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ORIGINAL PAPER

The Response to Postnatal Stress: Amino Acids Transporters and PKC Activity

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Abstract It is well known that animals exposed to stressful stimuli during their early life develop different neurological disorders when they become adults. In this study, we evaluated the effect of acute cold stress on γ-aminobutyric acid (GABA) and L-Serine (L-Ser) transporters in vitro, using the uptake of [^{3}H]-GABA and [^{3}H]L-Ser by synaptosomes-enriched fractions isolated from rat cerebral cortex during postnatal development. GABA and L-Ser uptake studies in vitro will be used in this investigation as a colateral evidence of changes in the expression of transporters of GABA and L-Ser. We observed that the maximum velocity (V_{max}) in L-Ser and GABA uptake after stress session increased in all stages studied. In contrast, K_m values of L-Ser uptake enhancent in almost age calculated, excluding at PD21 after cold stress during development, at the same time as K_m (uptake affinity) values of GABA increased in just about age considered but not at PD5 compared with the control group. Finally we investigated the mechanism by which cells regulate the substrate affinity of L-Ser and GABA transporters. We demonstrated a significantly increase in total PKC activity to PD5 from PD21. Pretreatment with PKC inhibitor: staurosporine (SP) led to a restoration of control uptake in several postnatal-days suggesting a relationship between amino acids system and PKC activation. These findings suggest that a single exposure to postnatal cold stress at different periods after birth modifies both GABA and L-Ser transporters and the related increase in total PKC activity could be intracellular events that participate in neuronal plasticity by early life stress, which could be relevant to function of transporters in the adult rat brain.

Keywords Acute cold stress · GABA · L-Serine · Uptake · PKC activity · Postnatal development · Transporters

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Introduction

The adaptability of living organisms and their capacity to construct new operating conditions in response to imposed external or internal stimuli enables continuation of life under adverse situations [1]. During postnatal development, the central nervous system (CNS) is highly sensitive to the effects of drugs, stressors and environment [1–3].

While GABA is the main inhibitory transmitter in the adult brain, GABAergic transmission is excitatory during early postnatal development. This different action of GABA results from a reversed chloride concentration gradient with higher intracellular chloride concentration in immature neurons [4–6]. The GABA driving force is strongly depolarizing during the first postnatal week [5, 7–9]. GABA in the adult and the developing brain promotes cell migration, synaptogenesis and regulates processing of the information provided by the internal and the external environment [10, 11]. Sequential formation of GABAergic synapses is thought to be crucial for constructing the stereotypic neural networks during brain development.

L-Serine (L-Ser) acts as an essential neurotrophic factor promoting the axonal growth and the survival of neurons [12, 13]. This small neutral amino acid is synthesized from the glycolytic intermediate 3-phosphoglycerate [14, 15].

Additionally, L-Ser serves as a precursor for the synthesis of neuroactive amino acids such as glycine and D-Serine (D-Ser) [12, 13, 16, 17].

The tight control of the extracellular levels of GABA and L-Ser is crucial for the right function and development of the central synapses and neural circuits. The extracellular concentrations of the both amino acids are largely regulated by transporter proteins expressed in the plasma membrane of both neurons and glial cells. GABA uptake highly selective Na^+-dependent manner [10, 18, 19], while L-Ser, low selectively which can be either Na^+-dependent or Na^+-independent [20, 21]. At least four high affinity GABA transporters (GAT), named GAT-1, GAT-2, GAT-3 and BGT-1 are expressed in mammalian CNS [10]. In neonatal cortex, the only GAT abundantly expressed is GAT-3 [22, 23] and GABA uptake is inhibited by β-alanine [24] suggesting that the extracellular GABA levels at birth are modulated mainly by GAT-3 mediated transport. Uptake of L-Ser occurs through several transport systems, including Na^+-dependent transporters such as neutral amino acid transporter system ASC and system A, and Na^+-independent transporters system L and system asc [20, 21]. System ASC transporters ASCT1 and ASCT2 have been identified and cloned from humans and mice [25–27].

It was demonstrated that in the adult brain PKC regulates the activity and cell surface expression of GABA transporters (GATs) [22, 28–31]. The PKC family of serine-threonine kinases consists of at least 12 different isoforms [32]. Most PKC isoforms are stimulated by the second messenger diacylglycerol and are found in brain as well as in peripheral tissues [33–35]. However, the γ isoform is specific for the CNS [29, 34, 35]. On the contrary, the regulation of L-Serine transport has been poorly investigated.

