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Research report

Gestational and postnatal malnutrition affects sensitivity of young rats to picrotoxin and quinolinic acid and uptake of GABA by cortical and hippocampal slices

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Abstract

It is widely known that a complex interaction between excitatory and inhibitory systems is required to support the adequate functioning of the brain and that significant alterations induced by early protein restriction are complex, involving many systems. Based on such assumptions, we investigated the effects of maternal protein restriction during pregnancy and lactation followed by offspring protein restriction on some GABAergic and glutamatergic parameters, which mediate inhibitory and excitatory transmission, respectively. The sensitivity of young malnourished rats to convulsant actions of the $GABA_A$ receptor antagonist picrotoxin (PCT; s.c.) and to *N*-methyl-D-aspartate (NMDA) receptor agonist quinolinic acid (QA; i.c.v) and also γ-amino-*n*-butyric acid (GABA) and glutamate uptake by cortical and hippocampal slices were evaluated in P25 old rats. Early protein malnutrition induced higher sensitivity to picrotoxin, which could be associated with the observed higher GABA uptake by cortical, and hippocampal slices in malnourished rats. In contrast, we observed lower sensitivity to quinolinic acid in spite of unaltered glutamate uptake by the same cerebral structures. Picrotoxin enhanced GABA uptake in hippocampus in well- and malnourished rats; however, it did not affect cortical GABA uptake. Our data corroborate our previous report, showing that malnutrition depresses the glutamatergic activity, and point to altered modulation of GABAergic neurotransmission. Such findings allow us to speculate that malnutrition may affect the excitatory and inhibitory interaction.

Theme: Development and regeneration
Topic: Nutritional and prenatal factors

Keywords: Protein malnutrition; GABA and glutamate uptake; Cerebral cortex; Hippocampus; Picrotoxin; Quinolinic acid

1. Introduction

Nutritional inadequacy is among the main non-genetic factors affecting brain development [43]. In the framework of such nutritional inadequacy, early protein malnutrition adversely affects the central nervous system (CNS) maturation, resulting in long-lasting or even permanent deleterious effects [33,42]. In rodents, such abnormalities are manifested in different brain regions [37]. In early postnatal undernourished infants, for example, Cordero et al. [12] showed a decrease in the arborization and span of the basilar

Abbreviations: AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate; GAD, Glutamate descarboxilase; GAT, GABA transporter; mIPSCs, Miniature inhibitory postsynaptic currents; NMDA, *N*-methyl-D-aspartate; PCT, Picrotoxin; QA, Quinolinic acid

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dendrites, indicating that undernutrition during the first months of postnatal life could affect the growth of pyramidal cells. In humans, there is sound evidence, mostly epidemiological, that malnutrition could contribute to raise the prevalence of epilepsy in children in developing countries [22,28].

Although most nutrients may affect brain maturation, proteins appear to be the most critical ones [43]. Prenatal protein malnutrition causes alterations on developmental time course of dentate granule cell birth in the dentate gyrus [16,17], on the morphology of hippocampal cells [20,21], on the number and distribution of neurotransmitters receptors [24] and on the performance of rats in behavioral tasks involving hippocampus [65], and increases the frequency of miniature inhibitory postsynaptic current (mIPSC) in CA1 pyramidal cells [36]. Besides such alterations, it is also known that protein deprivation affects cathecolaminergic, cholinergic, serotoninergic [72], glutamatergic [49] and GABAergic [36,56,57] neurotransmission systems.

Once brain functions depend on the dynamic interaction between excitatory (e.g., glutamatergic) and inhibitory (e.g., GABAergic) inputs, the modulation of γ-amino-*n*-butyric acid (GABA) release by glutamatergic and/or GABAergic terminals containing glutamate receptors is of utmost importance for the maintenance of the balance between excitation and inhibition. For instance, the glutamate receptor-induced modulation of GABAergic synaptic transmission has been thought of as being involved in glutamate-induced seizures and neuronal damage [5]. Saransaari and Oja [50] showed that the ionotropic receptor agonist kainate, *N*-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) increase the receptor-mediated basal GABA release with no effect on K^+-stimulated GABA release.

There are accumulating evidences for the GABAergic system sensitivity to malnutrition. Steiger et al. [56,57] showed that prenatal protein malnutrition selectively alters $GABA_A$ receptor mRNA levels in rat hippocampus and septum. Early undernutrition significantly influenced characteristics of [^{3}H]GABA binding in rat brain cerebral cortex and hippocampus [64] and delayed developmental pattern of glutamate descarboxilase (GAD) activity [18]. Changes in reactivity to drugs acting via $GABA_A$ receptor such as ethanol [8,13] and benzodiazepines [1,9,67] induced by early undernutrition raises the question of whether this insult could affect the action of other therapeutic compounds on brain tissue.

