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Blockade of 5-Ht_3 receptors in the septal area increases Fos expression in selected brain areas

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ABSTRACT

Serotonin is widely distributed throughout the brain and is involved in a multiplicity of visceral, cognitive and behavioral responses. It has been previously shown that injections of different doses of ondansetron, a 5-HT_3 receptor antagonist, into the medial septum/vertical limb of the diagonal band complex (MS/vDB) induce a hypertensive response in rats. On the other hand, administration of m-CPBG, a 5-HT_3 agonist, into the MS/vDB inhibits the increase of blood pressure during restraint stress. However, it is unclear which neuronal circuitry is involved in these responses. The present study investigated Fos immunoreactive nuclei (Fos-IR) in different brain areas following the blockade of 5-HT_3 receptors located in the MS/vDB in sham and in sinoaortic denervated (SAD) rats. Ondansetron injection into the MS/vDB increases Fos-IR in different brain areas including the limbic system (central amygdala and ventral part of the bed nucleus of the stria terminalis), hypothalamus (medial parvocellular parts of the paraventricular nucleus, anterodorsal preoptic area, dorsomedial hypothalamic nucleus), mesencephalon (ventrolateral periaqueductal gray region) and rhombencephalon (lateral parabrachial nucleus) in sham rats. Barodenervation results in higher Fos expression at the parvocellular and magnocellular part of the paraventricular nucleus, the lateral parabrachial nucleus, the central nucleus of amygdala, the locus coeruleus, the medial part of the nucleus of the solitary tract, the rostral ventrolateral medulla and the caudal ventrolateral medulla following 5-HT_3receptor blockade in the MS/vDB. Based on the present results and previous data showing a hypertensive response to ondansetron injected into the MS/vDB, it is reasonable to suggest that 5-HT_3receptors in the MS/vDB exert an inhibitory drive that may oscillate as a functional regulatory part of the complex central neuronal network participating in the control of blood pressure.

1. Introduction

Serotonin is widely distributed throughout the brain and participates in the control of a variety of visceral, cognitive and behavioral responses that are mediated by 15 different subtypes of receptors (Hoyer et al., 2002; Peters et al., 2013). The brain serotoninergic system has long been recognized as an important component of the adaptive mechanisms to stress (Graeff et al., 1996; Chaouloff et al., 1999; Carrasco and Van de Kar, 2003). The primary responses to stress are activation of the hypothalamic–pituitary–adrenocortical (HPA) axis and changes in the activity of the autonomic nervous system, leading to an increase in circulating glucocorticoids and in blood pressure and heart rate (Carrasco and Van de Kar, 2003; Ulrich-Lai and Herman, 2009). Under non-stress conditions, cardiovascular function may also be modulated by inhibitory or stimulatory drives exerted by the brain serotonin pathways on autonomic nervous system activity (Ramage and Villalón, 2008; Nalivaiko and Sgoifo, 2009). This has already been shown in several studies with a variety of serotonergic actions including sympathoexcitatory and inhibitory effects associated with hypertensive or hypotensive responses. The nature of the responses observed appears to depend on the type of serotonin receptor studied and the anatomical location of the brain serotonergic component (Frishman and Grewall, 2000; Ramage, 2001).

Studies carried out in this laboratory have shown that central serotonin pathways exert a tonic inhibitory effect mediated by 5-HT_3 receptors on blood pressure in rats that appears to result from a sympathoinhibitory-related mechanism (Ferreira et al., 2004). In

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addition, central 5-HT_3 receptors have been shown to be important for cardiovascular responses to stress (Ferreira et al., 2004; Urzedo-Rodrigues et al., 2011).

The medial septum/vertical limb of the diagonal band complex (MS/vDB) is a brain site that is involved with the genesis, expression and control of visceral emotional responses (Brady and Nauta, 1953) and participates in cardiovascular regulation (Calaresu et al., 1976; Tavares and Corrêa, 2003; Tavares et al., 2007). This region contains 5-HT_3 receptors (Kilpatrick et al., 1987; Chen and Lawrence, 2000) and receives serotonergic neurons originating in the medial raphe nucleus (McQuade and Sharp, 1997). Previous data from our laboratory have shown that 5-HT_3 receptor blockade in the MS/vDB promotes a hypertensive response that appears to depend on angiotensinergic activity at this site and may involve sympathetic activity. In addition, the administration of m-CPBG, an agonist of $5HT_3$, into the MS/vDB inhibits the increase in blood pressure during restraint stress (Urzedo-Rodrigues et al., 2011).

Although the MS/vDB is part of the limbic structures responsible for coping with stress and participates in the control of cardiovascular function, it is unclear which brain circuitries are involved in the responses to 5-HT_3 receptor activity in this area. Our hypothesis is that serotonin released in the MS/vDB may bind to 5-HT_3 receptors and activate a downstream brain circuitry that modulates blood pressure. To investigate this issue further, the present study used Fos expression to identify the brain areas activated following blockade of 5-HT_3 receptors in the MS/vDB. Since a hypertensive response was found in a previous study following blockade of the 5-HT_3 receptors in the MS/vDB and since it is believed that this increase in blood pressure could increase Fos expression (Miura et al., 1994; Graham et al., 1995), the present study also analyzed the pattern of Fos expression in the brain of sinoaortic denervated (SAD) rats to exclude the effect of the increase in blood pressure per se on Fos expression.

