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BED NUCLEUS OF THE STRIA TERMINALIS α_1- AND α_2-ADRENOCEPTORS DIFFERENTIALLY MODULATE THE CARDIOVASCULAR RESPONSES TO EXERCISE IN RATS

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Abstract—Dynamic exercise evokes sustained blood pressure and heart rate (HR) increases. Although it is well accepted that there is a CNS mediation of cardiovascular adjustments during dynamic exercise, information on the role of specific CNS structures is still limited. The bed nucleus of the stria terminalis (BST) is involved in exercise-evoked cardiovascular responses in rats. However, the specific neurotransmitter involved in BST-related modulation of cardiovascular responses to dynamic exercise is still unclear. In the present study, we investigated the role of local BST adrenoceptors in the cardiovascular responses evoked when rats are submitted to an acute bout of exercise on a rodent treadmill. We observed that bilateral microinjection of the selective α1-adrenoceptor antagonist WB4101 into the BST enhanced the HR increase evoked by dynamic exercise without affecting the mean arterial pressure (MAP) increase. Bilateral microinjection of the selective α2-adrenoceptor antagonist RX821002 reduced exercise-evoked pressor response without changing the tachycardiac response. BST pretreatment with the nonselective β-adrenoceptor antagonist propranolol did not affect exercise-related cardiovascular responses. BST treatment with either WB4101 or RX821002 did not affect motor performance in the open-field test, which indicates that effects of BST adrenoceptor antagonism in exercise-evoked cardiovascular responses were not due to changes in motor activity. The present findings are the first evidence showing the involvement of CNS adrenoceptors in cardiovascular responses during dynamic exercise. Our results indicate an inhibitory influence of BST α1-adrenoceptor on the exercise-evoked HR response. Data also point to a facilitatory role played by the activation of BST α2-adrenoceptor on the pressor response to dynamic exercise.

Key words: central nervous system, noradrenergic neurotransmission, adrenoceptors, dynamic exercise, treadmill, open-field test.

Physical exercise requires a higher blood flow in working muscles in order to match the increase in metabolic demand (Waldrop et al., 1996; Rowell, 1997). Therefore, heart rate (HR), cardiac output and arterial pressure increase during exercise (Waldrop et al., 1996; Rowell, 1997). Moreover, sympathetic-mediated vasoconstriction diverts blood away from the kidneys and splanchnic regions, thus redistributing the blood flow to active muscles (Amaral and Michelini, 1997; Rowell, 1997; Miki et al., 2003). These cardiovascular adjustments are controlled by the CNS through several neural mechanisms. The central command is a feed-forward mechanism originating in telencephalic regions that involves a parallel activation of brain stem and spinal circuits responsible for the control of locomotion as well as cardiovascular activity during exercise (Raven et al., 2002; Williamson et al., 2006). Cardiovascular responses during exercise are also driven by neural feedback from working muscles, which are stimulated by mechanical changes or metabolic products originating in active muscles (Kaufman and Forster, 1996; Fisher and White, 2004). Cardiovascular changes during exercise are accompanied by a resetting of baroreflex toward higher blood pressure values (Rowell and O'Leary, 1990; Raven et al., 2006). It has been proposed that the increase in sympathetic nerve activity and arterial pressure observed during exercise depends on an upward shift of the operating point of the arterial baroreflex (Ludbrook and Potocnik, 1986; DiCarlo and Bishop, 1992). Although the neural mechanisms are well established, the specific nuclei and CNS neural pathways involved in cardiovascular responses to exercise are still poorly understood.

The bed nucleus of the stria terminalis (BST) is localized in the rostral prosencephalon, and is associated with autonomic and neuroendocrine functions (Dunn, 1987; Gelsema and Calaresu, 1987; Hatam and Nasimi, 2007; Alves et al., 2009; Crestani et al., 2009b). Previous results from our laboratory showed that bilateral microinjection of the unspecific neurotransmitter blocker $CoCl_2$ into the BST of rats reduced both pressor and tachycardiac responses evoked by an acute bout of exercise on a rodent treadmill (Crestani et al., 2010b). These results indicated a role of the BST in cardiovascular adjustments during dynamic exercise in rats. However, due to the nonselective blockade of local neurotransmission caused by $CoCl_2$ (Kretz, 1984; Lomber, 1999), the specific neurotransmitter involved in the BST-related modulation of cardiovascular responses to exercise is yet unknown.

Previous evidence has suggested an involvement of the CNS noradrenergic pathway in adjustments observed during exercise (Lambert and Jonsdottir, 1998; Higa-Taniguchi et al., 2007). It has been reported that running on a treadmill markedly activates neurons in A1, A2 and A6

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Abbreviations: ACSF, artificial cerebrospinal fluid; BST, bed nucleus of the stria terminalis; CVLM, caudal ventrolateral medulla; HR, heart rate; MAP, mean arterial pressure; NTS, nucleus of the tractus solitarius; PAP, pulsatile arterial pressure.

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noradrenergic neurons in the brain stem of rats (Timofeeva et al., 2003; Ohiwa et al., 2006). Exercise on the treadmill also enhances noradrenaline release and turnover rate in the CNS (Pagliari and Peyrin, 1995; Kitaoka et al., 2010; Takatsu et al., 2010). Moreover, administration of the selective α_2-adrenoceptor agonist clonidine attenuates increases in arterial pressure and HR elicited during static muscle contraction when injected either intracisternally, intrathecally, into the cerebral aqueduct or microdialyzed into the L7 dorsal horn of the spinal cord of anesthetized cats (Williams, 1985; Williams et al., 1987; Hill and Kaufman, 1991; Ally et al., 1996). Although the above results suggest involvement of the central noradrenergic pathway in cardiovascular adjustments during exercise, its role in cardiovascular responses to dynamic exercise has never been investigated.

