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5-HT_{1A} autoreceptor modulation of locomotor activity induced by nitric oxide in the rat dorsal raphe nucleus

L.B. Gualda¹, G.G. Martins¹, B. Müller¹, F.S. Guimarães² and R.M.W. Oliveira¹

¹Departamento de Farmacologia, Universidade Estadual de Maringá, Maringá, PR, Brasil

²Departamento de Farmacologia, Faculdade de Medicina de Ribeirão Preto, Universidade de São Paulo, Ribeirão Preto, SP, Brasil

Abstract

The dorsal raphe nucleus (DRN) is the origin of ascending serotonergic projections and is considered to be an important component of the brain circuit that mediates anxiety- and depression-related behaviors. A large fraction of DRN serotonin-positive neurons contain nitric oxide (NO). Disruption of NO-mediated neurotransmission in the DRN by NO synthase inhibitors produces anxiolytic- and antidepressant-like effects in rats and also induces nonspecific interference with locomotor activity. We investigated the involvement of the 5-HT_{1A} autoreceptor in the locomotor effects induced by NO in the DRN of male Wistar rats (280-310 g, N = 9-10 per group). The NO donor 3-morpholinylsulfonamide hydrochloride (SIN-1, 150, and 300 nmol) and the NO scavenger S-3-carboxy-4-hydroxyphenylglycine (carboxy-PTIO, 0.1-3.0 nmol) were injected into the DRN of rats immediately before they were exposed to the open field for 10 min. To evaluate the involvement of the 5-HT_{1A} receptor and the N-methyl-D-aspartate (NMDA) glutamate receptor in the locomotor effects of NO, animals were pretreated with the 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 8 nmol), the 5-HT_{1A} receptor antagonist *N*-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-*N*-2-pyridinyl-cyclohexanecarboxamide maleate (WAY-100635, 0.37 nmol), and the NMDA receptor antagonist DL-2-amino-7-phosphonoheptanoic acid (AP7, 1 nmol), followed by microinjection of SIN-1 into the DRN. SIN-1 increased the distance traveled (mean ± SEM) in the open-field test (4431 ± 306.1 cm; $F_{7,63} = 2.44$, $P = 0.028$) and this effect was blocked by previous 8-OH-DPAT (2885 ± 490.4 cm) or AP7 (3335 ± 283.5 cm) administration ($P < 0.05$, Duncan test). These results indicate that 5-HT_{1A} receptor activation and/or facilitation of glutamate neurotransmission can modulate the locomotor effects induced by NO in the DRN.

Key words: Dorsal raphe nucleus; Nitric oxide; Serotonin; 5-HT_{1A} receptor; Locomotor activity

Introduction

Inhibition of the nitric oxide (NO) signaling pathway in the brain has been reported to induce anxiolytic- and antidepressant-like effects in animal models. Systemic administration of the NO synthase (NOS) inhibitor *N*-methyl-L-arginine ester (L-NAME) (1,2) or the more selective neuronal NOS (nNOS) inhibitor 7-nitroindazole (2) produces anxiolytic-like effects in the elevated plus maze. The same compounds and the soluble guanylyl cyclase inhibitors methylene blue (3) dose-dependently reduce immobility time in the forced swimming test, a preclinical behavioral method for detecting antidepressant-like activity of drugs.

The behavioral effects of NOS inhibitors appear to depend on endogenous serotonin (5-hydroxytryptamine, 5-HT). Low doses of L-NAME that are ineffective at 3 mg/

kg potentiate the behavioral effects of imipramine and fluoxetine but not reboxetine, a norepinephrine reuptake inhibitor, in the forced swimming test (4). A functional interaction between 5-HT receptor subtypes and NO has been recently proposed. Ulak et al. (5) have shown that depletion of endogenous 5-HT or the antagonism of 5-HT₂ receptor prevented the antidepressant-like effect of 1-(2-trifluoromethylphenyl)-imidazole (TRIM), an nNOS inhibitor, implying that the antidepressant effects of TRIM might be mediated by an interaction with 5-HT₂ receptors. 5-HT₂ receptors have been shown to mediate an increase in the hippocampal NOS activity induced by imipramine withdrawal in rats (6). Furthermore, hippocampal NOS is required for the functional activity of 5-HT_{1A} receptors in

Correspondence: R.M.W. Oliveira, Departamento de Farmacologia, Universidade Estadual de Maringá, Av. Colombo, 5790, 87020-900 Maringá, PR, Brasil. Fax: +55-44-3261-4999. E-mail: rmmwoliveira@uem.br

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modulating anxiety-related behaviors (7). These findings suggest that nNOS may serve as a downstream signaling enzyme of the 5-HT neurotransmitter system (7).

