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ORIGINAL PAPER

Postnatal Nitric Oxide Inhibition Modifies Neurotensin Effect on ATPase Activity

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Abstract We have previously showed that peptide neurotensin inhibits neuronal Na^+, K^+-ATPase activity, an effect which involves high affinity neurotensin receptor. Nitric oxide (NO) acts as a neurotransmitter or as a neuromodulator when it is synthesized by neuronal nitric oxide synthase. Neurotensin effect on Na^+, K^+-ATPase activity was evaluated in cortical synaptosomal membranes isolated from rats injected at 3, 4 and 5 postnatal days with saline (control) or N (ω)-nitro-L-arginine methyl esther (L-NAME), a nitric oxide synthase inhibitor. Assays were carried out at two stages: juvenile (35 days) and adult (56 days) ages**.** In an open field task, results recorded in juvenile rats markedly differed from those obtained in adult rats. The presence of neurotensin at 3.5×10^{-8}–3.5×10^{-6} M concentration decreased 16–34% Na^+, K^+-ATPase activity in membranes purified from control animals. At variance, the peptide failed to alter this enzyme activity in membranes obtained after L-NAME treatment. After administration of L-NAME, [^{3}H]-ouabain binding to membranes isolated from adult male rats decreased 64% in the presence of 1.0×10^{-6} M neurotensin, a peptide concentration which only slightly decreased binding to membranes isolated from juvenile rats. It is postulated that early postnatal NO dysfunction may exert a permanent change in neurotensin system that influence later Na^+, K^+-ATPase response to neurotensin.

Keywords Neurotensin · Na^+, K^+-ATPase activity · Nitric oxide synthase · Nitric oxide · [^{3}H]-ouabain binding

Introduction

Neurotensin, a 13-amino acid peptide first isolated from bovine hypothalamus [1], is widely distributed in brain and peripheral tissues of several mammalian species including man [2, 3]. In the central nervous system (CNS), neurotensin acts as a neurotransmitter or a neuromodulator mainly in the dopaminergic transmission pathways [4].

Neurotensin plays a role in nociception, hypothermia, muscle relaxation and control of anterior pituitary hormone secretion of nigrostriatal and mesolimbic routes [5]. This peptide binds to two membrane receptors, corresponding to NTS1 and NTS2 receptors [6, 7]. Neurotensin physiological effects seem to involve an interaction with high affinity sites (NTS1 receptor) which can be blocked by SR 48692, a selective non-peptide receptor antagonist [8].

Nitric oxide (NO) acts as an intercellular messenger. NO is synthesized from L-arginine via Ca^{2+} activation of nitric oxide synthase (NOS), an enzyme that requires NADPH as coenzyme and tetrahydrobiopterin as cofactor [9]. Three

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distinct forms of NOS have been identified: an inducible form (iNOS) which is present in glia; a neuronal form (nNOS) which is widespread in distribution throughout the CNS; and an endothelial enzyme (eNOS). NO may exert physiologic roles in vision, feeding behaviour, nociception, olfaction, learning and memory [9].

NO influences the maturation of neurons and synaptogenesis during neuronal development [10–13]. Therefore, a disturbance in NO release (as determined by improper migration of NADPH-d neurons) could interfere with the maturation of cortical (and other brain areas) neurons as well as with their functional connections. This potentially could lead to a dysfunctional CNS exhibiting itself later in life as schizophrenia [14]. Therefore, postnatal administration to rats with L-NAME, a nitric oxide synthase inhibitor, is an experimental model to induce schizophrenia symptoms [14].

We have previously studied the effect of neurotensin on Na^+, K^+-ATPase activity, to observe that the peptide inhibits the enzyme present in cortical synaptosomal membranes. This effect is entirely prevented by the presence of NTS1 receptor antagonist SR 48692 or pretreatment with dopaminergic D2 antagonist haloperidol [15, 16].

The purpose of the present study was to investigate potential regulation of ATPase activity by neurotensin in rats which had been early administered with NOS inhibitor L-NAME.

Experimental Procedure

Animals and Drugs

Male and female Sprague–Dawley rats were used in this study. Studies described were conducted in accordance with the Guide for Care and Use of Laboratory Animals provided by the National Institutes of Health, USA. All animals were housed in a light-controlled and temperature-controlled facility, with free access to food and water. Six or eight pups from three litters were treated. On postnatal days 3, 4, and 5 rat pups were injected s.c. with either vehicle (saline solution) or 10 mg/kg L-NAME dissolved in saline solution. When indicated, a dose of 100 mg/kg L-NAME dissolved in saline solution was administered. The pups were returned to their mothers and no further manipulations were made until the postnatal day 35 (juvenile rats) and 56 (adult rats) [14]. At that time, the animals were decapitated; cerebral cortices were harvested, homogenized and subjected to differential and sucrose gradient centrifugation to obtain synaptosomal membrane fractions [17]. For each L-NAME treated pooled pups, controls from saline injected pups were processed in parallel throughout.