Concerning the glutamatergic system, Tonkiss et al. [66] provided behavioral evidence that the excitatory amino acid system may be altered in prenatally protein malnourished rats. Fiacco et al. [25] found increased density of hippocampal kainate receptors but normal density of NMDA and AMPA receptors in a rat model of prenatal protein malnutrition. Those authors [25] observed increases in [^{3}H]kainate binding density that may be compensatory to the decreased glutamate release accounted to mossy fiber plexus reduction reported at these ages in prenatally malnourished rats by Cintra et al. [11]. On the other hand, our laboratory showed that pre- and postnatal nutritional insult depressed some parameters of the glutamatergic system in 75-day-old rats, decreasing the sensitivity to QA-induced seizures, reducing the Na^+-independent glutamate binding in neural cell membranes, and decreasing the basal (but not K^+-stimulated) glutamate release from synaptossomal preparations [49].

Considering (i) the accumulating evidence for significant alterations in the GABAergic system in response to malnutrition; (ii) the data from our laboratory on the effect of protein malnutrition on the glutamatergic system; (iii) the fact that brain functions are based on the dynamic interaction between the excitatory and inhibitory systems, which, in their turn, are involved in many neurological disorders, including seizures and epilepsy, the present study was undertaken to evaluate the effects of gestational and postnatal malnutrition on some parameters of the GABAergic and glutamatergic systems. Accordingly, we evaluated the sensitivity of P25 old rats to the convulsing actions of the GABAergic antagonist picrotoxin and of quinolinic acid (QA), which is an over stimulator of the glutamatergic system. We also evaluated the GABA and glutamate uptake by cortical and hippocampal slices.

2. Experimental procedures

2.1. Chemicals

GABA and glutamic acid were from Sigma (St. Louis, MO, USA). 4-Amino-*n*-[2.3-^{3}]butyric acid ([^{3}H]GABA) (specific activity 94 Ci/mmol) and L-[2,3-^{3}H] glutamic acid ([^{3}H]Glu) (specific activity 49 Ci/mmol) were from Amersham International (Berkingshamshire,UK). All other reagents used were of analytical grade.

2.2. Animals and diets

Female Wistar rats were mated and maintained at 22 °C on a controlled 12:12 h light–dark cycle. A group of pregnant rats (G_{25}—control group) was fed a 25% casein, whereas other group (G_7) was fed a 7% casein diet, a high quality protein, which avoids additional effects of protein of low quality [23,75]. Both diets, given at libitum, were supplemented with 0.15% L-methionine, were isocaloric and contained similar amounts of fat (15%), cellulose (1%), minerals (4%) and vitamins (1.5%), as recommended by the Association of Official Analytical Chemists [30] and were maintained during the gestation and lactation (Table 1). After birth (postnatal 1—the day of birth was counted as postnatal day 0), litters size were adjusted to

Table 1
Nutritional composition of diets

Component (g/kg/diet)	Casein Diet	
	25%	7%
Casein (87% protein)[a]	287	80.5
Fat (soybean oil)	150	150
Carbohydrate (corn starch)	501.5	708
Salt mix[b]	40	40
Vitamin mix[c]	10	10
Non-nutritive fiber	10	10

Salt and vitamin compositions are according to Horwitz [30]. Energy for both diets: 4.3 kcal/g diet in both diets.

[a] Casein, purity 87% (from Farmaquímica, Porto Alegre, Brazil) supplemented with 0.15% L-methionine (from Merck, Rio de Janeiro, Brazil).

[b] Mineral mixture (from Roche, São Paulo, Brazil) mg/100 g of ration: NaCl, 557; KI, 3.2; KH_2PO_4, 1556; $MgSO_4$, 229; $CaCO_3$, 1526; $FeSO_4 \cdot 7H_20$, 108; $MnSO_4 \cdot H_2O$, 16; $ZnSO_4 \cdot 7H_2O$, 2.2; $CuSO_4 \cdot 5H_2O$, 1.9; $CoCl_2 \cdot 6H_2O$, 0.09.

