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ANGIOTENSINERGIC NEUROTRANSMISSION IN THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS MODULATES THE PRESSOR RESPONSE TO ACUTE RESTRAINT STRESS IN RATS

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Abstract—We tested the hypothesis that the angiotensinergic neurotransmission, specifically in the paraventricular nucleus of the hypothalamus (PVN), is involved in the cardiovascular modulation during acute restraint stress (RS) in rats. The intravenous pretreatment with the angiotensin AT1 receptor antagonist losartan (5 mg/kg) inhibited the pressor response to RS, but did not affect the concomitant RS-evoked tachycardiac response. Because similar effects were observed after the PVN pretreatment with $CoCl_2$, and considering the high density of angiotensin receptors reported in the PVN, we studied the effect of the pretreatment of the PVN with either losartan or the angiotensin-converting enzyme (ACE) inhibitor lisinopril on the RS-evoked cardiovascular response. The bilateral microinjection of losartan (0.5 nmol/100 nL) or lisinopril (0.5 nmol/100 nL) into the PVN inhibited the RS-related pressor response without affecting the tachycardiac response, suggesting that the PVN angiotensinergic neurotransmission modulates the vascular component of the stress response. Finally, to exclude the possibility that centrally injected drugs could be leaking to the circulation and acting on peripheral vascular receptors, we tested the effect of the intravenous pretreatment with either losartan (0.5 nmol/animal) or lisinopril (0.5 nmol/animal), assuming the hypothesis of a total spread of drugs from the CNS to the peripheral circulation. When animals were pretreated with such doses of either losartan or lisinopril, the cardiovascular RS-evoked response was not affected, thus indicating that even if there were a complete leakage of the drug to the periphery, it would not affect the cardiovascular response to RS. This observation favors the idea that the effect of the intravenous injection of 5 mg/kg of losartan on the RS-related cardiovascular response would be explained by an action across the blood–brain barrier, possibly in the PVN. In conclusion, the results suggest that an angiotensinergic neurotransmission in the PVN acting on AT1-receptors modulates the vascular component of the RS-evoked cardiovascular response.

Key words: angiontensin AT1 receptor, angiotensin-converting enzyme, paraventricular nucleus of the hypothalamus, restraint stress.

INTRODUCTION

Acute restraint (RS) is an unavoidable stress model, which is known to elicit several emotional and autonomic responses. These autonomic responses include mean arterial pressure (MAP) and heart rate (HR) increases, skeletal muscle vasodilatation and cutaneous vasoconstriction that leads to a drop in skin temperature and is followed by body temperature increase (Barron and Van Loon, 1989; Irvine et al., 1997; Bhatnagar et al., 1998; McDougall et al., 2000; Vianna and Carrive, 2005; Tavares and Correa, 2006). Besides the well-evidenced involvement of both circulating and brain catecholamines in the mediation of the autonomic response elicited by RS, there is also evidence pointing to the participation of central and peripheral renin-angiotensin systems (RAS) in stress (Saavedra and Benicky, 2007).

The paraventricular nucleus of the hypothalamus (PVN) is in close proximity to the walls of the third ventricle, being divided into parvocellular and magnocellular neurons that regulate autonomic and neuroendocrine functions (Swanson and Kuypers, 1980). The PVN is connected to limbic structures (Canteras et al., 1995; Risold and Swanson, 1997; Ongur et al., 1998), which are involved in the mediation of behavioral responses (Leibowitz, 1978; Norrholm et al., 2005; Blume et al., 2008), as well as with brainstem structures involved in cardiovascular regulation (Kuypers and Maisky, 1975; Coote et al., 1998; Shafton et al., 1998; Pyner and Coote, 2000). Its electrical or chemical stimulation with *N*-methyl-D-aspartic or glutamate has been reported to cause cardiovascular responses, which are characterized by increases in blood pressure (BP), HR and sympathetic nerve activity (Ciriello and Calaresu, 1980; Kannan et al., 1989; Li et al., 2001; Busnardo et al., 2009).

There is evidence indicating a PVN involvement in the modulation of sympathetic responses related to the

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Abbreviations: ACE, angiotensin-converting enzyme; ACSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; HR, heart rate; MAP, mean arterial pressure; PAP, pulsatile arterial pressure; PVN, paraventricular nucleus of the hypothalamus; RAS, renin-angiotensin systems; RS, acute restraint stress.

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exposure to stressful conditions (Coote et al., 1998). Also, PVN neurons were reported to be activated during the exposure to stressful stimuli (Imaki et al., 1998; Day et al., 2005; Girotti et al., 2006). The role of the PVN in cardiovascular modulation during stressful situations, such as the RS, is well established (Tavares et al., 2009; Busnardo et al., 2010).

