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RESEARCH PAPER

Both α_1 and α_2-adrenoceptors mediate the cardiovascular responses to noradrenaline microinjected into the bed nucleus of the stria terminal of rats

CC Crestani, FHF Alves, LB Resstel and FMA Corrêa

Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Ribeirão Preto, SP, Brazil

Background and purpose: We have previously shown that noradrenaline microinjected into the bed nucleus of stria terminalis (BST) elicited pressor and bradycardiac responses in unanaesthetized rats. In the present study, we investigated the subtype of adrenoceptors that mediates the cardiovascular response to noradrenaline microinjection into the BST.
Experimental approach: Cardiovascular responses following noradrenaline microinjection into the BST of male Wistar rats were studied before and after BST pretreatment with different doses of the selective α_1-adrenoceptor antagonist WB4101, the α_2-adrenoceptor antagonist RX821002, the combination of WB4101 and RX821002, the non-selective β-adrenoceptor antagonist propranolol, the selective β_1-adrenoceptor antagonist CGP20712 or the selective β_2-adrenoceptor antagonist ICI118,551.
Key results: Noradrenaline microinjected into the BST of unanaesthetized rats caused pressor and bradycardiac responses. Pretreatment of the BST with different doses of either WB4101 or RX821002 only partially reduced the response to noradrenaline. However, the response to noradrenaline was blocked when WB4101 and RX821002 were combined. Pretreatment with this combination also shifted the resulting dose-effect curve to the left, clearly showing a potentiating effect of this antagonist combination. Pretreatment with different doses of either propranolol or CGP20712 increased the cardiovascular responses to noradrenaline microinjected into the BST. Pretreatment with ICI118,551 did not affect cardiovascular responses to noradrenaline.
Conclusion and implications: The present results indicate that α_1 and α_2-adrenoceptors mediate the cardiovascular responses to noradrenaline microinjected into the BST. In addition, they point to an inhibitory role played by the activation of local β_1-adrenoceptors in the cardiovascular response to noradrenaline microinjected into the BST.
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Abbreviations: BST, bed nucleus of the stria terminalis; HR, heart rate; MAP, mean arterial pressure; PAP, pulsatile arterial pressure

Introduction

The bed nucleus of the stria terminal (BST) is a limbic structure that modulates autonomic, neuroendocrine and behavioural functions (Dunn, 1987; Casada and Dafny, 1991; Dunn and Williams, 1995). Previous studies have shown that BST is involved in cardiovascular control. BST stimulation, either electrical or chemical using excitatory amino acids, can elicit pressor as well as depressor responses in anaesthetized rats (Ciriello and Janssen, 1993; Gelsema *et al*., 1993; Dunn and Williams, 1995; Hatam and Nasimi, 2007). The microinjection of carbachol into the BST evoked pressor and bradycardiac responses in unanaesthetized rats (Alves *et al*., 2007). Moreover, it has also been demonstrated that the BST modulates baroreflex activity in rats (Crestani *et al*., 2006).

Cardiovascular responses have been observed after microinjections of noradrenaline into the nucleus tractus solitarii (Zandberg *et al*., 1979), septal area (Scopinho *et al*., 2006), amygdala (Ohta *et al*., 1991), cingulated cortex (Fernandes *et al*., 2003), paraventricular nucleus (Harland *et al*., 1989),

Correspondence: CC Crestani, Department of Pharmacology, School of Medicine of Ribeirão Preto, University of São Paulo, Bandeirantes Av., Ribeirão Preto, São Paulo 14090-090, Brazil.
E-mail: fmdacorr@fmrp.usp.br
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anterior (Poole, 1983), medial, lateral (Camargo *et al*., 1979) and posterior (Brezenoff and Jenden, 1969) hypothalamic areas. These results suggest that the noradrenergic system in the CNS can be involved in the modulation of CVS.

Noradrenergic synaptic terminations are present in the BST (Phelix *et al*., 1992). These terminations originate from neurons located in the A1, A2 and A5 brain stem nuclei (Byrum and Guyenet, 1987; Woulfe *et al*., 1988; Aston-Jones *et al*., 1999) as well as in the locus coeruleus (Moore, 1978). BST electrical stimulation evokes noradrenaline efflux into the BST (Palij and Stamford, 1992) that is regulated by local uptake (Palij and Stamford, 1992) and α_2-adrenoceptors (Palij and Stamford, 1993). Moreover, adrenoceptors have been identified in the BST (Sawada and Yamamoto, 1981; Matsui and Yamamoto, 1984), providing further evidence for the existence of noradrenergic neurotransmission in the BST.

