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Anxiety-like responses induced by nitric oxide within the BNST in mice: Role of CRF1 and NMDA receptors

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ABSTRACT

It has been shown that the bed nucleus of the stria terminalis (BNST) of rats contains nitrergic neurons, which are activated during animal exposure to aversive stimuli. The BNST is also populated by glutamatergic and corticotrophin releasing factor (CRFergic) neurons, which in turn are activated under stressful situations. Here we investigated the anxiogenic-like effects of intra-BNST injections of a nitric oxide (NO) donor, NOC-9 in mice. The role of CRFergic and glutamatergic systems on defensive behavior induced by NOC-9 was investigated with previous intra-BNST infusion of different doses of CP376395, a CRF type 1 receptor antagonist (CRF1), or AP-7, an NMDA (N-methyl-D-aspartate) receptor antagonist. Anxiety-like behavior was assessed immediately and 5 min after intra-BNST drug injection, exposing mice to a novel arena and to the elevated plus-maze (EPM; an anxiogenic situation). Results showed that NOC-9 provoked a short period (≈150 s) of freezing behavior in the novel arena and increased anxiety in the EPM. Both CP and AP-7 attenuated the anxiogenic-like effects of NOC-9 in the EPM without changing freezing behavior in the novel arena. When given alone (i.e. without prior intra-BNST injection of NOC-9), AP-7 (0.20 nmol), but not CP (0.75, 1.50, or 3.00 nmol), attenuated anxiety in mice exposed to the EPM. These results suggest that CRF1 and NMDA receptors located within the BNST differentially modulate aversive effects induced by NO production in this limbic forebrain structure.

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Introduction

Animal exposure to aversive stimuli induces fear- and anxiety-like behaviors (e.g., fight, flight, freezing and vocalization) as well as neuroendocrine (e.g., ACTH and corticosterone release) and autonomic (e.g., tachycardia, tachypnea, increased blood pressure and defecation) activations (Blanchard and Blanchard, 2003; Blanchard et al., 1993; Graeff, 1990). These defensive reactions are coordinated by various areas of the brain aversive system (Graeff, 1990). For instance, the midbrain periaqueductal gray, the hypothalamus, and the amygdaloid complex have been implicated in the modulation of behavioral, hormonal, and autonomic responses induced by dangerous and threatening situations (Bandler and Depaulis, 1991; Behbehani, 1995; Brandao et al., 2003; Carrive et al., 1997; Fanselow, 1991; Graeff et al., 1993; Lang et al., 1998; Lovick, 2000; Walker and Carrive, 2003).

The amygdaloid complex has been widely investigated as an important modulator site of defensive responses, such as freezing, the startle reflex, antinociception, and heart rate alteration (Rosen and Schulkin, 1998), and its extensive connections to the bed nucleus of the stria terminalis (BNST) have raised substantial interest in the identification of the role of the BNST in the modulation of anxiety-related responses (Alheid, 2003; Davis, 1998; Heimer, 2003; Sahuque et al., 2006; Schulkin et al., 2005; Ventura-Silva et al., 2012). For instance, electrical stimulation of the BNST elicits behavioral and endocrine changes that are similar to those induced by environmental stressors (Casada and Dafny, 1991; Dunn, 1987). In contrast, pharmacological inactivation of the BNST decreases the expression of conditioned or unconditioned responses induced by aversive situations (Sahuque et al., 2006).

Although many neurotransmitters (e.g., catecholamines, serotonin, and excitatory/inhibitory amino acids) have been implicated in the modulation of anxiety-like responses (Carobrez et al., 2001; Molchanov and Guimaraes, 2002; Vianna et al., 2001), the so-called atypical neurotransmitters, endocannabinoids and nitric oxide (NO), as well as the peptide corticotrophin-releasing factor (CRF) have more recently gained attention as important candidates in the modulation of emotional states (Esplugues, 2002; Fogaca et al., 2012; Guimaraes et al., 2005; Howlett et al., 2002; Piomelli, 2003). NO is a diffusible gas produced by the nitric oxide synthase (NOS) enzyme, through conversion of L-arginine to L-citrulline, using nicotinamide adenine dinucleotide phosphate (NADPH) and Ca^{2+} as co-factors (Lohse et al., 1998;