[c] Vitamin mixture (from Roche), mg/100 g of ration: Vitamin A, 4; Vitamin D, 0.5; Vitamin E, 10; Menadione, 0.5; Choline, 200; PABA, 10; Inositol, 10; Niacin, 4; Pantothenic acid, 4; Riboflavin, 0.8; Thiamin, 0.5; Pyridoxine, 0.5; Folic acid, 0.2; Biotin, 0.04; Vitamin B12, 0.003.

eight pups per dam, and at 21 days of age, offspring were weaned onto their respective dams diet until P25. The handling and care of the animals were conducted according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, School of Veterinary Medicine and Animal Science of the University of São Paulo, Brazil.

2.3. Body and brain weights

Body weights were recorded weekly and whole brain, cerebellum and hippocampus weights were recorded at P25.

2.4. Picrotoxin (PCT)-induced seizure

Picrotoxin (a racemic mixture of picrotin and the active agent picrotoxinin) is thought to bind within the ion channel [27,45], blocks the GABA-gated Cl^- channel [6] decreasing the mean channel open time [54] and the mean number of opening per burst [68]. The model of GABA-related convulsions is often used to evaluate the effectiveness of anticonvulsive drugs [46]; in this case, the model was used to evaluate the functional state of the GABA system following protein restriction. Picrotoxin was diluted in saline buffered to pH 7.4, and injected at P25 (1 mL/kg) subcutaneously (s.c.) in several doses. After injection, the animals were observed for 60 min and seizures were classified as: (1) minimal seizures characterized by clonuses of head muscles and forelimbs, with righting ability preserved, and (2) major seizures usually beginning with running followed by a loss of posture accompanied by the tonic phase and, after some seconds, occurrence of long-lasting (*status epilepticus*) clonic seizures of all limbs [69]. For dose–response curve of picrotoxin-induced seizures, only major seizures were considered. The seizure latency was considered the time for the first long-lasting (*status epilepticus*) clonic seizures of all limbs.

2.5. Quinolinic acid induced seizure

Quinolinic acid, an endogenous compound produced by the metabolism of tryptophan, which may over stimulate the glutamatergic system, is involved in the pathogenesis of various CNS disorders [58] including the etiology of epilepsy [29,44,58]. QA binds to NMDA channel receptors [59], allowing its activation [40], which increases the intracellular calcium levels [15]. Additionally, recent studies from our group (see also Stone [58]) have shown that QA affects several parameters of the glutamatergic system, once it: (i) decreases glutamate uptake by cultured astrocytes [63]; (ii) decreases glutamate uptake by synaptic vesicles from rat brain [62]; and (iii) stimulates synaptosomal glutamate release [63]. Accordingly, QA is used by our group for experimental over stimulation of the glutamatergic system [32,51,70].

Surgery was performed at P23 for both groups (G_{25} and G_7). The animals were anesthetized with ketamine (G_{25}=90 mg/kg; G_7=70 mg/kg) and xilasine (12 mg/kg for both groups) and placed in a stereotaxic apparatus. The skin of the skull was removed and a 27-gauge, 6-mm guide cannula was placed unilaterally at 1 mm above the lateral ventricle (AP: −0.5 mm; L: −1.5 mm; V: −3.0 mm from the skull surface). The cannula was fixed with acrylic cement, xylocain was applied around the cut and animals were allowed to recover for about 48 h. For intracerebroventricular (i.c.v.) quinolinic acid administration (1.0 μl) at P25, a 30-gauge, 7-mm cannula was fitted into the guide and connected by a polyethylene tube to a 5-μl Hamilton micro syringe. The tip of the infusion cannula protruded 1.0 mm beyond the guide cannula towards the lateral brain ventricle. The animals were observed after administration for 10 min in Plexiglas chambers for the occurrence of tonic–clonic seizures. After observations, methylene blue (0.5 μl) was injected and the animals without dye in the lateral brain ventricle were discarded.

2.6. [^{3}H]GABA uptake by cerebral cortex and hippocampus slices

In order to determine the adequate GABA concentration and incubation time for uptake assays, slices of hippocampus and parietal cortex were incubated with 25, 50 and 100 μM GABA for 5, 10, 15 and 30 min (data not shown). Based upon these previous data, the GABA concentration and incubation time used in the following GABA uptake assays were 25 μM and 15 min, respectively.