We have previously demonstrated that noradrenergic neurotransmission in the BST is involved in the modulation of the CVS (Crestani *et al*., 2007). Microinjection of noradrenaline into the BST of unanaesthetized rats elicited pressor and bradycardiac responses that were inhibited by systemic pretreatment with a vasopressin antagonist, suggesting that these responses are mediated by vasopressin release into the peripheral circulation (Crestani *et al*., 2007). In the same study, no cardiovascular responses were observed when noradrenaline was microinjected into the BST of urethane-anaesthetized animals.

Finally, although microinjection of noradrenaline into the BST of unanaesthetized rats was shown to evoke cardiovascular responses, the adrenoceptors involved in their mediation have not yet been identified. To address this question, we studied the effect of pretreatment of the BST, with selective antagonists of both α- and β-adrenoceptors, on the cardiovascular responses to the microinjection of noradrenaline into the BST of unanaesthetized rats.

Methods

Animals

Eighty 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 individually in plastic cages under standard laboratory conditions. They were kept under a 12-h light/dark cycle (lights on at 0630 h) and had free access to food and water. The Institution's Animal Ethics Committee authorized housing conditions and experimental procedures.

Surgical preparation

Four days before the experiment, rats were anaesthetized with tribromoethanol (250 mg kg^{-1} i.p.). After scalp anaesthesia with 2% lidocaine, the skull was exposed and a stainless steel guide cannula (26G) was implanted into the BST at a position 1 mm above the site of injection, using a stereotaxic apparatus (Stoelting, Wood Dale, IL, USA). Stereotaxic coordinates for cannula implantation into the BST were anteroposterior = +8.6 mm, lateral = 4.0 mm from the medial suture, ventral = −5.8 mm from the skull with a lateral inclination of 23° (Paxinos and Watson, 1997). Cannulae 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. to prevent infection and with the non-steroidal anti-inflammatory flunixine meglumine i.m. for post-operative 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 (HR) recording. The catheter consisted of a 4-cm piece of PE-10 heat-bound to a 13-cm piece of PE-50 (Clay Adams, Sparks, MD, USA). The catheter was tunnelled under the skin and exteriorized on the animal's dorsum. After surgery, the treatment with the anti-inflammatory drug was repeated.

Measurement of cardiovascular responses

After surgery, animals were kept in individual cages that were later used for transport to the experimental room. Animals were allowed 1 h to adapt to the conditions of the experimental room, such as sound and illumination, before blood pressure and HR recording were started. The experimental room was acoustically isolated and had constant background noise generated by an air exhauster. At least one additional 30-min period was allowed before the experiments were started. Care was taken to start injections when the blood pressure and especially the HR were observed to be stable. Pulsatile arterial pressure of freely moving animals was recorded using an HP-7754A preamplifier (Hewlett Packard, Palo Alto, CA, USA) and an acquisition board (MP100A; Biopac Systems Inc., Santa Barbara, CA, USA) connected to a personal computer. Mean arterial pressure (MAP) and HR values were from pulsatile arterial pressure recordings and were processed online.

Drug microinjection into the BST

Noradrenaline, WB4101, RX821002, propranolol, CGP20712 and ICI118,551 were dissolved in artificial cerebrospinal fluid (composition in mM: NaCl, 100; Na_3PO_4, 2; KCl, 2.5; $MgCl_2$, 1; $NaHCO_3$, 27; $CaCl_2$, 2.5; pH = 7.4) before microinjection into the BST. The needles (33 G, Small Parts, Miami Lakes, FL, USA) used for microinjection into the BST were 1 mm longer than the guide cannulae and were connected to a 2-μl syringe (7002H, Hamilton, USA) through PE-10 tubing. Needles were carefully inserted into the guide cannulae without restraining the animals and drugs were injected in a final volume of 100 nl. Needles were removed after a 20-s period.

Experimental protocols

A dose of 10 nmol of noradrenaline (Crestani *et al*., 2007) was used to study the effect of BST pretreatment with artificial cerebrospinal fluid or with different antagonists on the cardiovascular response to noradrenaline. Each animal

received three microinjections into the BST. An initial injection of noradrenaline was performed as a control. Twenty-four hours later, a second injection (artificial cerebrospinal fluid or one dose of adrenoceptor antagonist) was made into the BST, which was followed 10 min later by a third injection (noradrenaline).

