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RESEARCH PAPER

Both α_1- and β_1-adrenoceptors in the bed nucleus of the stria terminalis are involved in the expression of conditioned contextual fear

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BACKGROUND AND PURPOSE

The bed nucleus of the stria terminalis (BNST) is a limbic structure that is involved in the expression of conditioned contextual fear. Among the numerous neural inputs to the BNST, noradrenergic synaptic terminals are prominent and some evidence suggests an activation of this noradrenergic neurotransmission in the BNST during aversive situations. Here, we have investigated the involvement of the BNST noradrenergic system in the modulation of behavioural and autonomic responses induced by conditioned contextual fear in rats.

EXPERIMENTAL APPROACH

Male Wistar rats with cannulae bilaterally implanted into the BNST were submitted to a 10 min conditioning session (6 footshocks, 1.5 ma/ 3 s). Twenty-four hours later freezing and autonomic responses (mean arterial pressure, heart rate and cutaneous temperature) to the conditioning box were measured for 10 min. The adrenoceptor antagonists were administered 10 min before the re-exposure to the aversive context.

KEY RESULTS

L-propranolol, a non-selective β-adrenoceptor antagonist, and phentolamine, a non-selective α-adrenoceptor antagonist, reduced both freezing and autonomic responses induced by aversive context. Similar results were observed with CGP20712, a selective β_1-adrenoceptor antagonist, and WB4101, a selective α_1-antagonist, but not with ICI118,551, a selective β_2-adrenoceptor antagonist or RX821002, a selective α_2-antagonist.

CONCLUSIONS AND IMPLICATIONS

These findings support the idea that noradrenergic neurotransmission in the BNST via α_1- and β_1-adrenoceptors is involved in the expression of conditioned contextual fear.

Abbreviations

ACTH, adrenocorticotropic hormone; BNST, bed nucleus of the stria terminalis; CeA, central nucleus of the amygdala; CER, conditioned emotional responses; CVLM, caudal ventrolateral medulla; HPA, hypothalamo–pituitary–adrenal axis; HR, heart rate; MAP, mean arterial pressure; NTS, nucleus of the solitary tract

Introduction

The bed nucleus of the stria terminalis (BNST) is a highly heterogeneous and complex limbic structure that is associated with autonomic, neuroendocrine and behavioural functions (Dunn, 1987; Casada and Dafny, 1991; Dunn and Williams, 1995). It has reciprocal connections with the medial and central nuclei of the amygdala (Dong *et al.*, 2001a). Moreover, the BNST receives projections from the hippocampus, the basolateral complex of amygdala and the medial prefrontal cortex (Dong *et al.*, 2001b; Vertes, 2006) and sends projections to the hypothalamus and brainstem areas (Herman *et al.*, 1994; Alheid, 2003), supporting the idea of its regulatory role for defensive responses.

Several studies have shown that the BNST is critically involved in the expression of anxiety-like responses (Walker *et al.*, 2003; Davis *et al.*, 2010), including conditioned emotional responses (CER) and cardiovascular responses to aversive situations (Resstel *et al.*, 2008; Crestani *et al.*, 2009). In terms of the CER, re-exposure to aversively conditioned context induced in the BNST an increase in the expression of the Fos-protein, a marker for neuronal activation (Beck and Fibiger, 1995). Moreover, lesion of the BNST mitigates the conditioned responses observed in the conditioned contextual fear model (Sullivan *et al.*, 2004). Furthermore, our group reported that reversible inactivation of the BNST neurotransmission using cobalt chloride ($CoCl_2$), a non-selective synaptic blocker, attenuated both cardiovascular and behavioural responses induced by conditioned contextual fear (Resstel *et al.*, 2008). However, the neurotransmitters involved in these responses are not yet entirely known.

The BNST is considered a major target for noradrenergic innervation in the brain (Swanson and Hartman, 1975; Moore, 1978). Neuroanatomical studies showed that most of the noradrenergic fibre input to BNST arises from the A1 and A2 medullary cell groups crossing through the ventral noradrenergic bundle (Aston-Jones *et al.*, 1999; Delfs *et al.*, 2000). Several stressful stimuli are known to activate central noradrenergic neurons (Morilak *et al.*, 2005). In this context, evidence points to an important role of the BNST noradrenergic signalling in the modulation of aversive responses (Forray and Gysling, 2004). In fact, noradrenaline release in the BNST is increased under aversive situations (Pacak *et al.*, 1995; Fuentealba *et al.*, 2000; Cecchi *et al.*, 2002; Fendt *et al.*, 2005). In addition, the exposure to the conditioned fear paradigm increased the activity of noradrenergic projections to the BNST and noradrenaline depletion in this structure prevented neuroendocrine and behavioural responses induced by fear stimuli (Onaka and Yagi, 1998).

Finally, although these data show that the BNST was involved in the expression of the CERs and that BNST noradrenergic system was involved in the modulation of aversive responses, which adrenoceptors could be involved in CERs has not been investigated yet. Hence, the objective of this study was to test the hypothesis that noradrenergic neurotransmission in the BNST is involved in the modulation of autonomic and behavioural responses induced by conditioned contextual fear, and to evaluate the participation of α- and β-adrenoceptors in these responses.

Methods

Animals

All animal care and experimental procedures conformed to the Brazilian Society of Neuroscience and Behavior guidelines for the care and use of laboratory animals, which are in compliance with UK animal regulation, and were approved by the Institution's Animal Ethics Committee (process number: 119/2010). The experiments were performed using male Wistar rats weighing 230–270 g (total of 164 animals). 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 3 days before experimental manipulations (habituation, conditioning and testing) in plastic cages with free access to food and water under a 12 h light/dark cycle (lights on at 06:30 h). All experimental protocols are reported in accordance with the ARRIVE guidelines for reporting experiments involving animals (McGrath *et al.*, 2010).

Stereotaxic surgery

Seven days before the experiment, the rats were anaesthetized with 2,2,2-tribromoethanol (250 mg·kg^{-1} i.p.; Sigma-Aldrich, St. Louis, MO, USA) and fixed in a stereotaxic frame (Stoelting, Kiel, WI, USA). After scalp anaesthesia with 2% lidocaine, the skull was surgically exposed, and stainless steel guide cannulae (26G) were implanted bilaterally into the BNST (anteroposterior = +8.6 mm from interaural; lateral = +4.0 mm from the medial suture, vertical = −5.5 mm from the skull with a lateral inclination of 23°) (Paxinos and Watson, 1997). Cannulae were fixed to the skull with dental cement and a metal screw. An obturator inside the guide cannulae prevented obstruction. After surgery, the animals received a polyantibiotic (0.27 g·kg^{-1} i.m.; Pentabiotico®, Fort Dodge, Campinas, Brazil) to prevent infection and a non-steroidal anti-inflammatory agent (0.025 g·kg^{-1} s.c.; Banamine®, Schering Plough, Rio de Janeiro, Brazil) for post-operative analgesia.

Fear conditioning and testing

Habituation, conditioning and testing were carried out in 23 × 20 × 21 cm footshock chambers. The chambers had a grid floor composed of 18 stainless steel rods (2 mm in diameter), spaced 1.5 cm apart and wired to a shock generator (Insight, Ribeirão Preto, Brazil). They were cleaned with 70% ethanol before and after use. Habituation session started 7 days after the stereotaxic surgery and consisted of one 10 min-long pre-exposure to the footshock chamber. No shock was given during the pre-exposure. In the conditioning shock session, performed 4 h after the habituation session, animals were divided into two experimental groups: non-conditioned and conditioned. The non-conditioned group was exposed to the footshock chamber for 10 min, but no shock was delivered. The conditioned group was subjected to a shock session consisting of six electrical 1.5 mA/3 s footshocks delivered at pseudorandom intervals (ranging from 20 to 60 s) (Resstel *et al.*, 2008; Gomes *et al.*, 2012). The animals were allowed to explore the chamber prior to shock delivery for 2 min. Twenty-four hours after the conditioning session, the rats were anesthetized with 2,2,2-tribromoethanol (250 mg·kg^{-1} i.p.), and a catheter (a 4 cm PE-10 segment heat-bound to a 13 cm PE-50 segment, Clay Adams, Parsippany, NJ, USA) was

implanted into the femoral artery for blood pressure and heart rate recording. The catheters were tunnelled under the skin and exteriorized on the animal's dorsum.

