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European Journal of Pharmacology 503 (2004) 35–42

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Locus coeruleus activity in perinatally protein-deprived rats: effects of fluoxetine administration

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Received 18 December 2003; received in revised form 26 August 2004; accepted 1 September 2004
Available online 12 October 2004

Abstract

We have previously described an increased locus coeruleus activity in perinatally protein-deprived rats. Since locus coeruleus dysfunction has been involved in different types of anxiety disorders and considering the modulating action of serotonergic transmission on locus coeruleus activity, we assessed the effect of fluoxetine, a selective serotonin reuptake inhibitor (SSRI), on locus coeruleus activity as measured by the firing rate and the number of spontaneously active cells/track. Repeated fluoxetine administration reduced locus coeruleus activity in both control and protein-deprived rats, although the reduction was greater in protein-deprived rats. Dose–response curves for the inhibitory effect of clonidine showed subsensitivity of α_2-adrenergic autoreceptors in protein-deprived rats, a phenomenon reversed by fluoxetine treatment. Dose–response curves for the inhibitory effect of 2,5-dimethoxy-4-iodoamphetamine (DOI) were similar in both groups of rats. Following fluoxetine administration, subsensitivity to this effect developed in control but not in protein-deprived rats. Extracellular noradrenaline level in the prefrontal cortex, as measured by microdialysis procedure, was higher in protein-deprived rats compared to controls, and this difference was reduced after fluoxetine administration. A challenge with yohimbine increased the extracellular noradrenaline level in control but not in protein-deprived rats, suggesting subsensitivity of α_2-adrenergic autoreceptors in early protein malnourished animals. These results stress the complexity of plastic changes induced by early protein malnutrition and sustain the hypothesis that perinatally protein-deprived rats may represent a useful animal model for screening antipanic agents.

Keywords: Perinatal protein malnutrition; Locus coeruleus activity; Fluoxetine; Prefrontal cortex noradrenaline; Panic disorder

1. Introduction

Undernutrition at perinatal age, during the period of rapid brain growth, produces long-lasting or permanent alterations in different neuronal systems. This may account for changes in reactivity to pharmacological treatments described by different authors (see Almeida et al., 1996), as well as for behavioral and cognitive deficits reported in children with a history of severe undernutrition (Stoch and Smythe, 1976). As regards the catecholaminergic system, it has been noted that adult rats that had been undernourished at a perinatal age showed a significant increase in brain noradrenaline and dopamine turnover, together with an increased brain tyrosine hydroxylase activity and normal levels of brain noradrenaline (Marichich et al., 1979). A significant reduction of brain α- and β-adrenoceptor binding was also described in perinatally malnourished rats (Keller et al., 1982). In addition, subsensitivity to the effects of noradrenaline at the cardiovascular level and in other sympathetically innervated organs was found (Del Basso et al., 1983; Keller et al., 1984). In agreement with these data, we recently reported that the spontaneous activity of locus coeruleus noradrenergic neurons from adult rats submitted to a similar protein deprivation schedule, measured by the firing rate and the number of spontaneously active cells per track, was significantly higher than in controls (Nasif et al., 2001).

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doi:10.1016/j.ejphar.2004.09.007

The locus coeruleus is the major nucleus of the noradrenergic system in the brain. The neuronal pathway from this nucleus, which projects to different areas of the brain, is thought to mediate the physiological and behavioral responses to pain and fear, anxiety-like animal behavior. In locus coeruleus neurons, α_2-adrenergic autoreceptors, receptors for endogenous opiates, gamma aminobutyric acid (GABA), glycine, serotonin (5-HT) and adrenaline exert an inhibitory modulatory action on this nucleus, while acetylcholine, substance P and glutamate exert an excitatory modulatory effect (Charney et al., 1984). A modulatory effect on locus coeruleus activity by neuropeptide Y and galanin, which coexist with noradrenaline in noradrenergic locus coeruleus neurons, has also been postulated (Holets, 1988; Goddard and Charney, 1997). However, an increasing amount of clinical and experimental evidence supports the hypothesis that functional abnormalities of the locus coeruleus may be involved in the pathophysiological state of anxiety, principally in panic disorders (Charney et al., 1984; Goddard and Charney, 1997). Panic disorder is a type of anxiety that has been associated with hyperreactivity of noradrenergic functions, while the supramolecular $GABA_A$ receptor complex may be involved in the etiology of the generalized anxiety disorder (Hayes and Kirstoff Kirkwood, 1989). In this regard, a thorough study of patients suffering from panic attacks and agoraphobia demonstrated that the administration of yohimbine, an α_2-adrenoceptor antagonist that increases noradrenergic activity, induces higher plasma levels of the noradrenaline metabolite 3-methoxy-4-hydroxyphenylglycol (MHPG), as well as increased blood pressure and pulse rate, plus subjective and somatic symptoms when compared with its effects in healthy subjects (Charney et al., 1984). These neuronal abnormalities have a close resemblance to the changes in the noradrenergic system described in adult rats as a consequence of perinatal malnutrition, thus it is possible to hypothesize that they may represent the neurobiological basis of the pathophysiology of panic disorders. In agreement with this supposition, desipramine, a tricyclic antidepressant used in the treatment of panic disorders, was able to normalize the increased locus coeruleus activity in protein-deprived rats (Nasif et al., 2001). Furthermore, antipanic agents, such as propranolol, desipramine and phenelzine, that did not exhibit any anxiolytic effect in normal rats when assessed in conventional tasks (File and Johnston, 1987; Johnston and File, 1988) showed a significant anticonflict effect in perinatally protein-deprived rats when assessed in the elevated plus-maze test (Laino et al., 1993).

