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Lithium attenuates behavioral and biochemical effects of neuropeptide S in mice

A.A. Castro [a], T.S. Casagrande [a], M. Moretti [a], L. Constantino [b], F. Petronilho [b], G.C.B. Guerra [e], G. Calo' [c], R. Guerrini [d], F. Dal-Pizzol [b], J. Quevedo [a], E.C. Gavioli [a,e,*]

[a] Laboratório de Neurociências, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil
[b] Laboratório de Fisiopatologia Experimental, Programa de Pós-Graduação em Ciências da Saúde, Universidade do Extremo Sul Catarinense, Criciúma, SC, Brazil
[c] Department of Experimental and Clinical Medicine, Section of Pharmacology and Neuroscience Center, University of Ferrara, Ferrara, Italy
[d] Department of Pharmaceutical Science and Biotechnology Center, University of Ferrara, Ferrara, Italy
[e] Base de Pesquisas em Farmacologia, Departamento de Biofísica e Farmacologia, Centro de Biociências, Universidade Federal do Rio Grande do Norte, Natal, RN, Brazil

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ABSTRACT

Neuropeptide S (NPS) and its receptor NPSR comprise a recently deorphaned G-protein-coupled receptor system. There is a body of evidence suggesting the involvement of NPS in wakefulness, anxiety, locomotor activity and oxidative stress damage. Considering that mood stabilizers block the stimulatory effect of psychostimulants in rodents, the present study aimed to investigate the effects of the pretreatment with lithium and valproate on the hyperlocomotion evoked by NPS. Another relevant action induced by lithium and valproate is the neuroprotection against oxidative stress. Thus, aiming to get further information about the mechanisms of action of NPS, herein we evaluated the effects of NPS, lithium and valproate, and the combination of them on oxidative stress damage. Behavioral studies revealed that the pretreatment with lithium (100 mg/kg, i.p.) and valproate (200 mg/kg, i.p.) prevented hyperlocomotion evoked by NPS 0.1 nmol. Importantly, the dose of valproate used in this study reduced mouse locomotion, although it did not reach the statistical significance. Biochemical analyses showed that lithium attenuated thiobarbituric reactive species (TBARS) formation in the striatum, cerebellum and hippocampus. NPS per se reduced TBARS levels only in the hippocampus. Valproate did not significantly affect TBARS levels in the brain. However, the combination of mood stabilizers and NPS blocked, instead of potentiate, the neuroprotective effects of each one. No relevant alterations were observed in carbonylated proteins after all treatments. Altogether, the present findings suggested that mainly the mood stabilizer lithium evoked antagonistic effects on the mediation of hyperlocomotion and protection against lipid peroxidation induced by NPS.

1. Introduction

Neuropeptide S (NPS) is a 20-amino acid peptide recently identified in the brain and peripheral tissues of distinct species of vertebrates [43]. NPS has a high sequence homology among mammalian species, including a highly conserved N-terminal serine [31]. NPS is the endogenous ligand of a G-protein-coupled receptor named NPSR receptor [43]. In cells expressing the recombinant NPSR receptor, NPS increases Ca^{2+} mobilization, intracellular cAMP formation and phosphorylation of extracellular signal-regulated kinase (ERK1/2) [43,32].

The NPSR is widely expressed in the discrete regions of mammalian brain, and higher levels were found in cortex, hypothalamus, amygdala, endopiriform nucleus, subiculum, and nuclei of the thalamic midline, while low levels were found in basal ganglia [42]. In contrast, NPS precursor mRNA is found highly expressed only in a cluster of neurons located between the locus coeruleus and Barrington's nucleus [43]. This pattern of expression is consistent with the behavioral responses evoked by the injection of NPS in rodents, such as hyperlocomotion [43,34,37,33,21,5], anxiolysis [43,21,33,39,28], wakefulness [43,33], reduction of food consumption [1,39,6,7], antinociception [23], and reestablishment of alcohol-seeking behavior [4].

The mechanism by which NPS evokes hyperlocomotion in rodents is still not completely elucidated. Recently, distinct research groups demonstrated that the stimulatory effects of NPS are blocked by the administration of NPSR antagonists [3,14,27]. Interestingly, Paneda et al. [28] showed that the corticotrophin releasing factor signaling via CRF_1 receptors seems to be mediating the hyperlocomotory action of NPS. In fact, this effect of NPS is blocked by the selective CRF_1 antagonist antalarmin and, no longer evident in $CRF_1(-/-)$ mice [28].