To study the involvement of BST adrenoceptors, each animal received one dose of the selective α_1-adrenoceptor antagonist WB4101 (10, 15 or 25 nmol); the selective α_2-adrenoceptor antagonist RX821002 (10, 15 or 25 nmol); the non-selective β-adrenoceptor antagonist *dl*-propranolol (5, 15 or 25 nmol); the selective β_2-adrenoceptor antagonist ICI118,552 (2.5, 7.5 or 12.5 nmol) or the selective β_1-adrenoceptor antagonist CGP20712 (2.5, 7.5 or 12.5 nmol) microinjected into the BST.

Histological determination of the microinjection site

At the end of the experiment, animals were anaesthetized with urethane (1.25 g kg^{-1} i.p.) and 100 nl of 1% Evan's Blue dye was injected into the BST as a marker of the injection site. Animals were submitted to intracardiac perfusion with saline (0.9% NaCl) followed by 10% formalin. Brains were removed and post-fixed for 24 h at 4 °C and 40-μm sections were cut using a cryostat (CM 1900; Leica, Wetzlar, Germany). Brain sections were stained with 0.5% cresyl violet for optical microscopy analysis. The placement of the microinjection needles was determined by analysing serial sections and are presented according to the rat brain atlas of Paxinos and Watson (1997).

Drugs

The following drugs were used: noradrenaline-HCl (Sigma, St Louis, MO, USA); dl-propranolol (Sigma); urethane (Sigma); WB4101 (2-(2,6-dimethoxyphenoxyethyl)aminomethyl-1,4-benzodioxane hydrochloride; Tocris, Ellisvalle, MO, USA); RX 821002 (2-(2,3-dihydro-2-methoxy-1,4-benzodioxin-2-yl)-4,5-dihydro-1*H*-imidazole hydrochloride; Tocris); ICI118,551 ((±)-1-[2,3-dihydro-7-methyl-1*H*-inden-4-yl)oxy]-3-[(1-methyl-ethyl)amino]-2-butanol hydrochloride; Tocris); CGP20712 (1-[2-((3-carbamoyl-4-hydroxy)phenoxy)ethylamino]-3-[4-(1-methyl-4-trifluoromethyl-2-imidazolyl)phenoxy]-2-propanol dihydrochloride; Tocris); tribromoethanol (Aldrich, St Louis, MO, USA); streptomycins and penicillins (Pentabiotico, Fort Dodge, Brazil); flunixine meglumine (Banamine, Schering Plough, Brazil).

Statistical analysis

Mean arterial pressure and HR baselines were compared using Student's paired *t*-test (before treatment vs after treatment). Percentages of response inhibition by BST pretreatment with α-adrenoceptor antagonists and percentages of response increase by BST pretreatment with β-adrenoceptor antagonists were analysed utilizing non-linear regression analysis. Maximum inhibition evoked by the BST pretreatment with α-adrenoceptor antagonists was evaluated using one-way ANOVA followed by the Bonferroni test. $P<0.05$ was assumed as statistically significant.

Results

Effect of pretreatment of the BST with WB4101 or/and RX821002 on the cardiovascular responses to noradrenaline microinjected into the BST

Vehicle. BST pretreatment with artificial cerebrospinal fluid ($n=5$) did not affect the pressor (33 ± 3 vs 35 ± 3 mm Hg; $P>0.05$) and the bradycardiac (−35 ± 6 vs −32 ± 5; $P>0.05$) response to noradrenaline microinjected into the BST of unanaesthetized rats.

WB4101. Microinjection of the selective α_1-adrenoceptor antagonist WB4101 into the BST did not affect baseline values of MAP (98 ± 3 vs 99 ± 4 mm Hg; $P>0.05$) or HR (341 ± 12 vs 345 ± 5 b.p.m.; $P>0.05$). BST pretreatment with WB4101 (10, 15 and 25 nmol; $n=4$ for each dose) caused a dose-related reduction in the pressor ($r^2=0.87$, $d.f.=9$, $P<0.05$) and the bradycardiac ($r^2=0.89$, $d.f.=9$, $P<0.05$) responses to noradrenaline microinjected into the BST (Figure 1). The higher dose of WB4101 caused only a partial reduction in the pressor (38 ± 2%) and bradycardiac (38 ± 3%) responses to noradrenaline microinjected into the BST.

RX821002. Microinjection of the selective α_2-adrenoceptor antagonist RX821002 into the BST did not affect baseline values of MAP (98 ± 2 vs 97 ± 4 mm Hg; $P>0.05$) or HR (332 ± 14 vs 341 ± 8 b.p.m.; $P>0.05$). Pretreating the BST with RX821002 (10, 15 and 25 nmol; $n=4$ for each dose) caused a dose-related reduction in the pressor ($r^2=0.81$, $d.f.=9$, $P<0.05$) and the bradycardiac ($r^2=0.80$, $d.f.=9$, $P<0.05$) responses to noradrenaline microinjected into the BST (Figure 1). The higher dose of RX821002 caused only a partial reduction in the pressor (35 ± 4%) and bradycardiac (37 ± 3%) responses to noradrenaline. The maximal reduction of the cardiovascular response to noradrenaline microinjected into the BST that was observed after BST pretreatment with RX821002 was not significantly different from the maximal reduction of the response caused by BST pretreatment with WB4101.