Considering that serotonergic transmission exerts a modulatory effect on locus coeruleus activity, we evaluated the effect of fluoxetine, a selective serotonin reuptake inhibitor (SSRI) widely used in the treatment of panic disorders, on locus coeruleus activity in perinatally protein-deprived rats compared with controls. With this purpose, the spontaneous activity of locus coeruleus noradrenergic neurons was assessed using single unit recording by measuring the firing rate and the number of spontaneously active cells per track. In addition, dose–response curves for the inhibitory effect of the α_2-adrenoceptor agonist clonidine and the $5\text{-}HT_2$ receptor agonist 2,5-dimethoxy-4-iodoamphetamine (DOI) on locus coeruleus activity were performed in control and protein-deprived animals. Furthermore, in order to evaluate the effect of fluoxetine administration on the functional state of the central noradrenergic system, extracellular noradrenaline levels in the prefrontal cortex were measured in both groups under basal conditions and after fluoxetine administration, by means of the microdialysis procedure.

2. Materials and methods

2.1. Animals

A protein deprivation schedule, as previously described, was used (Borghese et al., 1998). Briefly, pregnant female Wistar rats from our own colony were divided into two groups after 14 days of pregnancy, then housed in polyethylene cages and fed on isocaloric diets containing 24% and 8% casein for control and protein-deprived rats, respectively. Diet composition is indicated below. Litters from both groups were culled to eight pups. After weaning at 30 days, pups were fed on the same diet consumed by their dams until 40 days of age (end of deprivation period). Thereafter, both groups were fed on balanced standard chow (Cargill, Buenos Aires, Argentina) for at least 30 days prior to assays (period of nutritional recovery). Animals were maintained at 22 ± 2 °C under a 12-h light–dark cycle (lights on at 07:00 AM) with food and water ad libitum. In all experimental groups, subjects came from different litters in order to avoid sibling replication. Male control and protein-deprived rats, weighing 270–320 and 250–300 g, respectively, were used in the present experiments. Operators followed the European Community guidelines for the use of experimental animals. Experimental protocols were approved by the Ethics Committee of our School.

Diet composition (g %). For control rats: casein 24.00; sucrose 44.45; corn starch 9.90; hydrogenated vegetable oil 15.00; corn oil 1.00; vitamin mix 1.25; minerals 4.00; DL-methionine 0.40. For protein-deprived rats: casein 8.00; sucrose 56.95; corn starch 13.40; hydrogenated vegetable oil 15.00; corn oil 1.00; vitamin mix 1.25; minerals 4.00; DL-methionine 0.40.

2.2. Drugs and treatments

Fluoxetine (kindly provided by Gador Laboratories, Buenos Aires, Argentina) was injected daily (5 mg/kg, i.p.) for 5 days. Electrophysiological experiments were

performed the day following the last injection. Microdialysis for noradrenaline determination was performed 2 days after the last injection. Controls from both groups (control and protein-deprived) received saline in the same volume as fluoxetine in treated rats (1 ml/kg, i.p.). Clonidine (Catapresan, Boehringer-Ingelheim Laboratories, Argentina), was diluted in saline (5 μg/ml) for i.v. administration. DOI (2,5-dimethoxy-4-iodoamphetamine) from Sigma-Aldrich (St. Louis, USA) was dissolved in saline (50 μg/ml) for i.v. administration. Yohimbine (kindly provided by Sidus Laboratories, Buenos Aires, Argentina) was dissolved in distilled water (2.5 mg/ml) for i.p. administration.

