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RESEARCH PAPER

Blockade of tachykinin NK_3 receptor reverses hypertension through a dopaminergic mechanism in the ventral tegmental area of spontaneously hypertensive rats

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BACKGROUND AND PURPOSE
Intracerebroventricularly injected tachykinin NK_3 receptor (R) antagonists normalize mean arterial blood pressure (MAP) in spontaneously hypertensive rats (SHR). This study was pursued to define the role played by NK_3R located on dopamine neurones of the ventral tegmental area (VTA) in the regulation of MAP in SHR.

EXPERIMENTAL APPROACH
SHR (16 weeks) were implanted permanently with i.c.v. and/or VTA guide cannulae. Experiments were conducted 24 h after catheterization of the abdominal aorta to measure MAP and heart rate (HR) in freely behaving rats. Cardiovascular responses to i.c.v. or VTA-injected NK_3R agonist (senktide) and antagonists (SB222200 and R-820) were measured before and after systemic administration of selective antagonists for D_1R (SCH23390), D_2R (raclopride) or non-selective D_2R (haloperidol), and after destruction of the VTA with ibotenic acid.

KEY RESULTS
I.c.v. or VTA-injected SB222200 and R-820 (500 pmol) evoked anti-hypertension, which was blocked by raclopride. Senktide (10, 25, 65 and 100 pmol) elicited greater increases of MAP and HR when injected in the VTA, and the cardiovascular response was blocked by R-820, SCH23390 and haloperidol. VTA-injected SB222200 prevented the pressor response to i.c.v. senktide, and vice versa, i.c.v. senktide prevented the anti-hypertension to VTA SB222200. Destruction of the VTA prevented the pressor response to i.c.v. senktide and the anti-hypertension to i.c.v. R-820.

CONCLUSIONS AND IMPLICATIONS
The NK_3R in the VTA is implicated in the maintenance of hypertension by increasing midbrain dopaminergic transmission in SHR. Hence, this receptor may represent a therapeutic target in the treatment of hypertension.

Abbreviations
aCSF, artificial cerebrospinal fluid; BP, blood pressure; DA, dopamine; DMSO, dimethylsulphoxide; HR, heart rate; MAP, mean arterial blood pressure; NKA, neurokinin A; NKB, neurokinin B; NK_1R, tachykinin NK_1 receptor; NK_2R, tachykinin NK_2 receptor; NK_3R, tachykinin NK_3 receptor; SHR, spontaneously hypertensive rats; SP, substance P; WKY, Wistar Kyoto; VTA, ventral tegmental area

Introduction

The ventral tegmental area (VTA), the origin of the mesolimbic and mesocortical (A10) dopaminergic pathways, is traditionally known for its implication in the regulation of locomotor activity, stress responses, reinforcement and reward mechanisms (Le Moal and Simon, 1991; Swanson *et al.*, 2000; Hyman and Malenka, 2001). The literature from the 1990s suggests a role for the VTA and the mesolimbic dopaminergic system in cardiovascular homeostasis in link with the defence reaction and the somatosensory input (Van den Buuse, 1998). The mesolimbic dopaminergic system is involved in diurnal blood pressure (BP) and heart rate (HR) regulation (Sei *et al.*, 1999; Sakata *et al.*, 2002). The discharge rate of dopaminergic neurones in the VTA seems to be regulated by inputs from arterial baroreceptors, probably through a relay in the nucleus tractus solitarius (Kirouac and Ciriello, 1997). The stimulation of the VTA is also associated with the modulation of the circulatory and pressor effects of vasopressin (Van den Buuse and Catanzariti, 2000). Electrical stimulation of the rat VTA or microinjection of a substance P (SP) analogue produces increases of BP and HR through the activation of dopamine D_1 and D_2 receptors (R), and vasopressin release (Cornish and Van den Buuse, 1995).

In a further recent study, the VTA was proposed as a potential target for tachykinin receptors in cardiovascular regulation (Deschamps and Couture, 2005). Increases of BP, HR and stereotypic behaviours are elicited by the intra-VTA injection of physiological doses (pmol) of tachykinin NK_1, NK_2 and NK_3 receptor agonists in freely moving rats. These responses are blocked in a selective manner by their respective antagonist and by systemic treatment with the D_1R antagonist, SCH23390, but not with the D_2R antagonist, raclopride. Furthermore, the cardiovascular response to tachykinin agonists is ascribed to enhanced cardiac output following the stimulation of cardiac β_1-adrenoceptors. This study is consistent with the increased firing rate of A10 dopamine (DA) cells induced by the application of NK_1, NK_2 and NK_3 receptor agonists in the VTA (Overton *et al.*, 1992), and the presence of NK_1 and NK_3 receptors on dopaminergic and non-dopaminergic neurones in the VTA as revealed by confocal and electron microscopy (Chen *et al.*, 1998; Lessard *et al.*, 2007; 2009). The VTA is rich in NK_3 binding sites (Dam *et al.*, 1990; Stoessl and Hill, 1990; Ding *et al.*, 1996; Langlois *et al.*, 2001) and is particularly sensitive to the NK_3R agonist senktide (Seabrook *et al.*, 1995; Deschamps and Couture, 2005). SP and senktide injected into the VTA enhance levels of DA and its metabolite dihydroxyphenylacetic acid, DA turnover in the prefrontal cortex and nucleus accumbens (Elliott *et al.*, 1986; Cador *et al.*, 1989; Overton *et al.*, 1992; Marco *et al.*, 1998), and behaviour consistent with mesolimbic DA activation (Eison *et al.*, 1982; Elliott and Iversen, 1986; Deschamps and Couture, 2005). SP, neurokinins A and B are found in the VTA (Deutch *et al.*, 1985; Kalivas *et al.*, 1985; Warden and Young, 1988), and SP immunoreactive axon terminals make direct synaptic contact with DA neurones in the VTA (Tamiya *et al.*, 1990). SP projecting fibres in the VTA originate from the nucleus accumbens and the habenular nucleus (Cuello *et al.*, 1978; Lu *et al.*, 1998).

In a recent study, we reported that i.c.v. injection of NK_3R antagonists reverses hypertension in spontaneously hypertensive rats (SHR) and does not affect resting blood pressure in Wistar-Kyoto rats (WKY) (Lessard *et al.*, 2004). Conversely, the inhibition of brain NK_1R and NK_2R fails to affect BP in SHR and WKY. Whereas these findings support a role for brain tachykinin NK_3R in the maintenance of hypertension in SHR, the underlying mechanism of the anti-hypertensive effect of NK_3R antagonists remains elusive. We showed that it persists after bilateral nephrectomy and is not accompanied by changes in plasma levels of vasopressin (Lessard *et al.*, 2004).

Based on all these revised findings, the VTA may represent a strategic site for the central anti-hypertensive effect of NK_3R antagonists in SHR. This is in keeping with the increased central DA activity, which contributes substantially to the hypertension in SHR (Van den Buuse, 1997; Amenta *et al.*, 2001). An up-regulation of D_1R and D_2R in the nucleus accumbens, one major neuronal projection of the VTA, was reported in young SHR (Kirouac and Ganguly, 1993; Vaughan *et al.*, 1999; Russell, 2003).

The present pharmacological study was undertaken to test the hypothesis that tachykinin NK_3R in the VTA contributes to the maintenance of high arterial BP by interacting with the mesocorticolimbic dopaminergic system in freely behaving SHR. This was achieved by determining the cardiovascular response to the selective NK_3R agonist (senktide) and antagonists (SB222200 and R-820) injected i.c.v., or into the VTA, before and after inhibition of D_1R and D_2R (with SCH23390 and raclopride, respectively) or after destruction of the VTA with ibotenic acid (IBO). Data suggest that tachykinin NK_3R located in the VTA is subjected to persistent tonic activation, which increases midbrain dopaminergic transmission and thereby contributes to hypertension in SHR.

Methods

Animal source and care

Male SHR (16 weeks) were purchased 1 week prior to experiments from Charles River (St. Constant, Quebec, Canada). They were housed two per cage under a 12 h light–dark cycle in a room with controlled temperature (22°C) and humidity (40%). Food (Charles River Rodent) and tap water were available *ad libitum*. The care of animals and research protocols conformed to the guiding principles for animal experimentation as enunciated by the Canadian Council on Animal Care and approved by the Animal Care Committee of our University.

Animal preparation

Before each surgery, rats received the antibiotics trimethoprim and sulphadiazine (Tribrissen 24%, 30 mg kg^{-1}, s.c., Schering Canada Inc., Pointe Claire, Quebec, Canada) and the analgesic ketoprophen (anafen, 10 mg kg^{-1}, s.c., Merial Canada Inc., Baie d'Urfé, Quebec, Canada). They were anaesthetized with isoflurane (3%) and then positioned in a stereotaxic apparatus (David Kopf Instrumentation, Tujunga, CA, USA) with the incisor bar set at 0.0 mm in the interaural line for i.c.v and at 3.3 mm below the interaural line for VTA implantation. The skull was exposed, cleaned and a hole was drilled to implant a 23-gauge stainless-steel guide cannula into the left (contralateral) or right (ipsilateral) lateral brain ventricle (coordinates: 0.6 mm posterior to the bregma, 1.3 mm lateral to the midline, 3.0 mm ventral from the skull surface), and/or 2 mm above the left or right VTA (coordinates: 5.3–5.6 mm posterior to the bregma, 0.7 mm lateral to the midline, 7.0 mm ventral from the skull surface) according to Paxinos and Watson (1998). The guide cannula was fixed with two screws and dental cement to the skull. Then the skin was replaced and sutured. Finally, a stylet (31-gauge stainless-steel) was inserted into the guide cannula to prevent loss of CSF and to avoid its obstruction. Animals were housed in individual plastic cages (40 × 23 × 20 cm) in the same controlled conditions and allowed to recover.