Among the numerous neural inputs to the BST, noradrenergic synaptic terminals are prominent in the BST (Phelix et al., 1992). In fact, the BST is one of the major targets of noradrenergic innervation in the brain (Swanson and Hartman, 1975; Moore, 1978). 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). Previous studies have pointed out the presence of adrenoceptors and noradrenaline transporters in that nucleus (Matsui and Yamamoto, 1984; Egli et al., 2005; Crestani et al., 2008b). BST-noradrenergic neurotransmission has been suggested to modulate the cardiovascular and baroreflex activity in resting animals (Crestani et al., 2007, 2008a). Noradrenaline microinjection into the BST has been reported to cause arterial pressure and HR changes (Crestani et al., 2007). It has also been reported that activation of BST α_1-adrenoceptors modulates baroreflex activity in a similar manner to that observed during exercise (Crestani et al., 2008a).

Given the involvement of BST-noradrenergic neurotransmission in cardiovascular control, we hypothesized an involvement of BST adrenoceptors in the control of exercise-related cardiovascular adjustments in rats. To test this hypothesis, we investigated the effect of microinjection into the BST of selective adrenoceptor antagonists in pressor and tachycardiac responses elicited by an acute bout of exercise on a rodent treadmill. Moreover, in order to address whether changes in exercise-evoked cardiovascular responses induced by pharmacological manipulation of BST noradrenergic neurotransmission were not due to an indirect effect caused by an alteration in motor activity, we studied whether adrenoceptor blockade in the BST affects the performance motor in the open-field test.

EXPERIMENTAL PROCEDURES

Ethical approval and animals

Experimental procedures were carried out following protocols approved by the Ethical Review Committee of the School of Medicine of Ribeirão Preto, which complies with the Guiding Principles for Research Involving Animals and Human Beings of the American Physiological Society. Fifty-six male Wistar rats weighing 170–190 g were used in the present experiments. 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 under a 12:12 h light-dark cycle (lights on between 6:00 AM and 6:00 PM) and had free access to water and standard laboratory food.

Surgical preparation

When animal body weight reached 230–250 g, and 5 days before the trial, rats were anesthetized with tribromoethanol (250 mg/kg, i.p.). After scalp anesthesia with 2% lidocaine the skull was exposed and stainless-steel guide cannulas (26 G) were bilaterally 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: AP=+8.6 mm from interaural; L=4.0 mm from the medial suture, V=−5.8 mm from the skull with a lateral inclination of 23° (Paxinos and Watson, 1997). Cannulas were fixed to the skull with dental cement and one metal screw. After surgery, the animals received a poly-antibiotic (Pentabiotico®, Fort Dodge, Brazil), with streptomycins and penicillins, to prevent infection, and a nonsteroidal anti-inflammatory, flunixine meglumine (Banamine®, Schering Plough, Brazil), for post-operation analgesia.

One day before the trial (Braga et al., 2000; De Angelis et al., 2006; Higa-Taniguchi et al., 2009; Crestani et al., 2010b), rats were anesthetized with tribromoethanol (250 mg/kg, i.p.) and a catheter was inserted into the abdominal aorta through the femoral artery, for arterial pressure and HR recording. The catheter was tunneled under the skin and exteriorized on the animal's dorsum. After surgery, the nonsteroidal anti-inflammatory flunixine meglumine (Banamine®, Schering Plough, Brazil) was administered for post-operation analgesia.

Measurement of cardiovascular responses

On the day of the experiment, the arterial cannulas 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 Inc, Goleta, CA, USA) connected to a personal computer. Mean arterial pressure (MAP) and HR values were derived from pulsatile arterial pressure recordings and were processed online.

Drug 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 cannulas and were connected to a hand-driven 2 μl syringe (7002-KH, Hamilton Co., Reno, NV, USA) through PE-10 tubing. Needles were carefully inserted into the guide cannulas without restraining the animals and drugs were injected in a final volume of 100 nl (Alves et al., 2010; Crestani et al., 2010b). After a 30 s period, the needle was removed and inserted into the second guide cannula for microinjection into the contralateral BST. Drugs microinjected into the BST were dissolved in artificial cerebrospinal fluid (ACSF) (ACSF composition: 100 mM NaCl; 2 mM Na_3PO_4; 2.5 mM KCl; 1 mM $MgCl_2$; 27 mM $NaHCO_3$; 2.5 mM $CaCl_2$; pH=7.4).

Experimental protocol

Effect of ACSF, WB4101, RX821002 or propranolol microinjection into the BST on dynamic exercise-induced cardiovascular changes. Before surgical preparation, the animals ran daily on the rodent treadmill for at least 1 week at a speed of 0.3–0.8 km/h and 0% grade for 10 min. This procedure aimed to select the animals for their ability to walk on the treadmill and to familiarize them to exercise on the treadmill. No electrical stimulation was used to induce the animals to run.

On the trial day, animals were brought to the experimental room in their home cages. Animals were allowed 1 h to adapt to

the conditions of the experimental room, such as sound and illumination, before starting MAP and HR 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. At least one additional 30 min period was allowed for baseline MAP and HR recording. In the sequence, the first group received bilateral microinjection of vehicle (ACSF, 100 nl, $n=7$) into the BST (Crestani et al., 2006, 2010b); the second group received bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 (10 nmol/100 nl, $n=7$) into the BST (Crestani et al., 2008a,b); the third group received bilateral microinjection of the selective α_2-adrenoceptor antagonist RX821002 (10 nmol/100 nl, $n=6$) into the BST (Crestani et al., 2008a,b); and the fourth group received bilateral microinjection of the nonselective β-adrenoceptor antagonist propranolol (10 nmol/100 nl, $n=5$) into the BST (Crestani et al., 2008a,b). Ten minutes later, the animals were submitted to an acute exercise test on the rodent treadmill. The test consisted of exercise at 0.8 km/h for 6 min (Dufloth et al., 1997; Higa-Taniguchi et al., 2009; Crestani et al., 2010b), which corresponds to about 70% of the rats' maximum running capacity on the treadmill. Each animal received only one microinjection per brain side. An untreated group ($n=5$), with no guide cannula in the brain, was also included.