2.3. Electrophysiological procedures

Rats were anesthetized with chloral hydrate (400 mg/kg, i.p.). Additional anesthetic doses were administered through a dorsal tail vein when needed. Techniques used for extracellular single-unit recording have been described in detail elsewhere (Ramírez and Wang, 1986). Briefly, rats were mounted onto a stereotaxic frame, their skulls were exposed, a hole was drilled and the recording electrode was lowered into the locus coeruleus (1.1–1.3 mm posterior to lambda, 1.1–1.3 mm lateral to midline suture, 5.5–6.5 mm below the dura; atlas of Paxinos and Watson, 1986) by means of a hydraulic microdrive. The number of spontaneously active cells per track (an average five track per animal) and their firing rate were assessed. The firing rate was obtained from the counted cells which displayed a signal/noise ratio of 2:1 or more. Locus coeruleus noradrenergic neurons displayed the following pattern: (a) positive–negative action potentials lasting approximately 2 ms, often with a notch between the initial segment and the somatodendritic spike component; (b) a firing rate of 0.5–3.0 spikes/s; and (c) a burst of firing followed by a quiescent period in response to pinching of the contralateral paw. These properties fulfil electrophysiological criteria for identification of locus coeruleus noradrenergic cells (Cederbaum and Aghajanian, 1977). Electrode potentials were passed through a high-impedance amplifier and displayed on an oscilloscope. Electrical signals were passed through a window discriminator and monitored on an audio amplifier. Integrated histograms generated by the analogical output of the window discriminator were computed and stored for later computer analysis. Dose–response curves for the inhibitory effect of clonidine and DOI on locus coeruleus activity were recorded by injecting successive i.v. doses of these drugs at 3-min intervals and the percentage of spikes suppressed was assayed during this period. Only one cell per rat was tested with clonidine or DOI. At the end of each experiment, the cell location was marked by passing a 25-μA cathodal current through the recording electrode for 15 min and a spot of Fast Green dye was deposited. Rats were then perfused with phosphate-buffered 10% formalin solution. Serial frozen sections, 50-μm-thick, were sliced and the dye spot was microscopically traced.

2.4. Microdialysis procedure

The microdialysis procedure has previously been described in detail (Nasif et al., 2000). Briefly, control or protein-deprived rats were anesthetized with chloral hydrate (400 mg/kg, i.p.) and placed on a Stoetling stereotaxic frame. AN69 HF dialysis fiber (Hospal, Bologna, Italy) was transversally inserted into the prefrontal cortex (coordinates vs. Bregma: A+3.2, V−2.4; atlas of Paxinos and Watson, 1986). Rats were then placed in individual acrylic bowls and left to recover for at least 24 h. The dialysis membranes were perfused with Ringer's solution (NaCl 147 mM; KCl 4 mM; $CaCl_2$ 1.7 mM; $MgCl_2$ 0.8 mM; pH 7.2) at a constant rate of 1.2 μl/min. After an equilibration period, samples of the dialysate were collected every 30 min into vials containing 1 μl $HClO_4$ (0.1 M), to prevent oxidation of noradrenaline. Vials were kept at 4 °C in a refrigerated fraction collector. Immediately after collection, the dialysate was analyzed using high-performance liquid chromatography (HPLC). Control levels were defined as an average of these baseline levels with correction for the dead volume. At the end of the experiments, animals were killed by decapitation, brains were removed and the position of the dialysis probe track was histologically verified.

2.5. Monoamine assay

The amount of noradrenaline in the collected fraction was analyzed on an HPLC system equipped with a reverse-phase column (ultrasphere C18, Beckman) and electrochemical detection. The HPLC system consisted of a BAS LCD-4 electrochemical detector with a glass-carbon electrode and pump (Spectra Series P200). The potential was set at 600 mV (vs. Ag/AgCl, reference electrode). The mobile phase containing 75 mM NaH_2PO_4, 0.5 mM sodium dodecyl sulfate, 20 μM EDTA, 0.04% triethylamine, 15% methanol and 3% acetonitrile (pH 5.7) was filtered and pumped throughout the system at a flow rate of 1.0 ml/min by a Spectra Series P200 pump. Peaks from the HPLC system were displayed, integrated and stored using Peak Simple II Data System (SRI Inst., CA, USA). Quantification was made by comparing the peak heights of the samples to those of a standard curve.

