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Flight reactions to nitric oxide in the inferior colliculus of rats depend on NMDA receptor activation

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Abstract

The dorsolateral periaqueductal grey (dlPAG) is proposed to play a role in the elaboration of defensive behaviors. Nitric oxide (NO) donors, injected into this region, induce flight reactions. The reactions have also been observed after electrical or chemical stimulation of the inferior colliculus (IC). The enzyme responsible for NO formation, neuronal nitric oxide synthase (nNOS), is expressed in the IC. The aims of this study were to investigate if NO donors injected into the IC would also cause aversive reactions and if these reactions would involve activation of NMDA receptors. The results showed that 3-morpholinosylnomine hydrochloride (SIN-1; 300 nmol), an NO donor, injected into the central nucleus but not into the dorsal cortex of the IC (CIC and DCIC, respectively) of male Wistar rats induced flight reactions characterized by galloping and jumps. Pretreatment (10 min) with methylene blue (MB; 100 or 200 nmol), a guanylate cyclase (GC) inhibitor, partially inhibited this flight reaction, decreasing the number of jumps. 8-Bromo-cGMP (8-Br-GMP), a membrane-permeable cGMP analogue, increased the number of contralateral turnings. Pretreatment (10 min) with the NMDA receptor antagonist amino-7-phosphonoheptanoic acid (AP7; 2 nmol) completely prevented the effects of SIN-1. It is concluded that NO may induce aversive reactions in the CIC and that these reactions depend on NMDA receptor activation. They may also partially involve facilitation of GC activity.

Keywords: Aversion; Glutamate; Anxiety; AP7; SIN-1; Flight reaction

1. Introduction

The inferior colliculus (IC) is a primary acoustic structure anatomically divided into an external and dorsal cortex (DCIC) and into a central nucleus (CIC) (Faye-Lund and Osen, 1985). It is proposed to be involved in the processing of acoustic information leading to aversive responses (for a review, see Brandão et al., 1999). It would act as a filter for sounds that require immediate and explosive defensive reactions, such as those made by prey, predators or conspecifics (Casseday and Covey, 1996).

Electrical stimulation or microinjection of the GABA-A antagonist bicuculline into the IC of rats induces characteristic-aversive responses similar to the stimulation of the dorsal parts of the periaqueductal grey (PAG) such as arousal, freezing and escape behavior (Brandão et al., 1988). Accordingly, in rats, the GABA-A agonist muscimol increases the latency and decreases the frequency of learned switch-off responses to IC electrical stimulation (Melo et al., 1992). Moreover, microinjection of NMDA receptor agonists into this region also elicits a flight reaction characterized by running and jumps (Cardoso et al., 1994).

Glutamate binding to NMDA receptors causes calcium influx that may activate the calcium/calmodulin-dependent enzyme, neuronal nitric oxide synthase (nNOS) (Garthwaite et al., 1988, 1989). This enzyme synthesizes nitric oxide (NO) together with citrulline from L-arginine (Palmer et al., 1988). NO then diffuses to pre- and postsynaptic neurons (Edelman and Gally, 1992; Garthwaite, 1991; Snyder and Bredt, 1991) where it activates the enzyme guanylate cyclase (GC). This mediates the glutamate-linked enhancement of cGMP in the central nervous system (Bredt and Snyder, 1989; Garthwaite et al., 1988; Knowles et al., 1989). In addition, NO may enhance glutamate release in the brain in a reciprocal regulatory mechanism (Lin et al., 2000; Montague et al., 1994).

The nNOS is expressed in structures such as the IC, the dorsolateral periaqueductal grey (dlPAG), the amygdala and

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the hypothalamus (Vincent and Kymura, 1992). These regions are proposed to be part of a brain-aversive neural substrate related to the elaboration of defensive behaviors (Graeff, 1981, 1994). In the dlPAG, NO may be involved in the aversive action of glutamate (for a review, see De Oliveira et al., 2001). NO donors microinjected into this structure induce a flight reaction characterized by coordinated running and jumps with escape attempts (De Oliveira et al., 2000a). NOS inhibitors and GC antagonist, on the other hand, cause anxiolytic effects in the elevated plus-maze (De Oliveira and Guimarães, 1999; Guimarães et al., 1994).

Considering that nNOS is also expressed in the IC, the objectives of this work were to investigate if injections of an NO donor into this region would also induce aversive behaviors and verify if these reactions would involve GC and/or NMDA receptor activation.

