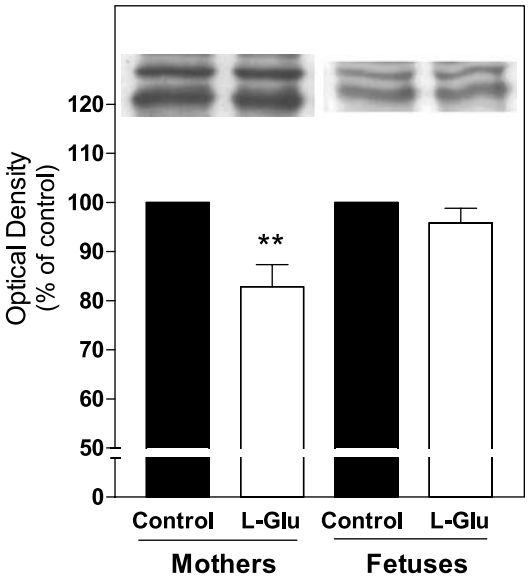

Fig. 5. Immunoblotting analysis of $\alpha G_{q/11}$ in plasma membranes from maternal and fetal brain. Fifty micrograms of plasma membranes from control and L-Glu-treated maternal and fetal rat brain were subjected to SDS/PAGE, electrophoretically transferred to nitrocellulose membrane and incubated with an isoenzyme-specific polyclonal antibody (anti-$\alpha G_{q/11}$) as described in Materials and methods. Data, expressed as percentage of control values, are mean ± SEM of 4–6 experiments performed using different plasma membranes isolations. $^{**}p < 0.01$ significantly different from control

was a significant decrease (≈20%) in maternal $G_{q/11}$ protein content in response to chronic agonist exposure. However, we did not find significant differences between bands corresponding to control and glutamate-treated fetal membranes. $G_{q/11}$ mRNA level was also studied by RT-PCR assays (Fig. 6) using specific primers. Results obtained after analysing the corresponding band show that chronic glutamate exposure produces an increase in mRNA levels coding $G_{q/11}$ in maternal brain. Again, there was no significant differences in the levels of $G_{q/11}$ mRNA between control and treated fetuses (Fig. 6).

L-Glu intake effect on the isoform PLCβ_1 and Group I mGluR-mediated phospholipase C stimulation

To fully investigate the $mGluR_1$ transduction pathway, we analysed PLCβ_1 isoform in maternal and fetal brain membranes by Western-blotting, using a specific antibody that recognized a protein that migrated as 150 kDa peptides on SDS polyacrilamide gels. Treatment of pregnant rats with glutamate significantly reduced the intensity of PLCβ_1 immunoreactivity by 20% in maternal brain membranes. This change was not accompanied by alterations in

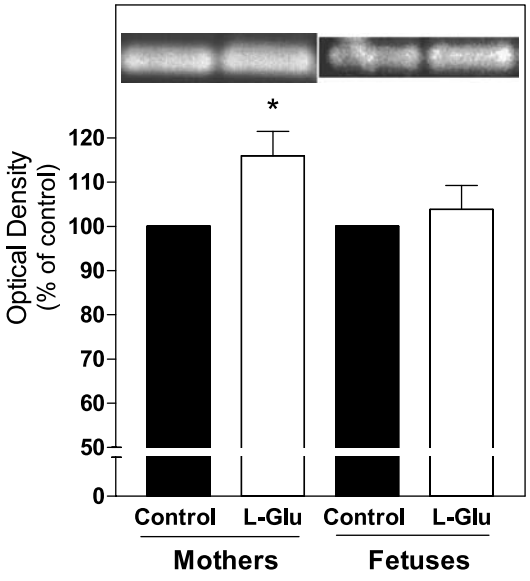

Fig. 6. Effect of chronic L-Glu treatment on $\alpha G_{q/11}$ expression detected by RT-PCR. After RNA isolation from control and L-Glu treated maternal and fetal brain, RT-PCR assays were performed using specific oligonucleotides to $\alpha G_{q/11}$ as described in Materials and methods. The histograms show the mean ± SEM of 5–9 experiments performed using different RNA isolations. *$p < 0.05$ significantly different from control

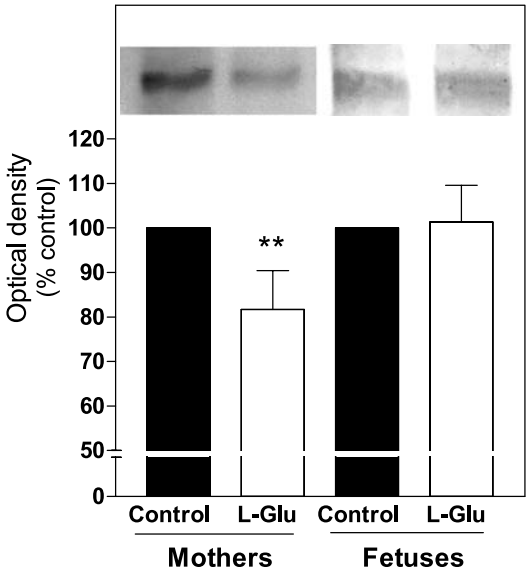

Fig. 7. Immunoblotting analysis of PLCβ_1 in plasma membranes from maternal and fetal brain. Fifty micrograms of plasma membranes from control and L-Glu-treated maternal and fetal rat brain were subjected to SDS/PAGE, electrophoretically transferred to nitrocellulose and incubated with an isoenzyme-specific monoclonal antibody (anti-PLCβ_1) as described in Materials and methods. Data, expressed as percentage of control values, are mean ± SEM of 6 experiments performed using different plasma membranes isolations. **$p < 0.01$ significantly different from control