Reagents were analytical grade. Ouabain, disodium ATP (grade I, prepared by phosphorylation of adenosine), haloperidol, L-NAME and neurotensin acetate were from Sigma Chemical Co., St. Louis, MO, USA. Peptide solutions in redistilled water were freshly prepared for each experiment. Dimethylsulfoxide (DMSO), used to dissolve haloperidol, was from J. T. Baker Chemical Co., Phillipsburg, NJ, USA. BCS Biodegradable counting scintillant was from Amersham Biosciences, UK, and [^{3}H]ouabain (specific radioactivity of 20.5 Ci/mmol) was from New England Nuclear, Du Pont, Boston, MA, USA.

Open Field Task

The testing chamber was a Formica container (45 × 45 × 40 cm), with its floor divided into nine squares (15 × 15 cm each). Each rat was placed in a corner facing the wall and allowed to explore the chamber for a period of 5 min. The number of squares crossed with the four paws from one square to another was indicative of locomotor activity. The rearing responses (standing on the hind feet) were also counted in the sessions. Between runs, the floor of the open field was cleaned with a 5% ethanol solution and dried with a cloth. An observer blind to the treatments recorded the measurements.

Preparation of Synaptosomal Membrane Fractions

Synaptosomal membranes from cerebral cortex were isolated by differential and sucrose gradient centrifugation as previously described in this laboratory [17]. In each experiment, lots of four or eight rats were processed. Half of the animals received L-NAME and half received the vehicle. Cerebral cortices were pooled and homogenized at 10% (w/v) in cold 0.32 M sucrose (neutralized to pH 7.0 with 0.2 M Tris base) using a Teflon glass homogenizer of the Potter–Elvehjem type. The homogenate was subjected to differential centrifugation to separate nuclear and mitochondrial fractions. The crude mitochondrial pellet was resuspended in redistilled water (pH 7.0 with 0.2 M Tris base) for the osmotic shock. A pellet containing mitochondria, synaptosomal membranes and myelin was separated by centrifugation at 20,000×g for 30 min, then resuspended in 0.32 M sucrose, layered on top of a gradient containing 0.8, 0.9, 1.0, and 1.2 M sucrose and centrifuged at 50,000×g for 2 h in a SW 28 rotor of an L8 Beckman ultracentrifuge. The fraction at the level of 1.0 M sucrose was collected, diluted with 0.16 M sucrose and spun down at 100,000g for 30 min to obtain the synaptosomal membrane fraction. Pellets were stored at −70° C and, prior to enzyme assay, resuspended by brief homogenization in redistilled water and used for 3 weeks without appreciable change in enzyme activities.

Enzyme Assays

ATPase activity was measured as described by Albers et al. [18]. Total ATPase activity was assayed in a medium containing 100 mM NaCl, 20 mM KCl, 3 mM $MgCl_2$, 0.20 M Tris–HCl buffer (pH 7.4) and 4 mM ATP. Mg^{2+}-ATPase activity was determined in a similar medium with no added Na^+ and K^+ and containing 1 mM ouabain. The difference between activities was taken to correspond to Na^+, K^+-ATPase. Before performing ATPase assay, samples of synaptosomal membrane fractions were preincubated with 0.20 M Tris–HCl buffer (pH 7.4) or 3.5×10^{-6} M neurotensin solution at 37°C for 10 min; incubation volume (µl) was 35:5 for buffer and membranes. When indicated, 1×10^{-6} M haloperidol dissolved in DMSO10% (v/v) was included during preincubation. Aliquots of preincubated fractions (3 µl) were distributed in two series of microtubes containing the respective medium (40 µl) for the assay of total- and Mg^{2+}-ATPase activities, and incubated at 37°C for 30 min. The reaction was stopped with 30% (w/v) trichloroacetic acid solution. ATPase activity was monitored by colorimetric determination of orthophosphate released [19]. In all enzyme assays, tubes containing enzyme preparations and assay media maintained at 0°C throughout the incubation period were used as blanks.

Isolation of Crude Cerebral Cortex Membranes

Cerebral cortices of three rats (1.8 g tissue) were pooled and homogenized for two 1-min periods in 0.32 M sucrose (neutralized to pH 7.0 with Tris base) and centrifuged at $900 \times g$ for 10 min; supernatants were spun down at 100,000*g* for 30 min in a L8-Beckman ultracentrifuge. Resultant pellets were resuspended in 0.16 M sucrose and distributed in 14 tubes for further spinning as above, and after discarding the supernatant, stored at −70°C. Prior to use, pellets were resuspended in redistilled water to achieve 10 mg protein per ml concentration and processed for [^{3}H]-ouabain binding assay.