* Corresponding author at: Universidade Federal do Rio Grande do Norte (UFRN), Centro de Biociências, Departamento de Biofísica e Farmacologia, 59072-970 Natal, RN, Brazil. Tel.: +55 84 3215 3419.
E-mail address: egavioli@hotmail.com (E.C. Gavioli).

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As mentioned before, NPS induces some effects such as arousal promoting action, and hyperlocomotion, similar to those described for psychostimulants, i.e. amphetamine and caffeine. Interestingly, the stimulatory effects of amphetamine were attenuated by the acute and chronic administration of mood stabilizers in rats [11] and mice [12]. In vitro studies suggest that classical mood-stabilizing drugs, such as lithium and valproate, indirectly alter dopaminergic neurotransmission, affect phosphoinositide signal pathway by the inhibition of receptor-coupled G-proteins, and protein kinase C, leading to down-regulation of PKC substrate (for a review see [16]). Additionally, lithium inhibits the cyclic AMP signaling pathway, by suppressing both stimulatory and inhibitory G-proteins, and also directly regulating adenylyl cyclase and its expression. Finally, mood stabilizers inhibit the activity of several enzymes including glycogen synthase kinase GSK-3 (for a review see [16,41,24]).

Numerous studies have demonstrated a robust neuroprotective effect for mood stabilizers under distinct experimental conditions, such as brain damage induced by glutamate, oxidative damage elicited by chronic administration of psychostimulants, ischemia-induced brain injury and others (for a review see [35,38]). Previously, our research group has demonstrated that NPS attenuates oxidative stress damage in the mouse brain, thus suggesting a putative role played by NPS–NPSR receptor system in modulating acute brain injury [5]. Altogether, mood stabilizers, such as lithium and valproate, could be considered ideal compounds to compare with the effects evoked by NPS under noxious stimulus. Additionally, they could give further information about the mechanism(s) by which NPSR signaling attenuates oxidative damage in the mouse brain.

On these bases, the present study was aimed to investigate the effects of the pretreatment with the mood stabilizers lithium and valproate on the hyperlocomotion evoked by NPS. Additionally, considering the well-characterized neuroprotective effects of mood stabilizers, the current study aimed to compare, under the same experimental conditions, the effects of NPS and the mood stabilizers lithium and valproate on lipid peroxidation and protein carbonyl formation in mouse brain structures, such as the cerebellum, striatum, cortex and hippocampus. The effects of the combined administration of lithium, valproate and NPS were also assessed in the current study, in order to investigate the mechanisms by which NPSR activation attenuates oxidative stress damage in the mouse brain.

2. Materials and methods

2.1. Animals

To develop this study, 85 naïve male CF-1 mice weighting 30–35 g obtained from FEPPS (Porto Alegre, Brazil) were kept under our animal house at least 1 week before testing. The animals were housed six per cage (30 cm × 19 cm × 13 cm) with food and water available ad libitum and were maintained on a 12-h light/dark cycle (lights on at 7:00 h). All experimental procedures involving animals were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and the Brazilian Society for Neuroscience and Behavior (SBNeC) recommendations for animal care. This study was approved by the local ethics committee (Comitê de Ética em Pesquisa da Universidade do Extremo Sul Catarinense; protocol no. 545/07). Experimental groups for behavioral studies consisted of 13–15 animals per group; all mice were used only once. All efforts were made to minimize animal suffering, and to reduce the number of animals used.

2.2. Drugs and treatment

The following drugs were used: NPS (synthesized by Dr. R. Guerrini, Department of Pharmaceutical Science and Biotechnology Center, University of Ferrara, according to published methods [34]), lithium chloride (Globe Química Ltda, Cosmopolis, SP, Brazil), and valproate (Abbott laboratories, São Paulo, Brazil). All drugs were dissolved in saline (NaCl 0.9%). In order to mimic a similar experimental condition, mice were injected by both via i.p. (10 ml/kg) and i.c.v. (2 μl/mouse) as follows: saline (saline i.p. + saline i.c.v.); NPS (saline i.p. + NPS 0.1 nmol i.c.v.); lithium (lithium 100 mg/kg i.p. + saline i.c.v.) and valproate (valproate 200 mg/kg i.p. + saline i.c.v.). Lithium, valproate or saline were injected intraperitoneally, in a volume of 10 ml/kg, 10 min prior i.c.v. injections, while NPS or saline were i.c.v. injected 5 min before behavioral testing.