Autonomic and behavioural responses evoked by re-exposure to aversively conditioned context were evaluated 48 h after conditioning. The test session consisted of a 10 min-long re-exposure to the footshock chamber without shock delivery. Animals were transferred from the animal room to the procedure room in their home cage. Cardiovascular recordings were initiated after a 60 min room adaptation period. Because animals can associate environmental cues with conditioning (Frank *et al.*, 2004), testing was performed in a different room from that used during the conditioning procedure. Mean arterial pressure (MAP) and heart rate (HR) were recorded using an HP-7754A amplifier (Hewlett Packard, Chicago, IL, USA) connected to a signal acquisition board (Biopac M-100, Goleta, CA, USA) and computer processed. Rats were tested one at a time. Five minutes after the BNST local microinjection, 5 min of baseline recording were recorded before animals were placed in the centre of the footshock chamber to record conditioned cardiovascular and behavioural responses that are evoked when animals are re-exposed to the context. Additionally, variations in the cutaneous temperature were also recorded. The cutaneous temperature of the tail was recorded with a thermal camera (Multi-Purpose Thermal Imager IRI 4010, InfraRed Integrated Systems Ltd. Northampton, UK) at a distance of 50 cm every minute for 5 min period before the beginning of the experiment and during the 10 min of exposure to the aversive context. The testing was conducted in a room kept at 26 ± 1°C, which is the thermoneutral zone for rats (Gordon, 1990).

Behavioural responses (freezing) were evaluated during the test by an experimenter who was unaware of the experimental groups, sitting 45 cm away from the footshock chamber. Freezing was defined as the complete absence of movement, except that of the flanks related to respiration, while the animal assumed a characteristic tense posture (Fanselow, 1980).

Drugs

The following drugs were used: L-propranolol (a non-selective β-adrenoceptor antagonist; Sigma-Aldrich), phentolamine (a non-selective α-adrenoceptor antagonist; Sigma-Aldrich), WB4101 (a selective α_1-adrenoceptor antagonist; Tocris Bristol, UK), RX821002 (a selective α_2-adrenoceptor antagonist; Tocris), CGP20712 (a selective β_1-adrenoceptor antagonist; Tocris) and ICI118,551 (a selective β_2-adrenoceptor antagonist; Tocris); receptor nomenclature follows Alexander *et al.*, (2011). All drugs were dissolved in sterile saline (vehicle). The solutions were prepared immediately before the tests and protected from the light during the experimental sessions.

Intra-BNST injection

Intra-BNST injections were performed with a thin dental needle (30 G, 0.3 mm OD; Terumo Dental Needle®, São Paulo, Brazil) introduced through the guide cannula until its tip was 1 mm below the cannula end, connected to a 2 μL syringe (7001 KH, Hamilton Co., Reno, NV, USA) through a PE-10 tubing. The needles were carefully inserted into the guide cannulae, and a volume of 100 nL was injected over a 30 s period with a rate of 200 nL·min^{-1} with the help of an infusion pump (Kd Scientific Inc., Holliston, MA, USA). In order to prevent reflux, the needles were left in place for a 30–45 s period after the end of each injection.

Experimental protocols

Experiment 1: Involvement of BNST adrenoceptors in the expression of conditioned contextual fear.

In independent experiments, non-conditioned and conditioned animals received bilateral injections of L-propranolol (7.5 or 12.5 nmol) or phentolamine (10 or 20 nmol) into the BNST before re-exposure to the conditioning chamber. Additionally, we also investigated if the combination of L-propranolol (7.5 nmol) and phentolamine (10 nmol) would potentiate the effect of the drugs alone in conditioned animals. For this experiment, the animals received intra-BNST injections of L-propranolol followed 5 min later by an injection of phentolamine, or vice versa. The doses were based on previous results from the literature (Crestani *et al.*, 2008a; Zhou *et al.*, 2010).

Experiment 2: Effects of the selective β_1-adrenoceptor antagonist CGP20712 and the selective β_2-adrenoceptor antagonist ICI118,551 injected into the BNST on conditioned contextual fear.

Based on the results obtained in the experiment 1, we performed this experiment to investigate the participation of BNST β_1- and β_2-adrenoceptors on expression of conditioned contextual fear. Conditioned animals received bilateral injections of vehicle, the β_1-adrenoceptor antagonist CGP20712 (4.5 nmol) or the β_2-adrenoceptor antagonist ICI118,551 (34.5 nmol) into the BNST before re-exposure to the conditioning chamber. Drugs doses were chosen based on the ratio of K_i values for L-propranolol (based on the dose of 7.5 nmol) to β_1- and β_2-adrenoceptors (K_i = 1 nM, both β-adrenoceptors), CGP20712 to the β_1-adrenoceptors (K_i = 0.6 nM) and ICI118,551 to the β_2-adrenoceptors (K_i = 4.6 nM) (Bylund *et al.*, 1994). Therefore, each one of the selective antagonists produced a similar, but selective, blockade of β-adrenoceptors, as did L-propranolol.

Experiment 3: Effects of the selective α_1-adrenoceptor antagonist WB4101 and the selective α_2-adrenoceptor antagonist RX821002 injected into the BNST on conditioned contextual fear.

Based on the results obtained in the experiment 1, we performed this experiment to investigate the participation of BNST α_1- and α_2-adrenoceptors on the expression of conditioned contextual fear. Conditioned animals received bilateral injections of vehicle, α_1-adrenoceptor antagonist WB4101 (1.7 nmol) or α_2-adrenoceptor antagonist RX821002 (2.3 nmol) into the BNST before re-exposure to the conditioning chamber. Drug doses were chosen based on the ratio of Ki values for phentolamine (based on the dose of 10 nmol) to α1 (Ki = 3.6 nM) and α2-adrenoceptors (Ki = 4.4 nM), WB4101 to the α1-adrenoceptors (Ki = 0.6 nM) and RX821002 to the α2- adrenoceptors (Ki = 1 nM) (Bylund *et al.*, 1994; Miralles *et al.*, 1993). Therefore, each one of the selective antagonists produced a similar, but selective, blockade of α–adrenoceptors, as did phentolamine.

Experiment 4: Effects of a combination of the CGP20712 and WB4101 injected into the BNST on conditioned contextual fear.

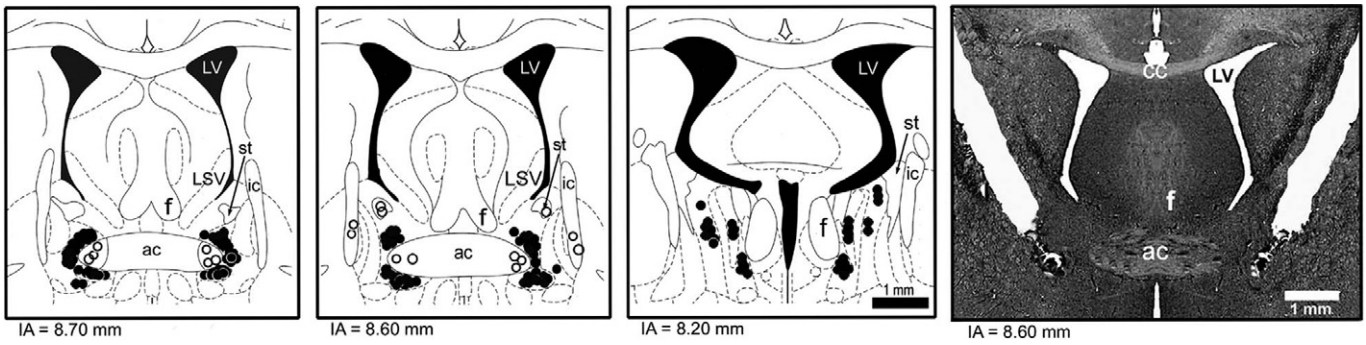

Figure 1

Representative photomicrograph of a coronal brain section from a rat showing bilateral microinjection sites in the BNST, and diagrammatic representation based on the rat brain atlas of Paxinos and Watson (1997), indicating the injection sites into and outside the BNST of all the animals used in the experiments. IA, interaural coordinate; ac, anterior commissure; cc, corpus callosum; f, fornix; ic, internal capsule; LV, lateral ventricle; LSV, lateral septal ventral; st, stria terminalis.

Based on the results obtained in experiments 2 and 3, we performed this experiment to investigate if the combination of CGP20712 and WB4101 would potentiate the effect of the drugs alone. The animals were divided into two groups: vehicle or CGP20712 + WB4101. The latter received intra-BNST injections of CGP20712 followed 5 min later by a second injection of WB4101, or vice versa. Five minutes after the last injection, the animals were placed in the centre of the footshock chamber.

