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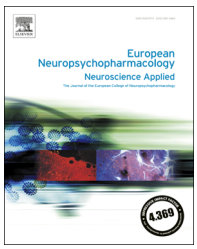

# Nitric oxide-cGMP-PKG signaling in the bed nucleus of the stria terminalis modulates the cardiovascular responses to stress in male rats

**Lucas Barretto-de-Souza[a,b], Mariane B. Adami[a], Leandro A. Oliveira[a,b], Lucas Gomes-de-Souza[a,b], Josiane O. Duarte[a], Jeferson Almeida[a,b], Carlos C. Crestani[a,b,*]**

[a]*Laboratory of Pharmacology, São Paulo State University (UNESP), School of Pharmaceutical Sciences, Araraquara, SP, Brazil*
[b]*Joint UFSCar-UNESP Graduate Program in Physiological Sciences, São Carlos, SP, Brazil*

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**Abstract**
The bed nucleus of the stria terminalis (BNST) constitutes an important component of neural substrates of physiological and behavioral responses to aversive stimuli, and it has been implicated on cardiovascular responses evoked by stress. Nevertheless, the local neurochemical mechanisms involved in BNST control of cardiovascular responses during aversive threats are still poorly understood. Thus, the aim of the present study was to assess the involvement of activation in the BNST of the neuronal isoform of the enzyme nitric oxide synthase (nNOS), as well as of signaling mechanisms related to nitric oxide effects such as soluble guanylate cyclase (sGC) and protein kinase G (PKG) on cardiovascular responses induced by an acute session of restraint stress in male rats. We observed that bilateral microinjection of either the nonselective NOS inhibitor Nω-Nitro-L-arginine methyl ester (L-NAME), the selective nNOS inhibitor Nω-Propyl-L-arginine (NPLA) or the sGC inhibitor 1H-[1,2,4]Oxadiazolo[4,3-a]quinoxalin-1-one (ODQ) into the BNST enhanced the tachycardic response and decreased the drop in tail cutaneous temperature evoked by acute restraint stress, but without affecting the increase on blood pressure. Bilateral BNST treatment with the selective PKG inhibitor KT5823 also facilitated the heart rate increase and decreased the drop in cutaneous temperature, in addition to enhancing the blood pressure increase. Taken together, these results provide evidence that NO released from nNOS and activation of sGC and PKG within the BNST play an

*Correspondence to: Laboratory of Pharmacology, Department of Natural Active Principles and Toxicology, School of Pharmaceutical Sciences, São Paulo State University - UNESP, Rodovia Araraquara-Jau Km 01, 14800-903 Araraquara, SP, Brazil. Fax: +55 16 3301 6980.
*E-mail address:* cccrestani@yahoo.com.br (C.C. Crestani).

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inhibitory influence on tachycardia to stress, whereas this signaling mechanism mediates the sympathetic-mediated cutaneous vasoconstriction.


## 1. Introduction

Physiological responses evoked during aversive situations are triggered by overlapping limbic circuits in the central nervous system (Dampney, 2015; Myers, 2017; Ulrich-Lai and Herman, 2009). The bed nucleus of stria terminalis (BNST) is a limbic structure located in the prosencephalon, which has been implicated in cardiovascular, neuroendocrine and behavioral responses to stress (Crestani et al., 2013; Davis et al., 2010). Specifically regarding the cardiovascular responses, it was demonstrated that reversible inactivation of the BNST enhanced the heart rate (HR) increase evoked by an acute session of restraint stress, but without affecting the blood pressure increase (Crestani et al., 2009). These results indicated an inhibitory role of the BNST on cardiac responses evoked by unconditioned aversive stimuli (Crestani et al., 2013). Conversely, a facilitatory role of the BNST on blood pressure and HR changes induced by contextual fear conditioning has been reported (Hott et al., 2012; Hott et al., 2017; Resstel et al., 2008). Taken together, these pieces of evidence suggested that role of the BNST regulating cardiovascular responses to stress is related to paradigm of aversive stimulus (e.g., conditioned versus unconditioned) (Crestani et al., 2013). Previous studies reported a role of local typical neurotransmitters in BNST control of cardiovascular responses to aversive threats, such as glutamate, acetylcholine and noradrenaline (Adami et al., 2017; Crestani et al., 2009; Gouveia et al., 2016; Hott et al., 2012, 2017). However, evidence of a possible involvement of atypical neurotransmitters, such as nitric oxide (NO), is still scarce.