The dose of NPS employed in the present study is able to induce hyperlocomotion in mice as described previously by our group [5]. Lithium and valproate at 100 and 200 mg/kg, respectively, prevented the hyperlocomotor effect of amphetamine in mice [12,19]. The i.c.v. injections were performed by employing a "free-hand" method under ether anesthesia according to the procedure described by Laursen and Belknap [20], and as previously reported by our research group [5].

2.3. Locomotor activity assay

An infrared beam array cage (Insight Equipments, Ribeirao Preto, Brazil) connected to a PC was used for assessing locomotor activity in mice. The infrared beam array cage consists of a cubicle made of clear Perspex (48 cm × 50 cm) surrounded by 50-cm high walls. Two facing blocks containing an infrared array record the horizontal activity, and a similar system assesses the vertical activity. The animals were gently placed on the center of the arena and they were allowed to explore the apparatus individually during a period of 30 min. All behavioral experiments were conduct in an illuminated room (300 lux in the apparatus center) and quiet room. Locomotor activity was recorded in the light cycle between 9:00 and 12:00 h. After the behavioral evaluation of each mouse, the arena was cleaned with 10% ethanol solution. Locomotor activity and number of rearings were assessed for each mouse individually. The total distance traveled (cm) by each animal was averaged into 5 min time bins.

Immediately after the behavioral procedure mice were sacrificed by decapitation, and brain structures were dissected (striatum, hippocampus, cerebellum and cortex), rapidly frozen, and stored at −80 °C until measurement of thiobarbituric acid reactive species (TBARS) and protein carbonyl contents.

During brain dissection, when any signs of cannula misplacement or cerebral hemorrhage were observed, that brain was discarded from biochemical assays and also statistical analysis; these brains represent less than 5% of the overall injected animals. Any dye was injected for checking cannula misplacement in the brain, the presence of a narrow "red line" formed into the brain exactly where cannula passed through was used to verify cannula placement.

2.4. Biochemical assays

2.4.1. Lipid peroxidation

Lipid peroxidation was measured by formation of thiobarbituric acid (TBA) reactive substances (TBARS) after the method of Esterbauer and Cheeseman [9]. After brain dissection, brain structures were washed with PBS, harvested and lysed. Thiobarbituric reactive species, obtained by acid hydrolysis of 1,1,3,3-tetra-ethoxy-propane (TEP), was used as the standard for the

quantification of TBARS. TBA 0.67% was added to each tube and vortexed. The reaction mixture was incubated at 90 °C for 20 min and the reaction was stopped by placing samples on ice. The optical density of each solution was measured in a spectrophotometer at 535 nm. Data were expressed as nmol of TBARS equivalents per mg of protein.

2.4.2. Protein carbonyl formation

Protein carbonyl content was measured in brain homogenates using 2,4-dinitrophenylhydrazine (DNPH) in a spectrophotometric assay [22]. Briefly, sample tissues were sonicated in ice-cold homogenization buffer containing phosphatase and protease inhibitors (200 nM calyculin, 10 μg/ml leupeptin, 2 μg/ml aprotinin, 1 mM sodium orthovanadate, and 1 μM microcystin-LR) and centrifuged at 1000 × *g* for 15 min to sediment insoluble material. Three hundred microliter aliquots of the supernatant containing 0.7–1.5 mg of protein were treated with 300 μl of 10 mM DNPH, dissolved in 2 M HCl, and compared with 2 M HCl alone (reagent blank). Samples then were incubated for 1 h at room temperature in the dark and stirred every 10 min. Samples were precipitated with trichloroacetic acid (final concentration of ~20%) and centrifuged at 16,000 × *g* at 4 °C for 15 min. The pellet was washed three times with 1 ml of ethanol/ethyl acetate (1:1, v/v). Each time, the pellet was lightly vortexed and left exposed to the washing solution for 10 min before centrifugation (16,000 × *g* for 5 min). The final pellet was dissolved in 1 ml of 6 M guanidine in 10 mM phosphate buffer–trifluoroacetic acid, pH 2.3, and the insoluble material was removed by centrifugation at 16,000 × *g* for 5 min. Absorbance was recorded in a spectrophotometer at 370 nm for both DNPH-treated and HCl-treated samples. Protein carbonyl levels were expressed as nmol of carbonyl per mg of protein.

Protein was estimated in all the fractions according to the method of Lowry et al. [25]. Bovine serum albumin was used as standard.