Three days later, the animals were re-anaesthetized with isoflurane and two siliconized PE-10 catheters (one PE-10 connected to PE-60), filled with physiological saline and 100 iu mL^{-1} heparin sodium salt, were inserted into the abdominal aorta through the left femoral artery for BP recording and into the left femoral vein for drug administration respectively. They were passed subcutaneously to emerge at the back of the neck and secured with tape. During the following days, the animals were observed closely. Those which lost more than 20% of their body weight or had clear signs of cerebral haemorrhage, atypical behaviour or weaknesses were killed with CO_2 inhalation. Experimental protocols were initiated 24–48 h after the last vascular surgery in conscious and freely moving rats (Deschamps and Couture, 2005). Successful VTA and i.c.v. implantation were confirmed in all rats used in the study after post-mortem examination.

Measurement of cardiovascular parameters

During all experiments, continuous direct recordings of arterial BP and HR were made on a high-performance data acquisition system PowerLab 8/30 with LabChart Pro ML870P and ML228 Bridge Amp (ADInstruments Inc, Colorado Springs, CO, USA). Mean arterial blood pressure (MAP) was calculated from systolic (S) and diastolic (D) blood pressure values [(S – D) ÷ 3 + D = MAP].

The cardiovascular response was measured 1 h after the rats were transported to an isolated and quiet testing room. Rats remained in their resident cage, but the top grid containing the food and tap water was removed prior to and up to 1 h post-injection. When BP and HR were stable, a 31-gauge stainless-steel injector connected to a 5 µL Hamilton microsyringe via a PE-10 tubing catheter was inserted into the guide cannula, which was accessible without handling the rat. Five minutes later, injection was made i.c.v. or directly into the VTA of undisturbed, freely moving rats. All solutions were freshly prepared and injected (volume of 1 µL i.c.v.; 0.5 µL VTA and 0.1 mL kg^{-1} i.v.) over a period of 1 min. The injector was removed from the guide cannula 1 min after injection to prevent any possible leakage of the injectate.

Experimental protocols

Cardiovascular effects of i.c.v and VTA administered tachykinin NK_3R antagonists. This series of experiments was aimed at determining the anti-hypertensive effect of centrally administered NK_3R antagonists (500 pmol). SHR were administered the selective tachykinin NK_3R antagonist i.c.v. (SB222200, n = 8) (Sarau *et al.*, 2000) 1 h after the vehicle [artificial cerebrospinal fluid (aCSF), n = 8]. The inactive enantiomer of SB222200 (SB222201, n = 10) was also injected as control for specificity in a separate group of SHR. The selection of this dose of SB222200 was based on a previous dose-response curve, which established that 500 pmol SB222200 i.c.v. caused the optimal anti-hypertensive effect in SHR (Lessard *et al.*, 2004). For comparison, SHR implanted with a VTA cannula were administered aCSF and 1 h later SB222200 (500 pmol, n = 5).

In two separate groups of SHR, R-820 (500 pmol) was injected either i.c.v. (n = 5) or directly into the VTA (n = 4), and the anti-hypertensive responses

were measured for up to 3 days or until they were back to baseline values. The dose and duration of recording were based on a previous i.c.v. study done in SHR (Lessard *et al.*, 2004).

Effects of systemic treatments with the D_1 and D_2 receptor antagonists against SB222200 and R-820. This series of experiments was designed to determine the contribution of DA and its receptors to the anti-hypertensive effects of NK_3R antagonists. SHR that have received SB222200 (500 pmol, 24 h previously) or R-820 (500 pmol, 72 h previously) by the i.c.v. or the VTA route were given the D_1R antagonist SCH23390 (0.2 mg kg^{-1}, i.v.) (Kirouac and Ciriello, 1997; Gioanni *et al.*, 1998) or the D_2R antagonist raclopride (0.16 mg kg^{-1}, i.v.) (Millan *et al.*, 1998) 30 min prior to a second injection of the NK_3R antagonist. SB222200 and R-820 (500 pmol) were re-injected again either 24 h (SB222200) or 72 h (R-820) later to assess whether any blockade observed in the presence of the DA antagonist could be reversed. Each rat was injected with only one DA antagonist and one NK_3R antagonist either i.c.v or into the VTA.

Chronic anti-hypertensive effect of i.c.v. R-820. SHR (n = 4) were injected daily with R-820 (500 pmol, i.c.v.) to see whether its anti-hypertensive effect could be maintained for a period of 5 days. MAP and HR were measured at the peak time within the 5 h post-injection period every day. The recording was pursued for up to 2 days after cesssation of the treatment.

Dose–response curves to i.c.v. or VTA microinjection of the NK_3R agonist senktide. Two groups of SHR were used on 4 consecutive days; the first group was implanted with an i.c.v. cannula (n = 6) and the second group with a VTA cannula (n = 6). Each group was administered aCSF, either i.c.v. or directly into the VTA, followed by 10 pmol senktide 60 min later, and then increasing doses of 25, 65 and 100 pmol were administered on the following 3 days at 24 h intervals. This protocol and the doses selected were based on our previous studies in which the cardiovascular effects of senktide, injected either i.c.v. or into the VTA, were characterized in normotensive rats (Cellier *et al.*, 1997; Deschamps and Couture, 2005).

Blockade of the effects of senktide by the tachykinin NK_3R antagonist in the VTA. In this series of experiments, the dose of 25 pmol senktide was selected on the basis of its maximal cardiovascular response after VTA injection. SHR (n = 4) were administered a microinjection of aCSF into the VTA and followed 60 min later by a single dose of senktide (25 pmol). Twenty-four hours later, senktide was re-injected again 1 h after the VTA injection of the tachykinin NK_3R antagonist R-820 (500 pmol) (Regoli *et al.*, 1994). The reversal of the inhibition was tested 24 h later.

Effects of systemic treatments with dopamine D_1 and D_2 receptor antagonists against i.c.v. senktide. In this series of experiments, the dose of 65 pmol senktide was selected on the basis of its maximal pressor response upon i.c.v. injection. Three groups of SHR were initially injected i.c.v. with a single dose of senktide (65 pmol). One hour later, they were injected with either the D_1R antagonist SCH23390 (0.2 mg kg^{-1}, i.v. n = 6), the D_2R antagonist raclopride (0.16 mg kg^{-1}, i.v. n = 6) or the non-selective D_2R antagonist haloperidol (10 mg kg^{-1}, s.c. n = 4) (Nsimba *et al.*, 1997; Miyamoto *et al.*, 2005), followed 30 min later by senktide (65 pmol). The following day (24 h later), senktide (65 pmol) was re-injected alone to assess the reversal of any blockade observed in the presence of antagonist on the preceding day. In one additional group of SHR (n = 4), raclopride and SCH23390 were administered alone, 24 h apart, to assess their direct cardiovascular effects. A second group of SHR (n = 4) served to evaluate the direct cardiovascular effect of haloperidol. Pilot experiments have shown that the cardiovascular response to i.c.v. senktide (65 pmol) is reproducible when two injections are given at 1 h apart.

Blockade of i.c.v. senktide by SB222200 injected into the VTA of SHR and reciprocally blockade of VTA SB222200 by i.c.v. senktide. This experiment aimed to determine, firstly, the contribution of VTA NK_3R to the i.c.v. pressor response to senktide, and secondly, the possibility that the anti-hypertensive effect of SB222200 injected into the VTA could be prevented by an ipsilateral i.c.v. injection of the agonist senktide. Rats were injected with senktide (65 pmol, i.c.v. n = 4) (right side). Two hours later, SB222200 (500 pmol) was injected into the ipsilateral VTA, and senktide was re-injected i.c.v. 1 h later to evaluate the inhibition of the pressor response to senktide. On the second day, SB222200 (500 pmol, n = 4) was injected into the right VTA to assess its anti-hypertensive effect. On the third day, senktide (65 pmol) was injected i.c.v. (right side) 1 h after injection of SB222200 into the right VTA to see if it could block the anti-hypertensive effect of SB222200, which starts 1 h post-injection. In another group of rats (n = 4), senktide was injected i.c.v. on the contralateral side (left side) of VTA injection of SB222200 (right side) and the rest of the protocol was similar to that described previously.

Effect of ipsilateral and contralateral lesions of the VTA with IBO on the cardiovascular response to senktide and R-820. IBO has been shown to be an effective agent for producing circumscribed lesions when local, discrete brain lesions are desired. This method works reliably without apparent damage to passing or underlying fibres and, in contrast to kainic acid, a similar excitatory amino acid toxin, without causing distant lesion effects in the brain (Guldin and Markowitsch, 1981; 1982).

Senktide (65 pmol) was given i.c.v. to two groups of sham-operated rats ($n = 4$) and to rats subjected to ipsilateral ($n = 4$) or contralateral ($n = 4$) lesions of the VTA, induced by administration of IBO (1 µg in 0.5 µL) injected directly into the VTA, 5 days earlier. Twenty-four hours after senktide injection, R-820 (500 pmol) was given i.c.v. to the four groups of SHR.