In the present work we have examined, using synaptosomal fractions prepared from neonatal cerebral cortex if the effect of postnatal acute cold stress on the transporters of GABA and L-Ser could be correlated with change in PKC activity. All parameters measured for [^{3}H]L-Ser uptake were compared to the corresponding parameters obtained for the accumulation of [^{3}H]-GABA. High affinity transport system would be beneficial for L-Ser to act as a neurotrophic factor in rhe CNS. To analyze the presence of such transporter with high affinity, we used [^{3}H]L-Ser at low micromolar concentration in this study [20, 43].

Since PKC has been described as important enzyme in mediating cellular transduction mechanisms and in the regulating neuronal plasticity, the out coming findings of our work could be useful to explain how PKC activity would be intracellular events that would be involved in the regulation of transporters during development.

Experimental Procedures

Animals

Male Wistar rats were studied at postnatal days 5, 7, 13, 21 (PD5, PD7, PD13, PD21, respectively). They were housed under constant temperature and a 12-hour light–dark cycle. They were kept in an acclimatized animal room (21–23°C) with ad libitum access to dry food and tap water. Stress application usually started at 10 a.m. They were divided in two groups: control and stressed groups. Control rats moved to a separated cage without their mother. Stressed rats were exposed to 4°C during 1 h without their mother. All animal procedures were performed in accordance to our institutional guidelines after obtaining the permission of the Laboratory Animal Committee and with the U.S. National Institute of Health Guide for the Care and Use of Laboratory Animals (NHI publication N° 80-23/96). All efforts were made to minimize suffering of animals and to reduce the number of animals used.

Preparation of Tissue Samples

At the end of the stress period, animals were immediately killed by decapitation. The brains were removed from the cranial cavity; the cerebral cortex was dissected on a Petri dish at 0°C, according to Glowinski and Iversen [36] and homogenized with a glass-PTFE homogenizer in 15 volumes of 0.32 M sucrose. The homogenates were centrifuged at 800g for 10 min, the pellet was discarded and the supernatant was centrifuged at 20.000g for 20 min. The pellet (P2 = crude synaptosomal fraction) was suspended with a glass-PTFE homogenizer in fresh 0.32 M sucrose and again centrifuged at 20.000g for 20 min. The procedure was repeated three times; the resulting pellet was re-suspended and the suspension was used in uptake experiments within 5 h after preparation [20].

GABA and L-Serine Transporters In Vitro

GABA and L-Ser transporters we studied using the uptake of [^{3}H]-GABA or [^{3}H]L-Ser by fresh synaptosomes fractions of rat cerebral cortex. Both preincubation and incubation were carried out at 30°C to limit the metabolism of [^{3}H]-GABA or [^{3}H]L-Ser and the consequent loss of the ^{3}H-label in the form of [^{3}H]-H_2O leaking out of the tissue [37]. The technique provides accurate and reproducible data and can be employed to measure kinetic parameters such as K_m and V_{max}, ionic requirements of the transporters.

Uptake experiments were carried out using fresh synaptosomes fractions originating from 20 mg of tissue (wet

weight) per 1 ml of incubation medium. This consisted of 125 mM NaCl, 3.5 mM KCl, 1.5 mM $CaCl_2$, 1.2 mM $MgSO_4$, 1.25 mM KH_2PO_4, 25 mM $NaHCO_3$, 10 mM HEPES and 10 mM D-glucose, pH adjusted to 7.4. The tissue was first preincubated for 5 min at 30°C, followed by addition of pure 10 nM radiolabeled substrate ([^{3}H]-GABA or [^{3}H]L-Ser) and subsequent incubation for 1–40 min (in time course studies) or for 3 min (in kinetics studies). Each sample was exposed to an unequal concentration of GABA and L-Ser; 5 concentrations from 0.1 to 1,000 μM for GABA and 5 concentrations from 25 to 200 μM for L-Ser were used to determine either maximal velocity or affinity values for two transporters. The incubation was terminated by vacuum-filtration through Whatman glass fiber-filters (type D) and three rapid washes with isotonic saline solution (at 2–4°C). Parallel experiments were always performed without any incubation as 0 time to obtain radioactivity not specifically taken up into brain preparations for all radiolabeled substrates used. Protein content was estimated by the technique of Lowry et al. [38] using bovine serum albumin as standard.

In order to study the influence of PKC in regulating uptake activity of GABA and L-Ser, the effects of in vitro pretreated the homogenates with PKC inhibitor staurosporine (SP) (10^{-9} M) were analyzed. SP was added to the re-suspended pellet after first centrifugation (800*g* for 10 min).

PKC Activity

The soluble (cytosolic) and particulate (membrane) fractions were obtained as previously described by Genaro and Bosca [39]. PKC enzyme was purified by filtration through a DE 52 column (3.5 × 0.5 cm). The enzyme was eluted in a buffer containing 120 mM NaCl, 10 mM *β*-mercaptoethanol, 0.5 mM EGTA and 10 mM HEPES (pH 7.4).

