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The ventral tegmental area as a putative target for tachykinins in cardiovascular regulation

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1 Tachykinin receptor agonists and antagonists were microinjected into the ventral tegmental area (VTA) to study the relative participation of the three tachykinin receptors in cardiovascular regulation in freely behaving rat.

2 Selective agonists (1–100 pmol) for NK_1 ([Sar^9, Met $(O_2)^{11}$]SP), NK_2 ([β-Ala^8]NKA (4–10)) and NK_3 (senktide) receptors evoked increases in blood pressure, heart rate (HR) along with behavioural manifestations (face washing, sniffing, head scratching, rearing, wet dog shake). At 1 pmol, NK_1 and NK_3 agonists did not affect behaviour and blood pressure but only HR.

3 Tachykinin agonists-induced cardiovascular responses were selectively and reversibly blocked by the prior injection of antagonists for NK_1 receptors (LY 303870 ((*R*)-1-[*N*-(2-methoxybenzyl)acetyl-amino]-3-(1H-indol-3-yl)-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane), 5 nmol), NK_2 receptors (SR 48968 ([(*S*)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl]benzamide]), 250 pmol) and NK_3 receptors (SB 235375 ((−)-(*S*)-*N*-(α-ethylbenzyl)-3-(carboxymethoxy)-2-phenylquinoline-4-carboxamide), 25 nmol). With the exception of the NK_2 agonist, most behavioural effects were also blocked by antagonists.

4 Tachykinin agonists-induced cardiovascular responses were inhibited by intravenous (i.v.) treatments with antagonists for D_1 dopamine receptor (SCH23390, 0.2 mg kg^{-1}) and β_1-adrenoceptor (atenolol, 5 mg kg^{-1}) but not for D_2 dopamine receptor (raclopride, 0.16 mg kg^{-1}). Behavioural responses were blocked by SCH23390 only.

5 The present study provides the first pharmacological evidence that the three tachykinin receptors in the rat VTA can affect the autonomic control of blood pressure and HR by increasing midbrain dopaminergic transmission. This mechanism may be involved in the coordination of behavioural and cardiovascular responses to stress and noxious stimulation.
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Abbreviations: aCSF, artificial cerebrospinal fluid; ANOVA, analysis of variance; DA, dopamine; DMSO, dimethylsulphoxide; HR, heart rate; i.c.v., intracerebroventricular; i.p., intraperitoneal; MAP, mean arterial blood pressure; NKA, neurokinin A; NKB, neurokinin B; NTS, nucleus tractus solitarius; s.c., subcutaneous; SP, substance P

Introduction

A role for tachykinins has been suggested in the central autonomic control of blood pressure and in cardiovascular defence reactions (Unger *et al*., 1988; Couture *et al*., 1995; Culman & Unger, 1995). The intracerebroventricular (i.c.v.) injection of substance P (SP), neurokinin A (NKA) and neurokinin B (NKB) or receptor selective agonists in freely moving rats leads to increases in mean arterial blood pressure (MAP) and heart rate (HR), and this is accompanied by typical behaviours such as face washing, sniffing, grooming and wet dog shakes (Takano *et al*., 1990; Itoi *et al*., 1992; Tschöpe *et al*., 1992; Cellier *et al*., 1997; 1999). The exact site and mechanism of action of tachykinins in the central autonomic control of blood pressure remain unclear. Thus far, the anterior hypothalamus represents the primary putative target for tachykinins (Itoi *et al*., 1991; 1994), although a role for the nigrostriatal dopaminergic system was highlighted in recent pharmacological studies in normotensive and spontaneously hypertensive rats (Lessard & Couture 2001; Lessard *et al*., 2003).

A potential strategic site for tachykinins in cardiovascular regulation is the ventral tegmental area (VTA) (A10 dopamine cell group) located in the midbrain, medially to the substantia nigra. The main neuronal dopaminergic projections of the mesocortical–mesolimbic system from the VTA are to the prefrontal cortex, the nucleus accumbens of the striatum and the amygdala (Swanson, 1982; Oades & Halliday, 1987). Endogenous SP, NKA and tachykinin NK_3 receptors are present in the VTA (Deutch *et al*., 1985; Kalivas *et al*., 1985; Warden & Young, 1988; Ding *et al*., 1996; Shughrue *et al*., 1996; Chen *et al*., 1998; Lu *et al*., 1998; Lessard *et al*., 2003). The distribution of NK_3 receptor immunoreactive neurons completely overlaps that of tyrosine hydroxylase neurons in this area (Chen *et al*., 1998) and SP immunoreactive axon

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terminals make direct synaptic contact with dopaminergic neurons in the VTA (Tamiya *et al*., 1990). SP projecting fibres in the VTA originate from the nucleus accumbens and the medial habenular nucleus (Cuello *et al*., 1978; Lu *et al*., 1998). Application of NK_1, NK_2 and NK_3 receptor agonists in the VTA increases the firing rate of A10 dopamine cells (Overton *et al*., 1992). Also, SP and NK_3 agonists cause increases in the levels of dopamine, its metabolite dihydroxyphenylacetic acid (DOPAC) and dopamine turnover in the prefrontal cortex and nucleus accumbens (Elliott *et al*., 1986a, b; Cador *et al*., 1989; Marco *et al*., 1998), as well as behavioural effects consistent with mesolimbic dopaminergic activation (Eison *et al*., 1982).

Electrical stimulation of the VTA or microinjection of a stable analogue of SP (DiMe-C7 or [pGlu^5, MePhe^8, Sar^9]-SP (5–11)) in the rat VTA produces increases in blood pressure and HR. The DiMe-C7-mediated effects result from central D_1 and D_2 dopamine receptors activation and vasopressin release (Cornish & van den Buuse, 1994; 1995; van den Buuse, 1998). Despite the wealth of anatomical, biochemical and behavioural evidence for a regulatory function of tachykinins on the mesolimbic dopaminergic system, little is known regarding the relative participation of the three tachykinin receptors in the cardiovascular function of the VTA.

Therefore, this study tested the hypothesis that tachykinin receptors may affect blood pressure and HR at the level of the VTA through central dopamine and the activation of the sympathetic nervous system in freely behaving rats. This was achieved with a pharmacological approach using selective tachykinin agonists and antagonists microinjected unilaterally in the VTA and by systemic treatment with selective antagonists for D_1 (SCH23390) and D_2 (raclopride) dopamine receptors and for β-adrenoceptors (atenolol). The agonists and nonpeptide antagonists used were: [Sar^9, Met $(O_2)^{11}$]SP (Regoli *et al*., 1988) and LY 303870 ((*R*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1H-indol-3-yl)-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane) (Iyengar *et al*., 1997; Cellier *et al*., 1999) for NK_1 receptors, [β-Ala^8]NKA (4–10) (Rovero *et al*., 1989) and SR 48968 ([(*S*)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl)butyl]-benzamide]) (Advenier *et al*., 1992; Emonds-Alt *et al*., 1992) for NK_2 receptors, and senktide (Wormser *et al*., 1986) and SB 235375 ((−)-(*S*)-*N*-(α-ethylbenzyl)-3-(carboxymethoxy)-2-phenylquinoline-4-carboxamide) (Hay *et al*., 2002) for NK_3 receptors. This literature indicates that the tachykinin antagonist's chosen are appropriate for rat tachykinin receptors. Behavioural activity induced by the agonists was measured in parallel to the cardiovascular changes to provide insights into possible relationship between these centrally mediated responses.

Methods

Animal source and care

Male Wistar rats (300–350 g) were purchased 3–5 days prior to experiments from Charles River, St Constant, Québec, Canada and 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

Male Wistar rats ($n=189$) were anaesthetized with an intraperitoneal (i.p.) injection of 65 mg kg^{-1} sodium pentobarbitone (Somnotol; MTC Pharmaceuticals, Cambridge, Ontario, Canada). Before each surgery, rats received a mixture of Trimethoprim and Sulphadiazine (tribrissen 24%, 5 and 26 mg kg^{-1}, respectively, subcutaneous (s.c.) Schering Canada Inc., Montréal, Québec, Canada) and Ketoprophen (anafen, 10 mg kg^{-1}, s.c., MERIAL Canada Inc., Montréal, Québec, Canada). Also, to avoid core hypothermia during the surgery, rats were installed with a rectal thermometer connected to a heating pad calibrated at 35°C. Animals were then positioned in a stereotaxic frame (David Kopf Instrumentation, Tujunga, CA, U.S.A.) with the incisor bar set at 3.3 mm below the interaural line. The skull was exposed, cleaned and a hole was drilled above the right VTA (coordinates: 5.3 mm posterior to the bregma, 0.7 mm lateral to the midline, 7.0 mm ventral from the skull surface; Paxinos & Watson, 1998). A 23 G stainless-steel guide cannula was implanted 2 mm above the VTA to prevent its damage and fixed with two screws and dental cement to the skull. Then, the skin was repositioned and sutured. Finally, stylet (31 G stainless steel) was inserted into the guide cannula to prevent loss of cerebrospinal fluid and haemorrhage. Animals were housed in individual plastic cage ($40\times23\times20\,cm^3$) in the same controlled conditions and allowed to recover for 5–7 days. Afterwards, the rats were reanaesthetized with sodium pentobarbitone (65 mg kg^{-1}, i.p.) and an intravascular siliconized (Sigmacote, Sigma, St Louis, MO, U.S.A.) polyethylene tubing PE-50 catheter (Intramedics, Clay Adams, NJ, U.S.A.), filled with physiological saline and 100 i.u. ml^{-1} heparin sodium salt (Sigma, St Louis, MO, U.S.A.), was inserted into the abdominal aorta *via* the femoral artery. Then, the catheter was tunnelled s.c. and exteriorized at the back of the neck for direct blood pressure recording. Recovery from anaesthesia was monitored closely under a warming lamp to maintain the body temperature of the animals. Thereafter, rats were housed individually in polyethylene cages with a top grid and returned to their resident room. On the subsequent days, rats that lost more than 25% of their body weight or had clear signs of cerebral haemorrhage, atypical behaviour or weaknesses were immediately humanely killed with an overdose of pentobarbitone. Experimental protocols were initiated 24–48 h after the last surgery in conscious and freely moving rats.

