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Lateral septal area alpha(1)-and alpha(2)-adrenoceptors differently modulate baroreflex activity in unanaesthetized rats

Research Paper

Lateral septal area α_1- and α_2-adrenoceptors differently modulate baroreflex activity in unanaesthetized rats

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The lateral septal area (LSA) is a limbic structure involved in autonomic, neuroendocrine and behavioural responses. An inhibitory influence of the LSA on baroreflex activity has been reported; however, the local neurotransmitter involved in this modulation is still unclear. In the present study, we verified the involvement of local LSA adrenoceptors in modulating cardiac baroreflex activity in unanaesthetized rats. Bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 (10 nmol in a volume of 100 nl) into the LSA decreased baroreflex bradycardia evoked by blood pressure increases, but had no effect on reflex tachycardia evoked by blood pressure decreases. Nevertheless, bilateral administration of the selective α_2-adrenoceptor antagonist RX821002 (10 nmol in 100 nl) increased baroreflex tachycardia without affecting reflex bradycardia. Treatment of the LSA with a cocktail containing WB4101 and RX821002 decreased baroreflex bradycardia and increased reflex tachycardia. The non-selective β-adrenoceptor antagonist propranolol (10 nmol in 100 nl) did not affect either reflex bradycardia or tachycardia. Microinjection of noradrenaline into the LSA increased reflex bradycardia and decreased the baroreflex tachycardic response, an opposite effect compared with those observed after double blockade of α_1- and α_2-adrenoceptors, and this effect of noradrenaline was blocked by local LSA pretreatment with the cocktail containing WB4101 and RX821002. The present results provide advances in our understanding of the baroreflex neural circuitry. Taken together, data suggest that local LSA α_1- and α_2-adrenoceptors modulate baroreflex control of heart rate differently. Data indicate that LSA α_1-adrenoceptors exert a facilitatory modulation on baroreflex bradycardia, whereas local α_2-adrenoceptors exert an inhibitory modulation on reflex tachycardia.

The baroreflex is an important mechanism for moment-to-moment regulation of arterial pressure (Sved & Gordon, 1994). Furthermore, it has been proposed that a resetting of baroreflex activity towards higher blood pressure values mediates, at least in part, autonomic and cardiovascular changes during physical exercise and stress situations (DiCarlo & Bishop, 1992; Dampney *et al.* 2008; Crestani *et al.* 2010*b*). Some recent evidence has also suggested a role of the baroreflex in cardiovascular adjustments to exercise training (Ceroni *et al.* 2009).

The role of medullary structures in the baroreflex is well documented (Spyer, 1981; Sved & Gordon, 1994; Michelini, 2007). However, several supramedullary limbic structures are also involved in baroreflex responses (Dampney, 1994; Nosaka, 1996; Dampney *et al.* 2008), such as the medial prefrontal cortex (Sevoz-Couche *et al.* 2006), the bed nucleus of the stria terminalis, the periaqueductal grey area (Pelosi *et al.* 2007), hypothalamic regions (Sevoz-Couche *et al.* 2006; Crestani *et al.* 2009), the insular cortex (Alves *et al.* 2009; Nagai *et al.* 2010),

the diagonal band of Broca (Crestani *et al.* 2008*c*), the amygdala (Quagliotto *et al.* 2008) and the lateral septal area (LSA; Scopinho *et al.* 2007).

The LSA is a limbic structure that integrates autonomic, neuroendocrine and behavioural responses (DeFrance *et al.* 1976). Electrophysiological studies have demonstrated that an electrical stimulation of the aortic depressor nerve alters the firing of LSA neurons (Miyazawa *et al.* 1988). Moreover, baroreflex-related cardiovascular changes evoked after bilateral carotid occlusion were decreased when occlusion was performed during septal stimulation in anaesthetized animals (Covian & Timo-Iaria, 1966). We have previously reported that bilateral microinjection of the non-selective synapse blocker cobalt chloride into the LSA enhanced both baroreflex bradycardia and tachycardia in unanaesthetized rats, thus suggesting an inhibitory influence of the LSA in the cardiac component of the baroreflex (Scopinho *et al.* 2007). Although these results support an involvement of the LSA in baroreflex responses, the specific local neurotransmitter involved in LSA-related modulation of baroreflex activity remains unknown.

The involvement of noradrenergic neurotransmission in control of baroreflex responses in several regions of the central nervous system has been documented (Hwang *et al.* 1998; Crestani *et al.* 2008*a*; Alves *et al.* 2009). The LSA has a dense network of noradrenergic neural terminations (Lindvall & Stenevi, 1978; Risold & Swanson, 1997; Antonopoulos *et al.* 2004). Moreover, microinjection of noradrenaline into the LSA evokes changes in arterial pressure and heart rate (Scopinho *et al.* 2006). However, although central noradrenergic pathways seem to play a role in baroreflex activity and there is evidence suggesting the existence of functional noradrenergic neurotransmission in the LSA, its involvement in baroreflex responses has not yet been evaluated. Therefore, the hypothesis of this work is that the noradrenergic neurotransmission present in the LSA is involved in baroreflex control of heart rate in unanaesthetized animals. To investigate this hypothesis, we analysed the reflex bradycardia in response to blood pressure increases as well as tachycardic responses to blood pressure decreases before and after microinjection of adrenoceptor agonist and antagonists into the LSA.

Methods

Ethical approval

Housing conditions and experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto, which comply with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society. All efforts were made to minimize animal suffering and to reduce the number of animals used.

Animals

Sixty-six male Wistar rats weighing 240–260 g were used. Animals were housed in plastic cages in a temperature-controlled room at 25°C in the Animal Care Unit of the Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo. They were kept with a 12 h–12 h light–dark cycle (lights on between 06.00 and 18.00 h) and had free access to water and standard laboratory food.

Surgical preparation

Four days before the experiment, rats were anaesthetized with tribromoethanol (250 mg kg^{-1}, I.P.). After induction of scalp anaesthesia with 2% lidocaine, the skull was exposed and stainless-steel guide cannulae (26 gauge) were bilaterally implanted into the LSA at a position 1 mm above the site of injection, using a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Co-ordinates for cannula implantation into the LSA were as follows: anteroposterior +9.2 mm from interaural, lateral +2.5 mm from the medial suture, and ventral −4.4 mm from the skull, with a 22 deg lateral inclination (Paxinos & Watson, 1997). Cannulae were fixed to the skull with dental cement and one metal screw. After surgery, the animals received a broad-spectrum antibiotic mixture (Pentabiotico®; Fort Dodge, Campinas, Brazil), with streptomycins and penicillins, to prevent infection, and the non-steroidal anti-inflammatory flunixin meglumine (Banamine®; Schering Plough, Cotia, Brazil) for postoperative analgesia.