Histology

At the end of the experimental protocol, the rats were killed by CO_2 inhalation and then immediately injected with 0.5 µL of Evans Blue dye (Sigma-Aldrich Canada Ltd, Oakville, Ontario, Canada) in the VTA. The brains were removed and fixed with 10% (vv^{-1}) buffered formalin (Fisher Scientific Inc., Ontario, Canada) and 20% (wv^{-1}) sucrose until the piece was floating (around 2 weeks) on the surface (Carson, 1992). Coronal sections (40 µm, cut on a freezing microtome) were mounted on glass slides and stained with cresyl violet for histological examination of the microinjection site. The injection site was confirmed by the presence of a black spot in the VTA with no evidence of haemorrhage or necrosis (Figure 1).

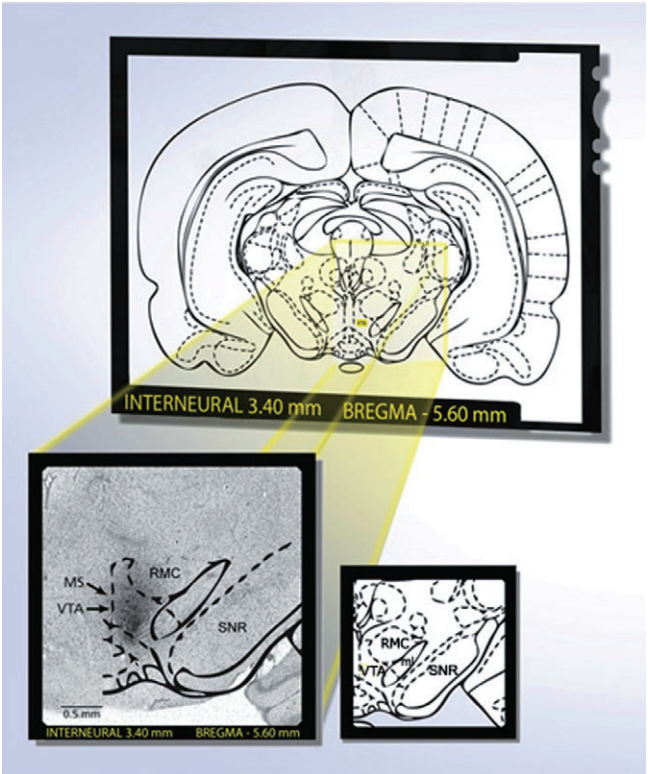

Figure 1

Identification of the VTA as a microinjection site following post-mortem histological examination of microinjected Evans's blue. A rat was considered to be correctly injected when a black spot was seen in the VTA without any evidence of haemorrhage or necrosis. Diagram was modified from the atlas of Paxinos and Watson (1998). VTA, ventral tegmental area; SNR, substantia nigra reticular; ml, medial lemniscus; RMC, red nucleus magnocellular; MS, microinjection site. Scale bar: 0.5 mm.

Drugs and solutions

The drug target nomenclature conforms to the *British Journal of Pharmacology's* Guide to Receptors and Channels (Alexander *et al.*, 2009). aCSF was purchased from Harvard Bioscience (Holliston, MA, USA.). Succinyl-[Asp^6,MePhe^8]-SP (6–11) (senktide) (MW: 842) was purchased from Bachem Bioscience Inc. (King of Prussia, PA, USA). Raclopride, haloperidol, IBO and heparin were purchased from Sigma-Aldrich Canada and SCH23390 from Tocris (Ellisville, MO, USA). Haloperidol was dissolved in ethanol and dimethylsulphoxide (DMSO), and diluted in saline (final solution contained 3% ethanol and 0.5% DMSO). SCH23390 and raclopride were dissolved in DMSO and diluted in saline (final solutions contained 3% DMSO). R-820 (3-indolylcarbonyl-Hyp-Phg-N (Me)-Bzl) (MW: 624.6) was a kind gift of the late Dr Jean-Luc Fauchère (Research Institute Servier, Paris, France) (Regoli *et al.*, 1994). SB222200 [(*S*)-(–)-*N*-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide (MW: 686.7) and its inactive enantiomer SB222201 were a donation from Dr Henry M. Sarau (GlaxoSmithKline, PA, USA.) (Sarau *et al.*, 2000). They were dissolved in DMSO and aCSF mixed with 2-hydroxypropyl-β-cyclodextrin (Sigma-Aldrich Canada) to obtain the desired solution (final solutions contained less than 15% DMSO and 20% 2-hydroxypropyl-β-cyclodextrin).

Statistical analysis of data

Results are expressed as the means ± SEM of values obtained from (n) rats. Statistical analysis of data was performed with Graph-Pad Prism software (GraphPad Software Inc., La Jolla, CA, USA). Time-course effects were analysed for statistical significance by a two-way analysis of variance (ANOVA) followed by a Bonferroni's test for multiple comparisons. A one-way ANOVA followed by a *post hoc* Dunnett's test was used for multiple comparisons with the same control group or by a *post hoc* Bonferroni's

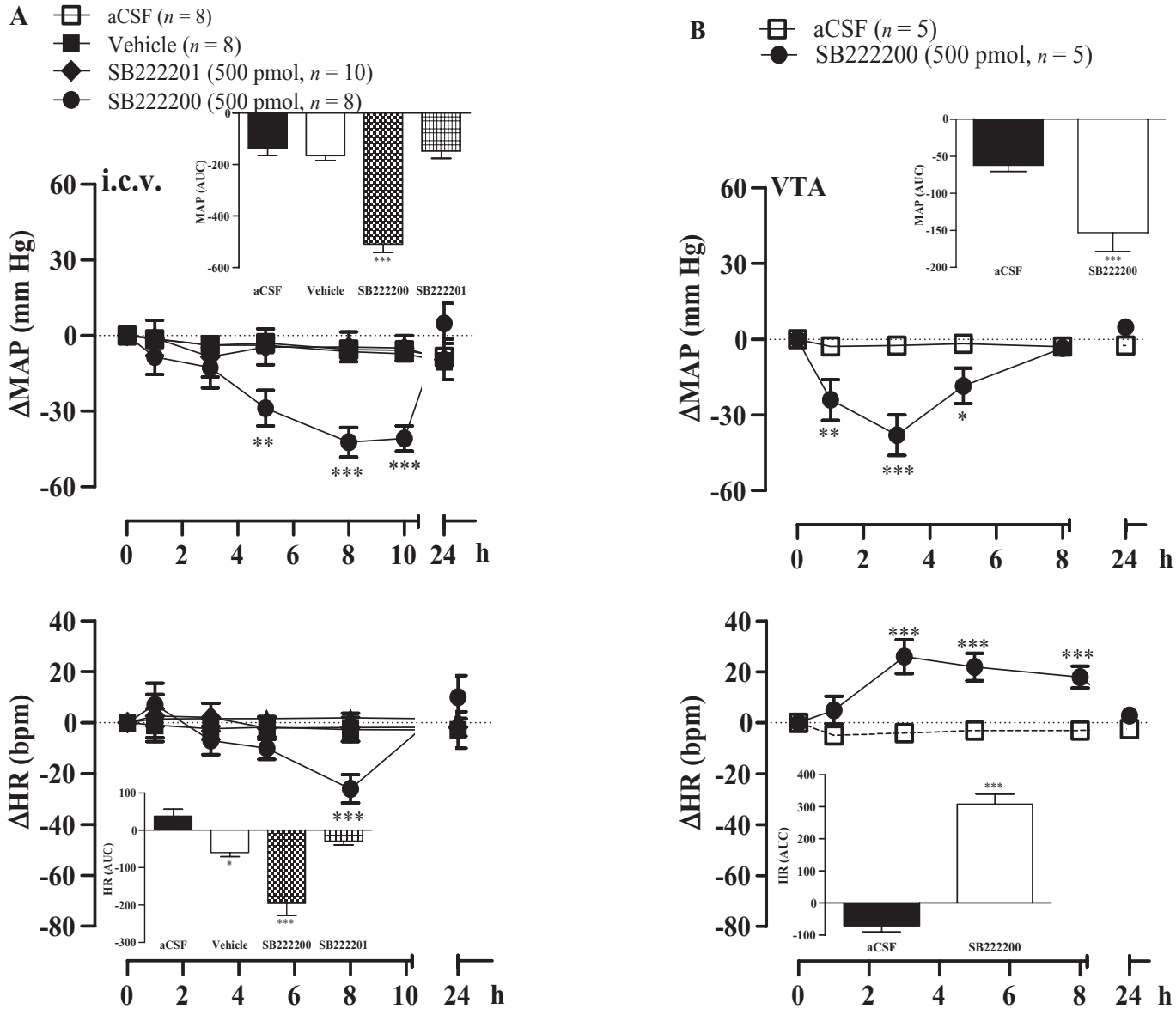

Figure 2

Time-course effects on changes in mean arterial blood pressure (ΔMAP) and heart rate (ΔHR) induced by i.c.v. (A) or ventral tegmental area (VTA) (B) injection of 500 pmol of SB222200 or its inactive enantiomer SB222201. Areas under the curves (AUC) were measured for a period of 0–10 h for i.c.v and 0–8 h for VTA (small insets). Values represent the mean ± SEM of *n* rats. Significant difference from aCSF is indicated by $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

test for comparison between groups [area under the curve (AUC)]. Only probability values (*P*) less than 0.05 were considered to be statistically significant.

