Autonomic Neuroscience: Basic and Clinical 160 (2011) 90–98

Contents lists available at ScienceDirect

Autonomic Neuroscience: Basic and Clinical

journal homepage: www.elsevier.com/locate/autneu

Cardiovascular effects of noradrenaline microinjected into the insular cortex of unanesthetized rats

Fernando H.F. Alves [a], Carlos C. Crestani [b], Leonardo B.M. Resstel [a], Fernando M.A. Correa [a,*]

[a] Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, 14049-900, Brazil
[b] Department of Natural Active Principles and Toxicology, School of Pharmaceutical Sciences, São Paulo State University, UNESP, Araraquara, SP, 14801-902, Brazil

ARTICLE INFO

Article history:
Received 11 August 2010
Received in revised form 28 October 2010
Accepted 2 November 2010

Keywords:
Prefrontal cortex
Blood pressure
Heart rate
Noradrenergic neurotransmission
Adrenoceptors
Vasopressin

ABSTRACT

The insular cortex (IC) has been reported to be involved in central cardiovascular control. In the present study, we investigated the cardiovascular responses evoked by microinjection of noradrenaline into the IC as well as the central and peripheral mechanisms involved in their mediation. Microinjection of noradrenaline into the IC (3, 7, 10, 15, 30 and 45 nmol/100 nL) caused long-lasting dose-related pressor and bradycardiac responses. The cardiovascular responses evoked by 15 nmol of noradrenaline were blocked by IC pretreatment with WB4101 or 5-methyl-urapidil, selective α_1-adrenoceptor antagonists. IC pretreatment with either the selective α_2-adrenoceptor antagonists RX821002 or the β-adrenoceptor antagonist propranolol did not affect noradrenaline cardiovascular responses. Noradrenaline cardiovascular responses were mimicked by microinjection of the selective α_1-adrenoceptor agonist phenylephrine into the IC, thus reinforcing the idea that α_1-adrenoceptors mediate cardiovascular responses to noradrenaline microinjected into the IC. The pressor response to noradrenaline microinjection was potentiated by i.v. pretreatment with the ganglion blocker pentolinium and inhibited by i.v. pretreatment with the selective V_1-vasopressin receptor antagonist dTyr$(CH_2)_5$(Me)AVP. The bradycardiac response to noradrenaline microinjection into the IC was abolished by pretreatment with either pentolinium or the V_1-vasopressin receptor antagonist, indicating its reflex origin. In conclusion, our results suggest that pressor response evoked by microinjection of noradrenaline into the IC involve the activation of IC α_1-adrenoceptors to cause the release of vasopressin into the circulation.

1. Introduction

It was assumed before the 1960s that rats did not have a cortical structure analogous to the prefrontal cortex of primates (Rose and Woolsey, 1948). However, Leonard (1969) described projections from the dorsal medial thalamic nucleus to two distinct frontal cortex regions of the rat brain that could be defined as prefrontal cortex. The prefrontal cortex is a limbic region, which integrates sensory and visceral information from peripheral receptors and has been implicated in control of autonomic activity (Anand and Dua, 1956; Delgado, 1960; Neafsey, 1990; Resstel and Correa, 2006). In rats, the prefrontal cortex has been anatomically divided into two main regions: the lateral prefrontal cortex or insular cortex (IC) and the medial prefrontal cortex (mPFC) (Krettek and Price, 1977; Leonard, 1969). The IC has been proposed to be involved in cardiovascular regulation (Allen and Cechetto, 1995; Alves et al., 2009a, 2010; Ruggiero et al., 1987). Its electrical or chemical stimulation has been reported to cause either pressor or depressor responses (Allen and Cechetto, 1995; Hardy and Holmes, 1988; Hardy and Mack, 1990; Ruggiero et al., 1987; Yasui et al., 1991). Moreover, it has also been reported that the IC modulates baroreflex activity in unanesthetized rats (Alves et al., 2009a,b).

Cardiovascular responses have been reported after noradrenaline microinjection into medullary structures such as the nucleus of the solitary tract (Zandberg et al., 1979) as well as into supramedullary structures such as the lateral septal area (Scopinho et al., 2006), the amygdala (Ohta et al., 1991), hypothalamic nuclei (Busnardo et al., 2009; Camargo et al., 1979; Harland et al., 1989) and the bed nucleus of the stria terminalis (BST) (Crestani et al., 2008, 2007). These results suggest that central noradrenergic pathways are involved in cardiovascular control.

There is evidence that noradrenergic terminals are present in the prefrontal cortex (Morrison et al., 1979). These terminals originate mainly in projections from the locus coeruleus (LC) and play an important role in the regulation of cortical function (Morrison et al., 1979; Sara and Segal, 1991; Ungerstedt, 1971). Moreover, noradrenergic neurotransmission in the prefrontal cortex has been implicated in modulation of the cardiovascular system in stress-induced situations (Funk and Stewart, 1996). We have previously reported that such noradrenergic neurotransmission in the IC is involved in the modulation of baroreflex activity (Alves et al., 2009a). However, the

* Corresponding author. Department of Pharmacology, School of Medicine of Ribeirão Preto, USP, Av. Bandeirantes 3900, 14049-900, Ribeirão Preto, São Paulo, Brazil. Tel.: +55 16 36023207; fax: +55 16 36332301.
E-mail address: fmdacorr@fmrp.usp.br (F.M.A. Correa).

1566-0702/$ – see front matter © 2010 Elsevier B.V. All rights reserved.
doi:10.1016/j.autneu.2010.11.002

involvement of IC noradrenergic neurotransmission in the control of arterial pressure and heart rate (HR) has never been systematically investigated. Here, we report on the cardiovascular effects evoked by noradrenaline microinjection into the IC. In addition, we studied the subtypes of adrenoceptor activated in the IC as well as the peripheral mechanism involved in the mediation of these cardiovascular effects.

2. Materials and methods

2.1. Animal preparation

Eighty-nine male Wistar rats weighing 230–270 g were used. Animals were kept in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. Rats were housed in plastic cages under standard laboratory conditions, with free access to food and water and under a 12 h light/dark cycle (lights on at 06:30 h). The Institution's Animal Ethics Committee approved the housing conditions and experimental procedures (process number: 167/2007).

Five days before the experiment, the rats were anesthetized with tribromoethanol (250 mg/kg, i.p.). After local anesthesia with 2% lidocaine, the skull was surgically exposed and stainless steel guide cannulas (26 G) were implanted unilaterally in the IC, using a stereotaxic apparatus (Stoelting, Wood Dale, Illinois, USA). Stereotaxic coordinates for cannula implantation in the IC were selected from the rat brain atlas of Paxinos and Watson (1997) and were: antero-posterior = +11.7 mm from interaural, lateral = 4.0 mm from the medial suture and vertical = −4.5 mm from the dorsal surface of the skull. Cannulas were fixed to the skull with dental cement and one metal screw. After surgery, the animals were treated with a poly-antibiotic preparation of streptomycins and penicillins (i.m., 0.27 mg/kg, Pentabiotico, Fort Dodge®, Campinas, SP, Brazil) to prevent infection, and with the non-steroidal anti-inflammatory flunixine meglumine (2.5 mg/kg, i.m.; Banamine®, Schering Plough, Cotia, SP, Brazil) for post-operative analgesia.

One day before the experiment, the rats were anesthetized with tribromoethanol (250 mg/kg, i.p.) and a catheter (a 4 cm segment of PE-10 heat-bound to a 13 cm segment of PE-50, Clay Adams, Parsippany, NJ, USA) was inserted into the abdominal aorta through the femoral artery, and later used for arterial pressure and HR recording. A second catheter was implanted into the femoral vein for infusion of saline, pentolininum or dTyr$(CH_2)_5$(Me)AVP. Both catheters were tunneled under the skin and exteriorized on the animal's dorsum. After surgery, the animals were treated with the non-steroidal anti-inflammatory flunixine meglumine (2.5 mg/kg, i.m.) for post-operative analgesia.

2.2. Measurement of cardiovascular responses

On the day of the experiment, the arterial cannula was connected to a pressure transducer. Pulsatile arterial pressure of freely moving animals was recorded with a HP-7754A preamplifier (Hewlett Packard, Palo Alto, CA) and an acquisition board (MP100A; Biopac Systems Inc., Santa Barbara, CA) connected to a computer. Mean arterial pressure (MAP) and HR values were derived from the pulsatile arterial pressure recordings and processed on-line. The sampling rate utilized to acquisition of the data was 200 Hz.