One day before the trial, rats were anaesthetized with tribromoethanol (250 mg kg^{-1}, I.P.), and a catheter was inserted into the abdominal aorta through the femoral artery for blood pressure and heart rate recording. A second catheter was implanted into the femoral vein for infusion of the vasoactive drugs. Both catheters were tunnelled under the skin and exteriorized on the animal's dorsum. After surgery, the non-steroidal anti-inflammatory flunixin meglumine (Banamine®) was administered for postoperative analgesia.

Measurement of cardiovascular responses

On the day of the experiment, the arterial cannulae were connected to a pressure transducer. The pulsatile arterial pressure was recorded using an HP-7754A preamplifier (Hewlett Packard, Palo Alto, CA, USA) and an acquisition board (MP100A; Biopac Systems, Goleta, CA, USA) connected to a personal computer. Mean arterial pressure

and heart rate values were derived from pulsatile arterial pressure recordings and were processed online.

Drug injection

Needles (33 gauge; Small Parts, Miami Lakes, FL, USA) that were used for microinjection into the LSA were 1 mm longer than guide cannulae and were connected to a hand-driven 1 μl syringe (7002H; Hamilton, Reno, NV, USA) through PE-10 tubing. Drugs or vehicle were injected in a final volume of 100 nl into the LSA.

Baroreflex assessment

The baroreflex was tested by intravenous infusion of either the selective α_1-adrenoceptor agonist phenylephrine (50 μg ml^{-1} at 0.32 ml min^{-1} kg^{-1}) or the nitric oxide donor sodium nitroprusside (70 μg ml^{-1} at 0.8 ml min^{-1} kg^{-1}), using an infusion pump (K.D. Scientific, Holliston, MA, USA; Head & McCarty, 1987; Scopinho *et al.* 2007). Phenylephrine and sodium nitroprusside caused incremental pressor or depressor responses, respectively. Infusions of vasoactive drugs were randomized, and the interval between infusions was approximately 5 min. Infusions lasted for 30–40 s, resulting in the injection of a total dose of 8–10 μg kg^{-1} of phenylephrine and 20–25 μg kg^{-1} of sodium nitroprusside.

Method of baroreflex evaluation

Heart rate values corresponding to mean arterial pressure variations, evoked by phenylephrine or sodium nitroprusside infusions, were determined. Paired values of mean arterial pressure and heart rate were plotted to generate sigmoid logistic functions for each rat, which were used to determine baroreflex activity (Head & McCarty, 1987; Scopinho *et al.* 2006; Crestani *et al.*

2010*a*). Baroreflex analysis using sigmoid curves were characterized by the following four parameters: (i) P1 (in beats per minute), the lower heart rate plateau, and P$_2$ (in beats per minute), the upper plateau; (ii) the heart rate range (in beats per minute), i.e. the difference between upper and lower plateau levels; (iii) maximal gain (G_{max}, in –beats per minute per millimetre of mercury), which is the maximal gain obtained from the calculation of the baroreflex gain across the full range of mean arterial pressure variations; and (iv) the median blood pressure (BP$_{50}$, in millimetres of mercury), which is the mean arterial pressure at 50% of the heart rate range (Head & McCarty, 1987; Zoccal *et al.* 2009; Crestani *et al.* 2010*b*). The instantaneous gain over the full range of mean arterial pressure variations was determined by taking the first derivative of the sigmoid logistic function (DiCarlo & Bishop, 1990; Maliszewska-Scislo *et al.* 2003). The delay in reflex bradycardia and tachycardia was about 1.2 s because of the integration factor of the recording system, which was about 500 ms, and the time of baroreflex synapse processing, which was 700 ms (Su *et al.* 1992).

Drugs

WB4101 (Tocris, Westwoods Business Park, Ellisville, MO, USA), RX821002 (Tocris), a cocktail containing WB4101 and RX821002, DL-propranolol hydrochloride (Sigma, St Louis, MO, USA) and noradrenaline hydrochloride (Sigma) were dissolved in sterile artificial cerebrospinal fluid [aCSF; composition (mM): NaCl, 100; Na$_3$PO$_4$, 2; KCl, 2.5; MgCl$_2$, 1; NaHCO$_3$, 27; and CaCl$_2$, 2.5; pH 7.4]. Phenylephrine hydrochloride (Sigma), sodium nitroprusside dihydrate (Sigma), tribromoethanol (Sigma) and urethane (Sigma) were dissolved in saline (0.9% NaCl). Flunixin meglumine (Banamine$^{®}$; Schering Plough) and a poly-antibiotic preparation of streptomycins and penicillins (Pentabiotico$^{®}$; Fort Dodge) were used as provided.
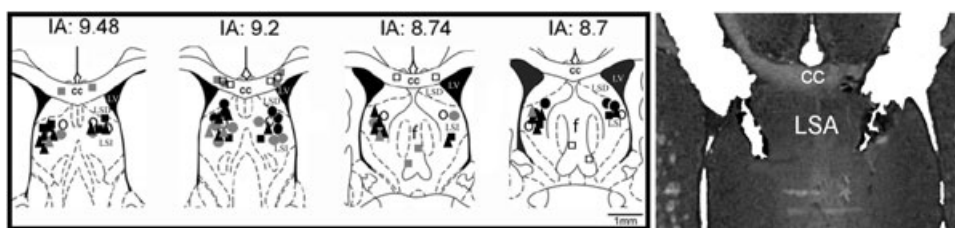

Figure 1. Injection sites in the lateral septal area.
Left panel shows diagrammatic representations based on the rat brain atlas of Paxinos & Watson (1997), indicating injection sites of artificial cerebrospinal fluid (aCSF; open circles), WB41001 (black circles), RX821002 (grey circles), the cocktail containing WB4101 and RX821002 (black triangles), propranolol (black squares) and noradrenaline (grey triangles) into the LSA, as well as injection sites of WB4101 (open squares) and RX821002 (grey squares) into structures surrounding the LSA. Abbreviations: cc, corpus callosum; f, fornix; IA, interaural co-ordinates; LSI, lateral septum intermediate; LSD, lateral septum dorsal; and LV, lateral ventricle. Right panel is a photomicrograph of a coronal brain section from one representative rat, showing bilateral injection sites in the lateral septal area.

Table 1. Parameters derived from sigmoidal baroreflex curves generated before or 10 min after bilateral microinjection of artificial cerebrospinal fluid (vehicle) into the lateral septal area (LSA)

Group	G_{max} (–beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	Range (beats min^{-1})	BP$_{50}$ (mmHg)
Before vehicle	2.7 ± 0.3	303 ± 5	433 ± 3	130 ± 6	93 ± 2
10 min after	2.9 ± 0.4	300 ± 6	427 ± 5	127 ± 5	89 ± 2
	$t = 0.4$	$t = 0.3$	$t = 1$	$t = 0.3$	$t = 1.2$

Values are means $\pm$ SEM.

Abbreviations: G_{max}, maximum gain; P1, lower heart rate plateau; P2, upper heart rate plateau; Range: difference between upper and lower plateau levels; BP$_{50}$, arterial pressure at 50% of the heart rate range; t, student's t test.