Results

Cardiovascular responses induced by i.c.v. or VTA-injected SB222200

The tachykinin NK_3R antagonist SB222200 (500 pmol) injected i.c.v. caused a significant anti-hypertensive effect from 5 to 10 h post-injection in comparison with vehicle values in SHR. MAP was back to hypertensive levels (184.1 ± 3.8 mm Hg) 24 h later (Figure 2A). The anti-hypertension induced by SB222200 peaked at 8 h (–42 ± 5.5 mm Hg, $P < 0.001$) and was associated with a significant bradycardia. In contrast, the inactive enantiomer of SB222200 (SB222201) did not affect MAP or HR in comparison with vehicle values.

The injection of SB222200 (500 pmol) into the VTA caused a more rapid decrease in MAP, which was significant at 1 h and peaked at 3 h post-injection in SHR. This anti-hypertensive effect of the NK_3R antagonist was resolved at 8 h and was associated with a significant increase in HR at 3 h and during the remaining recording period of 8 h (Figure 2B).

Effects of dopamine antagonists on anti-hypertensive responses induced by tachykinin NK_3R antagonists

Raclopride (0.16 mg kg^{-1}, i.v.) prevented the anti-hypertensive effect of i.c.v. SB222200 (500 pmol) and reduced the accompanying bradycardia (Figure 3A). Conversely, SCH23390 (0.2 mg kg^{-1}, i.v.) caused a small reduction in the cardiovascular

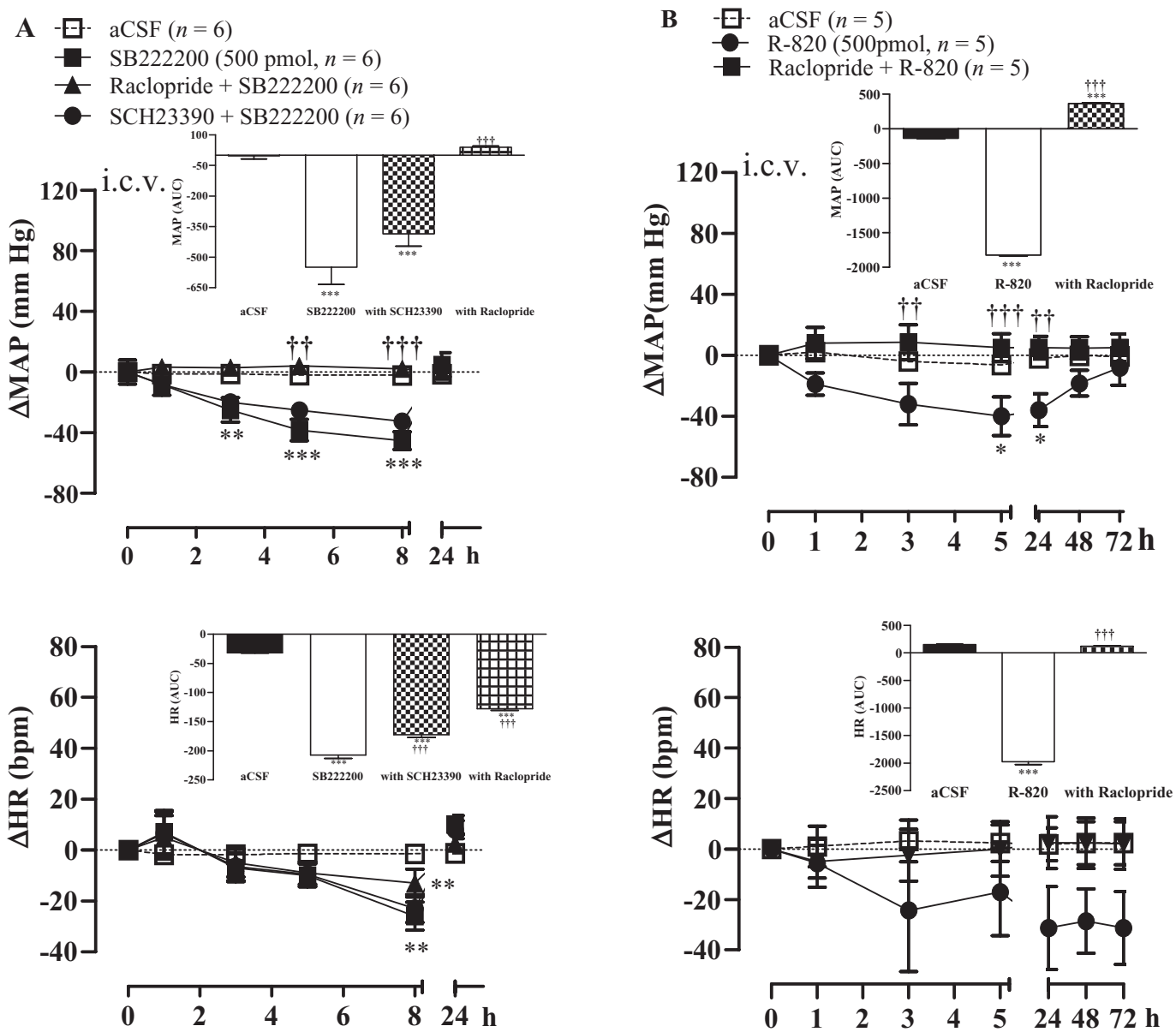

Figure 3

Time-course effects on changes in mean arterial blood pressure (ΔMAP) and heart rate (ΔHR) induced by i.c.v. injection of (A) SB222200 (500 pmol) and (B) R-820 (500 pmol) before and 30 min after treatment with the D_2R antagonist raclopride (0.16 mg kg^{-1}, i.v.) or the D_1R antagonist SCH23390 (0.2 mg kg^{-1}, i.v.). Areas under the curves (AUC) were measured for a period of 0–8 h (SB222200) or 0–72 h (R-820) as shown in small insets. Values represent the mean ± SEM of *n* rats. Significant difference from aCSF values (*) or NK_3R antagonist alone (†) is indicated by $*P < 0.05$, $**††P < 0.01$; $***†††P < 0.001$.

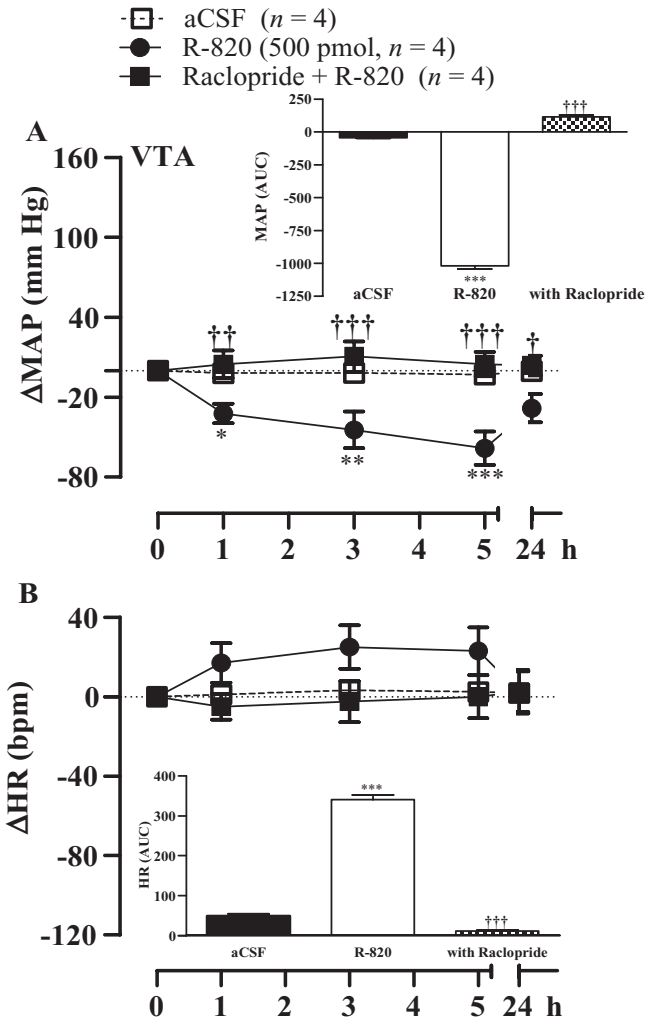

Figure 4

Time-course effects on changes in mean arterial blood pressure (ΔMAP) (A) and heart rate (ΔHR) (B) induced by R-820 (500 pmol) injected into the ventral tegmental area (VTA) before and 30 min after treatment with the D_2R antagonist raclopride (0.16 mg kg^{-1}, i.v.). Areas under the curves (AUC) were measured for a period of 0–5 h (small insets). Values represent the mean ± SEM of *n* rats. Significant difference from aCSF values (*) or R-820 (†) is indicated by *†$P < 0.05$; **††$P < 0.01$; ***†††$P < 0.001$.