PKC activity was assayed on both cytosolic and membrane preparations by measuring the incorporation of ^{32}P from [ATP-γ-^{32}P] into histone$_1$ (H_1). Incubations were performed for 30 min at 30°C in a final volume of 85 μl. Final concentrations of the assay were 25 μM ATP (0.4 μCi), 10 mM Mg_2 acetate, 5 mM *β*-mercaptoethanol, 50 μg H_1, 20 mM HEPES (pH 7.4), and unless otherwise indicated, 0.2 mM $CaCl_2$ and 10 mg/ml of phosphatidylserine vesicles. The incorporation of ^{32}P into H_1 was linear for at least 30 min. The reaction was stopped by the addition of 2 ml of ice-cold 5% trichloroacetic acid, 10 mM H_3PO_4. The radioactivity retained on GF/C glass-fiber filters after filtration was determined by counting the filters in 2 ml of scintillation fluid. PKC activity was determined after subtracting the incorporation of ^{32}P into H_1 in the absence of calcium and phospholipids.

Corticosterone Assay

Corticosterone plasma levels were determined in separated groups of animals (three to six neonates per experimental group) on postnatal day 5, 7, 13 and 21 by collecting trunk blood samples after decapitation. The experiments were always performed between 11:00 and 11:30 a.m. so as to avoid the influence of circadian rhythms.

Plasma was separated by centrifugation and stored at −70°C until high-perfomance liquid chromatography assay (HPLC) was performed according to Retana-Márquez et al. [40].

Statistical Analysis

Statistical analysis and line fitting was performed by GraphPad Prism (San Diego, CA, USA). The same software was used to prepare the Figs. 1, 2, 3, 4. Data were expressed as means ± SEM from four to six independent experiments, each performed with three animals per group (n). The effect of the cold stress was tested for significance using a single-factor analysis of variance (ANOVA). The post-hoc analyses to test the significance between individual means were performed according to Tukey [41]. A *P*-value below 0.05 was considered as significant.

Sources of Materials

[^{3}H]-GABA (specific activity: 28.7 Ci/mmol) and [^{3}H]L-serine (specific activity: 23 Ci/mmol) were from Perkin Elmer NEN Life Science Inc. (Boston, MA, USA). [^{32}P]-γ ATP (specific activity: 3,000 Ci/mmol) was also provided by Perkin Elmer. Dowex AG 50WX-8 was purchased from Bio-Rad (Hercules, CA, USA) and DE-52 from Whatman and Co (Maidstone, England). Other chemicals were purchased from Sigma Chemical Co. St. Louis, MO, USA.

Results

Stress Markers

As a validation of our stress model, we examined the contribution of this form of stress to the development of gastric ulceration as described by Landeira-Fernández [42]. Control group did not present any gastric ulceration. By contrast, we found that acute cold stress induced clear erosions in the gastric mucosa. Ulcerations were typically spherical or oblong, and bleeding (data not shown). The gastric ulcerations were observed after the rats were killed and their stomachs removed. Gastric mucosal injury induced by body can be enhanced when combined with cold stress session. This study had the purpose to examine