Effect of microinjection into the BST of ACSF, WB4101 or RX821002 in the open-field test. A different group of animals was submitted to the open-field test. This protocol aimed to study whether changes in cardiovascular responses to exercise induced by blockade of BST adrenoceptors were not due to an unspecific change in locomotor activity. For this, animals were divided into three experimental groups: the first group received bilateral microinjection of vehicle (ACSF, 100 nl, $n=6$) into the BST; the second group received bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 (10 nmol/100 nl, $n=6$) into the BST; and the third group received bilateral microinjection of the selective α_2-adrenoceptor antagonist RX821002 (10 nmol/100 nl, $n=6$) into the BST. Ten minutes after BST pharmacological treatment animals were individually placed on the centre of a circular arena (76.5 cm diameter surrounded by 49-cm-high walls) made of dark transparent plastic. The distance coursed by the animals was measured for 10 min. Motor activity in the open field was videotaped and later analyzed with AnyMaze software (Stoelting, Wood Dale, IL, USA), which detects and calculates the distance moved by the animals.

Histological determination of the microinjection sites

At the end of experiments, animals were anesthetized with urethane (1.25 g/kg, i.p.) and 100 nl of 1% Evan's Blue dye was injected into the BST as a marker of injection sites. They were then submitted to intracardiac perfusion with 0.9% NaCl followed by 10% formalin. Brains were removed and postfixed for 48 h at 4 °C and serial 40 μm-thick sections were cut with a cryostat (CM1900, Leica, Wetzlar, Germany). Sections were stained with 1% Neutral Red for light microscopy analysis. The actual placement of the microinjection needles was determined by analyzing serial sections and identified according to the rat brain atlas of Paxinos and Watson (1997).

Drugs

WB4101 (Tocris, Westwoods Business Park Ellisville, MO, USA), RX821002 (Tocris) and propranolol (Sigma, St. Louis, MO, USA) were dissolved in ACSF. Urethane (Sigma) and tribromoethanol (Sigma) were dissolved in saline (0.9% NaCl). Flunixine meglumine (Banamine®, Schering Plough, Brazil) and poly-antibiotic preparation of streptomycins and penicillins (Pentabiotico®, Fort Dodge, Brazil) were used as provided.

Statistical analysis

Data are presented as mean±SEM. Basal values of MAP and HR before and after BST pharmacological treatment were compared using paired Student's *t*-test. Time-course curves of MAP and HR changes and of distance traveled during the open-field test were compared using two-way ANOVA for repeated measurements (treatment vs. time), with repeated measures on the second factor, followed by Bonferroni's post test. Total distance travelled during the open-field test and MAP and HR baseline values of all experimental groups were compared using one-way ANOVA. Significance was set at $P<0.05$.

RESULTS

Determination of microinjection sites into the bed nucleus of the stria terminalis

A representative photomicrograph of a coronal brain section depicting bilateral microinjection sites in the BST of one representative animal is presented in Fig. 1. Diagrammatic representation showing microinjection sites of ACSF, WB4101, RX821002 and propranolol into the BST and WB4101 and RX821002 into structures surrounding the BST is also shown in Fig. 1.

Effect of microinjection into the BST of ACSF, WB4101, RX821002 or propranolol on dynamic exercise-induced cardiovascular changes

MAP ($F=0.4$, $P>0.05$) and HR ($F=0.9$, $P>0.05$) baseline values were similar in all experimental groups.

ACSF. Bilateral microinjection of ACSF ($n=7$) into the BST did not affect either MAP (99±3 vs. 98±3 mmHg, $t=1.1$, $P>0.05$) or HR (349±10 vs. 352±8 bpm, $t=0.6$, $P>0.05$) baseline values. Exercise-evoked cardiovascular responses in animals that received ACSF injected into the BST were not significantly different from those of the untreated group ($n=5$) (Table 1 and Fig. 2).

WB4101. Bilateral microinjection of the selective α_1-adrenoceptor antagonist WB4101 ($n=7$) into the BST did not affect either MAP (98±2 vs. 96±3 mmHg, $t=1$, $P>0.05$) or HR (366±8 vs. 360±6 bpm, $t=0.5$, $P>0.05$) baseline values. However, BST pretreatment with W4101 significantly increased the exercise-evoked tachycardiac response without affecting the pressor response, when compared with animals that received ACSF injected into the BST (Table 1 and Fig. 3). Microinjection of WB4101 into structures surrounding the BST ($n=4$), such as the anterior comissure, internal capsule or fornix did not affect either MAP ($F_{(1,171)}=0.3$; $P>0.05$) or HR ($F_{(1,171)}=0.1$; $P>0.05$) responses to exercise. Representative recordings showing the cardiovascular responses to exercise on the treadmill in animals treated with ACSF or WB4101 injected into the BST are presented in Fig. 4.