Histological determination of the injection sites

At the end of the experiments, the rats were anaesthetized with urethane (1.25 g·kg^{-1} i.p., Sigma-Aldrich) and 200 nL of 1% Evan's Blue dye was bilaterally injected into the BNST as an injection site marker. The chest was surgically opened, the descending aorta was occluded, the right atrium was severed and the brain was perfused with 10% formalin through the left ventricle. Brains were post-fixed for 24 h at 4°C, and 40 mm sections were cut using a cryostat (CM-1900, Leica, Weltzar, Germany). Serial brain sections were stained with 1% Neutral Red, and injection sites were determined using the rat brain atlas (Paxinos and Watson, 1997) as reference. As histological control, the animals subjected to the conditioned contextual fear that received L-propranolol (7.5 nmol) or phentolamine (10 nmol) outside the BNST were joined together in an additional group ('OUT').

Statistical analysis

Freezing was represented as percentage of the test period. Freezing was expressed as mean ± SEM and analysed using a two-way ANOVA with condition (conditioned or non-conditioned rats) and treatment as the two factors in the experiment 1; using one-way ANOVA for the analysis of experiments 2 and 3 and Student's *t*-test for the experiment 4. Animals that received L-propranolol (7.5 nmol) or phentolamine (10 nmol) outside the BNST were compared with vehicle non-conditioned or conditioned groups using Student's *t*-test.

MAP, HR and cutaneous temperature values were continuously recorded during the 5 min period prior and the 10 min period after the exposure to the footshock chamber. Data were expressed as mean ± SEM of MAP, HR or cutaneous temperature changes (respectively, Δ MAP, Δ HR and Δ Temperature) sampled at 1 min intervals. Points sampled during the 5 min prior to the exposure were used as control baseline values. The baseline values of MAP, HR and cutaneous temperature of the animals recorded before chamber re-exposure were compared by one-way ANOVA. In experiment 1, MAP, HR and cutaneous temperature changes were analysed separately to non-conditioned and conditioned groups using a two-way ANOVA, with treatment as the main independent factor and time as a repeated measurement. Additionally, two-way ANOVA was performed to compare these changes between non-conditioned and conditioned vehicle-treated animals. In experiments 2–4, the MAP, HR and cutaneous temperature changes were analysed using a two-way ANOVA, with treatment as the main independent factor and time as a repeated measurement. The results of experiments with the combination of either L-propranolol + phentolamine or CGP20712 + WB4101 were compared with the effect of isolated treatments on the freezing using Student's *t*-test and a two-way ANOVA to compare MAP, HR or cutaneous temperature changes, with treatment as the main independent factors and time as a repeated measurement. Results of statistical tests with $P < 0.05$ were considered significant.

Results

Diagrammatic representations showing BNST injection sites and a representative photomicrograph are presented in Figure 1.

Experiment 1: Involvement of BNST adrenoceptors in the expression of conditioned contextual fear

L-propranolol. Rats in the conditioned vehicle-treated group ($n = 8$) spent more time in freezing during the re-exposure to aversive context (condition factor: $F_{1,36} = 66.1$, $P < 0.001$, two-way ANOVA) when compared with non-conditioned

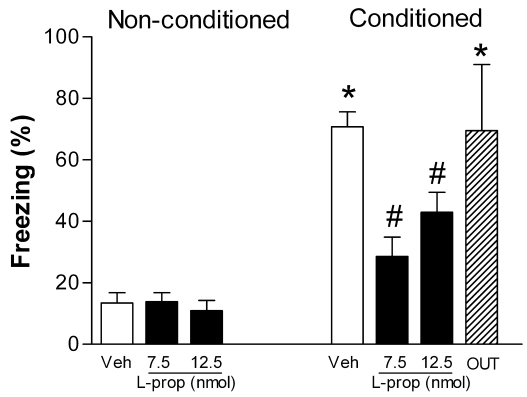

Figure 2

Effects of bilateral injection of saline (Veh) or L-propranolol (L-prop; 7.5 and 12.5 nmol) in non-conditioned (n = 6 per group) and conditioned animals (n = 8 per group) on the percentage of time spent in freezing behaviour. Values from rats with L-propranolol (7.5 nmol) injections outside the BNST were combined in an additional group (n = 5; OUT). Data shown are means ± SEM. *P < 0.05 compared with vehicle non-conditioned group. $^{\#}P$ < 0.05 compared with vehicle conditioning group, Bonferroni's *post hoc* test, except the 'OUT' group that was compared with vehicle non-conditioned or conditioned groups by Student's t-test.

groups (n = 6 per group; Figure 2). Moreover, there were significant effects of treatment ($F_{2,36}$ = 8.5, P < 0.001, two-way ANOVA) and interaction ($F_{2,36}$ = 8.4, P < 0.001, two-way ANOVA). L-propranolol (7.5 and 12.5 nmol, n = 8 per group) injections significantly reduced freezing of conditioned animals ($F_{2,21}$ = 13.3, P < 0.001, one-way ANOVA) as compared with vehicle-treated conditioned animals (Figure 2). L-propranolol injection into the BNST of non-conditioned animals did not affect freezing behaviour ($F_{2,15}$ = 0.2, P > 0.05, one-way ANOVA; Figure 2).

In terms of the autonomic responses, no differences were observed on baseline values of MAP, HR and cutaneous temperature among the groups recorded immediately before re-exposure to the chamber (Table 1). The analyses of the cardiovascular responses showed that re-exposure to a context previously paired with footshock induced a marked and sustained increase in both HR and MAP (time factor: MAP: $F_{14,315}$ = 22.1, P < 0.001 and HR: $F_{14,315}$ = 26.6, P < 0.001, two-way ANOVA; Figure 3). In the non-conditioned vehicle-treated group, re-exposure to the context increased both HR and MAP. However, these increases were significantly lower than those observed in the conditioned vehicle-treated group (MAP: $F_{1,180}$ = 79.2, P < 0.001 and HR: $F_{1,180}$ = 83.9, P < 0.001, two-way ANOVA). Similar to its effect on behavioural responses, L-propranolol (7.5 and 12.5 nmol) attenuated the MAP and HR increases in conditioned groups (treatment factor: MAP: $F_{2,315}$ = 56.5, P < 0.001 and HR: $F_{2,315}$ = 80.6, P < 0.001, two-way ANOVA) but had no effect on non-conditioned animals (treatment factor: MAP: $F_{2,225}$ = 1.6, P > 0.05 and HR: $F_{2,225}$ = 1.9, P > 0.05, two-way ANOVA; Figure 3). Regarding the cutaneous temperature, it was observed that the re-exposure to the aversive context induced a decrease in the cutaneous temperature (time factor: $F_{14,315}$ = 29.3, P < 0.001, two-way ANOVA; Figure 3). In the non-conditioned vehicle-treated group, re-exposure to the context decreased the cutaneous temperature. However, this decrease was significantly lower than that observed in the conditioned vehicle-treated group ($F_{1,180}$ = 37.3, P < 0.001, two-way ANOVA). L-propranolol (7.5 and 12.5 nmol) attenuated cutaneous temperature decreases in conditioned groups (treatment factor: $F_{2,315}$ = 104.7, P < 0.001, two-way ANOVA) but had no effect on non-conditioned animals (treatment factor: $F_{2,225}$ = 1.1, P > 0.05, two-way ANOVA; Figure 3).

Phentolamine. Animals in the conditioned vehicle-treated group (n = 7) spent more time freezing during the re-exposure to the aversive context (conditioned factor: $F_{1,36}$ = 65.4, P < 0.001, two-way ANOVA) when compared with those from the non-conditioned group (n = 5–7 per group; Figure 4). Moreover, there were significant effects of treatment ($F_{2,36}$ = 11.8, P < 0.01, two-way ANOVA) and interaction ($F_{2,36}$ = 7.4, P < 0.01, two-way ANOVA). Phentolamine (10 and 20 nmol; n = 8 per group) injections significantly reduced the freezing of conditioned animals ($F_{2,20}$ = 14.6, P < 0.01, one-way ANOVA) when compared with vehicle-treated conditioned animals (Figure 4). In non-conditioned animals, the injection of phentolamine did not produce any effect on the freezing behaviour ($F_{2,16}$ = 0.7, P > 0.05, one-way ANOVA; Figure 4). Regarding the autonomic responses to fear conditioning, phentolamine did not affect baseline values of MAP, HR or cutaneous temperature (Table 1). In the non-conditioned vehicle-treated group, re-exposure to the context increased both HR and MAP. However, these increases were significantly lower than those observed in the conditioned vehicle-treated group (MAP: $F_{1,165}$ = 136.1, P < 0.001 and HR: $F_{1,165}$ = 145.7, P < 0.001, two-way ANOVA). Phentolamine (10 and 20 nmol) attenuated the cardiovascular responses observed during the re-exposure to the aversive context (treatment factor: MAP: $F_{2,315}$ = 36.9, P < 0.001 and HR: $F_{2,315}$ = 90.1, P < 0.001, two-way ANOVA), but no effect was observed in non-conditioned animals (treatment factor: MAP: $F_{2,240}$ = 2.3, P > 0.05 and HR: $F_{2,240}$ = 1.1, P > 0.05, two-way ANOVA; Figure 5). Moreover, re-exposure to aversive context induced a decrease in the cutaneous temperature (time factor: $F_{14,315}$ = 65.5, P < 0.001, two-way ANOVA; Figure 5). In the non-conditioned vehicle-treated group, re-exposure to the context decreased the cutaneous temperature. However, this decrease was significantly lower than that observed in the conditioned vehicle-treated group ($F_{1,165}$ = 71.7, P < 0.001, two-way ANOVA). Phentolamine (10 and 20 nmol) attenuated cutaneous temperature decreases in conditioned groups (treatment factor: $F_{2,315}$ = 64.9, P < 0.001, two-way ANOVA), but had no effect on non-conditioned animals (treatment factor: $F_{2,240}$ = 2.5, P > 0.05, two-way ANOVA; Figure 5).