2.6. Statistical analysis

Statistical analysis for firing rate and cells/track was performed using two-way analysis of variance (ANOVA), and subsequent post hoc comparisons were carried out with the Newman–Keuls test. The ID_{50} values obtained from the inhibition curves for clonidine and DOI and the basal levels of noradrenaline were analyzed by one-way ANOVA. The microdialysis data for the effect of yohimbine were analyzed by two-way ANOVA for repeated measurements with diet

and treatment as between-subjects factor and time as within-subjects factor. Post hoc comparisons between groups were performed with a Newman–Keuls test. A P value of 0.05 or less was considered significant.

3. Results

3.1. Effects of fluoxetine administration on locus coeruleus activity in control and protein-deprived rats

Experiments carried out to evaluate locus coeruleus activity in control and protein-deprived rats demonstrate that perinatal protein malnutrition induces changes in the electrical activity of noradrenergic neurons, since protein-deprived rats showed a significant increase in the number of spontaneously active cells/track [$F(1,14)=41.38$; $P<0.001$] and in the firing rate [$F(1,53)=106.04$; $P<0.001$], compared with controls.

Fig. 1(A,B) shows the activity of locus coeruleus noradrenergic neurons obtained from control and protein-deprived rats after repeated saline or fluoxetine administration. Two-way ANOVA showed a significant interaction of diet and drug treatment, for both the number of spontaneously active cells/track [$F(1,29)=29.46$; $P<0.001$] and the firing rate [$F(1,132)=59.33$; $P<0.001$]. Post-hoc comparison showed that after repeated fluoxetine administration, a significant reduction in both the firing rate and the number of spontaneously active cells was observed in control and protein-deprived rats. In control rats the

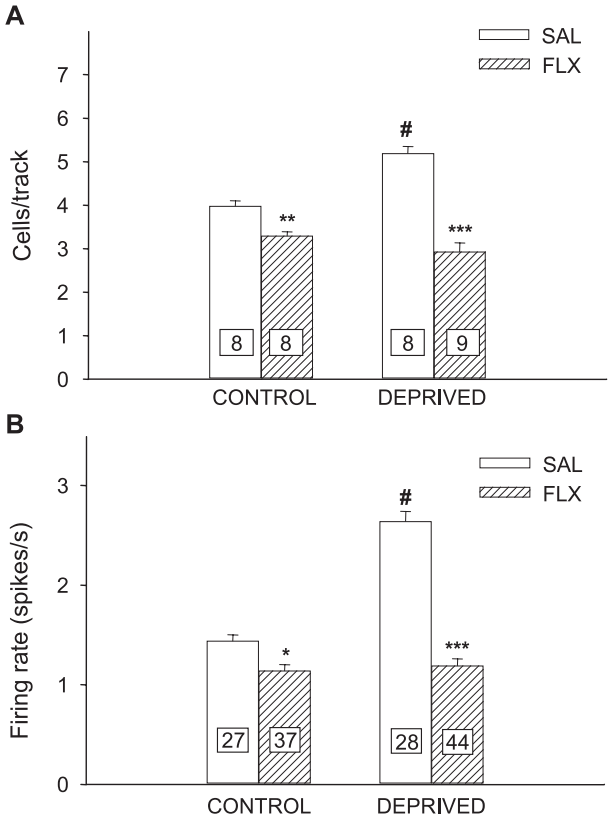

Fig. 1. Panel A shows means±S.E.M. of the number of locus coeruleus spontaneously active cells/track. The number of rats analyzed is displayed in each box. Panel B shows means±S.E.M. of the firing rate of locus coeruleus noradrenergic neurons. The number of neurons recorded is displayed in each box. The effect of 5 days of fluoxetine administration (5 mg/kg, i.p.) on the same variables is also shown. #$P<0.0001$ vs. Control-Saline; ***$P<0.0001$ vs. Protein-deprived-Saline; **$P<0.005$ vs. Control-Saline; *$P<0.05$ vs. Control-Saline (Newman–Keuls test).