RX821002. Bilateral microinjection of the selective α_2-adrenoceptor antagonist RX821002 ($n=6$) into the BST did not affect either MAP (100±3 vs. 99±4 mmHg, $t=0.4$, $P>0.05$) or HR (358±7 vs. 363±8 bpm, $t=1.5$, $P>0.05$) baseline values. However, pretreatment of the BST with RX821002 significantly reduced exercise-evoked pressor

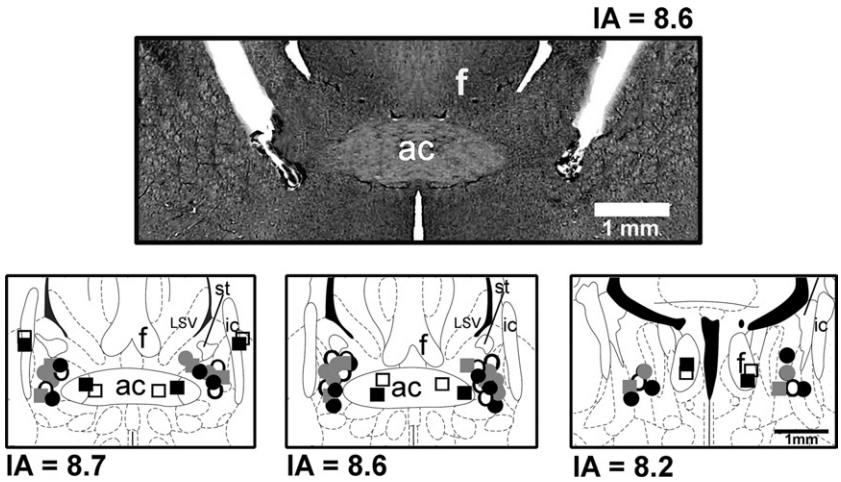

Fig. 1. Photomicrograph of a coronal brain section from one representative rat showing bilateral injection sites in the BST and a diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997), indicating injection sites of ACSF (○), WB4101 (●), RX821002 (●) and propranolol (■) into the BST, as well as WB4101 (■) and RX821002 (□) into structures surrounding the BST. ac, anterior commissure; IA, interaural coordinate; ic, internal capsule; LSV, lateral septal ventral; st, stria terminalis; f, fornix.

response without affecting the tachycardiac response, when compared with animals that received ACSF injected into the BST (Table 1 and Fig. 5). Microinjection of RX821002 into structures surrounding the BST (n=4), such as the anterior comissure, internal capsule or fornix did not affect either MAP ($F_{(1,171)}$=0.5; P>0.05) or HR ($F_{(1,171)}$=0.3; P>0.05) response to exercise. Representative recordings showing the cardiovascular responses to exercise on the treadmill in animals treated with ACSF or RX821002 injected into the BST are presented in Fig. 6.

Propranolol. Bilateral microinjection of the nonselective β-adrenoceptor antagonist propranolol (n=5) into the BST did not affect either MAP (96±2 vs. 97±2 mmHg, t=1.6, P>0.05) or HR (348±13 vs. 355±9 bpm, t=1.6, P>0.05) baseline values. Propranolol microinjection into the BST also did not affect cardiovascular responses to exercise on the treadmill (Fig. 7).

Effect of microinjection into the BST of ACSF, WB4101 or RX821002 in the open-field test

Bilateral microinjection of either WB4101 (n=6) (12±3 vs. 14±3 m, P>0.05) or RX821002 (n=6) (12±3 vs. 13±2 m, P>0.05) into the BST did not affect total distance travelled during the open-field test ($F_{(2,17)}$=0.1, P>0.05), when compared with animals treated with ACSF (n=6) (Fig. 8B). Time-course analysis of distance traveled during the open-field test also did not show a significant effect of BST adrenoceptor antagonism ($F_{(2,150)}$=1.1, P>0.05), but indicated a significant effect over time ($F_{(9,150)}$=50, P<0.0001) (Fig. 8A).

DISCUSSION

The present work brings the first direct evidence for the involvement of CNS adrenoceptors in cardiovascular responses observed during dynamic exercise. We have

Table 1. Statistical summary of time-course analysis of mean arterial pressure (ΔMAP) and heart rate (ΔHR) responses to dynamic exercise (0.8 km/h for 6 min). It was compared responses of groups vehicle (ACSF) (n=7) vs. untreatred (n=5), the selective α1-adrenoceptor antagonist WB4101 (n=7) vs. ACSF, the selective α2-adrenoceptor antagonist RX821002 (n=6) vs. ACSF and the nonselective β-adrenoceptor antagonist propranolol (n=5) vs. ACSF

	Treatment	Time	Interaction (treatment vs. time)
ACSF vs. untreated			
ΔMAP	$F_{(1,190)}$=1	$F_{(18,190)}$=25*	$F_{(18,190)}$=0.2
ΔHR	$F_{(1,190)}$=0.2	$F_{(18,190)}$=110*	$F_{(18,190)}$=0.7
WB4101 vs. ACSF			
ΔMAP	$F_{(1,228)}$=3	$F_{(18,228)}$=36*	$F_{(18,228)}$=0.5
ΔHR	$F_{(1,228)}$=343*	$F_{(18,228)}$=315*	$F_{(18,228)}$=12*
RX821002 vs. ACSF			
ΔMAP	$F_{(1,209)}$=177*	$F_{(18,209)}$=12*	$F_{(18,209)}$=6*
ΔHR	$F_{(1,209)}$=0.1	$F_{(18,209)}$=182*	$F_{(18,209)}$=1
Propranolol vs. ACSF			
ΔMAP	$F_{(1,190)}$=0.6	$F_{(18,190)}$=24*	$F_{(18,190)}$=0.3
ΔHR	$F_{(1,190)}$=1	$F_{(18,190)}$=130*	$F_{(18,190)}$=1.6

* P<0.05, two-way ANOVA followed by Bonferroni's post hoc test.