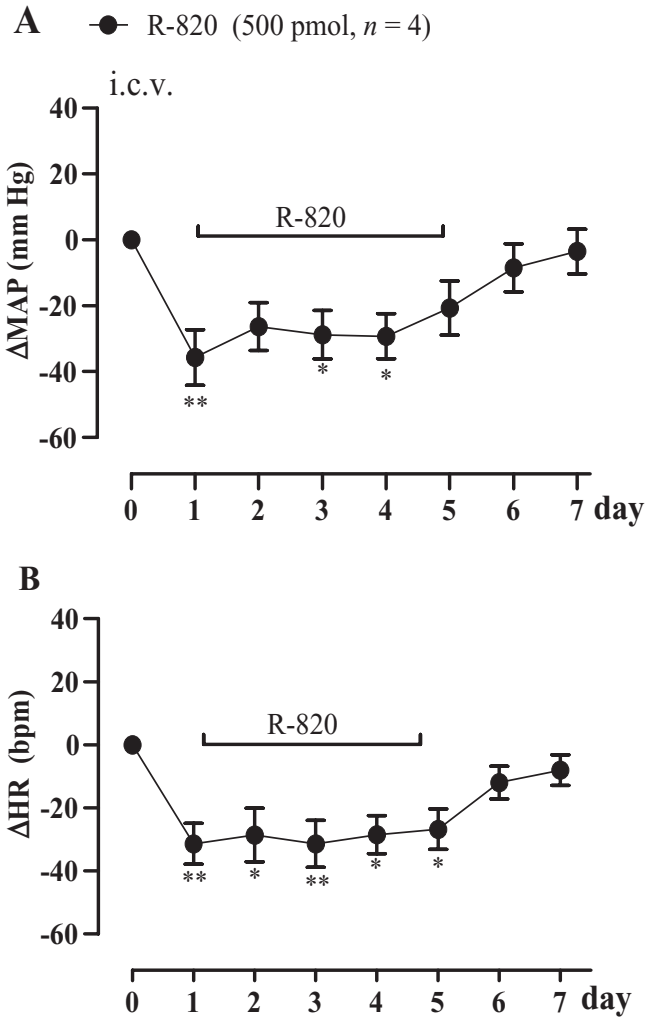

Figure 5

Time-course effects on changes in mean arterial blood pressure (ΔMAP) and heart rate (ΔHR) induced by i.c.v. injection of 500 pmol R-820, once daily for a period of 5 days in spontaneously hypertensive rats. Values represent the mean ± SEM of four rats. Significant difference from pre-injection values is indicated by *$P < 0.05$; **$P < 0.01$.

response to SB222200 (Figure 3A). The anti-hypertensive effect mediated by i.c.v. SB222200 was completely recovered 24 h later when it was re-injected in the absence of DA antagonists (data not shown). Likewise, the longer-lasting anti-hypertensive response and bradycardia induced by i.c.v. R-820 (500 pmol) were blocked by raclopride (0.16 mg·kg^{-1}, i.v.) (Figure 3B). A similar treatment with raclopride also prevented the more rapid, although shorter-lasting, anti-hypertensive response and the accompanying tachycardia elicited by R-820 injected into the VTA (Figure 4).

Chronic anti-hypertensive effect of i.c.v. R-820

In SHR, daily i.c.v. injection of R-820 (500 pmol) for 5 days led to a persistent decrease of both MAP and HR in comparison with the pre-administration values. MAP and HR values returned gradually to baseline hypertensive values within 48 h after cessation of the treatment with R-820 (Figure 5).

Comparison of the cardiovascular effect of i.c.v and VTA injected senktide

The i.c.v. injections of the tachykinin NK_3R agonist senktide (10–100 pmol) increased MAP and HR significantly but not dose-dependently during the first

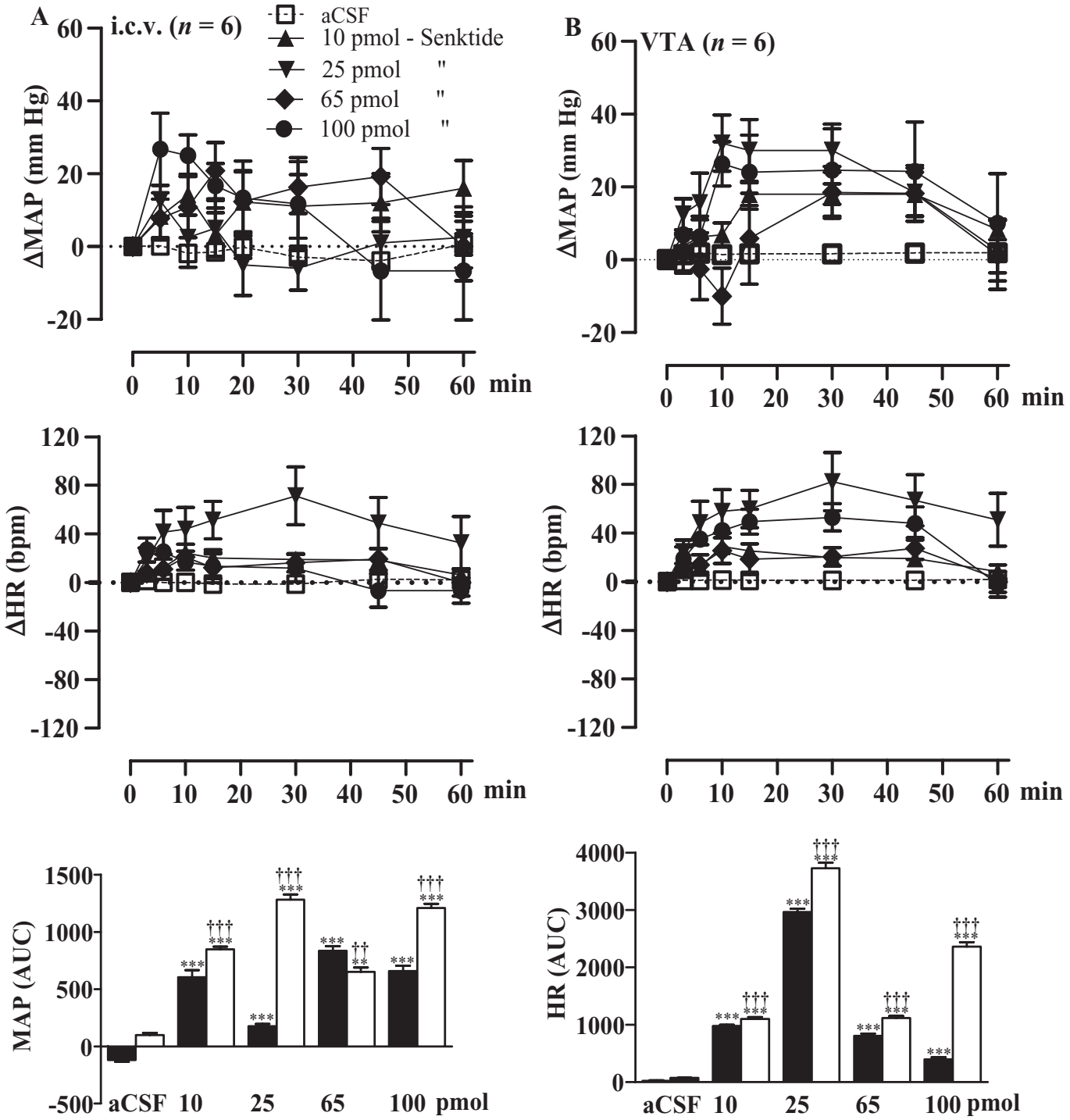

Figure 6

Time-course effects on changes in mean arterial blood pressure (ΔMAP) and heart rate (ΔHR) following i.c.v. (A) or ventral tegmental area (VTA) (B) injection of four increasing doses (10, 25, 65 and 100 pmol) of senktide in spontaneously hypertensive rats. Areas under the curves (AUC) were measured for a period of 0–1 h (open columns for VTA and solid columns for i.c.v.). Values represent the mean ± SEM of six rats per injection site. Significant difference from aCSF values (*) or i.c.v. (†) is indicated by **††P < 0.01; ***†††P < 0.001.

30–60 min post-injection in SHR. The pressor response was maximal at 65 pmol, while the tachycardiac response was greater in intensity and duration at 25 pmol (Figure 6A).

When senktide (10–100 pmol) was injected directly into the VTA of SHR, it caused larger increases of MAP and HR than when injected i.c.v. (Figure 6B). These effects were, however, not dose-

dependent and reached maximal values at 25 pmol for both parameters. The initial depressor response to senktide contributes to the smaller AUC for the MAP response at 65 pmol and may explain the absence of a dose-dependent cardiovascular effect.

Effect of the NK_3R antagonist, R-820, on the cardiovascular response to senktide in the VTA

The increase in MAP and HR induced by senktide (25 pmol) injected into the VTA was completely abolished by the prior administration into the VTA of the NK_3R antagonist R-820 (500 pmol, 1 h earlier) in SHR (Figure 7). The cardiovascular response to senktide was fully recovered 24 h later in the absence of R-820 (data not shown). When a microinjection of senktide (25 pmol, n = 6) was made outside the VTA, no cardiovascular effect was observed. Also, no changes in blood pressure occurred after microinjection of R-820 (500 pmol, n = 8) outside the VTA (data not shown).

Effects of dopamine receptor antagonists on the pressor response induced by i.c.v. senktide

The dopamine D_1R antagonist SCH23390 (0.2 mg kg^{-1}, i.v., 30 min earlier) blocked the pressor effect mediated by i.c.v. senktide (65 pmol). In contrast, the D_2R antagonist raclopride (0.16 mg kg^{-1}, i.v., 30 min previously) had a marginal effect on the pressor response induced by senktide (Figure 8A,C). The same treatment with haloperidol (10 mg kg^{-1}, s.c., 30 min previously) abolished the pressor effect induced by senktide (65 pmol) injected i.c.v. in SHR (Figure 8B,C). The MAP response to senktide was completely recovered when it was re-injected alone 24 h after treatment with the dopamine antagonists (data not shown).

At the dose used, the systemic administration of raclopride (0.16 mg kg^{-1}, i.v.) caused a transient increase in MAP, which was significant in comparison with saline between 5 and 15 min post-injection. SCH23390 (0.2 mg kg^{-1}, i.v.) had no significant effect on MAP during the first hour post-injection. Conversely, haloperidol (10 mg kg^{-1}, s.c.) had an anti-hypertensive effect, which was significant from 1 h up to 6 h post-injection in comparison with saline (Figure 9). The maximal decrease in MAP induced by haloperidol at 3 h (–33 ± 5.5 mm Hg, $P < 0.001$) was back to hypertensive levels 8 h later (184.1 ± 3.8 mm Hg).