2. Material and methods

2.1. Subjects

Male Wistar rats weighing 220–240 g at the beginning of each experiment were housed in pairs in a temperature-controlled room (24 ± 1 °C) under standard laboratory conditions with free access to food and water and a 12:12-h light/dark cycle (lights on at 6:30 a.m.). Procedures were conducted in conformity with the Brazilian Society of Neuroscience and Behavior Guidelines for the Care and Use of Laboratory Animals, which are in compliance with international laws and politics. All efforts were made to minimize animal suffering.

2.2. Drugs

3-Morpholinosylnomine hydrochloride (SIN-1; 300 nmol; RBI), amino-7-phosphonoheptanoic acid (AP7; 2 nmol; Ciba-Geigy), 8-Bromo-cGMP (8-Br-GMP; 225 nmol, Sigma) and methylene blue (MB; 100–200 nmol; Sigma) were dissolved in sterile isotonic saline. The solutions were prepared immediately before use. They were kept on ice and protected from the light during the experimental session. The doses were chosen based on previous studies that investigated the effects of these compounds in the dlPAG (De Oliveira et al., 2000a, 2001; De Oliveira and Guimarães, 1999; Guimarães et al., 1991).

2.3. Apparatus

The experiments were carried out in a circular open arena (72 cm in diameter with a 50-cm high Plexiglas wall). The arena was placed in a sound-attenuated, temperature-controlled (25 ± 1 °C) room, illuminated with three 40-W fluorescent bulbs placed 4 m over the apparatus.

2.4. Surgery

Rats were anesthetized with 2.5% 2,2,2-tribromoethanol (10 mg/kg ip) and fixed in a stereotaxic frame. A stainless steel guide cannula (0.7-mm OD) was implanted unilaterally on the right side aimed at the CIC (coordinates: AP = − 1.0 mm from lambda, L = 1.5 mm, D = 3.5 mm) or cortical (coordinates: AP = − 1.0 mm from lambda, L = 1.8 mm, D = 2.5 mm, at an angle of 20°) nucleus of the IC. The cannula was attached to the bones with stainless steel screws and acrylic cement. A stiletto inside the guide cannulas prevented obstruction.

2.5. Procedure

Seven days after surgery, the animals were randomly assigned to one of the treatment groups. Intracerebral injections were performed with a thin dental needle (0.3-mm OD) introduced through the guide cannula until its tip was 1.0 mm below the cannula end. A volume of 0.2 μl (1.0 μl for MB 200 nmol) was injected in 20 s using a microsyringe (Hamilton, USA) controlled by an infusion pump (Kd Scientific, USA). A polyethylene catheter (PE 10) was interposed between the upper end of the dental needle and the microsyringe (De Oliveira et al., 2000a). The rats were placed in the open arena immediately after the last injection and the exploratory behavior was videotaped for 10 min. Afterwards, the Ethovision software (Version 1.9; Noldus, The Netherlands) analyzed the distance moved. Episodes of galloping (fast running alternating stance and swing movements of anterior and posterior limb pairs), jumping (upward movements) and turning behavior (360° turnings) were manually recorded to evaluate the flight reactions.

Five experiments were performed: (1) Rats ($n = 7$ per group) received injections of saline or SIN-1 into the cortical nucleus of the IC. (2) The animals ($n = 6–8$ per group) received injections of these same compounds (saline and SIN-1) into the CIC. (3) The animals received injections of saline ($n = 5$) or 8-Br-GMP ($n = 6$) into the CIC. (4) They received injections into the CIC of saline or MB (100 nmol) followed by saline or SIN-1 10 min later ($n = 8–10$ per group); an additional group ($n = 6$) was treated with MB (200 nmol) followed by SIN-1 (300 nmol). (5) Rats received injections into the CIC of saline or AP7, followed by saline or SIN-1 10 min latter ($n = 7$ per group). In all experiments, each rat was placed in the open arena immediately after the SIN-1 injection.

2.6. Histology

After the behavioral tests, the rats were sacrificed under deep urethane anesthesia. They were perfused through the left ventricle of the heart with isotonic saline followed by 10% formalin solution. After that, a dental needle was inserted through the guide cannula and 0.2 μl of fast green

was injected. The brains were removed and after a minimum period of 3 days immersed in 10% formalin solution, 50-μm sections were obtained in a Cryostat (Cryocut 1800). The injection sites were identified in diagrams from the atlas of Paxinos and Watson (1997) and representative sites can be seen in Fig. 1. Rats that received injections outside the aimed area were excluded from analysis.