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Mayer et al., 1991). Among the three main NOS isoforms, neuronal NOS (nNOS) is the constitutive form expressed in neurons (de Oliveira et al., 2000). Proaversive effects (e.g., fight and flight reactions) have been demonstrated after injection of NO donors [e.g., SIN-1 (3-morpholinosylnomine hydrochloride) and NOC-9 (6-(2-hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-hexanamine)] into the periaqueductal gray (PAG) in rats and mice (Guimaraes et al., 2005; Miguel et al., 2012). However, while SIN-1 produces peroxynitrite, a substance that can provoke cytotoxic effects and cause other NO-independent cellular effects (Del Carlo and Loeser, 2002; Morot Gaudry-Talarmain et al., 1997), NOC-9, which is relatively stable at alkaline pH (>10.0), releases NO at physiological pH (7.4) without producing peroxynitrite (Ambalavanan et al., 1999; Del Carlo and Loeser, 2002; Seccia et al., 1996). Conversely, intra-PAG injection of nNOS inhibitors, guanylate cyclase inhibitors, and an NO scavenger provokes anxiolytic-like effects in rats exposed to the elevated plus-maze (EPM) (Guimaraes et al., 1994). In mice, intra-PAG injection of a highly selective and potent nNOS inhibitor, NΩ-propyl-L-arginine (NPLA), attenuates defensive behavior in the rat exposure test, a prey–predator interaction test (Carvalho-Netto et al., 2009) and blocks NMDA (N-methyl-D-aspartate)-induced anxiogenic-like effects in the EPM (Nunes-de-Souza et al., 2010).

The anxiogenic effects of NO may be related to its interference with the release of neurotransmitters, such as acetylcholine, GABA, dopamine, serotonin, glutamate, and CRF in distinct brain areas related to the defensive response (de Oliveira et al., 2000; Moreira and Guimaraes, 2004; Moreira et al., 2004). For instance, Guimaraes et al. (2005) have demonstrated that glutamate receptor antagonists are able to attenuate the proaversive effect of NO donors in the PAG. We have also observed that blocking the CRF type 1 receptor (CRF1) within the mouse PAG attenuates the proaversive effects produced by local injection of NOC-9, a NO donor (Miguel et al., 2012).

Very few studies have investigated the role of nitrergic transmission within the BNST in the context of aversive reactions. Previous studies have demonstrated that the nitrergic neurons located within the BNST are activated by aversive stimuli in rats (Beijamini and Guimaraes, 2006; Guimaraes et al., 2005). Moreover, glutamatergic (McElligott and Winder, 2008) and CRFergic neurons have been identified in the BNST (Cummings et al., 1983; Ju et al., 1989; Morin et al., 1999; Sakanaka et al., 1987; Swanson et al., 1983). However, while the anxiogenic-like effects of NO seem to be modulated by NMDA-glutamate and CRF1 receptors within other limbic brain structures (e.g., PAG, inferior colliculus) (de Araujo Moreira et al., 2003; Guimaraes et al., 2005; Miguel et al., 2012; Miguel and Nunes-de-Souza, 2008), it remains unclear whether these interactions play a role within the BNST on the modulation of anxiety-like responses. Taken together, these lines of evidence suggest that the facilitation of nitrergic neurotransmission in the BNST may induce anxiogenic-like behavior, which may in turn be modulated by CRF and NMDA-glutamate receptors. In this paper, we investigate the effects of intra-BNST injection of NOC-9, a NO donor, on the behavior of mice exposed to a novel arena and to the EPM. The effects of intra-BNST injection of CRF1 or NMDA receptor antagonists, n-(1-ethylpropyl)-3,6-dimethyl-2-(2,4,6-trimethylphenoxy)-4-pyridinamine hydrochloride (CP376395) and 2-amino-7-phosphonoheptanoic acid (AP-7), on the behavioral effects induced by local injection of NOC-9 are also investigated.

Materials and methods

Ethics

Experimental protocols were conducted according to the ethical principles of the Brazilian College of Animal Experimentation (COBEA) and approved by the local Research Ethics Committee (CEP/FCF/CAr-UNESP: protocol number 09/2011).

