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Blockade of central vasopressin receptors reduces the cardiovascular response to acute stress in freely moving rats

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Abstract

To investigate the contribution of central vasopressin receptors to blood pressure (BP) and heart rate (HR) response to stress we injected non-peptide selective V_{1a} (SR49059), V_{1b} (SSR149415), V_2 (SR121463) receptor antagonists, diazepam or vehicle in the lateral cerebral ventricle of conscious freely moving rats stressed by blowing air on their heads for 2 min. Cardiovascular effects of stress were evaluated by analyzing maximum increase of BP and HR (MAX), latency of maximum response (LAT), integral under BP and HR curve ($\int$), duration of their recovery and spectral parameters of BP and HR indicative of increased sympathetic outflow (LF_{BP} and LF/HF_{HR}). Moreover, the increase of serum corticosterone was measured. Exposure to air-jet stress induced simultaneous increase in BP and HR followed by gradual decline during recovery while LF_{BP} oscillation remained increased as well as serum corticosterone level. Rats pre-treated with vasopressin receptor antagonists were not sedated while diazepam induced sedation that persisted during exposure to stress. V_{1a}, V_{1b} and V_2 receptor antagonists applied separately did not modify basal values of cardiovascular parameters but prevented the increase in $\int_{BP}$. In addition, V_{1b} and V_2 receptor antagonists reduced BP_{MAX} whereas V_{1a}, V_{1b} antagonist and diazepam reduced HR_{MAX} induced by exposure to air-jet stress. All drugs shortened the recovery period, prevented the increase of LF_{BP} without affecting the increase in serum corticosterone levels. Results indicate that vasopressin receptors located within the central nervous system mediate, in part, the cardiovascular response to air-jet stress without affecting either the neuroendocrine component or inducing sedation. They support the view that the V_{1b} receptor antagonist may be of potential therapeutic value in reducing arterial pressure induced by stress-related disorders.

Keywords: V_{1a}; V_{1b}; V_2; Vasopressin; Stress; Blood pressure

1. Introduction

Growing evidence from experimental and clinical studies indicates that chronic psychological stress contributes to development and progression of cardiovascular disease (Rozanski et al., 1999, 2005). Mechanisms such as excessive adrenergic activation, endothelial dysfunction, hypercorticosteronaemia and ovarian dysfunction have been invoked (Rozanski et al., 1999, 2005). Furthermore, acute stress was reported to trigger myocardial ischemia, promote arrhythmogenesis and fatal outcome in coronary disease (Mittleman et al., 1995). However, little is known about the central mechanisms that mediate the cardiovascular responses to psychological stress.

In the brain, several neuropeptides play a key role in the regulation of neuroendocrine and behavioural response to stress. In particular, corticotropin-releasing factor (CRF) and vasopressin (VP), originating from the hypothalamic paraventricular nucleus (PVN), were found to control adrenocorticotropin (ACTH) release. Polymorphism in the promoter structure of the VP gene and VP receptor genes has been associated with genetic susceptibility to stress and

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psychopathology (Landgraf, 2006), and central vasopressin contributes to cardiovascular regulation (Berecek and Swords, 1990; Okada et al., 2002). However, the role of central vasopressin receptors in the autonomic adjustment of the cardiovascular system to psychological stress is poorly understood.

Vasopressin exerts its effects by stimulation of three types of membrane bound G protein linked receptors: V_{1a}, V_{1b} and V_2. They have been cloned and characterized in terms of their primary structure, gene localization, mRNA distribution and pharmacology (Ostrowski et al., 1992, 1994; Hirasawa et al., 1994; Hernando et al., 2001; Thibonnier et al., 2001; Serradeil-Le Gal et al., 2005). Most of the central effects of VP have been attributed to central V_{1a} receptors, while V_{1b} receptors have been mainly implicated in the neuroendocrine response to stress, including ACTH release (Griebel et al., 2002; Landgraf, 2006). Recently, V_2 receptor mRNA was also found in the brain of adult rats and related to antinociception (Yang et al., 2007).

The aim of the present study was to investigate the contribution of central vasopressin V_{1a}, V_{1b} and V_2 receptors in the mediation of the cardiovascular response to acute psychological stress in conscious freely moving rats. Preliminary results of this study were reported at the ESGCO Conference in Jena (Stojičić et al., 2006) and the Focused Meeting on New Developments in Stress Physiology – From Gene to Man in Bristol (Japundžić-Žigon et al., 2006).

2. Methods

All experimental procedures in this study conformed to European Communities Council Directive of 24 November 1986 (86/609/EEC) and the School of Medicine, University of Belgrade Guidelines on Animal Experimentation.

2.1. Animals

Outbred male Wistar rats (imported from the University of Bristol's animal facility and bred at the animal facility of the Institute of Pharmacology, School of Medicine, University of Belgrade) weighing 330 ± 10 g were used. Rats were housed individually in Plexiglas cages ($25 \times 25 \times 25$ cm) with food pellets (Veterinarski Zavod, Subotica, Serbia) and tap water ad libitum, in controlled laboratory conditions (temperature 21 ± 1 °C, relative humidity 60 ± 5%, and 12:12 h light:dark cycle). The number of animals per experimental group was calculated according to the variability of the parameters in the control group of rats, using statistical software "Power Sample Size Calculation" available at http://biostat.mc.vanderbilt.edu/twiki/bin/view/Main/PowerSampleSize, for power of 90% and type I error probability of 0.05. At the end of experiment rats were euthanized by an overdose of thiopentone sodium (200 mg/kg, i.p.).

2.2. Surgery

Rats were submitted to two surgical interventions 5 days apart. The first surgery was performed under ketamine xylazine anesthesia (0.4 ml 10% ketamine i.p. plus 0.1 ml 2% xylazine i.p. per animal). The rat was mounted in a stereotaxic frame and a guide cannula (G22) was positioned 4 mm beneath the skull in the right lateral cerebral ventricle (at AP = −1.08 mm from Bregma and LAT = 1.5 mm from the midline) and fixed with dental cement. The skin above was sutured and sprayed with antibiotics (neomycin plus bacitracyn) and the guide was plugged with a stainless steel pin.

A second surgical procedure was performed under halothane anesthesia (4% for induction and 2% via mask) equilibrated by air, using Univentor 400 anesthesia unit (TSE Systems, Bad Homburg, Germany). A polyethylene catheter (PE, ED 0.90 mm, ID 0.58 mm) filled with heparinized (50 IU/ml) saline was inserted in the left femoral artery and tunneled subcutaneously to exit at the back of the neck. Postoperatively rats received one injection of penicillin (30 IU, i.m.) and of metamizol (200 mg/kg i.m.) for pain relief, and were allowed 48 h to recover before being submitted to different experimental protocols.

The position of the intracerebroventricular cannula was verified at the end of each experiment. Rats were anesthetized by sodium thiopentone (80 mg/kg, i.p.) and methylene blue (1 mg/ml) was then injected in a volume of 5 μl (i.c.v.) to verify access to the ventricle. Rats in which no blue coloration of the cerebral ventricles could be observed postmortem were excluded from the study.

2.3. Experimental protocol

Every day experiments were started at 11:00 A.M. in a quiet surrounding, 1 h after the rat has been connected to arterial catheter and intracerebroventricular needle catheter extensions to avoid direct animal manipulation during experimentation. The arterial line was then connected to a pressure transducer (Isotec™, Harvard Apparatus, HSE, Freiburg, Germany) for direct measurement of pulsating arterial blood pressure. For i.c.v injections the tip of the needle protruded 0.5 mm below the tip of the guide cannula into the lateral ventricle, and a needle was connected to a PE extension (ED 1.09 mm, ID 0.38 mm) and a 50 μl Hamilton syringe prefilled with drug solution. Rats were then submitted to different experimental protocols.