Previously, we have shown that a PVN inhibition by local injection of the nonselective synapse inhibitor $CoCl_2$ markedly reduced the RS-evoked pressor response, without affecting the tachycardiac response (Tavares et al., 2009; Busnardo et al., 2010), thus suggesting that a local PVN neurotransmission is involved in the neural pathway that controls the RS-evoked pressor response.

The angiotensinergic neurotransmission in the PVN has been suggested to exert excitatory effects on the cardiovascular system (Li et al., 2006). Besides, a RS-evoked increase in the expression of angiotensin AT1-receptors in the PVN has been reported (Castren and Saavedra, 1988), thus suggesting an activation of the angiotensinergic neurotransmission in the PVN during exposure to aversive conditions.

Regardless of the above evidence, the role of PVN angiotensin neurotransmission in the modulation of stress-evoked cardiovascular responses has not yet been elucidated. Therefore, in the present study, we evaluated the involvement of the angiotensinergic neurotransmission of the PVN in the RS-evoked cardiovascular response. For this purpose, the PVN was bilaterally treated with either the angiotensin AT1 receptor antagonist losartan or the angiotensin-converting enzyme inhibitor lisinopril, and we verified their effects on stress-evoked physiological responses.

EXPERIMENTAL PROCEDURES

Ethical approval

Housing conditions and experimental procedures were approved by the University of São Paulo Animal Ethical Committee (N° 074/2012), which complies with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society.

Subjects

Male Wistar rats weighing approximately 250 g were used in the present experiment. Animals were housed in plastic cages in a temperature-controlled room (25 °C) in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. Animals were kept under a 12:12-h light–dark cycle (lights on between 06:00 am and 6:00 pm). Animals had free access to water and standard laboratory food, except during the experimental period.

Surgical procedures

Five days before experiments, animals were anesthetized with tribromoethanol (250 mg/kg i.p., SIGMA, St. Louis, Missouri, USA) and their heads were fixed to a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). The skull was surgically exposed and trepanned with a dental drill at 1.9 mm from the medial line and 7.2 mm anterior to the interaural line (Paxinos and Watson, 1986). Stainless steel guide cannulas (24 G, 13 mm-long) were bilaterally lowered 7 mm from the skull, at a 12° angle. Guide cannulas were positioned 1 mm above the intended stimulation sites and fixed to the skull with a metal screw and dental cement. After surgery, animals received a poly-antibiotic (Pentabiotico®, Fontoura-Wyeth, SP, Brazil, 80.000 UI i.m.), with streptomycins and penicillins, to prevent infection and the nonsteroidal anti-inflammatory flunixine meglumine (Banamine®, Schering Plough, RJ, Brazil) for post operation analgesia.

One day before the trial, animals were anesthetized with tribromoethanol (250 mg/kg i.p.) and a polyethylene catheter was implanted into the femoral artery for cardiovascular recording and into the femoral vein for drug administration when it was necessary. The catheter was exposed on the dorsum of the animal and attached to the skin. After surgical procedures, animals were injected with the nonsteroidal anti-inflammatory flunixine meglumine (Banamine®, Schering Plough, RJ, Brazil; 2.5 mg/kg s.c.).

Cardiovascular recording

On the day of the experiment, the arterial cannulas were connected to a pressure transducer. The pulsatile arterial pressure (PAP) was recorded using an amplifier (model 7754A, Hewlett Packard, Palo Alto, CA, USA) coupled to a computerized acquisition system (MP100A, Biopac, Santa Barbara, CA, USA). MAP and HR values were derived from the PAP data using the Acknowledge III software (Biopac, USA). The MAP was calculated according to the equation: diastolic pressure + (systolic − diastolic)/3. The HR (beats/min, bpm) was calculated from PAP peak intervals integrated every 6 s.

Acute restraint stress

Animals were submitted to restraint by placing each rat into a plastic cylindrical restraint tube (diameter 6.5 cm, length 15 cm), ventilated by holes (1-cm diameter) that comprised approximately 20% of the tube surface. Restraint lasted for 60 min. Immediately after the end of the exposure to the stress, rats were returned to their home cages. Each rat was submitted to only one session of restraint in order to avoid habituation.

Drugs and solutions

The angiotensin AT1 receptor antagonist losartan (Merck, Sharp & Dohme, Rahway, NJ, USA) and angiotensin-converting enzyme inhibitor lisinopril (Merck, Sharp & Dohme, Rahway, NJ, USA) were dissolved in artificial cerebrospinal fluid (ACSF) that had the following composition: NaCl 100 mM, Na_3PO_4 2 mM, KCl 2.5 mM, $MgCl_2$ 1.0 mM, $NaHCO_3$ 27 mM, $CaCl_2$ 2.5 mM, pH 7.4 when were administrated centrally or these drugs were dissolved in saline (NaCl 0.9%) when were