2.3. Drug injection into the IC

The needles (33 G, Small Parts, Miami Lakes, FL, USA) used for microinjection into the IC were 1 mm longer than the guide cannula and were connected to a 1 μL syringe (7001-KH, Hamilton, Reno, NV, USA) through PE-10 tubing. The needle was carefully inserted into the guide cannula without restraining the animals and drugs were injected in a final volume of 100 nL over a 10 s period.

2.4. Experimental protocols

After surgery, animals were kept in individual cages that later were used in their transport to the experimental room. Animals were allowed 60 min to adapt to the conditions of the experimental room, such as sound and illumination, before starting arterial pressure and HR recordings. The experimental room was acoustically isolated and had constant background noise produced by an air exhauster to minimize sound interference within the experimental room. At least another 30 min period of basal recording of MAP and HR was allowed before beginning experiments. Care was taken to start injections only when blood pressure and HR values had stabilized.

2.4.1. Cardiovascular responses to the microinjection of different doses of noradrenaline into the IC of unanesthetized rats

Different doses of noradrenaline were microinjected into the IC of unanesthetized rats. The doses used were 3, 7, 10, 15, 30, or 45 nmol in 100 nL (n = 5 for 7, 10, 15 and 30 nmol and n = 4 for 3 and 45 nmol). To plot the dose–response curve, each animal received up to two microinjections of noradrenaline into the IC. The interval between microinjections was 24 h in order to avoid the tachyphylaxis phenomenon and the dose microinjected in the first and second day was different. The ordering of the injections was alternated to randomize experimental variable. For each dose in the curve, it was calculated the mean of the effect produced in different animals. These values were further plotted to produce the dose–effect curves.

The dose of 15 nmol of noradrenaline, that approximates the ED_{50}, was used when studying effects of different treatments on the cardiovascular responses to noradrenaline microinjected into the IC. A different group of animals was used to verify a possible tachyphylaxis phenomenon. In this protocol, microinjections of noradrenaline (15 nmol/100 nL, n = 5) into the IC were repeated 30 min apart.

2.4.2. Study of the local adrenoceptor involved on cardiovascular response to noradrenaline microinjection into the IC

A group of animals was used to study the IC adrenoceptor involved in noradrenaline responses. Twenty four hours after the first noradrenaline microinjection, different group of animals received artificial cerebrospinal fluid (ACSF) (100 nL, n = 5) (Alves et al., 2009a,b); selective α_1-adrenoceptor antagonists WB4101 (15 nmol/100 nL, n = 5) (Alves et al., 2009a) or 5-methyl-urapidil (15 nmol/100 nL, n = 5); the selective α_2-adrenoceptor antagonist RX821002 (15 nmol/100 nL, n = 5) (Alves et al., 2009a) or the nonselective β-adrenoceptor antagonist propranolol (15 nmol/100 nL, n = 5) (Alves et al., 2009a) injected into the IC. Noradrenaline was again injected into the IC 10 min later. To confirm the involvement of α_1-adrenoceptor in the mediation of responses to noradrenaline into the IC, the selective α_1-adrenoceptor agonist phenylephrine (200 nmol/100 nL, n = 5) (Correa and Peres-Polon, 1995) was microinjected into the IC of a group of animals.

2.4.3. Study of the peripheral mechanisms involved in the meditation of the cardiovascular responses to noradrenaline microinjected into the IC

A group of rats were used to study the peripheral mechanisms involved in the meditation of the cardiovascular responses to noradrenaline microinjected into the IC. These experiments were also carried out over 2 days. On day 1, noradrenaline (15 nmol/100 nL) was microinjected into the IC as control. On the second day, noradrenaline was injected into the IC 10 min after i.v. treatment with saline (n = 5), the ganglion blocker pentolinium (5 mg/kg, n = 5) (Alves et al., 2007; Moriguchi et al., 1998) or the V_1-vasopressin receptor antagonist dTyr$(CH_2)_5$(Me)AVP (50 μg/kg, n = 5) (Alves et al., 2007).

2.5. Histological determination of the microinjection site

At the end of the experiment, animals were anesthetized with urethane (1.25 g/kg i.p.) and 100 nL of 1% Evan's blue dye was microinjected into the IC as a marker of the microinjection site. Animals were submitted to intracardiac perfusion with 0.9% NaCl followed by 10% formalin. The brains were removed and post-fixed for 24 h at 4 °C, and 40-μm sections were cut with a cryostat (CM-1900; Leica, Wetzlar, Germany). Brain sections were stained with 1% neutral red for light microscopic analysis. Microinjection sites were identified in serial sections according to the rat brain atlas of Paxinos and Watson (1997).

2.6. Drugs

Noradrenaline HCl (SIGMA, St. Louis, MO, USA), dl-propranolol (SIGMA); WB4101 (TOCRIS, Ellisvalle, MO, USA); RX821002 (TOCRIS); 5-methyl-urapidil (RBI, Natick, MA, USA) and phenylephrine (SIGMA) were dissolved in artificial cerebrospinal fluid (ACSF), which had the following composition (in mmol/L): 100 NaCl; 2 Na_3PO_4; 2.5 KCl; 1.0 $MgCl_2$; 27 $NaHCO_3$; 2.5 $CaCl_2$ (pH = 7.4). Pentolinium bitartrate (SIGMA), dTyr$(CH_2)_5$(Me)AVP (Peninsula Laboratories, King of Prussia, PA, USA), tribromoethanol (SIGMA) and urethane (SIGMA) were dissolved in saline (NaCl 0.9%). Flunixine meglumine (Banamine®, Schering Plough, Brazil) and poly-antibiotic preparation of streptomycins and penicillins (Pentabiotico®, Fort Dodge, Brazil) were used as provided.

2.7. Statistical analysis

Data are presented as means ± S.E.M. Basal values of MAP and HR before and after pharmacological treatment were compared using paired Student's t-test. Nonlinear regression analysis was used to compare MAP and HR responses after microinjection of different doses of noradrenaline into the IC. Two-way ANOVA for repeated measurements (treatment vs. time) was used to compare effects of the treatments on MAP and HR responses observed after microinjection of noradrenaline into the IC. $P<0.05$ was assumed to be statistically significant.

3. Results

3.1. Cardiovascular responses to the microinjection of different doses of noradrenaline into the IC of unanesthetized rats

Microinjection of ACSF (n = 5) into the IC did not affect either MAP (97 ± 1 vs. 100 ± 2 mm Hg, t = 1.7, P>0.05) or HR (358 ± 11 vs. 352 ± 13 bpm, t = 0.3, P>0.05) baseline values. However, microinjection of noradrenaline (3, 7, 10, 15, 30 and 45 nmol/100 nL) into the IC of unanesthetized rats (basal MAP = 99 ± 1 and basal HR = 369 ± 16, n = 28) caused long-lasting dose-related pressor and bradycardiac responses (Fig. 1). Nonlinear regression analysis indicated a significant correlation between doses and either pressor ($r^2 = 0.91$, df = 26, $P<0.05$) or bradycardiac ($r^2 = 0.92$, df = 26, $P<0.05$) responses. The ED_{50} was approximately 15 nmol for cardiovascular responses. A typical recording showing the cardiovascular effects evoked by the microinjection of noradrenaline (15 nmol/100 nL) into the IC is presented in Fig. 2A.

The cardiovascular responses to the microinjection of noradrenaline (15 nmol/100 nL) (n = 5) into the IC were decreased when a second microinjection of noradrenaline was made 30 min later (MAP: $F_{(1,392)} = 30$, $P<0.0001$ and HR: $F_{(1,392)} = 32$, $P<0.0001$) (Fig. 3). However, responses were not affected when noradrenaline was microinjected 24 h after the first injection (Fig. 3).