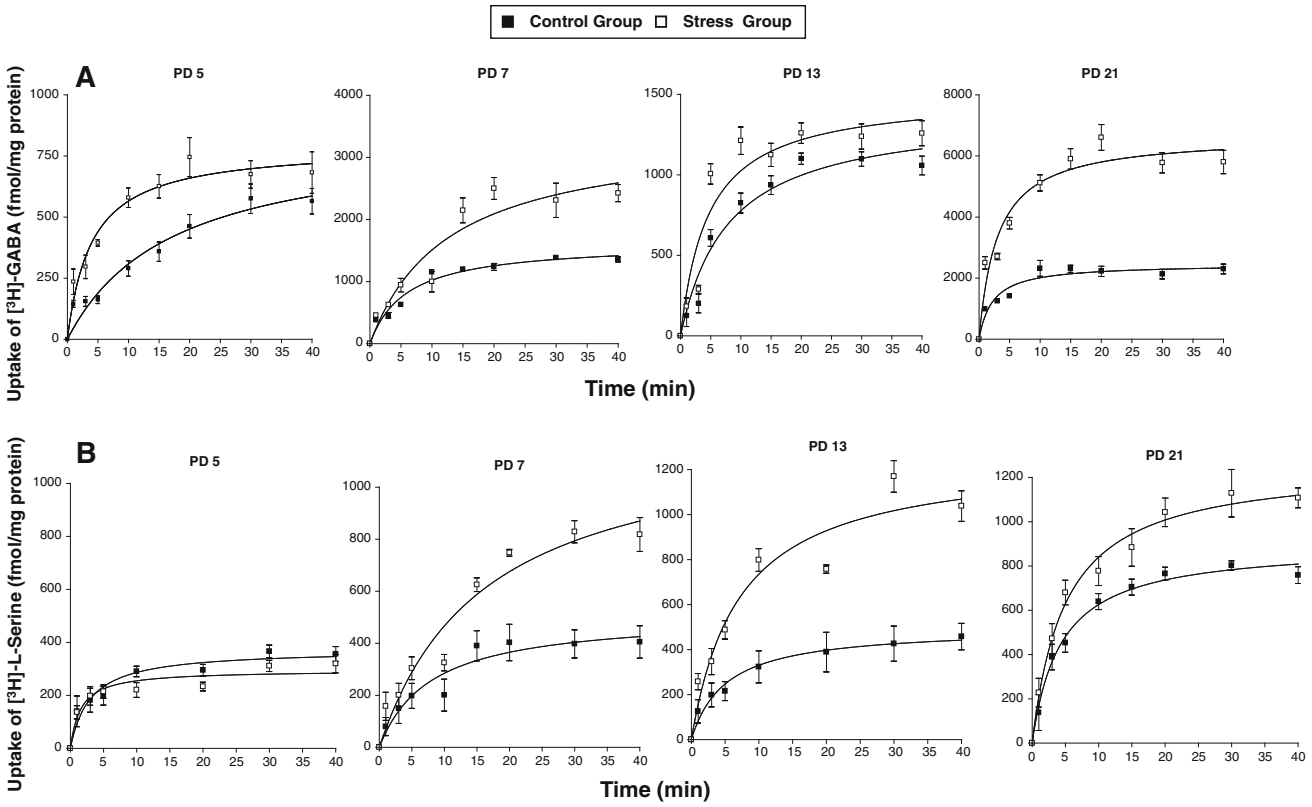

Fig. 1 **a** Time course of [^{3}H]-GABA uptake in unstressed and acutely cold stressed rats. Synaptosomes were incubated with 10 nM [^{3}H]-GABA over 40 min in the presence of 125 mM NaCl at 30°C. Values are the mean ± SEM in 6–7 separate experiments done in triplicate, while SEM was within the symbols in the figure. **b** Time course of [^{3}H]L-Ser uptake in unstressed and acutely cold stressed rats. Synaptosomes were incubated with 10 nM [^{3}H] L-Ser over 40 min in the presence of 125 mM NaCl at 30°C. Values are the mean ± SEM in 6–7 separate experiments done in triplicate, while SEM was within the *symbols* in the figure

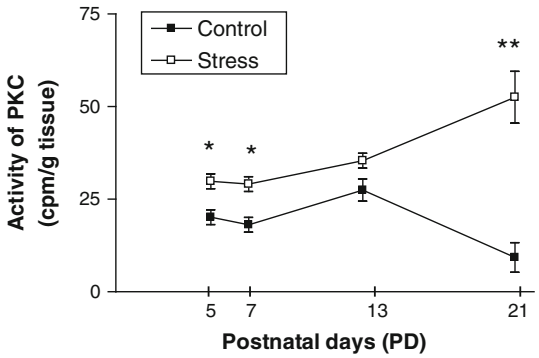

Fig. 2 : PKC activity in the cerebral cortex of control and stresses animals. Animals were exposed to acute cold stress and cerebral cortex was removed and homogenized. PKC activity was analyzed at PD5, PD7, PD13 and PD21. Values shown are the means ± SEM of 4–6 experiments, each one performed with three animals per group. *$P < 0.05$; *$P < 0.01$ with respect to control group

the contribution of each of this form of stress on the development of gastric ulceration and regulation of body temperature. Three independent groups of neonates were exposed for: (a) 1 h to cold stress alone; (b) maternal separation and acute cold stress; (c) maternal separation alone. Control animals were not exposed to any form of stress. Results indicated that neonates separated of their mother alone were not sufficient to induce gastric ulceration or changes in body temperature. On the other hand, cold-stress exposure in conjunction with maternal separation induced the same amount of stomach erosions and hypothermia. Therefore, it appears that maternal separation does not play an important role on gastric ulceration induced by the cold-stress procedure. These findings suggest that hypothermia resulting from cold exposure as a deleterious effect on gastric ulceration. It is concluded that acute cold stress is a useful procedure for the study of the underlying mechanisms involved in stress induced ulceration. These results indicated that neonates of all ages studied were stressed by exposure to 4°C for 1 h.