PLCβ_1 protein level in fetal membranes (Fig. 7). Despite of reduction in PLCβ_1 content in maternal brain in response to chronic glutamate exposure, the steady state

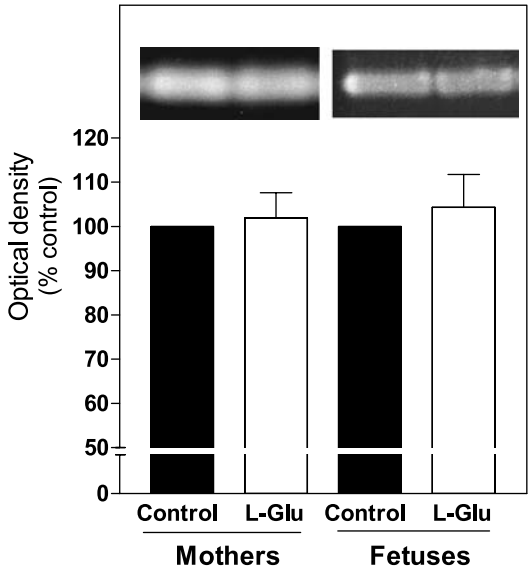

Fig. 8. Effect of chronic L-Glu treatment on PLCβ_1 expression detected by RT-PCR. After RNA isolation from control and L-Glu treated maternal and fetal brain, RT-PCR assays were performed using specific oligonucleotides to PLCβ_1 as described in Materials and methods. The histograms show the mean ± SEM of 4 experiments performed using different RNA isolations

level of PLCβ_1 mRNA transcript was unchanged, suggesting that a post-transcriptional mechanism may be involved. Again, glutamate treatment did not modify PLCβ_1 mRNA level in fetal brain (Fig. 8).

Besides, we evaluated the effect of chronic glutamate exposure on PLC activity. Firstly, we measured the basal and GTPγS-stimulated PLC activity in brain membranes from control and glutamate-treated mothers, using [^{3}H]PIP_2 as a exogenous substrate. The data in Fig. 9 shows that basal PLC activity was significantly reduced in glutamate-treated membranes. The stimulation of PLC activity through G-protein with 100 μM GTPγS, a non-hydrolizable GTP analogue, was also lower in treated than in control-membranes. To verify whether this was due to the lower basal activity detected in this group, we calculated the percentage of stimulation by GTPγS above the control values in both groups. The results obtained confirmed that the stimulation with the GTP analogue was lower in glutamate-treated group ($117 \pm 3\%$ versus $140 \pm 8\%$, $p < 0.01$). Taken together, these data support the idea that the decrease in basal and GTPγS-mediated PLC activity detected after chronic treatment can be due to the down-regulation of the PLCβ_1 isoform and the $\alpha G_{q/11}$ subunits. Secondly, we assayed the functional consequences of $mGluR_1$ down-regulation observed in L-Glu treated group. As Fig. 9 shows, the stimulation of PLC activity mediated by non-selective (1 mM L-Glu) and selective

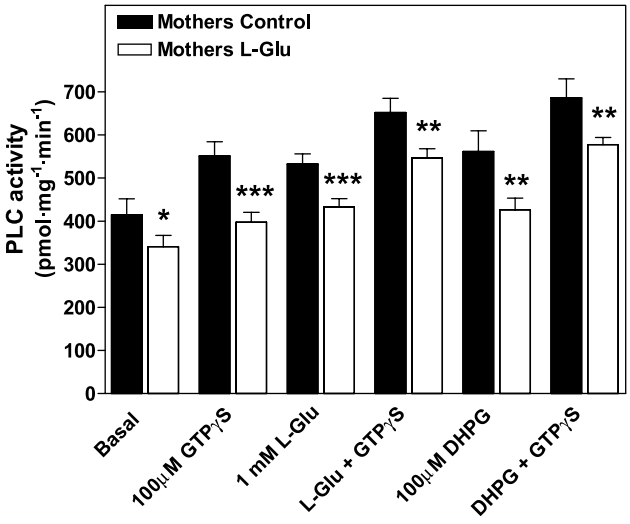

Fig. 9. Effect of chronic L-Glu treatment on phospholipase C activity in plasma membranes from maternal brain. The PLC activity was measured as described in Materials and methods using [^{3}H]PIP_2 as exogenous substrate in basal conditions and after stimulation with 100 μM GTPγS, 1 mM L-Glu or 100 μM DHPG. Additionally, we assayed the 100 μM GTPγS plus 1 mM L-Glu or 100 μM DHPG-mediated PLC stimulation. Data are mean $\pm$ SEM of 3–4 experiments performed in duplicated using different plasma membranes isolations. $^{*}p<0.05$, $^{**}p<0.01$ and $^{***}p<0.001$ significantly different from control