Nitric oxide has been involved in the release of several neurotransmitters. In the hippocampus and dorsal raphe nucleus (DRN), NO provides a tonic stimulus to maintain elevated levels of 5-HT release. Thus, nNOS inhibitors have been reported to cause an increase in hippocampal 5-HT efflux in the rat ventral hippocampus after local or systemic administration, whereas the NO precursor L-arginine had the opposite effect (8). These effects, however, may be region-dependent. When infused into the DRN, nNOS inhibitors decrease local extracellular 5-HT while increasing it in the frontal cortex (9).

The DRN is the origin of ascending serotonergic projections and is considered to be an important component of the brain circuit that mediates anxiety- and depression-related behaviors (10). 5-HT_{1A} receptors are expressed presynaptically in the DRN and act as inhibitory somatodendritic autoreceptors. A large fraction of DRN 5-HT-positive neurons also contain nNOS (11), suggesting that NO could interfere with DRN function. We recently showed that disruption of NO-mediated neurotransmission by the NOS inhibitors L-NAME and 7-nitroindazole in the DRN produced anxiolytic- and antidepressant-like effects in rats (2). However, the effects occurred within a limited dose range, with low doses causing anxiolytic-like effects and high doses decreasing locomotor activity. These results suggest that NO-related drugs administered into the DRN may also induce the nonspecific interference of locomotor activity.

In the present study, we investigated the involvement of 5-HT_{1A} receptors in the locomotor effects of NO administered into the DRN. Nitric oxide may increase glutamate release in the central nervous system, and 5-HT neurons in the DRN receive excitatory glutamatergic inputs from forebrain areas, hypothalamic nuclei and the lateral habenula (12). Therefore, we also tested the effects of pretreatment with DL-2-amino-7-phosphonoheptanoic acid (AP7), a glutamate *N*-methyl-D-aspartate (NMDA) receptor inhibitor, on the effects of NO in the DRN.

Material and Methods

Animals

Male Wistar rats (N = 127) weighing 280-310 g (77-91 days) were transported to a colony room adjacent to the test laboratory 48 h before surgery. They were housed 5 per cage under a 12/12-h light/dark cycle (lights on at 7:00 am) at 23 ± 1°C and given free access to food and water.

The experimental procedures used here were conducted in accordance with the Brazilian Society of Neuroscience and Behavior Guidelines for the Care and Use of Laboratory Animals, which comply with international guidelines and were approved by the Animal Ethics Committee of Universidade Estadual de Maringá, Paraná (CEEAA/037/2007). All

efforts were made to minimize animal suffering.

Experimental procedure

The animals were anesthetized with sodium pentobarbital (45 mg/kg, *ip*) and fixed in a stereotaxic frame. A stainless steel guide cannula (outer diameter: 0.7 mm) was implanted in the DRN using the following coordinates: anterior/posterior: ±1.4 mm from lambda, lateral: 3.7 mm, ventral: 6.2 mm (13). The guide cannula was inserted into the right hemisphere of the brain at a 36° angle to the vertical plane to avoid the sagittal sinus. The tip of the guide cannula was positioned 1 mm above the DRN. After the surgical procedures, animals were housed 5 to a cage until behavioral testing.

Six to 7 days after surgery, the animals were transported to the test laboratory and were not disturbed for at least 1 h prior to testing. They were then randomly assigned to one of the treatment groups (N = 9-10/group).