Microinjection of noradrenaline (15 nmol/100 nL) into structures surrounding the IC, such as primary motor cortex (MAP before: 98 ± 3 and after: 99 ± 3 mm Hg, t = 3, P>0.05; and HR before: 343 ± 12 and after: 341 ± 12 bpm, t = 1.2, P>0.05; n = 3) and forceps minor of the

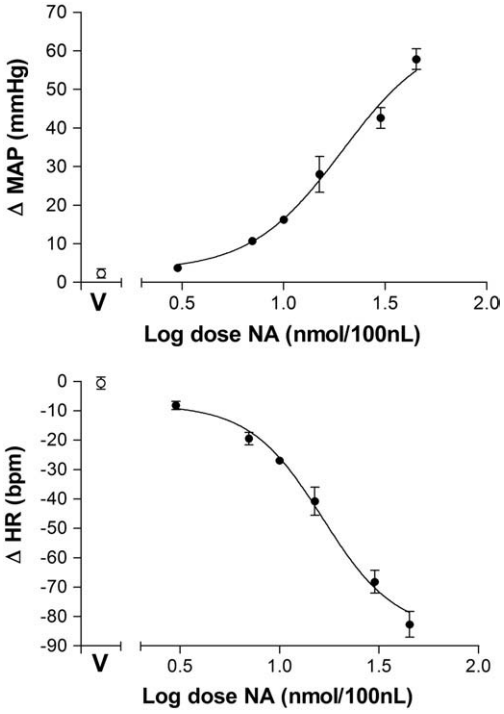

Fig. 1. Changes in mean arterial pressure (ΔMAP) and heart rate (ΔHR) evoked by noradrenaline microinjection (3, 7, 10, 15, 30, or 45 nmol/100 nL) into the IC of unanesthetized rats (n = 5 for 7, 10, 15 and 30 nmol and n = 4 for 3 and 45 nmol). Points represent means and bars the SEM. Dose–response curves were generated by nonlinear regression analysis and were statistically significant (ΔMAP, $r^2 = 0.91$ and ΔHR, $r^2 = 0.92$).

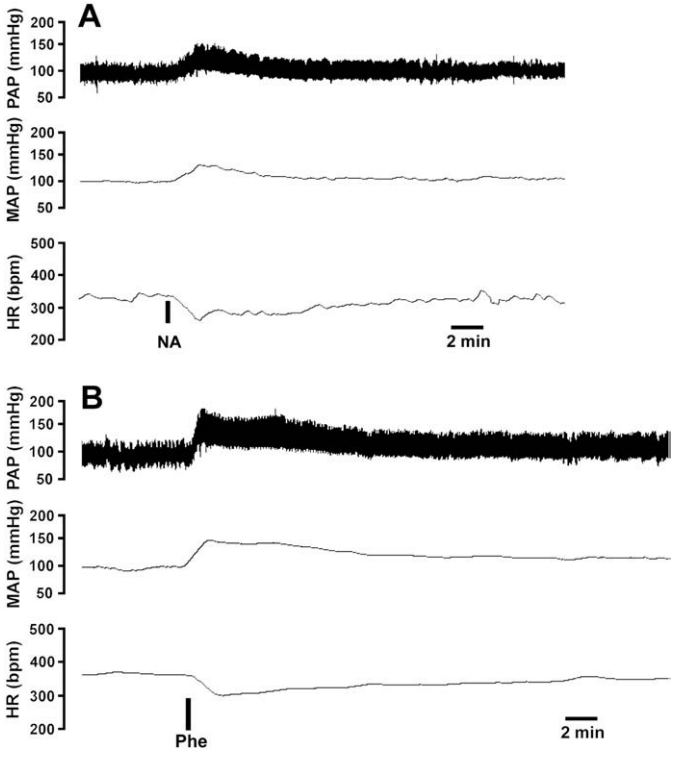

Fig. 2. A — Typical recordings showing mean arterial pressure (MAP), pulsatile arterial pressure (PAP) and heart rate (HR) changes in response to 15 nmol/100 nL of noradrenaline microinjected into the IC of one unanesthetized rat. B — Typical recordings showing MAP, PAP and HR changes in response to 200 nmol/100 nL of phenylephrine microinjected into the IC of one unanesthetized rat.

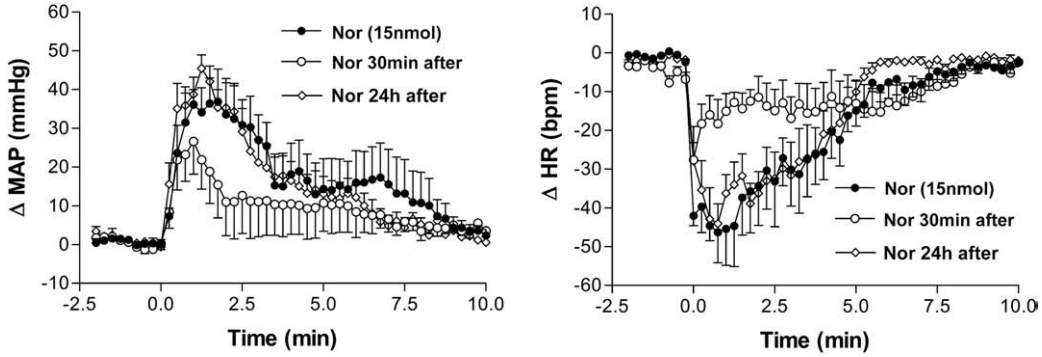

Fig. 3. Time course of the change of mean arterial pressure (ΔMAP) and heart rate (ΔHR) evoked by noradrenaline (15 nmol/100 nL) microinjection into the IC 30 min and 24 h after the first microinjection of noradrenaline. Noradrenaline injections were made at time 0. Points represent the mean and bars the SEM; two-way ANOVA followed by Bonferroni's post hoc test.

corpus callosum (MAP before: 96 ± 1 and after: 100 ± 3 mm Hg t = 1.5, P > 0.05; and HR before: 359 ± 15 and after: 357 ± 17 bpm; t = 0.9, P > 0.05; n = 3) had no effect on either MAP or HR.

3.2. Effect of IC pretreatment with ACSF, WB4101, 5-methyl-urapidil, RX821002 or propranolol on cardiovascular responses to the microinjection of noradrenaline into the IC of unanesthetized rats

3.2.1. ACSF

IC pretreatment with ACSF (n = 5) did not affect either the pressor (43 ± 2 vs. 45 ± 3 mm Hg, t = 0.3, P > 0.05) or the bradycardiac (50 ± 2 vs. 49 ± 3 bpm, t = 0.2, P > 0.05) response to noradrenaline microinjected into the IC of unanesthetized rats.

3.2.2. WB4101

The microinjection of the selective α_1-adrenoceptor antagonist WB4101 (n = 5) into the IC did not affect either MAP (103 ± 3 vs. 100 ± 3 mm Hg, t = 0.6, P > 0.05) or HR (356 ± 7 vs. 359 ± 11 bpm, t = 0.3; P > 0.05) baseline values. However, cardiovascular responses to the microinjection of noradrenaline into the IC were significantly reduced after IC treatment with WB4101 (Fig. 4A). Two-way ANOVA indicated a significant effect of IC treatment with WB4101 on cardiovascular effects of noradrenaline (MAP: $F_{(1,392)} = 278$, P < 0.0001; and HR: $F_{(1.392)} = 237$, P < 0.0001); a significant effect over time (MAP: $F_{(48,392)} = 10$, P < 0.0001; and HR: $F_{(48,392)} = 25$, P < 0.0001) and an interaction between treatment and time (MAP: $F_{(48,392)} = 8$, P < 0.0001; and HR: $F_{(48,392)} = 19$, P < 0.0001) (Fig. 4A).

