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Modulation of arterial pressure by P2 purinoceptors in the paraventricular nucleus of the hypothalamus of awake rats

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ABSTRACT

In the present study we evaluated the role of purinergic mechanisms in the PVN on the tonic modulation of the autonomic function to the cardiovascular system as well on the cardiovascular responses to peripheral chemoreflex activation in awake rats. Guide-cannulae were bilaterally implanted in the direction of the PVN of male Wistar rats. Femoral artery and vein were catheterized one day before the experiments. Chemoreflex was activated with KCN (80 μg/0.05 ml, iv) before and after microinjections of P2 receptors antagonist into the PVN. Microinjection of PPADS, a non selective P2X antagonist, into the PVN ($n=6$) produced a significant increase in the baseline MAP (99 ± 2 vs 112 ± 3 mmHg) and HR (332 ± 8 vs 375 ± 8 bpm) but had no effect on the pressor and bradycardic responses to chemoreflex activation. Intravenous injection of vasopressin receptors antagonist after microinjection of PPADS into the PVN produced no effect on the increased baseline MAP. Simultaneous microinjection of PPADS and KYN into the PVN ($n=6$) had no effect in the baseline MAP, HR or in the pressor and bradycardic responses to chemoreflex activation. We conclude that P2 purinoceptors in the PVN are involved in the modulation of baseline autonomic function to the cardiovascular system but not in the cardiovascular responses to chemoreflex activation in awake rats.

1. Introduction

The peripheral chemoreflex plays a critical role in the regulation of ventilatory, cardiovascular and behavioral responses to arterial blood hypoxemia [low PO_2 (Franchini and Krieger, 1993; Barros et al., 2002; Reddy et al., 2005)]. Considering the key role of the chemoreceptors in cardiorespiratory modulation, we are focused on characterizing the neurotransmitters involved in the processing of the cardiorespiratory responses to peripheral chemoreflex activation in the nucleus tractus solitarius (NTS) and paraventricular nucleus of hypothalamus [PVN (Haibara et al., 1995, 1999; Braga et al., 2007; Cruz et al., 2008; Cruz and Machado, 2009; Granjeiro and Machado, 2009)]. In a recent study, we showed the involvement of the intermediate and caudal commissural subnuclei of the NTS in the central pathway of the peripheral chemoreflex (Cruz et al., 2010) and we also showed that glutamatergic and purinergic mechanisms are part of the complex neurotransmission system of the sympathoexcitatory component of the chemoreflex at the commissural NTS level (Braga et al., 2007).

With respect to the involvement of other areas in the brain with the processing of the cardiovascular responses to chemoreflex activation, we also showed that hypothalamic areas take part in the sympathoexcitatory responses to peripheral chemoreflex activation in awake rats because bilateral lesion of the PVN produced a significant reduction in the pressor response to chemoreflex activation (Olivan et al., 2001). We also documented the involvement of the pre-autonomic PVN neurons projecting to rostral ventro-lateral medulla (RVLM) in the peripheral chemoreflex pathway (Cruz et al., 2008) and also that GABAergic, nitrergic and glutamatergic mechanisms in the PVN have an important role in the maintenance of tonic autonomic modulation to the cardiovascular system but not in the cardiovascular responses to peripheral chemoreflex activation in awake rats (Cruz and Machado, 2009). Considering that the neurotransmitter/neuromodulators of the chemoreflex at PVN are not completely characterized and also that there is evidence for a role for ATP in the PVN (Cham et al., 2006), the aim of the present study was to evaluate the role of ATP and P2 purinoceptors in the PVN in the modulation of the autonomic influences on the cardiovascular system as well as in cardiovascular responses to chemoreflex activation in awake rats. To reach these goals PPADS, a P2 receptors antagonist, was microinjected bilaterally into the PVN of awake rats and we evaluated the changes in the basal MAP and HR and in the cardiovascular responses to chemoreflex activation.

2. Material and methods

2.1. Animals

The experiments were performed on male Wistar rats weighing 300–320 g from the Animal Care of the University of São Paulo,

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campus of Ribeirão Preto, and kept on 12-h light–dark cycle, with food and water *ad libitum*. All the experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals and Ethical Principles for Animal Experimentation established by the Brazilian Committee for Animal Experimentation and approved by the Ethics Committee on Animal Experimentation of the School of Medicine of Ribeirão Preto, University of São Paulo (protocol 039/2005).

2.2. Placement of guide-cannulae in the direction of PVN

Rats were deeply anesthetized with tribromoethanol [250 mg/kg ip (Aldrich Chemical Company, Inc., Milwaukee, WI, USA)], and placed in a stereotaxic apparatus (David Kopf, Tujunga, CA, USA). Bilateral guide-cannulae were implanted in the direction to PVN. A longitudinal incision in the skin of the head was made and bregma and lambda were maintained at the same level. Using a drill, a little hole in the skull was made for perpendicular introduction of the 15-mm-long stainless steel guide-cannulae (22 gauge, Small Parts, Miami Lakes, FL, USA) following the coordinates of the atlas by Paxinos and Watson (1986): −1.2 mm posterior to the bregma, ±0.4 mm lateral to the midline and −4.8 mm ventral to the dura. The tip of the guide-cannulae was positioned ~3.0 mm above the dorsal aspect of the PVN. The guide-cannulae were fixed to the skull with methacrylate and watch screws and then closed with occluders which were removed just before the experimentation. The animals were allowed 4–5 days for a complete recovery from stereotaxic surgery.