Additionally, the injection of L-propranolol or phentolamine into structures surrounding the BNST was unable to prevent freezing (Figures 2 and 4) and autonomic changes (data not shown) induced in conditioned animals by aversive re-exposure to the context.

L-propranolol + phentolamine. Animals in the conditioned vehicle-treated group (n = 6) spent more time freezing during the re-exposure to the aversive context (condition factor: $F_{1,19}$ = 48.0, P < 0.001, two-way ANOVA) when compared with those in the non-conditioned group (n = 5–6 per group;

Table 1

Basal values of MAP and HR of non-conditioned and conditioned animals after treatment with adrenoceptor antagonists or vehicle

Group	N (animals)	MAP (mmHg)	HR (bpm)	Temperature (°C)
Non-conditioned				
Vehicle	6	93 ± 2	344 ± 13	31.9 ± 1.3
L-prop, 7.5 nmol	6	98 ± 3	349 ± 18	31.2 ± 0.9
L-prop, 12.5 nmol	6	97 ± 4	387 ± 16	31.7 ± 1.1
		$F_{2,12} = 0.8$	$F_{2,12} = 1.9$	$F_{2,17} = 0.1$
Vehicle	5	102 ± 5	360 ± 24	30.8 ± 2.3
Phento, 10 nmol	7	109 ± 6	366 ± 18	31.5 ± 1.2
Phento, 20 nmol	7	104 ± 2	357 ± 22	30.6 ± 0.6
		$F_{2,12} = 0.5$	$F_{2,12} = 0.03$	$F_{2,18} = 0.13$
Vehicle	5	102 ± 5	360 ± 24	32 ± 0.6
L-prop, 7.5 nmol + Phento, 10 nmol	7	107 ± 6	359 ± 23	31.8 ± 0.8
		$t = 0.67$	$t = 0.05$	$t = 0.18$
Conditioned				
Vehicle	8	94 ± 2	328 ± 9	31.1 ± 0.4
L-prop, 7.5 nmol	8	110 ± 8	351 ± 7	31.8 ± 1.1
L-prop, 12.5 nmol	8	105 ± 3	350 ± 10	30.5 ± 0.7
OUT	5	95 ± 2	318 ± 11	31.5 ± 0.9
		$F_{3,18} = 2.8$	$F_{3,18} = 2.9$	$F_{3,27} = 0.5$
Vehicle	7	95 ± 2	324 ± 9	31.7 ± 1
Phento, 10 nmol	8	102 ± 2	371 ± 15	32.1 ± 0.7
Phento, 20 nmol	8	99 ± 3	348 ± 11	31 ± 1.1
OUT	4	96 ± 2	342 ± 10	31.5 ± 0.9
		$F_{3,19} = 1.5$	$F_{3,19} = 2.5$	$F_{3,26} = 0.25$
Vehicle	7	98 ± 2	349 ± 6	32 ± 0.4
L-prop, 7.5 nmol + Phento, 10 nmol	8	99 ± 5	377 ± 9	31.6 ± 0.8
		$t = 0.15$	$t = 2.6$	$t = 0.42$

The values in the table represent the means ± SEM. No significant difference in the values within each group was observed;. one-way ANOVA. L-prop, L-propranolol; Phento, phentolamine.

Figure 6). Moreover, there were significant effects of treatment ($F_{1,19}$ = 49.4, P < 0.001, two-way ANOVA) and interaction ($F_{1,19}$ = 37.2, P < 0.001, two-way ANOVA). The combination of phentolamine (10 nmol) and L-propranolol (7.5 nmol) significantly reduced the freezing (n = 6, t = 4.4, P < 0.01) when compared with vehicle-treated conditioned animals (Figure 6). Moreover, this treatment potentiated the attenuation of freezing when compared with isolated treatments (L-propranolol: t = 2.3, P < 0.05 and phentolamine: t = 2.9, P < 0.05). In non-conditioned animals, the combination of phentolamine and L-propranolol did not produce any effect on the freezing behaviour (t = 0.8, P > 0.05; Figure 6).

There were no differences among the groups in baseline values of MAP, HR or cutaneous temperature (Table 1). Similarly to behavioural responses, the combination of phentolamine and L-propranolol attenuated autonomic changes in conditioned groups (treatment factor: MAP: $F_{1,150}$ = 105.5, P < 0.001; HR: $F_{1,150}$ = 101.8, P < 0.001, cutaneous temperature: $F_{1,150}$ = 44.1, P < 0.001, two-way ANOVA; Figure 7).

Moreover, the attenuation of autonomic responses evoked by the combination of both drugs was more effective than that observed with isolated treatments with L-propranolol or phentolamine, except for the potentiated attenuation of the cutaneous temperature induced by the combination when compared with the effect of phentolamine alone (L-prop: MAP: $F_{1,180}$ = 0.4, P > 0.05, HR: $F_{1,180}$ = 0.9, P > 0.05, cutaneous temperature: $F_{1,165}$ = 1.2, P > 0.05; Phento: MAP: $F_{1,180}$ = 0.2, P > 0.05 and HR: $F_{1,180}$ = 1.7, P > 0.05, cutaneous temperature: $F_{1,165}$ = 8.7, P > 0.05).

Experiment 2: Effects of the selective β_1-adrenoceptor antagonist CGP20712 and the selective β_2-adrenoceptor antagonist ICI118,551 injected into the BNST on conditioned contextual fear

Injection of CGP20712 (n = 5) into the BNST significantly reduced the freezing of conditioned animals ($F_{2,15}$ = 18.3, P < 0.001; Bonferroni test, P < 0.05) compared with