Interaction between i.c.v. senktide and VTA SB222200 in SHR

The pressor response evoked by i.c.v. senktide (65 pmol) was blocked when SB222200 (500 pmol) was injected into the ipsilateral VTA, 1 h prior to a

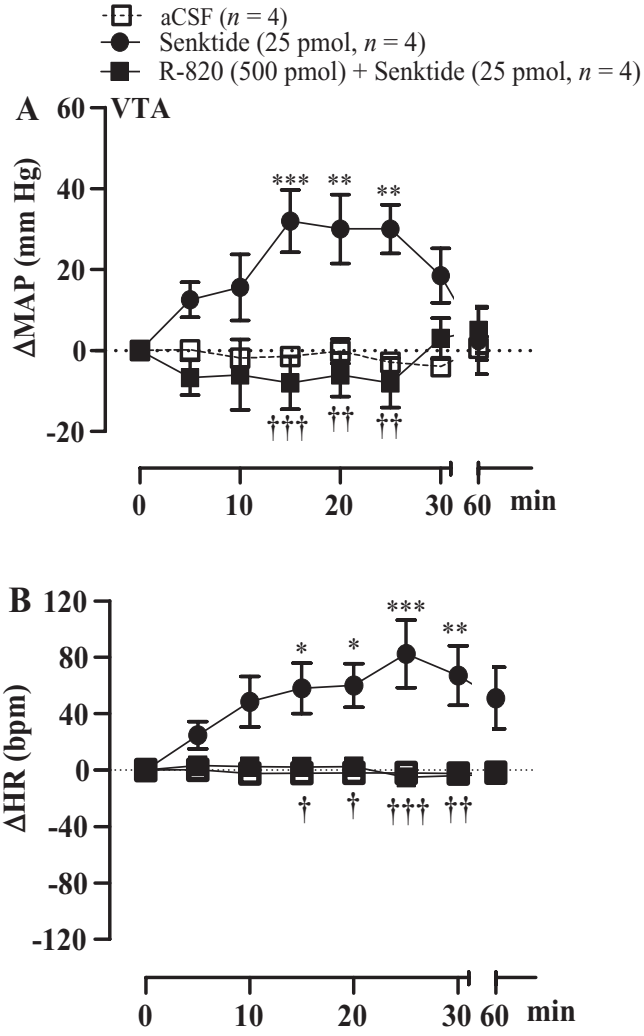

Figure 7

Time-course effects on changes in mean arterial blood pressure (ΔMAP) and heart rate (ΔHR) produced by senktide (25 pmol) injected into the ventral tegmental area (VTA) prior to (first day) and 1 h after VTA injection of R-820 (second day). Each point represents the mean ± SEM of four rats. Significant difference from aCSF (*) or senktide (†) values is indicated by *†$P < 0.05$; **††$P < 0.01$; ***†††$P < 0.001$.

second injection of i.c.v. senktide (Figure 10A,E). In contrast, when SB222200 was injected into the VTA under the same conditions, the pressor response to senktide injected i.c.v. on the contralateral side was little affected (Figure 10B,F). Moreover, the anti-hypertensive effect, which occurred from 1 to 5 h after VTA injection of SB222200 (500 pmol), was blocked when senktide was injected i.c.v. on the ipsilateral side (Figure 10C,E), yet it was only slightly reduced when senktide was injected i.c.v. on the contralateral side (Figure 10D,F). This suggests that i.c.v. senktide diffused chiefly to the ipsilateral VTA to

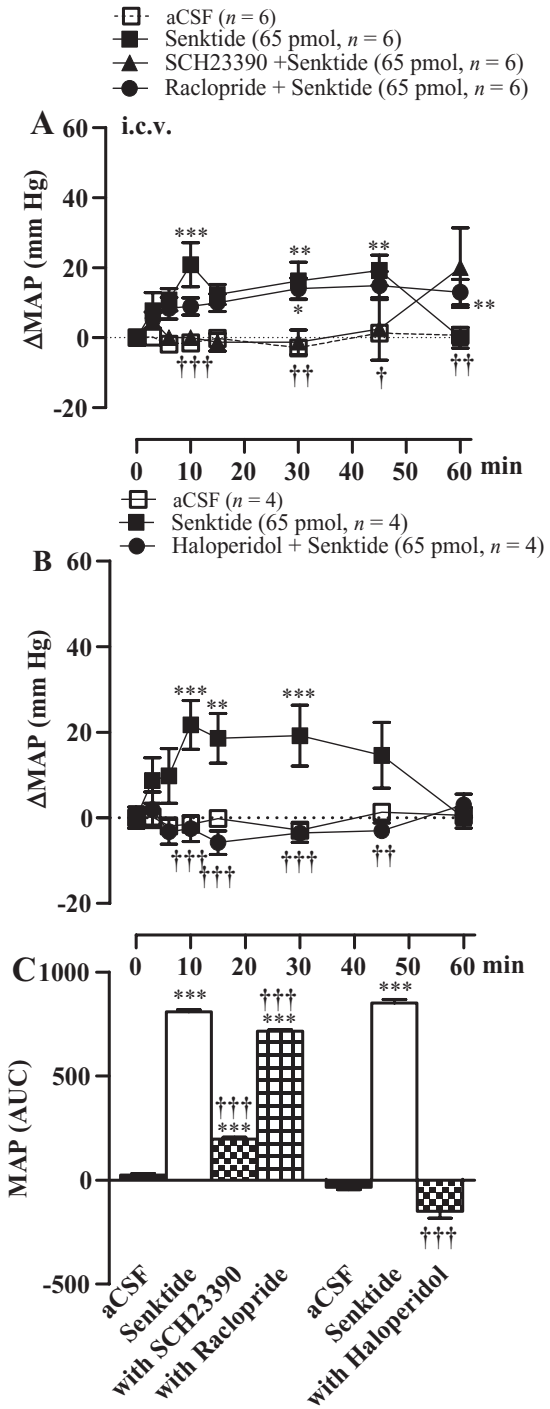

Figure 8

Time-course effects on changes in mean arterial blood pressure (ΔMAP) produced by i.c.v. senktide 65 pmol (baseline: 161 ± 5 mmHg) prior to and after treatment with (A) the D_1R antagonist SCH23390 (0.2 mg kg^{-1} i.v.) and the D_2R antagonist raclopride (0.16 mg kg^{-1} i.v.) (baseline: 167 ± 4 mm Hg); and (B) the non-selective D_2R antagonist haloperidol (10 mg kg^{-1} s.c.) (baseline: 164 ± 2.4 mm Hg) in spontaneously hypertensive rats. In (C), the areas under the curves (AUC) measured for a period of 0–1 h are shown. Values represent the mean ± SEM of *n* rats. Significant difference from aCSF (*) or senktide (†) values is indicated by *†$P < 0.05$; **††$P < 0.01$; ***†††$P < 0.001$.

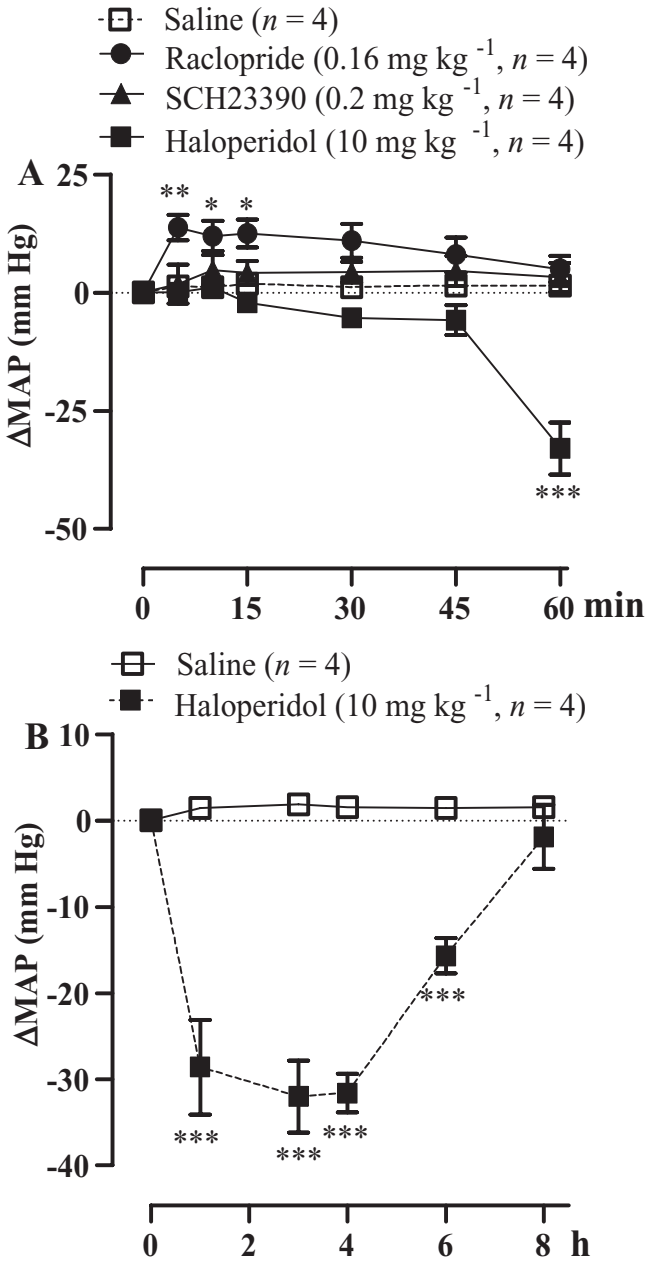

Figure 9

Time-course effects on changes in mean arterial blood pressure (ΔMAP) produced by treatments with the D_1R antagonist SCH23390 (0.2 mg kg^{-1} i.v.), D_2R antagonist raclopride (0.16 mg kg^{-1} i.v.) and the non-selective D_2R antagonist haloperidol (10 mg kg^{-1} s.c.) in spontaneously hypertensive rats. Different time scales were used: in (A) (min), and (B) (h).Values represent the mean ± SEM of four rats. Significant difference from saline is indicated by *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

cause its pressor effect and to prevent the anti-hypertensive effect of SB222200. This also suggests that microinjected SB222200 into the VTA had limited diffusion to the contralateral VTA.