In a previous study (Milutinović et al., 2006), we have shown that the lowest dose of vasopressin injected i.c.v. that increased SBP was 50 ng/5 μl. Therefore, the in vivo efficiency of vasopressin receptor antagonists injected intracerebroventricularly in blocking the hypertensive effect of vasopressin (50 ng/5 μl, i.c.v.) was investigated in a separate study in conscious rats. We found that the V_{1a} antagonist prevented the hypertensive effect of vasopressin at both 100 ng/5 μl and 500 ng/5 μl, V_{1b} antagonist was only effective at 500 ng/5 μl, while the V_2 antagonist (100 ng/5 μl and 500 ng/5 μl, i.c.v.) did not modulate the SBP increase induced by vasopressin. Increasing the dose over 500 ng (i.c.v.) of vasopressin antagonists was not considered as it would interfere with functional selectivity.

Diazepam, a $GABA_A$ agonist, with established anxiolytic activity that reduces the cardiovascular response to acute stress in humans (Ashton, 1994), was employed as a positive control. The anxiolytic dose of diazepam (i.c.v.) in rats was established in a separate group of experiments ($n = 6$ each). Since there is no clear-cut separation between doses of benzodiazepines that produce anxiolysis and sedation (Atack, 2005), we employed the dose of diazepam that produced motor sedation in rats. We found that 100 ng of diazepam (i.c.v.) produced loss of spontaneous exploratory activity in four of the six treated rats while 500 ng of diazepam (i.c.v.) affected all six treated rats. This sedative effect lasted for 30 min following intracerebroventricular administration.

Six experimental protocols were planned. Protocol 1 investigated the effect of air-jet stress on cardiovascular and endocrine response of freely moving rats that received 5 μl of saline (i.c.v.). Based on a previously published protocol (Barrès et al., 2004), acute stress was initiated by blowing air (1 bar) for 2 min through a tube (ID 4 mm) from the top of the cage directed towards the top of the head of rat. In protocols 2, 3 and 4, we investigated the effect of air-jet stress on cardiovascular and endocrine response of rats under blockade of V_{1a} or V_{1b} or V_2 receptors. Both non-peptide and selective V_{1a}, V_{1b} and V_2 vasopressin receptor antagonists were injected into separate rats 3 min prior to stress at two dose levels, 100 ng (i.c.v.) and 500 ng (i.c.v.). Protocol 5 was a "positive control" in which rats were pre-treated with 100 ng and 500 ng of diazepam (i.c.v.) while protocol 6 was introduced to rule out the possible effect of DMSO on recorded parameters. Six rats were exposed to air-jet after being pre-treated with 5 μl of 10% (vol/vol) DMSO (i.c.v.). All protocols consisted of two recording sessions separated by a few minutes. The first session comprised a 15-min recording of baseline values of cardiovascular parameters. At the end of the first recording session, saline (5 μl, protocol 1), or V_{1a} receptor antagonist (100 ng/5 μl, 500 ng/5 μl, protocol 2) or V_{1b} receptor antagonist (100 ng/5 μl, 500 ng/5 μl, protocols 3) or V_2 receptor antagonist (100 ng/5 μl, 500 ng/5 μl, protocol 4) or diazepam (100 ng/5 μl, 500 ng/5 μl, protocols 5), or 10% (vol/vol) DMSO (5 μl, protocol 6), was injected intracerebroventricularly. Two minutes was allowed for the dispersion of the vehicle or

drug in the cerebrospinal fluid. Then, a second recording session was started. The effect of vehicle/drug was recorded for 3 min before exposure to air-jet stress was initiated. The second recording session was ended after complete normalization of BP and HR. In addition, one experimental group of rats ($n = 12$) exposed to air-jet stress received 500 ng/5 µl of V_{1a} antagonist intravenously (i.v.) or 5 µl 10% (vol/vol) DMSO (i.v.) (part of protocol 2).

2.4. Quantitative measurement of corticosterone in the serum of rats

In consideration of the circadian rhythmicity of corticosterone release, blood samples were collected at the same time of the day, around 12:00 h. Blood could not be retrieved immediately after air-jet exposure since loss of even small volumes of blood may interfere with cardiovascular short-term variability (Japundžić-Žigon, 1998). Therefore, blood (1 ml) was collected from the arterial femoral catheter after the end of the second BP recording session. Time delay between air-jet exposure and blood sample retrieval corresponded to the duration of the recovery of BP and HR. This was not considered significant since the endocrine response to stress is slower than that mediated neurally. Blood samples were centrifuged at 2600 rpm at +4 °C for 10 min and stored at −20 °C. Serum corticosterone concentration was assessed by Coat-A-Count Rat Corticosterone solid-phase ^{125}I radioimmunoassay (Siemens, Germany).

2.5. Cardiovascular signal processing and analysis

Arterial blood pressure was recorded using an Isotec™ transducer and TAM A amplifier (Harvard Apparatus, Freiburg, Germany) digitized at 1000 Hz and via an interface (BNC2110, National Instruments, UnoLux, Belgrade) relayed to a PC equipped with a custom written software for acquisition and spectral analysis. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were derived from the arterial blood pressure. After linear interpolation at 20 Hz, SBP, DBP and HR were analyzed in the time and frequency domain. To evaluate effect of stress on the cardiovascular system five parameters were considered: (1) maximal increase in SBP, DBP and HR (SBP_{MAX}, DBP_{MAX} and HR_{MAX}); (2) latency of the maximal increase of SBP, DBP and HR (LAT_{SBP}, LAT_{DBP} and LAT_{HR}); (3) the integral under SBP, DBP and HR curves ($\int_{SBP}$, $\int_{DBP}$ and $\int_{HR}$) during exposure to air-jet stress; (4) duration of the recovery period when SBP, DBP and HR returned to basal values (before stress); and (5) the low-frequency (LF) oscillation of SBP, DBP (LF_{SBP} and LF_{DBP}) spectra and low-frequency to high-frequency spectral ratio of HR (LF/HR_{HR}); the latter was indicative of alterations in the sympathovagal balance to the heart. These cardiovascular parameters are illustrated in Fig. 1.

Time spectral analysis was performed using a fast Fourier transform (FFT) algorithm on 10 overlapping 1024 point time series each (51.4 s at 20 Hz sampling rate) involving in total 205-s registration period. BP and HR segments for FFT analysis were subjected to nine-point Hanning window and linear trend removal. Spectra were analyzed up to 3 Hz. The sum of the moduli under the volume (modulus vs. time) for 10 FFT segments was calculated for the whole spectrum (total volume, TV: 0.0195–3 Hz), in three frequency ranges: very low frequency (VLF: 0.0195–0.195 Hz), low frequency (LF: 0.195–0.8 Hz) and high frequency (HF: 0.8–3 Hz), as well as LF/HF index for the HR spectrum. Frequency boundaries were chosen on the basis of visual detection of frequency peaks and clustering frequencies: in the VLF range 0.1 Hz peak is associated with the spontaneous arterial myogenic activity (Janssen, 1998), the LF range (0.35 Hz peak) is related to the sympathetic activity and the HF range of 1.2 Hz peak is coupled to respiration (Japundžić et al., 1990).

2.6. Drugs

Both non-peptide and selective vasopressin V_{1a} (SR49059), V_{1b} (SSR149415) and V_2 (SR121463) receptor antagonists were donated by Dr Claudine Serradeil-Le Gal from Exploratory Research Department of Sanofi Synthélabo (Toulouse, France). Diazepam was donated by Dr Aleksandar Mitić from ICN Galenika (Belgrade, Serbia) and halothane by Jugoremedia (Belgrade, Serbia). Ketamine and xylazine were purchased from Richter Pharma (Wels, Austria) and Ceva Santé Animal (Budapest, Hungary), respectively. Metamizol and penicillin were purchased from Hemofarm (Vršac, Serbia). V_{1a} (SR49059) and V_{1b} (SSR149415) receptor antagonists were dissolved in 10% (vol/vol) DMSO and 5% (vol/vol) DMSO, respectively. The V_2 (SR121463) receptor antagonist and diazepam were dissolved in pyrogen free saline.