WB4101 and RX821002. Microinjection of the combination of WB4101 + RX821002 ($n=15$) into the BST did not affect baseline values of MAP (95 ± 2 vs 97 ± 1 mm Hg; $P>0.05$) and HR (349 ± 8 vs 355 ± 12 b.p.m.; $P>0.05$). Pretreating the BST with WB4101 + RX821002 (5, 10, 15 and 25 nmol of each; $n=3$ for 5 nmol and $n=4$ for the other doses) caused a dose-related reduction in the pressor ($r^2=0.91$, $d.f.=10$, $P<0.05$) and bradycardiac ($r^2=0.99$, $d.f.=10$, $P<0.05$) responses to noradrenaline microinjected into the BST (Figure 1). The higher dose of the combination of WB4101 and RX921002 inhibited the pressor (89 ± 4%) and bradycardiac (88 ± 3%) responses to noradrenaline. Maximum block evoked by BST pretreatment with the combination of WB4101 and RX82002 was more marked than that induced by BST pretreatment with each antagonist alone (MAP: $F=76$, $P<0.0001$; HR: $F=94$, $P<0.0001$).

Representative recordings showing the cardiovascular response to the microinjection of noradrenaline into

the BST, and the effect of pretreatment of different animals with WB4101, RX821002 or the combination of WB4101 + RX821002 on this response are presented in Figure 2.

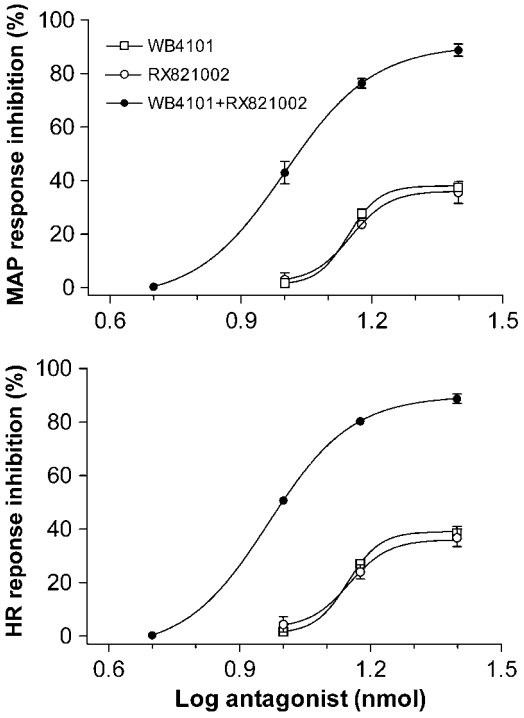

Figure 1 Inhibition of MAP and HR responses (expressed as %) to the microinjection of noradrenaline (10 nmol 100 nl^{-1}) into the BST after pretreatment of the BST with different doses of the α_1-adrenoceptor antagonist WB4101 (10, 15 and 25 nmol; $n=4$ for each dose), the α_2-adrenoceptor antagonist RX821002 (10, 15 and 25 nmol; $n=4$ for each dose) and WB4101 + RX821002 (5, 10, 15 and 25 nmol of each drug; $n=3$ for 5 nmol and $n=4$ for the other doses). Curves were generated by nonlinear regression analysis. Symbols represent means and vertical lines indicate the s.e.mean. BST, bed nucleus of stria terminalis; HR, heart rate; MAP = mean arterial pressure.

Effect of pretreatment of the BST with propranolol, CGP20712 or ICI118,551 on the cardiovascular responses to noradrenaline microinjected into the BST

Propranolol. Microinjection of the non-selective β-adrenoceptor antagonist dl-propranolol into the BST did not affect baseline values of MAP (100 ± 4 vs 102 ± 5 mm Hg; $P>0.05$) and HR (334 ± 8 vs 337 ± 13 b.p.m.; $P>0.05$). Pretreating the BST with propranolol (2.5, 7.5 and 12.5 nmol; $n=4$ for each dose) caused a dose-related increase of the pressor ($r^2=0.85$, $d.f.=9$, $P<0.05$) and the bradycardiac ($r^2=0.87$, $d.f.=7$, $P<0.05$) responses to noradrenaline microinjected into the BST (Figure 3). Maximum increases were 64 ± 7 and 56 ± 4% for the pressor and the bradycardiac responses, respectively.