The animals were divided into four groups: G_{25}–vehicle; G_{25}–picrotoxin; G_7–vehicle; G_7–picrotoxin. They were injected at P25 with vehicle or picrotoxin s.c. (lowest

concentration that produced convulsion in 100% of the animals), killed by decapitation 15, 25 or 60 min after s.c. administration of picrotoxin or vehicle, the brains were immediately removed and submerged in Hank's balanced salt solution (HBSS), pH 7.2. Parietal cerebral cortices were dissected and coronal slices (0.4 mm) were obtained from the parietal area using a Mc Illwain tissue chopper. Slices were transferred to multiwell dishes and washed with 1.0 ml HBSS. The same procedure was undertaken for the hippocampal GABA uptake assay, except that hippocampal dissection and slices incubation were performed only 60 min after s.c. administration. The uptake assay was assessed by adding 25 μM [^{3}H]GABA in 300 μL HBSS, at 37 °C. Incubation was stopped after 15 min by three ice-cold washes with 1 ml HBSS immediately followed by addition of 0.5 N NaOH, which was kept overnight. Aliquots of lysate were taken for determination of intracellular content of [^{3}H]GABA trough scintillation counting. Sodium independent uptake was determined by using *N*-methyl-D-glucamine instead of sodium chloride, being subtracted from the total uptake to obtain the sodium-dependent uptake. The experiments were done in triplicate.

2.7. [^{3}H]Glutamate uptake by cerebral cortex and hippocampus slices

The animals were divided into six groups: G_{25}–naive; G_{25}–vehicle; G_{25}–quinolinic acid; G_7–naive; G_7–vehicle; G_7–quinolinic acid.

They were killed by decapitation at P25 10 min after i.c.v. QA (lowest concentration which produces convulsions in 100% of the animals) or vehicle administration. The brains were immediately removed and submerged in HBSS. Parietal cerebral cortices and hippocampi were dissected as described for the GABA uptake assay. Glutamate uptake assay, performed according to our previous reports [26] was assessed by adding 100 μM [^{3}H]Glu [61] in 300 μL HBSS, at 37 °C. Incubation was stopped after 7 min for cortex and 5 min for hippocampus by two ice-cold washes with 1 mL HBSS, immediately followed by addition of 0.5 N NaOH, which was kept overnight. Intracellular [^{3}H]Glu content was determined by scintillation counting and sodium-dependent uptake was obtained by subtraction of sodium-independent uptake from the total uptake (as for GABA uptake assay). Experiments were performed in triplicate.

Table 3
Effect of pre- and postnatal malnutrition on brain, cerebellum and hippocampus weight in P25 rats

Group	Whole brain (mg)	Cerebellum (mg)	Hippocampus (mg)
G_{25}	1245.0 ± 38.7	235.0 ± 13.7	74.8 ± 2.0
G_7	799.0 ± 50.8*	175.0 ± 7.3*	66.4 ± 1.4*

Data are means ± S.E.M. (n = 14).
* Differs from control ($P < 0.05$, Student's t-test).

2.8. Protein determination

Protein was assessed using the Peterson method [47]. Briefly, this method consists in a simplification of the protein assay method described by Lowry et al. [35], which include sodium dodecylsulfate in the presence of NaOH and allows for immediate solubilization from proteolipids.

2.9. Statistical analysis

Seizures data were expressed as % of animals presenting seizures, and latency data were expressed as mean ±S.E.M. in seconds for the first long-lasting clonic seizures (*status epilepticus*) of all limbs. Body and cerebral weights, litter size and glutamate and GABA uptake data were expressed as mean±SEM. Statistical analysis of the data was performed by (i) Fisher's exact test for comparison of occurrence seizures; (ii) Pearson's Correlation Coefficient for dose-dependent latency for seizures; (iii) ANOVA for multiple groups comparison, followed by the Duncan's post hoc test for the neurotransmitters uptake and (iv) Student's t-test for latency time for seizures between dose groups, body and cerebral structure weights. $P<0.05$ was considered significant.

3. Results

3.1. Body and brain weight

At postnatal day 1, the mean weight of rats in the malnourished group was significantly lower than control ($P<0.05$). This was evident through the pre-weaning period up to P25 ($P<0.01$) (Table 2). Likewise, at P25, whole cerebral, cerebellum and hippocampus from malnourished group weighed significantly ($P<0.05$) less (36%, 25% and 11%, respectively) than from controls (Table 3). The mean litter size did not differ significantly between the groups

Table 2
Effect of early malnutrition on body growth of developing rats (body weight in grams)

Group	P1	P7	P14	P21	P25
G_{25}	6.25 ± 0.10	16.02 ± 0.27	31.77 ± 0.42	49.03 ± 0.65	67.37 ± 0.79
G_7	5.41 ± 0.09*	9.13 ± 0.18**	13.14 ± 0.63**	19.03 ± 0.92**	23.17 ± 0.87**

Data are means ± S.E.M. (n = 40).
* Differs from control at the same age at $P < 0.05$ (Student's t-test).
** Differs from control at the same age at $P < 0.01$ (Student's t-test).

with 10.90±0.37 pups/litter in the G_{25} and 9.90±0.83 pups in the G_7 group (n=20 in each group).