Measurement of cardiovascular parameters

During all experiments, continuous direct recordings of pulse blood pressure and HR were made, respectively, with a Statham pressure Transducer (P23ID) and a cardiac tachometer (model 7P4) (triggered by the arterial blood pressure pulse) coupled to a Grass polygraph (model 79; Grass Instruments Co., Quincy, MA, U.S.A.). Cardiovascular responses were measured 1 h after the rats were transported to an isolated and quiet testing room, where only the experimenter had access. Rats remained in their resident cage, but the top grid was removed and they had no more access to the food and water for about 3–5 h, which corresponds to the

duration of the experiments. When resting blood pressure and HR were stable, a 31 G stainless-steel injector connected to a Hamilton microsyringe (5 μl Fisher Scientific Ltd, Montréal, Québec, Canada) with a PE-10 tubing was inserted into the guide cannula without handling the rat. The injector extended 2 mm beyond the guide cannula (17 mm) to penetrate in the VTA. Microinjections were made into the VTA of resting and freely behaving rats. All solutions were freshly prepared and injected (volume of 0.2 μl) 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.

Measurement of behavioural parameters

Behavioural activity was measured as reported previously (Picard *et al*., 1994). Briefly, during every consecutive period of 15 s, a score of 1 or 0 was given systematically depending on whether the animal showed the specific type of behaviour or not, whatever its frequency, intensity or duration during that period. Summation of scores for the first 30 min period following VTA injection provided the behavioural scores for face washing, sniffing, head scratching, digging and rearing. The maximal theoretical score was 120 (15 s intervals $\times$ 30 min). Wet dog shake behaviour was measured according to the number of episodes or frequency during the first 30 min period.

Histology

At the end of the experimental protocol, the rats were killed with an overdose of sodium pentobarbitone and received immediately 0.1 μl of Evans Blue dye (Sigma, St Louis, MO, U.S.A.) in VTA. The brains were removed and fixed with 10% ($v\,v^{-1}$) formol and 20% ($w\,v^{-1}$) sucrose. Coronal sections (40 μm, cut on freezing microtome) were mounted on glass slides and stained with cresyl violet for histological examination of the microinjection sites. Rats that showed any evidence of haemorrhage or extensive necrosis ($n=31$) were excluded from the study.

Experimental protocols

Experiment 1: dose–response curves to VTA microinjection of selective tachykinin agonists Two different groups of rats were used to construct the entire dose–responses curves with agonists: the first group was used for the doses of 1, 10 and 25 pmol, whereas the second group was used for the highest doses of 50, 100, 200 and 1000 pmol. Rats initially received a VTA microinjection of artificial cerebrospinal fluid

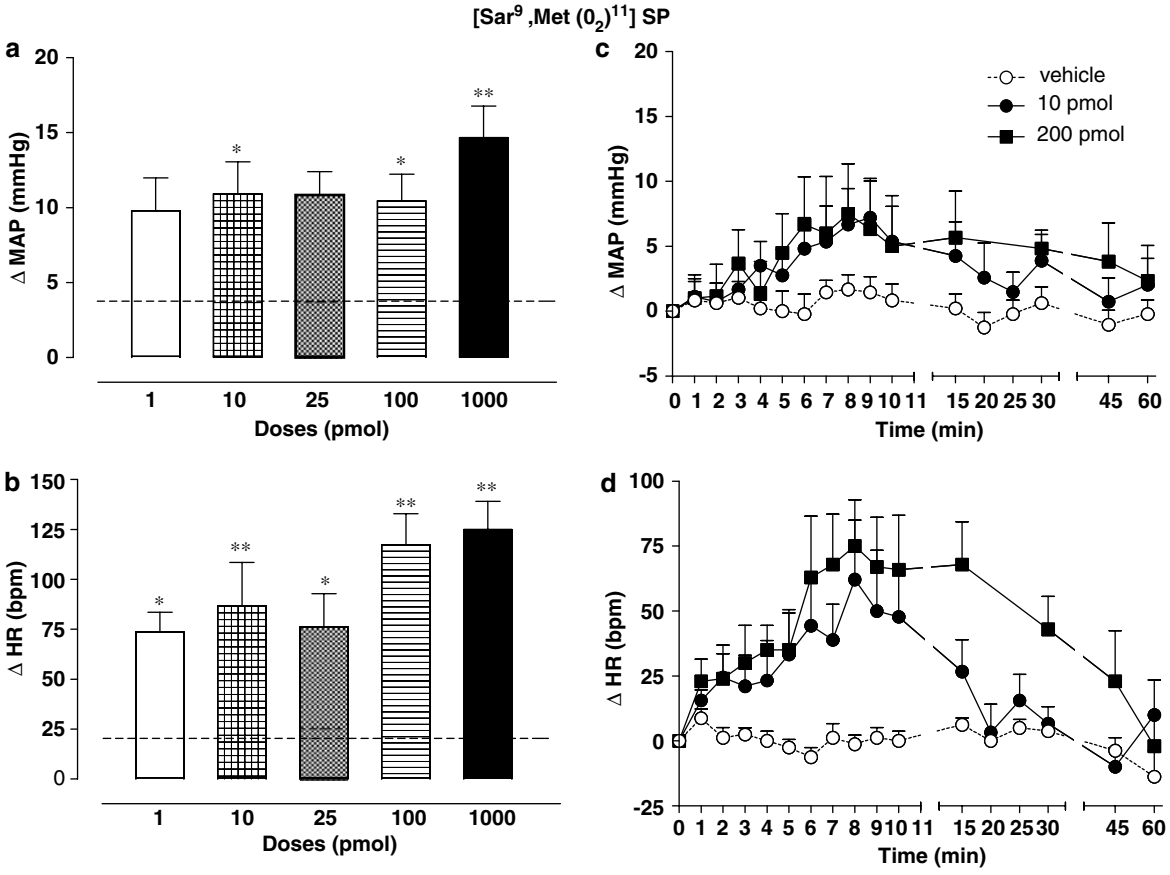

Figure 1 Maximal changes (a, b) and time-course effects (c, d) on mean arterial pressure (Δ MAP, a and c) and heart rate (Δ HR, b and d) following five increasing doses (1–1000 pmol, $n=18$) of [Sar^9, Met $(O_2)^{11}$] SP in the VTA. Values represent the mean $\pm$ s.e.m. of (n) rats. Comparison to vehicle values (dotted line) is indicated by $^*P<0.05$, $^{**}P<0.01$ (a, b). Statistical comparisons in (c) are 10 pmol ($F(1,16)=20.50$, $P<0.001$), 200 pmol ($F(1,19)=37.11$, $P<0.001$), and in (d) are 10 pmol ($F(1,16)=41.78$, $P<0.001$), 200 pmol ($F(1,19)=108.9$, $P<0.001$). Basal values were: MAP, 103.8 $\pm$ 4.1 mmHg; HR, 375 $\pm$ 18 b.p.m.

(aCSF) followed 60 min later by a single dose of either [Sar^9, Met $(O_2)^{11}$]SP (1–25 pmol ($n=8$), 50–1000 pmol ($n=10$)), [β-Ala^8]NKA (4–10) (1–25 pmol ($n=10$), 50–1000 pmol ($n=6$)) and senktide (1–25 pmol ($n=8$), 50–1000 pmol ($n=9$)). Each rat was selected randomly and injected with only one of the three agonists for the remainder of the protocol. For each group, increasing doses of [Sar^9, Met $(O_2)^{11}$]SP, [β-Ala^8]NKA (4–10) or senktide were given at 24 h intervals so the rats were used on 3–4 consecutive days.

Experiment 2: effects of VTA microinjection of selective tachykinin antagonists Rats that had previously (24 h) received 10 pmol of [Sar^9, Met $(O_2)^{11}$]SP or 25 pmol of either [β-Ala^8]NKA (4–10) or senktide were given VTA microinjection of the NK_1 antagonist LY 303870 (5 nmol against [Sar^9, Met $(O_2)^{11}$]SP ($n=8$) or senktide ($n=6$)), the NK_2 antagonist SR 48968 (250 pmol against [β-Ala^8]NKA (4–10) ($n=14$) or [Sar^9, Met $(O_2)^{11}$]SP ($n=6$)) and NK_3 antagonist SB 235375 (25 nmol against senktide ($n=6$) or [β-Ala^8]NKA (4–10) ($n=8$)). Then, the agonist was injected in the VTA 15 min later (25 pmol of NK_2 or NK_3 agonist) or 30 min later (10 pmol of NK_1 agonist). The agonists were reinjected alone 24 h later to assess the reversibility of any blockade observed in the presence of antagonist on the preceding day. This protocol also permitted to determine the selectivity of the blockade by each antagonist.

To control for specific blockade with the antagonist and to exclude any possibility that the blockade was not the consequence of receptor desensitization, the vehicle of LY 303870 (8% DMSO + 10% of 2-hydroxypropyl-β-cyclodextrin mixed with aCSF, $n=6$) and of SB 235375 (aCSF, $n=5$) or the opposite (R) enantiomer of SR 48968 (SR 48965 ([(*R*)-*N*-methyl-*N*-[4-acetylamino-4-phenylpiperidino-2-(3,4-dichlorophenyl) butyl]benzamide]), $n=8$) were injected prior to the agonists as described above with the antagonists. Doses of agonists in these protocols represent the minimal doses