2.7. Statistical analysis

The distance moved in the arena during 10 min was analyzed by a repeated measure multivariate analysis of variance (MANOVA) with time (1–10 min) as the within-subject and drug as the between-subject factors. The degrees of freedom of the within-subject factors were corrected by the Huynh–Feldt epsilon. When variances among groups were not homogenous, the raw data were log transformed (with the addition of a constant value of 1). In case of a significant Drug × Time interaction, post hoc comparisons were performed by *t* test or one-way analysis of variance (ANOVA) followed by the Duncan or Student's *t* tests, as appropriate. The total number of jumps and turnings and the total time of galloping were analyzed by the Kruskal–Wallis or Mann–Whitney tests, as appropriate. Differences were considered significant at the $P < .05$ level.

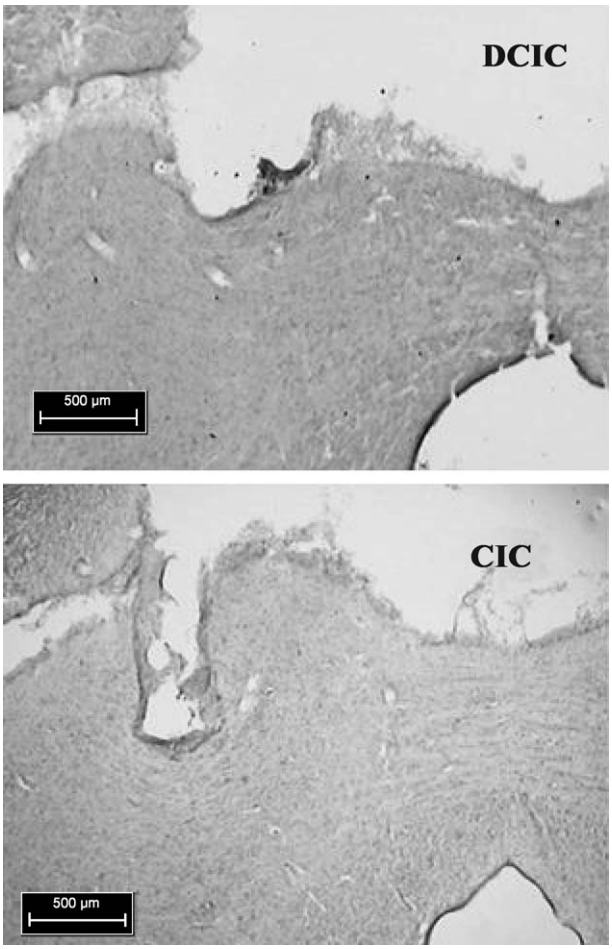

Fig. 1. Injection sites in the DCIC and CIC.

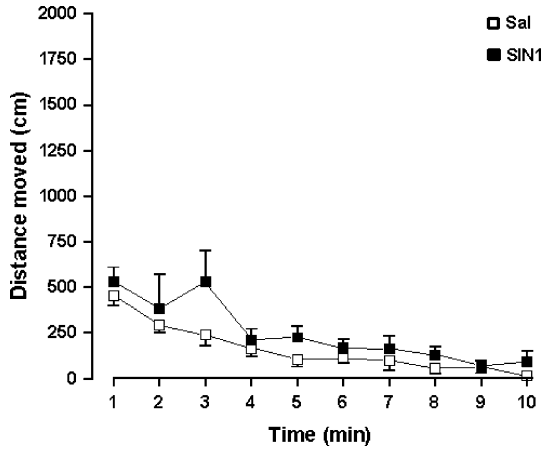

Fig. 2. Effects of saline (SAL, $n = 7$) or SIN-1 300 nmol ($n = 7$) microinjected into the cortical nucleus of the IC on the distance moved in the circular arena. Each point represents the mean distance moved in 1 min ± S.E.M. There was no difference between groups.

3. Results

Administration of SIN-1 into the cortical nucleus of the IC did not change distance moved in the arena [$F(1,12) = 2.08$, NS; Fig. 2] nor the decrease in exploratory behavior that occurred along time [time factor, $F(3.3,39.7) = 18.8$, $P < .001$; Drug × Time, $F(3.3,39.7) = 1.21$, NS]. It also did not induced galloping or jumping behavior ($P > .05$; Table 1).