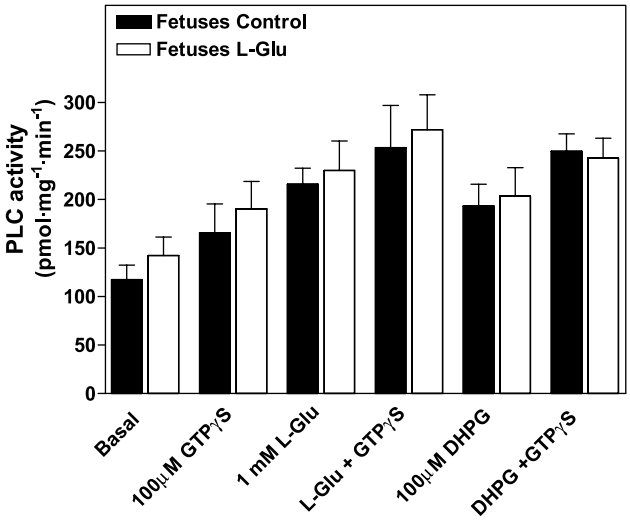

Fig. 10. Effect of chronic L-Glu treatment on phospholipase C activity in plasma membranes from fetal brain. The PLC activity was measured as described in Materials and methods using [^{3}H]PIP_2 as exogenous substrate in basal conditions and after stimulation with 100 μM GTPγS, 1 mM L-Glu or 100 μM DHPG. Additionally, we assayed the 100 μM GTPγS plus 1 mM L-Glu or 100 μM DHPG-mediated PLC stimulation. Data are mean $\pm$ SEM of 3–4 experiments performed in duplicated using different plasma membranes isolations

(100 μM DHPG) group I mGluR agonists were significantly lower in L-Glu-treated group, accordingly with the loss of $mGluR_1$ detected in these membranes. Similar to GTPγS-mediated PLC stimulation, the percentage of stimulation by these agonists were in both cases significantly lower in L-Glu-treated (L-Glu stimulation: $127 \pm 4\%$, $p<0.05$; DHPG stimulation: $125 \pm 2\%$, $p<0.001$) than control membranes (L-Glu stimulation: $137 \pm 5\%$; DHPG stimulation: $144 \pm 3\%$), indicating that chronic glutamate treatment caused a desensitization in Group I-mGluR

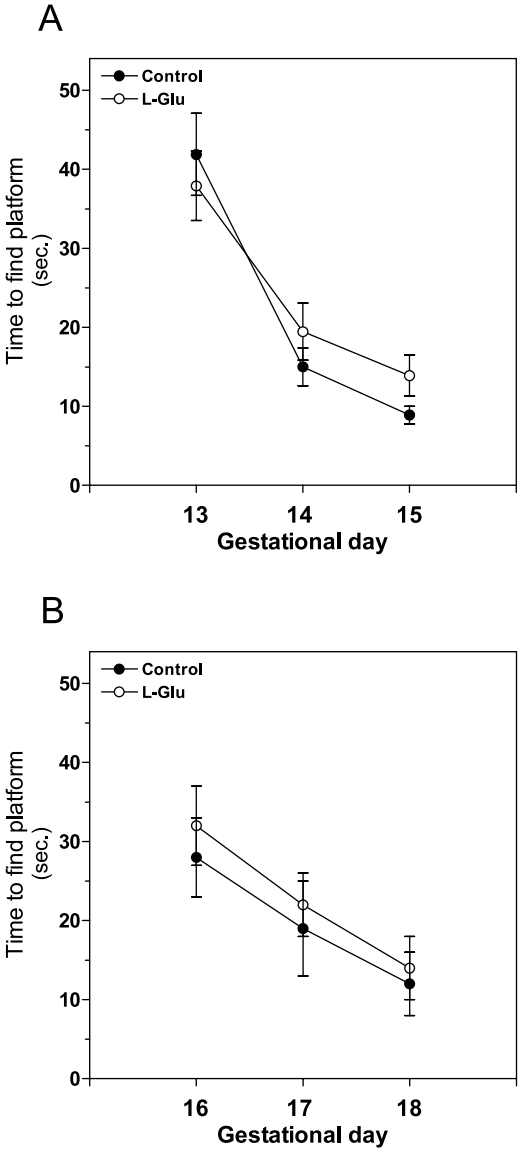

Fig. 11. Effect of chronic L-Glu treatment on the latency to find the platform in the Morris water maze. L-Glu treated ($n=4$) and control ($n=4$) rats were trained during the gestational days 13, 14 and 15 in the hidden variant of the water maze with four trials per day (A). The three following days were trained in the visible variant (B) with the same number of trials per day, as described in Materials and methods. The average time to find the platform was plotted against gestational day. ANOVA with repeated measures did not reveal any significant differences between control and L-Glu treated rats

transduction pathway in maternal brain. Thirdly, the agonist-mediated PLC stimulation was potentiated in the presence of GTPγS. However, this potentiation was significantly lower in L-Glu treated animals.