Experimental protocols

Animals were brought to the experimental room in their home cages. Animals were given 60 min to adapt to the conditions of the experimental room, such as sound and illumination, before starting arterial pressure and heart rate recordings. The experimental room had controlled temperature (25°C) and was acoustically isolated from the main laboratory. Constant background noise was generated by an air exhauster to minimize sound interference within the experimental room. Before beginning the experiments, another 30 min period of basal recording of arterial pressure and heart rate was allowed.

Effect of adrenoceptor blockades in the LSA on cardiac baroreflex responses in unanaesthetized rats. Animals used in this study were divided into the following seven experimental groups: the first group received bilateral microinjection of aCSF (vehicle, 100 nl on each side, $n = 5$) into the LSA (Scopinho *et al.* 2007, 2008); the second group received bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 (10 nmol in 100 nl on each side, $n = 5$) into the LSA (Scopinho *et al.* 2006; Crestani *et al.* 2008*b*); the third group received unilateral microinjection of WB4101 (10 nmol in 100 nl, $n = 5$) in the left side of the LSA; the fourth group received bilateral microinjection of the selective α_2-adrenoceptor antagonist RX821002 (10 nmol in 100 nl on each side, $n = 5$) into the LSA (Scopinho *et al.* 2006; Crestani *et al.* 2008*b*); the fifth group received unilateral microinjection of RX821002 (10 nmol in 100 nl, $n = 5$) in the left side of the LSA; the sixth group received bilateral microinjection of 100 nl of a cocktail containing WB4101 [10 nmol (100 nl)$^{-1}$] and RX821002 [10 nmol (100 nl)$^{-1}$; $n = 5$] into the LSA; and the seventh group received bilateral microinjection of the non-selective β-adrenoceptor antagonist propranolol (10 nmol in 100 nl on each side, $n = 5$) into the LSA (Crestani *et al.* 2008*b*). Animals in all experimental groups received two sets of phenylephrine and sodium nitroprusside infusions,
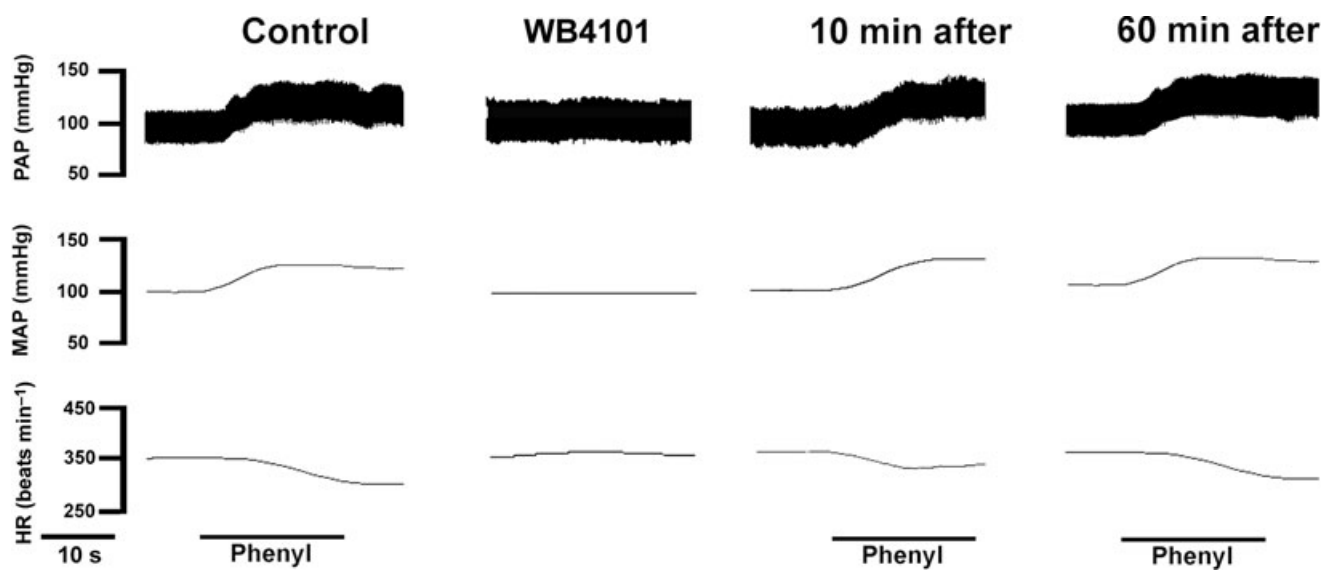

Figure 2. Recordings from one representative animal, illustrating the reflex bradycardia in response to blood pressure increase caused by intravenous infusion of phenylephrine (Phenyl) before and 10 or 60 min after bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 into the LSA
Abbreviations: MAP, mean arterial pressure; PAP, pulsatile arterial pressure; and HR, heart rate. Note the decrease in bradycardiac response 10 min after LSA treatment with WB4101.

in a random order, before pharmacological treatment of the LSA to determine control values of the baroreflex activity. In the sequence, phenylephrine and sodium nitroprusside infusions were repeated, in a random order, 10 and 60 min after LSA pharmacological treatments.

Effect of microinjection of noradrenaline into the LSA on cardiac baroreflex responses in unanaesthetized rats. In this protocol, animals received unilateral infusion of noradrenaline (10 nmol in 100 nl, $0.4 \mu l \, min^{-1}$) or vehicle (aCSF, 100 nl, $0.4 \mu l \, min^{-1}$) into the LSA (Scopinho *et al.* 2006) during the I.V. infusion of phenylephrine and sodium nitroprusside (Alves *et al.* 2009). Animals were divided into the following four experimental groups: the first group received local unilateral microinjection of vehicle (aCSF, 100 nl) 10 min before vehicle infusion (aCSF, 100 nl, $0.4 \mu l \, min^{-1}$) into the LSA ($n = 6$; Veh + Veh); the second group received local unilateral microinjection of a cocktail containing WB4101 $[10 \, nmol \, (100 \, nl)^{-1}]$ and RX821002 $[10 \, nmol \, (100 \, nl)^{-1}]$ 10 min before vehicle infusion (aCSF, 100 nl, $0.4 \mu l \, min^{-1}$) into the LSA ($n = 5$; (WB/RX +Veh); the third group received local unilateral microinjection of vehicle (aCSF, 100 nl) 10 min before noradrenaline infusion (10 nmol in 100 nl, $0.4 \mu l \, min^{-1}$)

into the LSA ($n = 5$; Veh + Nor); and the fourth group received local unilateral microinjection of 100 nl of the cocktail containing WB4101 and RX821002 10 min before noradrenaline infusion (10 nmol in 100 nl, $0.4 \mu l \, min^{-1}$) into the LSA ($n = 5$; WB/RX + Nor).