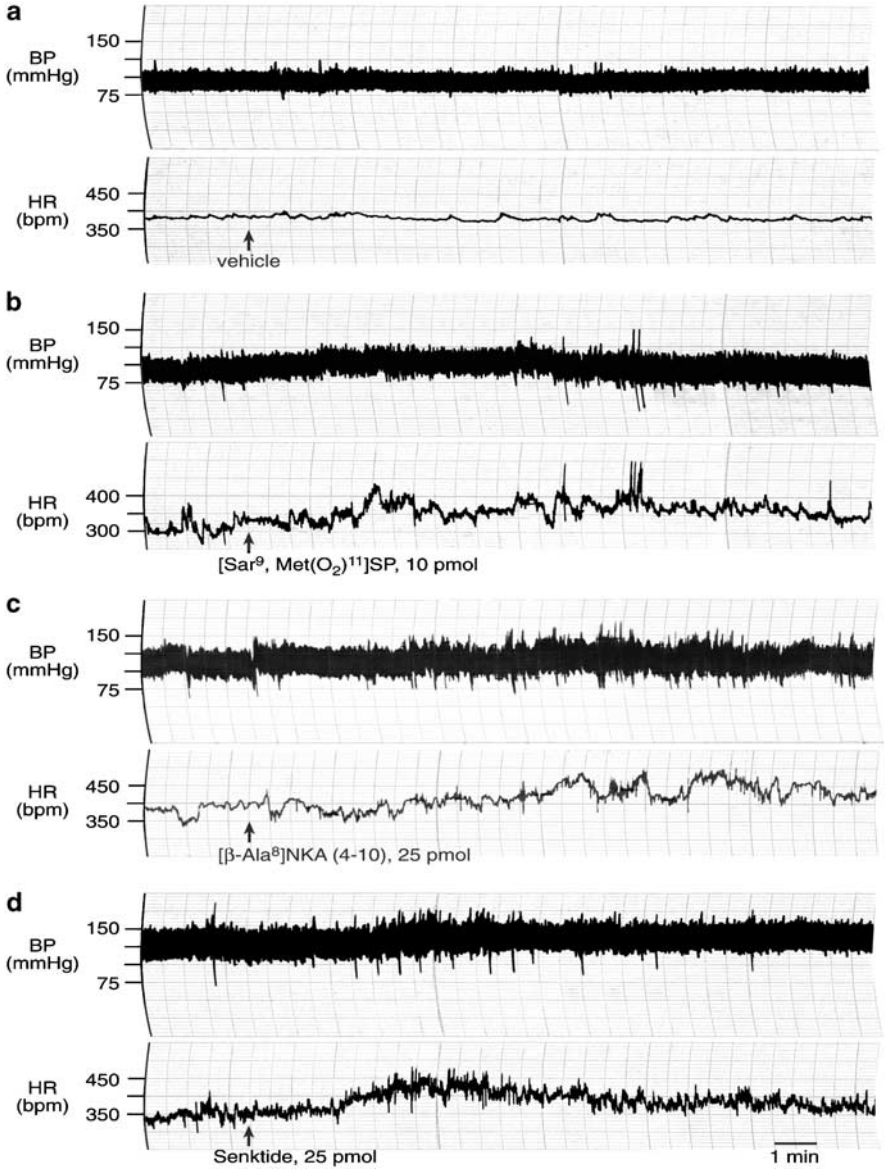

Figure 2 Original traces showing blood pressure (BP) and heart rate (HR) changes induced by VTA microinjection of (a) vehicle (aCSF), (b) [Sar^9, Met $(O_2)^{11}$]SP (10 pmol), (c) [β-Ala^8]NKA (4–10) (25 pmol) and (d) senktide (25 pmol) in freely moving rats.

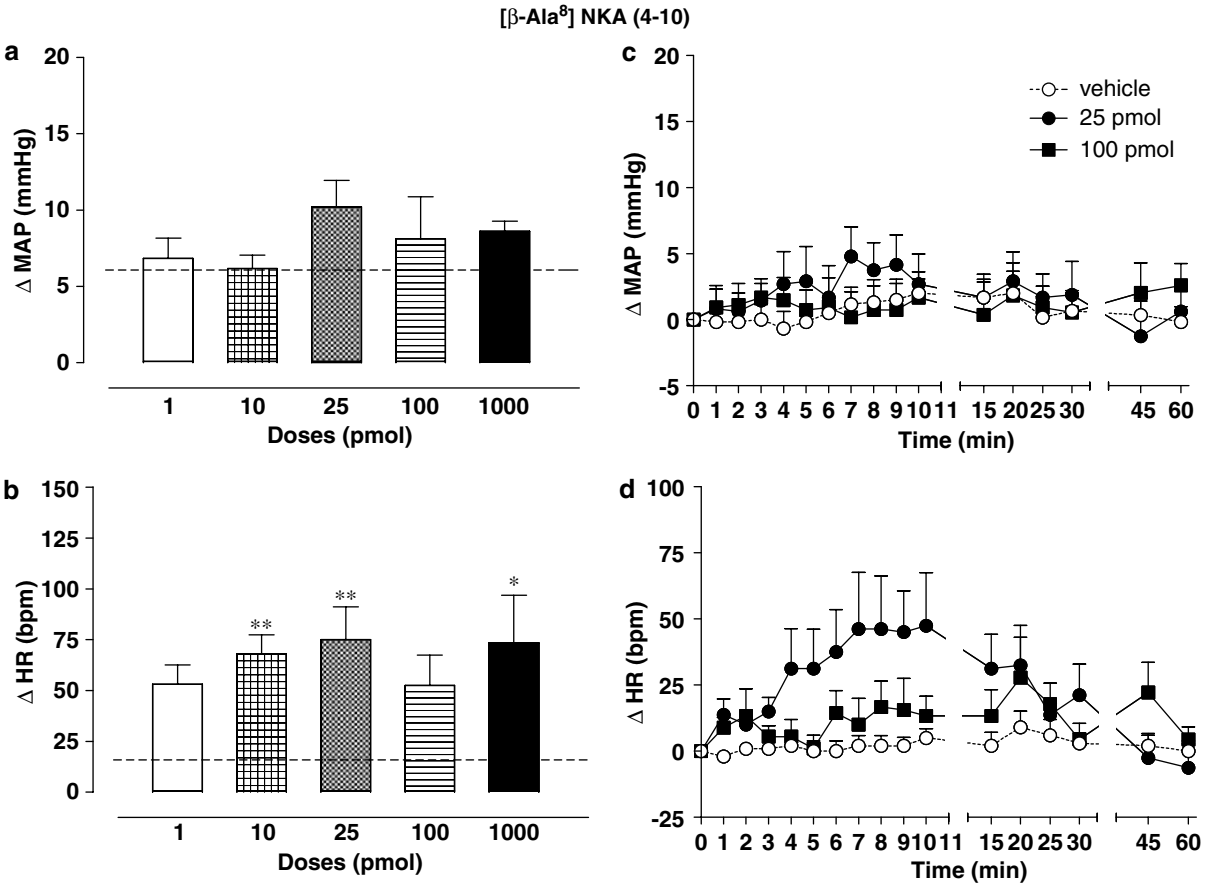

Figure 3 Maximal changes (a, b) and time-course effects (c, d) on mean arterial pressure (Δ MAP, a and c) and heart rate (Δ HR, b and d) following five increasing doses (1–1000 pmol, $n=16$) of [β-Ala^8]NKA (4–10) in the VTA. Values represent the mean ± s.e.m. of (n) rats. Comparison to vehicle values (dotted line) is indicated by $^*P<0.05$, $^{**}P<0.01$ (a, b). Statistical comparisons in (c) are 25 pmol ($F(1,17)=6.08$, $P<0.014$), 100 pmol ($F(1,17)=1.19$, $P>0.05$), and in (d) are 25 pmol ($F(1,17)=52.81$, $P<0.001$), 100 pmol ($F(1,17)=22.60$, $P<0.001$). Basal values were: MAP, 105.2 ± 2.5 mmHg; HR, 372 ± 10 b.p.m.

displaying significant cardiovascular effects on the pre-established dose–response curves. The doses of LY 303870 and SB 235375 were based on a series of preliminary experiments that showed potent inhibition without side effects at 5 and 25 nmol, respectively. Lower doses of antagonists provided only partial blockade. The dose of SR 48968 (250 pmol) was based on a previous study that reported an effective inhibition of the intranigral effects of 25 pmol [β-Ala^8]NKA (4–10) in the same animal model (Lessard & Couture, 2001).

Experiment 3: effects of VTA microinjection of glutamic acid A separate group of rats ($n=5$) received a VTA microinjection of aCSF followed 60 min later by a single dose of glutamic acid (500 nmol) to excite selectively neuronal cell bodies (Goodchild *et al*., 1982). Microinjection of this excitatory amino acid into the VTA caused cardiovascular depressor effect through the stimulation of dopamine neurons in alpha-chloralose-anaesthetized, paralysed and artificially ventilated rats (Kirouac & Ciriello, 1997a).

Experiment 4: effects of systemic treatments with dopamine and β_1-adrenergic receptor antagonists Rats that had previously (24 h) received microinjection of 25 pmol of either [Sar^9, Met $(O_2)^{11}$]SP, [β-Ala^8]NKA (4–10) or senktide were given intravenous (i.v.) injection of the D_1 dopamine antagonist SCH23390 (0.2 mg kg^{-1}, $n=6$–7 for each agonist) (Kirouac & Ciriello, 1997a; Gioanni *et al*., 1998), the D_2 dopamine antagonist raclopride (0.16 mg kg^{-1}, $n=3$ for each agonist) (Millan *et al*., 1998) and the β_1-adrenoceptor antagonist atenolol (5 mg kg^{-1}, $n=5$–8 for each agonist) (Lessard & Couture, 2001). Then, the tachykinin agonists were injected at the same dose in the VTA 15–20 min and 24 h later.

Drugs and solutions

The aCSF was purchased from Harward Bioscience (Massachusetts, U.S.A.). [Sar^9, Met $(O_2)^{11}$]SP (MW: 1392.9), [β-Ala^8]NKA (4–10) (MW: 780.9) and succinyl-[Asp^6, MePhe^8]SP(6–11) (senktide) (MW: 842) were all purchased from Bachem Bioscience Inc. (King of Prussia, PA, U.S.A.). The nonpeptide NK_1 antagonist LY 303870 (MW: 686.7) was obtained from Eli Lilly (Indianapolis, IN, U.S.A.), the nonpeptide NK_2 antagonist SR 48968 (MW: 552.5) and its opposite enantiomer SR 48965 were provided by

Dr X. Emonds-Alt (Sanofi Recherche, Montpellier, France). The nonpeptide NK_3 antagonist SB 235375 (MW: 437) was obtained from Dr H.M. Sarau (GlaxoSmithKline, PA, U.S.A.). [Sar^9, Met $(O_2)^{11}$]SP and SB 235375 were dissolved directly in aCSF, whereas the other agonists and antagonists were solubilized in dimethyl sulphoxide (DMSO; Fisher, Montréal, Québec, Canada), and aCSF mixed with 2-hydroxypropyl-β-cyclodextrin (Sigma, St Louis, MO, U.S.A.) was added to obtain the desired solution (final solutions contained less than 15% DMSO and 20% 2-hydroxypropyl-β-cyclodextrin). Glutamic acid, atenolol and raclopride were purchased from Sigma (St Louis, MO, U.S.A.) and SCH23390 from Tocris (Ellisville, MO, U.S.A.). Atenolol and glutamic acid were dissolved directly in saline and aCSF, respectively, while SCH23390 and raclopride were dissolved in DMSO and completed in saline (final solutions contained 3% DMSO). In all experiments, vehicle was tested as control and no significant effects were seen on any parameters when compared to baseline values. Stock solutions (1–10 mg ml^{-1}) of agonists and antagonists were stored in aliquot of 20–100 μl at -20°C until use.