However, when the drug was injected into the CIC, the animals displayed flight reactions characterized by a significant increase in galloping ($P < .05$) and jumps ($P < .05$; Table 1). There were significant effects of drug [$F(1,12) = 24.2$, $P < .001$; Fig. 3] and Drug × Time interac-

Table 1
Mean ± S.E.M. time of galloping and number of jumps observed over a period of 10 min after microinjection into the IC of saline (SAL), SIN-1 (300 nmol), AP7 (2 nmol) or MB (100 nmol)

Region	Treatment	Galloping (s)	Jumps
Cortical nucleus of the IC	SAL ($n = 7$)	0	0
	SIN-1 ($n = 7$)	0	0
CIC	SAL ($n = 6$)	0	0
	SIN-1 ($n = 8$)	94.8 ± 16.0 *	6.6 ± 2.0 *
	SAL + SAL ($n = 8$)	0	0
	MB + SAL ($n = 8$)	0	0
	SAL + SIN-1 ($n = 10$)	106.8 ± 21.0 *	9.4 ± 2.2 *
	MB + SIN-1 ($n = 10$)	77.6 ± 30.3 *	3.1 ± 1.4 #
	SAL + SAL ($n = 7$)	0	0
	AP7 + SAL ($n = 7$)	0	0
	SAL + SIN-1 ($n = 7$)	82.4 ± 14.9 *	6.9 ± 1.6 *
	AP7 + SIN-1 ($n = 7$)	0 #	0 #

* $P < .05$ compared to SAL + SAL.
\# $P < .05$ compared to SAL + SIN-1 (Mann–Whitney test).

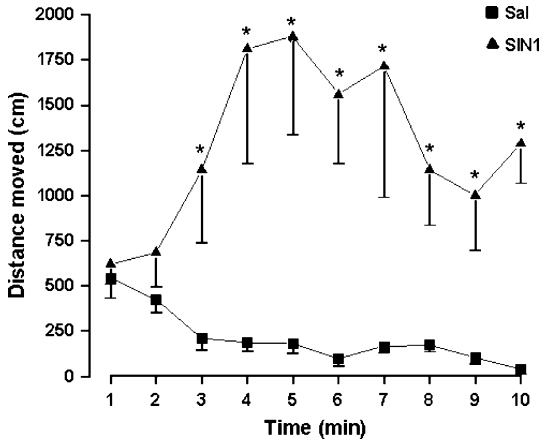

Fig. 3. Effects of saline (SAL, $n=6$) or SIN-1 300 nmol ($n=8$) microinjected into the CIC on the distance moved in the circular arena. Each point represents the mean distance moved in 1 min ± S.E.M. Asterisks signal significant difference from the saline-treated group detected by t test, $P<.05$.

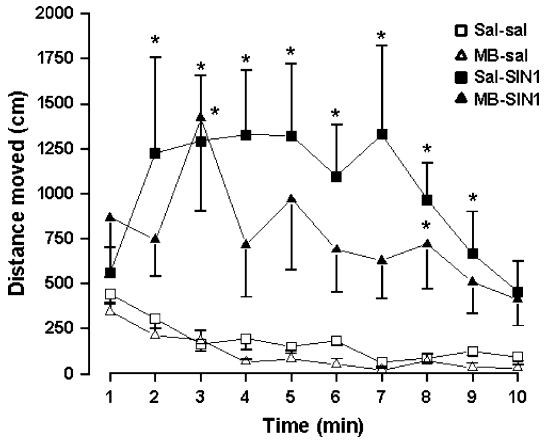

Fig. 5. Effects of saline + saline ($n=8$), MB 100 nmol + saline ($n=8$), saline + SIN-1 300 nmol ($n=10$) or MB + SIN-1 ($n=10$) microinjected into the CIC on the distance moved in the circular arena. Each point represents the mean distance moved in 1 min ± S.E.M. Asterisks signal significant difference from the saline + saline (ANOVA followed by the Duncan test, $P<.05$).

tion [$F(6,79)=4.78$, $P<.01$] in the distance moved in the arena. SIN-1 significantly increased this parameter at the 3rd, 4th, 5th, 6th, 8th, 9th and 10th minutes of the t test ($P<.05$).

Administration of 8-Br-GMP into the CIC failed to significantly increase the distance moved in the arena [$F(1,9)=0.32$, NS; Fig. 4]. The drug tended, however, to increase this parameter in the first minute of analysis [Drug × Time interaction, $F(4.58,41.18)=2.83$, $P=.03$, $t(df=9)=2.06$, $P=.07$]. It also significantly increased the total number of contralateral turnings (saline = 0 ± 0, 8Br-GMP = 3.7 ± 1.4, Mann–Whitney, $P=.013$). This effect was absent at the beginning of the analysis and became significant 8 min after drug injection ($P<.05$).