[^{3}H]-Ouabain Binding

[^{3}H]-ouabain binding was carried out by a filtration assay. Binding was performed in a medium (0.5 ml final volume) consisting of 3 mM $MgCl_2$, 2 mM H_3PO_4, 0.25 mM sucrose, 0.25 mM EDTA, 30 mM imidazol-HCl buffer (pH 7.4) [20], 250 μg cerebral cortex membrane protein, 45 nM [^{3}H]-ouabain, and 1.0×10^{-6} M neurotensin dissolved in redistilled water to achieve the concentrations indicated. After incubation at 37°C for 60 min, samples were filtered under vacuum on GF/B filters positioned in a Millipore multifilter and rinsed twice with 2 ml of ice-cold 30 mM imidazol-HCl buffer, pH 7.4. Filters were transferred to vials and after addition of 10 ml OptiPhase "Hisafe" 3; radioactivity was quantified in a Beckman Coulter-LS 6500 scintillation counter with 64% efficiency. Specific binding was calculated by substracting binding found in the presence of 100 µM unlabelled ouabain. Non-specific binding accounted for less than 10% of total membrane-bound radioactivity.

Protein Measurement

Protein content in synaptosomal membrane fractions was determined by the method of Lowry et al. [21], using bovine serum albumin as standard.

Data Analyses

Open field data were presented as mean values (±SD) from N measurements; N indicates the number of animals tested (6–8) from two or three litters which had been processed in different occasions. Statistical significance of difference versus control, between time periods and between doses was determined by Repeated measures ANOVA, followed by Bonferroni Comparison test. Data for Na^+, K^+-ATPase activity were expressed as µmol released inorganic phosphate per mg protein per hour or as per cent activity versus control (mean ± SD). Statistical analyses were performed using one-sample Student's *t* test or by two tailed Student *t* test. A probability level indicative of $P < 0.05$ was considered significant.

Results

The effect of NOS inhibition by L-NAME administration was tested on the behavior of Sprague–Dawley rats in the open field as well as on Na^+, K^+-ATPase response to neurotensin.

Exploratory behaviour of juvenile rats was tested for three 5 min trials after administration of NOS inhibitor L-NAME. Both the number of crosses and rearings recorded in the controls at the second and third periods were roughly 50% of values observed for the first period. At the first period, 10 mg/kg and 100 mg/kg L-NAME dose-dependently enhanced the number of crosses which decreased at the second and third periods. In general, the number of crosses were higher after L-NAME treatment *versus* the control (Fig. 1A). On performing ANOVA statistical analyses between dosis, differences were recorded at the first period for the number of crosses and rearings. At the second period, no significant differences were obtained whereas at the third period differences were recorded only for the number of crosses (Fig. 1A, B). The number or