Subjects

Subjects were 360 male Swiss mice (Univ. Estadual Paulista — UNESP, SP, Brazil) weighing 25–35 g at testing. Mice were housed in groups of 10 per cage (size: 41 × 34 × 16 cm) and maintained on a 12-h light–dark cycle (lights on at 07:00 a.m.) in a temperature-controlled environment (23 ± 2 °C). Food and water were available ad libitum, except during brief testing periods. All mice were naïve at the beginning of the experiments and were used only once.

Drugs

Drugs used were NOC-9 [6-(hydroxy-1-methyl-2-nitrosohydrazino)-N-methyl-1-hexanamine], a diazeniumdiolate (NONOate), quick-releasing NO donor (Tocris Cookson Inc., Ballwin, USA). NOC-9 spontaneously dissociates in a pH-dependent, first-order process with half-lives of 1 min and 3 min at 37 °C and 22–25 °C, respectively (pH 7.4), to liberate 2 mol of NO per mole of parent compound (Hrabie et al., 1993; Keefer et al., 1996). The main physiological pathway for the degradation of nitric oxide acts via the heme group (Seregelyes et al., 2004). The NO diffuses very quickly throughout the membranes to the lumen of the vessels, where it reacts with hemoglobin (Hb) forming nitrates and methemoglobin (metHb) (Guix et al., 2005). Other drugs were CP 376395 [n-(1-ethylpropyl)-3,6-dimethyl-2-(2,4,6-trimethylphenoxy)-4-pyridinamine hydrochloride], a CRF1 receptor antagonist (Tocris Cookson Inc., Ballwin, USA) and AP-7 (2-amino-7-phosphonoheptanoic acid), a NMDA glutamatergic receptor antagonist. Doses were based on pilot and previous studies (Nunes-de-Souza et al., 2010; Sahuque et al., 2006). The NOC-9 was dissolved in a TRIS solution, pH 10.00. The rational for dissolving NOC-9 in a pH 10.00 solution was to prevent NO formation until the drug reached the cerebral issue, where it would be produced at physiological pH (7.4) (Seccia et al., 1996). The other drugs were dissolved in 0.9% physiological saline, which was used as a control.

Surgery and microinjection

Each mouse was bilaterally implanted with 7-mm stainless steel guide cannulae (26-gauge; Insight Equipamentos Científicos Ltd., Brazil) under anesthesia with intraperitoneal injection of ketamine (100 mg/kg) plus xylasin (10 mg/kg). Guide cannulae were fixed to the skull with dental acrylic and jeweler's screws. The stereotaxic coordinates for the BNST were 0.5 mm anterior to bregma, ±3.0 mm lateral to the midline, and 3.2 mm ventral to the skull surface (Paxinos and Franklin, 2001), with the guide cannulae angled 32°. Guide cannulae were positioned +1.0 mm dorsally to the structure. A dummy cannula (33 gauge, stainless steel wire; Fishtex Industry and Commerce) was inserted into each guide cannula to reduce the incidence of occlusion. Immediately after surgery, animals received an intramuscular injection of penicillin-G benzathine (Pentabiotic, 56.7 mg/kg in a 0.1 mL volume; Fort Dodge, Campinas, SP, Brazil) and a subcutaneous injection of the anti-inflammatory analgesic Banamine (0.5 mg/kg flunixin meglumine in a volume of 0.3 mL).

Five to seven days after surgery, different solutions were bilaterally injected into the BNST through microinjection units (33-gauge stainless steel cannula; Insight Equipamentos Científicos Ltd., Brazil) that extended 1.0 mm beyond the tip of the guide cannula. Each microinjection unit was attached to a 2-μl Hamilton microsyringe via polyethylene tubing (PE-10). The microinjection procedure consisted of gently restraining the animal, removing the dummy cannula, inserting the injection unit in situ and proceeding with the microinjection, leaving the needle for a further 30 s after the microinjection was completed. The drug/vehicle volume delivered was 0.2 μL/side. Confirmation of a successful procedure was obtained by monitoring the movement of a small air bubble in the PE-10 tubing.

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