Pretreatment (10 min) with 100 nmol of MB did not prevent the increase in distance moved induced by 300 nmol of SIN-1 in the CIC [Drug × Time interaction, $F(24,252)=1.59$, Duncan test, $P<.05$; Fig. 5]. The total number of jumps were reduced ($P<.05$; Table 1), but the total time of galloping was not significantly changed ($P>.05$; Table 1). In the additional group of animals that received 200 nmol of MB, the drug also failed to reduce the distance moved induced by SIN-1 (MB + SIN-1 = 9663.8 ± 2340 cm, saline + SIN-1 = 10,245.32 ± 1790 cm) but decreased the number of jumps 4 min after drug injection (MB + SIN-1 = 0.17 ± 0.17, saline + SIN-1 = 1.6 ± 0.45, $P=.031$, Mann–Whitney).

AP7 administered before (10 min) SIN-1 completely prevented the increase in distance moved along the session [drug factor, $F(3,24)=4.88$, $P<.01$, Duncan test, $P<.05$; Fig. 6]. AP7 also prevented jumping ($P<.05$; Table 1) and

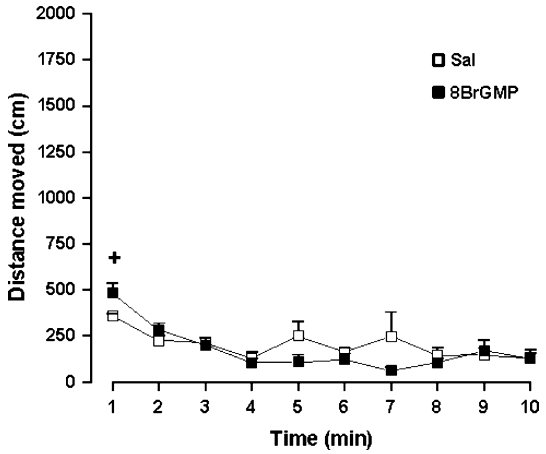

Fig. 4. Effects of saline (SAL, $n=5$) or 8-Br-GMP (225 nmol, $n=6$) microinjected into the CIC on the distance moved in the circular arena. Each point represents the mean distance moved in 1 min ± S.E.M. There was a significant Drug × Time interaction (MANOVA, $P<.05$). The cross signals a trend for difference between the groups (t test, $P=.07$).

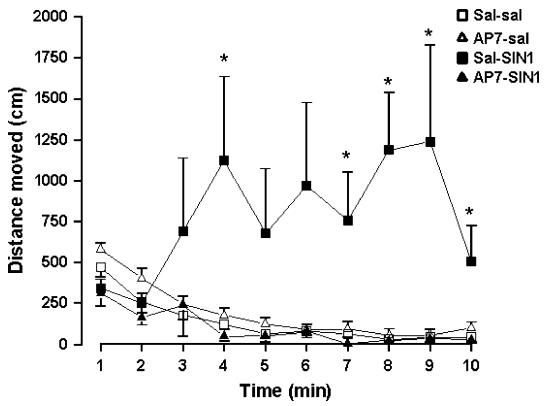

Fig. 6. Effects of saline + saline ($n=7$); AP7 2 nmol + saline ($n=7$); saline + SIN-1 300 nmol ($n=7$); AP7 + SIN-1 ($n=7$) microinjected into the CIC on the distance moved in the circular arena. Each point represents the mean distance moved in 1 min ± S.E.M. Saline + SIN-1 group was significantly different from the other groups along the session (MANOVA followed by the Duncan test, $P<.05$).

galloping behavior ($P < .05$; Table 1) induced by SIN-1 in the CIC.

4. Discussion

Microinjection of the NO donor SIN-1 into the CIC, but not into the cortical nucleus of the IC of rats, induced long-lasting (10 min) increase in the distance moved, galloping and jumps. The latter two behaviors are proposed as important ethological parameters for measuring flight reactions in rodents (Vargas and Schenberg, 2001).

In a previous work, we showed that SIN-1, in a dose-dependent manner, was also able to induce similar flight reactions when injected into the dlPAG (De Oliveira et al., 2000a). In both studies, there was approximately 3-min latency for the beginning of the reaction. This is compatible with the slow NO release produced by this compound through the formation of the intermediate compound SIN-1C (Feelish et al., 1989; Southam and Garthwaite, 1991). Corroborating this possibility, the response elicited by another drug that rapidly releases NO, DEA/NO (Southam and Garthwaite, 1991), showed a much smaller latency and duration (De Oliveira et al., 2000a).