2.7. Statistics

Results are expressed as mean ± standard error of the mean. Statistical significance in the same experimental group was assessed using repeated measures one-way ANOVA followed by a *post hoc* Bonferroni test while statistical significance between groups was evaluated using *t* test for unpaired observations, in GraphPad Prism 4 software (GraphPad Software Inc., San Diego, CA, USA). Statistical significance was considered at $p < 0.05$.

3. Results

3.1. Cardiovascular response to air-jet stress in freely moving rats

All rats ($n = 6$ in each group) pre-treated with 5 µl of saline (i.c.v.) or 5 µl of 10% (vol/vol) DMSO (i.c.v. or i.v.) exposed to 2 min air-jet exhibited a complex behavioural response involving emotional (fear), motor (startle, escape) and autonomic components (micturition, defecation). This was accompanied by immediate and simultaneous rise in SBP, DBP and HR (Fig. 1A, B). The maximum SBP (LAT_{SBP}), DBP (LAT_{DBP}) and HR (LAT_{HR}) responses were reached at the same time, about the middle of the exposure time (Figs. 1A, B and 2C–6C). The intensity of the cardiovascular response was also documented by the increase of the integral under SBP and DBP curve ($\int_{SBP}$, $\int_{DBP}$; Figs. 1A–6A) and HR curve ($\int_{HR}$; Figs. 1B and 2A–6A). After cessation of exposure to the air-jet SBP, DBP (Figs. 1A and 2D–6D) and HR (Figs. 1B and 2D–6D) recovered simultaneously in about 5 min. The recovery of SBP and DBP was accompanied by an increase in LF_{SBP} and LF_{DBP} oscillation (Figs. 1A and 2E–6E) while no change in LF/HF_{HR} (Figs. 1B and 2E–6E) or any other component of SBP, DBP and HR spectra, was observed. In the sera of stressed rats, a significant increase of corticosterone concentration was detected (Fig. 7).

3.2. Air-jet stress after blockade of central V_{1a} receptors

Pre-treatment of rats with the V_{1a} receptor antagonist (100 ng and 500 ng, i.c.v.) did not affect basal values of cardiovascular parameters (Fig. 2) or spontaneous exploratory activity. All rats exposed to air-jet stress exhibited typical behavioural response described previously except that the cardiovascular response was modified: the increase in $\int_{SBP}$ and $\int_{DBP}$ (Fig. 2A) was abolished; the HR_{MAX} increase was reduced at 500 ng of the drug (Fig. 2B); the recovery period of SBP, DBP and HR was shortened (Fig. 2D) and the increase in LF_{SBP} and LF_{DBP} (Fig. 2E) was prevented. No change in LF/HF_{HR} (Fig. 2E) or any other component of SBP, DBP and HR spectra was noted. The air-jet evoked increase in serum corticosterone was similar to that seen in non-treated rats (Fig. 7).

V_{1a} antagonist (500 ng) applied intravenously prior to stress did not modify basal BP, HR of rats and had a minor effect on cardiovascular parameters during exposure to the air-jet and recovery time: it prevented only the increase of $\int_{SBP}$, $\int_{DBP}$ (Fig. 3A) and LF_{SBP} and LF_{DBP} oscillation (Fig. 3E), respectively.

3.3. Air-jet stress after blockade of central V_{1b} receptors

The V_{1b} antagonist (100 ng and 500 ng, i.c.v.) neither modified basal values of BP and HR (Fig. 4), nor did it produce motor sedation as all rats exhibited a typical behavioural response (startle, escape, micturition and defecation) during exposure to air-jet stress. However, at both doses the V_{1b} receptor antagonist prevented the air-jet stress evoked increase of $\int_{SBP}$ and $\int_{DBP}$ (Figs. 1C, 4A), reduced SBP_{MAX} and DBP_{MAX} (Fig. 1C, 4B), and at 500 ng (i.c.v.), it reduced the HR_{MAX} response (Figs. 1D, 4B). At 100 ng and 500 ng V_{1b} receptor antagonist shortened the recovery period of SBP, DBP and HR (Fig. 4D) and inhibited the appearance of sympathetically mediated LF_{SBP} and LF_{DBP} (Fig. 4E) oscillation. In rats pre-treated with the V_{1b} receptor antagonist no change in other frequency component of SBP, DBP and HR spectrum was noted. The corticosterone response of rats pre-treated with V_{1b} antagonist and exposed to air-jet was not modified (Fig. 7).

3.4. Air-jet stress after blockade of central V_2 receptors

The V_2 receptor antagonist (100 ng and 500 ng, i.c.v.) did not influence basal values of cardiovascular parameters (Fig. 5) nor did it produce motor sedation so rats exposed to air-jet stress exhibited typical emotional, motor and autonomic reaction to air-jet exposure. In contrast, the V_2 receptor antagonist prevented the increase of $\int_{SBP}$ and $\int_{DBP}$ (Fig. 5A) and at 100 ng reduced SBP_{MAX} and DBP_{MAX} (Fig. 5B). At both doses V_2 receptor antagonist shortened the recovery period of SBP, DBP and HR (Fig. 5D) and inhibited the increase of LF_{SBP} and LF_{DBP} oscillation (Fig. 5E). V_2 receptor antagonist did not modulate LF/HF_{HR} (Fig. 5E) or any other component of BP and HR spectra. Fig. 7 illustrates that V_2 antagonist fails to modify the serum corticosterone response to stress.