3.2.3. 5-Methyl-urapidil

The microinjection of the selective α_1-adrenoceptor antagonist 5-methyl-urapidil (n = 5) into the IC did not affect either MAP (95 ± 2 vs. 100 ± 3 mm Hg, t = 1.1, P > 0.05) or HR (384 ± 12 vs. 362 ± 11 bpm, t = 1, P > 0.05) baseline values. However, cardiovascular responses to the microinjection of noradrenaline into the IC were significantly reduced

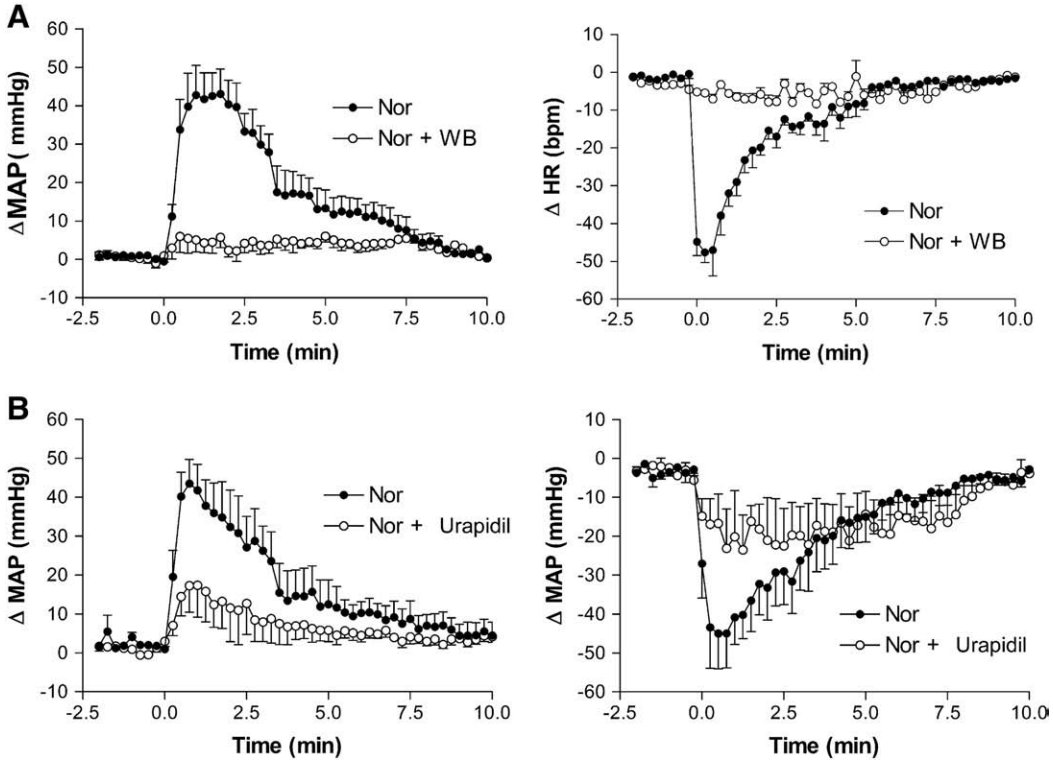

Fig. 4. Time course of the change of mean arterial pressure (ΔMAP) and heart rate (ΔHR) evoked by noradrenaline (15 nmol/100 nL) microinjection into the IC before or after IC pretreatment with selective α_1-adrenoceptor antagonists WB4101 (15 nmol/100 nL) or 5-methyl-urapidil. Noradrenaline injections were made at time 0. Points represent the mean and bars the SEM; two-way ANOVA followed by Bonferroni's post hoc test.

after IC treatment with 5-methyl-urapidil (Fig. 4B). Two-way ANOVA indicated a significant effect of the treatment with 5-methyl-urapidil on cardiovascular effects of noradrenaline (MAP: $F_{(1,392)}=74$, $P<0.0001$; and HR: $F_{(1.392)}=4$, $P<0.0001$); a significant effect over time (MAP: $F_{(48,392)}=6$, $P<0.0001$; and HR: $F_{(48,392)}=4$, $P<0.0001$) and an interaction between treatment and time (MAP: $F_{(48,392)}=1.5$, $P<0.0001$; and HR: $F_{(48,392)}=1.1$, $P<0.0001$) (Fig. 4B).

3.2.4. RX821002

Microinjection of the selective α_2-adrenoceptor antagonist RX821002 ($n=5$) into the IC did not affect either MAP (99 ± 3 vs. 100 ± 3 mm Hg, $t=0.2$, $P>0.05$) or HR (353 ± 9 vs. 366 ± 12 bpm, $t=2.1$, $P>0.05$) baseline values. IC pretreatment with RX821002 also did not change cardiovascular responses to the microinjection of noradrenaline into the IC (Fig. 5). Two-way ANOVA indicated no significant effect of RX821002 on the cardiovascular effects of noradrenaline (MAP: $F_{(1,392)}=0.2$, $P>0.05$; and HR: $F_{(1.392)}=0.2$, $P>0.05$), but indicated a significant effect over time (MAP: $F_{(48,392)}=26$, $P<0.0001$; and HR: $F_{(48,392)}=48$, $P<0.0001$) (Fig. 5).

3.2.5. Propranolol

The microinjection of the nonselective β-adrenoceptor antagonist propranolol ($n=5$) into the IC did not affect either MAP (96 ± 3 vs. 101 ± 4 mm Hg; $t=1.5$, $P>0.05$) or HR (356 ± 9 vs. 369 ± 16 bpm, $t=0.9$, $P>0.05$) baseline values. The IC pretreatment with propranolol also did not change cardiovascular responses to the microinjection of noradrenaline into the IC (Fig. 5). Two-way ANOVA indicated no significant effect of propranolol on the cardiovascular effects of noradrenaline (MAP: $F_{(1,392)}=0.2$, $P>0.05$; and HR: $F_{(1.392)}=2$, $P>0.05$), but indicated a significant effect over time (MAP: $F_{(48,392)}=26$, $P<0.0001$; and HR: $F_{(48,392)}=51$, $P<0.0001$) (Fig. 5).

3.3. Cardiovascular responses to microinjection of phenylephrine into the IC of unanesthetized rats

Microinjection of phenylephrine ($n=5$) into the IC (basal MAP: 95 ± 2 mm Hg and basal HR: 373 ± 8 bpm) caused pressor response ($\Delta MAP=49\pm4$ mm Hg, $t=9.7$, $P<0.05$), which was accompanied by bradycardia ($\Delta HR=-95\pm4$ bpm, $t=11$, $P<0.05$). A typical recording showing cardiovascular responses to the microinjection of phenylephrine into the IC of a representative animal is presented in Fig. 2B.

3.4. Effect of i.v. treatment with saline, pentolinium or $dTyr(CH_2)_5(Me)$ AVP on the cardiovascular responses to the microinjection of noradrenaline into the IC

3.4.1. Saline

The i.v. administration of saline ($n=5$) did not affect either pressor (43 ± 2 vs. 45 ± 3 mm Hg; $t=0.3$, $P>0.05$) or bradycardiac (-50 ± 2 vs. -49 ± 3 bpm; $t=0.2$, $P>0.05$) responses to the microinjection of noradrenaline into the IC.

3.4.2. Pentolinium

The i.v. pretreatment with the ganglion blocker pentolinium ($n=5$) significantly reduced baseline MAP values (98 ± 3 vs. 68 ± 3 mm Hg, $t=5.7$, $P<0.05$), but did not affect baseline HR values (364 ± 10 vs. 382 ± 9 bpm; $t=0.8$, $P>0.05$). Pretreatment with pentolinium potentiated the pressor response evoked by the microinjection of noradrenaline into the IC and blocked the bradycardiac response (Fig. 6). Two-way ANOVA indicated a significant effect of treatment with pentolinium on the cardiovascular effects of noradrenaline (MAP: $F_{(1.456)}=701$, $P<0.0001$; and HR: $F_{(1.456)}=852$, $P<0.0001$); a significant effect over time (MAP: $F_{(56.456)}=52$, $P<0.0001$; and HR: $F_{(56.456)}=28$, $P<0.0001$) and an interaction