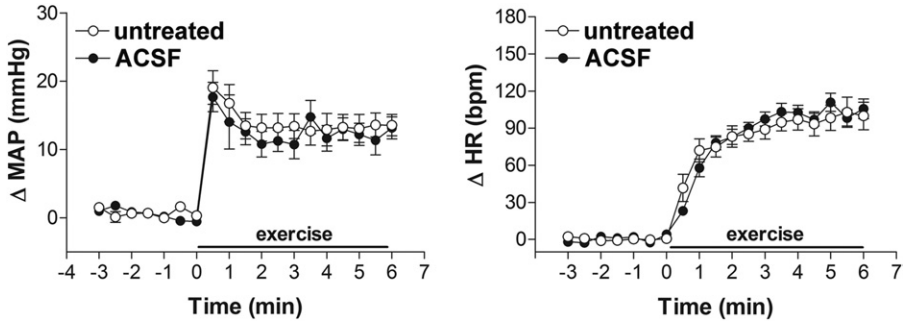

Fig. 2. Time-course of changes in mean arterial pressure (Δ MAP) and heart rate (Δ HR) during dynamic exercise on the treadmill (0.8 km/h for 6 min) of the untreated group (○, $n=5$), with no cannulas in the brain, and in rats that had received bilateral microinjection of vehicle (ACSF, ●, $n=7$) into the BST. The onset of exercise was at t=0. Circles represent the mean and bars the SEM. Microinjection of ACSF into the BST did not affect either MAP ($P>0.05$) or HR responses ($P>0.05$) to exercise on the treadmill.

shown that bilateral microinjection of WB4101, a selective α_1-adrenoceptor antagonist, into the BST enhanced exercise-evoked HR increase without affecting MAP response. Moreover, BST treatment with RX821002, a selective α_2-adrenoceptor antagonist, reduced MAP increase observed during dynamic exercise on the treadmill without changing tachycardiac response. However, bilateral microinjection into the BST of propranolol, a nonselective β-adrenoceptor antagonist, did not affect cardiovascular responses to exercise on the treadmill.

Dynamic exercise causes cardiovascular responses, which include increases in arterial pressure, HR and cardiac output, associated with decreased venous capacitance and redistribution of blood to different territories (regional vasoconstriction or vasodilatation), via neural, hormonal and local mechanisms (Winder et al., 1978; Wade, 1984; Waldrop et al., 1996; Michelini and Stern, 2009). We have observed pressor and tachycardiac response during dynamic exercise on the rodent treadmill. Cardiovascular responses reported in the present study were strictly related to exercise, and not due to exposure to a novel environment, since we have previously reported no fearful associations, including cardiovascular changes, when the animals were kept at rest on the treadmill (Crestani et al., 2010b).

Recently, we have demonstrated that $CoCl_2$-induced acute bilateral inhibition of BST neurotransmission greatly attenuated both pressor and tachycardiac responses evoked by exercise on the treadmill (Crestani et al., 2010b). However, due to the nonselective blockade of local neurotransmission caused by $CoCl_2$ (Kretz, 1984; Lomber, 1999), the possible neurotransmitter involved was not identified. The present work has demonstrated that blockade of α_2-adrenoceptors by bilateral microinjection of RX821002 into the BST is able to reduce exercise-evoked pressor response without changing HR response. This result suggests that local α_2-adrenoceptor mediates, at least in part, BST influence on MAP response during exercise. Although our results indicate a role of α_1-adrenoceptor in modulation of the tachycardiac response evoked by exercise, the blockade of α_1-adrenoceptor in the BST affected HR response in an opposite manner to that observed after BST treatment with $CoCl_2$. Therefore, further experiments are necessary to clarify the neurotransmitter and the receptors in the BST which are involved in its influence on tachycardiac response to exercise. However, the enhancement in exercise-evoked HR increase after the blockade of BST α_1-adrenoceptors led to the interesting observation of a reserve in the cardiac response to our exercise protocol. Moreover, this result indicates an impor-

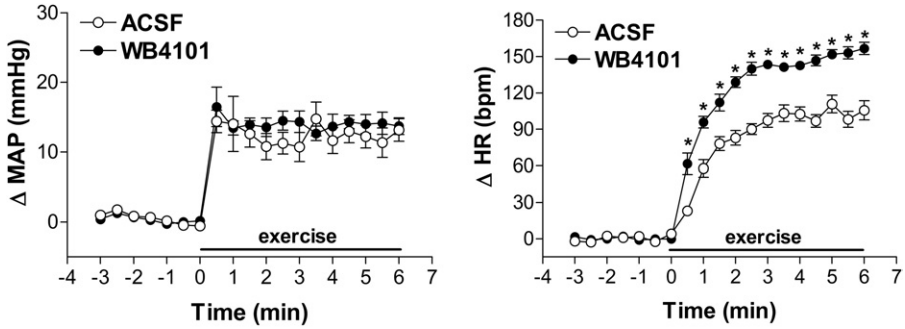

Fig. 3. Time-course of changes in mean arterial pressure (Δ MAP) and heart rate (Δ HR) during dynamic exercise on the treadmill (0.8 km/h for 6 min) in rats that had received bilateral microinjection of vehicle (ACSF, ○, $n=7$) or the selective α_1-adrenoceptor antagonist WB4101 (●, $n=7$) into the BST. The onset of exercise was at t=0. Circles represent the mean and bars the SEM, * $P<0.05$ compared with ACSF, two-way ANOVA (treatment vs. time) followed by Bonferroni's post test. Microinjection of WB4101 into the BST enhanced the HR increase evoked by dynamic exercise ($P<0.0001$) without affecting the pressor response ($P>0.05$).