For the microinjection procedure, a thin dental needle (outer diameter: 0.3 mm) was introduced through the guide cannula using a microsyringe (Hamilton, USA) controlled by an infusion pump (Kd Scientific, USA). A PE10 polyethylene catheter was interposed between the upper end of the dental needle and the microsyringe. The displacement of an air bubble inside the polyethylene tubing was used to monitor the microinjection process. The needle was held in place for an additional 1 min to maximize diffusion away from the injector tip. The animals received injections of saline or drugs into the DRN and were immediately placed in the center of an open-field apparatus (70 x 70 x 40 cm) for 10 min. Their behavior was videotaped and the distance traveled by them was determined by the Ethovision XT[®] software (Noldus Inc., Netherlands). For combined treatments, the animals received injections of saline or drugs, followed 10 min later by saline or the NO donor 3-morpholinopropanolamine (SIN-1). For all microinjections, including the first and second injections, the infused volume was 0.3 µL delivered over 30 s.

Drugs

The NO donor SIN-1 (150 and 300 nmol), the NO scavenger S-3-carboxy-4-hydroxyphenylglycine (carboxy-PTIO, 0.1-3.0 nmol), the 5-HT_{1A} agonist 8-hydroxy-2-(di-*n*-propylamino)tetralin (8-OH-DPAT, 8 nmol), the 5-HT_{1A} receptor antagonist *N*-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-*N*-2-pyridinyl-cyclohexanecarboxamide maleate (WAY-100635, 0.37 nmol), and the glutamate NMDA receptor antagonist AP7 (1 nmol) were dissolved in sterile isotonic saline. The drugs were purchased from Sigma Chemicals (USA). The solutions were prepared immediately before use. The doses of 8-OH-DPAT and WAY-100635 were chosen on the basis of studies showing no locomotor effects induced by these compounds when injected into the DRN (14,15). The doses of SIN-1 (2,16), carboxy-PTIO (17) and AP7 (16) were based on previous studies using direct administration

of these compounds into the dorsolateral periaqueductal gray matter (DLPAG).

Histology

After behavioral analysis, the rats were sacrificed by deep urethane anesthesia and were perfused through the left ventricle of the heart with isotonic saline followed by a 10% formalin. A dental needle was then inserted through the guide cannula, and 0.3 μ L methylene blue was injected. The brains were removed and immersed in a 10% formalin for a minimum of 3 days. Coronal brain sections (50 μ m) were then obtained with a cryostat (Cryocut 1800, Leica, Germany) and stained with cresyl violet. The injection sites were identified using diagrams from the atlas of Paxinos and Watson (13). Animals with injection sites located outside the DRN were excluded from the main statistical analysis but were combined in a group denoted "OUT" in Figure 1.

Statistical analysis

Data regarding the distance traveled by the rats were analyzed by one-way analysis of variance (ANOVA; Figure 1). For combined treatments (Figure 2), data were analyzed by a two-way ANOVA, with the main factors being the first and second injections. In case of a significant interaction, the groups were compared by one-way ANOVA. *Post-hoc* comparisons were performed by the Duncan test. For ethical reasons, to minimize the number of animals used in this experiment, the same control (saline/saline) and SIN-1 (saline/SIN-1) groups were included in the experiment presented in Figure 2. The level of significance was set at $P < 0.05$.

Results

Effects of SIN-1 and carboxy-PTIO on the distance traveled by rats in the open field

SIN-1, an NO donor, at 150 or 300 nmol significantly increased the distance traveled by the rats in the open field ($F_{2,31} = 11.28$, $P = 0.002$; Figure 1). No significant effect on distance traveled was detected after carboxy-PTIO administration into the DRN ($F_{2,35} = 2.06$; $P = 0.13$; Figure 1). Animals that received SIN-1 or carboxy-PTIO outside the DRN (OUT) did not differ from controls ($F_{6,70} = 3.51$, $P > 0.05$; Figure 1).

Pretreatment with 8-OH-DPAT or AP7 blocked the effects of SIN-1 on locomotor activity in rats

As expected, SIN-1 increased the distance traveled compared to all groups of the combined treatment (Figure 2), except for the WAY-100635 + saline and WAY-100635 + SIN-1 groups ($F_{7,63} = 2.47$, $P < 0.028$; $P < 0.05$, Duncan test). ANOVA detected a significant interaction between the first and the second injection ($F_{3,63} = 2.76$, $P = 0.049$). The effects of SIN-1 were prevented by pretreatment with the selective 5-HT_{1A} receptor agonist 8-OH-DPAT or the