2.5. Statistical analysis

Biochemical data are presented as mean ± SD of 5–6 animals/group, and behavioral data are expressed as mean ± SEM of 13–15 animal/group. Statistical analyses for behavioral tests were estimated using two-way ANOVA followed by the Duncan test. Importantly, when sample values did not assume a Gaussian distribution, as happened to all biochemical findings, data were analyzed by the non-parametric Kruskal–Wallis test followed by the Dunn's test. In all comparisons, statistical significance was set at $P < 0.05$.

3. Results

3.1. Locomotor activity

In the spontaneous locomotor activity, two-way ANOVA revealed that the i.c.v. administration of NPS displayed a trend to affect cumulative distance traveled by mice (Figs. 1 and 2;

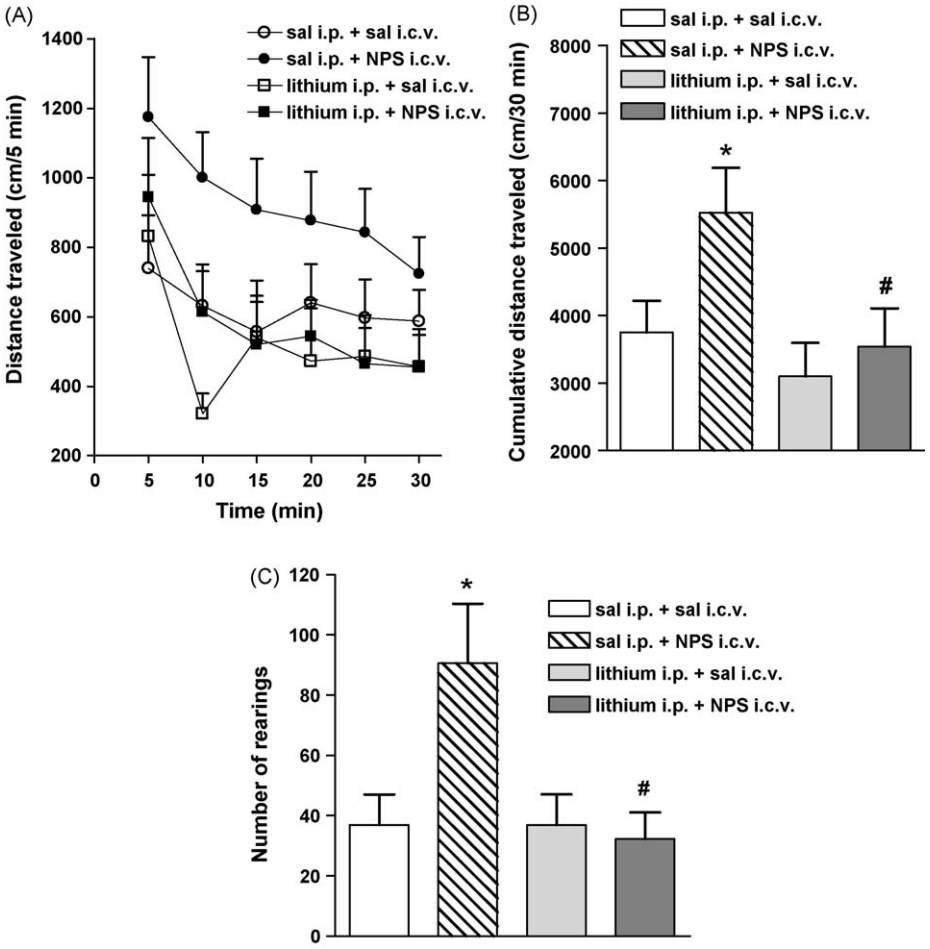

Fig. 1. Effects of the treatment with lithium (100 mg/kg, i.p., 15 min before testing) and neuropeptide S (NPS 0.1 nmol, i.c.v., 5 min before testing) on the distance moved (A and B) and number of rearings (C) by mice. Locomotor and explorative behavior was assessed in infrared beam array cages during 30 min. Data are shown as mean ± SEM (13–15 mice/group). $^{*}P < 0.05$ vs. control group and $^{\#}P < 0.05$ vs. NPS group, according to two-way ANOVA followed by the Duncan test.

