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Dissociation in control of physiological and behavioral responses to emotional stress by cholinergic neurotransmission in the bed nucleus of the stria terminalis in rats

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ABSTRACT

The bed nucleus of the stria terminalis (BNST) is a forebrain structure implicated in physiological and behavioral responses to emotional stress. However, the local neurochemical mechanisms mediating the BNST control of stress responses are not fully known. Here, we investigated the involvement of BNST cholinergic neurotransmission, acting via muscarinic receptors, in cardiovascular (increase in blood pressure and heart rate and fall in tail skin temperature) and neuroendocrine (increase in plasma corticosterone) responses and behavioral consequences (anxiogenic-like effect in the elevated plus-maze) evoked by acute restraint stress in rats. Bilateral microinjection into the BNST of either the choline uptake inhibitor hemicholinium-3 (3 nmol/100 nl) or the muscarinic receptor antagonist methylatropine (3 nmol/100 nl) enhanced the heart rate increase and inhibited the anxiogenic-like effect observed in the elevated plus-maze evoked by restraint. However, neither hemicholinium-3 nor methylatropine affected the increase in blood pressure and plasma corticosterone levels and the fall in tail skin temperature. Facilitation of local cholinergic signaling by microinjection of the acetylcholinesterase inhibitor neostigmine (0.1 nmol/100 nl) into the BNST reduced restraint-evoked pressor and tachycardiac responses and the fall in tail cutaneous temperature, without affecting the increase in plasma corticosterone. All effects of neostigmine were completely abolished by local BNST pretreatment with methylatropine. These findings indicate an opposite role of BNST cholinergic neurotransmission, acting via local muscarinic receptor, in control of cardiovascular responses (inhibitory influence) and emotional consequences (facilitatory influence) evoked by restraint stress. Furthermore, present findings provide evidence that BNST control of neuroendocrine responses to stress is mediated by mechanisms others than local cholinergic signaling.

1. Introduction

A coordinated and complex set of physiological changes is generated during aversive threats, which constitute important adaptive responses maintaining the homeostasis (Dampney et al., 2008; Danese and McEwen, 2012; Sterling, 2012; Ulrich-Lai and Herman, 2009). Cardiovascular changes include blood pressure and heart rate (HR) increases; hemodynamics alterations characterized by vasodilatation in skeletal muscle and vasoconstriction in splanchnic, renal and cutaneous beds; and modulation of baroreflex activity (Blessing, 2003; Crestani et al., 2010; Dos Reis et al., 2014; Schadt and Hasser, 1998). The vasoconstriction in skin territory leads to a drop in the cutaneous temperature (Oliveira et al., 2015; Vianna and Carrive, 2005). Activation of the hypothalamic–pituitary–adrenocortical (HPA) axis is a characteristic neuroendocrine response to stress (Dickerson and Kemeny, 2004; Ulrich-Lai and Herman, 2009). In addition to physiological responses, stressful events also evoke emotional changes that can be identified in rodents through analysis of anxiety- and depression-like behaviors (Busnardo et al., 2013; Padovan and Guimaraes,

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2000; Sevgi et al., 2006). Although importance of stress responses, the neurobiological mechanisms involved in physiological and behavioral changes to aversive threats is poorly understood.

The brain triggers the responses during emotional stress through action of several neurochemical mechanisms (Joels and Baram, 2009; Ulrich-Lai and Herman, 2009). The bed nucleus of the stria terminalis (BNST) is a limbic forebrain structure implicated in control of cardiovascular and neuroendocrine functions and behavioral responses (Crestani et al., 2013; Davis et al., 2010). Both an inhibitory and facilitatory role of the BNST in cardiovascular and neuroendocrine responses to stress has been reported, depending on the type of aversive stimulus and the BNST region investigated (for review, see Crestani et al. (2013)). Regarding the behavioral reactivity to stress, it was demonstrated an involvement of the BNST in reduction of food intake (Ohata and Shibasaki, 2011) and anxiogenic effect evoked by aversive threats (Cecchi et al., 2002; Khoshbouei et al., 2002). Although these results indicate a role of the BNST in physiological and behavioral responses to stress, the local neurochemical mechanisms involved in this control was not fully elucidated.