ICI118,551. Microinjection of the selective β_2-adrenoceptor antagonist ICI118,551 into the BST did not affect baseline values of MAP (99 ± 4 vs 98 ± 3 mm Hg; $P>0.05$) and HR (341 ± 7 vs 336 ± 6 b.p.m.; $P>0.05$). Pretreatment of the BST with different doses of ICI118,551 (2.5, 7.5 and 12.5 nmol; $n=4$ for each dose) did not affect the pressor ($r^2=0.01$, $d.f.=7$, $P>0.05$) or the bradycardiac ($r^2=0.02$, $d.f.=7$, $P>0.05$) responses to noradrenaline microinjected into the BST (Figure 3).

CGP20712. Microinjection of the selective β_1-adrenoceptor antagonist CGP20712 into the BST did not affect baseline values of MAP (100 ± 4 vs 97 ± 4 mm Hg; $P>0.05$) and HR (339 ± 10 vs 345 ± 8 b.p.m.; $P>0.05$). Pretreating the BST with different doses of CGP20712 (2.5, 7.5 and 12.5 nmol; $n=4$ for each dose) caused a dose-related increase of the pressor ($r^2=0.81$, $d.f.=9$, $P<0.05$) and the bradycardiac ($r^2=0.79$, $d.f.=9$, $P<0.05$) responses to noradrenaline microinjected into the BST (Figure 3). Maximum increases were 63 ± 5 and 52 ± 4% for the pressor and the bradycardiac responses, respectively.

Figure 4 shows a representative recording illustrating the effect of the pretreatment of the BST with CGP20712 on the cardiovascular response to noradrenaline. A representative photomicrograph of one coronal brain section showing the

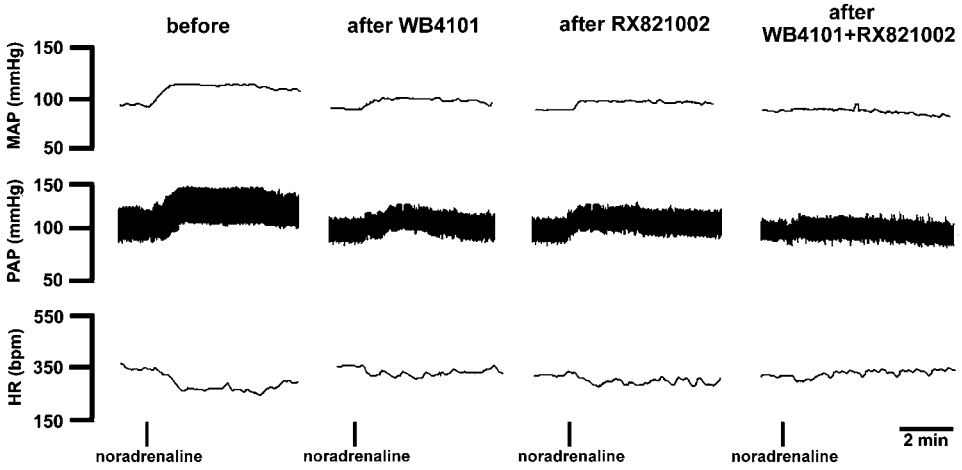

Figure 2 Typical recordings showing MAP, PAP and HR changes in response to noradrenaline (10 nmol 100 nl^{-1}) microinjected into the BST before and after pretreatment of the BST in different animals with WB4101 (25 nmol), RX821002 (25 nmol) or WB4101 + RX821002 (25 nmol). BST, bed nucleus of stria terminalis; HR, heart rate; MAP, mean arterial pressure; PAP, pulsatile arterial pressure.