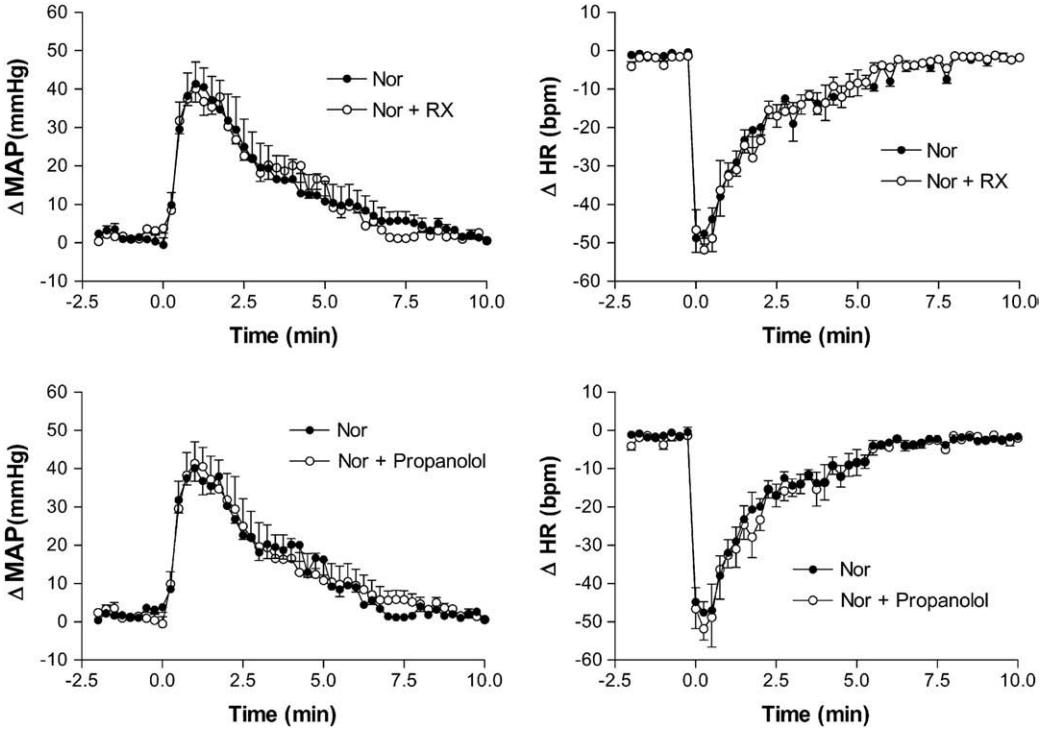

Fig. 5. Time course of the change of mean arterial pressure (ΔMAP) and heart rate (ΔHR) evoked by noradrenaline (15 nmol/100 nL) microinjection into the IC before or after IC pretreatment with the selective α_2-adrenoceptor antagonist RX821002 (15 nmol/100 nL) or the nonselective β-adrenoceptor antagonist propranolol (15 nmol/100 mL). Noradrenaline injections were made at time 0. Points represent the mean and bars the SEM; two-way ANOVA followed by Bonferroni's post hoc test.

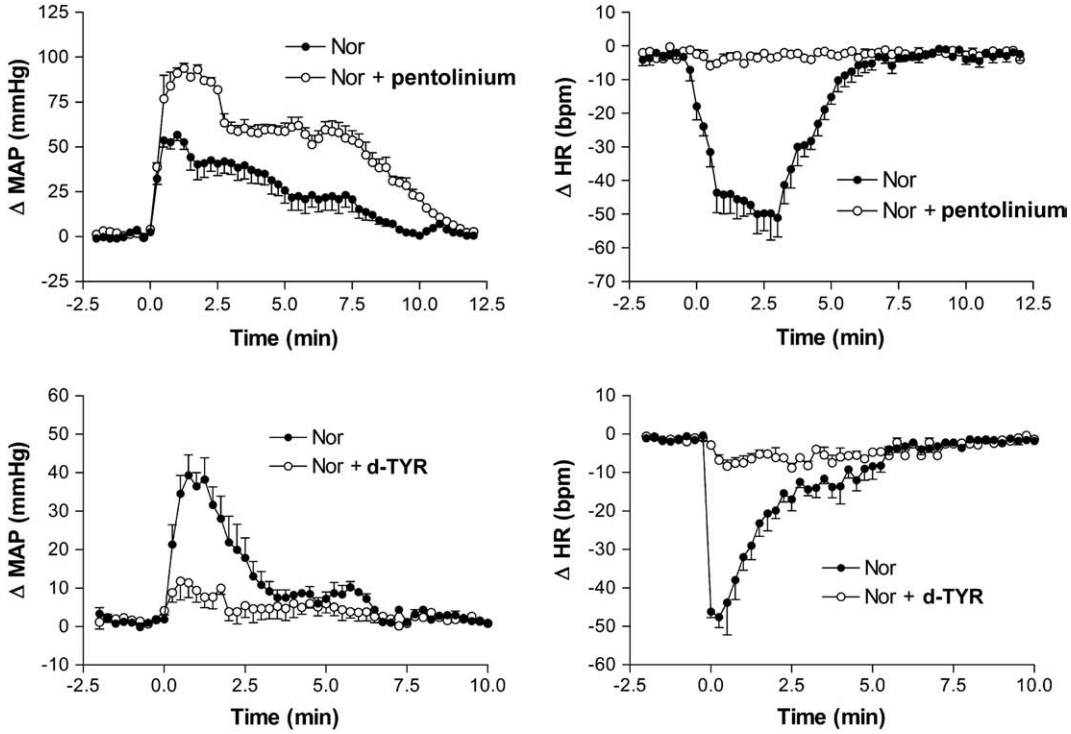

Fig. 6. Time course of the change of mean arterial pressure (ΔMAP) and heart rate (ΔHR) evoked by noradrenaline (15 nmol/100 nL) microinjection into the IC before or after intravenous pretreatment with pentolinium (5 mg/kg) or dTyr$(CH_2)_5$(Me)AVP (dTyr, 50 μg/kg). Noradrenaline microinjections were made at time 0. Points represent the mean and bars the SEM; two-way ANOVA followed by Bonferroni's post hoc test.

between treatment and time (MAP: $F_{(56.456)} = 5$, $P<0.0001$; and HR: $F_{(56.456)} = 24$, $P<0.0001$) (Fig. 6).

3.4.3. dTyr$(CH_2)_5$(Me)AVP

The i.v. pretreatment with the selective V_1-vasopressin receptor antagonist dTyr$(CH_2)_5$(Me)AVP ($n = 5$) did not affect either MAP (102 ± 3 vs. 97 ± 3 mm Hg, $t = 1.3$, $P>0.05$) or HR (368 ± 12 vs. 352 ± 9 bpm, $t = 0.6$, $P>0.05$) baseline values. Cardiovascular responses to the microinjection of noradrenaline into the IC were significantly reduced after i.v. treatment with the V_1-vasopressin receptor antagonist (Fig. 6). Two-way ANOVA indicated a significant effect of treatment with dTyr$(CH_2)_5$(Me)AVP on the cardiovascular effects of noradrenaline (MAP: $F_{(1.392)} = 120$, $P<0.0001$; and HR: $F_{(1.392)} = 294$, $P<0.0001$); a significant effect over time (MAP: $F_{(48.392)} = 14$, $P<0.0001$; and HR: $F_{(48.392)} = 29$, $P<0.0001$) and an interaction between treatment and time (MAP: $F_{(48.392)} = 6$, $P<0.0001$; and HR: $F_{(48.392)} = 18$, $P<0.0001$) (Fig. 6).

A photomicrograph of a coronal brain section showing a representative microinjection site into the IC is presented in Fig. 7. A diagrammatic representation modified from Paxinos and Watson (1997) that indicates microinjection sites of ACSF, noradrenaline, WB4101, 5-methyl-urapidil, RX821002 and propranolol into the IC and noradrenaline into areas surrounding the IC is also presented in Fig. 7.

4. Discussion

In the present study, we report that noradrenaline microinjection into the IC of unanesthetized rats caused dose-related pressor and bradycardiac responses. These responses were reduced by IC pretreatment with either selective α_1-adrenoceptor antagonists WB4101 or 5-methyl-urapidil. Moreover, the IC microinjection of the selective α_1-adrenoceptor agonist phenylephrine evoked pressor and bradycardiac responses similar those observed after noradrenaline microinjection. The systemic treatment with the ganglion blocker pentolinium potentiated the pressor response evoked by noradrenaline and blocked the bradycardiac response, whereas systemic treatment with the selective V_1-vasopressin receptor antagonist dTyr$(CH_2)_5$(Me)AVP reduced both pressor and bradycardiac responses to noradrenaline microinjected into the IC.

Noradrenergic neural terminals have been identified in the IC (Ungerstedt, 1971). These IC innervations are mainly originated from noradrenergic cells grouped in the LC, which is a small noradrenergic nucleus (designated as A6), that contains ≈1500 neurons projecting to several areas of the forebrain, including the IC (Morrison et al., 1979; Ungerstedt, 1971). The IC noradrenergic synapses are geometrically orderly throughout the lateral neocortex and that organization differs considerably from that of other cortical afferents. Two noteworthy features of the noradrenergic innervation are the high density of fibers throughout all cortical layers and the predominantly tangential orientation of axons within the cortical gray matter (Morrison et al., 1978).

