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α1 and α2-adrenoceptors in the medial amygdaloid nucleus modulate differently the cardiovascular responses to restraint stress in rats

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ABSTRACT

Medial amygdaloid nucleus (MeA) neurotransmission has an inhibitory influence on cardiovascular responses in rats submitted to restraint, which are characterized by both elevated blood pressure (BP) and intense heart rate (HR) increase. In the present study, we investigated the involvement of MeA adrenoceptors in the modulation of cardiovascular responses that are observed during an acute restraint. Male Wistar rats received bilateral microinjections of the selective α1-adrenoceptor antagonist WB4101 (10, 15, and 20 nmol/100 nL) or the selective α2-adrenoceptor antagonist RX821002 (10, 15, and 20 nmol/nL) into the MeA, before the exposure to acute restraint. The injection of WB4101 reduced the restraint-evoked tachycardia. In contrast, the injection of RX821002 increased the tachycardia. Both drugs had no influence on BP increases observed during the acute restraint. Our findings indicate that α1 and α2-adrenoceptors in the MeA play different roles in the modulation of the HR increase evoked by restraint stress in rats. Results suggest that α1-adrenoceptors and α2-adrenoceptors mediate the MeA-related facilitatory and inhibitory influences on restraint-related HR responses, respectively.

1. Introduction

Stress elicits both autonomic and behavioral responses, which involve an activation of the hypothalamo-pituitary–adrenal axis as well as the sympathetic nervous system [1]. Such responses are modulated by several limbic structures in the central nervous system [2–7]. Among these structures, the amygdaloid complex is known to modulate stress-related behavior and is connected with hypothalamic and brainstem areas that are involved with cardiovascular regulation [8–10]. In particular, the medial amygdaloid nucleus (MeA) of the amygdaloid complex is involved in the modulation of stress related responses and in cardiovascular control [1,11]. Electrical stimulation of the MeA was reported to evoke increased blood pressure and heart rate [12].

Stress is known to evoke cardiovascular changes that are characterized by moderate hypertension and intense tachycardia [4,7,13–20]. Pressor responses evoked by foot-shock in spontaneously hypertensive rats increased blood pressure and heart rate with contextual fear conditioning [6,21], while with exposure of borderline hypertensive rats to acute noise stress [22] they were reduced after electrolytic lesion of the amygdala, thus suggesting an involvement of the amygdala in the modulation of cardiovascular responses caused by stress. Moreover, increased c-fos expression in the MeA was observed after exposure to a number of stressors, such as a novel environment, swimming, social interaction and acute restraint stress [23,24].

MeA inhibition with muscimol was reported to attenuate the pressor response evoked during acute restraint [19]. Also, previous work from our laboratory showed that bilateral microinjection of the unspecific neurotransmitter blocker Cobalt chloride (CoCl2) into the MeA caused enhanced restraint-related tachycardiac response [4]. These data suggest the involvement of this area in cardiovascular modulation during stress.

The MeA receives substantial noradrenergic innervations originating in the A6 neurons in the locus coeruleus (LC) and other groups of noradrenergic neurons located in the lateral tegmental area [25,26]. Furthermore, studies of expression of mRNA and binding detected the presence of noradrenergic receptor subtypes α and β in the MeA [27,28], suggesting the existence of the noradrenergic system in this area.

Noradrenaline (NA) is a neurotransmitter with an important role in central cardiovascular regulation and is an important mediator in several structures of the central nervous system. Cardiovascular responses were observed after microinjection of NA into brain

Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; BP, blood pressure; HR, heart rate; MAP, mean arterial pressure; MeA, medial amygdaloid nucleus; PAP, pulsatile arterial pressure; RS, restraint stress.

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regions involved in the modulation of the cardiovascular system, such as the nucleus of the solitary tract (NTS); [29], the medial prefrontal cortex (CPFM); [30], the periaqueductal gray area (PAG); [31], the lateral septal area (ASL); [32], the bed nucleus of the stria terminal (BST); [33], the supraoptic nucleus (SON); [34] and the MeA [35].

Studies using microdialysis have shown increased levels of NA in several forebrain regions of the limbic system, mainly in the MeA of animals subjected to stress situations [1,36–40]. Although the noradrenergic system activation occurs in the MeA during stress, the role of this activation in the mediation of stress-induced cardiovascular responses has not yet been evaluated.

Several studies in the literature support the idea that acute restraint is an unavoidable aversive stimulus eliciting sustained BP and HR increase [41–46]. In this way, in the present study, we tested the hypothesis that noradrenergic neurotransmission within the MeA mediates the cardiovascular responses evoked by restraint stress. For that purpose, we pretreated the MeA with adrenoceptor antagonists and submitted the rats to acute restraint, after which we observed the cardiovascular responses evoked by this stressor.