PLC activity was also assayed in membranes from fetal brain. The results obtained indicated that neither basal activity nor GTPγS-, agonist- or agonist plus GTPγS-mediated PLC stimulation changed in response to chronic L-Glu treatment (Fig. 10).

Spatial learning in the Morris water maze

To evaluate whether chronic L-Glu treatment impaired the spatial learning task, we determined the time taken to find the escape platform in the hidden platform version of the water maze. Spatial learning in this version was carried out in control and L-Glu treated group rats during three consecutive days (GD 13, 14 and 15) in blocks of 4 trials per day. This number of trials was enough because the learning rate progressed rapidly. As shown in Fig. 11, the learning curves were similar in both groups without statistical differences in the escape latencies between control and L-Glu treated groups, indicating that chronic gestacional glutamate intake did not impair the learning task.

Additionally, spatial learning performance in the visible platform task was carried out in control and L-Glu treated group rats during the three following days (GD 16, 17 and 18) in blocks of 4 trials per day. Similar to the hidden platform version, the final escape latencies were not significantly different between control and treated groups, suggesting that chronic gestational treatment with L-Glu did not change neither sensorimotor nor motivational factors that might influence performance in the spatial learning task.

Discussion

The present work describes the effect of chronic glutamate treatment during gestacional period on $mGluR_1$ transduction pathway in both maternal and fetal rat brain. The present results demonstrate that $mGluR_1$ signalling pathway is desensitized in maternal brain in response to chronic Glu exposure, likely as a consequence of the down-regulation detected in $mGluR_1$, $G_{q/11}$ and PLCβ_1 proteins. However, the long term treatment with L-Glu failed to affect mGluRs transduction pathway components in fetal brain. Thus, $mGluR_1$, $G_{q/11}$ and PLCβ_1 remained unchanged in this tissue following treatment with L-Glu.

Chronic intake of L-Glu during gestational period results in Group I-mGluR desensitization in maternal brain

The data presented in Fig. 2 clearly indicate that chronic gestational treatment with L-Glu produces a decrease in the number of mGluR in plasma membranes from maternal brain that is accompanied by a loss of the receptor affinity. Apart from others subtypes that could be involved, the results obtained by Western Blot show that $mGluR_1$ contributes to this effect. These results agree with the down-regulation of mGluR and $mGluR_5$ observed in C6 cells (Albasanz et al., 2002) and in astrocytes (Balazs et al., 1997) respectively, after prolonged treatment with mGluR agonists. Similar results with loss of $mGluR_{1a}$ in membrane surface have also been shown in HEK 293 cells following agonist exposure (Mundell et al., 2003; Dale et al., 2001; Mundell et al., 2001). The down-regulation of $mGluR_1$ subtype appears to be relevant because it is associated with significant functional consequences. Thus, glutamate-induced down-regulation of $mGluR_1$ produced a decrease in the group I mGluR-mediated stimulation of the PLC activity. This desensitization is not surprising because it is a process frequently observed following the exposure of GPCRs to agonists (Zimmer et al., 2000; Ferguson, 2001). In particular, agonist-induced desensitization of Group I mGluR has been reported in numerous preparations *in vitro* such as hippocampal slices, primary neuronal culture, astrocytes, synaptosomes and a variety of recombinant systems (De Blasi et al., 2001). However, to our knowledge, to date the regulation of mGluR signalling has been performed in *in vitro* systems. In this sense, our study is specially interesting because we show for the first time a loss of Group I mGluR responsiveness after chronic L-glutamate administration.

The data presented in this work seem to indicate that glutamate crosses the blood brain barrier (BBB) and evokes a down-regulation in the three components of Group I mGluR transduction pathway. In this sense, it has been recently postulated that L-Glu can be taken up from plasma into brain by two transporters: system X and a second one located at the choroid plexus epithelium (Smith, 2000). Under physiological conditions, system X is saturated, mediating small glutamate fluxes into brain. These properties are consistent with several studies in male rats that indicate that the elevations of plasma glutamate after systemic or oral administration of L-Glu does not alter the concentration of glutamate in brain interstitial fluid (Bogdanov et al., 1996; Price et al., 1981). However, the data obtained in the present work agree well with the desensitization widely detected

after prolonged exposure of GPCRs to agonist. These discrepancies could be attributed to differences in the gender of the animals. Thus, different authors have found sex-related alterations in the permeability of the BBB (Saija et al., 1990; Minami et al., 2002). Furthermore, it is important to indicate that although the BBB limits the uptake of glutamate from the circulation in most regions of the brain, there are some few brain areas that do not contain a BBB (circumventricular organs) and do allow rapid L-glutamate uptake from the circulation (Hawkins et al., 1995). Supporting this, [^{3}H]Glu administration to maternal mice was followed by a marked increase in [^{3}H]Glu in the CNS (Yu et al., 1997).