Corticosterone Plasma Levels

Corticosterone plasma concentrations (mean ± SEM) at 11:00–11:30 a.m. were, respectively at PD5 in controls groups 2.2 ± 0.4 μg/dl and stress groups 4.4 ± 0.4 μg/dl

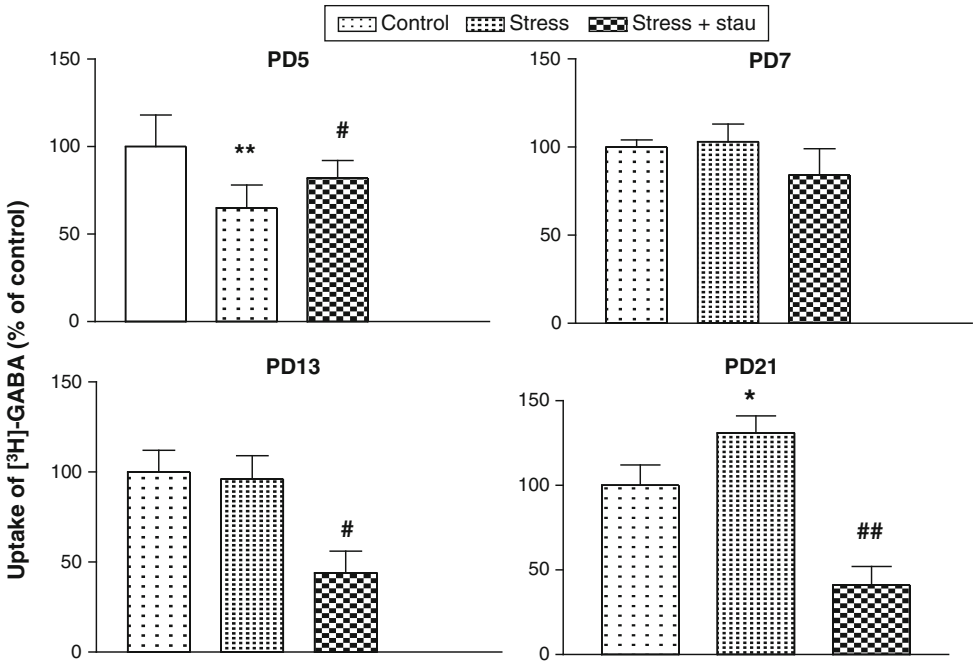

Fig. 3 Effect of PKC inhibitor (starurosporine) on GABA uptake in cerebral cortex of control and acutely stressed rats. Values are the mean ± SEM in 6–7 separate experiments done in triplicate. * $P < 0.05$, ** $P < 0.01$ compared to controls; # $P < 0.05$, ## $P < 0.01$ compared to stressed group non-staurosporine treatment rats