Statistical analysis of data

Results are expressed as means $\pm$ s.e.m. of (n) rats. Maximal effects were analysed for statistical significance by a one-way analysis of variance (ANOVA) followed by a Dunnett test for multiple comparisons with one control group. The time course of the effects was analysed with a two-way ANOVA. Behavioural values were evaluated with the Kruskal–Wallis nonparametric test and the paired Student's t-test. Only probability values (P) less than 0.05 were considered to be statistically significant.

Results

Cardiovascular effects of the NK_1 agonist, [Sar^9, Met $(O_2)^{11}$]SP

The effects of five increasing doses of [Sar^9, Met $(O_2)^{11}$]SP on MAP and HR are shown in Figure 1. The NK_1 agonist produced significant increases ($P<0.05$) in MAP and HR when compared to vehicle values (aCSF). The maximum rises in MAP were significant at 10 pmol (11 ± 2 mmHg, $n=8$), 100 pmol (10 ± 2 mmHg, $n=10$) and 1000 pmol (15 ± 2 mmHg, $n=10$). The maximum increases in HR evoked by [Sar^9, Met $(O_2)^{11}$]SP were significant at all doses: 1 pmol (74 ± 10 b.p.m.), 10 pmol (87 ± 22 b.p.m.), 25 pmol (76 ± 17 b.p.m.), 100 pmol (117 ± 16 b.p.m.) and 1000 pmol (125 ± 14 b.p.m.). The effect of 10 and 200 pmol [Sar^9, Met $(O_2)^{11}$]SP peaked at 8 min postinjection for MAP and HR. Although the pressor response

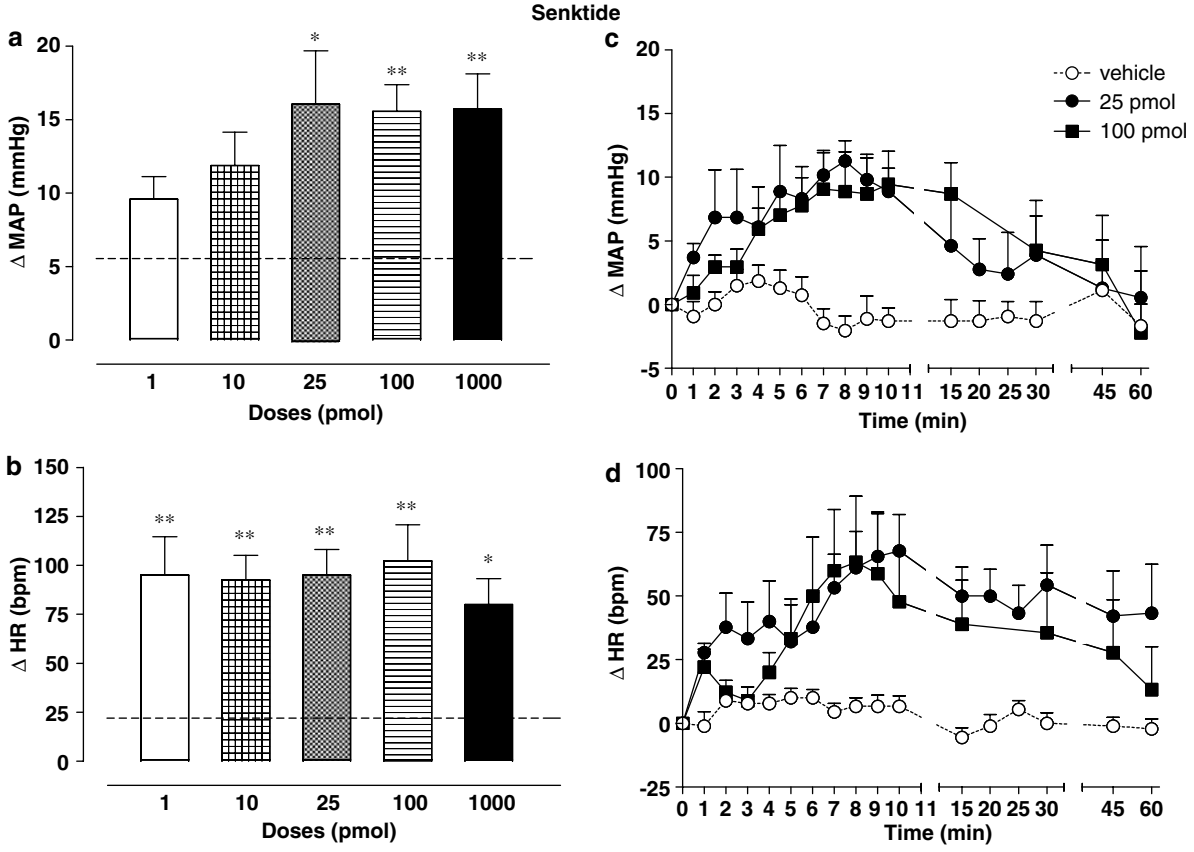

Figure 4 Maximal changes (a, b) and time-course effects (c, d) on mean arterial pressure (Δ MAP, a and c) and heart rate (Δ HR, b and d) following five increasing doses (1–1000 pmol, $n=17$) of senktide in the VTA. Values represent the mean $\pm$ s.e.m.of (n) rats. Comparison to vehicle values (dotted line) is indicated by $^*P<0.05$, $^{**}P<0.01$ (a, b). Statistical comparisons in (c) are 25 pmol ($F(1,15)=50.77$, $P<0.001$), 100 pmol ($F(1,17)=41.42$, $P<0.001$), and in (d) are 25 pmol ($F(1,15)=102.8$, $P<0.001$), 100 pmol ($F(1,17)=51.28$, $P<0.001$). Basal values were: MAP, 102.9 ± 6.2 mmHg; HR, 354 ± 18 b.p.m.

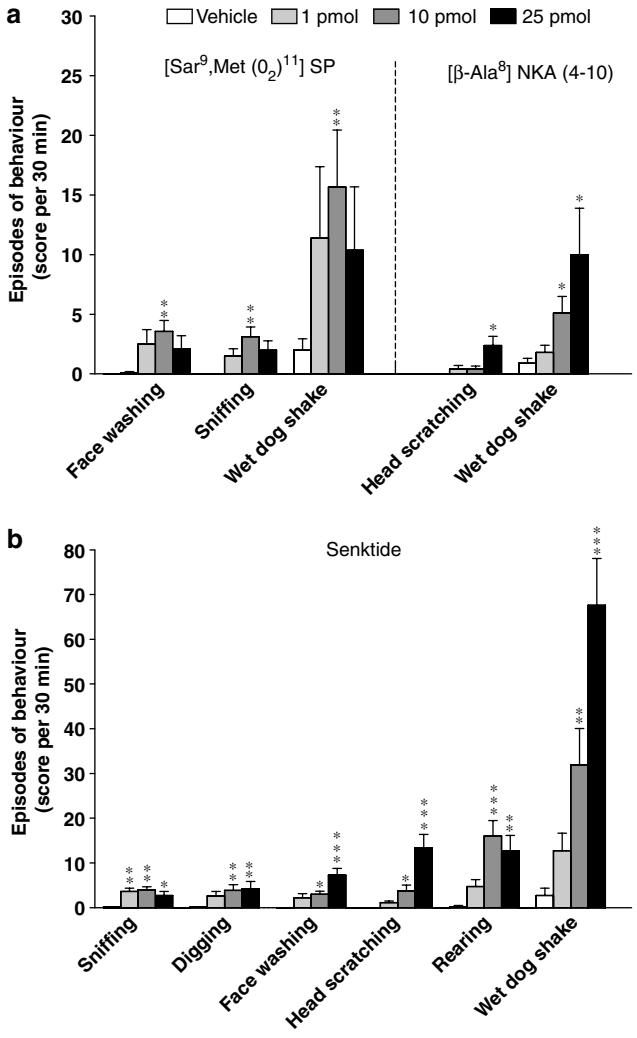

Figure 5 Bar graphs showing changes in behavioural activity induced by VTA microinjection of [Sar^9, Met $(O_2)^{11}$]SP (a, left), [β-Ala^8]NKA (4–10) (a, right) and senktide (b) at 1, 10 and 25 pmol during the first 30 min postinjection period in conscious rats (same rats than Figures 1, 3 and 4). Each bar represents the mean $\pm$ s.e.m. (n values are given in the previous legends). Comparison to vehicle values is indicated by $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$.

was already maximal at 10 pmol, the tachycardia was slightly greater and more prolonged at 200 pmol (Figures 1c, d and 2b).

Cardiovascular effects of the NK$_2$ agonist, [β-Ala^8]NKA (4–10)

The effects of five increasing doses of [β-Ala^8]NKA (4–10) on MAP and HR are shown in Figure 3. [β-Ala^8]NKA (4–10) had a weak but significant effect on MAP at 25 pmol; the time-course effect was significant when compared with vehicle (aCSF + 10% DMSO), yet maximal values did not reach statistical significance (Figure 3a and c). The NK_2 agonist increased significantly HR when compared with vehicle values, but not at all doses since the agonist caused also bradycardia at 1 pmol (-30 b.p.m. in 1/10 rat), 10 pmol (-33 ± 3 b.p.m. in 3/10 rats), 25 pmol (-33 ± 3 b.p.m. in 3/8 rats), 100 pmol (-55 ± 15 b.p.m. in 2/10 rats) and 1000 pmol (-45 ± 9 b.p.m. in 4/6 rats). Thus, the maximum rise in HR produced by [β-Ala^8]NKA (4–10) was not significant at 1 and 100 pmol, yet it was significant at 10 pmol (68 ± 9 b.p.m., $n=10$), 25 pmol (75 ± 16 b.p.m., $n=10$) and 1000 pmol (73 ± 23 b.p.m., $n=6$). The tachycardia produced at 25 pmol reached its maximum at 7 min postinjection (Figures 2c and 3d). A larger dose (100 pmol) caused a smaller tachycardia because of the predominant bradycardia. The effect of 25 pmol was reproduced after the preinjection of 100 pmol of [β-Ala^8]NKA (4–10). Moreover, the effect of the agonist at 100 pmol was similarly small when injected for the first time to naïve animals (data not shown), excluding therefore the possibility that the lower tachycardia elicited by the NK_2 agonist at higher doses is due to a desensitization mechanism caused by the prior injections of agonist.