Histological procedure

At the end of the experiments, rats were anaesthetized with urethane ($1.25 \, g \, kg^{-1}$, I.P.), and 100 nl of 1% Evan's Blue dye was injected bilaterally in the LSA to mark injection sites. The chest was surgically opened, the descending aorta occluded, the right atrium severed, and the brain perfused with 10% formalin through the left ventricle. Brains were postfixed for 24 h at $4°C$, and $40-\mu m$-thick sections were cut using a cryostat (CM-1900; Leica, Wetzlar, Germany). Sections were stained with 1% Neutral Red, and injection sites were identified according to the rat brain atlas of Paxinos & Watson (1997).

A representative photomicrograph of a coronal brain section depicting bilateral microinjection sites in the LSA of one representative animal is presented in Fig. 1. Also, diagrammatic representations showing microinjection sites of aCSF, WB4101, RX821002, the cocktail containing WB4101 and RX821002, propranolol and noradrenaline
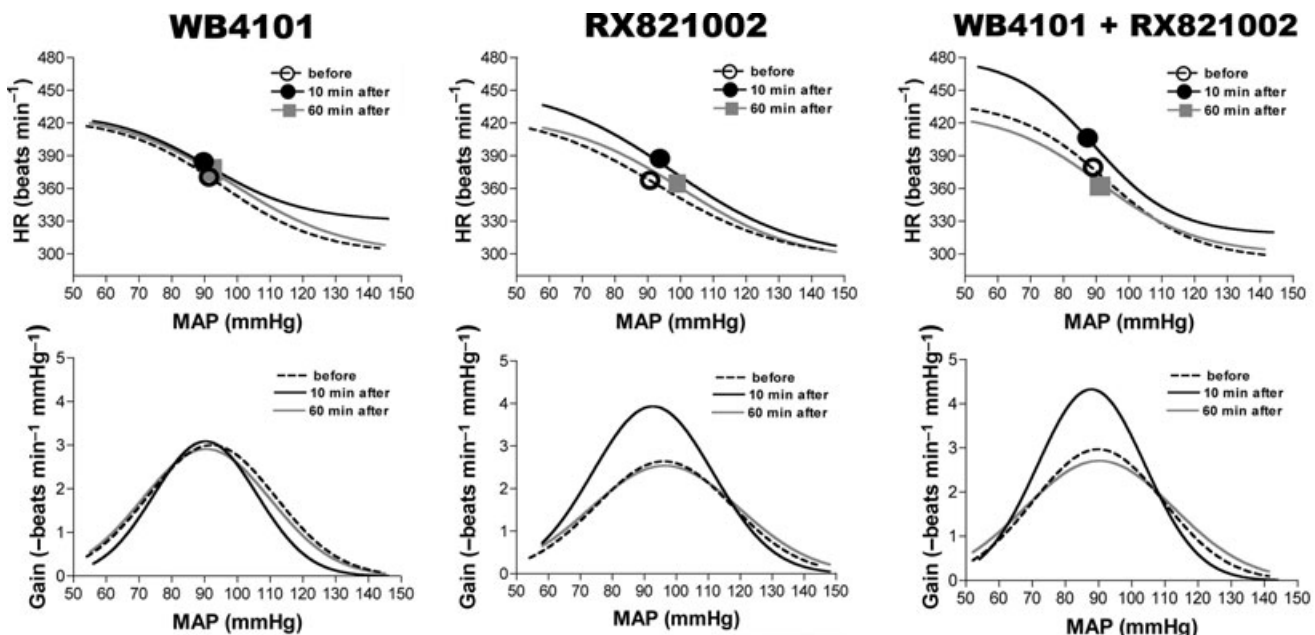

Figure 3. Effect of alpha-adrenoceptors blockade in the lateral septal area on cardiac baroreflex response.
Top panles show sigmoid baroreflex curves correlating mean arterial pressure (MAP) and heart rate (HR) before and 10 or 60 min after bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 (before, $r^2 = 0.88$; 10 min after, $r^2 = 0.78$; and 60 min after, $r^2 = 0.84$), the selective α_2-adrenoceptor antagonist RX821002 (before, $r^2 = 0.90$; 10 min after, $r^2 = 0.84$; and 60 min after, $r^2 = 0.89$) or a cocktail containing WB4101 and RX821002 into the LSA (before, $r^2 = 0.92$; 10 min after, $r^2 = 0.86$; and 60 min after, $r^2 = 0.93$). Symbols on curves indicate the respective median blood pressure (BP_{50}). Bottom panels show instantaneous gain of the respective baroreflex curve in the respective top panels. Increases or decreases in mean arterial pressure were induced by intravenous infusion of phenylephrine or sodium nitroprusside, respectively.

Table 2. Parameters derived from sigmoidal baroreflex curves generated before and 10 or 60 min after bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101, the selective α_2-adrenoceptor antagonist RX821002, or a cocktail containing WB4101 and RX821002 (WB + RX) into the LSA

Group	G_{max} (–beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	Range (beats min^{-1})	BP$_{50}$ (mmHg)
Before WB4101	3.8 ± 0.7	306 ± 6	429 ± 8	122 ± 6	89 ± 3
10 min after	3.5 ± 1.2	$327 \pm 4*$	430 ± 9	$103 \pm 8*$	88 ± 2
60 min after	3.0 ± 0.4	309 ± 6	438 ± 8	130 ± 6	89 ± 4
	$F = 0.3$	$F = 5$	$F = 0.2$	$F = 4$	$F = 0.1$
Before RX821002	3 ± 0.5	294 ± 6	429 ± 5	135 ± 5	91 ± 4
10 min after	$4.5 \pm 0.4*$	297 ± 8	$453 \pm 3*$	$156 \pm 6*$	96 ± 3
60 min after	3.1 ± 0.3	293 ± 6	427 ± 5	134 ± 7	98 ± 3
	$F = 5$	$F = 0.2$	$F = 11$	$F = 5$	$F = 1$
Before WB + RX	2.9 ± 0.6	299 ± 5	443 ± 8	144 ± 6	89 ± 1
10 min after	4.9 ± 0.7	$318 \pm 6*$	$482 \pm 11*$	$164 \pm 7*$	86 ± 3
60 min after	3.0 ± 0.5	300 ± 2	432 ± 5	132 ± 5	90 ± 1
	$F = 3$	$F = 5$	$F = 11$	$F = 8$	$F = 1$

Values are means $\pm$ SEM. *$P < 0.05$, significantly different from values before the respective treatment, one-way ANOVA followed by Dunnett's *post hoc* test.

into the LSA, as well as WB4101 and RX821002 into structures surrounding the LSA are also shown in Fig. 1.