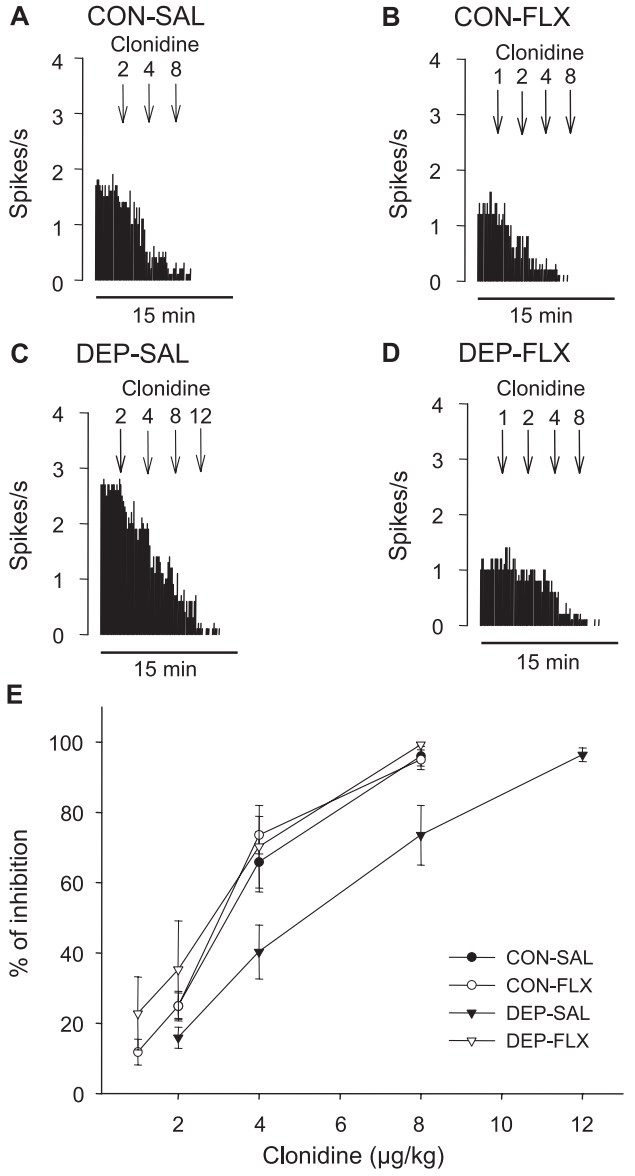

Fig. 2. Integrated firing rate histograms showing the effects of cumulative i.v. doses of the α_2-adrenoceptor agonist clonidine on representative locus coeruleus neurons from control and protein-deprived rats treated for 5 days with saline (A and C) or 5 mg/kg, i.p., of fluoxetine (B and D). Arrows indicate time of injection. The injected cumulative doses of clonidine (μg/kg) are indicated above the arrows. Panel E shows dose–response curves for the inhibitory effect of clonidine on the firing rate of locus coeruleus noradrenergic neurons in control and protein-deprived rats treated for 5 days with saline or fluoxetine (5 mg/kg, i.p.); $n=7$ for all groups.

reduction in the firing rate was only 20.8%, compared to a reduction of 54.9% in protein-deprived rats. Also, the number of spontaneously active cells fell by 17.3% in control rats, compared to 43.6% in protein-deprived rats.

3.2. Effects of fluoxetine administration on clonidine inhibition of locus coeruleus activity in control and deprived rats

In order to evaluate whether the increased locus coeruleus noradrenergic activity detected in protein-deprived rats is the consequence of changes in the sensitivity of α_2-autoreceptors, the ability of administered clonidine to suppress locus coeruleus noradrenergic activity was studied by means of dose–response curves. Fig. 2(A–D) shows representative firing rate histograms illustrating the effects of intravenous administration of the α_2-adrenoceptor agonist clonidine in control and protein-deprived rats after treatment for 5 days with saline or fluoxetine. As can be seen in Fig. 2(E), perinatal protein malnutrition diminished the sensitivity of the somatodendritically located α_2-adrenoceptors: ID_{50}=3.48±0.50 μg/kg for control rats and ID_{50}=5.43±0.56 μg/kg for protein-deprived animals [$F(1,12)$=7.83; P<0.05]. Fluoxetine administration (5 mg/kg, i.p., daily for 5 days) did not modify the dose–response curve for the clonidine inhibitory effect in control rats (ID_{50}=3.29±0.42 μg/kg), but was able to reverse the diminished sensitivity observed under basal conditions in protein-deprived rats (ID_{50}=2.97±0.94 μg/kg).