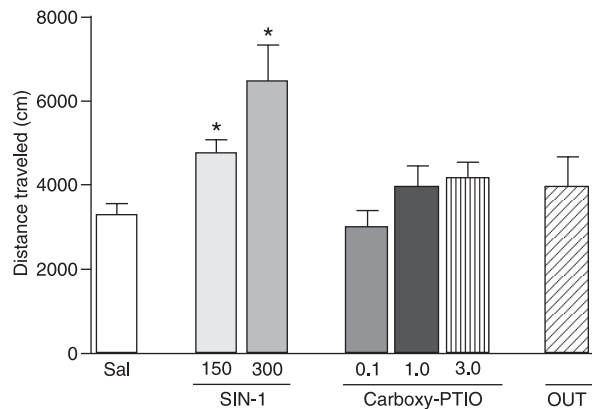


Figure 1. Effects of intra-DRN injection of the NO donor SIN-1 (150 and 300 nmol) or carboxy-PTIO (0.1-3.0 nmol) on the distance traveled by rats in the open field for 10 min. The drugs were administered immediately before testing. Rats whose injections of SIN-1 or carboxy-PTIO were outside the DRN ($N = 15$) are reported in the OUT group. The height of the columns indicate the means, and the bars represent the SEM ($N = 9-10$ /group). DRN = dorsal raphe nucleus; NO = nitric oxide; SIN-1 = 3-morpholinylsodium hydrochloride; carboxy-PTIO = S-3-carboxy-4-hydroxyphenylglycine. * $P < 0.05$ compared to the saline (Sal) group (ANOVA followed by the Duncan test).

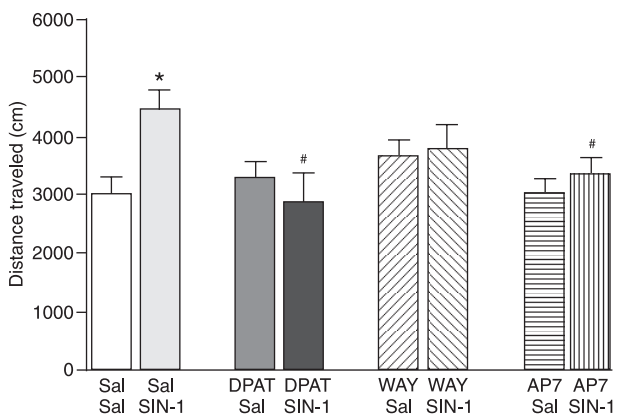


Figure 2. Effects of pretreatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT (DPAT, 8 nmol), the 5-HT_{1A} receptor antagonist WAY-100635 (WAY, 0.37 nmol) and the glutamate NMDA receptor antagonist AP7 (1 nmol) 10 min before SIN-1 (150 nmol) administration into the DRN in rats tested in the open field for 10 min. The height of the columns indicate the means, and the bars indicate the SEM ($N = 9-10$ /group). 8-OH-DPAT = 8-hydroxy-2-(di-*n*-propylamino)tetralin; WAY-100635 = *N*-(2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl)-*N*-2-pyridinyl-cyclohexanecarboxamide maleate; AP7 = DL-2-amino-7-phosphonoheptanoic acid; SIN-1 = 3-morpholinylsodium hydrochloride; DRN = dorsal raphe nucleus. * $P < 0.05$ compared to the saline + saline (Sal + Sal) group (ANOVA followed by the Duncan test); # $P < 0.05$ compared to the saline + SIN-1 (Sal + SIN-1) group (ANOVA followed by the Duncan test).

glutamate NMDA receptor antagonist AP7 before SIN-1 administration ($P < 0.05$, Duncan test). These results suggest that 5-HT_{1A} receptors in the DRN may mediate the effects of NO on locomotor activity. The effects of SIN-1 were also prevented by pretreatment with AP7 ($P < 0.05$, Duncan test). Since AP7 is a glutamate NMDA receptor antagonist, SIN-1 may also act by facilitating glutamate release in the DRN. No significant effect was detected with WAY-100635 pretreatment ($P > 0.05$, Duncan test).

Discussion

Nonspecific suppression of rat locomotor activity by NO-related drugs has been reported previously. NOS inhibitors, such as L-NAME (2) and methylene blue (3), reduce spontaneous locomotor activity in a dose-dependent way and may induce catalepsy in rodents (18). The latter effect involves NOS inhibition in the striatum. However, other brain areas have been shown to be involved in the systemic effects of NOS inhibitors on locomotor activity. Spiacci Jr. et al. (2) showed that NOS inhibitors injected into the DRN decreased, whereas the cyclic guanosine monophosphate (cGMP) analog 8-Br-cGMP dose-dependently increased, locomotor activity in rats, implicating NO neurotransmission in the DRN in the modulation of locomotor behavior.