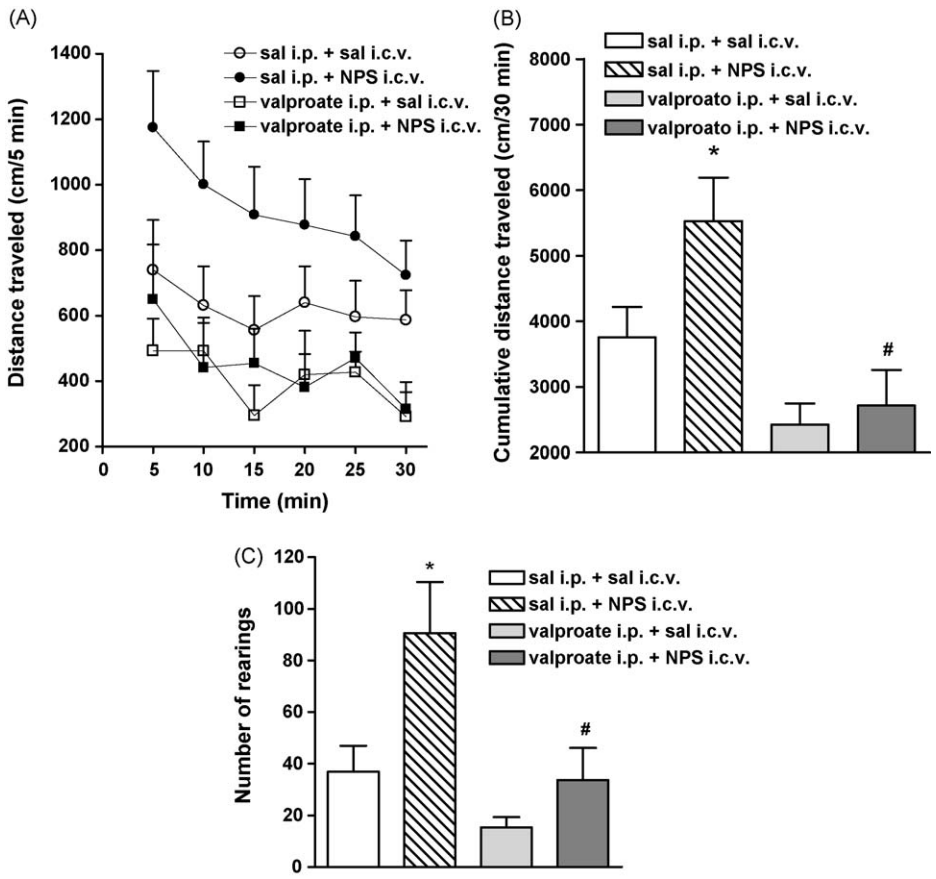

Fig. 2. Effects of the treatment with valproate (200 mg/kg, i.p., 15 min before testing) and neuropeptide S (NPS 0.1 nmol, i.c.v., 5 min before testing) on the distance moved (A and B) and number of rearings (C) by mice. Locomotor and explorative behavior was assessed in infrared beam array cages during 30 min. Data are shown as mean ± SEM (13–15 mice/group). $^{*}P < 0.05$ vs. control group and $^{\#}P < 0.05$ vs. NPS group, according to two-way ANOVA followed by the Duncan test.

$F(1,77) = 3.64$, $P = 0.06$). Additionally, two-way ANOVA showed a statistically significant effect induced by the pretreatment with mood stabilizers on mouse cumulative distance traveled (Figs. 1 and 2; $F(2,77) = 8.09$, $P = 0.0006$). Post hoc analysis indicated that NPS-treated mice displayed a statistical significant increase of cumulative distance moved compared with control group (Figs. 1 and 2B; $P = 0.02$). In addition, the pretreatment with lithium and valproate did not affect per se the mouse cumulative distance moved (Figs. 1 and 2B; $P = 0.43$ and $P = 0.12$, respectively). However, post hoc analysis revealed that the pretreatment with lithium and valproate significantly prevented the hyperlocomotion induced by NPS injection (Figs. 1 and 2B; $P = 0.01$ and $P < 0.001$, respectively). There was no interactive effect of mood stabilizers pretreatment and NPS administration on spontaneous locomotor activity of mice ($P = 0.29$).