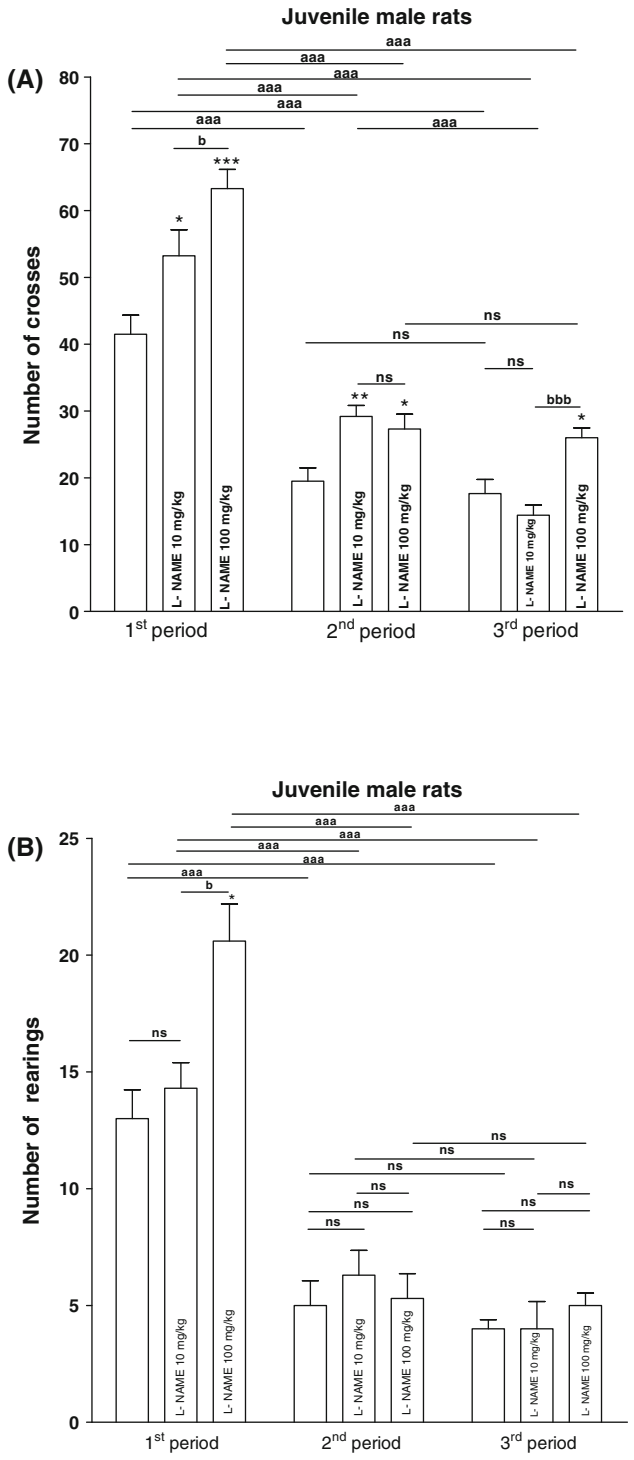

Fig. 1 Open field exploration of juvenile male rats. Effect of postnatal administration of 10 or 100 mg/kg L-NAME on the behavioural profile of juvenile male rats in the open field during 5 min exposures. *Bars* represent mean values (±SD) from 8 rats. **A** Number of crosses; **B** number of rearings. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$ treated versus control, by repeated measures ANOVA followed by Bonferroni comparison test. $^{aaa}P < 0.001$ between time periods, by repeated measures ANOVA followed by Bonferroni comparison test. $^{b}P < 0.05$; $^{bbb}P < 0.001$, between dosis, by repeated measures ANOVA followed by Bonferroni comparison test

rearings were significantly increased only with the dose of 100 mg/kg L-NAME at the first period whereas no changes were recorded at the other two periods or with 10 mg/kg L-NAME dose (Fig. 1B).

The dose of 100 mg/kg L-NAME lead in the adulthood to a reduction of the number of crosses at the first and second periods but an enhancement at the third period (Fig. 2A) In adult rats, the number of rearings remained unaltered or tended to decrease by L-NAME treatment (Fig. 2B).

The activities of ATPases were assayed in cortical synaptosomal membranes isolated from juvenile Sprague–Dawley rats of either sex. Absolute values in μmol inorganic phosphate released per mg protein per hour for Na^+, K^+- and Mg^{2+}-ATPase activities after L-NAME administration failed to differ from those recorded after administration of saline solution (Table 1).

The presence of 3.5×10^{-8}–3.5×10^{-6} M neurotensin inhibited 16–34% and 16–39% Na^+, K^+-ATPase activity in cerebral cortex synaptosomal membranes isolated from control juvenile male (Fig. 3) and juvenile female (Fig. 4) rats, respectively. Such peptide concentration range failed to modify this enzyme activity in membranes isolated from either male or female juvenile rats early administered with 10 mg/kg L-NAME (Figs. 3, 4). Mg^{2+}-ATPase activity remained unaltered in either case (data not shown).

Additional assays carried out in synaptosomal membranes isolated from adult male rats showed 33% enzyme inhibition by 3.5×10^{-6} M neurotensin, an effect entirely prevented by postnatal administration of 10 mg/kg L-NAME (Fig. 5). Here again, Mg^{2+}-ATPase activity remained unaltered (data not shown).

In another set of experiments, assays were carried out in the presence of haloperidol, known to block dopaminergic receptor. In membranes isolated from adult rats which had been injected postnatally with 100 mg/kg L-NAME, Na^+, K^+-ATPase activity remained unaltered in the presence of 3.0×10^{-6} M neurotensin, 3.0×10^{-6} M haloperidol (dissolved in DMSO 10%) or neurotensin plus haloperidol. In control membranes isolated from adult rats which had been injected postnatally with saline solution, 3.0×10^{-6} M neurotensin decreased 24% Na^+, K^+-ATPase activity either in the absence or presence of haloperidol. This antipsychotic did not modify Na^+, K^+-ATPase activity or neurotensin effect on the enzyme (Fig. 6). Additional assays showed that the presence of 10% DMSO failed to modify basal Na^+, K^+-ATPase activity (data not shown).

To further explore neurotensin effect on Na^+, K^+-ATPase, the K^+ site of the enzyme was evaluated by assaying [^{3}H]-ouabain binding to CNS membranes in juvenile and adult rats. Addition of 1.0×10^{-6} M neurotensin decreased 15% [^{3}H]-ouabain binding to CNS membranes from control juvenile rats but failed to alter