Previous results, performed in our laboratory, have demonstrated that activation of IC α_1-adrenoceptors induce an inhibitory influence on the cardiac component of the baroreflex (Alves et al., 2009a). Moreover, it has been reported that IC noradrenergic neurotransmission modulates the cardiovascular responses associated with stress situations (Funk and Stewart, 1996). These results provide evidences of a relevant involvement of IC noradrenergic neurotransmission in central cardiovascular control. Therefore, to investigate the subtype of adrenoceptor involved with mediation of cardiovascular responses evoked by microinjection of noradrenaline into the IC, the IC was pretreated with either selective α_1-adrenoceptor antagonists WB4101 or 5-methyl-urapidil; the selective α_2-adrenoceptor antagonist RX821002 or the nonselective β-adrenoceptor antagonist propranolol. The local IC pretreatment with either WB4101 or 5-methyl-urapidil significantly reduced the peak and length of the pressor and bradycardiac responses evoked by noradrenaline microinjection into the IC, thus indicating that these responses depend mainly on local α_1-adrenoceptor. IC pretreatment with either

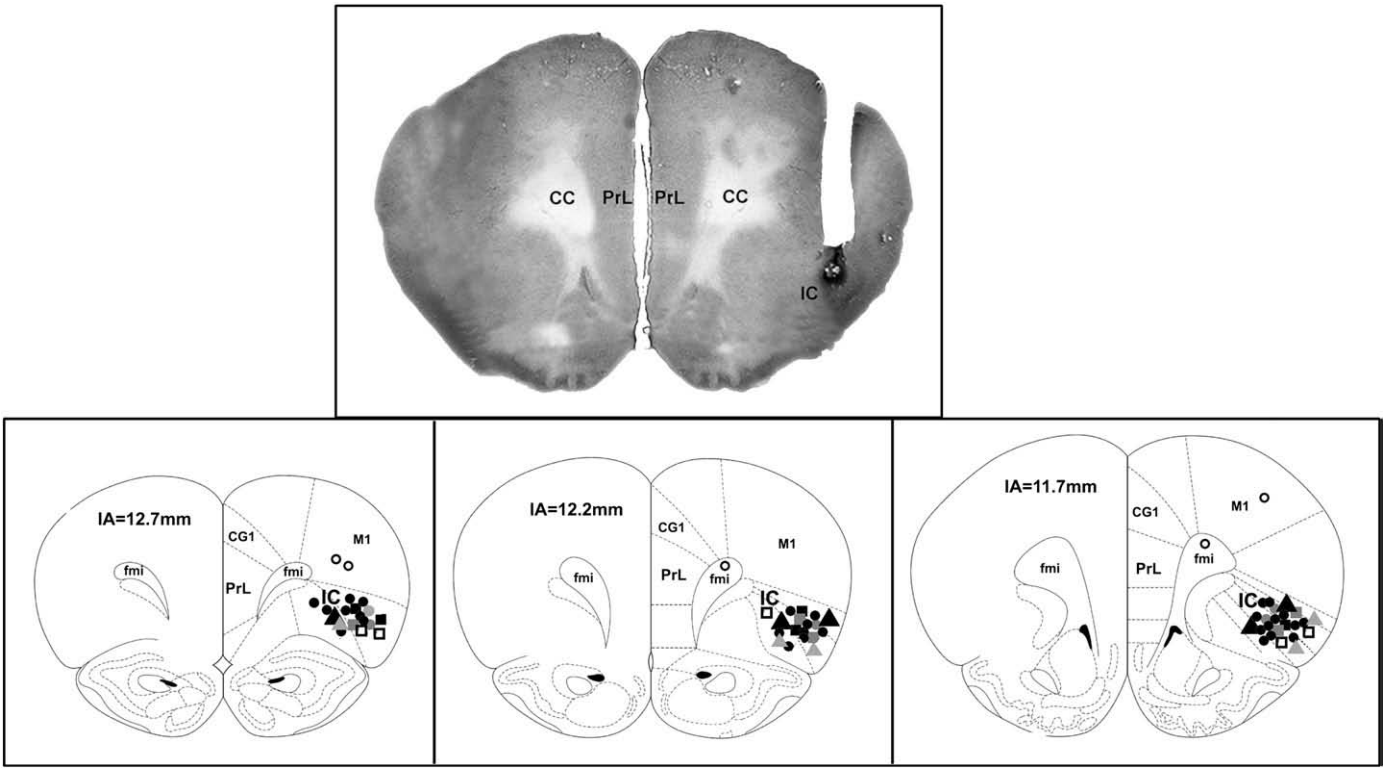

Fig. 7. A photomicrograph of a coronal brain section, from one representative rat, which shows the injection site in the IC. Diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997), which indicates injection sites into the IC of ACSF (gray circles), noradrenaline (filled circles), WB4101 (filled squares), RX821002 (gray squares), 5-methyl-urapidil (open squares), phenylephrine (gray triangles) or propranolol (filled triangles) and noradrenaline into structures surrounding the IC (open circles). Cg1 — cingulate cortex, area; PrL — prelimbic cortex, M1 — primary motor cortex; IC — insular cortex, cc — corpus callosum, forceps minor of the corpus callosum (fmi).

RX821002 or propranolol did not affect the cardiovascular changes evoked by noradrenaline, thus reinforcing the idea of an involvement of α_1-adrenoceptor. Therefore, these results suggest a preferential mediation via activation of local α_1-adrenoceptors on the cardiovascular responses evoked by microinjection of noradrenaline into the IC.

Similar doses of the antagonists WB4101 and RX821002 have been previously used to identify the subtype of α-adrenoceptors which were involved in the mediation of pressor responses to noradrenaline microinjection in specific central nervous system (CNS) structures (Fernandes et al., 2003; Scopinho et al., 2006; Busnardo et al., 2009). The pressor response to local microinjection of noradrenaline into the mPFC or lateral septal area was blocked by the pretreatment with WB4101, but not by the pretreatment with RX821002, thus suggesting an involvement of local α_1-adrenoceptors (Fernandes et al., 2003; Scopinho et al., 2006). On the other hand, pressor response to noradrenaline microinjection into the hypothalamic supraoptic nucleus (SON) was inhibited after local pretreatment with RX821002, but not by treatment with WB4101 (Busnardo et al., 2009), which points to an involvement of α_2-adrenoceptors. Also, their use allowed us to identify a situation in which both α-adrenoceptors subtypes were involved in the mediation of pressor responses to noradrenaline microinjected into the BST (Crestani et al., 2008). These observations indicate that the dose of WB4101 and RX821002 used in the present study is selective and effective in demonstrating the predominance of either α_1- or α_2-adrenoceptor in responses involving noradrenergic neurotransmission.

To confirm the involvement of local IC α_1-adrenoceptor on the noradrenaline cardiovascular responses, it was performed in the IC local administration of the selective α_1-adrenoceptor agonist phenylephrine. The administration of phenylephrine into the IC evoked similar changes on MAP and HR compared to that observed after noradrenaline microinjection, thus reinforcing the idea that α_1-adrenoceptors mediate cardiovascular responses to noradrenaline microinjected into the IC. It is important to note that the dose of phenylephrine was higher than that of noradrenaline, once that it has been reported that noradrenaline is approximately 10-times more potent than phenylephrine to evoke cardiovascular responses when microinjected in the CNS (Correa and Peres-Polon, 1995).

Changes in arterial pressure, HR and renal sympathetic nerve activity (RSNA) have been reported after chemical stimulation of the IC (Butcher and Cechetto, 1995). The IC has been proposed to modulate the sympathetic nervous activity through a mandatory synapse in the ventrolateral medulla, since it was reported that the increase in RSNA evoked by IC stimulation was completely abolished after ventrolateral medulla treatment with the nonselective synapses blocker $CoCl_2$ (Butcher and Cechetto, 1995; Cechetto and Chen, 1992). Consequently, the possible involvement of the sympathetic nervous system in the mediation of the pressor response observed after the microinjection of noradrenaline into the IC should be studied.

Systemic pretreatment with ganglion blocker has been traditionally used as a pharmacological tool to reduce sympathetic nervous activity. In the present study, systemic treatment with pentolinium significantly lowered basal levels of arterial pressure, confirming its effectiveness in reducing sympathetic activity. The variation of MAP (ΔMAP) evoked by IC treatment with noradrenaline was enhanced after ganglion blockade. However, the increase on ΔMAP was similar to change in MAP baseline evoked by pentolinium treatment. Therefore, the increase in ΔMAP caused by microinjection of noradrenaline into the IC in pentolinium-treated animals may be attributed to the initial reduction in MAP baseline caused by the ganglion blockade.