Concerning the number of rearings, two-way ANOVA revealed that the i.c.v. administration of NPS displayed a trend to affect this behavior (Figs. 1 and 2C; $F(1,77) = 3.62$, $P = 0.06$). Additionally, two-way ANOVA indicated a statistically significant effect induced by the pretreatment with lithium and valproate on the number of rearings performed by mice (Figs. 1 and 2C; $F(2,77) = 7.11$, $P = 0.002$). Duncan post hoc test revealed that the i.c.v. injection of NPS increased the number of rearings compared with control (Figs. 1 and 2C; $P = 0.01$), while the pretreatment with lithium 100 mg/kg and valproate 200 mg/kg did not affect per se this behavioral parameter (Figs. 1 and 2C; $P = 0.60$ and $P = 0.12$, respectively). The Duncan post hoc test also indicated that the pretreatment with lithium and valproate prevented the increase in the number of rearings evoked by NPS (Figs. 1 and 2C; $P = 0.003$ for both treatment). There was no interactive effect of mood stabilizers pretreatment and NPS administration on the number of rearings displayed by mice ($P = 0.13$).

3.2. Biochemical assays

The content of TBARS equivalents in the striatum, cortex, cerebellum and hippocampus of mice treated with lithium, valproate, NPS and the combination of mood stabilizers and NPS was illustrated in Fig. 3. The acute administration of lithium reduced peroxidation of polyunsaturated fatty acids in the mouse cerebellum (KW = 13.61, $P = 0.004$; Fig. 3, top panel), hippocampus (KW = 13.81, $P = 0.003$) and striatum (KW = 10.33, $P = 0.016$). The injection of NPS reduced in a significant manner the content of TBARS equivalents only in the hippocampus (KW = 13.81, $P = 0.003$; Fig. 3, top panel). Interesting enough, the pretreatment with lithium blocked the protective effects of NPS in the hippocampus (KW = 13.81, $P = 0.003$; Fig. 3, top panel). In the same way, the administration of NPS attenuated the reduction in TBARS equivalent levels induced by lithium in the striatum (KW = 10.33, $P = 0.016$; Fig. 3, top panel) and cerebellum (KW = 13.61, $P = 0.004$).

Unlike lithium, the administration of valproate did not alter, in a significant manner, the TBARS contents in the mouse brain (Fig. 3, bottom panel). However, the pretreatment with valproate blocked the reduction of lipid peroxidation induced by NPS in the mouse hippocampus (KW = 9.99, $P = 0.019$; Fig. 3, bottom panel). No alterations in lipid peroxidation were observed in the cortex after all treatments ($P > 0.05$; Fig. 3, top and bottom panels).

The effects of lithium, valproate, NPS and the combination of mood stabilizers and NPS administration on carbonylated protein

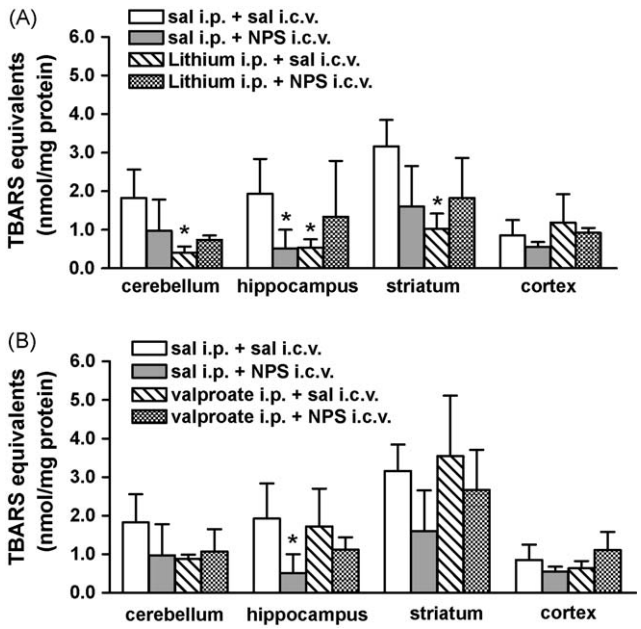

Fig. 3. Effects of the pretreatment with lithium (100 mg/kg; top panel), valproate (200 mg/kg; bottom panel), or saline in mice i.c.v. injected with neuropeptide S (NPS 0.1 nmol) or saline on thiobarbituric acid reactive species (TBARS) formation in homogenate tissues of cerebellum, hippocampus, striatum, and cortex. Thiobarbituric acid reactive species were assessed 35 min after i.c.v. injection by a spectrophotometric assay. Data are shown as mean ± SD. (5–6 mice/group). *$P < 0.05$ vs. control group, according to Kruskal–Wallis followed by the Dunn's test.