3.2. Picrotoxin-induced seizures

In order to investigate the effect of malnutrition on the convulsive effect of picrotoxin, a seizure response-curve for picrotoxin was performed, both in control and malnourished rats. Picrotoxin was injected s.c. in a dose range (3.2, 4.0, 4.8, 5.6 and 6.4 mg/kg), as shown in Fig. 1A. The lowest dose that induced *status epilepticus* in 100% of rats was different in each group: 4.8 mg/kg in malnourished rats and 5.6 mg/kg in control rats (P<0.05). The convulsion latency was also lower in the malnourished group (P<0.05) (Fig. 1B). Moreover, both groups displayed a dose-dependent latency time (r=0.723, P<0.001, G_{25}; r=0.386, P<0.05, G_7) (Fig. 1B).

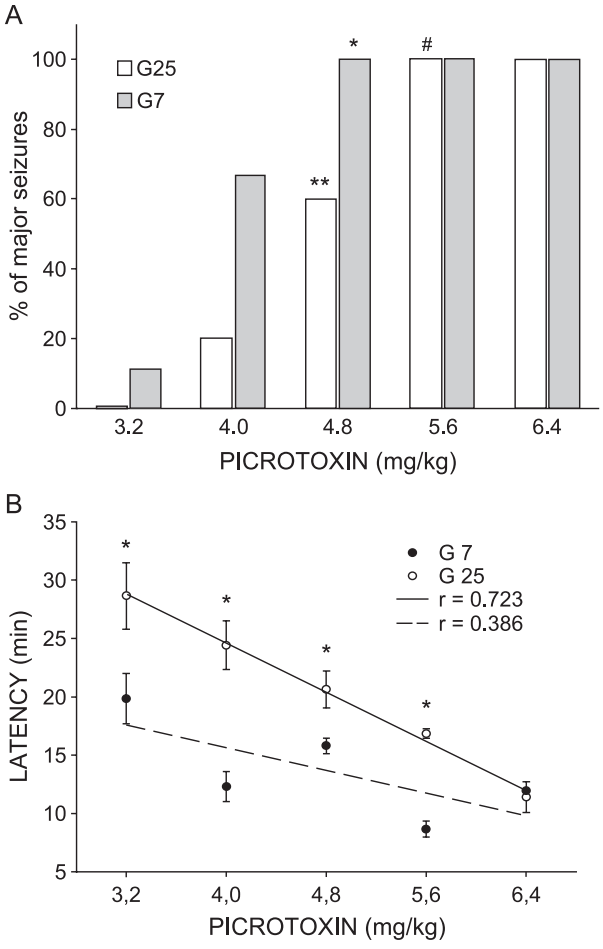

Fig. 1. (A) Picrotoxin-induced seizures. Rats were injected with picrotoxin and observed for 60 min for the presence of major seizures. n=9–10 animals/group. $^{\#}P$<0.05 as compared with G_{25} (3.2, 4.0, 4.8 mg/kg). *P<0.05 as compared with G_7 (3.2 and 4.0 mg/kg). **P<0.05 as compared with G_7 (4.8 mg/kg). Data are expressed as percentage of rats that presented major seizures and statistical analysis of the data was performed by Fisher's exact test. (B) Latency for picrotoxin-induced seizures. Rats were injected with picrotoxin and the seizure latency was considered the time for the first long-lasting (*status epilepticus*) clonic seizures of all limbs. n=9–10 animals/group. Latency was expressed as mean±S.E.M. *Significantly different from G_7, for the same dose (P<0.05, Student's t-test). Solid (——) and short dash (- - - - -) lines indicate the Pearson's correlation coefficient for dose-dependent latency for seizures.