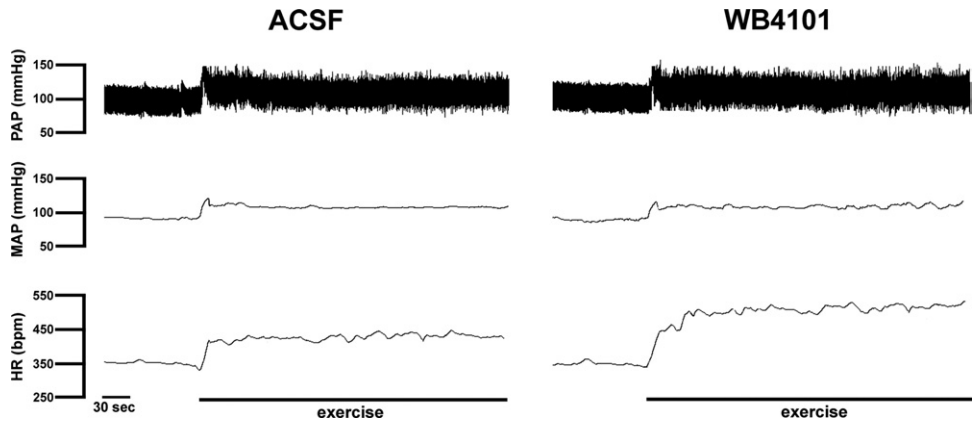

Fig. 4. Recording from representative animals illustrating changes in pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) observed during dynamic exercise on the treadmill after BST treatment with vehicle (ACSF) or the selective α_1-adrenoceptor antagonist WB4101. Note the increase in the HR response to exercise in the animal that received WB4101 injected into the BST.

tant physiological meaning of BST α_1-adrenoceptor in the control of cardiovascular activity during exercise, since activation of this receptor counteracts excessive cardiac activation. Thus, BST α_1-adrenoceptor plays an important role in achieving fine tuning of the cardiac response during exercise, thus ensuring the functional state stabilization of the cardiac activity during exercise since the amplitude of the response is reduced.

We have observed that animals treated with either WB4101 or RX821002 injected into the BST behaved in a similar manner in the open-field test as compared to rats treated with vehicle, indicating that blockade of adrenoceptors in the BST did not influence motor performance. Although connections between the BST and CNS locomotor regions have been reported (Dong et al., 2001; Dong and Swanson, 2004), previous studies from our group and other laboratories also reported absence of effects in the open-field test after BST electrolytic lesion or chemical ablation in both male and female rats (Schulz and Canbeyli, 2000; Pezuk et al., 2006, 2008; Resstel et al., 2008; Crestani et al., 2010a,b). These results suggest that changes in cardiovascular responses to dynamic exercise observed in the present study after BST pharmacological treatment is due to a direct interference in autonomic control, and not to an indirect effect caused by an alteration in motor activity.

According to current theory, circulatory control during exercise is governed by the CNS through several neural mechanisms (Raven et al., 2002; Fisher and White, 2004). Central command is a feed-forward mechanism originating in higher brain centers that involves the parallel activation of brainstem and spinal circuits responsible for the control of locomotion as well as cardiovascular activity during exercise (Raven et al., 2002; Fisher and White, 2004; Williamson et al., 2006). Neuroimaging and immunohistochemical studies have indicated that the neural pathway of central command appears to encompass regions of the cerebral cortex and hypothalamus involved in control of autonomic functions, such as the insular cortex, medial prefrontal cortex, paraventricular nucleus of the hypothalamus and lateral hypothalamus (Timofeeva et al., 2003; Williamson et al., 2006; Williamson, 2010), which interact with other structures involved in locomotor and cardiovascular integration during exercise (Raven et al., 2002; Williamson et al., 2006). Connections between the BST and these cortical and hypothalamic structures were previously described (Yasui et al., 1991; Dong et al., 2001; Vertes, 2004). In this way, it has been proposed that the BST could

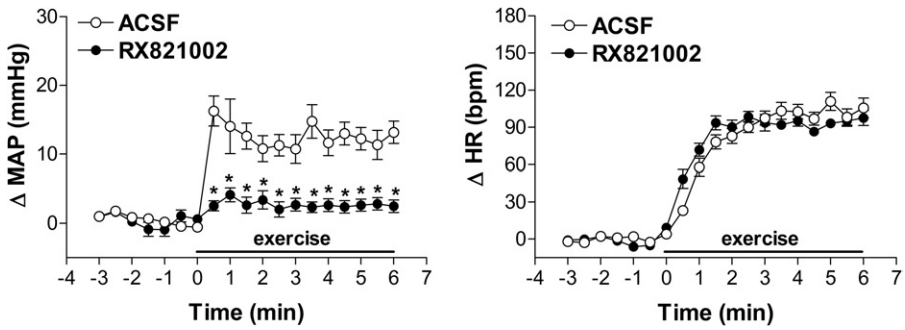

Fig. 5. Time-course of changes in mean arterial pressure (Δ MAP) and heart rate (Δ HR) during dynamic exercise on the treadmill (0.8 km/h for 6 min) in rats that had received bilateral microinjection of vehicle (ACSF, ○, $n=7$) or the selective α_2-adrenoceptor antagonist RX821002 (●, $n=6$) into the BST. The onset of exercise was at t=0. Circles represent the mean and bars the SEM, * $P<0.05$ compared with ACSF, two-way ANOVA (treatment vs. time) followed by Bonferroni's post test. Microinjection of RX821002 into the BST reduced the MAP increase evoked by dynamic exercise ($P<0.0001$) without affecting the tachycardiac response ($P>0.05$).