Table 1
Effects of the i.p. administration of lithium (100 mg/kg), or valproate (200 mg/kg), and the i.c.v. administration of neuropeptide S (NPS 0.1 nmol) or saline on protein carbonyl formation (measured in nmol/mg protein) in homogenate tissues of cerebellum, hippocampus, striatum, and cortex of mice assessed by a spectrophotometric assay.

Treatments	Cortex	Cerebellum	Striatum	Hippocampus
Sal i.p. + sal i.c.v.	1.10 ± 0.60	7.19 ± 7.66	4.31 ± 2.53	2.63 ± 1.52
Sal i.p. + NPS i.c.v.	1.58 ± 1.02	2.36 ± 2.50	4.51 ± 2.78	3.09 ± 3.21
Lithium i.p. + sal i.c.v.	0.54 ± 0.71	2.19 ± 1.89	4.75 ± 5.85	8.13 ± 4.10*
Lithium i.p. + NPS i.c.v.	1.56 ± 2.22	1.56 ± 2.22	5.19 ± 5.08	0.92 ± 1.06#
Valproate i.p. + sal i.c.v.	0.17 ± 0.12	0.76 ± 0.63	1.33 ± 0.52	0.18 ± 0.09
Valproate i.p. + NPS i.c.v.	0.33 ± 0.18	0.22 ± 0.35*	2.46 ± 1.71	0.47 ± 0.70

Data are shown as mean ± SD of 5 mice/group.
* $P < 0.05$ vs. control group, according to Kruskal–Wallis followed by the Dunn's test..
# $P < 0.05$ vs. lithium + saline group, according to Kruskal–Wallis followed by the Dunn's test.

levels in the mouse brain are shown in Table 1. The injection of lithium and NPS did not affect per se protein carbonyl formation in the cerebellum, striatum and cortex ($P > 0.05$; Table 1). However, the administration of lithium increased the carbonylated protein contents in the hippocampus, and NPS (which was inactive per se) was able to attenuate the increase in protein carbonyl formation induced by lithium in this brain structure (KW = 10.41, $P = 0.015$; Table 1). The treatment with valproate did not affect this biochemical parameter at the brain structures analyzed. However, the combined injection of valproate and NPS caused a significant reduction in protein carbonyl formation only in the cerebellum compared to control group (KW = 14.68, $P = 0.002$; Table 1).

4. Discussion

The results of the present study demonstrated that the pretreatment with mood stabilizers prevented the behavioral outcomes evoked by NPS in mice, i.e. hyperlocomotion and increase in the number of rearings. Additionally, our findings also indicated that NPS and lithium, but not valproate, reduced oxidative stress damage in the mouse brain. Interestingly, we observed that the protective effects of lithium and NPS against lipid peroxidation disappeared when these drugs were administered together. The same holds true for valproate, which prevented the protective effects of NPS against oxidative stress damage in the mouse hippocampus. Thus, assuming that lithium and valproate do not bind to the NPSR, it might be proposed that mood stabilizers blocked signaling pathways, which are mediating hyperlocomotion and protection against oxidative stress damage evoked by NPS.

In this study we observed a stimulatory effect of NPS at 0.1 nmol on mouse locomotion and exploration. Additionally, the dose of lithium employed in the present study did not affect mouse spontaneous locomotion and explorative behavior per se. Regarding the effects of valproate alone on mouse behavior, a nonstatistical significant reduction on mouse locomotor activity was observed, i.e. it was approximately 35% less compared to control. Despite the sedative effects of valproate, the pretreatment with this drug prevented the NPS-induced mouse locomotion and exploration. Thus, our behavioral findings suggest that the mood stabilizers lithium and valproate seem to exert a functional antagonist action against the stimulatory effects of NPS in mice.

Mood stabilizers are widely used to treat bipolar disorder in humans. In rodents, one of the most common rodent models with which the mood-stabilizing action of lithium and valproate are studied requires the induction of hyperactivity by a stimulant, such as amphetamine [11,12,26]. Amphetamine-induced hyperlocomotion is, at least in part, related to the stimulation of dopaminergic and noradrenergic systems. Very recently, Raiteri and colleagues [30] have shown that NPS can selectively inhibit the evoked release of 5-HT and noradrenaline, while NPS at relatively high concentrations only weakly reduced the overflow of dopamine and acetylcholine in the mouse frontal cortex. These effects on neurotransmitter release may explain in part the anxiolytic-like behavior of NPS, but it is not enough for explaining the hyperlocomotion evoked by NPS.