Long term glutamate intake during gestacional period do not modify $mGluR_1$ mRNA levels in maternal brain, in spite of the decreased observed in this subtype by Western-Blot. These results seem to indicate that the down regulation observed in $mGluR_1$ following chronic treatment with glutamate is not due to variations on the rate of synthesis, but due to an internalization mechanism through intracellular organelles. Supporting this hypothesis, different authors have demonstrated the endocytosis of mGluRs through clathrin-coated vesicles (Albasanz et al., 2002; Mundell et al., 2001) or via clathrin-independent pathway (Fourgeaud et al., 2003).

The study of the mechanisms that regulate mGluRs signalling has been widely focused on the events that take place at the level of receptor. Thus, it is known that mGluRs share the molecular mechanisms involved in the desensitization of classical GPCRs. In particular, several authors have shown that phosphorylation of Group I mGluR by both second messenger-dependent protein kinases and G protein coupled receptor kinases results in an attenuation of receptor responsiveness or desensitization (Gereau and Heinemann, 1998; Dale et al., 2000; Sallese et al., 2000). However, apart from receptor phosphorylation, there are others mechanisms that can attenuate signalling by GPCRs. These operate at the level of heterotrimeric G-protein and at diverse downstream steps of the signalling pathway (Bohm et al., 1997). Surprisingly, the studies of the regulation of mGluR carried out to date have not examined the role played by the heterotrimeric G-protein and the effector system in these processes.

In this sense, the results obtained in the present work are specially important because they indicate that the down-regulation of the $\alpha G_{q/11}$ subunits and the PLCβ_1 isoform are also involved in the desensitization of Group I mGluRs *in vivo*. Thus, this down-regulation was accompanied by a decrease in GTPγS-stimulated and basal PLC activities. These data agree with the reduction in the corresponding heterotrimeric G protein and the effector system reported by others authors following long term treatment with agonists of muscarinic receptor. Thus, in SH-SY5Y neuroblastoma cells, prolonged activation of a PLC-coupled muscarinic receptor with the agonist oxotremorine-M produced a down-regulation of muscarinic receptor, $\alpha G_{q/11}$ and PLCβ_1 isoform (Sorensen et al., 1998). Similar results have also been reported in CHO cells, where the chronic treatment with the agonist carbachol resulted in down-regulation of both αG_q and αG_{11} subunits (Mullaney et al., 1993; Svoboda and Milligan, 1994).

Decreases in $\alpha G_{q/11}$ and PLCβ_1 proteins are not associated with decreases in their corresponding mRNA levels, suggesting the implication of post-transcriptional regulatory mechanisms, such as internalization. In the same way that mGluRs, several authors have demonstrated the presence of αG_{11} and the isoform PLCβ_1 in clathrin-coated vesicles (Petrou and Tashjian, 1998; Martín et al., 1991a, b, 1993).

Spatial learning is not impaired by chronic gestational L-Glu intake

The hippocampus participates in the learning and processing of spatial information (Pouzet et al., 2002). The abundance of mGluR in this tissue suggested a role for these receptors in the process of spatial learning. Supporting this hypothesis, several authors showed that Group I mGluRs are necessary in the dentate gyrus (Riedel and Reymann, 1993) and CA1 (Breakwell et al., 1996) for the long term potentiation (LTP), a potential cellular mechanism for the learning and memory (Bashir et al., 1993). Since then, additional studies have corroborated the participation of mGluRs in spatial learning. For example, using mutant mice lacking $mGluR_1$ (Conquet et al., 1994) or $mGluR_5$ (Lu et al., 1997), it was described severe spatial learning deficits in the Morris water maze. Furthermore, a recent study have also shown that deficits in signal transduction mediated by Group I in the hippocampus, induced by a decrease in the PLCβ_1 isoform, performed poorly on the spatial learning tasks (Nicolle et al., 1999). All these results contrast with our findings, where the down-regulation of $mGluR_1/G_{q/11}$/PLCβ_1 and the corresponding Group I mGluR desensitization was not accompanied by impairment in the spatial learning processes. This discrepancy could be due to the fact that our data are obtained from full brain. In this sense, additional studies may help to determine if Group I mGluR signalling is unaltered in hippocampus following chronic glutamate intake.

Chronic intake of L-Glu during gestational period does not affect Group I-mGluR responsiveness in fetal brain

Another interesting finding in this work is the absence of effect in all parameters analyzed in fetal brain following chronic maternal treatment with L-Glu. Thus, the number of mGluR, in particular $mGluR_1$, and the subunits $\alpha G_{q/11}$ and the isoform $PLC\beta_1$ remain unchanged after chronic treatment. Additionally, we have not observed any difference neither in basal PLC activity nor Group I- or $G_{q/11}$-mediated PLC stimulation between control and treated groups.