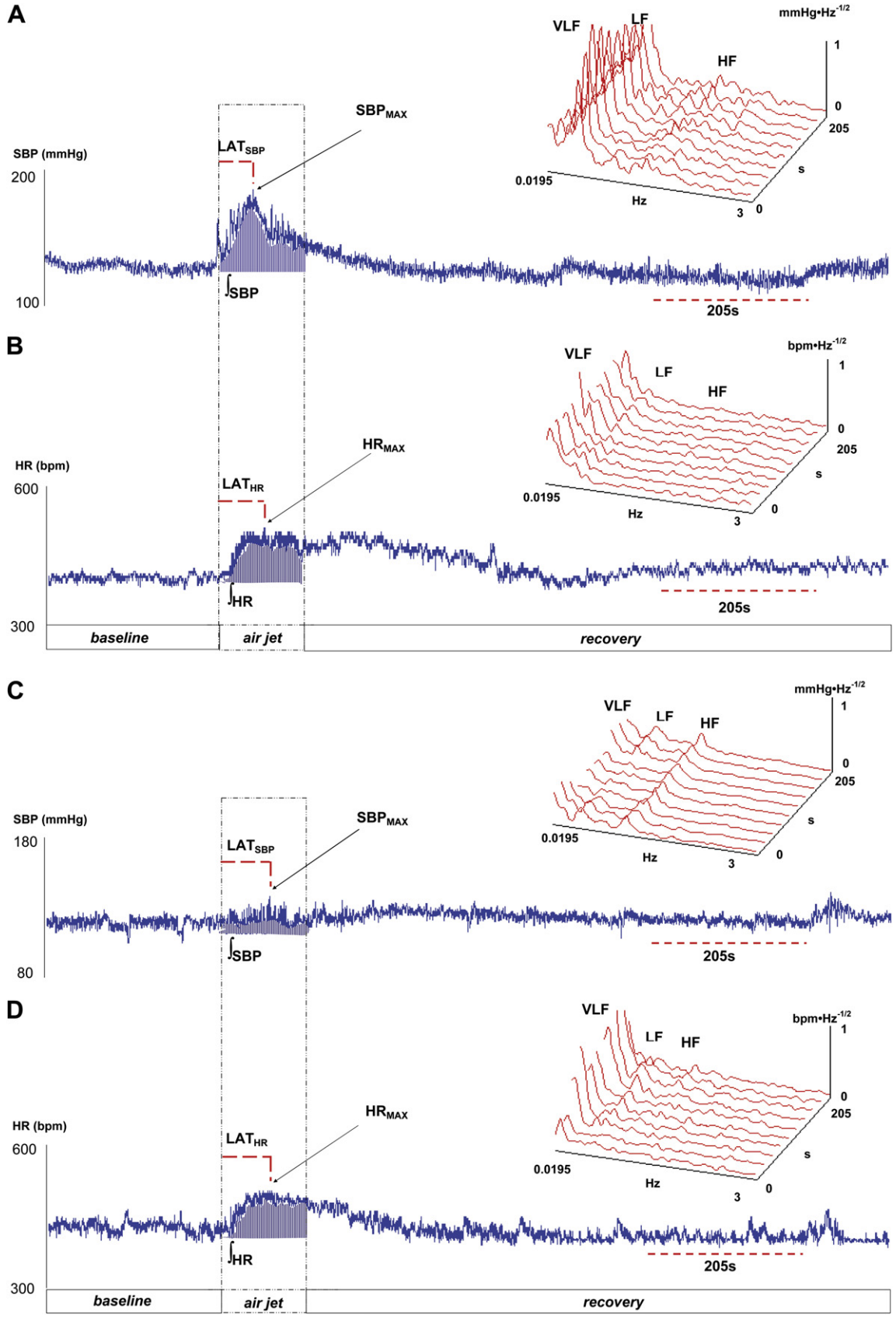
3.5. Air-jet stress after diazepam

Diazepam applied to rats before stress at 100 ng and 500 ng (i.c.v.) induced loss of spontaneous exploratory activity but did not affect basal values of BP and HR (Fig. 6A, B). During exposure to air-jet all rats remained sedated without exhibiting startle, escape, micturition and defecation. Diazepam pre-treatment, at both doses, significantly reduced the HR_{MAX} response (Fig. 6B), shortened the recovery period of SBP, DBP and HR (Fig. 6D) and prevented the appearance of LF_{SBP} and LF_{DBP} (Fig. 6E) oscillation. The HR variability response of rats pre-treated with diazepam and exposed to air-jet stress was comparable to the response of non-treated rats (Fig. 6E). In diazepam pre-treated rats, the increase of serum corticosterone was similar to the increase measured in rats without treatment (Fig. 7).

4. Discussion

The main finding of this study is that central vasopressin (V_{1a}, V_{1b} and V_2) receptors are involved in mediating the cardiovascular response to air-jet stress that accompanies complex behavioural reaction. During exposure to air-jet stress, pre-treatment of rats with vasopressin V_{1a}, V_{1b} and V_2 receptor antagonists (i.c.v.) reduced the intensity of BP response (Figs. 2A, B–5A, B) and, at higher doses of the V_{1a} and V_{1b} receptor antagonist, also reduced the HR_{MAX} response (Figs. 2B and 4B). In addition, V_{1a}, V_{1b} and V_2 receptor antagonists shortened the duration of the recovery period of both BP and HR (Figs. 2D, 4D and 5D) and prevented the appearance of a sympathetically mediated LF_{BP} oscillation (Figs. 2E, 4E and 5E). The results also indicate that vasopressin receptor antagonists neither produce sedation nor modulate serum corticosterone increase (Fig. 7).

It is well known that stressful challenges elicit specific patterned activation of central pathways leading to a characteristic autonomic and neuroendocrine pattern of response that is peculiar to the stressor. Air-jet stress is a model of acute environmental stress associated with complex behavioural reaction (emotional, motor and autonomic), vigorous cardiovascular response and ACTH release (Engelmann et al., 1996; Barrès et al., 2004). In our experiments, air-jet induced a fear-like response associated with immediate and simultaneous increase of BP and HR as well as serum corticosterone levels. Enhanced sympathetic outflow to the blood vessels was still observed after cessation of exposure to air-jet stress as indicated by the increase of LF_{BP} fast oscillation. The fast oscillations of BP and HR reflect autonomic adjustment of the cardiovascular system to internal and external perturbations (Japundžić-Žigon, 1998). The low-frequency (LF) oscillations of HR reflect both cardiac sympathetic and vagal outflow whereas respiratory related high-frequency (HF) oscillation of HR was found to reflect vagal control of the heart rate. Hence, it is generally accepted that LF/HF_{HR} may serve as an index of cardiac sympathovagal balance (Parati et al., 1995). Although we failed to detect changes in the LF/HF_{HR} index we did observe an increase in LF_{BP} variability associated with vascular sympathetic outflow (Japundžić et al., 1990). LF_{BP} increase associated with acute air-jet stress was suggested to be due to the feed-forward increase in sympathetic activity (Barrès et al., 2004). In our experiments the LF_{BP} increase was prevented by vasopressin receptor antagonists and diazepam, suggesting that although differential mechanism are involved, they all reduce air-jet stress evoked increase in central sympathetic outflow to blood vessels. Similarly, in a model of acute stress in rats induced by immobilization, we found that sympathetically mediated increase in LF_{BP} were accompanied by increase in HF_{BP} oscillations that involved central vasopressin mechanisms (Milutinović et al., 2006). However, exposure to air-jet in rats was associated with abrupt rise of BP and HR as well as a strong motor response, both of which preclude a spectral analysis. Only during recovery, when the animals were stationary, we were able to perform a spectral analysis of BP and HR but even then we did not observe an increase in HF_{BP} oscillation.


A
SBP (mmHg)
200
100
SBP_MAX
LAT_SBP
∫SBP
205s
VLF
LF
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mmHg•Hz^-1/2
1
0
205
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HR (bpm)
600
300
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LAT_HR
∫HR
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baseline
air jet
recovery
C
SBP (mmHg)
180
80
SBP_MAX
LAT_SBP
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recovery

Based on electrophysiological, pharmacological, central neural pathway tracing and early gene expression studies, several brainstem and hypothalamic regions mediating the behavioural and neuroendocrine response to air-jet stress have been determined (Morin et al., 2001; Dampney and Horiuchi, 2003; McDougall et al., 2005). These include: the nucleus of the solitary tract (NTS), caudal (CVLM) and rostral ventrolateral medulla (RVLM), periaqueductal gray matter (PAG), raphe pallidus (RPa) in the midbrain, lateral hypothalamic region, dorsomedial hypothalamic nucleus (DMH) and paraventricular nucleus (PVN) in the hypothalamus. Labelling vasopressin mRNA receptor distribution and using immunocytochemistry approaches, in most of these areas V_{1a} and V_{1b} receptors were found: the cortex, the hypothalamic supraoptic nucleus (SON) and PVN, PAG, the limbic system, and lower brainstem areas such as, NTS, RVLM and spinal cord intermediolateral cell column associated with cardiovascular regulation (Ostrowski et al., 1992, 1994; Hirasawa et al., 1994; Hernando et al., 2001). Recently, V_2 receptor mRNA was identified in PAG of adult rats and associated with antinociception (Yang et al., 2007). In the present experiment, i.c.v. injection of selective and non-peptide vasopressin V_{1a}, V_{1b} and V_2 receptor antagonists into conscious rats before exposure to air-jet did not affect BP and HR, in line with current knowledge (Berecek and Swords, 1990; Engelmann et al., 1996; Okada et al., 2002; Milutinović et al., 2006). However, they all effectively reduced the intensity of the BP response during exposure to air-jet stress and shortened both BP and HR recovery time. Bearing in mind that vasopressin receptor antagonists were injected in the lateral cerebral ventricle of rats, they most likely acted most at structures near the ventricular system, such as PVN and PAG. Moreover, the major target of DMH neuron projections is the parvocellular part of PVN (ter Horst and Luiten, 1986; Fontes et al., 2001), which is in turn a major source of direct hypothalamic projections to RVLM, NTS and spinal preganglionic neurons (Dampney and Horiuchi, 2003). PAG is also a relay nucleus for a number of descending fibers from DMH (da Silva et al., 2006), and PAG neurons directly project to RVLM (Wang and Lovick, 1993). Therefore, a possibility is that the V_{1a}, V_{1b} and V_2 receptor antagonists in our experiments acted, respectively, at V_{1a} and V_{1b} receptors in PVN and V_2 receptors in the PAG to suppress onward transmission to the RVLM and the spinal cord preganglionic neurons, thereby depressing the sympathetically mediated increase in BP. Furthermore, our results indicate that the effect of vasopressin antagonists on BP response induced by exposure to air-jet is specific and due to the selective blockade of vasopressin receptor subtypes since diazepam, $GABA_A$ agonist currently used to treat acute stress, had no effect on BP response during exposure to air-jet stress. Diazepam significantly reduced HR_{MAX} during exposure to air-jets, probably due to general depressant action on autonomic cardiovascular function. Nevertheless, diazepam and vasopressin receptor antagonists acted similarly in the recovery period, shortened HR and BP recovery time and prevented LF_{BP} increase, suggesting that diazepam may have acted to block $GABA_A$ receptors on RVLM projecting PVN neurons described by Zahner et al. (2007). Furthermore, a possibility is that a PVN vasopressinergic output relays via the RVLM and elevates levels of reactive oxygen species, e.g. superoxide, locally. Superoxide in the RVLM was found to underlie the pressor response evoked by emotional stress in rabbits (Mayorov, 2007). Moreover, stimulation of V_1 receptors was found to increase superoxide levels in arteries (Li et al., 2003) which could be a potential source of superoxide in RVLM that acts in paracrine fashion to stimulate the sympathoexcitatory neurons.