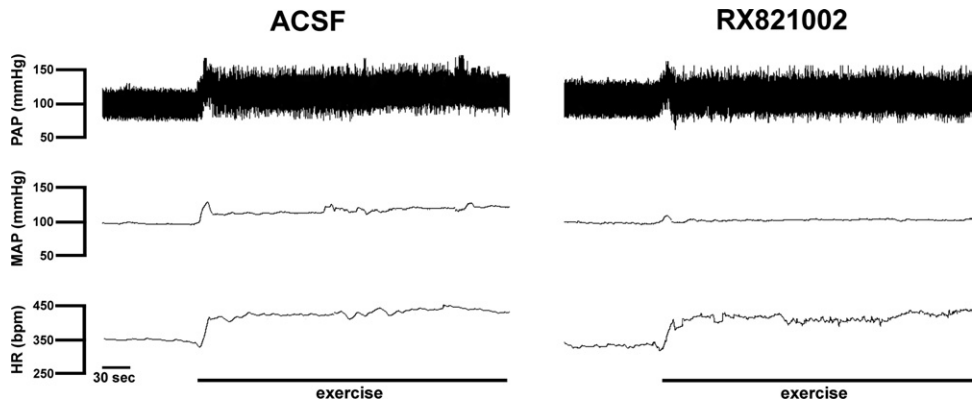

Fig. 6. Recording from representative animals illustrating changes in pulsatile arterial pressure (PAP), mean arterial pressure (MAP) and heart rate (HR) observed during dynamic exercise on the treadmill after BST treatment with vehicle (ACSF) or the selective α_2-adrenoceptor antagonist RX821002. Note the decrease in the arterial pressure response to exercise in the animal that received RX821002 injected into the BST.

be a relay in the neural circuitry of cardiovascular control, connecting telencephalic structures to autonomic regions in the hypothalamus and brainstem (Ulrich-Lai and Herman, 2009). Therefore, control of exercise-evoked cardiovascular responses by BST adrenoceptors proposed in the present study can occur through a modulation of signals arising from cortical structures to the BST.

Cardiovascular adjustment during exercise is also driven by type III and IV muscle afferent activity from exercising muscles, which provide feedback regarding the mechanical and metabolic conditions within those muscles (Kaufman and Forster, 1996; Fisher and White, 2004; Potts, 2006). Although medullary structures appear to be the primary pathway involved in the feedback control from active muscles, supramedullary nuclei may play a modulating role that can affect the reflex control of autonomic activity during exercise (Waldrop and Stremel, 1989; Kaufman and Forster, 1996; Li, 2004). Studies in the literature have shown that static muscle contraction activates brain stem regions consisting of noradrenergic cells (Li et al., 1998), thus indicating that the reflex from active muscles involves central noradrenergic pathways. Therefore, BST noradrenergic neurotransmission could also be part of the pathway of feedback control from active muscle receptors. However, although the activation of α_2-adrenoceptor in the CNS elicited by administration of clonidine, a selective α_2-adrenoceptor agonist, decreases the pressor and tachycardiac response evoked by static muscle contraction (Williams, 1985; Ally et al., 1996), it has been documented that the selective α_2-adrenoceptor antagonist yoimbine does not affect the cardiovascular responses evoked by static exercise when injected either into the lateral ventricle or cerebral aqueduct (Williams et al., 1987; Ally et al., 1996). These results indicate absence of a tonic influence of α_2-adrenoceptor on the neurons regulating the cardiovascular responses to static exercise. In this way, a possible involvement of BST noradrenergic neurotransmission in the pathway of feedback control from active muscle receptors is not mediated by activation of local α_2-adrenoceptors.

It has been reported that the baroreflex stimulus–response curve resets during exercise, with a vertical upward shift on the response arm and a lateral rightward shift to higher operating pressures (Rowell and O'Leary, 1990; DiCarlo and Bishop, 2001; Raven et al., 2006; Dampney et al., 2008). It has been proposed that the central command and reflex mechanism from active muscles may exert its effects on cardiovascular parameters by changing baroreflex activity (Raven et al., 2002; Potts, 2006). Previous results from our laboratory indicated that BST noradrenergic neu-

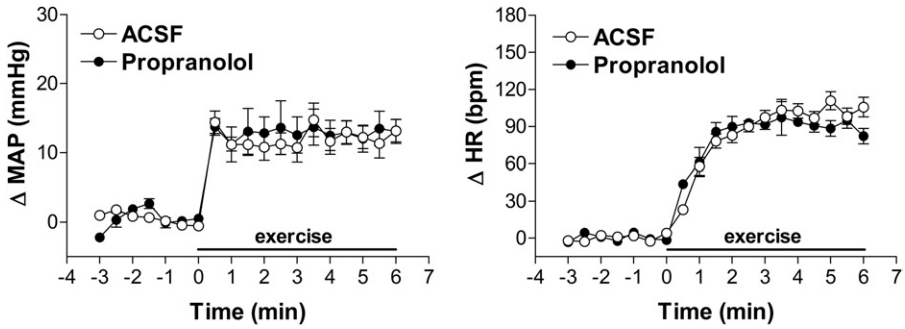

Fig. 7. Time-course of changes in mean arterial pressure (Δ MAP) and heart rate (Δ HR) during dynamic exercise on the treadmill (0.8 km/h for 6 min) in rats that had received bilateral microinjection of vehicle (ACSF, ○, $n=7$) or the nonselective β-adrenoceptor antagonist propranolol (●, $n=5$) into the BST. The onset of exercise was at t=0. Circles represent the mean and bars the SEM. Microinjection of propranolol into the BST did not affect either MAP ($P>0.05$) or HR responses ($P>0.05$) to exercise on the treadmill. Two-way ANOVA (treatment vs. time).