The NO is synthesized from l-arginine by three isoforms of the enzyme nitric oxide synthase (NOS), which are called neuronal (nNOS), endothelial (eNOS) and induced (iNOS) (Alderton et al., 2001). The nNOS is proposed as the major isoform involved in the synthesis of NO in the brain (Garthwaite, 2008; Huang et al., 1993; Zhou and Zhu, 2009), and is widely expressed in mammalian encephalon (Bredt et al., 1991; Garthwaite, 2008; Vincent and Kimura, 1992). The activation of the soluble guanylate cyclase (sGC) enzyme, which converts guanosine triphosphate (GTP) to cyclic guanosine monophosphate (cGMP), has been described as the main pathway related to NO effects (Garthwaite, 2008). Despite the report of other signaling pathways (Craven and Zagotta, 2006; Rybalkin et al., 2003), the activation of the protein kinase G (PKG) is a prominent effector mechanism of cGMP (Garthwaite, 2008; Hofmann et al., 2006).

Neurons capable of synthesizing NO were identified within the BNST (Vincent and Kimura, 1992), and these neurons are activated by aversive stimuli (Guimaraes et al., 2005). Furthermore, systemic administration of nNOS inhibitors attenuated the neuronal activation in the BNST induced by an aversive stimulus (Silva et al., 2012), thus providing evidence that nNOS in the BNST is activated during stress. Previous studies evidenced a role of nNOS within the BNST in the control of baroreflex activity (Alves et al., 2009) and of cardiovascular responses to contextual fear conditioning (Hott et al., 2017). However, an involvement of signaling mechanisms related to NO effects, such as cGMP-PKG pathway, in BNST control of cardiovascular function has never been investigated. Furthermore, evidence of a role of BNST nitrergic neurotransmission in control of cardiovascular responses to unconditioned aversive stimulus is missing. Therefore, this study aimed to evaluate the hypothesis that activation of nNOS, sGC and PKG within the BNST modulate the cardiovascular responses evoked by an acute session of restraint stress in male rats.

## 2. Experimental procedures

### 2.1. Animals

Male Wistar rats with body weight ranging from 240 to 260 g were used. The animals were supplied by the animal breeding facility of the UNESP (Botucatu, SP, Brazil). The animals had free access to granulated feed and water, and were submitted to alternating light/dark cycles (lights on between 7:00 h a.m. and 7:00 h p.m.). All experimental procedures were carried out following protocol approved by Ethical Committee for Use of Animals (CEUA) of the School of Pharmaceutical Science/UNESP (protocol # 10/2013), which complies with Brazilian and international guidelines for animal use and welfare.

### 2.2. Surgical preparation

Five days before the trial, animals were anesthetized with tribromoethanol (250 mg/kg, i.p.), scalp was anesthetized with 2% lidocaine, and the skull was exposed. Then, using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA), stainless-steel cannulas (26G, 12 mm long) were bilaterally implanted into the BNST. Stereotaxic coordinates were: antero-posterior = +8.6 mm from interaural; lateral = 4.0 mm from the medial suture, ventral = −5.8 mm from the skull, with a lateral inclination of 23° (Paxinos and Watson, 1997). Dental cement was used to fix cannulas to the skull. After surgery, the rats were treated with a poly-antibiotic containing streptomycins and penicillins to prevent infection (560 mg/mL/kg, i.m.) and the nonsteroidal anti-inflammatory flunixine meglumine to provide post-operation analgesia (0.5 mg/mL/kg, s.c.).

One day before the experiment, animals were again anesthetized with tribromoethanol (250 mg/kg, i.p.) and a polyethylene cannula (a 4 cm segment of PE-10 bound to a 13 cm segment of PE-50) (Clay Adams, Parsippany, New Jersey, USA) was implanted into the abdominal aorta via the femoral artery for cardiovascular recording. The catheter was tunneled under the skin and exteriorized on the animal's dorsum. After the surgery, the nonsteroidal