Cardiovascular effects of the NK$_3$ agonist, senktide

The effects of five increasing doses of senktide on MAP and HR are shown in Figure 4. The NK_3 agonist induced pressor responses and tachycardia, which were significant ($P<0.05$) compared with vehicle values (aCSF + 10% DMSO). The maximal pressor responses produced by senktide were significant at 25 pmol (16 ± 4 mmHg, $n=8$), 100 pmol (16 ± 2 mmHg, $n=9$) and 1000 pmol (16 ± 2 mmHg, $n=9$). The maximum rises in HR were significant at all doses: 1 pmol (95 ± 19 b.p.m.), 10 pmol (92 ± 13 b.p.m.), 25 pmol (95 ± 13 b.p.m.), 100 pmol (102 ± 18 b.p.m.) and 1000 pmol (80 ± 13 b.p.m.). Thus, maximal increases in MAP and HR were obtained at 25 and 1 pmol, respectively. The pressor and tachycardic responses induced by 25 and 100 pmol senktide peaked at 8–9 min postinjection and displayed similar time-course effect at both doses (Figures 2d and 4c, d).

Behavioural responses to tachykinin agonists

The injection of [Sar^9, Met $(O_2)^{11}$]SP and [β-Ala^8]NKA (4–10) into the VTA increased behavioural activity, but to a lower extent than senktide (Figure 5). While the maximal responses were obtained at 10 pmol for [Sar^9, Met $(O_2)^{11}$]SP (face washing = sniffing < wet dog shake), [β-Ala^8]NKA (4–10) increased dose-dependently head scratching and wet dog shake, reaching its nadir at 25 pmol (head scratching < wet dog shake). Senktide increased in a dose-dependent manner face washing, head scratching and wet dog shake with a maximal effect at 25 pmol. The rearing behaviour was already maximal at 10 pmol as sniffing and digging behaviours whose effects were smaller. The three tachykinin agonists failed to cause additional behavioural effects when injected at doses of 100 and 1000 pmol (data not shown). At the lowest dose of 1 pmol, [Sar^9, Met $(O_2)^{11}$]SP and senktide had no significant effect on behaviour, while HR was significantly increased and reached its maximal value with senktide. This suggests a dissociation of the HR and behavioural response to NK_1 and NK_3 agonists. At higher doses, tachykinin agonists caused behavioural and cardiovascular changes with a similar time course.

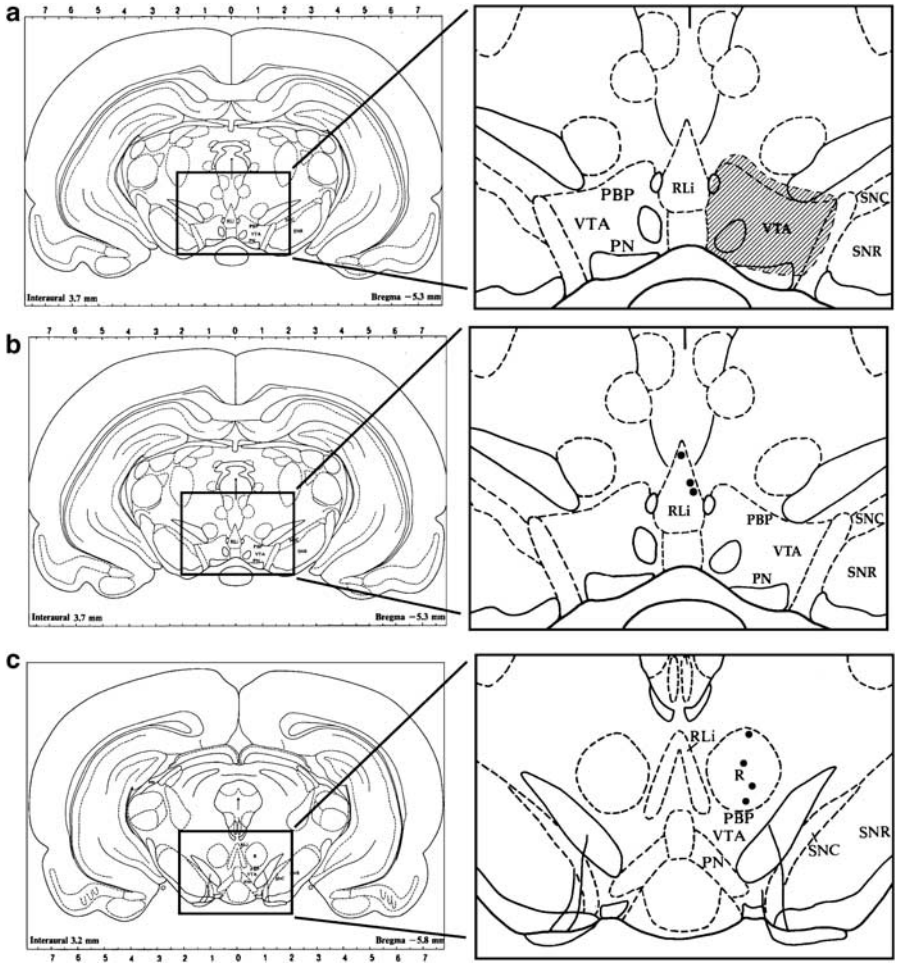

Figure 6 Diagrams showing the sites of injection of tachykinin agonists and antagonists. Successful injections in the VTA are represented by hatched bars ($n=189$) (a). As control, [Sar^9, Met $(O_2)^{11}$]SP (10 pmol), [β-Ala^8]NKA (4–10) (25 pmol) and senktide (25 pmol) were also injected in the rostral linear nucleus raphe (Rli) ($n=3$) (b) and in the red nucleus ($n=4$) (c). Points indicate the injection sites in (b) and (c). Diagrams were adapted from the Atlas of Paxinos & Watson (1998). Abbreviations: PBP, parabrachial pigmented nucleus; PN, paranigral nucleus; SNC, substantia nigra compact; SNR, substantia nigra reticular; VTA, ventral tegmental area.

Effects of tachykinin agonists injected outside the VTA

When [Sar^9, Met $(O_2)^{11}$]SP (10 pmol), [β-Ala^8]NKA (4–10) (25 pmol) or senktide (25 pmol) were injected outside the VTA in the rostral linear nucleus raphe ($n=3$) (Figure 6b) or in the red nucleus ($n=4$) (Figure 6c), variable and no significant cardiovascular and behavioural effects were measured in comparison with vehicle values. All results included in the study ($n=189$) were found within the VTA (Figure 6a).

Effects of LY 303870 on cardiovascular responses induced by [Sar^9, Met $(O_2)^{11}$]SP and senktide

The effects of the tachykinin NK_1 antagonist LY 303870 and its vehicle on [Sar^9, Met $(O_2)^{11}$]SP or senktide-induced cardiovascular responses are shown in Figure 7 and Tables 1 and 2. The MAP and HR responses to [Sar^9, Met $(O_2)^{11}$]SP (10 pmol) were completely abolished by 5 nmol LY 303870 (Figure 7a). The effects of [Sar^9, Met $(O_2)^{11}$]SP on MAP and HR were partly back 24 h after LY 303870 injection. On the other hand, the cardiovascular responses induced by 10 pmol senktide were not significantly altered by 5 nmol LY 303870 (Table 1). Also, the vehicle of LY 303870 (aCSF + 8% DMSO and 10% of 2-hydroxypropyl-β-cyclodextrin) had no effect on the pressor response and the tachycardia induced by [Sar^9, Met $(O_2)^{11}$]SP administered 30 min or 24 h later (Table 2). LY 303870 or its vehicle had no direct effect on MAP and HR when compared to aCSF (data not shown).

Effects of SR 48968 on cardiovascular responses induced by [β-Ala^8]NKA (4–10) and [Sar^9, Met $(O_2)^{11}$]SP

Effects of the NK_2 antagonist SR 48968 and its inactive enantiomer SR 48965 on the cardiovascular changes to [β-Ala^8]NKA (4–10) and [Sar^9, Met $(O_2)^{11}$]SP are shown in Figure 7 and Tables 1 and 2. The increases in MAP and HR induced by [β-Ala^8]NKA (4–10) at 25 pmol were completely abolished by 250 pmol SR 48968 and the responses to the