2.3. Catheterization

One day before the experiment (4–5 day after stereotaxic procedures), while rats were deeply anesthetized [tribromoethanol (250 mg/kg ip)] a catheter (PE-10 connected to PE-50; Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery for measurement of pulsatile arterial pressure (PAP), mean arterial pressure (MAP), and heart rate (HR). A second catheter was inserted into the femoral vein for systemic administration of potassium cyanide (KCN) and anesthetic injection [Thiopental Sodium, 0.01 ml/100 mg iv (Abbott Laboratórios do Brasil Ltda., São Paulo, Brazil)] just before brain perfusion. The catheters were tunneled subcutaneously and exteriorized in the back of the neck to prevent the rat pull-out the catheters. On the subsequent day, the catheter in the femoral artery was flushed with heparinized saline to prevent clotting and then connected to a computer-driven data recording and analyzing system (Power Lab, ADInstruments, NSW, Australia) using a pressure transducer (MLT 0380, ADInstruments, NSW, Australia) connected to an amplifier (Bridge Amp, ML221, ADInstruments, NSW, Australia) for recording of PAP, MAP and HR.

2.4. Chemoreflex activation

The peripheral chemoreflex was activated by intravenous injection of KCN (80 μg/0.05 ml/rat; Merck, Darmstadt, Germany) before and 1, 3, 10 and 30 min after microinjections into the PVN. The changes in MAP and HR after chemoreflex (KCN, iv) activation were quantified at the peak of the responses.

2.5. Microinjections into the PVN

A 33-gauge needle (Small Parts, Miami Lake, FL, USA) 3.5 or 3.6 mm longer than the guide-cannulae, was connected by PE-10 tubing to a 1-μl Hamilton syringe (Hamilton Co, Reno, NV, USA) through PE-10 polyethylene tubing filled with the drug solution. After removal of the occluder from the guide-cannulae, the needle for microinjection was carefully inserted into the guide-cannulae and manual injection was performed 30 s later. For bilateral microinjections, it was initially performed in one side, the needle was withdrawn and repositioned in the contralateral side, and the second injection was performed. The volume microinjected in each side in all experimental protocols was 50 nl.

2.6. Drugs

All drugs solution microinjected into the PVN were diluted in saline (0.9% NaCl) and sodium bicarbonate was added to the solutions to adjust the pH to a range from 7.0 to 7.4. A mixture of PPADS and KYN was prepared in order to keep in the total volume of solution the same concentration used for microinjection of PPADS (0.25 nmol/50 nl) or KYN (7.2 nmol/50 nl).

2.7. Histology

At the end of each experiment, for histological verification of microinjections sites, Evan's blue dye solution [2% (50 nl)] was bilaterally microinjected into the PVN. The animals were euthanized with an overdose of thiopental sodium and perfused with a solution of 0.9% saline followed by 10% formaldehyde. The brains were removed and stored in 10% formaldehyde for 2 days. Tissue containing the PVN was cut in serial transversal sections 15-μm-thick followed by Nissl stain procedures. Microinjections sites were identified using bright-field microscopy. The histology was considered positive when the microinjection sides reached bilaterally the medial and/or posterior PVN. The microinjections performed in areas outside of the PVN were used as a control misplaced microinjections group (Table 1).

2.8. Data analysis

The baseline values of the cardiovascular parameters were averaged before and in different times after bilateral microinjections. Changes in MAP and HR during stimulation of chemoreflex were calculated as the difference between the peak and the baseline values of MAP and HR. The data were analyzed by the one-way ANOVA test, followed by Tukey's post hoc test. All results are presented as means ± SEM. Differences were considered significant when $P<0.05$.

3. Experimental protocols

All studies were performed in awake freely moving rats. The experimental protocol for the evaluation of PVN purinoceptors involvement in the cardiovascular responses to chemoreflex activation was performed before (control) and 1, 3, 10 and 30 min after the bilateral microinjection of PPADS [Pyridoxal phosphate-6-azo (benzene-2,4-disulfonic acid tetrasodium salt hydrate — P2 antagonist Sigma-Aldrich, St. Louis, MO, USA) 0.25 nmol/50 nl (Braga et al., 2007)] or the solution of PPADS (0.25 nmol/50 nl) and KYN [kynurenic acid, a nonselective glutamate ionotropic receptors antagonist (Sigma Chemical, St. Louis, MO, USA, 7.2 nmol/50 nl), Cruz and Machado, 2009].

As a control to a possible nonspecific effect of volume microinjections, an experimental group ($n=6$) was performed in which the rats received bilateral microinjections of sterile saline 0.9% (50 nl, vehicle) as a volume control (Table 1).

4. Results

4.1. Effect of microinjection of PPADS into PVN on baseline MAP and HR and on cardiovascular responses to chemoreflex activation

The tracings of Fig. 1 shows baseline MAP, PAP, HR and changes on these parameters in response to chemoreflex activation before and after bilateral microinjections of PPADS into PVN. P2 purinoceptor antagonist microinjected into the PVN ($n=6$) produced a significant