3.3. Effects of fluoxetine administration on DOI inhibition of locus coeruleus activity in control and protein-deprived rats

The inhibitory effect of the 5-HT_2 receptor agonist DOI on locus coeruleus noradrenergic activity was assessed by means of dose–response curves for its inhibitory effect on the firing rate (Fig. 3). In saline-treated control rats, the DOI ID_{50} was 21.38±2.20 μg/kg. Fluoxetine administration significantly diminished the sensitivity to this inhibitory effect since the ID_{50} increased to 40.51±7.05 μg/kg [difference between saline and fluoxetine-treated control rats: $F(1,10)$=8.05; P<0.05]. Protein-deprived rats did not exhibit changes in the sensitivity of the 5-HT_2 receptors, since the ID_{50} for DOI in saline-treated protein-deprived rats (28.70±6.12 μg/kg) was not significantly different from that in saline control rats (21.38±2.20 μg/kg). The ID_{50} for DOI obtained after fluoxetine administration in protein-deprived rats (ID_{50} 23.08±2.82 μg/kg) did not significantly differ from that of saline-treated animals, indicating that in protein-deprived rats fluoxetine treatment was unable to diminish the sensitivity of 5-HT_2 receptors.

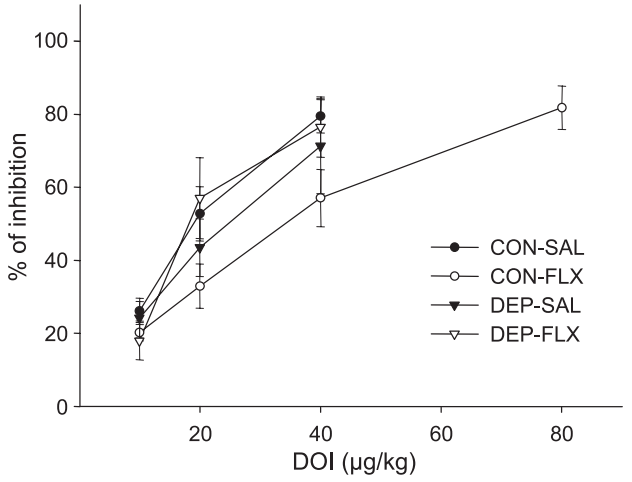

Fig. 3. Dose–response curves for the inhibitory effect of the 5-HT_2 agonist DOI on the firing rate of locus coeruleus noradrenergic neurons in control and protein-deprived rats treated for 5 days with saline or fluoxetine (5 mg/kg, i.p.); n=6 for all groups.

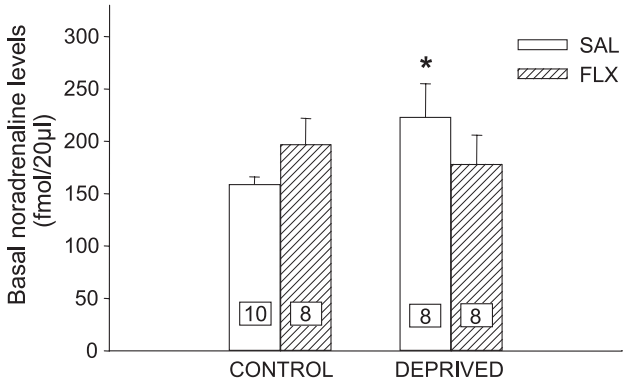

Fig. 4. Means±S.E.M. of the basal extracellular noradrenaline levels in the prefrontal cortex of control and protein-deprived rats treated for 5 days with saline or fluoxetine (5 mg/kg, i.p.). The number of rats analyzed is displayed in each box. *P<0.05 vs. Control-Saline (one-way ANOVA).

3.4. Effects of fluoxetine administration on the extracellular noradrenaline level in the prefrontal cortex of control and protein-deprived rats

Extracellular noradrenaline concentrations in the prefrontal cortex under basal conditions, i.e., after saline injections, were higher in protein-deprived rats (223±32 fmol/20 μl) than in controls (159±7 fmol/20 μl) [$F(1,16)$=5.48, P<0.05]. The difference in the extracellular noradrenaline level between control and protein-deprived rats disappeared after fluoxetine administration (Fig. 4). A challenge with yohimbine, an α_2-adrenoceptor antagonist, induced a significant increase in the extracellular noradrenaline level in saline-treated control rats, an effect that was blunted by repeated fluoxetine administration. In saline-treated protein-deprived rats, yohimbine showed a tendency to increase noradrenaline levels (not statistically significant). In protein-deprived rats previously treated with fluoxetine, the effect of yohimbine on the extracellular noradrenaline level did not differ