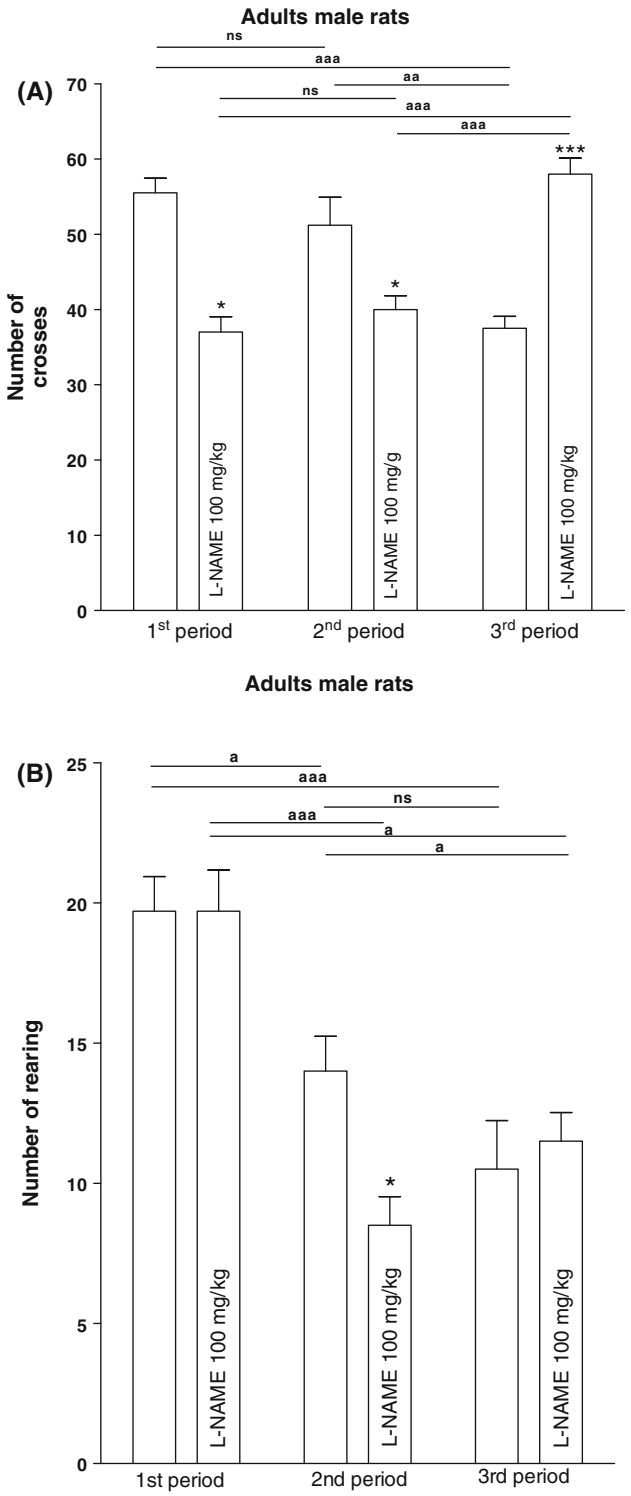

Fig. 2 Open field exploration of adult male rats. Effect of postnatal administration of 100 mg/kg L-NAME on the behavioural profile of adult male rats in the open field during 5 min exposures. *Bars* represent mean values (±SD) from 6 to 8 rats. **A** Number of crosses; **B** number of rearings. $^{*}P < 0.05$; $^{***}P < 0.001$ versus control, by repeated measures ANOVA followed by Bonferroni comparison test. $^{a}P < 0.05$; $^{aa}P < 0.01$; $^{aaa}P < 0.001$ between time periods, by repeated measures ANOVA followed by Bonferroni comparison test. *ns* non-significant difference

binding to membranes isolated from control adult rats. After 10 mg/kg L-NAME administration only a trend was recorded in juvenile rats whereas 64% decrease was obtained in adult rats (Fig. 7).

Discussion

The purpose of this study was to test potential involvement of nitrergic system in the modulation of Na^+, K^+-ATPase by neurotensin. Sprague–Dawley rats were postnatally administered with NOS inhibitor L-NAME. Open field task and ATPase assays were carried out in juvenile and adult rats. Results indicated that L-NAME treatment modified animal behavior as well as Na^+, K^+-ATPase response to neurotensin.

Evidences indicate that inhibition of nNOS by administration of L-NAME during the early postnatal period results in plastic changes that begin to manifest after 35 days [14]. For this reason, herein L-NAME was administered to rats at days 3–5 after birth and assays were carried out at two stages: 35 and 56 days old. Results indicated that adult rats had a different behavior versus juvenile rats after postnatal treatment with L-NAME.

Exploratory behavior is fundamental to the rodent nature and the open field task is currently used to test drugs for neurological diseases, injury, drug abuses or neurotoxicity [22]. Habituation is a simple form of learning which has been used to study behavioral consequences resulting from brain lesions or pharmacological manipulations. Present results showed that the number of crosses in the open field task for control animals is lower in a second and third period of exposure, in agreement with data from the literature [23, 24]. After the treatment with L-NAME a dose dependent increase in the number of crosses and rearing was recorded at the first period in juvenile rats, which may be associated with positive symptoms of the schizophrenia [25]. In adult rats the number of crosses decreased in the first two periods and increased in the third one in comparison to control animals, suggesting a greater latency to recognize a novel environment which may be due to learning disabilities by L-NAME treatment. Neonatal L-NAME treatment to rats leads to a deficit in social interaction when male animals are placed in an environment with other foreign animals, which is lower in the adult than that in the young animals. This behavior has been related to negative symptoms observed in the schizophrenia [26].