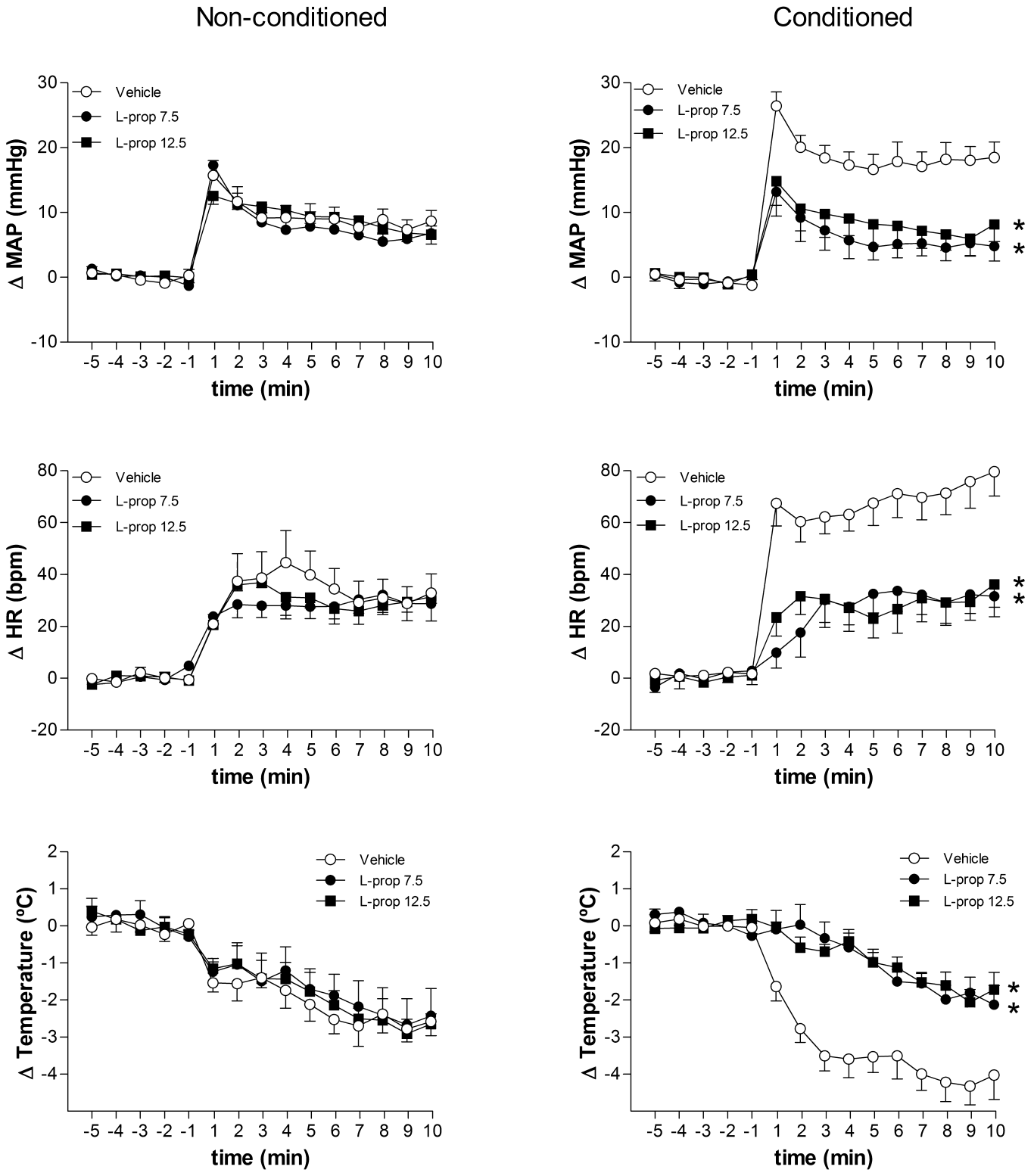

Figure 3

Time course of the bilateral injection of saline (Veh) or L-propranolol (L-prop; 7.5 and 12.5 nmol) into the BNST on mean arterial pressure (Δ MAP) and heart rate (Δ HR) increases and cutaneous temperature (Δ Temperature) decrease in non-conditioned (n = 6 per group) and conditioned animals (n = 8 per group). Data shown are means ± SEM. *P < 0.05 over the whole footshock chamber exposure period compared with vehicle-treated animals, Bonferroni's *post hoc* test.

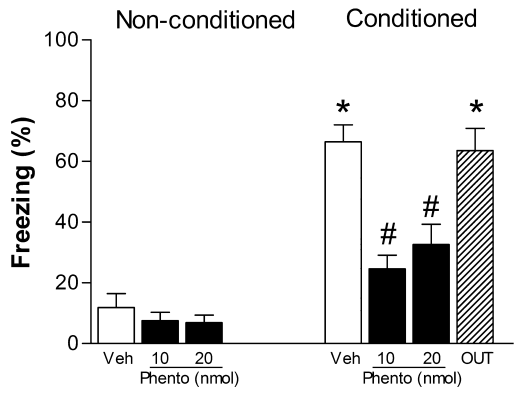

Figure 4

Effects of bilateral injection of saline (Veh) or phentolamine (Phento; 10 and 20 nmol) in non-conditioned (n = 5–7 per group) and conditioned animals (n = 8 per group) on the percentage of time spent in freezing behaviour. Values from rats with phentolamine (10 nmol) injections outside the BNST were combined in an additional group (n = 4; OUT). Data shown are means ± SEM. *P < 0.05 compared with vehicle non-conditioned group. $^{\#}P$ < 0.05 compared with vehicle conditioning group, Bonferroni's *post hoc* test, except the 'OUT' group that was compared with vehicle non-conditioned or conditioned groups by Student's t-test.

vehicle-treated conditioned animals (n = 6; Figure 8). No effect was observed after ICI118,551 (n = 7; Bonferroni test, P > 0.05). Concerning autonomic changes, the injection of either CGP20712 or ICI118,551 into the BNST did not affect baseline values of MAP, HR and cutaneous temperature (data not shown). Re-exposure to the context previously paired with footshock induced a sharp and sustained drop in the tail temperature during the test (time factor: $F_{14,225}$ = 31.4, P < 0.001, two-way ANOVA). There was a significant reduction in autonomic responses evoked after treatment with CGP20712 (treatment factor: MAP: $F_{2,225}$ = 106.3, P < 0.001; HR: $F_{2,225}$ = 37.6, P < 0.001; cutaneous temperature: $F_{2,225}$ = 61.3, P < 0.001, two-way ANOVA) during re-exposure to the conditioning box (Figure 8). However, ICI118,551 did not affect autonomic responses during re-exposure to aversive context (P > 0.05 for all parameters; Figure 8).

Experiment 3: Effects of the selective α_1-adrenoceptor antagonist WB4101 and the selective α_2-adrenoceptor antagonist RX821002 injected into the BNST on conditioned contextual fear

Injection of WB4101 (n = 5) into the BNST significantly reduced the freezing of conditioned animals ($F_{2,12}$ = 18.4, P < 0.001, one-way ANOVA) compared with vehicle-treated conditioned animals (n = 5; Figure 8). No effect was observed after RX821002 (n = 6, P > 0.05). Injection of either WB4101 or RX821002 into the BNST did not affect baseline values of MAP, HR and cutaneous temperature (data not shown). Re-exposure to the context previously paired with the footshock induced a marked drop in the cutaneous temperature, which lasted for the entire duration of the test (time factor: $F_{14,210}$ = 12.1, P < 0.001, two-way ANOVA). Analysis of the autonomic responses during the re-exposure to the conditioning box showed significant decreases after the treatment with WB4101 (treatment factor: MAP: $F_{2,210}$ = 28.7, P < 0.001; HR: $F_{2,210}$ = 27.9, P < 0.001; cutaneous temperature: $F_{2,210}$ = 10.7, P < 0.001, two-way ANOVA). RX821002 was unable to attenuate autonomic changes observed during the re-exposure to the aversive context (P > 0.05 for all parameters; Figure 8).

Experiment 4: Effects of a combination of the CGP20712 and WB4101 injected into the BNST on conditioned contextual fear

The combination of CGP20712 + WB4101 into the BNST significantly reduced the freezing of conditioned animals (n = 6, t = 7.8, P < 0.001) when compared with vehicle-treated conditioned animals (n = 6; Figure 8). However, there was no difference when compared with individual treatments (CGP20712: t = 0.2, P > 0.05 and WB4101: t = 0.6, P > 0.05). As observed with the behavioural responses, the combination of the CGP20712 and WB4101 attenuated the changes in MAP, HR and cutaneous temperature observed during re-exposure to the aversive context (treatment factor: MAP: $F_{1,135}$ = 45.9, P < 0.001; HR: $F_{1,135}$ = 73.5, P < 0.001; cutaneous temperature: $F_{1,135}$ = 22.6, P < 0.001; two-way ANOVA; Figure 8). Moreover, HR and cutaneous temperature results after the combination were significantly different when compared with those of the injection of CGP20712 or WB4101 alone (CGP20712–MAP: $F_{1,135}$ = 1.8, P > 0.05; HR: $F_{1,135}$ = 4.6, P < 0.05; cutaneous temperature: $F_{1,135}$ = 2.3, P < 0.05 and WB4101–MAP: $F_{1,135}$ = 1.6, P > 0.05; HR: $F_{1,135}$ = 31.6, P < 0.001; cutaneous temperature: $F_{1,135}$ = 26.9, P < 0.001).

Discussion

The present study investigated the involvement of the noradrenergic neurotransmission in the BNST in the expression of behavioural and autonomic responses induced by conditioned contextual fear. We observed that intra-BNST injection of non-selective α- or β-adrenoceptor antagonists attenuated freezing and autonomic responses induced by aversive context. Our results also showed that α_1- and β_1-adrenoceptors were specifically involved in these responses. These results extend previous findings suggesting the involvement of the BNST in the contextual fear paradigm (Sullivan *et al.*, 2004; Resstel *et al.*, 2008); and, to our knowledge, this study is the first to provide evidence on the role of α_1- and β_1-adreneceptors within the BNST in the expression of conditioned contextual fear.