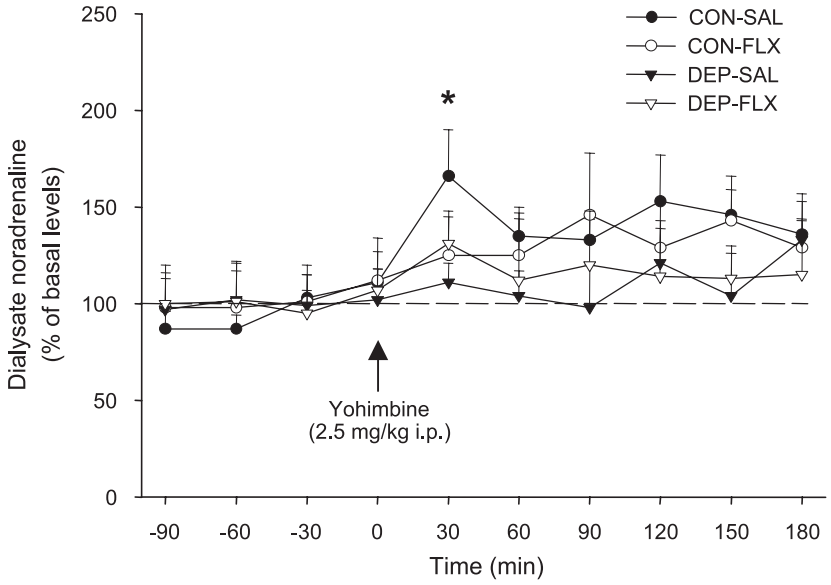

Fig. 5. Effect of a challenge with yohimbine (2.5 mg/kg, i.p.) on the extracellular noradrenaline levels in the prefrontal cortex of control and protein-deprived rats treated for 5 days with saline or fluoxetine (5 mg/kg, i.p.); n=5 for all groups. *P<0.05 vs. basal levels (Newman–Keuls test).

from that of the saline-treated protein-deprived group (Fig. 5).

4. Discussion

In agreement with a previous report from our laboratory (Nasif et al., 2001), adult rats that had been protein-deprived at a perinatal age showed an increased activity of locus coeruleus neurons, compared with controls, when they were evaluated by measuring the number of spontaneously active cells/track and its firing rate. This result appears reasonable, considering the alterations of the noradrenergic system induced by perinatal protein malnutrition previously described, such as increased brain noradrenaline turnover rate, increased tyrosine hydroxylase activity, normal levels of endogenous noradrenaline (Marichich et al., 1979) and reduced density of brain α- and β-adrenoceptors (Keller et al., 1982). These events suggest a higher neurotransmitter release as a consequence of perinatal malnutrition. In support of this interpretation, determination of extracellular noradrenaline levels in the prefrontal cortex by microdialysis under basal conditions showed a significant increase in noradrenaline levels in protein-deprived rats compared with controls, an effect that disappeared after fluoxetine administration. Repeated administration of fluoxetine, a SSRI with therapeutic efficacy in the treatment of anxiety and depressive disorders, including panic disorders, was able to reduce the firing rate and the number of spontaneously active cells/track in both control and protein-deprived rats. In this regard, a reduction in locus coeruleus activity has been reported following repeated SSRI administration in normal rats (Szabo et al., 1999, 2000). The decrease in neuronal activity following fluoxetine administration was more evident in protein-deprived rats than in controls.

Dose–response curves for the inhibitory effect of clonidine, performed to evaluate the functional state of α_2-adrenergic autoreceptors, confirmed a previous report that described a diminished sensitivity to the inhibition induced by clonidine of locus coeruleus activity in protein-deprived animals. Such an effect was reversed by fluoxetine administration to values comparable to those of controls, whereas this treatment did not have any effect in control animals. Additional experimental evidence that demonstrates a diminished sensitivity of α_2-adrenoceptors comes from experiments carried out to assess extracellular noradrenaline levels in the prefrontal cortex, which receives noradrenergic terminals from the locus coeruleus, before and after fluoxetine administration. A challenge with yohimbine induced in control rats a significant increase in extracellular noradrenaline levels, an effect that was blunted after fluoxetine treatment. Although yohimbine is not a selective α_2-adrenoceptor antagonist, the effect observed on the extracellular noradrenaline levels may be attributed to α_2-adrenoceptor blockade. Evidence for this comes from different reports. Thus, idazoxan, a selective α_2-adrenoceptor antagonist, increases the extracellular noradrenaline level in the prefrontal cortex (Cuadra and Giacobini, 1995; Thomas et al., 1998), and such an effect disappeared after chronic sertraline administration (Thomas et al., 1998). However, considering that yohimbine, in addition to its antagonist action at α_2-adrenoceptors, exerts antagonist actions at 5-HT_{1B}, 5-HT_{1D} and D_2 sites and a partial agonist action at 5-HT_{1A} sites (Millan et al., 2000), the possibility of yohimbine affecting extracellular noradrenaline levels through these receptors cannot be ruled out. In our experiments in protein-deprived rats, extracellular

noradrenaline levels were not modified after a challenge with yohimbine, neither under basal conditions nor after repeated fluoxetine treatment.