2. Materials and methods

2.1. Subjects

Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto (no. 057/2009). Male Wistar rats weighing 250–280 g were used in the present experiment. Animals were housed individually in plastic cages in a temperature-controlled room (25 °C) at the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto. Animals were kept under a 12:12 h light–dark cycle (lights on at 06:00). Animals had free access to water and standard laboratory food, except during the experimental period. Rats were transported to the experiment room and remained in their own cages until the experimental restraint procedure. Experiments were performed during the morning period to minimize possible circadian rhythm interferences.

2.2. Surgical preparation

Animals were anesthetized with tribromoethanol (250 mg/kg i.p.) and their heads were fixed to a stereotaxic apparatus (Stoelting, USA). The skull was surgically exposed and trepanned with a dental drill at a point located 3.4 mm from midline and 6.2 mm anterior to the interaural line, according to the rat brain atlas of Paxinos and Watson [47]. Bilateral stainless steel guide cannulae (26G, 15 mm-long) were lowered 8.0 mm from the skull. Guide cannulae were positioned 1 mm above the intended injection sites and fixed to the skull by a metal screw and dental cement. Animals were allowed to recover for 48 h before a polyethylene catheter was implanted into the femoral artery under anesthesia, for chronic recording of arterial BP and HR. The catheter was exposed on the dorsum of the animals and attached to the skin, allowing arterial pressure recording of unanesthetized rats 24 h after surgery.

2.3. Drug microinjection into the MeA

Injections were performed in a volume of 100 nL. For microinjections, we used a 1 μL syringe (KH7001, Hamilton, USA) connected to a 33G injection needle (Small Parts Inc, FL, USA) by PE-10 polyethylene tubing. The injection needle was 1.0 mm longer than the guide cannula.

2.4. Measurement of cardiovascular responses

Pulsatile arterial pressure (PAP) was recorded using an amplifier (model 7754A, Hewlett Packard, USA) coupled to a computerized acquisition system (MP100, Biopac, USA). Mean arterial pressure (MAP) and HR were derived from PAP data using the AcqKnowledge 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 that were integrated each 6 s.

2.5. Drugs utilized in the experimental procedures

The following drugs were used: Vehicle artificial cerebrospinal fluid (ACSF) had the following composition: 100 mM NaCl; 2 mM Na 3 PO 4; 2.5 mM KCl; 1 mM MgCl 2; 27 mM NaHCO 3; 2.5 mM CaCl 2 and pH = 7.4), RX821002 (α2-adrenoceptor antagonist), WB4101 (α1-adrenoceptor antagonist) urethane (Sigma, USA), tribromoethanol (Aldrich, USA), streptomycins and penicillins (Pentabiotico, Fort Dodge, Brazil), flunixine meglumine (Banamine, Schering Plough, Brazil).

2.6. Experimental procedure: acute restraint

Animals were transported to the experimental room in their home cages. They were allowed a 1 h period to adapt to the conditions of the experimental room, such as sound and illumination, before starting blood pressure and heart rate recording. The experimental room was acoustically isolated and had a constant background noise generated by an air exhauster. At least another 20 min period was allowed for baseline recording before experiments were initiated. After recording baseline values, bilateral microinjections of drugs or vehicle were made into the MeA, each animal receiving only one microinjection per brain side. Care was taken to start injection whenever a stable blood pressure and especially a stable heart rate recording were observed. The injection needle was slowly introduced through the guide cannula without touching or restraining the animals. Ten min later, the animals were submitted to restraint, which was initiated by putting animals into a small plastic cylindrical restraining tube (diameter = 6.5 cm and length = 15 cm). Restraint lasted for 60 min and immediately after, the animals were returned to their cages. Each animal was submitted to one session of restraint in order to prevent habituation.

Animals were divided into seven experimental groups: (1) aCSF group, vehicle microinjected into the MeA, (2) WB4101 dose 10 nmol group, (3) WB4101 dose 15 nmol group, (4) WB4101 dose 20 nmol group, (5) RX821002 dose 10 nmol group, (6) RX821002 dose 15 nmol group and (7) RX821002 dose 20 nmol group. The vehicle and all drugs were microinjected bilaterally into the MeA.

2.7. Histological determination of the microinjection sites

At the end of the experiments, animals were anesthetized with urethane (1.25 g/kg i.p.) and 100 nL of 1% Evan's blue was injected into the brain as a marker at the injection site. Animals were submitted to intracardiac perfusion with 0.9% NaCl followed by 10% formalin. Brains were removed, and post-fixed for 48 h at 4 °C and serial 40 μm-thick sections were cut with a cryostat (CM1900, Leica, Germany). Sections were stained with 1% violet cresyl for optical microscopy analysis. The actual placement of the microinjection needles was determined by analyzing serial sections, according to the rat brain atlas of Paxinos and Watson [47].