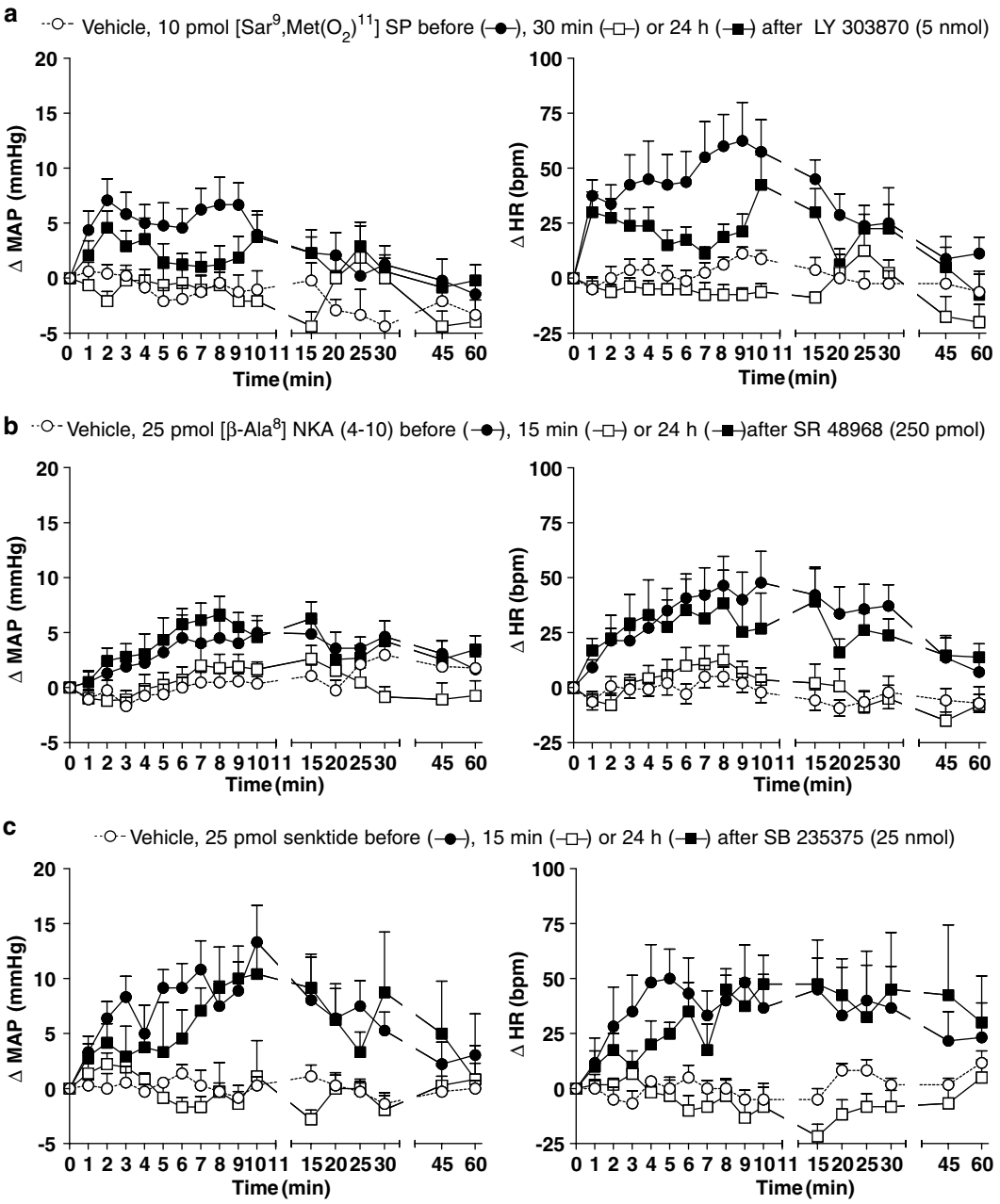

Figure 7 Time-course effects on changes in mean arterial pressure (Δ MAP, left) and heart rate (Δ HR, right) responses induced by (a) [Sar^9, Met $(O_2)^{11}$]SP (10 pmol, $n=8$), (b) [β-Ala^8]NKA (4–10) (25 pmol, $n=14$), (c) senktide (25 pmol, $n=6$) injected in the VTA before or after their respective antagonist. Values represent the mean ± s.e.m. of (n) rats. Statistical comparisons to the agonist before treatment with antagonist are (a) MAP ($F(1,15)=67.33$, $P<0.001$), HR ($F(1,15)=154$, $P<0.001$); (b) MAP ($F(1,27)=39.12$, $P<0.001$), HR ($F(1,27)=101$, $P<0.001$); and (c) MAP ($F(1,11)=99.54$, $P<0.001$); HR ($F(1,11)=87.58$, $P<0.001$). Basal values were: (a) MAP, 98.5 ± 5.8 mmHg; HR, 381 ± 17 b.p.m., (b) MAP, 100.8 ± 2.8 mmHg; HR, 372 ± 16 b.p.m., and (c) MAP, 97.5 ± 7.4 mmHg; HR, 403 ± 21 b.p.m.

agonist were entirely back to preantagonist values 24 h later (Figure 7b). In contrast, the same treatment with SR 48968 failed to significantly alter the cardiovascular effects produced by 25 pmol [Sar^9, Met $(O_2)^{11}$]SP (Table 1). Also, the inactive enantiomer, SR 48965 (250 pmol), failed to affect both the MAP and HR responses to [β-Ala^8]NKA (4–10) (25 pmol) (Table 2). SR 48968 and SR 48965 or their vehicles had no direct effect on MAP and HR when compared to aCSF (data not shown).

Effects of SB 235375 on cardiovascular responses induced by senktide and [β-Ala^8]NKA (4–10)

The effects of the tachykinin NK_3 antagonist SB 235375 and its vehicle on the cardiovascular responses evoked by senktide and [β-Ala^8]NKA (4–10) are shown in Figure 7 and Tables 1 and 2. The increases in MAP and HR induced by 25 pmol of senktide were abolished by 25 nmol SB 235375 (Figure 7c). These cardiovascular effects of senktide were back to

Table 1 Selectivity of the inhibitions by the tachykinin antagonists

Treatment	*Δ MAP* (mmHg)	*Δ HR* (b.p.m.)	n
A. LY 303870 (5 nmol)			
Vehicle	6.1±1.3	22±4	6
Senktide, 10 pmol (before)	15.6±1.7**	107±8**	6
Senktide, 10 pmol (30 min after)	15.6±1.7**	125±17**	6
B. SR 48968 (250 pmol)			
Vehicle	4.7±1.0	15±3	6
[Sar^9, Met $(O_2)^{11}$]SP, 25 pmol (before)	9.4±1.6	82±14**	6
[Sar^9, Met $(O_2)^{11}$]SP, 25 pmol (15 min after)	12.0±2.0*	117±16**	6
C. SB 235375 (25 nmol)			
Vehicle	3.5±0.6	20±3	8
[β-Ala^8]NKA (4–10), 25 pmol (before)	10.4±1.4**	66±10**	8
[β-Ala^8]NKA (4–10), 25 pmol (15 min after)	10.0±1.7**	78±15**	8

Data are means±(*n*) rats. Statistical comparison to vehicle values is indicated by $*P<0.05$, $**P<0.01$. Statistical comparison to the agonist before treatment with antagonist is not significantly different in (A), (B) and (C).

Table 2 Influence of antagonist vehicles or enantiomer on the cardiovascular responses to agonists

Treatment	*Δ MAP* (mmHg)	*Δ HR* (b.p.m.)	n
A. LY 303870 – vehicle			
Vehicle	3.3±0.9	17±3	6
[Sar^9, Met $(O_2)^{11}$]SP, 10 pmol (before)	12.2±0.6*	70±6*	6
[Sar^9, Met $(O_2)^{11}$]SP, 10 pmol (30 min after)	14.2±2.9**	82±19**	6
[Sar^9, Met $(O_2)^{11}$]SP, 10 pmol (24 h after)	13.1±2.3**	77±15**	6
B. SR 48965			
Vehicle	5 .4±1.1	20±7	8
[β-Ala^8]NKA (4–10), 25 pmol (before)	9.8±2.7	76±18*	8
[β-Ala^8]NKA (4–10), 25 pmol (15 min after)	8.1±1.8	75±15*	8
[β-Ala^8]NKA (4–10), 25 pmol (24 h after)	13.6±2.3*	76±19*	7
C. SB 235375 – vehicle			
Vehicle	4.3±0.9	16±4	5
Senktide, 25 pmol (before)	12.3±2.4	86±17*	5
Senktide, 25 pmol (15 min after)	14.0±4.3	92±17*	5
Senktide, 25 pmol (24 h after)	18.0±1.9**	140±28**	5

Data are means±(*n*) rats. Statistical comparison to vehicle values is indicated by $*P<0.05$, $**P<0.01$. Statistical comparison to the agonist before treatment with the antagonist – vehicle (A and C) or with the enantiomer SR 48965 (B) is not significantly different.

preantagonist values when the agonist was reinjected alone 24 h later. Although SB 235375 at 25 nmol was effective against senktide, it failed to affect the changes in MAP and HR induced by 25 pmol [β-Ala^8]NKA (4–10) (Table 1). Also, the vehicle of SB 235375 (aCSF) did not alter significantly the MAP and HR responses elicited by senktide injected either 15 min or 24 h later (25 pmol) (Table 2). SB 235375 had no direct effect on MAP and HR when compared to aCSF (data not shown).

Effects of tachykinin antagonists on tachykinin agonists-induced behavioural activity

The effects of LY 303870, SR 48968 and SB 235375 against the changes of behavioural activity induced by their respective agonist ([Sar^9, Met$(O_2)^{11}$]SP, [β-Ala^8]NKA (4–10) and senktide) are shown in Figure 8. The sniffing behaviour induced by [Sar^9, Met$(O_2)^{11}$]SP (10 pmol) was blocked by LY 303870 (5 nmol), and this behaviour was not completely restored 24 h later. The face washing and wet dog shake behaviours induced by the NK_1 agonist were no longer significant in rats pretreated with LY 303870 (Figure 8a). Head scratching was not significantly increased by 25 pmol [β-Ala^8]NKA (4–10) in this group of rats on day 1 (prior to antagonist) or in the presence of NK_2 antagonist. SR 48968 (250 pmol) failed to alter wet dog shakes induced by the agonist (Figure 8a). SB 235375 (25 nmol) inhibited completely and in a reversible manner (24 h later) the majority of behavioural manifestations evoked by 25 pmol senktide (face washing, digging, rearing and wet dog shake) (Figure 8b). None of the antagonists caused behavioural changes when administered alone (data not shown).