This lack of effect correlates well with reproduction and teratology studies carried out in rats and rhesus monkeys, which demonstrated that the increase observed in maternal plasma glutamate level after infusion or oral administration of this aminoacid was not accompanied by changes in glutamate level in fetal plasma (Walker and Lupien, 2000).

In this way, it is possible that the placental barrier may play an important role in the lack of effects detected in fetal brain after chronic L-Glu exposure. This observation agrees with previous published reports that indicated that the transport of glutamate across the primate placenta was little or nonexistent (Stegink et al., 1975). Recently, this data have been corroborated in ovine, where it has been shown that, in addition to not crossing the placental barrier, glutamate is taken up from the fetal circulation by the placenta (see for a review Battaglia, 2000).

In summary, results presented above indicate a down-regulation and desensitization of mGluR from maternal brain after chronic glutamate intake of pregnant rats without variation on fetal brain, suggesting cerebral affectation of pregnant rat by glutamate oral consumption. Therefore, particular attention should be paid to the glutamate consumption during pregnancy.

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References

Alagarsamy S, Marino MJ, Rouse ST, Gereau RW 4th, Heinemann SF, Conn PJ (1999) Activation of NMDA receptors reverses desensitization of mGluR5 in native and recombinant systems. Nat Neurosci 2: 234–240

Albasanz J, Fernandez M, Martin M (2002) Internalization of metabotropic glutamate receptor in C6 cells through clathrin-coated vesicles. Brain Res Mol Brain Res 99: 54–66

Balazs R, Miller S, Romano C, de Vries A, Chun Y, Cotman CW (1997) Metabotropic glutamate receptor mGluR5 in astrocytes: pharmacological properties and agonist regulation. J Neurochem 69: 151–163

Bashir ZI, Bortolotto ZA, Davies CH, Berretta N, Irving AJ, Seal AJ, Henley JM, Jane DE, Watkins JC, Collingridge GL (1993) Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. Nature 363: 347–350

Battaglia FC (2000) Glutamine and glutamate exchange between the fetal liver and the placenta. J Nutr 130: 974S–977S

Bogdanov MB, Tjurmina OA, Wurtman RJ (1996) Consumption of a high dietary dose of monosodium glutamate fails to affect extracellular glutamate levels in the hypothalamic arcuate nucleus of adult rats. Brain Res 736: 76–81

Bohm SK, Grady EF, Bunnett NW (1997) Regulatory mechanisms that modulate signalling by G-protein-coupled receptors. Biochem J 322: 1–18

Breakwell NA, Rowan MJ, Anwyl R (1996) Metabotropic glutamate receptor dependent EPSP and EPSP-spike potentiation in area CA1 of the submerged rat hippocampal slice. J Neurophysiol 76: 3126–3135

Catania MV, Aronica E, Sortino MA, Canonico PL, Nicoletti F (1991) Desensitization of metabotropic glutamate receptors in neuronal cultures. J Neurochem 56: 1329–1335

Chomczynski P, Sacchi N (1987) Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. Anal Biochem 162: 156–159

Claing A, Laporte SA, Caron MG, Lefkowitz RJ (2002) Endocytosis of G protein-coupled receptors: roles of G protein-coupled receptor kinases and beta-arrestin proteins. Prog Neurobiol 66: 61–79

Compton RP, Hood WF, Monahan JB (1990) Evidence for a functional coupling of the NMDA and glycine recognition sites in synaptic plasma membranes. Eur J Pharmacol 188: 63–70

Conquet F, Bashir ZI, Davies CH, Daniel H, Ferraguti F, Bordi F, Franz-Bacon K, Reggiani A, Matarese V, Conde F (1994) Motor deficit and impairment of synaptic plasticity in mice lacking $mGluR_1$. Nature 372: 237–243

Czech DA, Nielson KA, Laubmeier KK (2000) Chronic propranolol induces deficits in retention but not acquisition performance in the water maze in mice. Neurobiol Learn Mem 74: 17–26

Dale LB, Bhattacharya M, Anborgh PH, Murdoch B, Bhatia M, Nakanishi S, Ferguson SS (2000) G protein-coupled receptor kinase-mediated desensitization of metabotropic glutamate receptor 1A protects against cell death. J Biol Chem 275: 38213–38220

Dale LB, Bhattacharya M, Seachrist JL, Anborgh PH, Ferguson SS (2001) Agonist-stimulated and tonic internalization of metabotropic glutamate receptor 1a in human embryonic kidney 293 cells: agonist-stimulated endocytosis is beta-arrestin1 isoform-specific. Mol Pharmacol 60: 1243–1253

De Blasi A, Conn PJ, Pin J, Nicoletti F (2001) Molecular determinants of metabotropic glutamate receptor signaling. Trends Pharmacol Sci 22: 114–120

Ferguson SS (2001) Evolving concepts in G protein-coupled receptor endocytosis: the role in receptor desensitization and signaling. Pharmacol Rev 53: 1–24