Statistical analysis

Data are expressed as means $\pm$ SEM. Mean arterial pressure and heart rate baseline values before and after pharmacological treatment of the LSA, as well as baroreflex parameters from animals bilaterally treated with aCSF and propranolol and unilaterally with WB4101 and RX821002 were compared using Student's paired *t* test. Differences among sigmoid curves parameters from animals bilaterally treated with WB4101, RX821002, the cocktail containing WB4101 and RX921002, and unilaterally with noradrenaline were analysed using one-way ANOVA followed by Dunnett's *post hoc* test. A value of $P < 0.05$ was considered to be statistically significant.

Results

Effect of adrenoceptor blockades in the LSA on cardiac baroreflex responses in unanaesthetized rats

Vehicle. Bilateral microinjection of aCSF ($n = 5$) into the LSA did not affect either baseline mean arterial pressure (101 ± 3 *versus* 97 ± 3 mmHg, $P > 0.05$) or baseline heart rate (355 ± 16 *versus* 342 ± 14 beats min^{-1}, $P > 0.05$). Furthermore, the parameters of baroreflex function were not affected by LSA treatment with aCSF (Table 1).

Blockade of α_1-adrenoceptors. Bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 ($n = 5$) into the LSA did not affect either baseline mean arterial pressure (99 ± 2 *versus* 101 ± 1 mmHg, $P > 0.05$) or baseline heart rate (364 ± 5 *versus* 359 ± 4 beats min^{-1}, $P > 0.05$). Representative recordings showing the reflex

Table 3. Parameters derived from sigmoidal baroreflex curves generated before or 10 min after bilateral microinjection into structures surrounding the LSA (out) or unilateral microinjection into the LSA (unilateral) of the selective α_1-adrenoceptor antagonist WB4101 or the selective α_2-adrenoceptor antagonist RX821002

Group	G_{max} (–beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	Range (beats min^{-1})	BP$_{50}$ (mmHg)
Before WB4101 out	3.3 ± 0.5	303 ± 5	433 ± 8	130 ± 8	93 ± 2
10 min after	3.7 ± 0.2	302 ± 3	423 ± 3	121 ± 5	90 ± 1
	$t = 0.8$	$t = 0.2$	$t = 1.2$	$t = 1$	$t = 1.4$
Before RX821002 out	2.6 ± 0.3	305 ± 8	426 ± 9	121 ± 6	91 ± 4
10 min after	2.8 ± 0.3	299 ± 3	442 ± 11	143 ± 8	96 ± 3
	$t = 0.5$	$t = 0.8$	$t = 1.3$	$t = 2$	$t = 0.9$
Before WB4101 unilateral	2.9 ± 0.6	305 ± 5	446 ± 20	141 ± 18	102 ± 2
10 min after	2.7 ± 0.5	312 ± 6	433 ± 6	121 ± 5	96 ± 5
	$t = 0.2$	$t = 0.8$	$t = 0.6$	$t = 1$	$t = 1$
Before RX821002 unilateral	2.9 ± 0.6	309 ± 6	445 ± 20	136 ± 18	96 ± 5
10 min after	4.9 ± 0.7	300 ± 8	449 ± 12	149 ± 12	99 ± 1
	$t = 0.5$	$t = 1$	$t = 0.2$	$t = 0.6$	$t = 0.5$

Values are means $\pm$ SEM.

Abbreviation: *t*, Student's *t* test.

bradycardia evoked by blood pressure increases observed before and 10 or 60 min after bilateral treatment of the LSA with WB4101 are presented in Fig. 2. Non-linear regression analysis of baroreflex activity indicated that LSA treatment with WB4101 increased the lower (P1) plateau and decreased heart rate range (Fig. 3 and Table 2). Sixty minutes after treatment with WB4101, baroreflex parameters had returned to control values (Fig. 3 and Table 2).

Baroreflex activity was not affected by bilateral microinjection of WB4101 into structures surrounding the LSA ($n = 5$; Table 3). Moreover, unilateral microinjection of WB4101 ($n = 5$) into the LSA did not affect baroreflex control of heart rate (Table 3).

Blockade of α_2-adrenoceptors. Bilateral microinjection of the selective α_2-adrenoceptor antagonist RX821002 ($n = 5$) into the LSA did not affect either baseline mean arterial pressure (99 ± 4 *versus* 103 ± 5 mmHg, $P > 0.05$) or baseline heart rate (351 ± 9 *versus* 359 ± 11 beats min^{-1}, $P > 0.05$). Representative recordings showing the reflex tachycardia evoked by blood pressure decreases observed before and 10 or 60 min after LSA treatment with RX821002 are presented in Fig. 4. Non-linear regression analysis of baroreflex activity indicated that LSA treatment with RX821002 increased the upper plateau (P2), heart rate range and maximal gain (G_{max};

Fig. 3 and Table 2). Sixty minutes after treatment with RX821002, baroreflex parameters had returned to control values (Fig. 3 and Table 2).

The parameters of baroreflex function were not affected by either bilateral microinjection of RX821002 into structures surrounding the LSA ($n = 5$; Table 3) or unilateral microinjection of RX821002 ($n = 5$) into the LSA (Table 3).

Double blockade of α_1- and α_2-adrenoceptors. Bilateral microinjection of a cocktail containing the selective α_1-adrenoceptor antagonist WB4101 and the selective α_2-adrenoceptor antagonist RX821002 ($n = 5$) into the LSA did not affect baseline mean arterial pressure (99 ± 3 *versus* 96 ± 3 mmHg, $P > 0.05$) and baseline heart rate (338 ± 9 *versus* 375 ± 12 beats min^{-1}, $P > 0.05$). Non-linear regression analysis of baroreflex activity indicated that LSA treatment with the cocktail increased the lower (P1) and upper (P2) plateau and heart rate range (Fig. 3 and Table 2). Sixty minutes after treatment, baroreflex parameters returned to control values (Fig. 3 and Table 2).