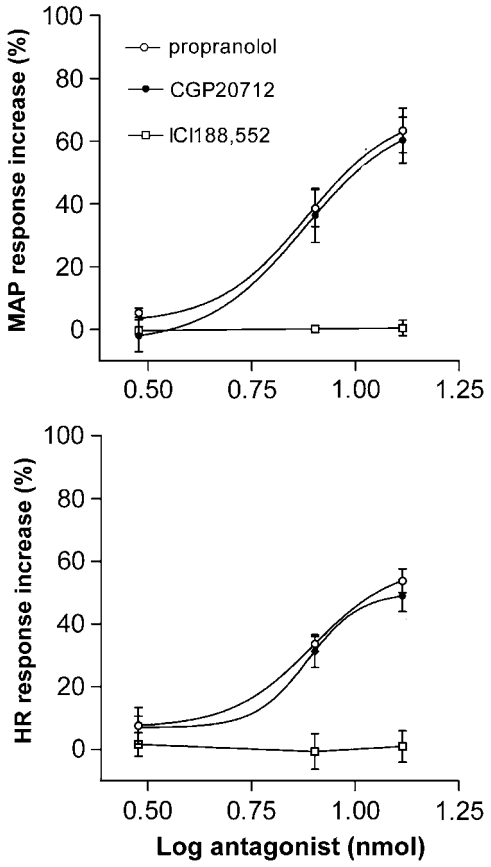

Figure 3 Increase of MAP and HR responses (expressed as %) to noradrenaline (10 nmol 100 nl^{-1}) microinjected into the BST caused by pretreatment of the BST with different doses of the nonselective β-adrenoceptor antagonist propranolol (2.5, 7.5 or 12.5 nmol; $n=4$ for each dose), the selective β_2-adrenoceptor antagonist ICI118,552 (2.5, 7.5 or 12.5 nmol; $n=4$ for each dose) or the selective β_1-adrenoceptor antagonist CGP20712 (2.5, 7.5 or 12.5 nmol; $n=4$ for each dose). Curves were generated by nonlinear regression analysis. Symbols represent means and vertical lines indicate the s.e.mean. BST, bed nucleus of stria terminalis; HR, heart rate; MAP, mean arterial pressure.

microinjection site in the BST and a diagrammatic representation of the BST (Paxinos and Watson, 1997) indicating the microinjection sites in the BST of all the animals used in the experiments are presented in Figure 5.

Discussion and conclusions

The BST is one of the major targets of noradrenergic innervation in the brain (Swanson and Hartman, 1975; Moore and Bloom, 1979). Noradrenergic terminals in the BST originate from noradrenergic neurons in the A1, A2 and A5 cell groups as well as in the locus coeruleus (Moore, 1978; Byrum and Guyenet, 1987; Woulfe *et al*., 1988; Aston-Jones *et al*., 1999). It has been shown previously that BST noradrenergic neurotransmission is involved in cardiovascular modulation (Crestani *et al*., 2007). Microinjection of noradrenaline into the BST evoked an MAP increase that was mediated by acute systemic vasopressin release (Crestani *et al*., 2007). The idea of functionally active noradrenergic

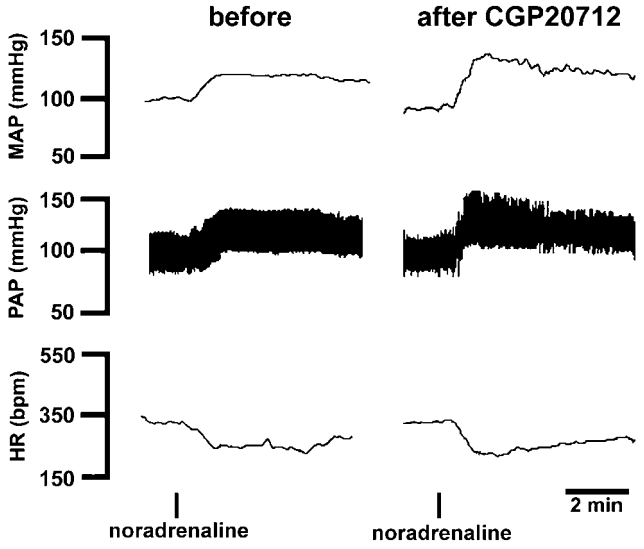

Figure 4 Typical recordings showing MAP, PAP and HR changes in response to noradrenaline (10 nmol 100 nl^{-1}) microinjected into the BST before and after BST pretreatment with CGP20712 (12.5 nmol). BST, bed nucleus of stria terminalis; HR, heart rate; MAP, mean arterial pressure; PAP, pulsatile arterial pressure.

neurotransmission in the BST was further supported by the observation that microinjection of tyramine, an indirectly acting sympathomimetic amine devoid of direct agonist activity, into the BST also caused pressor and bradycardiac responses, which were similar to those observed after the injection of noradrenaline into the BST (Crestani *et al*., 2007). In those experiments, as well as in the present study, the sites of noradrenaline injection were preferentially targeted to the anterior division of the BST. In this context, it is relevant to point out that neuronal lesions of the anterior BST caused by local injection of ibotenic acid resulted in attenuated expression of vasopressin mRNA in the magnocellular cell division of the paraventricular nucleus, without affecting that in the magnocellular neurons of the supraoptic nucleus (Choi *et al*., 2007). This observation indicates a possible direct neuronal link between the BST and the source of neuropituitary vasopressin in the magnocellular division of the paraventricular nucleus, which may constitute the neural substrate for the vasopressin-related cardiovascular effects observed after microinjection of noradrenaline into the BST.