Fourgeaud L, Bessis AS, Rossignol F, Pin JP, Olivo-Marin JC, Hemar A (2003) The metabotropic glutamate receptor mGluR5 is endocytosed by a clathrin-independent pathway. J Biol Chem 278: 12222–12230

Geha RS, Beiser A, Ren C, Patterson R, Greenberger PA, Grammer LC, Ditto AM, Harris KE, Shaughnessy MA, Yarnold PR, Corren J, Saxon A (2000) Review of alleged reaction to monosodium glutamate and outcome of a multicenter double-blind placebo-controlled study. J Nutr 130: 1058S–1062S

Gereau RW 4th, Heinemann SF (1998) Role of protein kinase C phosphorylation in rapid desensitization of metabotropic glutamate receptor 5. Neuron 20: 143–151

Guerineau NC, Bossu JL, Gahwiler BH, Gerber U (1997) G-protein-mediated desensitization of metabotropic glutamatergic and muscarinic responses in CA3 cells in rat hippocampus. J Physiol 500: 487–496

Hawkins RA, DeJoseph MR, Hawkins PA (1995) Regional brain glutamate transport in rats at normal and raised concentrations of circulating glutamate. Cell Tissue Res 281: 207–214

Herrera-Marschitz M, Goiny M, You ZB, Meana JJ, Engidawork E, Chen Y, Rodriguez-Puertas R, Broberger C, Andersson K, Terenius L, Hokfelt T, Ungerstedt U (1998) Release of endogenous excitatory amino acids in the neostriatum of the rat under physiological and pharmacologically-induced conditions. Amino Acids 14: 197–203

Kessler M, Terramani T, Lynch G, Baudry M (1989) A glycine site associated with N-methyl-D-aspartic acid receptors: characterization and identification of a new class of antagonists. J Neurochem 52: 1319–1328

Kimelberg HK, Pang S, Treble DH (1989) Excitatory amino acid-stimulated uptake of $^{22}Na^{+}$ in primary astrocyte cultures. J Neurosci 9: 1141–1149

Krupnick JG, Benovic JL (1998) The role of receptor kinases and arrestins in G protein-coupled receptor regulation. Annu Rev Pharmacol Toxicol 38: 289–319

Lu YM, Jia Z, Janus C, Henderson JT, Gerlai R, Wojtowicz JM, Roder JC (1997) Mice lacking metabotropic glutamate receptor 5 show impaired learning and reduced CA1 long-term potentiation (LTP) but normal CA3 LTP. J Neurosci 17: 5196–5205

Manzoni O, Fagni L, Pin JP, Rassendren F, Poulat F, Sladeczek F, Bockaert J (1990) (trans)-1-amino-cyclopentyl-1,3-dicarboxylate stimulates quisqualate phosphoinositide-coupled receptors but not ionotropic glutamate receptors in striatal neurons and Xenopus oocytes. Mol Pharmacol 38: 1–6

Martin M, Gonzalez-Calero G, Cubero A (1991a) Characterization of L-[^{3}H]glutamate binding sites in bovine brain coated vesicles. Eur J Pharmacol 207: 215–224

Martin M, Ros M, Gonzalez-Calero G, Cubero A (1991b) Presence of phospholipase C in coated vesicles from bovine brain. Dual regulatory effects of GTP-analogs. FEBS Lett 290: 22–26

Martin M, Sanz JM, Cubero A (1993) Characterization of metabotropic glutamate receptors coupled to a pertussis toxin sensitive G-protein in bovine brain coated vesicles. FEBS Lett 316: 191–196

Martin M, Albasanz JL, Fernandez M, Ros M (1998) Cross-talk between beta-adrenergic and metabotropic glutamate receptors in rat C6 glioma cells. Biochim Biophys Acta 1393: 186–192

Meldrum BS (2000) Glutamate as a neurotransmitter in the brain: review of physiology and pathology. J Nutr 130: 1007S–1015S

Minami T, Sakita Y, Ichida S, Dohi Y (2002) Gender difference regarding selenium penetration into the mouse brain. Biol Trace Elem Res 89: 85–93

Monaghan DT, Bridges RJ, Cotman CW (1989) The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annu Rev Pharmacol Toxicol 29: 365–402

Morris R (1984) Developments of a water-maze procedure for studying spatial learning in the rat. J Neurosci Methods 11: 47–60

Mullaney I, Mitchell FM, McCallum JF, Buckley NJ, Milligan G (1993) The human muscarinic M1 acetylcholine receptor, when express in CHO cells, activates and downregulates both Gq alpha and G11alpha equally and non-selectively. FEBS Lett 324: 241–245

Mundell SJ, Matharu AL, Pula G, Roberts PJ, Kelly E (2001) Agonist-induced internalization of the metabotropic glutamate receptor 1a is arrestin- and dynamin-dependent. J Neurochem 78: 546–551

Mundell SJ, Pula G, Carswell K, Roberts PJ, Kelly E (2003) Agonist-induced internalization of metabotropic glutamate receptor 1A: structural determinants for protein kinase C- and G protein-coupled receptor kinase-mediated internalization. J Neurochem 84: 294–304