Conditioned fear to context is evoked when re-exposing the animal to a context that has been previously paired with an aversive or unpleasant stimulus such as an electrical footshock (Fanselow, 1980; Antoniadis and McDonald, 1999). Animals subjected to this model show freezing behaviour and autonomic changes such as an increase in MAP and HR and a decrease in the cutaneous temperature (Blanchard and Blanchard, 1969; Carrive, 2000; Vianna and Carrive, 2005). We also observed that conditioned animals showed significant behavioural response (freezing), a marked increase in HR and MAP and a decrease in the cutaneous temperature, which remained stable throughout re-exposure to the context

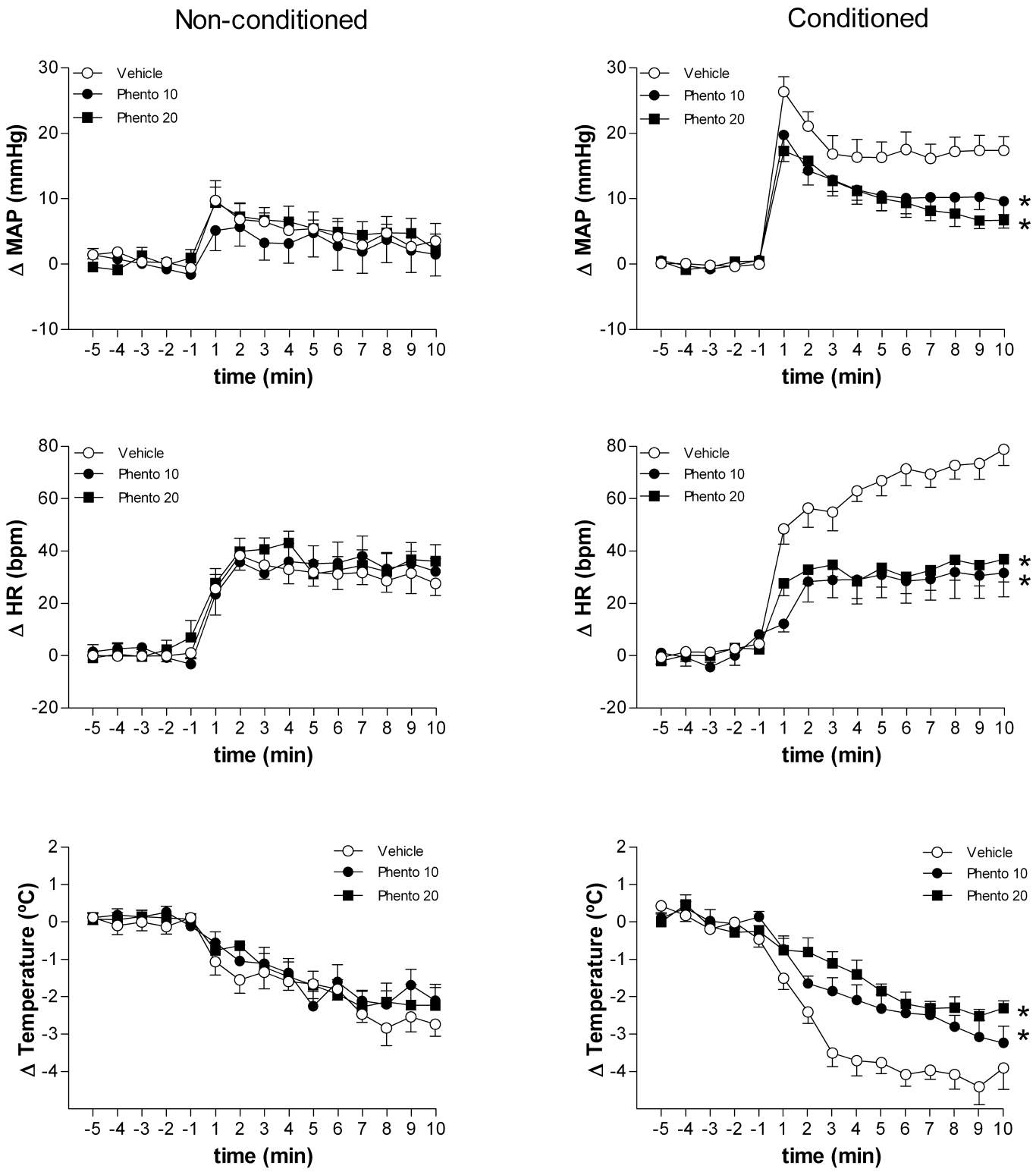

Figure 5

Time course of bilateral injection of vehicle or phentolamine (Phento; 10 and 20 nmol) into the BNST on Δ MAP and Δ HR increases and Δ Temperature decrease in non-conditioned (n = 5–7 per group) and conditioned animals (n = 8 per group). Data shown are means ± SEM. *P < 0.05 over the whole footshock chamber exposure period compared with vehicle-treated animals, Bonferroni's *post hoc* test.

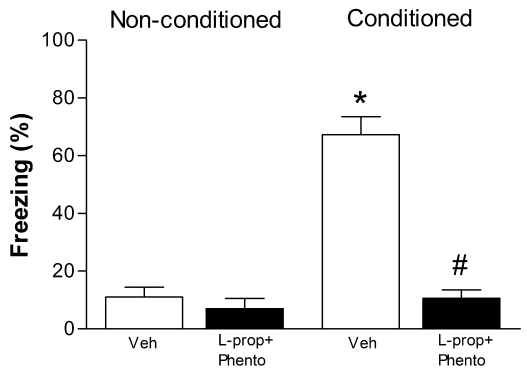

Figure 6

Effects of bilateral injection of saline (Veh) or phentolamine (Phento; 10 nmol) + L-propranolol (L-prop; 7.5 nmol) in non-conditioned (n = 5–6 per group) and conditioned animals (n = 6 per group) on the percentage of time spent in freezing behaviour. Data shown are means ± SEM. *P < 0.05 compared with vehicle non-conditioned group. $^{\#}P$ < 0.05 compared with vehicle conditioning group, Bonferroni's *post hoc* test.

that was previously paired with footshock. Furthermore, in non-conditioned animals, re-exposure to the chamber also increased the MAP and HR and decreased the cutaneous temperature. However, these changes were lower than those observed in conditioned vehicle-treated animals.

The observation that conditioned autonomic responses to the aversive conditioned context are associated with the conditioned freezing agrees with previous reports on behaviour-related autonomic responses (Carrive, 2000; Vianna and Carrive, 2005). Conditioned rats displayed prolonged freezing that consequently result in low locomotor activity. Longer freezing, reflecting increased conditioned fear of the context, was associated with activation of the sympathetic nervous system (McCarty and Kopin, 1978; Carrive, 2002). Non-conditioned animals showed significantly less freezing response than rats conditioned to the context, resulting in an increased locomotor activity associated with a concurrent increase of cardiovascular output. Somatomotor activity is positively correlated with MAP and HR, so that a greater locomotor activity leads to a higher cardiovascular response (Yancey and Overton, 1993; Nijsen *et al.*, 1998). Consequently, the autonomic responses observed in conditioned rats are mainly due to an increased sympathetic activation due to fear of the context (Carrive, 2002), while the autonomic responses of non-conditioned rats may derive from a combined lesser fear of the context and a greater activity-mediated increase of cardiovascular output. Nevertheless, our results support the contention that MAP, HR and tail cutaneous temperature are useful indices to evaluate the intensity of the response to a conditioned contextual fear stimulus, in agreement with previous papers.

Regarding the cutaneous temperature, we observed that re-exposure to aversive context induced a fall in the tail temperature down to room temperature (–3–4°C from baseline). Indeed, it has been reported that rats subjected to fear conditioning show an elevation in the body temperature and a marked decreases in the cutaneous temperature of the tail which is attributed to strong cutaneous vasoconstriction (Vianna and Carrive, 2005). In fact, the fear response includes a preparatory response to a more active reaction such as fight or flight and, as pointed out by Blessing (2003), vasoconstriction of the skin could be a protective mechanism to reduce blood loss in case of injury. Thus, the drop in the tail temperature observed by us may reflect a reduction in the tail blood flow caused by a cutaneous vasoconstriction.

Interestingly, behavioural and autonomic changes induced by the re-exposure to the aversive context were attenuated by the injection of L-propranolol or phentolamine, a non-selective β- and α-adrenoceptor antagonist, respectively, into the BNST, in conditioned animals. However, after individual treatments with these drugs the behaviour and autonomic responses were only partly reduced. Therefore, we investigated if the combination of L-propranolol and phentolamine would potentiate the attenuation of responses induced by the conditioned contextual fear. Although the combination reduced both autonomic and behavioural responses, the synergistic effect was only evident on the attenuation of the behavioural response. Additionally, the drug combination was more effective to attenuate changes observed in the cutaneous temperature when compared with the treatment with phentolamine alone. Concerning non-conditioned animals, as expected, no effect was observed in behavioural and autonomic responses after the administration of either L-propranolol or phentolamine. These data support an involvement of the BNST noradrenergic neurotransmission in the modulation of CER evoked by conditioned contextual fear. Based on these data, we performed additional experiments with selective adrenoceptor antagonists to evaluate which subtype of adrenoceptors was involved in these responses. For that, we used equipotent doses of selective α- and β-adrenoceptor antagonists based on the ratio of K_i values between non-selective and selective drugs (Miralles *et al.*, 1993; Bylund *et al.*, 1994). In addition to behavioural and cardiovascular responses, variations in cutaneous temperature were also recorded in the experiments using selective adrenoceptor antagonists.