In the present study, administration of SIN-1, an NO donor, into the DRN increased the distance traveled by rats in the open field. Pretreatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT or the NMDA receptor antagonist AP7 blocked the effects of SIN-1 on the locomotor activity of rats. Although a slight increase in locomotor activity was detected with WAY-100635 administration into the DRN, this effect was not statistically significant and did not modify the effects of SIN-1.

The activity of DRN neurons is under complex regulatory control mediated by the medial prefrontal cortex and lateral habenula, a process that involves excitatory glutamate inputs to serotonergic and γ -aminobutyric acid (GABA) neurons (19). The glutamatergic inputs are mediated, at least in part, by NMDA receptors, resulting in NO formation. Nitric oxide thus modulates the local release of glutamate, GABA, and 5-HT, having complex effects on DRN neuronal activity and implying interference of motor and emotion-related processes. The DRN innervates emotion-related areas such as the amygdala, hippocampus and DLPAG (19). It also sends 5-HT fibers to the striatum, a motor-related area where they can modulate motor function.

We showed that NO neurotransmission in the DRN mediates anxiolytic- and antidepressant-like effects in rats, which were followed by significant locomotor changes (2). The fact that the locomotor effects of SIN-1 were prevented by AP7, a glutamate NMDA receptor antagonist, suggests that SIN-1 may act by facilitating glutamate release in the DRN. Similar mechanisms have been implicated in the escape responses produced by SIN-1 administration into

the DLPAG. In this region, escape responses appear to be modulated also by 5-HT because these effects were inhibited by the 5-HT_{2A/2C} receptor agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) (20).

The present study demonstrated that the increased locomotor activity induced by SIN-1 administration into the DRN was prevented by pretreatment with the 5-HT_{1A} receptor agonist 8-OH-DPAT. Considering this evidence, it is possible that 5-HT_{1A} receptors in the DRN mediate the effects of NO on locomotor activity. Recently, it was demonstrated that administration of the 5-HT_{1A} receptor agonist 8-OH-DPAT into the DRN favored the expression of the escape, which was chemically induced by SIN-1 administration into the DLPAG while intra-DRN injection of the WAY-100635 did not change this defensive behavior (14). These findings support the view that DRN 5-HT_{1A} autoreceptors are under the tonic inhibitory influence of 5-HT. In this respect, an interesting interaction between NO and 5-HT has been demonstrated in the regulation of anxiety- and depressive-related behaviors. Zhang et al. (7) have shown that nNOS is required not only for the anxiolytic-like effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT but also for the anxiogenic-like effects of the 5-HT_{1A} receptor antagonist 1-(methoxyphenyl)-4-[4-(2-phthalimido)butyl]piperazine (NAN-190), indicating that NO may serve as a downstream signaling enzyme of 5-HT_{1A} receptor functions.

Contrary to expectations, however, administration of carboxy-PTIO failed to change the locomotor activity when injected into the DRN. It is possible that exogenous NO, delivered by SIN-1 was more effective in interfering with locomotion than the effect of the NO scavenger, carboxy-PTIO. The results of our study also revealed that pretreatment of rats with WAY-100635 did not affect locomotor activity or increase the effects of SIN-1. WAY-100635 slightly increased the distance traveled in the open field, but this effect was not statistically significant. The failure of WAY-100635 to modify the increased locomotor activity produced by SIN-1, however, is consistent with reports showing that WAY-100635 injected into the DRN at the same dose (0.37 nmol) presented a panicolytic-like effect without affecting locomotion (12) and did not change defensive behaviors induced by DLPAG stimulation (14). To better clarify the interaction between NO and 5-HT_{1A} receptors in the DRN, however, further experiments need to be conducted.

The present results indicate that exogenous NO administration into the DRN increases locomotor activity in rats, and this effect is mediated at least in part by 5-HT_{1A} receptor activation and/or facilitation of glutamate NMDA receptor-mediated neurotransmission in this region.

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