Effects of dopamine antagonists on tachykinin agonists-induced central effects

The effects of SCH23390 (0.2 mg kg^{-1}, i.v.), D_1 dopamine antagonist, on the cardiovascular responses induced by

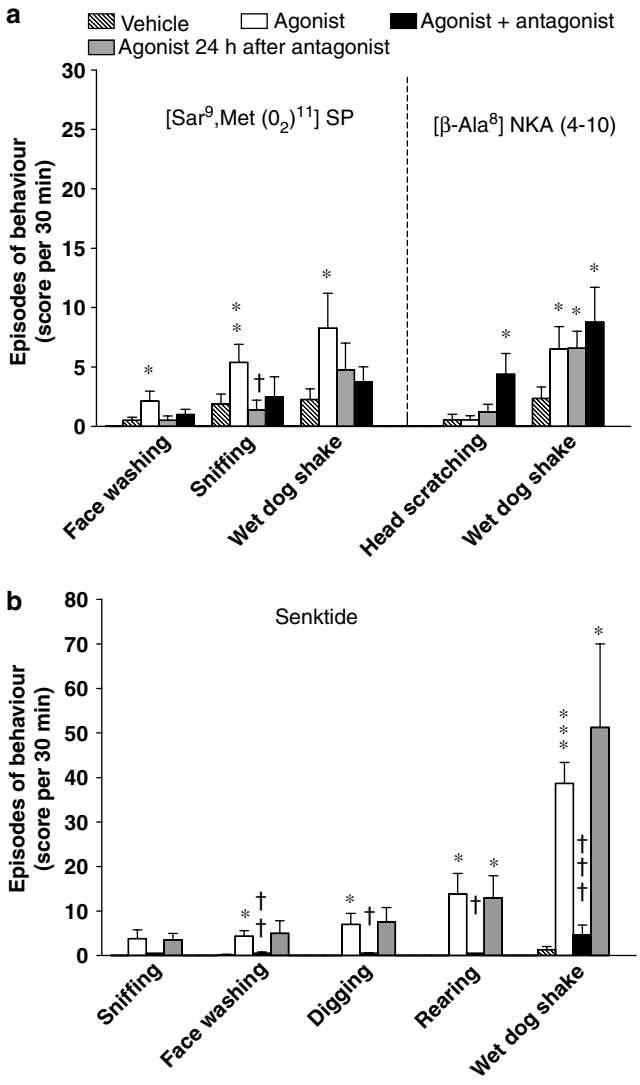

Figure 8 Bar graphs showing changes in behavioural activity induced by (a) [Sar^9, Met $(O_2)^{11}$]SP (10 pmol) and [β-Ala^8]NKA (4–10) (25 pmol) following the injection of their respective antagonist: LY 303870 (5 nmol) and SR 48968 (250 pmol) (b) senktide (25 pmol) following the injection of SB 235375 (25 nmol) during the first 30 min postinjection period in conscious rats (same rats than Figure 7). Each bar represents the mean $\pm$ s.e.m. (n values are given in Figure 7). Comparison to vehicle values is indicated by $^*P<0.05$, $^{***}P<0.001$ and to the agonist before treatment with antagonist by $^{\dagger}P<0.05$, $^{\dagger\dagger\dagger}P<0.001$.

tachykinin agonists (25 pmol) in the VTA are shown in Figure 9. SCH23390 blocked completely the tachycardia induced by [Sar^9, Met$(O_2)^{11}$]SP, [β-Ala^8]NKA (4–10) and senktide. This inhibition was reversible as the reinjection of agonists 24 h later caused the same HR responses than before the treatment with the D_1 dopamine antagonist. The latter also inhibited in a reversible manner the pressor response induced by senktide, but failed to affect significantly the response to [Sar^9, Met$(O_2)^{11}$]SP. The pressor response to [β-Ala^8]NKA (4–10) was not significant either in the absence or in the presence of SCH23390. With respect to behaviours, SCH23390 blocked wet dog shakes (score per 30 min) induced by [Sar^9, Met$(O_2)^{11}$]SP (vehicle: 1.9 ± 0.9; agonist NK_1: 10.4 ± 4.5, $P<0.05$; agonist + SCH23390: 3.4 ± 0.9, $n=7$) and senktide (vehicle: 2.0 ± 1.0; agonist NK_3: 24.5 ± 9.1, $P<0.05$; agonist + SCH23390: 5.0 ± 2.6, $P<0.05$, $n=7$), but not by [β-Ala^8]NKA (4–10) (vehicle: 0.6 ± 0.3; agonist NK_2: 8.7 ± 5.3; agonist + SCH23390: 6.1 ± 2.2, $n=6$). Rearing induced by senktide was also abolished by SCH23390 (vehicle: 0.0; senktide: 14.5 ± 5.4; senktide + SCH23390: 0.0, $P<0.001$, $n=7$). SCH23390 blocked all other behavioural manifestations induced by the three tachykinin agonists.

On the other hand, systemic administration of raclopride (0.16 mg kg^{-1}, i.v.), D_2 dopamine antagonist, failed to affect both the cardiovascular and behavioural responses induced by the administration of 25 pmol [Sar^9, Met$(O_2)^{11}$]SP, [β-Ala^8] NKA (4–10) or senktide in the VTA (data not shown).

Effects of β*-adrenoceptor antagonist on tachykinin agonists-induced central effects*

The effects of atenolol (5 mg kg^{-1}, i.v.), *β_1-adrenoceptor antagonist*, on the cardiovascular responses induced by tachykinin agonists (25 pmol) in the VTA are shown in Figure 10. Atenolol blocked completely the tachycardia induced by [Sar^9, Met$(O_2)^{11}$]SP, [β-Ala^8]NKA (4–10) and senktide. Whereas the pressor response induced by the two former agonists was reduced to levels not significantly different from vehicle values, the pressor response evoked by senktide was completely blocked by atenolol. This treatment with atenolol failed to affect significantly behavioural changes elicited by the three tachykinin agonists (data not shown).

Effects of glutamate in the VTA

As control experiments, glutamic acid (500 nmol) was injected in the VTA. Results presented in Figure 11 show that glutamic acid elicited increases of blood pressure and HR that peaked at 3 and 5 min, respectively, and behavioural activity (wet dog shake > sniffing > face washing > grooming > rearing > head scratching > digging). However, only HR response reached statistical significance when compared to vehicle values.

Discussion

The mesocorticolimbic dopaminergic system originating in the VTA is traditionally known for its implication in the regulation of locomotor activity, stress responses, reinforcement and rewards mechanisms (Le Moal & Simon, 1991; Swanson 2000; Hyman & Malenka, 2001). However, recent evidence highlights a putative role for this system in cardiovascular control (Kirouac & Ciriello, 1997a, b; van den Buuse, 1997; 1998; Vaughan *et al.*, 1999). The present study provides the first pharmacological evidence that the three tachykinin receptors may exert a neuromodulatory role in cardiovascular regulation in this midbrain area through a dopaminergic mechanism. Previous studies reported cardiovascular changes with fairly high doses (10 nmol) of DiMe-C7 in the VTA (Cornish & van den Buuse, 1994; 1995; van den Buuse, 1998). In contrast, the present data show cardiovascular responses with physiological doses (1–25 pmol) of tachykinin NK_1, NK_2 and NK_3 receptor agonists in freely behaving rats. Moreover, both the cardiovascular and behavioural effects elicited by agonists were selectively and reversibly blocked by their respective receptor antagonist. As

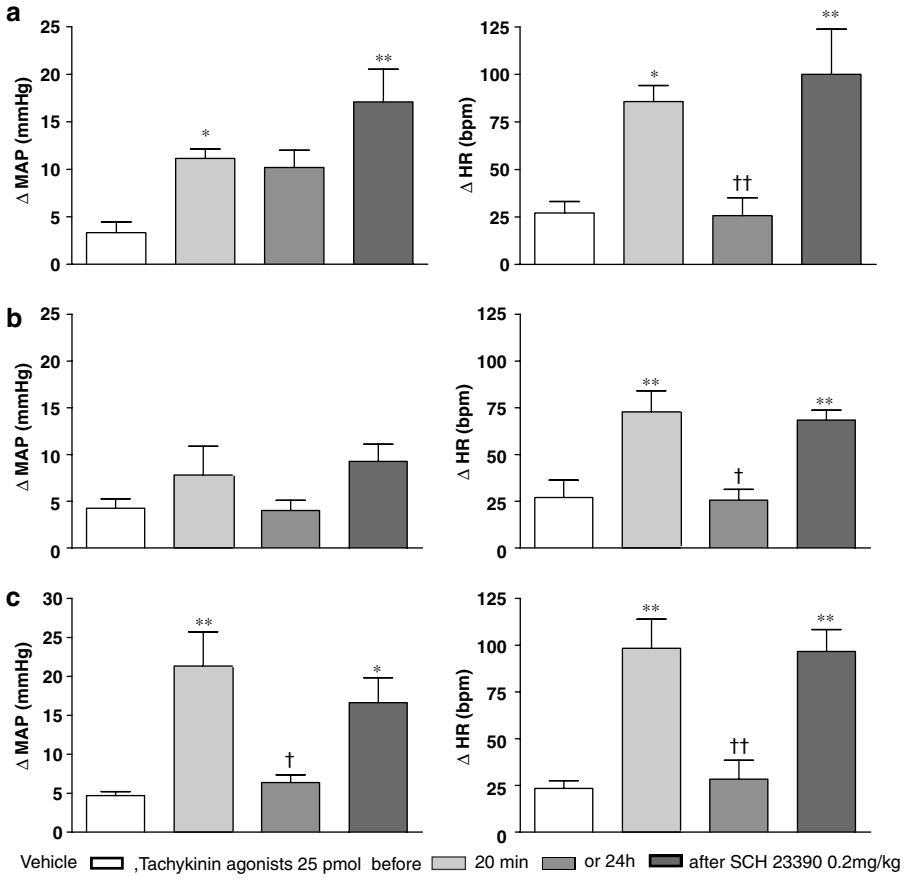

Figure 9 Maximal changes on mean arterial pressure (Δ MAP, left) and heart rate (Δ HR, right) following the injection of 25 pmol [Sar^9, Met $(O_2)^{11}$]SP (a), [β-Ala^8]NKA (4–10) (b) and senktide (c) in the VTA before and after (20 min and 24 h) i.v. administration of the D_1 dopamine receptor antagonist SCH 23390 (0.2 mg kg^{-1}). Values represent the mean ± s.e.m. of six to seven rats. Statistical comparisons to vehicle values (*) or to agonist before SCH 23390 (†) are indicated by $*^\dagger P<0.05$, $**^{\dagger\dagger} P<0.01$.

the effects of tachykinin agonists were similar to those evoked by glutamic acid, it is likely that they derived from neuronal cell activation.

Blockade of the cardiovascular and behavioural responses with SCH23390 provides direct evidence that dopamine release and the subsequent activation of D_1 dopamine receptors are involved in the central effects of tachykinin agonists. The likelihood that tachykinins can affect the mesocorticolimbic system was based on the presence of endogenous tachykinins and NK_3 receptors in the VTA (Deutch *et al*., 1985; Kalivas *et al*., 1985; Warden & Young, 1988; Ding *et al*., 1996; Shughrue *et al*., 1996; Chen *et al*., 1998; Lu *et al*., 1998; Langlois *et al*., 2001; Lessard *et al*., 2003), and by microinfusion in the VTA of NK_1 and NK_3 agonists, which increased the levels of dopamine and/or its metabolite, dihydroxyphenylacetic acid (DOPAC), in the prefrontal cortex, nucleus accumbens and striatum (Deutch *et al*., 1985; Elliott *et al*., 1986a, b; Marco *et al*., 1998). Evidence suggests a dissimilar cellular localization of NK_1 and NK_3 receptors in the VTA. While one study suggested the presence of NK_1 receptors on nondopaminergic inhibitory neurons in the VTA (Lejeune *et al*., 2002), NK_3 receptors were shown to be located on dopaminergic neurons (Chen *et al*., 1998).