Blockade of β-adrenoceptors. Bilateral microinjection of the non-selective β-adrenoceptor antagonist propranolol ($n = 5$) into the LSA did not affect either baseline mean arterial pressure (102 ± 2 *versus* 99 ± 2 mmHg, $P > 0.05$) or baseline heart rate (351 ± 10 *versus* 363
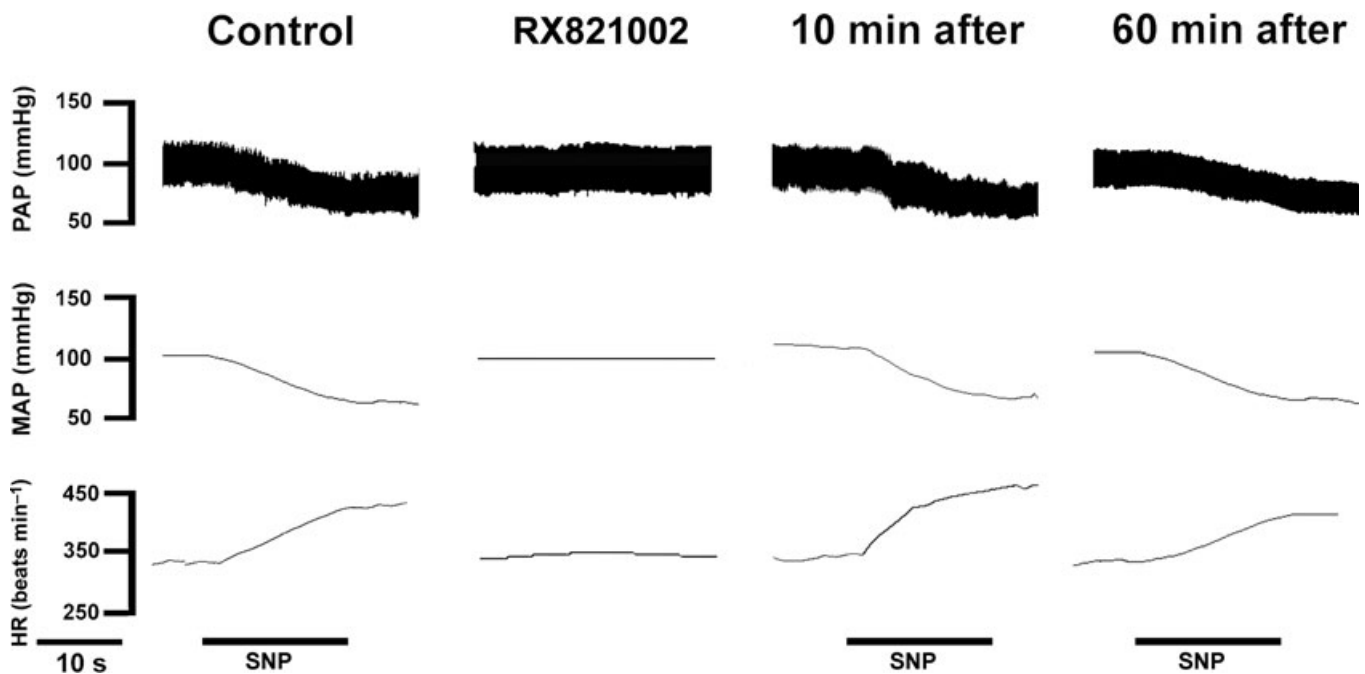

Figure 4. Recordings from one representative animal illustrating the reflex tachycardia in response to blood pressure decrease caused by intravenous infusion of sodium nitroprusside (SNP) before and 10 or 60 min after bilateral microinjection of the selective α_2-adrenoceptor antagonist RX821002 into the LSA
Abbreviations are as in the legend to Fig. 2. Note the increase in tachycardic response 10 min after LSA treatment with RX821002.

± 6 beats min^{-1}, $P > 0.05$). Non-linear regression analysis of baroreflex activity indicated that LSA treatment with propranolol did not affect the baroreflex control of heart rate (Fig. 5 and Table 4).

Effect of microinjection of noradrenaline into the LSA on cardiac baroreflex responses in unanaesthetized rats

Unilateral infusion of noradrenaline into the LSA did not affect either baseline mean arterial pressure (104 ± 2 *versus* 103 ± 1 mmHg, $P > 0.05$) or baseline heart rate (345 ± 6 *versus* 341 ± 9 beats min^{-1}, $P > 0.05$). Unilateral microinjection of a cocktail containing the selective α_1-adrenoceptor antagonist WB4101 and the selective
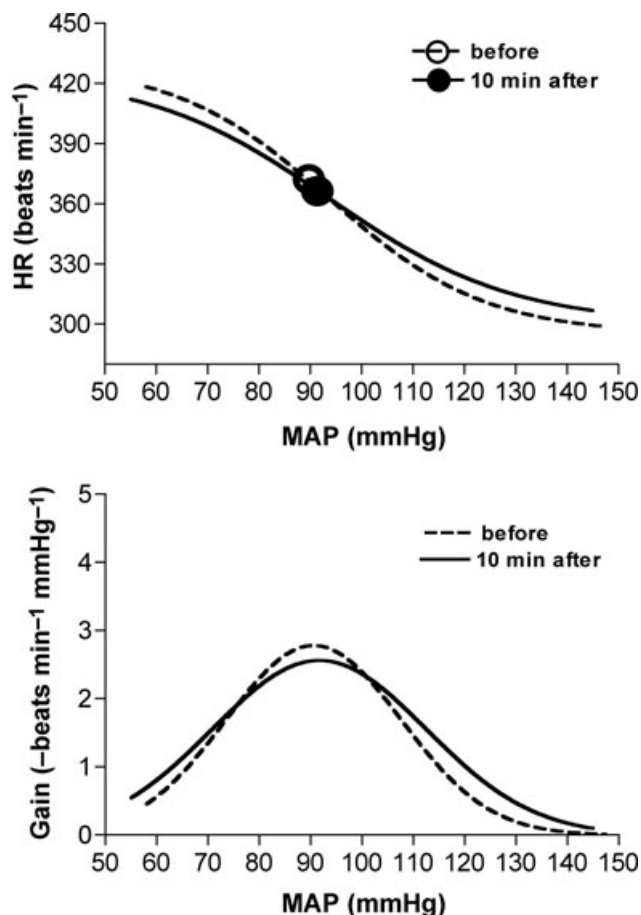

Figure 5. Effect of beta-adrenoceptors blockade in the lateral septal area on cardiac baroreflex response.
Top panel shows sigmoid baroreflex curves correlating mean arterial pressure (MAP) and heart rate (HR) before ($r^2 = 0.85$) and 10 min after ($r^2 = 0.72$) bilateral microinjection of the non-selective β-adrenoceptor antagonist propranolol into the LSA. Symbols on curves indicate the respective BP$_{50}$. Bottom panel shows instantaneous gain of the baroreflex curve in the top panel. Increases or decreases in mean arterial pressure were induced by intravenous infusion of phenylephrine or sodium nitroprusside, respectively.

α_2-adrenoceptor antagonist RX821002 into the LSA also did not affect baseline mean arterial pressure (97 ± 3 *versus* 99 ± 4 mmHg, $P > 0.05$) and baseline heart rate (349 ± 6 *versus* 335 ± 8 beats min^{-1}, $P > 0.05$). Non-linear regression analysis of baroreflex activity indicated that noradrenaline administration into the LSA decreased lower (P1) and upper (P2) plateau and increased the median arterial pressure (BP$_{50}$; Fig. 6 and Table 5). Unilateral microinjection of the cocktail of α-adrenoceptor antagonists did not affect baroreflex activity (Fig. 6 and Table 5); however, the effect of noradrenaline was blocked by LSA pretreatment with the cocktail (Fig. 6 and Table 5).