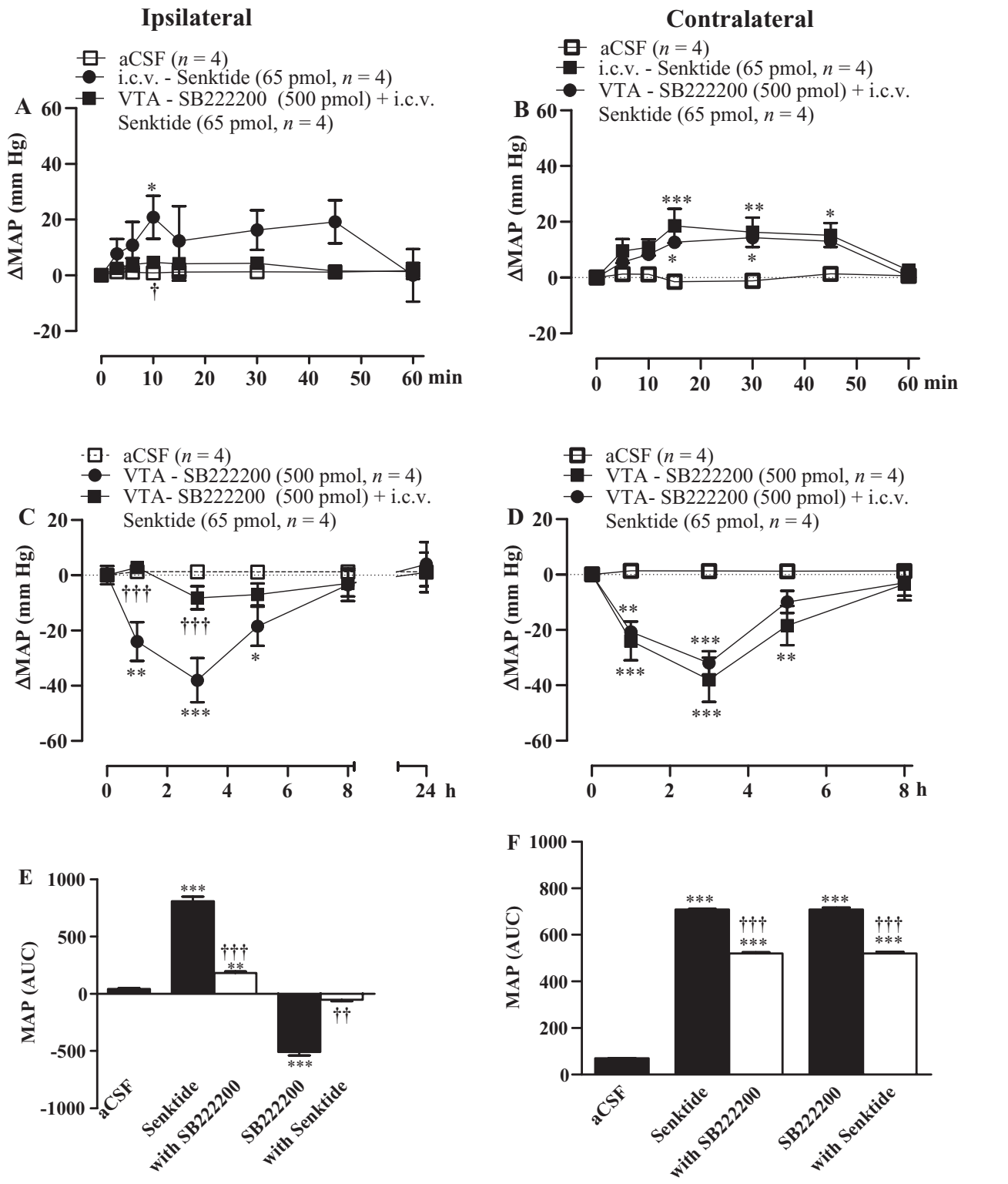

Figure 10

Time-course effects on changes in mean arterial blood pressure (ΔMAP) produced by senktide injected i.c.v. on the (A) ipsilateral and (B) contralateral sides before and after SB222200 (500 pmol) injected into the ventral tegmental area (VTA). The anti-hypertensive effect elicited by the VTA injection of SB222200 (500 pmol) was measured before and after i.c.v. injection of senktide (65 pmol) on the ipsilateral (C) and contralateral (D) side in spontaneously hypertensive rats. Areas under the curves (AUC) for MAP are shown in (E and F). Values represent the mean ± SEM of *n* rats. Significant difference from aCSF (*) and senktide alone or SB222200 alone (†) is indicated by *†P < 0.05; **††P < 0.01; ***†††P < 0.001.

Effect of ipsilateral and contralateral lesions of the VTA with IBO on the cardiovascular response to senktide and R-820

The pressor response induced by i.c.v. senktide (65 pmol) in sham-operated SHR was absent in rats subjected to ipsilateral lesion of the VTA with IBO (Figure 11A,E). One day after the senktide experiment, R-820 (500 pmol) was given i.c.v. to both groups of rats. Only the sham-operated rats displayed the typical anti-hypertensive response to R-820 (Figure 11C,E). Conversely, the pressor response induced by i.c.v. senktide (65 pmol) in sham-operated SHR was similar to that observed in rats subjected to contralatereal lesion of the VTA with IBO (Figure 11B,F). One day after the senktide experiment, the anti-hypertensive effect of i.c.v. R-820 (500 pmol) was very little affected by the contralateral lesion of the VTA (Figure 11D,F).

Discussion

This study extends our earlier work showing that tachykinin NK_3R antagonists exert dose-dependent and reversible anti-hypertensive effects when injected intracerebrally in SHR (Lessard *et al.*, 2004). The present results demonstrate that this anti-hypertensive effect is mediated by the VTA and the mesocorticolimbic dopaminergic pathway. This statement is based on four findings: (i) the anti-hypertensive effect of NK_3R antagonists injected i.c.v. was reproduced with a faster onset when drugs were injected directly into the VTA; (ii) the cardiovascular responses elicited by i.c.v. or VTA administration of NK_3R agonist and antagonists were blocked by dopamine receptor antagonists; (iii) the i.c.v. pressor effect of the NK_3R agonist was prevented following the inhibition of NK_3R in the ipsilateral VTA, and vice versa, the anti-hypertensive effect of SB222200 injected into the VTA was blocked by an ipsilateral i.c.v injection of senktide; and (iv) the chemical destruction of the ipsilateral VTA with IBO eliminated completely the cardiovascular response induced by i.c.v. senktide and R-820. Hence, these findings suggest that the VTA and its dopaminergic projections in the brain are of primary importance in the cardiovascular effects associated with i.c.v. injections of an NK_3R agonist and antagonist.

I.c.v. injection of senktide increases HR and blood pressure in normotensive rats (Cellier *et al.*, 1997) and guinea pigs (Roccon *et al.*, 1996). This cardiovascular response is blocked by the prior i.c.v. injection of the NK_3R antagonist R-820 in normotensive rats (Cellier *et al.*, 1997) and has been ascribed to the activation of the sympatho-adrenal system (Nagashima *et al.*, 1989; Roccon *et al.*, 1996). The cardiovascular response to senktide in the VTA, also blocked by R-820 (present study), is mediated by the sympathetic nervous system and the subsequent increase of cardiac output, as it has been shown to be abolished by i.v. treatment with atenolol, a β_1-adrenoceptor antagonist (Deschamps and Couture, 2005). The latter study showed that the cardiovascular response to senktide in the VTA is similar to that caused by glutamic acid, which is consistent with neuronal cell activation. Moreover, the cardiovascular response to a central injection of senktide in SHR is probably mediated by DA release because it was abolished after treatment with SCH23390 and haloperidol. The lack of effect of raclopride suggests the involvement of D_1R rather than D_2R, as reported previously in normotensive rats (Deschamps and Couture, 2005). This is congruent with the release of DA in the nucleus accumbens following the activation of NK_3R by senktide in the VTA (Spooren *et al.*, 2005) and with the localization of NK_3R on dopaminergic neurones in the VTA (Chen *et al.*, 1998; Lessard *et al.*, 2007; 2009).