We accept that we cannot exclude a role for V_{1a} and V_{1b} receptors within limbic structures involved in mediating the emotional component to air-jet stress. We did not measure this but since behaviour may not always convey emotional changes we cannot rule out this possibility. For instance, intraseptal application of the V_{1b} antagonist (SSR149415) has been shown to induce antidepressive behaviour (Griebel et al., 2002; Serradeil-Le Gal et al., 2005; Stemmelin et al., 2005), while down regulation of V_{1a} receptors in the septum was also reported to reduce anxiety (Landgraf et al., 1995). Recently Antunes et al. (2006) have reported that spinal V_{1a} receptors mediate the sympathoexcitatory response to acute salt loading. Therefore the V_{1a} receptor antagonists in our study could have also acted at the spinal level to block V_{1a} receptors and contribute to the reduction of sympathetic outflow to blood vessels and BP. Moreover, we cannot totally reject the possibility that vascular V_{1a} receptors contribute, at least in part, to the BP increase during exposure to air-jet stress. V_{1a} receptor antagonist applied i.c.v. could leak in the systemic circulation to block vascular V_{1a} receptors. When we applied 500 ng of V_{1a} antagonist i.v., it had a minor effect on BP changes supporting central rather than peripheral action, in line with the finding of Dobruch et al. (2005).

An interesting finding is that pre-treatment of rats with diazepam or any of the vasopressin receptor antagonists, did not affect the endocrine response to air-jet stress as witnessed by the comparable increase in serum corticosterone in all experimental groups. As discussed previously, patterns and modes of vasopressin release during stress depend upon the type of stressors. Generally, in rodents, active coping strategy is

Fig. 1. Typical tracing of SBP and HR response of one freely moving rat exposed to air-jet stress without treatment and another freely moving rat pre-treated with V_{1b} receptor antagonist. Panels A and B compare air-jet stress evoked increase in SBP and HR, respectively, in a control rat. Note the gradual decline in these variables during recovery. Panels C and D show that the air-jet stress evoked cardiovascular response ($\int_{SBP}$, SBP_{MAX}, LF_{SBP} and HR_{MAX}) is attenuated significantly after pre-treatment with the V_{1b} receptor antagonist (500 ng, i.c.v.). Note also the shortening of the recovery period. In this and the following figures: SBP_{MAX} (maximum increase of SBP during exposure to air-jet); DBP_{MAX} (maximum increase of DBP during exposure to air-jet); LAT_{SBP} (latency of SBP maximum increase); LAT_{DBP} (latency of DBP maximum increase); $\int_{SBP}$ (integral under SBP curve during exposure to air-jet); $\int_{DBP}$ (integral under DBP curve during exposure to air-jet); HR_{MAX} (maximum increase of HR during exposure to air-jet); LAT_{HR} (latency of HR maximum increase); $\int_{HR}$ (integral under HR curve during exposure to air-jet); VLF (very low frequency); LF (low frequency) and HF (high frequency).

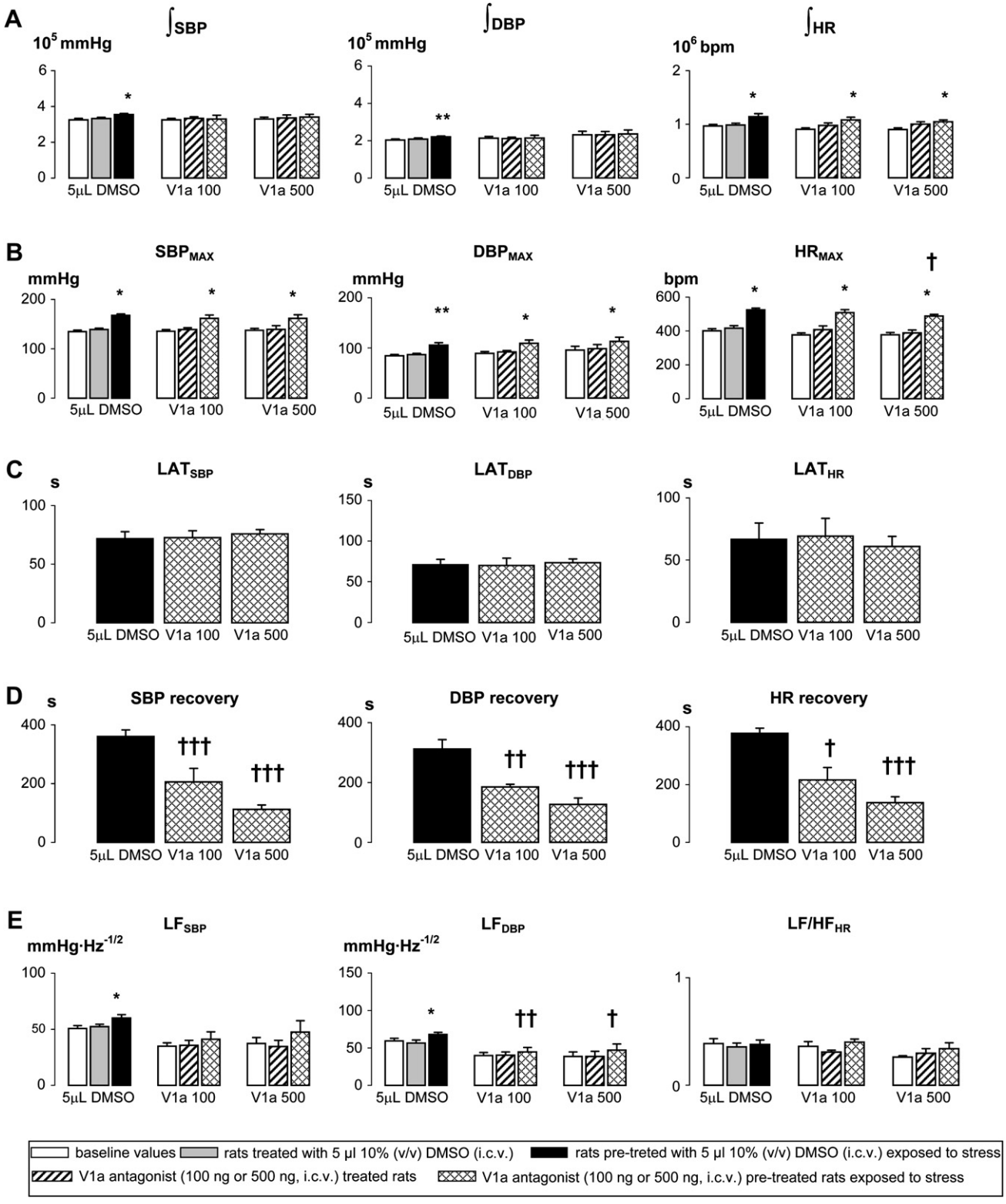

Fig. 2. V_{1a} receptor antagonism centrally reduces the cardiovascular response to air-jet stress. Panels A and B show that V_{1a} receptor antagonist (i.c.v.) does not modulate basal values of SBP, DBP and HR, but it prevents air-jet stress evoked increase in $\int_{SBP}$, $\int_{DBP}$ (A) and HR_{MAX} (at 500 ng, B) without modifying the latency of the maximal changes in SBP, DBP and HR (C). Moreover, V_{1a} receptor antagonist (i.c.v.) shortened the recovery period of SBP, DBP and HR (D) and prevents air-jet stress evoked increase in LF_{SBP} and LF_{DBP}. There was no change in the sympathovagal balance at the heart, as depicted in the unaffected LF/HF_{HR} oscillation (E). Each bar represents mean value of six experiments ± s.e.m. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. baseline value (open bar). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. DMSO plus air-jet stress (black bar).