Discussion

We have previously reported that acute bilateral inhibition of LSA neurotransmission using the non-selective synaptic blocker cobalt chloride affected cardiac baroreflex responses (Scopinho *et al.* 2007). Temporary inhibition of LSA neurotransmission enhanced both baroreflex tachycardia and bradycardia (Scopinho *et al.* 2007). This observation indicated a tonic inhibitory influence of the LSA on the heart rate response to either an increase or a decrease in arterial pressure. However, due to the non-selective blockade of local neurotransmission caused by cobalt chloride (Kretz, 1984), the possible neurotransmitter involved was not elucidated. The present work has demonstrated that blockade of α_2-adrenoceptors by bilateral microinjection of RX821002 into the LSA was able to increase reflex tachycardia without changing baroreflex bradycardia. This result suggests that local noradrenergic neurotransmission within the LSA through activation of α_2-adrenoceptors mediates, at least in part, the influence of LSA on baroreflex tachycardia. The present data corroborate those of Covian & Timo-Iaria (1966), who reported an inhibitory influence of LSA stimulation on baroreflex responses to carotid artery occlusion in anaesthetized cats. Although our results indicate a role of α_1-adrenoceptors in the modulation of reflex bradycardia, the blockade of α_1-adrenoceptors in the LSA affected the heart rate response to an increase in arterial pressure in the opposite manner to that observed after LSA treatment with cobalt chloride. Therefore, further experiments are necessary to clarify the neurotransmitter and receptors within the LSA that are involved in its influence on baroreflex bradycardia. However, the present results suggest that LSA α_1-adrenoceptors play a facilitatory role in baroreflex bradycardia.

Microinjection of noradrenaline into the LSA evoked opposite effects in baroreflex activity compared with that following double blockade of α_1- and α_2-adrenoceptors. The effects of noradrenaline were blocked by LSA pretreatment with a cocktail containing α_1- and α_2-

Table 4. Parameters derived from sigmoidal baroreflex curves generated before or 10 min after bilateral microinjection of the non-selective β-adrenoceptor antagonist propranolol into the LSA

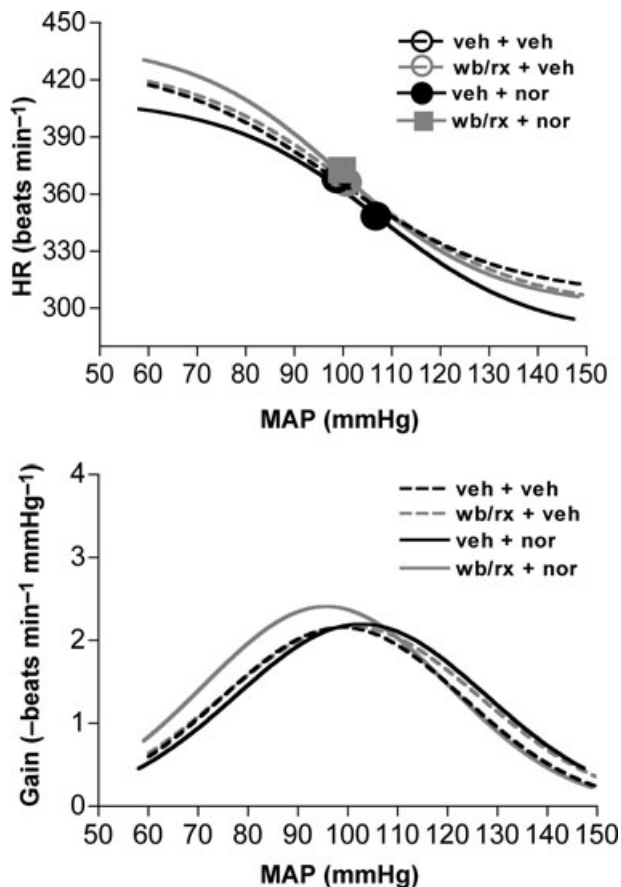| Group	G_{max} (–beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	Range (beats min^{-1})	BP$_{50}$ (mmHg)
Before propranolol	2.9 ± 0.5	295 ± 6	434 ± 8	139 ± 8	89 ± 3
10 min after	2.8 ± 0.4	303 ± 8	423 ± 7	120 ± 12	92 ± 5
	$t = 0.6$	$t = 0.8$	$t = 1$	$t = 1.2$	$t = 0.5$

Values are means $\pm$ SEM.
Abbreviation: t, Student's t test.

adrenoceptor antagonists. Although bilateral blockades were necessary to show tonic action of α-adrenoceptors on baroreflex activity, the unilateral activation of these receptors with exogenous noradrenaline was enough to

Figure 6. Effect of noradrenaline microinjection into the lateral septal area on cardiac baroreflex response.
Top panel shows sigmoid baroreflex curves correlating mean arterial pressure (MAP) and heart rate (HR) obtained during unilateral infusion of noradrenaline or vehicle into the LSA in animals that received vehicle (artificial cerebrospinal fluid) 10 min before vehicle infusion (Veh + Veh, $r^2 = 0.85$), a cocktail containing WB4101 and RX821002 10 min before vehicle infusion (WB/RX + Veh, $r^2 = 0.90$), vehicle 10 min before noradrenaline infusion (Veh + Nor, $r^2 = 0.89$) and the cocktail containing WB4101 and RX821002 10 min before noradrenaline infusion (WB/RX + Nor, $r^2 = 0.90$). Symbols on curves indicate the respective BP$_{50}$. Bottom panel shows instantaneous gain of the baroreflex curve shown in the top panel. Increases or decreases in mean arterial pressure were induced by intravenous infusion of phenylephrine or sodium nitroprusside, respectively.

show a downward shift of baroreflex function, consistent with the upward shift evoked by the blockades. Baroreflex changes following LSA treatment with noradrenaline reinforce the idea that activation of local α-adrenoceptors in the LSA modulates the baroreflex control of heart rate.

It has been reported that electrical stimulation of the aortic depressor nerve alters the firing of LSA neurons (Miyazawa *et al.* 1988), and hypotension increases the number of Fos-immunoreactive neurons, a marker of neuronal activity, in the LSA (Graham *et al.* 1995). These results suggest that signals arising from arterial baroreceptors can activate the LSA. The LSA receives a dense catecholaminergic innervation arising via the medial forebrain bundle from brainstem noradrenergic cell groups located in the nucleus tractus solitarii, which is the first synaptic relay of the baroreflex pathway within the central nervous system; ventrolateral medulla and locus coeruleus (Vertes, 1988; Antonopoulos *et al.* 2004). Fibres from the aortic arch innervate an area of the nucleus tractus solitarii rich in catecholamine-containing neurons (Katz & Karten, 1979). Moreover, baroreflex loading or unloading increases the number of Fos-immunoreactive neurons in brainstem nuclei containing noradrenergic cells (McKitrick *et al.* 1992; Li & Dampney, 1994). Thus, peripheral information conveyed by the baroreceptors could enter the LSA through central noradrenergic pathways.