Acetylcholine is released in several limbic structures during aversive stimuli (Mark et al., 1996; Nail-Boucherie et al., 2000). Indeed, convergent evidence has indicated a role of this signaling mechanism in controlling stress responses in several limbic structures (Bhatnagar et al., 1997; Fortaleza et al., 2009; Helm et al., 2004; Kubo et al., 2003). Cholinergic terminals as well as muscarinic and nicotinic cholinergic receptors were detected within the BNST (Clarke et al., 1985; Guo et al., 2012; Ruggiero et al., 1990; Spencer et al., 1986; Wamsley et al., 1984). Activation of muscarinic receptors seems to be the primary mechanism mediating acetylcholine actions in the BNST. For instance, modulation of firing activity of BNST neurons by acetylcholine is inhibited by muscarinic receptor antagonists, without being affected by nicotinic receptor blockers (Casada and Dafny, 1993; Guo et al., 2012). Moreover, microinjection of cholinergic agonists into the BNST evokes blood pressure increase and reduces baroreflex activity via muscarinic receptors (Alves et al., 2007; Nasimi and Hatam, 2011). A role of BNST cholinergic neurotransmission in control of neuroendocrine functions through local muscarinic receptors has also been described (Alves et al., 2011; Crestani et al., 2013).

Activation of muscarinic cholinergic receptors within the BNST evokes physiological responses similar to those observed during stress (Alves et al., 2007; Nasimi and Hatam, 2011). However, a possible involvement of BNST cholinergic neurotransmission in stress responses has never been evaluated. Therefore, in the present study we evaluated the involvement of cholinergic signaling within the BNST, acting via local muscarinic receptors, in cardiovascular and neuroendocrine responses and anxiogenic-like effects evoked by acute restraint stress. Restraint stress is a widely utilized experimental model to study the emotional and behavioral responses to stress. It is an unavoidable and unconditioned aversive situation that leads to HPA axis activation; cardiovascular responses; and behavioral changes, such as reduced exploration of open arms of the elevated plus-maze (EPM) (Busnardo et al., 2013; Choi et al., 2007; Dos Reis et al., 2014; Padovan and Guimaraes, 2000).

2. Material and methods

2.1. Animals

Ninety-five male Wistar rats weighting 240–260 g (60-days-old) were used in the present study. Animals were obtained from the animal breeding facility of the Univ. Estadual Paulista–UNESP (Botucatu, SP, Brazil) and were housed in plastic cages in a temperature-controlled room (24 °C) in the Animal Facility of the Laboratory of Pharmacology/School of Pharmaceutical Sciences/UNESP. They were kept under a 12:12 h light–dark cycle (lights on between 7:00 h and 19:00 h) and had free access to water and standard laboratory food. Housing conditions and experimental procedures were approved by the Ethical Committee for Use of Animals of the School of Pharmaceutical Science/UNESP, which complies with Brazilian and international guidelines for animal use and welfare.

2.2. Surgical procedures

Five days before the trial, rats were anesthetized with tribromoethanol (250 mg/kg, i.p.) and their heads fixed to a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). After scalp anesthesia with 2% lidocaine the skull was exposed and stainless-steel guide cannulas (26G, 12 mm-long) were bilaterally implanted into the BNST at a position 1 mm above the site of injection. Stereotaxic coordinates for cannula implantation into the BNST 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). Cannulas were fixed to the skull with dental cement and one metal screw. Immediately after end of the surgery procedures, the animals received a streptomycin and penicillin polyantibiotic formulation to prevent infection (560 mg/ml/kg, i.m.) and the non-steroidal anti-inflammatory flunixine meglumine for post-operation analgesia (0.5 mg/ml/kg, s.c.).

One day before the experiment, rats were anesthetized with tribromoethanol (250 mg/kg, i.p.) and a catheter (Clay Adams, Parsippany, NJ, USA) filled with a solution of heparin (50UI/ml, Hepamax-S®, Blausiegel, Cotia, SP, Brazil) diluted in saline (0.9% NaCl) was inserted into the abdominal aorta through the femoral artery for cardiovascular recording and blood samples collection. The catheter was tunneled under the skin and exteriorized on the animal's dorsum. Immediately after end of the surgery procedures, the non-steroidal anti-inflammatory flunixine meglumine was administered for post-operation analgesia (0.5 mg/ml/kg, s.c.). The animals were kept in individual cages during the postoperative period and experimental procedures and did not show signs of pain or discomfort on the trial day, thus indicating a proper recovery from surgeries.

2.3. Blood pressure and heart rate recording

The cannula implanted into the femoral artery was connected to a pressure transducer (DPT100, Utah Medical Products Inc., Midvale, UT, USA). The pulsatile blood pressure was recorded using an amplifier (Bridge Amp, ML224, ADInstruments, Australia) and an acquisition board (PowerLab 4/30, ML866/P, ADInstruments, NSW, Australia). Mean arterial pressure (MAP) and heart rate (HR) values were derived from the pulsatile arterial pressure.

2.4. Cutaneous temperature measurement

The tail skin temperature was recorded using a thermal camera (IRI4010, Infra Red Integrated Systems Ltd., Northampton, UK). The temperature was measured on five points of the animal's tail and the mean value was calculated for each recording (Busnardo et al., 2013; Oliveira et al., 2015).

2.5. Plasma corticosterone measurement

Plasma corticosterone concentration was measured by radioimmunoassay. The method was adapted from that described