Na^+, K^+-ATPase activity is involved in the activity-dependent synaptic plasticity, as well as in memory and learning mechanisms [27]. Na^+, K^+-ATPase inhibition has been related to the impairment in learning and memory

Table 1 Basal Na^+, K^+-ATPase and Mg^{2+}-ATPase activities in cortical synaptosomal membranes isolated from juvenile male and female rats early administered with saline solution (Control) or 10 mg/kg L-NAME

	Na^+, K^+- ATPase activity (μmol Pi. mg $prot^{-1}$ h^{-1})		Mg^{2+}-ATPase activity (μmol Pi. mg $prot^{-1}$ h^{-1})	
	Control	L-NAME	Control	L-NAME
Male rats (n = 4)	25.1 ± 5.8	29.9 ± 9.8	13.9 ± 5.9	13.9 ± 5.2
Female rats (n = 4)	38.1 ± 8.5	39.8 ± 9.0	19.4 ± 3.2	17.4 ± 7.5

Results are mean values (±SD) from three enzyme assays carried out with membranes obtained in at least two different occasions

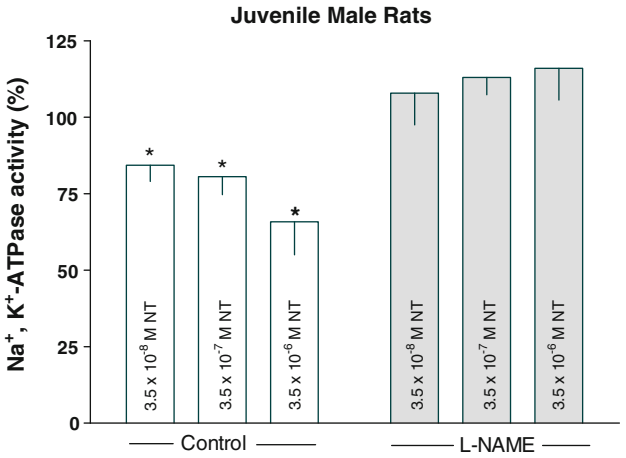

Fig. 3 Neurotensin effect on Na^+, K^+- ATPase actvity in cerebral cortex of juvenile male rats postnatally administered with vehicle (control) or 10 mg/kg Nω-nitro-L-arginine methyl ester (L-NAME). Cortical synaptosomal membranes were preincubated in the absence or presence of 3.5×10^{-8}–3.5×10^{-6} M neurotensin and assayed for ATPases. Results are expressed as percentage enzyme activity taking as 100% values obtained for each experiment in the absence of neurotensin. SD of four experiments performed per triplicate is indicated within the *bars*. $^*P < 0.05$ with respect to the control without neurotensin, by one sample Student *t* test

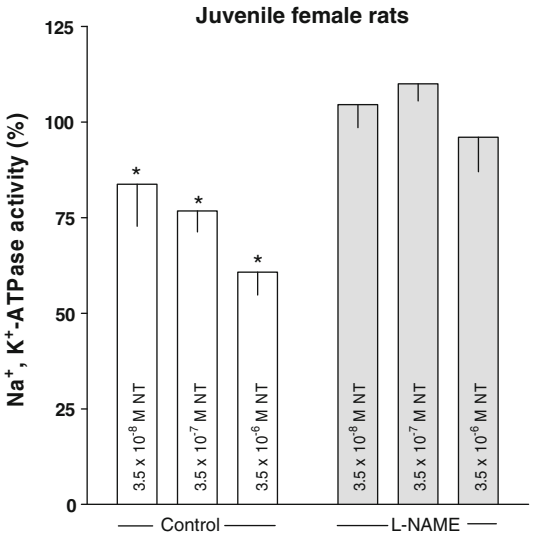

Fig. 4 Neurotensin effect on Na^+, K^+- ATPase actvity in cerebral cortex of juvenile female rats postnatally administered with vehicle or 10 mg/kg L-NAME. Cortical synaptosomal membranes were preincubated in the absence or presence of 3.5×10^{-8}–3.5×10^{-6} M neurotensin and assayed for ATPases. Results are expressed as percentage enzyme activity taking as 100% values obtained for each assay in the absence of neurotensin. SD of four experiments performed per triplicate is indicated within the bars. $^*P < 0.05$ with respect to the control without neurotensin, by one sample Student *t* test

observed after administration of enzyme inhibitor ouabain [28] or in pathophysiological conditions such as hyper- or hypothyroidism [29]. Previous studies carried out in synaptosomal membranes isolated from cerebral cortex of Wistar adult rats showed that neurotensin inhibits Na^+, K^+-ATPase activity whereas it fails to alter Mg^{2+}-ATPase activity [15]. Similar findings were presented herein in synaptosomal membranes isolated from cerebral cortex of Sprague–Dawley rats early administered with saline solution. Postnatal administration of L-NAME prevented further inhibition of synaptosomal membrane Na^+, K^+-ATPase activity by neurotensin.