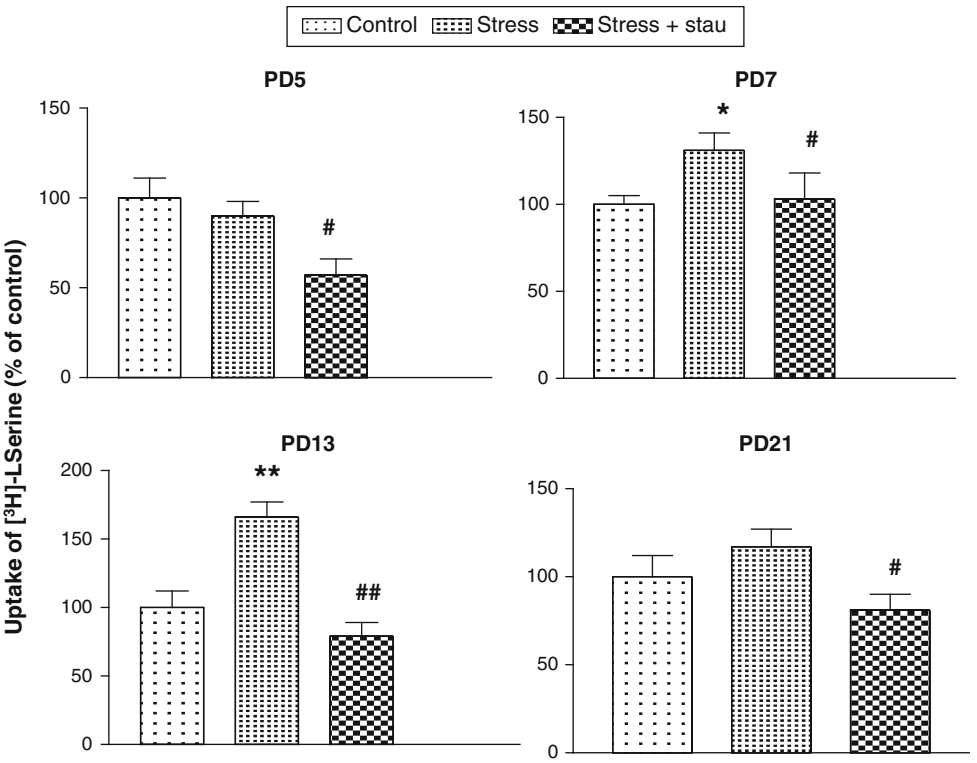

Fig. 4 Effect of PKC inhibitor (starurosporine) on L-Ser uptake in cerebral cortex of control and acutely stressed rats. Values are the mean ± SEM in 6–7 separate experiments done in triplicate. * $P < 0.05$, ** $P < 0.01$ compared to controls; # $P < 0.05$, ## $P < 0.01$ compared to stressed group non-staurosporine treatment rats

($P < 0.05$); at PD7 controls groups 2.0 ± 0.2 μg/dl and stress groups 3.5 ± 0.3 μg/dl ($P < 0.05$); PD13 controls groups 2.2 ± 0.3 μg/dl and stress groups 3.6 ± 0. 8 μg/dl ($P < 0.05$); PD21 controls groups 10.3 ± 2.9 μg/dl and acute stress group: 38.4 ± 5.2 μg/dl ($P < 0.01$).

Effect of Acute Cold Stress on GABA and L-Ser Transporters During Postnatal Development

The developmental time course of changes in uptake of GABA and L-Ser on the cerebral cortex from both cold

unstressed and acutely cold stressed rats was studied at PD5, PD7, PD13 and PD21. In a previous study, we determined that the optimal condition for GABA and L-Ser uptake was a medium rich in Na^+ at 30°C for all postnatal stages mentioned above including the adult age [43].

After the stress session, we noted different effects induced by cold stress on the uptake of GABA in the conditions described above. The profile for GABA uptake following acute cold stress was: PD5, PD21 and PD7 increase whereas PD13 no change (Fig. 1a).

Uptake of 10 nM [^{3}H]L-Ser followed a time course was little similar to that displayed by the uptake of [^{3}H]-GABA under the same experimental conditions, and remained linear from time 0 min to about 3 min at all ages considering for the study. The profile for L-Ser uptake following stress session was: PD5 no change while PD7, PD13, PD 21 increase (Fig. 1b).

Kinetics of [^{3}H]-GABA and [^{3}H]L-Ser

The transporters for L-Ser and GABA are influenced in different way by acute cold stress during development. Vmax of [^{3}H]-GABA in stressed animals showed an increased pattern in all of age studied compared with control group whereas the K_m values increased at almost ages except at PD5 (Table 1). This might be due the period of rapid synaptogenesis (between 12 and 21 days). The K_m values for all age groups tested after cold stress was applied in the 1.3 ± 0.1 to 2.7 ± 1.2 μM range compared with control values were in the 2.4 ± 0.2 to 2.3 ± 0.3 μM range. At PD13 we obtained the highest values of maximum velocity while at PD7 we can observe a low affinity site of GABA transporter (Table 1).

When we studied the uptake of [^{3}H]-L-Ser, we observed a same pattern for GABA, V_{max}, increased in all age from 173.9 ± 36.8 fmol/mg protein/min to PD21 from $150 \pm .24$ fmol/mg protein/min with which we would indicate an enhance in number of transporter. In addition, K_m values increased in almost stage of development studied from PD5 to PD13 [45, 48], there before this meaning a decreased affinity site of transporters (Table 1).

Cerebral Cortex PKC Activity Evaluation in Stressed Rats

At the light of previous studies we known that PKCs are key modulators of amino acid uptake [29, 31], so we evaluated if acute cold stress could produce any alteration in the activity of this enzyme. Acute postnatal stress induced an initial increment of cerebral cortex total PKC activity that reached the maximal value at PD21, susceptible with activation of the enzyme after postnatal cold stress (Fig. 2). Protein kinase modulators appear to implicate PKC-dependent regulation of GABA and L-Ser.

In order to analyze the PKC participation on GABA and L-Ser uptake, pretreatment with SP was performed. The data showed that under this condition, GABA uptake was restored near to the control values at PD5 (68%) (# $P < 0.05$ compared to stresssed group non-staurosporine treatment rats). At PD7, acute cold stress and SP had no effect on GABA uptake. Nevertheless, at PD13 and PD21, SP decreased the uptake level of GABA by 44 and 40%, correspondingly (# $P < 0.05$; ## $P < 0.01$ compared to stressed group non-staurosporine treatment rats). Administration in vitro of SP not only countered this effect but also decreased the uptake below the control values (# $P < 0.05$) (Fig. 3).