Baroreflex bradycardia and tachycardia are mainly mediated by cardiac parasympathetic and sympathetic stimulation, respectively (Head & McCarty, 1987; Sullebarger *et al.* 1990; Crestani *et al.* 2008*a*). Therefore, the present results suggest a facilitatory influence of LSA α_1-adrenoceptors on the parasympathetic component of the baroreflex, whereas activation of local α_2-adrenoceptors plays an inhibitory role on sympathetic baroreflex activity. Although there is no evidence for a direct connection between the LSA and autonomic nuclei in the medulla, the LSA sends fibres to prosencephalic nuclei known to be involved in control of autonomic activity, such as medial prefrontal cortex, bed nucleus of stria terminalis, diagonal band of Broca and hypothalamic regions (Swanson & Cowan, 1979; Risold & Swanson, 1997; Vertes, 2004). Thus, LSA α_1-adrenoceptors could modulate the parasympathetic component of the baroreflex by stimulating facilitatory inputs to cardiovagal neurons in the brainstem, whereas activation of local α_2-

Table 5. Parameters derived from sigmoidal baroreflex curves obtained during unilateral infusion of noradrenaline or artificial cerebrospinal fluid (vehicle) into the LSA in animals that received artificial cerebrospinal fluid (vehicle) 10 min before vehicle infusion (Veh + Veh), a cocktail containing WB4101 and RX821002 10 min before vehicle infusion (WB/RX + Veh), vehicle 10 min before noradrenaline infusion (Veh + Nor), or the cocktail containing WB4101 and RX821002 10 min before noradrenaline infusion (WB/RX + Nor)

Group	G_{max} (–beats min^{-1} mmHg^{-1})	P1 (beats min^{-1})	P2 (beats min^{-1})	Range (beats min^{-1})	BP$_{50}$ (mmHg)
Veh + Veh	2.5 ± 0.4	306 ± 5	427 ± 6	120 ± 4	97 ± 2
WB/RX + Veh	2.3 ± 0.3	300 ± 5	429 ± 3	129 ± 5	99 ± 1
Veh + Nor	2.4 ± 0.6	$285 \pm 5*$	$409 \pm 5*$	124 ± 8	$108 \pm 2*$
WB/RX + Nor	2.3 ± 0.7	302 ± 4	436 ± 7	134 ± 6	99 ± 1
	$F = 0.1$	$F = 3$	$F = 5$	$F = 1$	$F = 9$

Values are means $\pm$ SEM. *$P < 0.05$, significantly different from Veh + Veh group, one-way ANOVA followed by Dunnett's *post hoc* test. F = ANOVA.

adrenoceptors would modulate sympathetic baroreflex activity by stimulating inhibitory inputs to sympathetic neurons.

Electrophysiological studies have documented the presence of α_1- and α_2- adrenoceptors mediating opposite effects on membrane excitability of neurons within the LSA (Carette, 1999). Noradrenaline induces hyperpolarizations through activation of local α_2-adrenoceptors and depolarizations via local α_1-adrenoceptors in LSA neurons (Carette, 1999). Results reported in the present study suggest that LSA α_1- and α_2-adrenoceptors modulate different autonomic components of the baroreflex. Therefore, although coexistence of α_1- and α_2-adrenoceptors mediating opposite effects in the same neurons was sometimes observed in the LSA (Carette, 1999), the present study suggests that these receptors modulate cardiac baroreflex activity through an action in different neuronal pathways.

The facilitated tachycardic baroreflex response observed in the present study after the blockade of LSA α_2-adrenoceptors was also observed after inhibition of other limbic structures, such as periaqueductal grey (Pelosi *et al.* 2007) and diagonal band of Broca (Crestani *et al.* 2008a; Urzedo-Rodrigues *et al.* 2011). Moreover, similar reduction in baroreflex bradycardia observed after blockade of LSA α_1-adrenoceptors was reported after ablation of the medial prefrontal cortex (Resstel *et al.* 2004), insular cortex (Alves *et al.* 2009), lateral hypothalamus (Inui *et al.* 1995; Crestani *et al.* 2009) and hypothalamic paraventricular nucleus (Crestani *et al.* 2010a). These areas are connected with the LSA (Risold & Swanson, 1997), thus suggesting interrelation among them during baroreflex control of heart rate.

In addition to the tachycardic response, baroreflex unloading evokes an increase in vasopressin release into the circulation by hypothalamic magnocellular neurons (Dampney, 1994). It has been proposed that the central nervous system noradrenergic pathway is a critical component for modulation of vasopressin release by baroreceptors (Blessing & Willoughby, 1985; Dampney, 1994). We have previously reported that

microinjection of noradrenaline into the LSA causes cardiovascular responses through acute vasopressin release into the circulation (Scopinho *et al.* 2006). Vasopressin-mediated cardiovascular responses following noradrenaline microinjection into the LSA are mediated by activation of local α_1-adrenoceptors and magnocellular neurons in the paraventricular nucleus (Scopinho *et al.* 2006, 2008). Thus, although LSA treatment with the selective α_1-adrenoceptor antagonist does not change reflex tachycardia, we cannot exclude an involvement of this receptor in neuroendocrine response during blood pressure decreases.

Cardiovascular changes during physical exercise and stress situations are accompanied by a resetting of the baroreflex towards higher blood pressure values (DiCarlo & Bishop, 1992; Nosaka, 1996; Miki *et al.* 2003; Dampney *et al.* 2008; Crestani *et al.* 2010b). The change in the baroreflex control of heart rate during stress is characterized by an increase in baroreflex tachycardia and decrease in reflex bradycardia (Crestani *et al.* 2010b). It has been proposed that the change in baroreflex activity mediates, at least in part, autonomic and cardiovascular changes in response to stress and physical exercise (Nosaka, 1996; Dampney *et al.* 2008). Noradrenergic pathways in the central nervous system play an important role in stress- and exercise-evoked cardiovascular responses (Morilak *et al.* 2005; Alves *et al.* 2011). Moreover, it has been documented that the LSA modulates cardiovascular responses to stress and emotional threat situations (Kubo *et al.* 2002; Reis *et al.* 2010), possibly through activation of local adrenoceptors (Bondi *et al.* 2007). In this way, the present study provides initial evidence that LSA α-adrenoceptors could modulate baroreflex activity during stress and/or physical exercise. Double blockade of α_1- and α_2-adrenoceptors in the LSA evoked a similar change in baroreflex activity to that caused by stress and exercise, whereas an opposite effect was observed following local microinjection of noradrenaline. Therefore, activation of LSA α-adrenoceptors would counteract changes in baroreflex activity during threat situations and/or exercise, thus playing an inhibitory role in autonomic and

cardiovascular changes induced by these stimuli. However, further studies are necessary to clarify this issue.

In summary, the present results show that noradrenergic neurotransmission within the LSA modulates baroreflex control of heart rate in a complex way. Taken together, our results provide evidence that LSA α_1-adrenoceptors exert a facilitatory modulation on baroreflex bradycardia. Moreover, data indicate an inhibitory role played by LSA α_2-adrenoceptors on the tachycardic response to a decrease in blood pressure.

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