Nicolle MM, Colombo PJ, Gallagher M, McKinney M (1999) Metabotropic glutamate receptor-mediated hippocampal phosphoinositide turnover is blunted in spatial learning-impaired aged rats. J Neurosci 19: 9604–9610

Novelli A, Tasker RA (2000) On excitotoxicity. Amino Acids 19: 227–228

Ozawa S, Kamiya H, Tsuzuki K (1998) Glutamate receptors in the mammalian central nervous system. Prog Neurobiol 54: 581–618

Peavy RD, Conn PJ (1998) Phosphorylation of mitogen-activated protein kinase in cultured rat cortical glia by stimulation of metabotropic glutamate receptors. J Neurochem 71: 603–612

Petrou C, Tashjian AH Jr (1998) The thyrotropin-releasing hormone-receptor complex and G11alpha are both internalised into clathrin-coated vesicles. Cell Signal 10: 553–559

Pin JP, Acher F (2002) The metabotropic glutamate receptors: structure, activation mechanism and pharmacology. Curr Drug Target CNS Neurol Disord 1: 297–317

Pin JP, Galvez T, Prezeau L (2003) Evolution, structure, and activation mechanism of family 3/C G-protein-coupled receptors. Pharmacol Ther 98: 325–354

Pouzet B, Zhang WN, Feldon J, Rawlins JN (2002) Hippocampal lesioned rats are able to learn a spatial position using non-spatial strategies. Behav Brain Res 133: 279–291

Price MT, Olney JW, Lowry OH, Buchsbaum S (1981) Uptake of exogenous glutamate and aspartate by circumventricular organs but not other regions of brain. J Neurochem 36: 1774–1780

Riedel G, Reymann K (1993) An antagonist of the metabotropic glutamate receptor prevents LTP in the dentate gyrus of freely moving rats. Neuropharmacology 32: 929–931

Riedel G, Platt B, Micheau J (2003) Glutamate receptor function in learning and memory. Behav Brain Res 140: 1–47

Saija A, Princi P, D'Amico N, De Pasquale R, Costa G (1990) Aging and sex influence the permeability of the blood-brain barrier in the rat. Life Sci 47: 2261–2267

Sallese M, Salvatore L, D'Urbano E, Sala G, Storto M, Launey T, Nicoletti F, Knopfel T, De Blasi A (2000) The G-protein-coupled receptor kinase GRK4 mediates homologous desensitization of metabotropic glutamate receptor 1. FASEB J 14: 2569–2580

Sattler R, Tymianski M (2001) Molecular mechanisms of glutamate receptor-mediated excitotoxic neuronal cell death. Mol Neurobiol 24: 107–129

Schoepp DD, Johnson BG (1998) Selective inhibition of excitatory amino acid-stimulated phosphoinositide hydrolysis in the rat hippocampus by activation of protein kinase C. Biochem Pharmacol 37: 4299–4305

Smith QR (2000) Transport of glutamate and other amino acids at the blood-brain barrier. J Nutr 130: 1016S–1022S

Sorensen SD, Linseman DA, Fisher SK (1998) Down-regulation of phospholipase C-beta1 following chronic muscarinic receptor activation. Eur J Pharmacol 346: R1–R2

Stegink LD, Pitkin RM, Reynolds WA, Filer LJ Jr, Boaz DP, Brummel MC (1975) Placental transfer of glutamate and its metabolites in the primate. Am J Obstet Gynecol 122: 70–78

Svoboda P, Milligan G (1994) Agonist-induced transfer of the alpha subunits of the guanine-nucleotide-binding regulatory proteins Gq and G11 and of muscarinic m1 acetylcholine receptors from plasma membranes to a light-vesicular membrane fraction. Eur J Biochem 224: 455–462

Tiger G, Bjorklund PE, Cowburn RF, Garlind A, O'Neill C, Wiehager B, Fowler CJ (1990) Effect of monovalent ions upon G proteins coupling muscarinic receptors to phosphoinositide hydrolysis in the rat cerebral cortex. Eur J Pharmacol 188: 51–62

Vendite D, Sanz JM, Lopez-Alanon DM, Vacas J, Andres A, Ros M (1998) Desensitization of adenosine A1 receptor-mediated inhibition of adenylyl cyclase in cerebellar granule cells. Neurochem Res 23: 211–218

Walker R, Lupien JR (2000) The safety evaluation of monosodium glutamate. J Nutr 130: 1049S–1052S

Yu T, Zhao Y, Shi W, Ma R, Yu L (1997) Effects of maternal oral administration of monosodium glutamate at a late stage of pregnancy on developing mouse fetal brain. Brain Res 747: 195–206

Zimmer J, Kristensen BW, Jakobsen B, Noraberg J (2000) Excitatory amino acid neurotoxicity and modulation of glutamate receptor expression in organotypic brain slice cultures. Amino Acids 19: 7–21

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