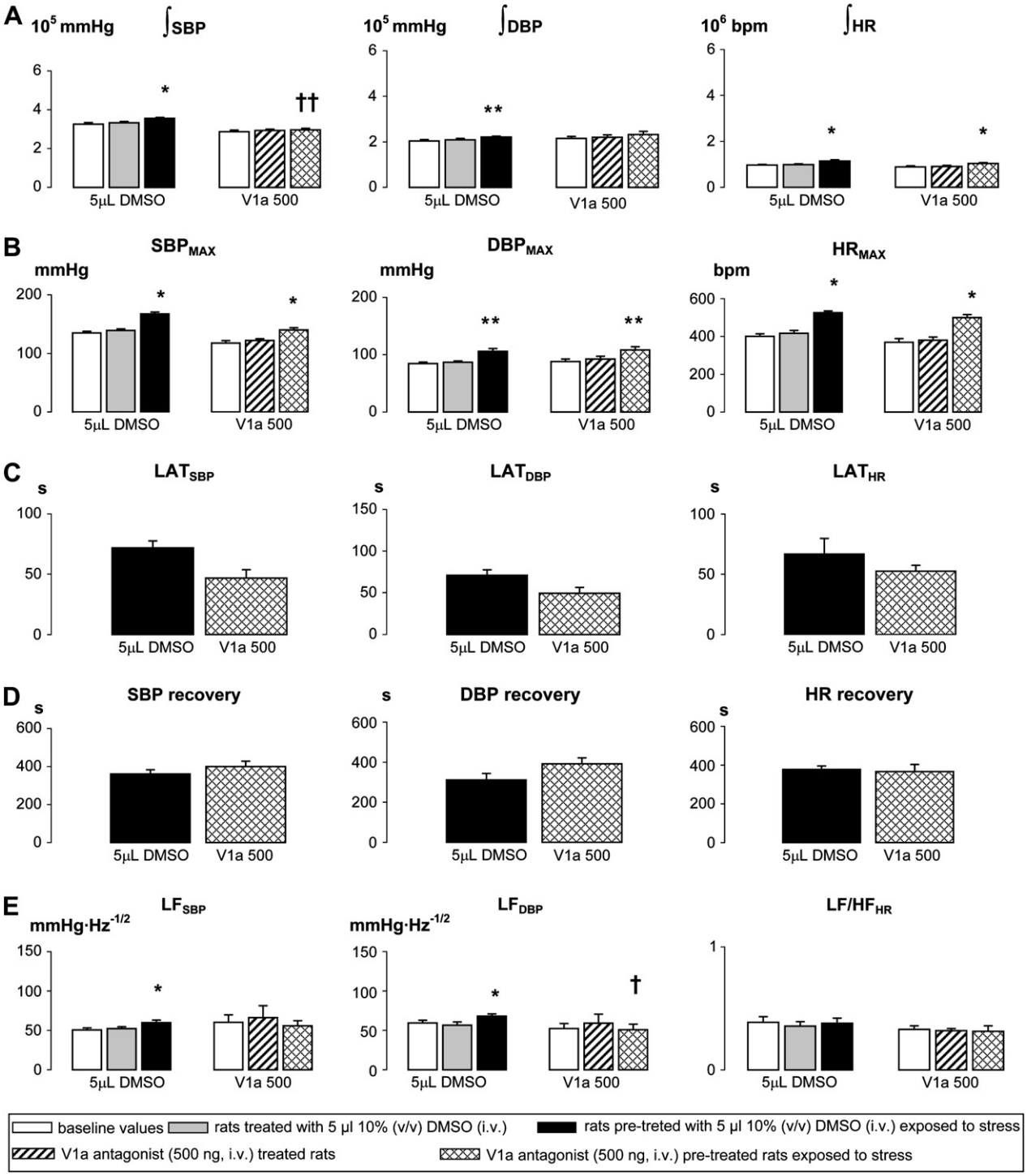

Fig. 3. Intravenous administration of V_{1a} receptor antagonist is less effective in reducing the cardiovascular response to air-jet stress. V_{1a} receptor antagonist (500 ng, i.v.) does not modulate basal values of SBP, DBP and HR (A, B). During exposure to air-jet stress the V_{1a} receptor antagonist prevents the increase in $\int_{SBP}$, $\int_{DBP}$ without affecting the $\int_{HR}$ (A), maximal response (B), latency (C) and the duration of recovery of SBP, DBP and HR (D). However, it prevented the sympathetically mediated increase in LF_{SBP} and LF_{DBP} oscillations evoked by air-jet stress without affecting the symapthovagal balance to the heart, as depicted by the unchanged LF/HF_{HR} oscillation response following V_{1a} receptor antagonist pre-treatment (E). Each bar represents mean value of six experiments ± s.e.m. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. baseline value (open bar). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$ vs. DMSO plus air-jet stress (black bar).

characterized by high locomotor activity, hyper-reactive sympathoadrenal system and hyporeactive hypothalamopituitary axes (Dampney and Horiuchi, 2003; McDougall et al., 2005). Acute environmental stress such as air-jet is known to activate the parvocellular part of the PVN to release CRH (Morin et al., 2001) in portal circulation and increase ACTH release in the systemic circulation. By measuring urine flow in rats, Hatton et al. (1991) showed that there is no increase of vasopressin release in the bloodstream of normotensive controls submitted to air-jet stress, and that this is even inhibited in borderline