L-Ser uptake was returned near to the control values at PD7, PD13 and PD21 (106, 80, 81% respectively; # $P < 0.05$; ## $P < 0.01$ compared to stressed group non-staurosporine treatment rats). At PD5 we showed a partial restitution by 57% compared to stress group without SP (Fig. 4). These results proposed that in this developmental stage postnatal stress induced changes in GABA and L-Ser uptake rates could be associated with PKC activation.

To asses if the inhibition of PKC could contribute to the restoration effect, we tested the effectiveness of SP of suppressing the activity of PKC in the corresponding

Table 1 Kinetic parameters for GABA and L-serine uptake during postnatal acute cold stress

	GABA				L-Serine			
	Control group		Stress group		Control group		Stress group	
Age (days)	V_{max} (fmol/mg protein/min)	K_m (μM)	V_{max} (fmol/mg protein/min)	K_m (μM)	V_{max} (fmol/mg protein/min)	K_m (μM)	V_{max} (fmol/mg protein/min)	K_m (μM)
PD5	36.7 ± 11.2	2.4 ± 0.2	77.5 ± 8.1**	1.3 ± 0.1**	90.4 ± 12.3	21.6 ± 11.2	173.9 ± 36.8**	41.5 ± 22**
PD7	110.4 ± 20	2.4 ± 0.4	380.9 ± 15.5**	9.8 ± 0.1**	51.9 ± 9.0	5.9 ± 0.1	157 ± 38.2**	34.9 ± 3.0**
PD13	161.2 ± 55	2.5 ± 0.3	745.3 ± 20.1**	2.7 ± 0.8*	160.8 ± 3.4	5.7 ± 3.3	391 ± 16.1**	40.1 ± 6.1**
PD21	180.2 ± 50	2.3 ± 0.3	461.3 ± 6.1**	2.7 ± 1.2*	101.9 ± 40	27.8 ± 9.3	150 ± 24.0**	0.9 ± 0.1**

All assays were done in the presence of 125 mM Na^+ at 30°C (see "Experimental Procedures"). Values are means ± SEM in 6–7 experiments done in triplicate. *$P < 0.05$; **$P < 0.01$ compared with the respectively control group (Tukey t after one way ANOVA yielded $P < 0.05$)

homogenates. We observed a significant inhibition of PKC activity by SP (data not shown).

In many cases, in acute regulation has been show that activity and cell surface expression of many neurotransmitter transporters undergo a dynamic trafficking between the cell surface and intracellular compartments [29]. These regulatory mechanisms allow rapid changes in membrane fraction of transporters and such adaptive processes could have a variety of physiological and pathological consequences. Second messenger systems involved in this rapid regulation include protein kinases and phosphatases. These signaling systems share the common characteristic of altering maximal transport velocity and/or cell surface expression, consistent with regulation of transporter trafficking. Arachidonic acid, reactive oxygen species, and nitric oxide also alter transporter function. In addition to post-translational modifications, cytoskeleton interactions and transporter oligomerization regulate transporter activity and trafficking. Furthermore, promoter regions involved in transporter transcriptional regulation have begun to be identified.

Discussion

Postnatal acute cold stress induces a series of early events in biological systems that occur instantly.

The effect of acute cold postnatal stress can provoke alterations in the uptake of GABA and L-Ser at different ages studied. However, we observed that the ionic dependence of these transport systems did not change due to the acute stress. Substitution of Na^+ for K^+ or Choline ion reduced [^{3}H] L-Ser uptake to about half of that observed at 125 mM of Na^+ at all postnatal ages studied (data not shown). In all cases, these transport proteins showed their maximum activity in presence of Na^+ at 30°C [21, 43]. Considering that GABA promotes the neuronal growth, migration, differentiation and survival prior to synapse formation [10, 44–46] and that L-Ser is a key neurotrophic factor [13, 16], it is clear that the maintenance of adequate concentrations of these molecules is crucial for the normal CNS development and function [10, 13, 21, 43]. Kinetic analysis of [^{3}H]L-Ser uptake has indicated the presence of at least two components-low and high affinity. Trophic activity has been detected at micromolar concentrations L-Ser of indicating that it is a transport system capable of operating at very low concentrations (characterized by low K_m or high affinity) that should be of primary interest [20].

Recent research from our laboratory, we have begun to study the consequences of repeated maternal separation and exposed to cold stress on brain development in order to determinate if the effects on GABAergic function are age-specific and time dependent. Rats' pups were separated from their mother plus cold exposure for 1 h at different stages of development during 20 days. These animals were allowed to a 30 days recovery period until adulthood. At the end of this period, the rats were killed by decapitation. We showed that the time course obtained for GABA uptake in repeated stressed animals decreased either on frontal cortex at PD5 and PD7 or hippocampus at PD13. These data would suggest the idea that modification affected by stressors during early life not disappear after a few hours or days, in which case they are relevant to study development′s dysfunction on the brain [47].