Sex differences were recorded after L-NAME treatment to neonatal rats. Male rats developed hypersensitivity to amphetamine in adulthood whereas female rats do not. At variance, female rats develop hypersensitivity to phencyclidine at both juvenile and adult ages whereas male animals do not. These results indicate male/female differences inherent to schizophrenia [14]. On the other hand, there are reports in the literature which show sex differences for brain Na^+, K^+-ATPase activity. To illustrate, synaptosomal Na^+, K^+-ATPase activity decreases with age in female rats whereas it remains elevated in males during aging [30]. Rat brain Na^+, K^+-ATPase activity decreases in males whereas it increases in females after treatment with sibutramin, a drug which had been used clinically in weight control, which acts on serotonergic metabolism [31]. For this reason, at the begining of our study we tested basal enzyme activity in juvenile male and female rats after 10 mg/kg L-NAME dose, to observe no sex differences for L-NAME treatment in preventing neurotensin effect on Na^+, K^+-ATPase activity.

Results carried out in membranes isolated from Wistar rats indicated that the presence of haloperidol in the assay fails to modify both basal Na^+, K^+-ATPase activity and neurotensin effect on the enzyme [16]. Present findings showed a similar behavior for membranes isolated from

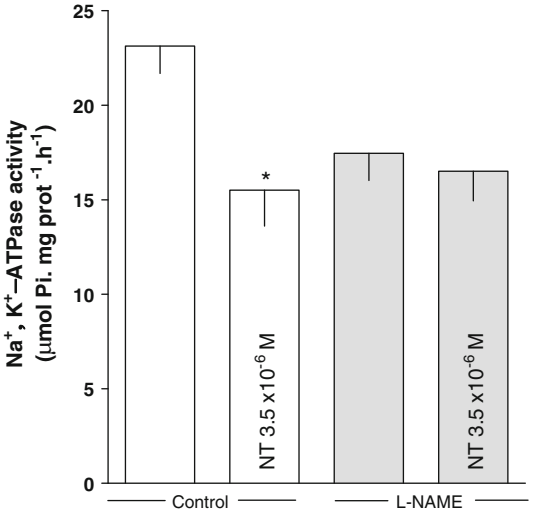

Fig. 5 Neurotensin effect on Na^+, K^+-ATPase actvity in cerebral cortex of adult male rats postnatally administered with vehicle or 10 mg/kg L-NAME. Cortical synaptosomal membranes were preincubated in the absence or presence of 3.5×10^{-6} M neurotensin and assayed for ATPases. Results are expressed as specific activity and are mean values from 3 experiments (±SD) performed per triplicate. $^*P < 0.05$ with respect to the control without neurotensin, by Student *t* test

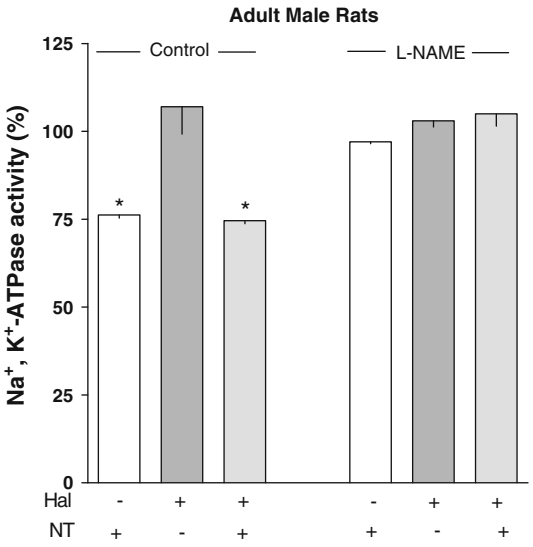

Fig. 6 Na^+, K^+-ATPase activity in cortical synaptosomal membranes in the presence of neurotensin and haloperidol. Membranes from cerebral cortex of adult male rats postnatally administered with vehicle or 100 mg/kg L-NAME were preincubated for 5 min with or without 3.0×10^{-6} M haloperidol (Hal), followed by 3.0×10^{-6} M neurotensin (NT), both dissolved in 10% (v/v) DMSO, for a further 5 min period. Values from 3, 4 experiments performed per triplicate were expressed as percentage enzyme activity (±SD), taking as 100% data obtained in the absence of additions. $^*P < 0.05$ with respect to the control without additions, by one sample Student *t* test

Sprague–Dawley rats, most likely suggesting that dopamine D2 receptors are hardly involved in the neurotensin effect on Na^+, K^+-ATPase activity.

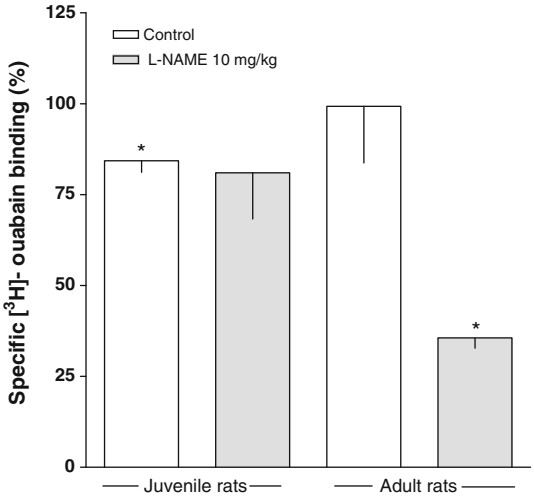

Fig. 7 Neurotensin effect on [^{3}H]-ouabain binding to central nervous system membranes of juvenile and adult male rats postnatally administered L-NAME. Rats were administered with saline (control) or 10 mg/kg L-NAME. [^{3}H]-ouabain binding was assayed in the presence of 1×10^{-6} M neurotensin. Results are expressed as percentage [^{3}H]-ouabain binding taking as 100% the value recorded in the absence of neurotensin. SD of 3, 4 experiments performed per triplicate is indicated within the *bars*. $^*P < 0.05$ with respect to the control without neurotensin, by one sample Student *t* test