2. Material and methods

2.1. Animals

Adult male Wistar rats (300 ± 10 g), kept under controlled light (lights on from 5 AM to 7 PM) and temperature (22 ± 2 °C) conditions, were used in these experiments. The animals had free access to tap water and laboratory chow (Nuvital Nutrientes Ltda., Curitiba, Brazil). A total of 35 rats were used. The experimental protocols were performed in accordance with the regulations for the care of laboratory animals and were approved by the Institution's Animal Ethics Committee (CEUA-ICS-UFBA # 025/2012).

2.2. Surgical procedures

The MS/vDB was cannulated under ketamine/xylazine (80/11.5 mg/kg i.p.) anesthesia five days before the experimental sessions. In brief, after positioning the rat in a stereotaxic apparatus (David Kopf Instruments, USA), a chronic 22-gauge guide cannula was implanted according to the following coordinates: anteroposterior = 0.5 mm anterior to the bregma; lateral = 0.0 mm; vertical = 5.0 mm below the skull (Paxinos and Watson, 1998). The guide cannula was positioned 1 mm above the intended microinjection site and fixed to the skull with metal screws and dental cement. After surgery, the animals were treated with an antibiotic combination of penicillin and streptomycin (Pentabiótico, Fort Dodge Ltda., Brazil; 0.2 ml/rat s.c.) and the analgesic/anti-inflammatory agent, flunixin meglumine (2.5 mg/kg s.c.).

Sinoaortic denervation (SAD) was performed according to Krieger's technique (Krieger, 1964). Briefly, 48 h before the experiments, the animals were anesthetized (ketamine/xylazine; 80/11.5 mg/kg i.p.) and the aortic baroreceptors were denervated under microscopic visualization (×25) by removing a 1 cm segment of the cervical sympathetic trunk, a section of the superior laryngeal nerve at its origin in the nodose ganglion, and resecting all the tissue surrounding the vagus nerve. In addition, the carotid bifurcation was stripped, removing the carotid branches from the neural fibers, the carotid chemoreceptors and the connective tissue. Sham denervation consisted of a similar surgical exposure but without sectioning any neural or vascular structures, and lasted 15–40 min. Immediately afterward, a catheter (PE50) filled with heparinized saline solution (100 U/ml) was inserted into the left carotid artery and the femoral vein and exteriorized at the nape of the animal's neck to permit blood pressure recording.

2.3. Drugs and microinjections

Ondansetron, a specific 5-HT_3 receptor antagonist (Gaster and King, 1997) was purchased from Sigma Chemical, Co., St. Louis, MO. The compound was dissolved in a sterile isotonic saline solution. Central injections were given using a Hamilton microsyringe connected through polyethylene tubing to a 30-gauge injector that was 1 mm longer than the guide cannula. A total volume of 200 nl was slowly injected (60 s) and the injector remained in the guide cannula for additional 60s.

2.4. Blood pressure recording

The animals were allowed to move around freely in their home cage while their blood pressure was continuously monitored through the carotid catheter connected to a blood pressure transducer (World Precision Instruments) whose signal was amplified and digitally recorded by an analog-to-digital interface (AqDados — application for data acquisition, Lynx Tecnologia Eletrônica Ltda., São Paulo, Brazil, version 7.0) and recorded (1 kHz) on a microcomputer for later analysis. Mean arterial pressure (MAP) was calculated from the systolic and diastolic pressure measurements, and heart rate (HR) was determined from pulsatile arterial pressure using the AcqKnowledge software program (version 3.5.7, developed by Biopac Systems, Inc., California, USA).

A bolus intravenous injection of phenylephrine (6 μg/kg; Sigma Chemical, Co., St. Louis, MO) was used to evaluate baroreflex sensitivity. Maximal changes in blood pressure and heart rate were recorded. In sham rats, phenylephrine elicited reflex bradycardia while in SAD rats the baroreflex control of heart rate was eliminated. A typical tracing of pulsatile arterial pressure and the corresponding heart rate confirming the efficacy of the barodenervation (Panel A) and the effect of the ondansetron injection into the MS/vDB SAD rats (Panel B) is shown in Fig. 11.

2.5. Histological procedure

The animals in all the study groups were anesthetized (thionembutal, 50 mg/kg, i.p.) and transcardially perfused with 400 ml of phosphate buffered saline (0.1 M PBS, pH 7.4) followed by 4% paraformaldehyde (pH 7.4). After these procedures, the brains were removed and stored overnight in the same fixative at 4 °C and then submerged in 30% sucrose solution for at least two days. The brains were serially sectioned at 40 μm in a cryostat. Two series of serial coronal tissue sections were obtained from each brain, *series I* being used for Fos immunohistochemistry and *series II* (adjacent to series I) counterstained with cresyl violet for neuroanatomical identification of brain areas and to determine the position of the cannula. A photomicrograph showing the typical appearance of the brain parenchyma surrounding the MS/vDB after cannulation and injection of Evans Blue dye is shown in Fig. 1. Only the data from the 28 animals in which the cannulas were strictly within the MS/vDB were analyzed and taken into consideration in the interpretation of the effects of ondansetron.