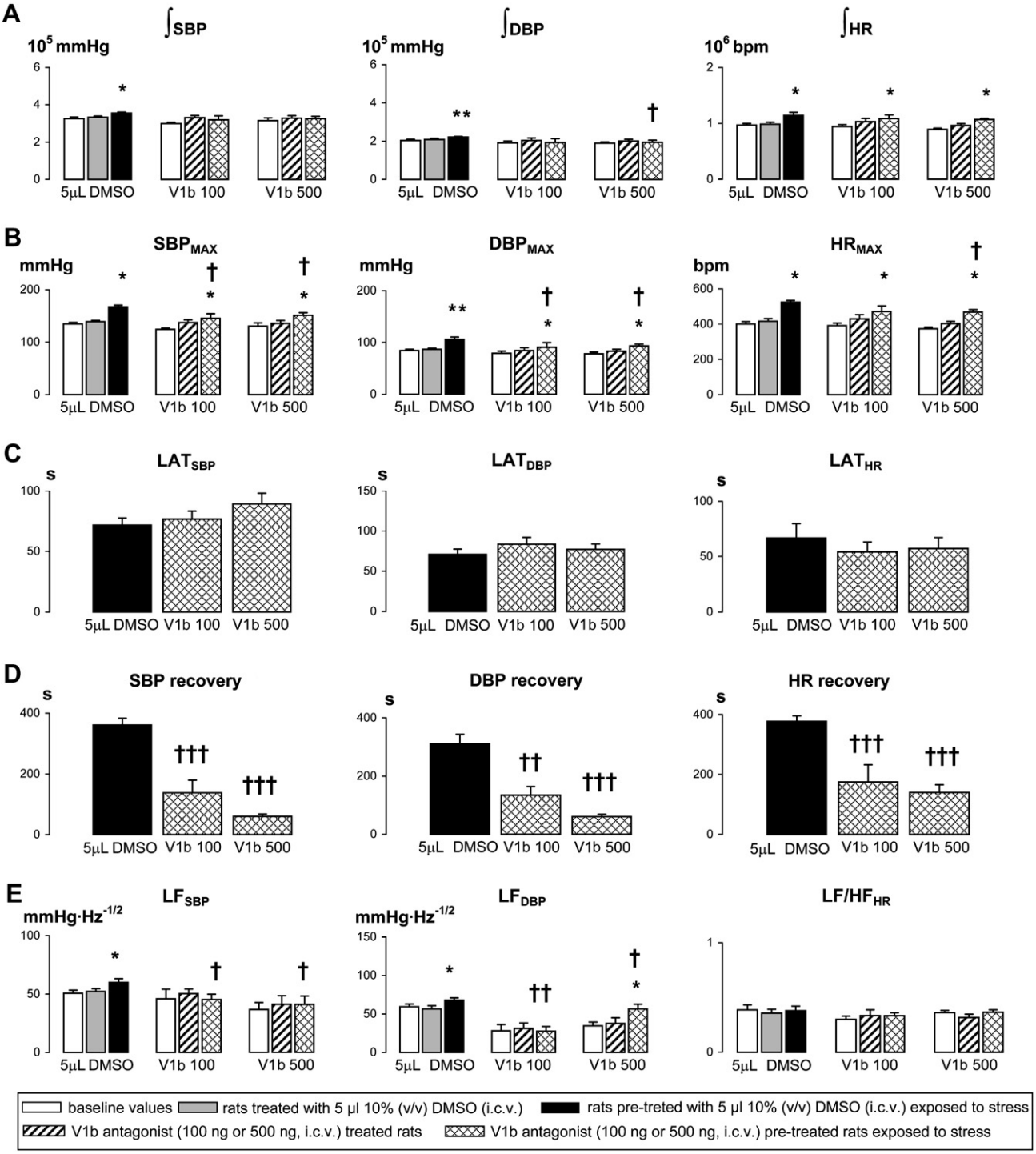

Fig. 4. V_{1b} receptor antagonism centrally reduces the cardiovascular response to air-jet stress. Panels A and B show that V_{1b} receptor antagonist (i.c.v.) does not modulate basal values of SBP, DBP and HR. During exposure to air-jet stress it prevents the increase in $\int_{SBP}$, $\int_{DBP}$ (A), reduces the SBP_{MAX}, DBP_{MAX} and HR_{MAX} responses (at 500 ng, B) but does not modify their latency (C). V_{1b} receptor antagonist shortened the recovery period of SBP, DBP and HR (D) and prevented the increase in LF_{SBP} and LF_{DBP} oscillations evoked by air-jet stress without affecting the symapthovagal balance at the level of the heart, as depicted in the unchanged LF/HF_{HR} oscillation (E). Each bar represents mean value of six experiments ± s.e.m.. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. baseline value (open bar). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. DMSO plus air-jet stress (black bar).

hypertensive rats. Other models of stress such as acute immobilization (Serradeil-Le Gal et al., 2005) and foot shock (Onaka et al., 1986) were found to increase vasopressin release in the systemic circulation. In the former, V_{1b} antagonist was found to reduce ACTH release in rats (Serradeil-Le Gal et al., 2005). However, in stress induced by forced swimming, in which vasopressin was not found to be released into the circulation (Wotjak et al., 1998), V_{1b} antagonist failed to modulate ACTH release (Ramos et al., 2006). Moreover, major modulation of ACTH release by vasopressin occurs during chronic

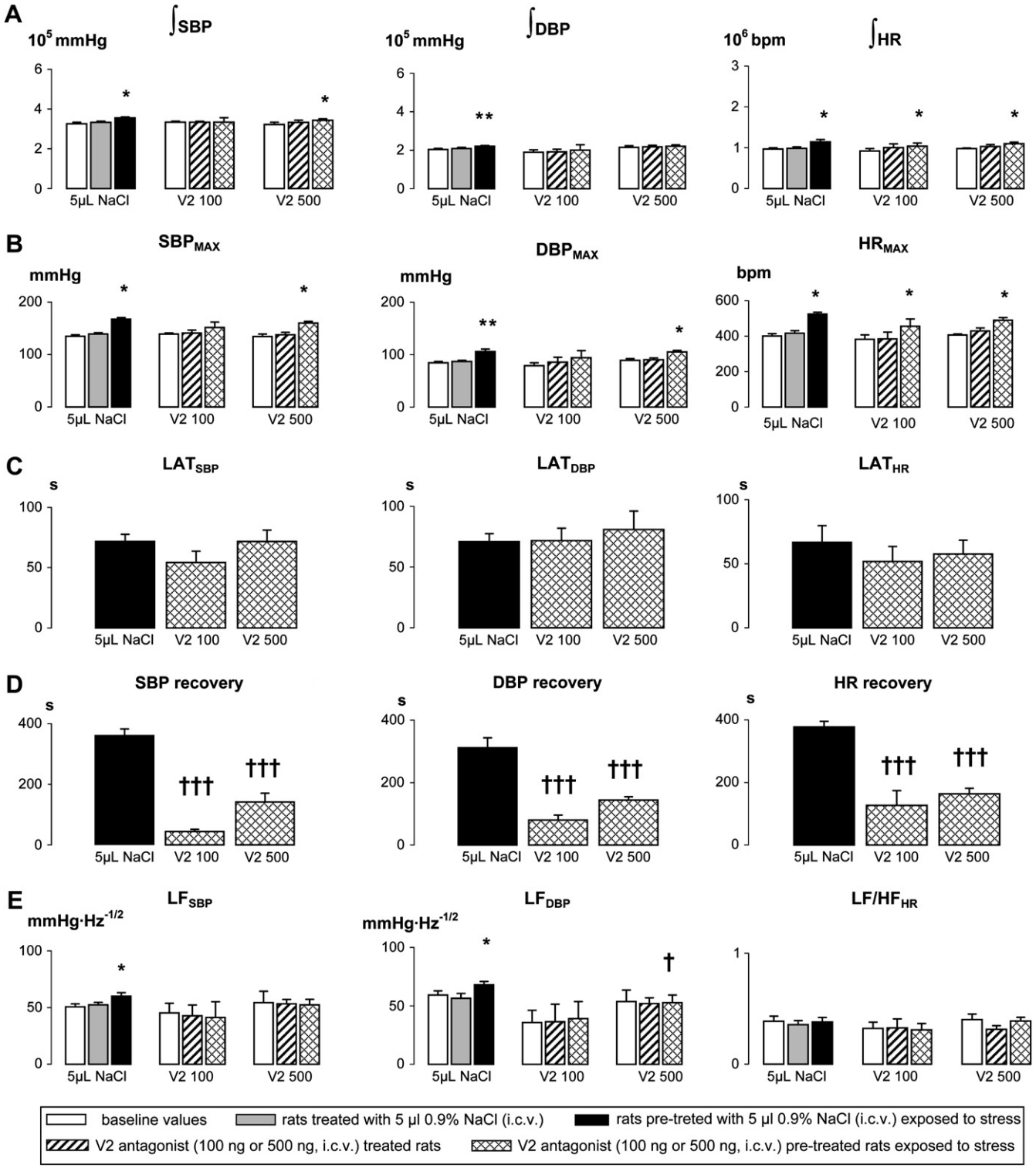

Fig. 5. V_2 receptor antagonism centrally reduces the cardiovascular response to air-jet stress. V_2 receptor antagonist applied i.c.v. does not modulate basal values of SBP, DBP and HR (A, B). However, during exposure to air-jet stress, it prevented the increase in $\int_{SBP}$, $\int_{DBP}$ without affecting $\int_{HR}$ (A), and reduced SBP_{MAX}, DBP_{MAX} (at 100 ng) without affecting the HR_{MAX} response (B). The V_2 receptor antagonist does not modify the latency of the maximal response of SBP, DBP and HR (C), but shortened their recovery period (D) and prevented the increase of LF_{SBP} and LF_{DBP} oscillations without affecting the symapthovagal balance to the heart, as revealed by an unaltered LF/HF_{HR} oscillation (E). Each bar represents mean value of six experiments ± s.e.m. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. baseline value (open bar). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. saline plus air-jet stress (black bar).