Similar increases in pressor responses caused by central injection of noradrenaline were reported after its injection intracerebroventricular (i.c.v.) (Correa et al., 1985), into the BST (Crestani et al., 2007), into the SON (Busnardo et al., 2009) or into the mPFC (Fernandes et al., 2003) of

pentolinium-treated rats, thus suggesting a humoral mediation. Under those conditions, the pressor effect of the central injection of noradrenaline was reported to be caused by vasopressin release into the circulation (Busnardo et al., 2009; Correa et al., 1985; Crestani et al., 2007; Fernandes et al., 2003).

In the CNS α_1-adrenoceptors have been shown to mediate vasopressin release by cathecolamines both *in vitro* (Randle et al., 1986; Yamashita et al., 1988) and *in vivo* (Brooks et al., 1986; Correa and Peres-Polon, 1995; Crestani et al., 2007). Attempting to verify whether circulating vasopressin was involved in the pressor response to noradrenaline microinjected into IC, we pretreated animals with the selective V_1-vasopressin receptor antagonist dTyr$(CH_2)_5$(Me)AVP. This vasopressin antagonist has been previously used to evidence a vasopressin involvement in the mediation of pressor responses caused by noradrenaline microinjections either i.c.v. or into specific CNS structures (Busnardo et al., 2009; Correa et al., 1985; Crestani et al., 2007), as well as after the intraseptal microinjection of acetylcholine (Peres-Polon and Correa, 1994) and the microinjection of L-glu into the diagonal band of Broca or the SON (Busnardo et al., 2007; Tavares and de Aguiar Correa, 2003). The peak and length of the pressor response to noradrenaline microinjection into the IC were significantly reduced after pretreatment with dTyr$(CH_2)_5$(Me)AVP, thus indicating that this response is mainly mediated by an action of the vasopressin. Thus, this result suggests that pressor response evoked by microinjection of noradrenaline into the IC involves acute release of vasopressin into the circulation.

The i.v. treatment with either pentolinium or dTyr(CH2)5(Me)AVP abolished the bradycardiac response to noradrenaline microinjected into the IC. Together, these data suggest a vagal baroreflex response, as a consequence of MAP increase. Vasopressin has been reported to cause positive chronotropic effect (Chiba, 1977), thus reinforcing the idea of a reflex bradycardia. Also, there was a good correlation between MAP increases and bradycardiac responses.

Vasopressin, also known as antidiuretic hormone, is a potent vasoconstrictor (Altura and Altura, 1984; Barer, 1961). Two different types of hypothalamic neurons, magnocellular and parvocellular, synthesize vasopressin. The magnocellular neurons are mainly located in the SON and paraventricular nucleus (PVN) of the hypothalamus. Each neuron gives rise to a single axon into the posterior pituitary gland, where its neurosecretory endings release vasopressin. Because the capillaries within the pituitary gland lack an effective blood-brain barrier, vasopressin is released in close proximity to the capillaries and easily enters the bloodstream (Leng et al., 1999). No direct connections were observed between the IC and either the PVN or the SON (Yasui et al., 1991), thus suggesting the existence of a relay in this pathway. Thus, the IC should be linked to the PVN or SON through an indirect projection. The microinjection of noradrenaline into the BST has been reported to cause pressure and bradycardiac responses, which are mediated by vasopressin release into the circulation (Crestani et al., 2008, 2007; Crestani et al., 2009). Also, neuronal connections have been identified between the IC and the BST (Yasui et al., 1991), as well as between the BST and the PVN and SON (Dong and Swanson, 2004). Considering this, the connection between the IC and the hypothalamus could be the BST. However, further studies are necessary to clarify the pathway involved in these responses.

The physiological role of this pressor pathway activated by the microinjection of noradrenaline into the IC is not yet clear. Vasopressin release is stimulated by plasma osmolality increase, arterial pressure decrease and by reduced cardiac filling or changes in blood volume (Goldsmith, 1988). Therefore, we may hypothesize that a noradrenergic mechanism in the IC can integrate cardiovascular and neuroendocrine control and could take part in fluid balance and cardiovascular adjustments to stress responses. This idea is favored by studies indicating that brainstem noradrenergic cell groups are activated during stress (Chen and Herbert, 1995; Dayas et al., 2001).

IC treatment with adrenoceptor antagonists did not affect either MAP or HR baseline values. Thus, although the present study supports the idea that an IC noradrenergic neurotransmission modulates the cardiovascular system, this neurotransmission is not involved in the tonic maintenance of either arterial pressure or HR. These results corroborate previous data indicating no changes in cardiovascular parameters after IC lesions, local synaptic blockade, blockade of glutamate receptors or blockade of adrenoceptors (Alves et al., 2009a,b; Zhang et al., 1998).

In summary, the administration of noradrenaline into the IC caused pressor responses, which was accompanied by bradycardia in unanesthetized rats. Our data suggest that bradycardiac response is a vagal baroreflex response, as a consequence of MAP increase. Because noradrenaline effects caused by activation of IC α_1-adrenoceptors were mediated by acute vasopressin release into the circulation, the results suggest that IC noradrenergic neurotransmission plays a role in the neural circuitry that regulates neuroendocrine and cardiovascular responses.

Acknowledgments

The authors wish to thank I.A.C. Fortunato, I.I.B. Aguiar and S.S. Guilhaume for technical help. Alves has a CNPq PhD Fellowship (870307/1997-5). The present research was supported by grants from the CNPq (306381/2003-6 and 505394/2003-0), FAPESP (06/57670-4) and FAEPA.

References

Allen, G.V., Cechetto, D.F., 1995. Neurotensin in the lateral hypothalamic area: origin and function. Neuroscience 69 (2), 533–544.
Altura, B.M., Altura, B.T., 1984. Actions of vasopressin, oxytocin, and synthetic analogs on vascular smooth muscle. Fed. Proc. 43 (1), 80–86.
Alves, F.H., Crestani, C.C., Resstel, L.B., Correa, F.M., 2007. Cardiovascular effects of carbachol microinjected into the bed nucleus of the stria terminalis of the rat brain. Brain Res. 1143, 161–168.
Alves, F.H., Crestani, C.C., Resstel, L.B., Correa, F.M., 2009a. Insular cortex alpha1-adrenoceptors modulate the parasympathetic component of the baroreflex in unanesthetized rats. Brain Res. 1295, 119–126.
Alves, F.H., Crestani, C.C., Resstel, L.B., Correa, F.M., 2009b. N-methyl-D-aspartate receptors in the insular cortex modulate baroreflex in unanesthetized rats. Auton. Neurosci. 147 (1–2), 56–63.
Alves, F.H., Crestani, C.C., Corrêa, F.M., 2010. The insular cortex modulates cardiovascular responses to acute restraint stress in rats. Brain Res. 1333, 57–63.
Anand, B.K., Dua, S., 1956. Circulatory and respiratory changes induced by electrical stimulation of limbic system (visceral brain). J. Neurophysiol. 19 (5), 393–400.
Barer, G.R., 1961. A comparison of the circulatory effects of angiotensin, vasopressin and adrenaline in the anaesthetized cat. J. Physiol. 156, 49–66.
Brooks, D.P., Share, L., Crofton, J.T., 1986. Central adrenergic control of vasopressin release. Neuroendocrinology 42 (5), 416–420.
Busnardo, C., Tavares, R.F., Antunes-Rodrigues, J., Correa, F.M., 2007. Cardiovascular effects of L-glutamate microinjection in the supraoptic nucleus of unanaesthetized rats. Neuropharmacology 52 (6), 1378–1384.
Busnardo, C., Tavares, R.F., Correa, F.M., 2009. Mechanisms involved in the pressor response to noradrenaline microinjection into the supraoptic nucleus of unanesthetized rats. Auton. Neurosci. 145 (1–2), 63–70.
Butcher, K.S., Cechetto, D.F., 1995. Autonomic responses of the insular cortex in hypertensive and normotensive rats. Am. J. Physiol. 268 (1 Pt 2), R214–R222.
Camargo, L.A., Saad, W.A., Machado, J.A., Rodrigues, G.N., 1979. Effects of noradrenergic stimulation of the medial and lateral hypothalamus on cardiovascular responses. Pharmacol. Res. Commun. 11 (2), 147–156.
Cechetto, D.F., Chen, S.J., 1992. Hypothalamic and cortical sympathetic responses relay in the medulla of the rat. Am. J. Physiol. 263 (3 Pt 2), R544–R552.
Chen, X., Herbert, J., 1995. Regional changes in c-fos expression in the basal forebrain and brainstem during adaptation to repeated stress: correlations with cardiovascular, hypothermic and endocrine responses. Neuroscience 64 (3), 675–685.
Chiba, S., 1977. Positive chronotropic and negative inotropic actions of vasopressin in isolated blood-perfused canine atrium. Jpn. Heart J. 18 (2), 221–224.
Correa, F.M., Magro, I.A., Peres-Polon, V.L., Antunes-Rodrigues, J., 1985. Mechanism of the CNS-mediated pressor response to intracerebroventricular injection of noradrenaline in unanaesthetized rats. Neuropharmacology 24 (9), 831–837.
Correa, F.M., Peres-Polon, V.L., 1995. Central alpha 1-adrenoceptors mediate the pressor response to intracerebroventricular injection of noradrenaline in unanesthetized rats. Neuropharmacology 34 (7), 793–798.
Crestani, C.C., Busnardo, C., Tavares, R.F., Alves, F.H., Correa, F.M., 2009. Involvement of hypothalamic paraventricular nucleus non-N-methyl-D-aspartate receptors in the