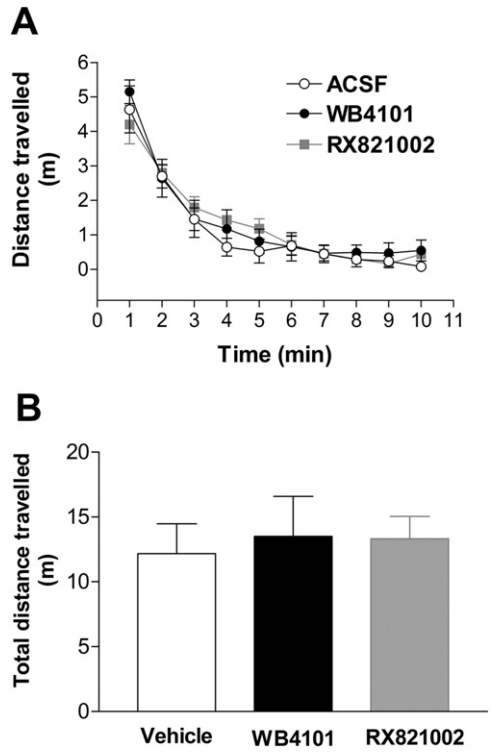

Fig. 8. (A) Time-course of the distance travelled when exposed to the open-field test by animals that received vehicle (ACSF, ○, n=6), the selective α_1-adrenoceptor antagonist WB4101 (●, n=6), or the selective α_2-adrenoceptor antagonist RX821002 (●, n=6) injected into the BST. Circles represent the means and bars the SEM. BST pharmacological treatment did not affect the motor performance in the open-field test (P>0.05). Two-way ANOVA (treatment vs. time). (B) Total distance travelled by rats exposed to the open-field test shown by animals treated with ACSF (white bar), WB4101 (black bar) or RX821002 (gray bar) injected into the BST. BST pretreatment with either WB4101 (P>0.05) or RX821002 (P>0.05) did not modify the rat locomotor activity in the open-field test, when compared with animals treated with ACSF. One-way ANOVA.

rotransmission, through activation of α_1-adrenoceptor, modulates the baroreflex activity in a similar manner to that observed during exercise (Crestani et al., 2008a). This result suggests that activation of BST α_1-adrenoceptors could facilitate cardiovascular responses to dynamic exercise through its modulation of baroreflex activity. However, results reported in the present study indicate an inhibitory influence of BST α_1-adrenoceptors on the exercise-induced HR increase. This evidence suggests that BST noradrenergic neurotransmission affects exercise-induced cardiovascular responses by a mechanism independent of baroreflex modulation. These results corroborate with previous data indicating that BST stimulation evokes similar cardiovascular responses in sham animals or those submitted to sinoaortic denervation (i.e. baroreflex denervation) (Dunn and Williams, 1998).

Both the sympathetic and the parasympathetic branches of the autonomic nervous system participate in the control of cardiovascular activity during dynamic exercise. Blockade of parasympathetic control of HR reveals that most of the initial response to exercise is attributable to the withdrawal of tonic vagal activity, whereas β-adrenergic blockade reveals the importance of augmented cardiac sympathetic activity during moderate and heavy exercise (Overton, 1993; Goldsmith et al., 2000). The BST sends direct projections to medullary structures involved with autonomic activity, such as the nucleus of the tractus solitarius (NTS), dorsal motor nucleus of the vagus, nucleus ambiguus and ventrolateral medulla (Gray and Magnuson, 1987; Dong and Swanson, 2004). In this way, it was demonstrated that ablation of the caudal ventrolateral medulla (CVLM) attenuated MAP and HR decreases elicited by BST stimulation (Giancola et al., 1993). The CVLM projects to and inhibits sympathetic premotor neurons in the rostral ventrolateral medulla, thus decreasing sympathetic preganglionic neuronal outflow (Sved et al., 2000). Previous evidence has also indicated an involvement of the NTS in cardiovascular control during exercise (Dufloth et al., 1997; Raven et al., 2006; Higa-Taniguchi et al., 2009). These results provide evidence of the neural substrate for the influence of BST α_1-adrenoceptor on exercise-related HR response. Thus, BST α_1-adrenoceptors could modulate the cardiac response during exercise by stimulating facilitatory inputs to vagal neurons and/or by stimulating inhibitory inputs to sympathetic medullary neurons. Connections from the BST to the medulla could also be the neural substrate for the facilitatory influence of BST α_2-adrenoceptors on the pressor response to exercise.

The existence of specific neuronal pathways controlling autonomic activity to different organs provides the structural substrate for differences between BST α_1- and α_2-adrenoceptors in modulating cardiovascular adjustments during dynamic exercise (Morrison, 2001). Therefore, subtypes of α-adrenoceptors in the BST may modulate the activity of specific neural pathways in the CNS, thus differentially affecting exercise-evoked pressor and tachycardiac responses. Present results corroborate with previous results that indicated specific actions of BST α_1- or α_2-adrenoceptors on cardiovascular control (Crestani et al., 2008a, 2009a). On the other hand, it was reported that cardiovascular responses evoked by microinjection of noradrenaline into the BST are mediated by activation of both α_1- and α_2-adrenoceptors in the BST (Crestani et al., 2008b). Because different experimental procedures were used in these studies with different response parameters being analyzed, it is possible that BST α_1- and α_2-adrenoceptors have similar or different roles depending on the stimulus.

BST treatment with adrenoceptor antagonists did not affect either MAP or HR baseline values. Therefore, although the present study supports the hypothesis that BST noradrenergic neurotransmission plays an important role in modulating the cardiovascular responses to dynamic exercise, this neurotransmission is not involved in the tonic maintenance of either arterial pressure or HR. These results corroborate previous data in the literature indicating no changes in cardiovascular parameters after blockade of either glutamatergic, cholinergic or adrenergic receptors in the BST (Alves et al., 2007; Hatam and Nasimi, 2007; Crestani et al., 2008a, 2009a).

CONCLUSION

In conclusion, the present results show that noradrenergic neurotransmission in the BST modulates cardiovascular adjustments during dynamic exercise in a complex way. Our data provide evidence of an inhibitory influence of BST α_1-adrenoceptors on exercise-evoked HR response. Moreover, the results point to a facilitatory role played by the activation of BST α_2-adrenoceptors on pressor response to dynamic exercise. These results provide the first direct evidence for the involvement of CNS adrenoceptors in cardiovascular responses observed during dynamic exercise.

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