As observed after L-propranolol or phentolamine, we also observed that the selective β_1-adrenoceptor antagonist CGP20712 and the selective α_1-adrenoceptor antagonist WB4101 when injected into the BNST also reduced the freezing time and the cardiovascular responses induced by conditioned contextual fear. Additionally, both CGP20712 and WB4101 attenuated the decrease in the tail temperature induced by aversive context. On the other hand, neither the selective β_2-adrenoceptor antagonist ICI118,551 nor the selective α_2-adrenoceptor antagonist RX821002 caused any effect, further reinforcing the idea that effects of both L-propranolol and phentolamine were mediated by respectively an antagonism of β_1- and α_1-adrenoceptors in the BNST. We also investigated if the combination of CGP20712 and WB4101 could potentiate the effect when compared with the treatment with these drugs alone and found that the combination was more effective in attenuating the increase in HR, compared with CGP20712 and WB4101 given individually, indicating a possible synergism between β_1- and α_1-adrenoceptors in the BNST for this effect. However, the combination did not potentiate the effects on the freezing time, the MAP and the cutaneous temperature that were induced by the treatments given separately. Although the

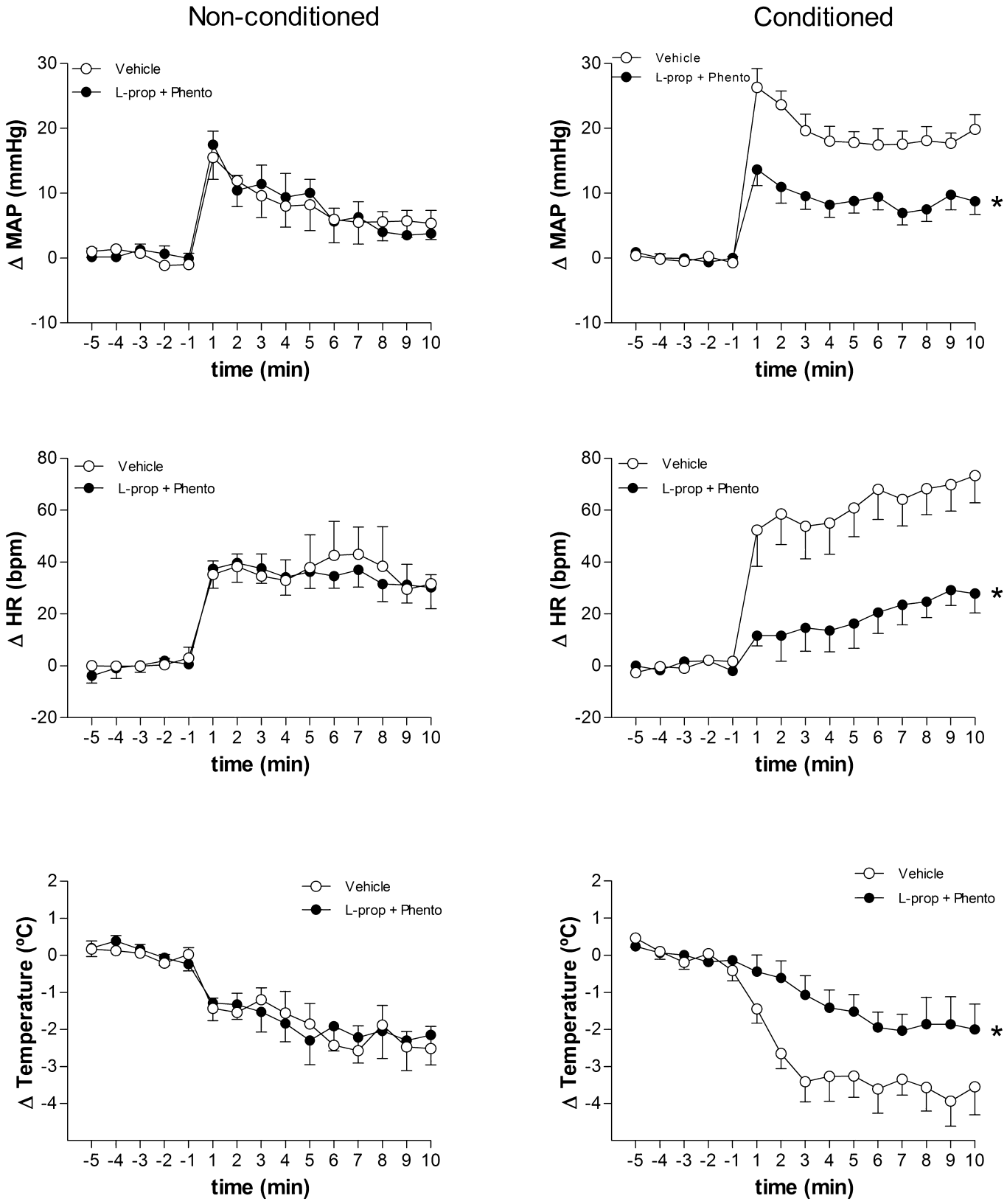

Figure 7

Time course of bilateral injection of saline (Veh) or phentolamine (Phento; 10 nmol) + L-propranolol (L-prop-; 7.5 nmol) on Δ MAP and Δ HR increases and Δ Temperature decrease in non-conditioned ($n = 5$–6 per group) and conditioned animals ($n = 6$ per group). Data shown are means ± SEM. *$P < 0.05$ over the whole footshock chamber exposure period compared to vehicle-treated animals, Bonferroni's *post hoc* test.

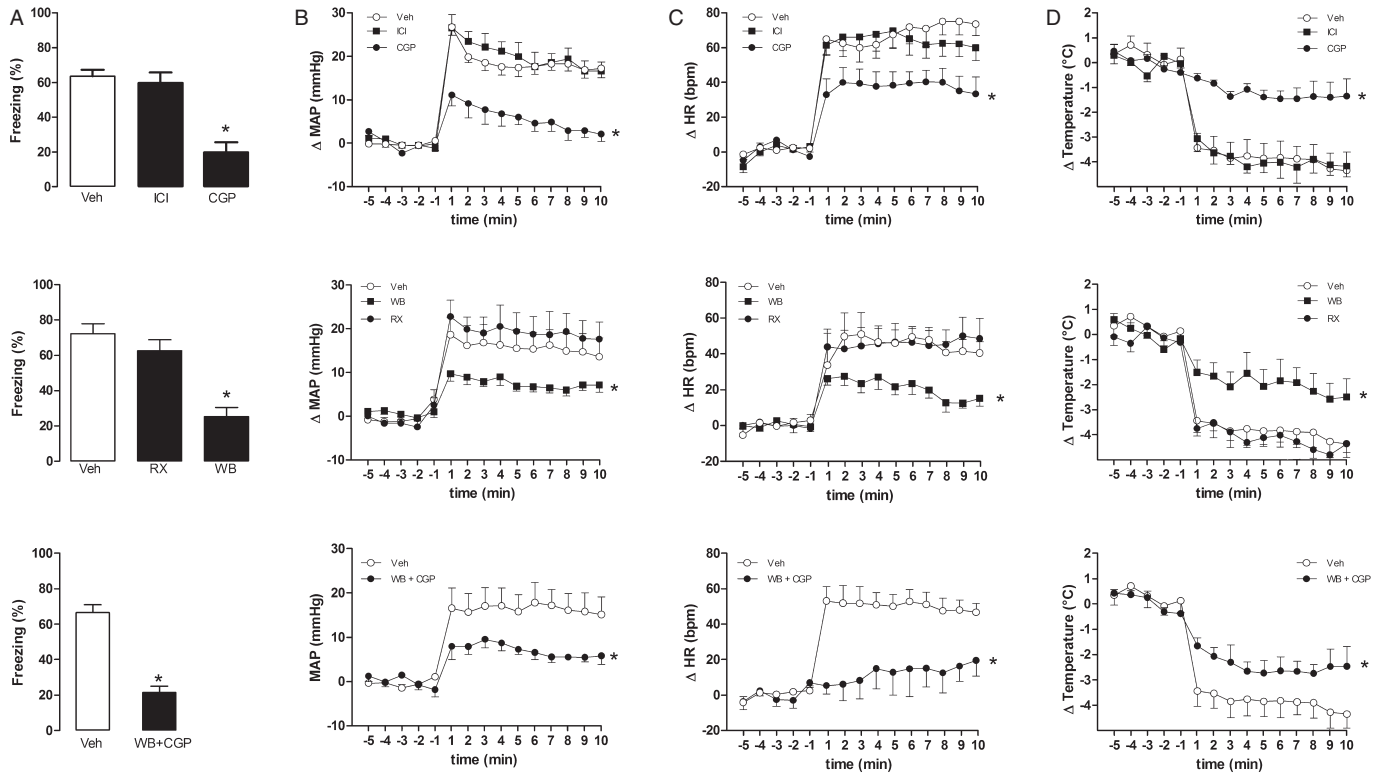

Figure 8

(A) Effects of bilateral injection of saline (Veh), CGP20712 (CGP, 4.5 nmol) or ICI118,551 (ICI, 34.5 nmol); saline (Veh), WB4101 (WB, 1.7 nmol) or RX821002 (RX, 2.3 nmol); and saline (Veh) or WB4101 (1.7 nmol) + CGP20712 (CGP, 4.5 nmol) in conditioned animals (n = 5-7/group) on the percentage of time spent in freezing behavior. Data shown are means ± SEM.. *P < 0.05 compared with vehicle conditioning group, Bonferroni's post hoc test. (B) Time course of mean arterial pressure (Δ MAP), (C) heart rate (Δ HR) and (D) cutaneous temperature (Δ Temperature) changes in conditioned animals. Data shown are means ± SEM.. *P < 0.05 over the whole footshock chamber exposure period compared to vehicle-treated animals, Bonferroni's post hoc test.