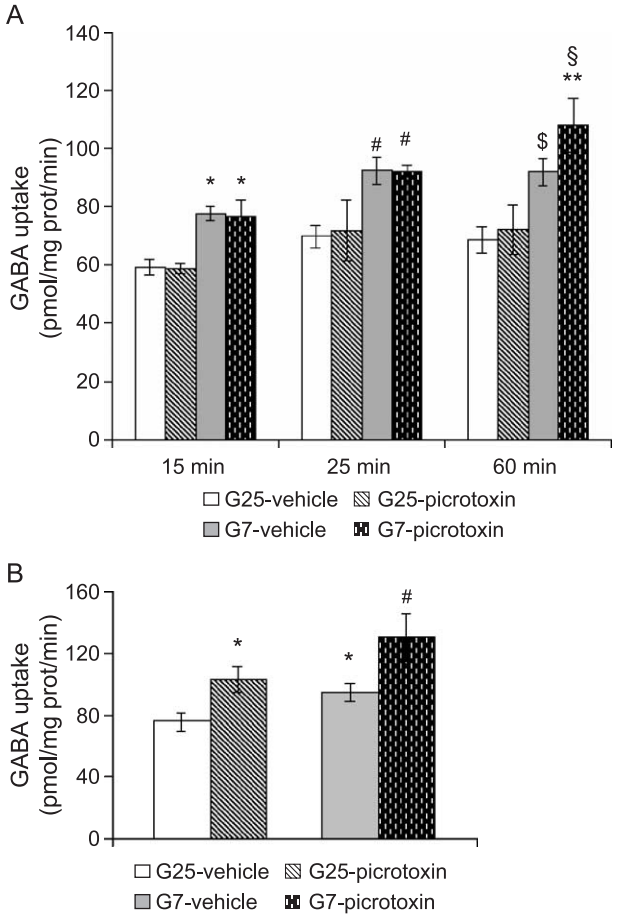

Fig. 2. (A) GABA uptake by cerebral parietal cortex slices of well- and malnourished rats. Data are represented as mean±S.E.M. (bars) for six to eight animals of which slices were incubated 15, 25 and 60 min after picrotoxin injection. Experiments were done in triplicate. Significance of differences (one-way ANOVA followed by Duncan's multiple range test): *P<0.05 compared with G_{25}–vehicle and –picrotoxin 15 min; $^{\#}P$<0.05 compared with G^{25}–vehicle and –picrotoxin 25 min;$^{\$}P$<0.05 compared with G_{25}–vehicle 60 min; **P<0.05 compared with G_{25}–vehicle and –picrotoxin 60 min; $^{\S}P$<0.05 compared with G_7–picrotoxin 15 min. (B) GABA uptake by hippocampus slices of well- and malnourished rats 60 min after picrotoxin or vehicle injection. Groups: G_{25}–vehicle; G_{25}–picrotoxin; G_7–vehicle; G_7–picrotoxin. Data are means±S.E.M. (bars) and obtained from three independent experiments, which were done in triplicate for eight animals per group. Significance of differences (one-way ANOVA followed by Duncan's multiple range test): *P<0.05 compared with G_{25}–vehicle; $^{\#}P$<0.05 compared with G_7– and G_{25}–vehicle.

3.3. [^{3}H]GABA uptake

GABA uptake was performed in order to verify whether malnutrition and/or picrotoxin administration influenced GABA uptake by slices from cerebral cortex and hippocampus.

In cerebral cortex, we observed that malnutrition increased the uptake (P<0.05) in all times spent after

vehicle/picrotoxin injection. Picrotoxin had no effect for any of the two groups (Fig. 2A). There was no difference in the GABA uptake for the same group at different incubation times except for the G_7–picrotoxin group, i.e., picrotoxin effect was higher in cerebral cortical slices of malnourished rats which were incubated 60 min after picrotoxin injection in comparison with those which were incubated 15 min after picrotoxin (Fig. 2A).

In hippocampal slices (incubated only 60 min after picrotoxin/vehicle injection), malnutrition also increased GABA uptake. In this structure, however, picrotoxin increased GABA uptake for both malnourished and well-nourished rats (Fig. 2B).

3.4. Quinolinic-acid-induced seizures

In order to investigate the effect of malnutrition on the convulsing effect of QA, a seizure response-curve for QA was performed (in a dose range of 36, 72, 145, 194 and 242 nmol), as shown in Fig. 3. The lowest QA dose causing seizures in all animals was higher ($P<0.05$) in malnourished (242 nmol) than in the control group (145 nmol).