Additionally, it could be concluded that the susceptibility to cold stress on the systems of GABA and L-Ser considered fluctuate across the postnatal ontogeny. For instance, while at PD13 a profound increase were observed for GABA uptake and L-Ser uptake between control and stressed animals. The comparatively similar uptake per mg protein at PD7 and PD13 should be connected to the switching from excitatory to inhibitory transmission in postnatal ontogeny on GABA system. The increment we observed in GABA uptake after stress session from PD5 to PD21 might be due the major period of forebrain synaptogenesis [4, 5, 8]. During development, when inhibitory and excitatory synapses are formed and refined, homeostatic mechanisms act to adjust inhibitory input in order to maintain neural activity within a normal range. As the brain matures, synaptogenesis slows and a relatively stable level of inhibition is achieved [48].

Postnatal stress influenced the transport constant (K_m) for GABA and L-Ser in stressed animals. The K_m values for the affinity of GABA uptake were increased at PD7 to PD21 while for L-Ser K_m values were enhanced from PD5 to PD13 ages compared with the control group.

One the most fascinating result of this study was the similar effect of postnatal stress development on the affinity of synaptosomal GABA and L-Ser transporters for their substrates. The reduced affinity of high affinity GABA transporters during the period of rapid synaptogenesis would increase the transporters' ability to buffer the extracellular GABA concentration. Under most physiological conditions, GABA uptake transporters rapidly remove synaptically released GABA from the extracellular space. By the way, we noted that opposite pattern described for the affinity of L-Ser uptake transporter at PD21. We found that affinity incremented in PD5, PD7 and PD13. On the contrary, V_{max} values for L-Ser and GABA increased at all ages considering in this studied. This finding agrees with emerging evidence that indicates that the non-essential amino acid L-Ser plays a significant role in neuronal development and function [43]. These results put forward that susceptibility to acute cold postnatal stress and the regulation mechanism involved in transporters function are regulated during postnatal development. Even though

Western blotting will be principal method, the [^{3}H]GABA or [^{3}H]L-Ser uptake studies in vitro we used as a colateral evidence of changes in the expression of GABA and L-Ser transporters.

Considering the duration of the stress session (1 h), it is obvious that the modifications observed in L-Ser uptake have no trophic actions. Since L-Ser is converted into D-Ser (a NMDA receptor co-agonist) by the enzyme serine racemase [17, 49] a link with glutamatergic neurotransmission could be suggested.

PKC has been described as important enzyme in mediating cellular transduction mechanism and in the regulation of neuronal plasticity. In the present work, we have found that postnatal stress induces changes in cerebral cortex PKC activity that in turn could participate in the long-term alterations observed in adult rats.

It is well documented that PKC plays a key role in regulating the activity of GATs [22, 29, 30]. The data presented here demonstrate that under conditions of acute cold stress there is a significant translocation of PKC from cytosol to the membrane, at 5, 7, 13 and 21 days postnatal. This suggests that PKC would be significantly involved in the initial processing of the stressful information at this age.

In order to study the role of PKC and its interdependency with the uptake changes, the activity of two amino acids were studied. We described that PKC inhibitor, SP, led to total restitution of control values the uptake of GABA at PD5 and PD7. At PD13 and PD21 another mechanism should be proposed for the regulation of GABA uptake. Guillet et al. [50] demonstrated that other protein kinases, such as protein kinase A and phosphatidylinositol-3-kinase, regulate the GABA transporter in cell cultures. These enzymes could be proposed as alternative regulators of the aminoacidergic response to acute stress. On the contrary, this inhibitor of PKC, returns to the control values the uptake levels of L-Ser at 7, 13 and 21 days of age. This also applies to changes in PKC activity as related to modifications in GABA and L-Ser uptake. We can speculate that PKC might be involucres in the response to cold stress in the aminoacidergic system, possibly by altering the extracellular levels of GABA and L-Ser. The specific isoform(s) involved in the initial response to stress should be determined in future experiments. With the studies in this work we can not see if there is a specific activation of one or more of brain isoforms of PKC, this will require further studies.

Taken together these observations, it is possible to conclude that GABA and L-Ser transporter (and therefore processing of stressful information) and PKC activation in the neonatal cerebral cortex is affected by a single exposure to cold stress. This enhances the perception of the cerebral cortex as a structure that participates significantly in the elaboration of the stress response [51].

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