Our results suggest that tonic activation of NK_3R in the VTA enhances the release of DA in the mesocorticolimbic system and contributes to high BP in SHR. This hypothesis is supported by the normalization of high BP induced by blockade of NK_3R in the VTA through a mechanism sensitive to DA inhibition. The persistent anti-hypertensive effect induced by the 5 day i.c.v. administration of R-820 suggests that NK_3R is under constant and chronic tonic activation in SHR. In addition, this experiment also substantiates the use of centrally acting NK_3R antagonists for the treatment of hypertension in this model. As the anti-hypertensive effect of NK_3R antagonists was prevented by raclopride but much less by SCH23390, it is concluded that this effect is chiefly mediated by D_2R. The suggestion of a greater basal release of tachykinins in the mesocorticolimbic system of SHR is consistent with the existence of endogenous tachykinins in the VTA (Kalivas *et al.*, 1985; Warden and Young, 1988; Tamiya *et al.*, 1990; Lu *et al.*, 1998) and the increased neurokinin B-like immunoreactive contents in the brain of SHR, the putative NK_3R endogenous ligand (Nagashima *et al.*, 1989). Because D_1R and D_2R are up-regulated in the nucleus accumbens in SHR (Kirouac and Ganguly, 1993; Vaughan *et al.*, 1999), it is tempting to speculate that the tonic activation of NK_3R by endogenous tachykinins in the VTA is facilitated by a hyperactive dopaminergic mesolimbic system. Reciprocally, tonic activation of NK_3R may contribute to the hyperactivity of the VTA dopaminergic neuronal pathway and thereby in the maintenance of hypertension in SHR. The affinity and densities of

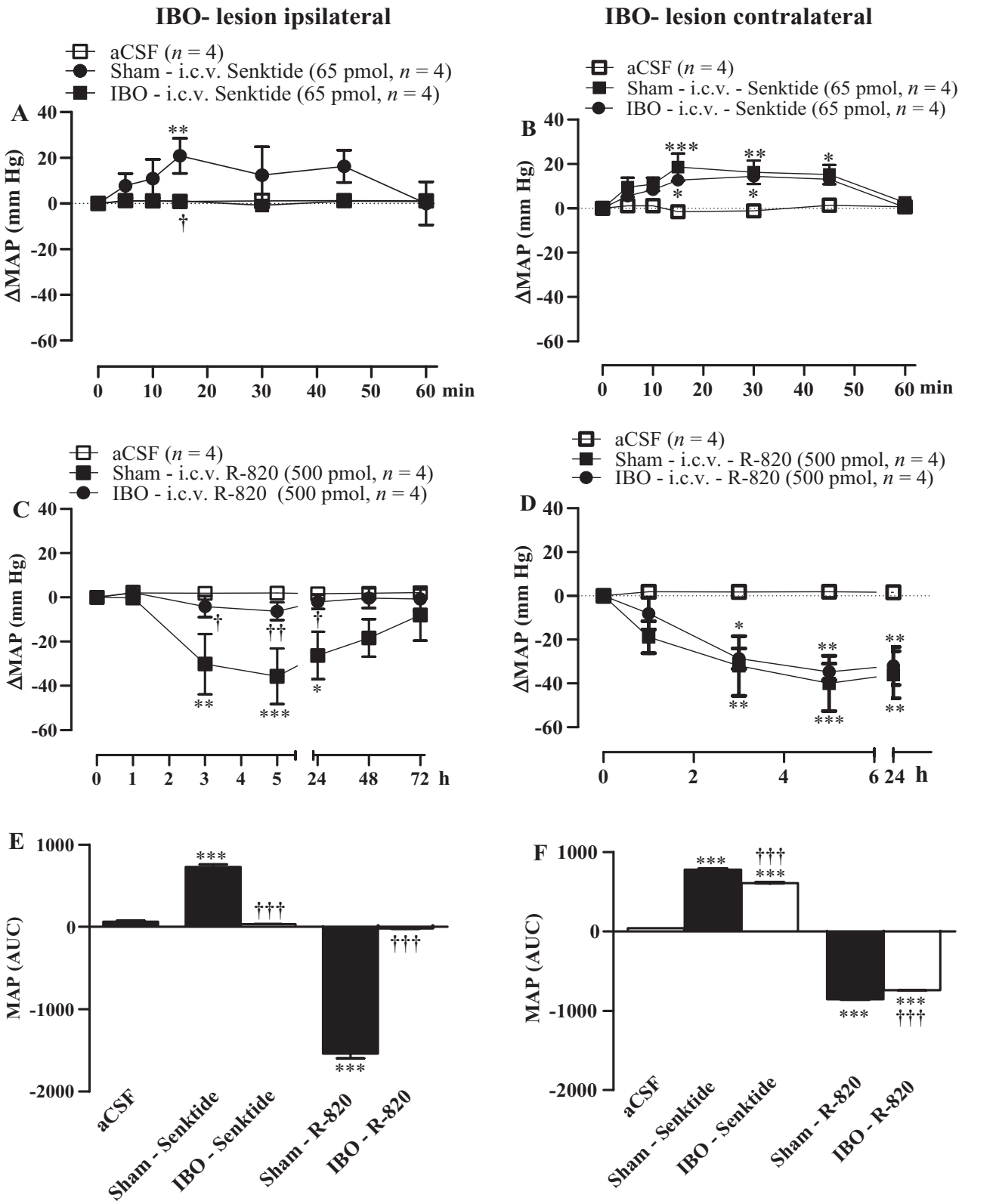

Figure 11

Time-course effects on changes in mean arterial blood pressure (ΔMAP) produced by i.c.v. injected 65 pmol senktide (A, B) or 500 pmol R-820 (C, D) in spontaneously hypertensive rats which underwent an ipsilateral (A,C) and contralateral (B,D) lesion of the ventral tegmental area with ibotenic acid (IBO), 5 days earlier or a sham-operation. Areas under the curves (AUC) for MAP are shown in (E,F). Each point represents the mean ± SEM of *n* rats. Significant difference from aCSF (*) or sham-operated rats (†) is indicated by *†$P < 0.05$; **††$P < 0.01$; ***†††$P < 0.001$.

specific NK_3R binding sites measured in the VTA and other midbrain nuclei are, however, not significantly different between SHR and WKY (Lessard *et al.*, 2003).

As i.c.v. injection of NK_3R antagonists has no significant effect on resting BP in normotensive rats, it was concluded that brain NK_3R did not exert a tonic autonomic control of BP in normotensive rats (Couture *et al.*, 1995; Cellier *et al.*, 1997; Lessard *et al.*, 2004; Deschamps and Couture, 2005). Nevertheless, the mesolimbic dopaminergic system is involved in the diurnal regulation of BP and HR (Sei *et al.*, 1999; Sakata *et al.*, 2002), and therefore, the VTA may represent an important site of action for tachykinins in cardiovascular regulation in normotensive subjects.

Whereas SB222200 and R-820 induced tachycardia when injected into the VTA, both antagonists induced a small bradycardia at the peak of the anti-hypertensive response after i.c.v. injection. The occurrence of tachycardia in response to a fall in BP suggests that the inhibition of NK_3R in the VTA did not interfere with the activation of baroreceptors, in contrast to the i.c.v. administration of NK_3R antagonists, and further suggests that the inhibition of the cardiac output or baroreflex activity is unlikely to be the mechanism of the anti-hypertensive effect of these antagonists. Although the increase in BP and HR induced by i.c.v and VTA-injected senktide has been ascribed to the activation of the sympathetic nervous system (Roccon *et al.*, 1996; Deschamps and Couture, 2005), the decrease in BP induced by i.c.v. injected SB222200 is not associated with changes in plasma catecholamine levels in SHR (Lessard *et al.*, 2004). However, the possibility that central tachykinin NK_3R antagonists may exert an inhibitory action on neuronal sympathetic vasomotor tone cannot be excluded.

The dissimilar time-course of the anti-hypertensive effect of R-820 and SB222200 was also observed in our previous study (Lessard *et al.*, 2004) and may be explained by the different physico-chemical features of these two compounds. SB222200, a much more lipophilic molecule than R-820, can diffuse more easily into the brain after i.c.v. injection. This could explain why SB222200 displays a quicker but less prolonged effect than R-820. The peripheral administration of these antagonists at 500 pmol in SHR had no effect on BP, supporting a central site of action (Lessard *et al.*, 2004) that has been identified in the present study as the VTA.

The anti-hypertensive effect of s.c. administered haloperidol in SHR was not shared by selective blockade of D_1R and D_2R, which caused either no effect or a transient pressor response. The non-selectivity of haloperidol versus SCH23390 and raclopride for DA receptors, and the putative inhibition of non-DA receptors can account for the pronounced anti-hypertensive response of haloperidol. Although data of the present study obtained with haloperidol support the conclusion drawn with the selective DA antagonist SCH23390 on the cardiovascular effect of senktide, they must be interpreted with caution. The reduction in MAP with haloperidol has also been observed in angiotensin II-hypertensive rats, while the same treatment had no effect in normotensive rats (De Brito Gariepy *et al.*, 2010). In addition, it has been suggested that haloperidol could be used to treat hypertensive crises caused by high-dose amphetamine or methamphetamine abuse (Angrist *et al.*, 2001).

Conclusion

This neuropharmacological study suggests that tachykinin NK_3R located in the VTA is subjected to persistent tonic activation, which increases midbrain dopaminergic transmission and thereby contributes to hypertension in SHR. Hence, these receptors may represent a therapeutic target in the treatment of arterial hypertension. Since NK_3R antagonists have been proposed as putative antipsychotics in the treatment of schizophrenia (Spooren *et al.*, 2005; Meltzer and Prus, 2006), understanding the functional interaction between NK_3R and the dopaminergic system in the central autonomic control of BP is of prime importance.

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Conflicts of interest

None.

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