pressor response to noradrenaline microinjected into the bed nucleus of the stria terminalis of unanesthetized rats. Eur. J. Neurosci. 29 (11), 2166–2176.
Crestani, C.C., Alves, F.H., Resstel, L.B., Correa, F.M., 2008. Both alpha1 and alpha2-adrenoceptors mediate the cardiovascular responses to noradrenaline microinjected into the bed nucleus of the stria terminal of rats. Br. J. Pharmacol. 153 (3), 583–590.
Crestani, C.C., Alves, F.H., Resstel, L.B., Correa, F.M., 2007. Cardiovascular effects of noradrenaline microinjection in the bed nucleus of the stria terminalis of the rat brain. J. Neurosci. Res. 85 (7), 1592–1599.
Dayas, C.V., Buller, K.M., Day, T.A., 2001. Medullary neurones regulate hypothalamic corticotropin-releasing factor cell responses to an emotional stressor. Neuroscience 105 (3), 707–719.
Delgado, J.M., 1960. Circulatory effects of cortical stimulation. Physiol. Rev. Suppl. 4, 146–178.
Dong, H.W., Swanson, L.W., 2004. Projections from bed nuclei of the stria terminalis, posterior division: implications for cerebral hemisphere regulation of defensive and reproductive behaviors. J. Comp. Neurol. 471 (4), 396–433.
Fernandes, K.B., Crippa, G.E., Tavares, R.F., Antunes-Rodrigues, J., Correa, F.M., 2003. Mechanisms involved in the pressor response to noradrenaline injection into the cingulate cortex of unanesthetized rats. Neuropharmacology 44 (6), 757–763.
Funk, D., Stewart, J., 1996. Role of catecholamines in the frontal cortex in the modulation of basal and stress-induced autonomic output in rats. Brain Res. 741 (1–2), 220–229.
Goldsmith, S.R., 1988. Baroreceptor-mediated suppression of osmotically stimulated vasopressin in normal humans. J. Appl. Physiol. 65 (3), 1226–1230.
Hardy, S.G., Holmes, D.E., 1988. Prefrontal stimulus-produced hypotension in rat. Exp. Brain Res. 73 (2), 249–255.
Hardy, S.G., Mack, S.M., 1990. Brainstem mediation of prefrontal stimulus-produced hypotension. Exp. Brain Res. 79 (2), 393–399.
Harland, D., Gardiner, S.M., Bennett, T., 1989. Paraventricular nucleus injections of noradrenaline: cardiovascular effects in conscious Long–Evans and Brattleboro rats. Brain Res. 496 (1–2), 14–24.
Krettek, J.E., Price, J.L., 1977. The cortical projections of the mediodorsal nucleus and adjacent thalamic nuclei in the rat. J. Comp. Neurol. 171 (2), 157–191.
Leng, G., Brown, C.H., Russell, J.A., 1999. Physiological pathways regulating the activity of magnocellular neurosecretory cells. Prog. Neurobiol. 57 (6), 625–655.
Leonard, C.M., 1969. The prefrontal cortex of the rat. I. Cortical projection of the mediodorsal nucleus. II. Efferent connections. Brain Res. 12 (2), 321–343.
Moriguchi, S., Ohzuru, N., Koga, N., Honda, K., Saito, R., Takano, Y., Kamiya, H., 1998. Central administration of a nitric oxide synthase inhibitor causes pressor responses via the sympathetic nervous system and the renin–angiotensin system in Wistar rats. Neurosci. Lett. 245 (2), 109–112.
Morrison, J.H., Grzanna, R., Molliver, M.E., Coyle, J.T., 1978. The distribution and orientation of noradrenergic fibers in neocortex of the rat: an immunofluorescence study. J. Comp. Neurol. 181 (1), 17–39.
Morrison, J.H., Molliver, M.E., Grzanna, R., 1979. Noradrenergic innervation of cerebral cortex: widespread effects of local cortical lesions. Science 205 (4403), 313–316.
Neafsey, E.J., 1990. Prefrontal cortical control of the autonomic nervous system: anatomical and physiological observations. Prog. Brain Res. 85, 147–165 discussion 165-146.
Ohta, H., Watanabe, S., Ueki, S., 1991. Cardiovascular changes induced by chemical stimulation of the amygdala in rats. Brain Res. Bull. 26 (4), 575–581.
Paxinos, G., Watson, C., 1997. The Rat Brain in Stereotaxic Coodinates, 2 ed. Sydney Academic Press.
Peres-Polon, V.L., Correa, F.M., 1994. Pressor effects of acetylcholine injected into the lateral septal area of conscious rats. Neuropharmacology 33 (12), 1537–1544.
Randle, J.C., Mazurek, M., Kneifel, D., Dufresne, J., Renaud, L.P., 1986. Alpha 1-adrenergic receptor activation releases vasopressin and oxytocin from perfused rat hypothalamic explants. Neurosci. Lett. 65 (2), 219–223.
Resstel, L.B., Correa, F.M., 2006. Involvement of the medial prefrontal cortex in central cardiovascular modulation in the rat. Auton. Neurosci. 126–127, 130–138.
Rose, J.E., Woolsey, C.N., 1948. Structure and relations of limbic cortex and anterior thalamic nuclei in rabbit and cat. J. Comp. Neurol. 89 (3), 279–347.
Ruggiero, D.A., Mraovitch, S., Granata, A.R., Anwar, M., Reis, D.J., 1987. A role of insular cortex in cardiovascular function. J. Comp. Neurol. 257 (2), 189–207.
Sara, S.J., Segal, M., 1991. Plasticity of sensory responses of locus coeruleus neurons in the behaving rat: implications for cognition. Prog. Brain Res. 88, 571–585.
Scopinho, A.A., Resstel, L.B., Antunes-Rodrigues, J., Correa, F.M., 2006. Pressor effects of noradrenaline injected into the lateral septal area of unanesthetized rats. Brain Res. 1122 (1), 126–134.
Tavares, R.F., de Aguiar Correa, F.M., 2003. Pressor effects of L-glutamate injected into the diagonal band of Broca of unanesthetized rats. Brain Res. 959 (2), 312–319.
Ungerstedt, U., 1971. Stereotaxic mapping of the monoamine pathways in the rat brain. Acta Physiol. Scand. Suppl. 367, 1–48.
Yamashita, H., Inenaga, K., Dyball, R.E., 1988. Thermal, osmotic and chemical modulation of neural activity in the paraventricular nucleus: in vitro studies. Brain Res. Bull. 20 (6), 825–829.
Yasui, Y., Breder, C.D., Saper, C.B., Cechetto, D.F., 1991. Autonomic responses and efferent pathways from the insular cortex in the rat. J. Comp. Neurol. 303 (3), 355–374.
Zandberg, P., De Jong, W., De Wied, D., 1979. Effect of catecholamine-receptor stimulating agents on blood pressure after local application in the nucleus tractus solitarii of the medulla oblongata. Eur. J. Pharmacol. 55 (1), 43–56.
Zhang, Z.H., Rashba, S., Oppenheimer, S.M., 1998. Insular cortex lesions alter baroreceptor sensitivity in the urethane-anesthetized rat. Brain Res. 813 (1), 73–81.