In contrast to previous results obtained in the dlPAG using similar doses of 8-bromo-cGMP (De Oliveira et al., 2001), this membrane-permeable analogue of cGMP failed to increase locomotion in the open arena. There was, however, a tendency to increase locomotion during the first postinjection minute. Also, the drug increased the number of contralateral turnings at the end of the session. Other studies have also reported increased turning behavior after stimulation of the dlPAG, the superior colliculus or the IC (Cardoso et al., 1994; Northmore et al., 1988; Molchanov and Guimarães, 1999). This behavioral change was not seen after SIN-1 administration into the CIC, perhaps because it is incompatible with the wild running and galloping behavior induced by this drug.

The GC inhibitor MB, which in a lower dose (30 nmol) significantly prevented the effects of SIN-1 in the dlPAG (De Oliveira et al., 2000a), could only partially inhibit the flight reaction induced by this drug in the CIC, even at very high doses (200 nmol). Together, these results suggest a limited role of cGMP-dependent mechanisms on the flight reactions induced by exogenously administered NO in the CIC. However, NOS neurons are much more densely distributed in the dlPAG than in the CIC (Vincent and Kymura, 1992). Since NO can diffuse rather easily, it is possible that the volume of the MB solution injected and the interval between the two injections were not sufficient to inhibit GC in the whole area reached by NO.

NO participation in aversion-related mechanisms has already been shown in the dlPAG (De Oliveira et al., 2000a) and in other brain areas through molecular techniques. Restraint stress increases nNOS mRNA expression in the dlPAG, hypothalamic paraventricular nucleus and medial amygdala (De Oliveira et al., 2000b) and increases NADPHd activity in the dlPAG and IC (Krukoff and Khalili, 1997). Increased c-*fos* expression is observed in these areas after injection of SIN-1 into the dlPAG (De Oliveira et al., 2000a). Although the flight reactions elicited from the CIC are described as less explosive than those induced from the dorsal PAG (Brandão et al., 1999), the flight reactions induced by SIN-1 in the CIC were quite similar to that induced in the dlPAG.

Despite the reciprocal connection between the IC and the PAG (Herrera et al., 1988), flight reactions induced by stimulation of the IC seem to occur by a mechanism that does not necessarily depend on this structure since extensive lesions of the dlPAG do not modify the response (Bagri et al., 1992; Maisonnette et al., 1996). Corroborating this possibility, the increase in the distance moved induced by SIN-1 in the CIC was greater than that observed when the compound was administered into the dlPAG (data not shown). This makes it unlikely that the drug effects in the CIC are due to NO diffusion to the latter region.

The amygdala may have a role in the elaboration of aversive reactions after stimulation of the CIC. These structures are connected through the medial geniculate body of the thalamus so that acoustic inputs may reach the amygdala bypassing the neocortex (LeDoux et al., 1990). Aversive responses to conditioned sound previously paired with electric footshock are disrupted by bilateral lesions of the IC but not of the auditory cortex (LeDoux et al., 1984). Moreover, lesions of the central nucleus of the amygdala reduce the aversiveness of the electrical stimulation of the IC (Maisonnette et al., 1996) while lesion of the telencephalon does not (Brandão et al., 1988). Hence, it is possible that these aversive reactions do not necessarily involve cortical analysis but are integrated in a very primitive level of subcortical organization (Tomaz et al., 1988; Lamprea et al., 2002).

Stimulation of glutamate NMDA receptors in the CIC causes flight reactions that are prevented by AP7, an NMDA receptor antagonist (Cardoso et al., 1994; Pandossio and Brandão, 1999). NO donors can increase glutamate release in brain structures such as the striatum (Guevara-Gusman et al., 1994) and hypothalamus (Prast et al., 1996). Moreover, NMDA-induced glutamate release in the striatum is inhibited by NOS inhibitors (Bogdanov and Wurtman, 1997). Similar inhibition was found in the cortex of nNOS knockout mouse (Kano et al., 1998). In our study, the aversive effects of SIN-1 were prevented by AP7. Therefore, it is possible that NO donors induce flight reactions in the CIC by facilitating glutamate release.

In summary, SIN-1 (an NO donor) injected into the CIC, but not into the cortical nucleus of the IC, induces flight reaction. This reaction was partially inhibited by MB but completely prevented by AP7, suggesting that NO may

induce aversive responses in this structure through glutamate-dependent mechanisms.