reasons for these results are not clear, we cannot rule out the possibility that CGP20712 and WB4101 alone produced a ceiling effect on the attenuation of freezing and MAP and that the BNST noradrenergic neurotransmission could have a direct influence on the autonomic nervous system, which is segregated from behavioural responses.

It has been previously shown that a noradrenergic neurotransmission in the BNST is involved in the cardiovascular control. The BNST sends direct projections to medullary structures with autonomic activity, such as the nucleus of the solitary tract (NTS), dorsal motor nucleus of the vagus, nucleus ambiguous and the ventrolateral medulla (Dong and Swanson, 2004). Furthermore, ablation of the caudal ventrolateral medulla (CVLM) attenuated MAP and HR decreases elicited by a BNST 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). A previous study from our group showed that α_1-adrenoceptors in the BNST exert a tonic influence on the parasympathetic component of the baroreflex in rats, in a similar manner to that observed during aversive situations, thus suggesting that an activation of BNST α_1-adrenoceptors could facilitate cardiovascular responses during the re-exposure to aversive context. The BNST modulation of baroreflex activity is similar to that observed during aversive situations (Crestani *et al.*, 2008a), thus suggesting that the BNST influence on the autonomic responses to contextual fear could depend on the baroreflex modulation by BNST α_1-adrenoceptors. However, in contrast to our results, Crestani *et al.* (2009) observed that the microinjection of WB14041 enhanced HR increase without affecting the blood pressure increase evoked by acute restraint stress, whereas RX821002 or propranolol did not affect restraint stress-related cardiovascular responses. Furthermore, we observed that treatment of the BNST with adrenoceptor antagonists did not induce any significant change in baseline values of both MAP and HR, in agreement with other reports indicating that the BNST noradrenergic neurotransmission is not involved in the tonic maintenance of cardiovascular responses (Crestani *et al.*, 2008b; 2009; Alves *et al.*, 2011). Therefore, although the noradrenergic neurotransmission within BNST is involved in cardiovascular control, the attenuation of the cardiovascular responses to aversive context could not depend only on direct cardiovascular effects but also on the attenuation of the emotional response.

A deregulation of noradrenergic transmission has been implicated in neuropsychiatric disorders such as depression and anxiety (Ressler and Nemeroff, 2000). Corroborating our findings, adrenoceptor antagonists reduce anxiety and fear.

For example, systemic administration of propranolol reduced contextual fear in rodents and humans (Grillon *et al.*, 2004; Ouyang and Thomas, 2005). Moreover, data from clinical studies have demonstrated that prazosin, a α_1-adrenoceptor antagonist, alleviated symptoms of post-traumatic stress disorder (Peskind *et al.*, 2003). Thus, our results suggest that the BNST could be involved in the effects of adrenoceptor antagonists after systemic administration, on fear conditioning. Furthermore, other brain structures that are directly connected with the BNST also seems to be involved in the adrenoceptor antagonists effects on fear conditioning, such as hippocampus, amygdala and medial prefrontal cortex (Lazzaro *et al.*, 2010; Do-Monte *et al.*, 2010a,b; Murchison *et al.*, 2011). Additionally, it is conceivable that adrenoceptors in the BNST could regulate the expression of contextual fear through the central nucleus of the amygdala (CeA), because this structure receives marked innervation from the BNST (Dong *et al.*, 2001a), and it is implicated in fear conditioning (Zimmerman *et al.*, 2007). Although the BNST and CeA are anatomically and neurochemically related and possess several functional similarities, Davis and co-workers have suggested a different role of the BNST and CeA in 'fear' versus 'anxiety' (Walker *et al.*, 2003; Davis *et al.*, 2010). From a biological view, 'anxiety' refers to an increased alertness due to a potential threat and needs cognitive appraisal, whereas 'fear' is elicited by an explicit threat (MacNaughton and Corr, 2004). There is much experimental evidence that the BNST is essential for sustained and potential threats (e.g. conditioned contextual fear), which are followed by long-duration responses, whereas the BNST is not necessary for short-duration responses that occur in response to threats with a clear offset (e.g. conditioned response to short-duration acoustic or luminous stimuli) (Walker *et al.*, 2003; Davis *et al.*, 2010). Based on such evidence, a role of the BNST has been proposed in anxiety, as opposed to fear, with a major involvement of the amygdala (Walker *et al.*, 2003; Davis *et al.*, 2010).

Several studies show a marked release of noradrenaline in the BNST induced by aversive situations, such as restraint stress (Pacak *et al.*, 1995; Cecchi *et al.*, 2002), opiate withdrawal (Fuentealba *et al.*, 2000), somatic and visceral pain-induce aversion (Deyama *et al.*, 2008; 2009) and exposure to predator odour (Fendt *et al.*, 2005). There is also considerable evidence that noradrenergic inputs to the BNST modulate the expression of neuroendocrine responses associated with stress, particularly those related to the activation of the hypothalamo–pituitary–adrenal axis (HPA) (Forray and Gysling, 2004). Cecchi *et al.* (2002) observed that pretreatment of the BNST with a α_1-adrenoceptor antagonist, but not with a mixture of β_1- and β_2-adrenoceptor antagonists, attenuated stress-induced rises in adrenocorticotropic hormone (ACTH), whereas both treatments were able to reduce the stress-induced anxiety-like behaviours on the elevated plus-maze (Cecchi *et al.*, 2002). Moreover, the infusion of the non-selective β-adrenoceptor antagonist L-propranolol or the selective β_1-adrenoceptor antagonist betaxolol into the BNST attenuated the morphine withdrawal-induced conditioned place aversion (Aston-Jones *et al.*, 1999; Delfs *et al.*, 2000; Cecchi *et al.*, 2007). In addition, whereas we did not observe effects with the injection of β_2-adrenoceptor antagonist ICI118,551, Deyama *et al.* (2008) showed that this drug microinjected into the BNST was able to attenuate the pain-induced aversion, suggesting the involvement of the BNST β_2-adrenoceptors in the negative affective component of pain.

Interestingly, several findings suggest that noradrenergic neurotransmission in the BNST exerts an inhibitory effect upon glutamate release through the activation of both α_1- and α_2-adrenoceptors (Forray *et al.*, 1997; Egli *et al.*, 2005; McElligott and Winder, 2008), whereas the β_1-adrenoceptor activation enhances the BNST glutamatergic neurotransmission (Nobis *et al.*, 2011), suggesting that these receptors could induce opposing effects on BNST glutamate release. On the other hand, Dumont and Williams (2004) observed that noradrenaline depolarized BNST GABAergic neurons to trigger an increase in the GABAergic inhibition during the acute opiate withdrawal, through α_1- and β-adrenoceptors. Although Dumont and Williams (2004) did not distinguish between β_1- and β_2-adrenoceptors, a similar mechanism could contribute to the aversive responses induced by conditioned contextual fear and to explain our results, thus suggesting that α_1- and β_1-adreneceptors are specifically involved in the conditioned contextual fear. Indeed, Radley *et al.* (2009) found that a selective ablation of GABAergic neurons in the BNST produced exaggerated emotional stress-induced HPA responses. Thus, it is possible to suggest an interaction between BNST noradrenergic neurotransmission and others neurotransmitters in the expression of conditioned contextual fear. However, this possibility remains to be tested.

In conclusion, the results of the present study showed that noradrenergic neurotransmission in the BNST, specifically the α_1- and β_1-adrenoceptors, are involved in the expression of responses induced by conditioned contextual fear.

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Statement of interest

None.

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