Evidences from the literature point to a relationship between neurotensinergic system and NO production. To illustrate, it is known that neurotensin activates tyrosine hydroxylase gene (regulator of catecholamine levels). In this process, NO production seems crucial and an interaction between neurotensin and tyrosine hydroxylase expression has been demonstrated [32]. Neurotensin potentiates NO production during co-stimulation with lipopolysaccharide and interferon gamma, though neurotensin alone fails to induce NO generation. Inducible NO synthase seems responsible for this up-regulation of NO generation [33]. On the other hand, SR 48692, a non-peptide antagonist for high affinity neurotensin receptor (NTS1), exerts diuretic action. Although neurotensin itself has no effect on urine output, this peptide diminishes diuretic action of SR 48692 in fed rats. The opposite is observed in fasted animals where neurotensin inhibits diuresis, an effect diminished by SR 48692. L-NAME alone has no effect on diuresis or on furosemide-stimulated diuresis but prevents diuretic action of SR 48692 in fed rats. These findings suggested a link between endogenous neurotensin, AVP and peripheral NO production in the regulation of renal excretion of water, Na^+, K^+ and Cl^- [34]. Present results also favour the view of a link between neurotensin and NO production.

It is known that hypoxia induces modifications of Na^+, K^+-ATPase activity and the hypothesis that NO production is involved in such changes has been advanced. However, studies carried out in newborn piglets after administration

of L-NAME have shown that inhibition of NO synthase fails to change the hypoxia-induced decrease of Na^+, K^+-ATPase activity [35]. Accordingly, present results indicated that basal Na^+, K^+-ATPase activity in cortical synaptosomal membranes isolated from Sprague–Dawley rats remained unaltered by NO synthesis inhibition after L-NAME administration. Most interestingly, the treatment entirely prevented further neurotensin effect on Na^+, K^+-ATPase activity.

To further study neurotensin effect on Na^+, K^+-ATPase activity, the ouabain binding site of the enzyme was evaluated by assaying [^{3}H]-ouabain binding to cortical membranes. In control or L-NAME treated juvenile rats the peptide slightly decreased [^{3}H]-ouabain binding. At variance, neurotensin failed to modify [^{3}H]-ouabain binding to membranes obtained from control adult rats whereas it highly diminished binding to membranes from L-NAME treated adult rats. A plausible explanation for this finding may be that the kinetics for ouabain binding depends on the different conformational states that Na^+, K^+-ATPase can assume in the presence of a ligand [36]. Where the ligand neurotensin, it may lead to a conformational change in the enzyme to a state of low affinity for ouabain.

One theory of the etiology of schizophrenia is that disturbances during brain development may be, at least in part, be responsible for the breakdown of normal neural control in later life [37–40]. It is known that the interference of NO formation during development may produce some symptoms of schizophrenia [26]. Present results recorded in an open field task in juvenile rats markedly differ from those in the adult rats. At variance, Na^+, K^+-ATPase response to neurotensin was similar at both ages. Therefore, it seems that there is no relationship between the effects of L-NAME early administration on animal behavior with the modulation of Na^+, K^+-ATPase activity by neurotensin.

Neurotensin added in vitro is able to decrease Na^+, K^+-ATPase activity of cerebral cortex synaptosomal membranes. This effect is entirely prevented by SR 48692, antagonist for high affinity neurotensin receptor (NTS1) [15]. Present results showed that inhibition of NO synthesis by postnatal administration of L-NAME to rats prevented further inhibition of synaptosomal membrane Na^+, K^+-ATPase activity by in vitro neurotensin addition. It is tempting to suggest that L-NAME administration alters neurotensin binding to its receptor (NTS1) and therefore no further response to neurotensin can be observed. To test this hypothesis, we studied [^{3}H]-neurotensin binding to cerebral cortex membranes after postnatal administration of 100 mg/kg L-NAME. Preliminary results showed 60% decrease in ligand binding, favouring the view that Na^+, K^+-ATPase activity inhibition by neurotensin occurs through NTS1 receptor (data not shown). This finding is in line with the suggestion that Na^+, K^+-ATPase response to neurotensin involves NTS1 receptor.

To conclude, results obtained showed that early administration of L-NAME produced biochemical changes concomitant with modifications of animal behaviour. Findings support the hypothesis of a relationship between nitrergic and neurotensinergic systems at CNS.

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