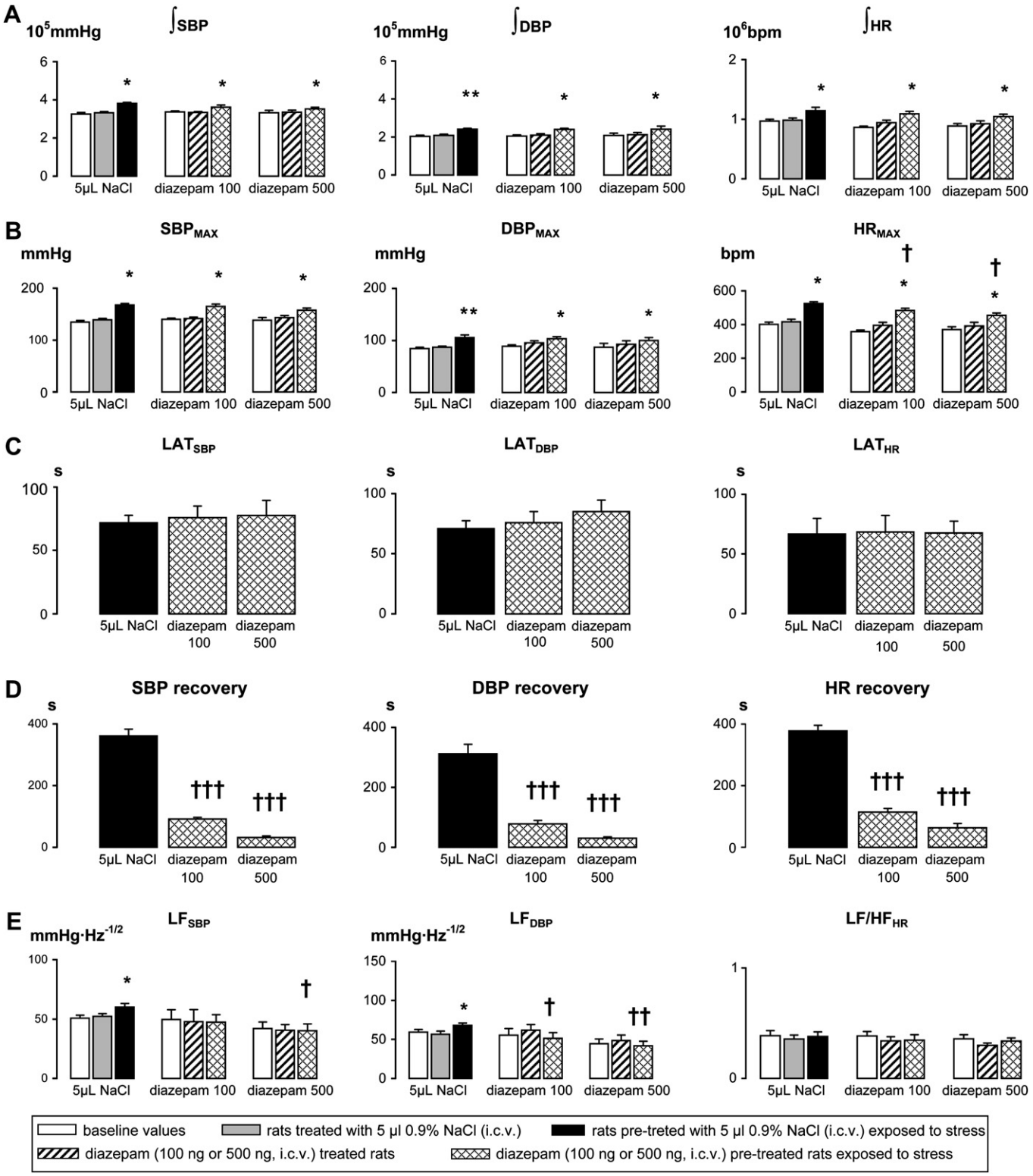

Fig. 6. Effects of air-jet stress on cardiovascular parameters in rats pre-treated with $GABA_A$ receptor agonist (i.c.v.). Diazepam applied i.c.v. does not modulate basal values of SBP, DBP and HR (A, B), but during exposure to air-jet stress, diazepam reduced HR_{MAX} (B) without modifying the latency of maximal response of SBP, DBP and HR (C). Diazepam also shortened the recovery period of SBP, DBP and HR (D) and prevented the increase in LF_{SBP} and LF_{DBP} oscillations without affecting the symapthovagal balance to the heart, as the LF/HF_{HR} oscillation was unaltered (E). Each bar represents mean value of six experiments ± s.e.m. $^{*}p < 0.05$, $^{**}p < 0.01$ vs. baseline value (open bar). $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, $^{\dagger\dagger\dagger}p < 0.001$ vs. saline plus air-jet stress (black bar).

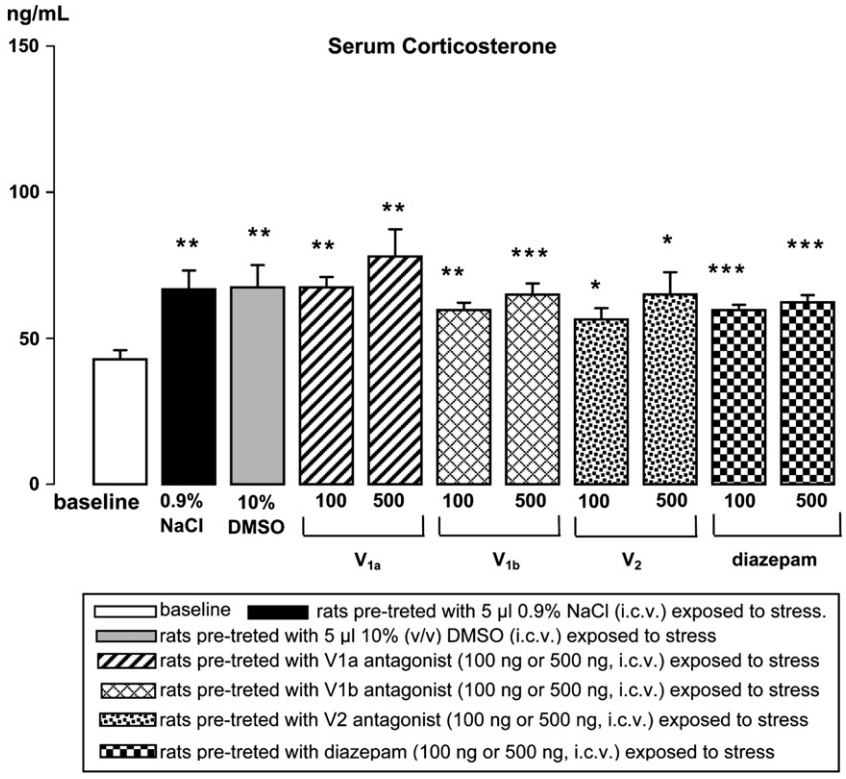

Fig. 7. Serum corticosterone concentration in rats exposed to air-jet stress without or with pre-treatment. Selective V_{1a}, V_{1b} and V_2 receptor antagonists and diazepam did not modify the air-jet stress evoked serum corticosterone response. Each bar represents mean value of six experiments ± s.e.m. $*p < 0.05$, $**p < 0.01$ $***p < 0.001$ vs. baseline value (open bar).

stress (Hauger and Aguilera, 1993). Therefore our finding that V_{1b} receptor antagonist does not modulate serum corticosterone increase in rats exposed to acute air-jet stress is in line with previous reports.

In conclusion, results of this study indicate that V_{1a}, V_{1b}, and V_2 vasopressin receptor antagonist attenuate the cardiovascular response to air-jet stress by acting at separate and/or common central structures and pathways without inducing sedation and without modulating corticosterone release. They also support the view that V_{1b} receptor antagonist may be of potential therapeutic value in reducing arterial pressure changes induced by stress-related disorders.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.neuropharm.2007.12.013.

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