3.5. [^{3}H]Glutamate uptake

Considering that astrocytic sodium-dependent glutamate transport is the most important process for maintaining extracellular glutamate concentrations below toxic levels [2,14] and that the QA-induced seizures is a pharmacological strategy to evaluate excitotoxicity by overstimulation of the glutamatergic system [58,62,63], here, we investigated the effect of malnutrition on [^{3}H]Glu uptake by slices from cerebral cortex and hippocampus, 10 min after QA i.c.v. injection. There was neither effect of malnutrition nor QA in any of those cerebral structures (Fig. 4).

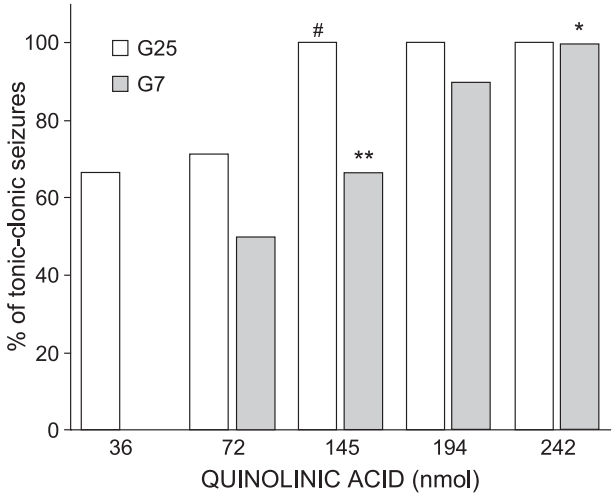

Fig. 3. Quinolinic-acid-induced seizures. Rats were injected with quinolinic acid and observed for 10 min for the presence of tonic–clonic seizures. n=8–10 animals/group. $^{\#}P<0.05$ as compared with G_{25} (72 and 36 nmol). $^{*}P<0.05$ as compared with G_7 (72 and 145 nmol). $^{**}P<0.05$ as compared with G_{25} (145 nmol). Data are expressed as percentage of rats that presented tonic–clonic seizures and statistical analysis of the data was performed by Fisher's exact test.

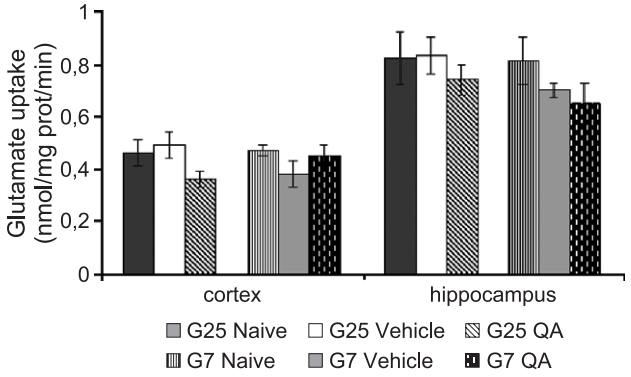

Fig. 4. Glutamate uptake by rat cerebral parietal cortex and hippocampus slices 10 min after QA or vehicle injection. Incubation time: 7 and 5 min, respectively. Groups: G_{25}–naive; G_{25}–vehicle; G_{25}–QA; G_7–naive; G_7–vehicle; G_7–QA. Data are means±S.E.M. and obtained from four independent experiments, which were done in triplicate for 12 animals/group for cortex uptake. Hippocampus uptake experiments were done also in triplicate for 6 animals/group in two independent experiments. There was no difference between groups in the same cerebral structure using one-way ANOVA followed by Duncan's multiple range test.

4. Discussion

Body and cerebral weight gain in malnourished animals is reduced by dams protein restriction during gestation and lactation periods followed by pups protein restriction [19,7] a finding which was also supported by what was observed in our model.

In this work, malnourished rats were more sensitive to the convulsing action of picrotoxin (Fig. 1A and B) than control rats. Several studies with recombinant receptors [38,55,71] or animal models with specific $GABA_A$ receptor gene-knockout or knock-in strategy [31,34,39,60] indicate that the subunit composition confers a unique pharmacological profile and that alterations in subunit-specific genes expression may contribute to alterations in $GABA_A$ receptor function [57]. Besides, the sensitivity to picrotoxin is notably enhanced in $GABA_A$ receptors lacking α-subunits; in contrast, α β γ-expressing receptors are relatively less sensitive to picrotoxin than β γ-expressing receptors [6]. Steiger et al. [56,57] showed selective changes in $GABA_A$ receptor mRNA levels in RNA derived from hippocampal formation and septum, but not from the neocortex in prenatally malnourished rats. Although differences in α-subunit mRNA expression in hippocampus were not found by Steiger et al. [57] in 30-day-old rats and only in adult rats, a significative β_2 upregulation was found in 30-day-old rats. In an attempt to explain the higher sensitivity of malnourished rats to picrotoxin, it is reasonable to speculate that protein malnutrition induced alterations in $GABA_A$ receptor subunit [56,57] and that the different profiles of picrotoxin inhibitory characteristics across several $GABA_A$