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References

Bagri A, Di Scala G, Sandner G. Wild running elicited by microinjections of bicuculline or morphine into the inferior colliculus of rats: lack of effect of periaqueductal grey lesions. Pharmacol Biochem Behav 1992; 41:727–32.

Bogdanov MB, Wurtman RJ. Possible involvement of nitric oxide in NMDA-induced glutamate release in the rat striatum: an in vivo microdialysis study. Neurosci Lett 1997;197–201.

Brandão ML, Tomaz C, Borges PCL, Coimbra NC, Bagri A. Defense reaction induced by microinjections of bicuculline into the inferior colliculus. Physiol Behav 1988;44:361–5.

Brandão ML, Anseloni VZ, Pandossio JE, De Araújo JE, Castilho VM. Neurochemical mechanisms of the defensive behavior in the dorsal midbrain. Neurosci Biobehav Rev 1999;23:863–75.

Bredt DS, Snyder SH. Nitric oxide mediates the glutamate-linked enhancement of cGMP levels in the cerebellum. Proc Natl Acad Sci U S A 1989;86:9030–6.

Cardoso SH, Coimbra NC, Brandão ML. Defensive reactions evoked by activation of NMDA receptors in distinct sites of the inferior colliculus. Behav Brain Res 1994;63:17–24.

Casseday JH, Covey E. A neuroethological theory of the operation of the inferior colliculus. Brain Behav Evol 1996;311–36.

De Oliveira RW, Guimarães FS. Anxiolytic effect of methylene blue microinjected into the dorsal periaqueductal gray matter. Braz J Med Biol Res 1999;32:1529–32.

De Oliveira RW, Del Bel EA, Guimarães FS. Behavioral and c-*fos* expression changes induced by nitric oxide donors microinjected into the dorsal periaqueductal gray. Brain Res Bull 2000a;51:457–64.

De Oliveira RW, Del-Bel EA, Mamede-Rosa ML, Padovan CM, Deakin JF, Guimarães FS. Expression of neuronal nitric oxide synthase mRNA in stress-related brain areas after restraint stress. Neurosci Lett 2000b; 289:123–6.

De Oliveira RW, Del Bel EA, Guimarães FS. Effects of excitatory amino acids and nitric oxide on flight behavior elicited from the dorsolateral periaqueductal gray. Neurosci Biobehav Rev 2001;25:679–85.

Edelman GM, Gally JÁ. Nitric oxide: linking space and time in the brain. Proc Natl Acad Sci U S A 1992;89:11651–2.

Faye-Lund H, Osen KK. Anatomy of the inferior colliculus in rat. Anat Embryol 1985;171:1–20.

Feelish M, Ostrowsky J, Noack E. On the mechanisms of NO release form sydnonimines. J Cardiovasc Pharmacol 1989;14(Suppl. 11):S13–22.

Garthwaite J. Glutamate, nitric oxide and cell–cell signalling in the nervous system. Trends Neurosci 1991;14:60–7.

Garthwaite J, Charles SL, Chess-Williams R. Endothelium-derived relaxing factor release on activation of NMDA receptor suggests role as intercellular messenger in the brain. Nature 1988;336:385–8.

Garthwaite J, Garthwaite G, Palmer RMJ, Moncada S. NMDA receptor activation induces nitric oxide synthesis from arginine in rat brain slices. Eur J Pharmacol 1989;172:413–6.

Graeff FG. Minor tranquilizers and brain defense systems. Braz J Med Biol Res 1981;14:239–65.

Graeff FG. Neuroanatomy and neurotransmitter regulation of defensive behavior and related emotions in mammals. Braz J Med Biol Res 1994;27:811–29.

Guevara-Gusman R, Emson PC, Kendrick KM. Modulation of in vivo striatal release by nitric oxide and cyclic GMP. J Neurochem 1994; 62:807–10.

Guimarães FS, Carobrez AP, De Aguiar JC, Graeff FG. Anxiolytic effect in the elevated plus-maze of the NMDA receptor antagonist AP7 microinjected into the dorsal periaqueductal grey. Psychopharmacology 1991;103:91–4.

Guimarães FS, Aguiar JC, Del-Bel EA, Ballejo G. Anxiolytic effect of nitric oxide synthase inhibitor microinjected into the dorsal central grey. NeuroReport 1994;5:1929–32.