In the present study, we evaluated the subtype of adrenoceptors involved in the mediation of the cardiovascular responses to the injection of noradrenaline into the BST. To investigate the possible involvement of α-adrenoceptors in the cardiovascular responses to noradrenaline, we pretreated the BST with either the selective α_1-adrenoceptor antagonist WB4101 or the selective α_2-adrenoceptor antagonist RX821002. Although the cardiovascular response to noradrenaline was dose-dependently affected by both pretreatments, only a partial blockade was observed. The response to noradrenaline was completely blocked only after combined pretreatment of the BST with WB4101 and RX821002. These data suggest that both α_1- and α_2-adrenoceptors may mediate the cardiovascular response to noradrenaline microinjected into the BST.

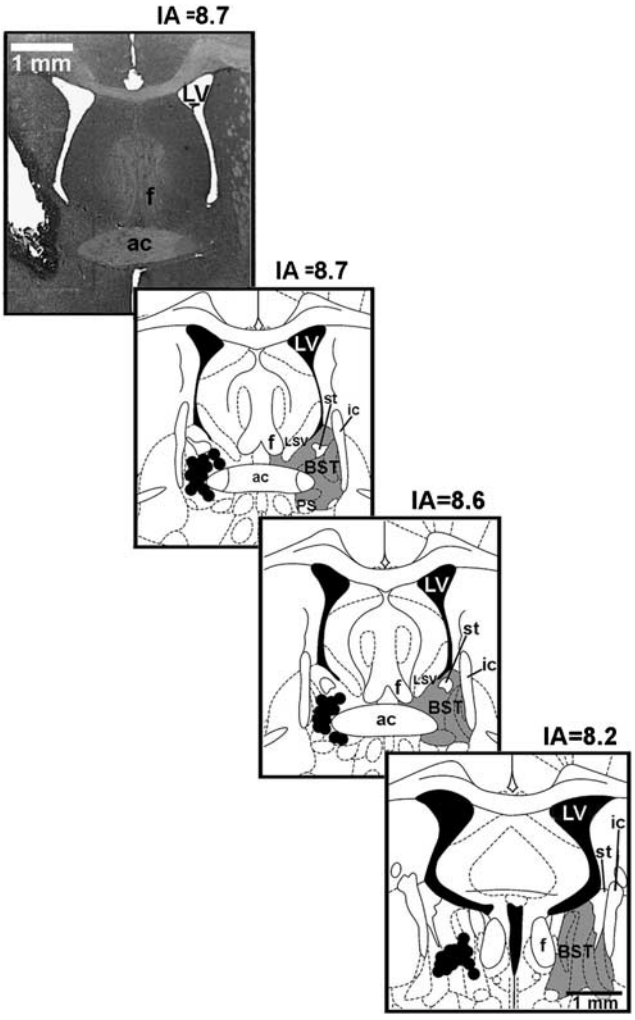

Figure 5 Photomicrograph and diagrammatic representation modified from the rat brain atlas of Paxinos and Watson (1997), indicating the sites of microinjection into the BST of all the animals used in the experiments. ac, anterior commissure; f, fornix; IA, interaural; ic, internal capsule; LSV, ventral lateral septal area; LV, lateral ventricle; PS, parastrial; st, stria terminalis.

The presence of α_1- and α_2-adrenoceptors in the BST has been demonstrated previously. Regardless of known differences between sub-areas of the BST, activation of α_2-adrenoceptors has been, preferentially, shown to cause postsynaptic inhibitory effects on BST neuronal activity (Sawada and Yamamoto, 1981; Matsui and Yamamoto, 1984). The noradrenergic transmission has been proposed to exert a tonic inhibition over the glutamatergic neurotransmission in the BST (Forray *et al*., 1999; Forray and Gysling, 2004; Egli *et al*., 2005). Activation of α_2-adrenoceptors and to a lesser extent of α_1-adrenoceptors in the BST has been found to inhibit K^+-induced glutamate release in that area, whereas their blockade significantly increased glutamate release (Forray *et al*., 1999). These results suggest that noradrenaline released in the anterior BST would play an inhibitory role on local glutamatergic neurotransmission. In addition, there is evidence that noradrenaline via activation of α_1-adrenoceptors depolarizes local GABAergic neurons to increase $GABA_A$ receptor-related inhibitory action in the BST (Dumont and Williams, 2004). The resulting interaction between α_1- and α_2-adrenoceptor effects could explain why complete inhibition of the pressor response to the microinjection of noradrenaline into the BST was only observed when both receptor subtypes were simultaneously blocked by pretreatment with WB4101 and RX821002. In addition to the complete blockade observed, the dose–inhibition curve was also shifted to the left, indicating a potentiating effect of the combined treatment with α_1- and α_2-adrenoceptor antagonists.