receptors subunit configurations [6,53] may underlie our findings. This higher sensitivity to picrotoxin in 25-day-old malnourished rats, in its turn, could be related to this upregulation. This interpretation, however, must be cautiously considered, since the regional and ontogenic differences showed by Steiger et al. [56,57] in malnourished rats may be reproduced in other brain structures and other factors may also be involved.

The higher basal GABA uptake levels on hippocampal and cerebral cortex slices induced by malnutrition (Fig. 2A and B) can be associated with the higher sensitivity to the convulsing action of picrotoxin (Fig. 1), since the higher uptake of GABA could decrease extracellular GABA levels, leading to the observed increase in the sensitivity to picrotoxin. In that respect, recently, Zhao et al. [76] have found increased picrotoxin-induced seizure in overexpressing GABA transporter (GAT)-1 transgenic mice, which suggests that GABA transporters might play an important role in epileptogenesis.

As picrotoxin increased hippocampal GABA uptake in both control and malnourished rats while it had no effect on GABA cortical uptake, we could assume that: (i) malnutrition affects different structures in many ways and/or intensity, with hippocampus being severely affected [3,4,10,33,43] and (ii) a distinct regional pattern of neuronal involvement in PCT-induced seizures is suggested [48,73].

Moreover, the developmental profile of GATs, which can contribute to determine the factors that regulate the development of GABA-mediated signaling, could also be considered. The reason for such an assumption is that the maturation of GAT-1, which appears to be the quantitatively prevalent GABA transporter [52], reaches adult pattern only at P30–45 [74] and early in the development GAT-3 probably accounts for the largest fraction of GABA transport in the neonatal cortex [41], whereas GAT-2 is low at early postnatal ages and did not change significantly during postnatal development [41]. This ontogenic pattern, which is under study in our group, may be altered by malnutrition that, in turn, could lead to the observed effects on GABA uptake in our study. Alternatively, malnutrition could have altered the number of functional transporters, which may be considered to explain the observed effects on GABA uptake.

In contrast to the higher sensitivity to the $GABA_A$ receptor antagonist picrotoxin (Fig. 1), malnutrition induced lesser sensitivity to the convulsant action of the NMDA-agonist QA (Fig. 3). Malnourished rats at P25 required higher QA doses to promote convulsing effect than well-nourished ones (Fig. 3). The findings from Rotta et al. [49] showing that chronic malnutrition decreases the sensitivity to QA-induced seizures at P75 denote that early nutritional insult on this parameter is not reverted by aging, when protein deprivation keeps on. Considering both studies, we cannot affirm that perinatal nutritional deprivation effect on this parameter is long lasting but that this period being decisive to unchain it.

The decreased glutamate binding in brain from P75-day-old malnourished rats also described by our group [49] points to a decrease in the glutamatergic activity which, in turn, could explain the lesser sensitivity of adult rats to QA. Consequently, this result may be considered as a possible explanation for our findings with P25-day-old malnourished rats, since reduced Na^+-independent [^{3}H]glutamate binding in cellular membranes were also observed by our group in P21-day-old malnourished rats (unpublished data).

The glutamate uptake was not affected by malnutrition (as well as observed by our group for 21-day-old rats in parietal cortex slices, unpublished data), nor by QA i.c.v injection both in cerebral parietal cortex and hippocampal slices (Fig. 4), apparently ruling out this parameter as a cause of the altered sensitivity to QA.

In this paper, we observed a distinct pharmacological profile induced by malnutrition, demonstrated by the higher sensitivity to the convulsing action of picrotoxin and lower sensitivity to the convulsing action of QA. Additionally, malnutrition increased the GABA uptake by cortical and hippocampal slices, with no effect on glutamate uptake. These results together and the knowledge that dynamic interaction of excitatory and inhibitory inputs are involved in brain functions, lead us to speculate that, by affecting this interaction, malnutrition could modify the sensitivity to drugs, including those used with therapeutic purposes. Further understanding of regulatory mechanism may provide pharmacological target by which neurotransmission may be manipulated providing novel therapeutic issues in neurological disorders in a context of malnutrition.

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