Herrera M, Sánchez Del Campo F, Ruiz A, Smith Agreda V. Neuronal relationships between the dorsal periaqueductal nucleus and the inferior colliculus (Nucleus comissularis) in the cat. A Golgi study. J Anat 1988;158:137–45.

Kano T, Shimizu-Sasamata M, Huang PL, Moskowitz MA, Lo EH. Effects of nitric oxide synthase gene knock out on neurotransmitter release in vivo. Neuroscience 1998;695–9.

Knowles RG, Palacios M, Palmer RMJ, Moncada S. Formation of nitric oxide from L-arginine in the central nervous system: a transduction mechanism for stimulation of the soluble guanylate cyclase. Proc Natl Acad Sci U S A 1989;86:5159–62.

Krukoff TL, Khalili P. Stress-induced activation of nitric oxide-producing neurons in the rat brain. J Comp Neurol 1997;377:509–19.

Lamprea MR, Cardenas FD, Vianna DM, Castilho VM, Cruz-Morales SE, Brandão ML. The distribution of Fos immunoreactivity in rat brain following freezing and escape responses elicited by electrical stimulation of the inferior colliculus. Brain Res 2002;186–94.

LeDoux JE, Sakaguchi A, Reis DJ. Subcortical efferent projections of the medial geniculate nucleus mediate emotional responses conditioned to acoustic stimuli. J Neurosci 1984;4:683–98.

LeDoux JE, Farb C, Ruggiero DA. Topographic organization of neurons in the acoustic thalamus that project to the amygdala. J Neurosci 1990; 10(4):1043–54.

Lin HC, Kang BH, Wan FJ, Huang ST, Tseng CJ. Reciprocal regulation of nitric oxide and glutamate in the nucleus tractus solitari of rats. Eur J Pharmacol 2000;407:83–9.

Maisonnette SS, Kawasaki MC, Coimbra NC, Brandão ML. Effects of lesions of the amygdaloid nuclei and substantia nigra on aversive responses induced by electrical stimulation of the inferior colliculus. Brain Res Bull 1996;40(2):93–8.

Melo LL, Cardoso SH, Brandão ML. Antiaversive action of benzodiazepines on escape behavior induced by electrical stimulation of the inferior colliculus. Physiol Behav 1992;51:557–62.

Molchanov ML, Guimarães FS. Defensive reaction induced by a metabotropic glutamate receptor agonist microinjected into the dorsal periaqueductal gray of rats. Braz J Med Biol Res 1999;32:1533–7.

Montague PR, Gancayco CD, Winn MJ, Marchase RB, Friedlander MJ. Role of NO production in NMDA receptor-mediated neurotransmitter release in cerebral cortex. Science 1994;263:973–7.

Northmore DP, Levine ES, Schneider GE. Behavior evoked by electrical stimulation of the hamster superior colliculus. Exp Brain Res 1988;73: 595–605.

Palmer RMJ, Ashton DS, Moncada S. Vascular endothelial cells synthesize nitric oxide from L-arginine. Nature 1988;333:664–6.

Pandossio JE, Brandão ML. Defensive reactions are counteracted by midazolam and muscimol and elicited by activation of glutamate receptor in the inferior colliculus. Psychopharmacolgy 1999;142:360–8.

Paxinos G, Watson C. The rat brain in stereotaxic coordinates. 3rd ed. San Diego: Academic Press; 1997.

Prast H, Lamberti C, Fisher H, Tran MH, Philippu A. Nitric oxide influences the release of histamine and glutamate in the rat hypothalamus. Naunyn-Schmiedeberg's Arch Pharmacol 1996;354:731–5.

Snyder SH, Bredt DS. Nitric oxide as a neuronal messenger. Trends Pharmacol Sci 1991;12:125–8.

Southam E, Garthwaite J. Comparative effects of some nitric oxide donors on cyclic cGMP levels in rat cerebellar slices. Neurosci Lett 1991;130: 107–11.

Tomaz C, Brandão ML, Bagri A, Carrive P, Schmitt P. Flight behaviour induced by microinjections of GABA antagonists into periventricular structures in the detelecephalated rats. Pharmacol Biochem Behav 1988;30:337–42.

Vargas LC, Schenberg LC. Long-term effects of clomipramine and fluoxetine on dorsal periaqueductal-grey evoked innate defensive behaviours of the rat. Psychopharmacology 2001;155:260–8.

Vincent S, Kymura H. Histochemical mapping of nitric oxide synthesis in the rat brain. Neuroscience 1992;46(4):755–84.