Considering that serotonergic transmission modulates locus coeruleus activity, it seemed interesting to evaluate the functional state of 5-HT_2 receptors in protein-deprived rats compared with controls. Dose–response curves for the locus coeruleus inhibitory effect of DOI, a 5-HT_2 agonist, revealed no differences between control and protein-deprived rats under basal conditions. After fluoxetine administration, a diminished sensitivity to the DOI inhibitory effect developed in control rats, an effect that has been attributed to the progressive desensitization of 5-HT_{2A} receptors (Szabo et al., 2000). In contrast, such an effect was absent in protein-deprived rats. It has been described that both 5-HT_{2A} and 5-HT_{2C} receptors exert modulatory inhibitory actions on noradrenergic transmission and that DOI exhibits equally potent agonist effects on 5-HT_{2A} and 5-HT_{2C} receptors (Gobert et al., 2000). Thus, it should be kept in mind that the effect observed after DOI administration in our experiments may be mediated by interactions at both subtypes of 5-HT_2 receptors (5-HT_{2A} and 5-HT_{2C} receptors). The observation that the diminished sensitivity to the DOI inhibitory effect on locus coeruleus activity was not found in protein-deprived rats indicates a deficiency in the adaptive neuronal process induced by adequate stimuli and/or treatments, which is frequently described in animals exposed to malnutrition early in life (Levitsky and Strupp, 1995).

At present, no conclusive evidence exists about the localization of the 5-HT_{2A} receptors involved in the modulation of locus coeruleus activity (Barnes and Sharp, 1999). In this regard, it has been reported that direct application of serotonergic compounds on the locus coeruleus does not alter its neuronal activity and that section of the forebrain, caudal to frontal cortex (rich in 5-HT_2 receptors), does not modify the effect of quipazine and ketanserin on locus coeruleus activity. However, their effects were reduced after bilateral or contralateral lesions of the prepositus hypoglossi, a major afferent to the locus coeruleus, indicating that this nucleus is responsible for the 5-HT_2 receptor modulation of locus coeruleus activity (Gorea et al., 1991). Furthermore, additional experimental evidence suggests that the prepositus hypoglossi provides an inhibitory synaptic input via a gabaergic interneuron (Ennis and Aston-Jones, 1989). With respect to this, it is interesting to note that perinatally malnourished rats are subsensitive to the anticonflict effect of diazepam (Brioni and Orsingher, 1988; Almeida et al., 1991).

The timing of neurogenesis and the number of neurons generated in the locus coeruleus of rats submitted to a prenatal protein deprivation schedule have been studied. Although protein-deprived animals in these experiments consumed a diet containing 6% casein, lower than that used in our study, no significant differences between protein-deprived and control animals in the total number of neurons generated or in the timing of their generation were detected (King et al., 1999). Accordingly, it is possible to conclude that the alterations induced by perinatal protein malnutrition on locus coeruleus function are a consequence of "functional alterations" rather than anatomical changes induced by prenatal protein deprivation. The observation that the increased locus coeruleus activity detected in protein-deprived rats could be normalized by pharmacological manipulations sustains this conclusion.

In summary, our results extend previous studies of plastic changes in the central nervous system induced by early undernutrition, showing that these changes may be reversed by pharmacological treatments, and demonstrate the complexity of the alterations induced by nutritional deprivation and their relevance in adulthood. Furthermore, the similarities in the noradrenergic alterations induced by perinatal undernutrition and those described in patients suffering from panic disorders, together with the fact that antidepressive drugs with therapeutic efficacy in the treatment of panic disorder showed a selective anticonflict effect in protein-deprived rats (Laino et al., 1993), supports the hypothesis that perinatally protein malnourished rats may represent a useful animal model for screening antipanic agents.

Acknowledgments

This work was supported by grants from Ministerio de Salud de la Nación, Secretaría de Ciencia y Tecnología (SECyT-UNC), Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) and Agencia Nacional de Promoción Científica y Tecnológica (FONCyT).

We are grateful to Miss Elsa R. Pereyra for her technical assistance.

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