Lithium and valproate do not directly affect the release of dopamine in the rodent brain [10,17], but it is likely that, mainly to lithium, the modification of dopamine-mediated behavioral changes involve alterations in receptor sensitivity or changed activation of downstream signaling pathways (for a review see [29]). The inhibitory effects of lithium on G-protein functions, in particular cAMP levels [13], which can be stimulated via NPSR activation [32], can be relevant for interpreting the present findings. Additionally, we cannot rule out the effects of mood stabilizers, both lithium and valproate, on ion flux [8]. In fact, the presence of lithium is related to alterations in IP_3/DAG/Ca^{2+} responses, while valproate is an inhibitor of voltage-dependent sodium and calcium channels [13,35,41]. By contrast, NPSR signaling activation increases Ca^{2+} intracellular mobilization in HEK293 cells [32]. These alterations affect neuronal firing and neurotransmitter release, and they could also be involved in the functional antagonist action of mood stabilizers against the stimulatory effects of NPS.

The neuroprotective effects of mood stabilizers were demonstrated under distinct experimental conditions. In fact, literature findings have shown that lithium and valproate attenuated: glutamate-induced lipid peroxidation and protein oxidation observed in rat cerebral cortex slices [36] and glioblastoma cells cultured [18], in vitro oxidative stress damage induced by $FeCl_3$ [40], ischemia-induced hippocampal damage in gerbils [2], and apoptotic cell death in brain induced by transient exposure of

infant mice to ethanol [44]. Interesting enough, a previous study has already shown that NPS administration attenuated brain damage caused by oxidative stress in the mouse brain [5]. Thus assuming that mood stabilizers (i.e. lithium and valproate) and NPS evoke neuroprotective effects in the rodent brain, herein we aimed to investigate, under the same experimental conditions, the effects of lithium, valproate and NPS in the oxidative brain damage to lipids and proteins in the mouse brain. The present study revealed that NPS significantly reduced lipid peroxidation in one region, the hippocampus, and in this region with a similar effect size to lithium. In contrast, lithium is also effective in attenuating brain damage in the cerebellum and striatum without any significant effect of NPS. By contrast, valproate did not evoke any protective effect under the present condition. Taken together, the current findings corroborate the view that NPS, similar to lithium, attenuates oxidative stress damage in the mouse brain. Further studies aiming to evaluate the effect of NPS treatment in distinct stimulus-induced neuronal injury are mandatory.

In the present study we also observed that the combined administration of lithium and NPS attenuated the protective effects of lithium in the cerebellum and striatum. Our findings also revealed that valproate, which did not induce protection against oxidative damage per se, prevented the effects of NPS on lipid peroxidation in the hippocampus. Therefore, supporting the view that mood stabilizers attenuate the protective effects of NPS instead of potentiate. Additionally, in the hippocampus, lithium and NPS induced protective effects when given alone, but not when both drugs were administered together, thus suggesting that lithium and NPS attenuated the protective effects of each other. It is known that mood stabilizers, such as lithium and valproate, share the ability to attenuate influx and intracellular accumulation of sodium, thus affecting significantly flux and intracellular ion concentrations [8]. By contrast, activation of NPS receptor signaling evokes Ca^{2+} mobilization [32]. It might be hypothesized again that the attenuation of oxidative damage by mood stabilizers and NPS can be mediated by alterations in ion flux.

Little literature information could support the acute neuroprotective effects of NPS. It is worth noting that oxidative stress results from an overproduction of reactive oxygen species which overwhelms the cellular antioxidant capacity. Either overproduction of reactive oxygen species or insufficient endogenous antioxidant defense can cause oxidative stress damage [15]. Previously, we have shown that the administration of NPS inhibited an increase in SOD activity, but had little effect on catalase activity [5], which could in part explain the effects of NPS in attenuating oxidative stress damage in the mouse brain.

In conclusion, our findings demonstrated that mood stabilizers, mainly lithium, prevented the hyperlocomotor effects of NPS in mice. Additionally, we observed that lithium and NPS, but not valproate, attenuated the oxidative stress damage to lipids in the mouse brain. The combined administration of mood stabilizers and NPS prevented the attenuation of oxidative stress damage of NPS. Taken together, the inhibitory effects of lithium on G-protein functions, i.e. cAMP formation [13], which can be stimulated via NPSR activation [32], might be relevant for interpreting the present findings. Additionally, ion flux alterations, particularly Ca^{2+}, could be mediating these data of NPS and mood stabilizers.

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