No significant effects on the field potentials evoked in the BST by electrical stimulation of the stria terminalis were observed after treatment of the BST with the β-agonist isoprenaline (Matsui and Yamamoto, 1984). Also, pretreatment of BST preparations with the non-selective β-adrenoceptor antagonist dichloroisoprenaline did not affect the postsynaptic inhibitory action of noradrenaline on the field potentials evoked in the BST by electrical stimulation of the stria terminalis (Matsui and Yamamoto, 1984), suggesting little involvement of the β-adrenoceptor in the response to noradrenaline in the BST. However, noradrenaline has also been shown to increase glutamatergic neurotransmission in BST preparations, in addition to its predominant inhibitory effect on BST neuronal firing (Egli *et al*., 2005). Such an effect would involve activation of β-adrenoceptors in addition to α_2-adrenoceptors, because treatment with the selective β_2-adrenoceptor ICI118,551 partially reduced the increase of BST glutamatergic transmission observed after local treatment with noradrenaline and the application of a β-agonist after noradrenaline resulted in further increased glutamatergic responses (Egli *et al*., 2005). There is also evidence that activation of α_1- and β-adrenoceptors depolarizes local GABAergic neurons in the BST, increasing $GABA_A$-ipsc (Dumont and Williams, 2004). The blockade of β-adrenoceptors in the BST has been shown to reduce the intensity of aversive symptoms associated with morphine or cocaine withdrawal (Aston-Jones *et al*., 1999; Dumont and Williams, 2004). Together, these results indicate a possible synergistic effect between α- and β-adrenoceptors in the BST.

To determine whether local β-adrenoceptors are involved in the cardiovascular responses to the microinjection of noradrenaline into the BST, we pretreated the BST with the non-selective β-adrenoceptor antagonist propranolol, the selective β_1-adrenoceptor antagonist CGP20712 or the selective β_2-adrenoceptor antagonist ICI118,551 before the injection of noradrenaline. The pressor and bradycardiac response to noradrenaline microinjected into the BST was increased up to 60% by pretreatment with propranolol or the β_1-adrenoceptor antagonist CGP20712 but unaffected by pretreatment with the selective β_2-antagonist ICI118,551. These data suggest that activation of local β_1-adrenoceptors in the BST may modulate the intensity of the cardiovascular responses caused by the activation of α_1- and α_2-adrenoceptors, evoked by the microinjection of noradrenaline into the BST.

The physiological role of the cardiovascular response mediated by acute vasopressin release, observed after microinjection of noradrenaline into the BST, is not clear. Although the effect of BST pretreatment with the different pharmacological antagonists on the release of vasopressin

that mediate the pressor response to the microinjection of noradrenaline into the BST was not evaluated in the present study, the results suggest that noradrenergic mechanisms in the BST could be involved in fluid balance adjustments to stress responses, integrating cardiovascular and neuroendocrine responses. This idea is favoured by studies indicating that brainstem noradrenergic cell groups are activated during stress (Chen and Herbert, 1995; Dayas *et al*., 2001) and that immobilization stress evokes a marked release of noradrenaline in the BST (Pacak *et al*., 1995; Cecchi *et al*., 2002). There is also considerable evidence that noradrenergic inputs to the BST modulate the expression of neuroendocrine responses associated with stress, particularly those related to the activation of the hypothalamo-pituitary-adrenal axis (Bruehl and Chung, 2004; Forray and Gysling, 2004). Pretreatment of the BST with an α_1-adrenoceptor antagonist, but not with β-adrenoceptor antagonists, has been found to attenuate stress-induced adrenocorticotropic hormone release, whereas both treatments were able to reduce the stress-induced, anxiety-like behavioural responses on the elevated plus-maze (Cecchi *et al*., 2002), indicating a broad functional role of the BST noradrenergic system.

In summary, the present results suggest that the pressor and bradycardiac response to noradrenaline microinjected into the BST involves activation of local α_1- and α_2-adrenoceptors in the BST. In addition, the results also indicate that activation of BST β_1-adrenoceptors has an inhibitory action on the cardiovascular responses to noradrenaline microinjected into that area and may modulate the intensity of the cardiovascular responses evoked by activation of BST α-adrenoceptors by the microinjection of noradrenaline into the BST.

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Conflict of interest

The authors state no conflict of interest.

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