The VTA may indeed represent a highly strategic site for tachykinins in cardiovascular regulation since the mesolimbic dopaminergic system is involved in diurnal blood pressure and HR regulation (Sei *et al*., 1999; Sakata *et al*., 2002). An upregulation of D_1 and D_2 dopamine receptors in the nucleus accumbens, one major neuronal projection of the VTA, was reported in young prehypertensive spontaneously hypertensive rat (Kirouac & Ganguly, 1993; Vaughan *et al*., 1999). Finally, electrophysiological evidence indicates that the activation of arterial baroreceptors alters the discharge rate of neurons in the VTA, likely through a relay in the NTS (Kirouac & Ciriello, 1997b).

Comparison of the cardiovascular effects of the three tachykinin agonists reveals that the NK_3 agonist induced at low doses the most prominent effects on mean arterial pressure and HR, suggesting that the VTA is particularly sensitive to the NK_3 agonist. This is congruent with the fact that the NK_3 receptors are the most concentrated tachykinin receptors in this brain area (Nakanishi, 1991; Ding *et al*., 1996). The cardiovascular responses to senktide reached a plateau at lower doses than the NK_1 agonist, [Sar^9, Met $(O_2)^{11}$]SP.

Thus far, there is no information regarding the presence or cellular localization of NK_2 receptors in the VTA. The expression of this receptor is relatively low in comparison to that of the two other tachykinin receptors in the CNS (Ribeiro-Da-Silva *et al*., 2000; Saffroy *et al*., 2001). This could

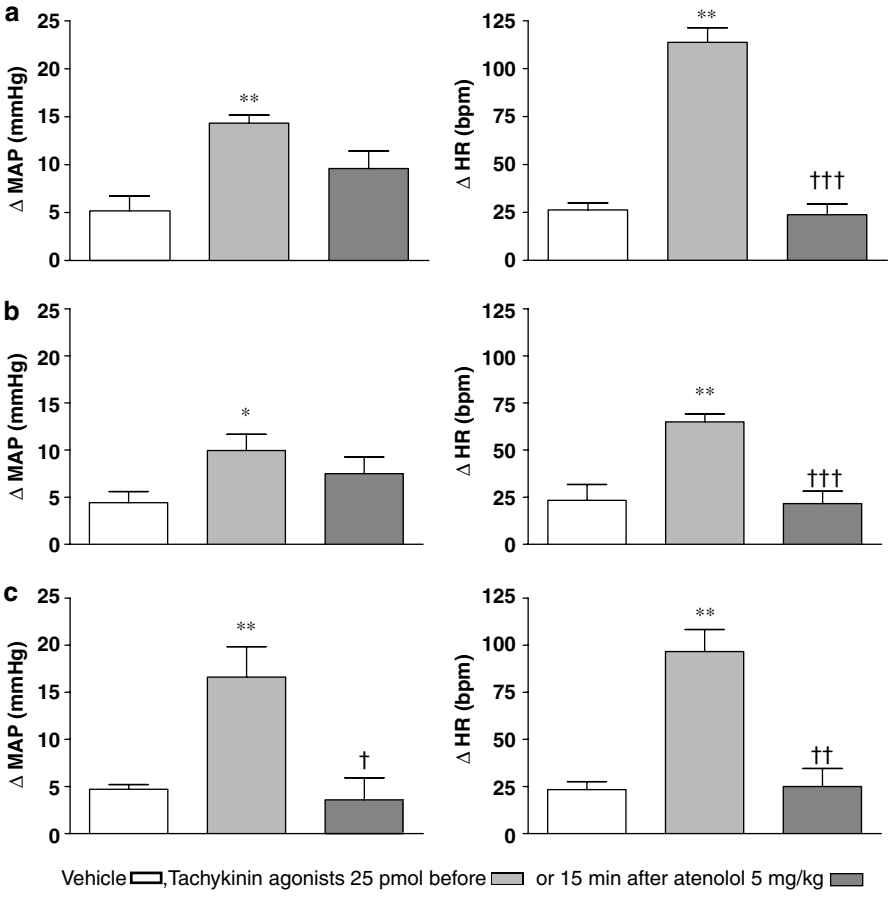

Figure 10 Maximal changes on mean arterial pressure (Δ MAP, left) and heart rate (Δ HR, right) following the injection of 25 pmol [Sar^9, Met $(O_2)^{11}$]SP ($n=8$) (a), [β-Ala^8]NKA (4–10) ($n=5$) (b) and senktide ($n=6$) (c) in the VTA before and 15 min after treatment with the β_1 adrenoceptor antagonist atenolol (5 mg kg^{-1}, i.v.). Values represent the mean ± s.e.m. of (n) rats. Statistical comparisons to vehicle values (*) or to agonist before atenolol (†) are indicated by $*^{\dagger}P<0.05$, $**^{\dagger\dagger}P<0.01$, $^{\dagger\dagger\dagger}P<0.001$.

account for the variable pressor and behavioural responses elicited by the NK_2 receptor agonist in this study. Nevertheless, the NK_2 antagonist abolished the cardiovascular effect induced by the NK_2 agonist in a selective and reversible manner. The amplitude of the tachycardia evoked by [β-Ala^8]NKA (4–10) was blunted in many rats by the occurrence of a significant bradycardia. This dual effect of the NK_2 agonist on HR (tachycardia or bradycardia or both) that contributes to the modest cardiovascular response may suggest the activation of the sympathetic and parasympathetic autonomic nervous system from the VTA.

Although the onset of the cardiovascular response to all agonists occurred within 1 min, the maximal effect peaked between 7 and 9 min postinjection. This slow developing response to the agonist is unlikely to be due to its diffusion outside the VTA on the basis of five arguments. First, control experiments with the same doses of tachykinin agonists did not elicit consistent cardiovascular/behavioural changes when injected around the VTA, including the red nucleus and the rostral linear nucleus raphe. Second, their injection in the substantia nigra, a nucleus located in the vicinity of the VTA, induced only increases in HR and no changes in blood pressure and behaviour (Lessard & Couture, 2001). Third, a large dose of DiMe-C7 (10 nmol) 2 mm above the VTA did not cause any cardiovascular/behavioural changes (Cornish & van den Buuse, 1995). In the latter study, the agonist was injected in a volume of 1 μl, which is five times greater than the volume (0.2 μl) used in our study. Fourth, the injection site for each rat was carefully confirmed by histological post-mortem examination. Fifth, glutamic acid, which is known to excite neuronal cell bodies and not fibres of passage (Goodchild *et al*., 1982), caused cardiovascular responses with a time course that resembled that of tachykinin agonists. Depressor responses with a faster onset were reported in alpha-chloralose-anaesthetized, paralysed and artificially ventilated rats with L-glutamate in the VTA (Kirouac & Ciriello, 1997a). Cardiovascular responses in opposite directions depend on the presence or not of anaesthesia as reported earlier (Kirouac & Ciriello, 1997a).

Senktide induced several behaviours, but the most frequent ones were rearing and particularly the wet dog shake, which is in agreement with previous studies on the i.c.v. injection of this agonist in rat (Picard *et al*., 1994; Cellier *et al*., 1997). Both rearing and wet dog shake induced by senktide were completely blocked by the D_1 dopamine receptor antagonist SCH23390 in the present study. Previous studies have reported that senktide-induced wet dog shake results from brain serotoninergic mechanisms (Stoessl *et al*., 1990), while the rearing caused by NK_3 receptor stimulation with DiMe-C7 in the VTA is related to dopaminergic activity since it was

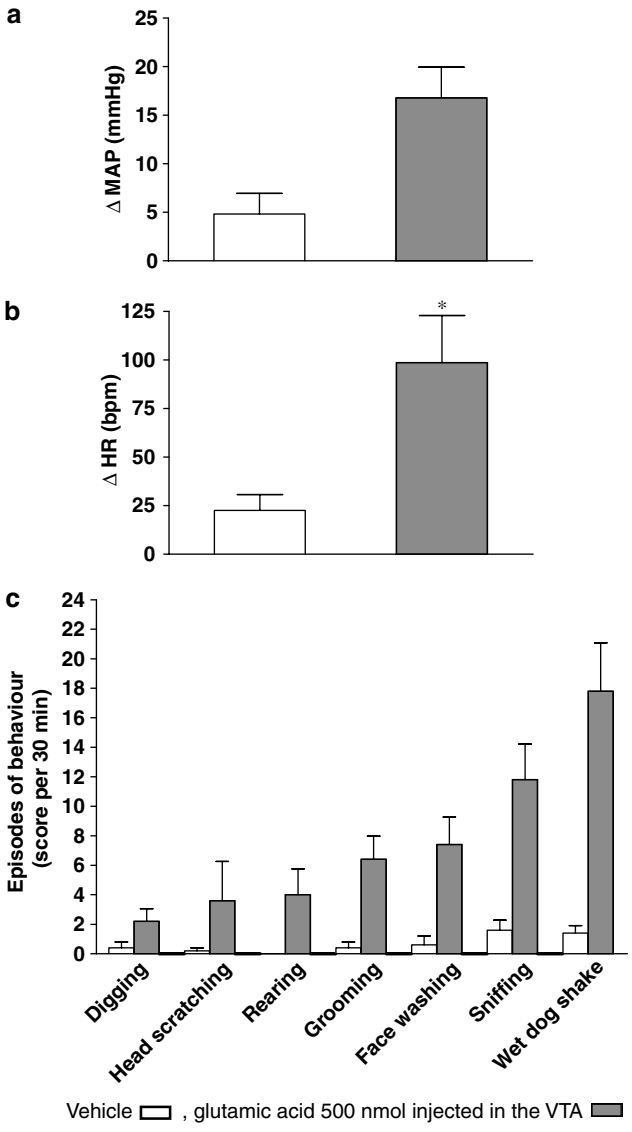

Figure 11 Maximal changes on mean arterial pressure (Δ MAP, a), heart rate (Δ HR, b) and behavioural activity (c) following the injection of glutamic acid (500 nmol) in the VTA. Values represent the mean ± s.e.m. of five rats. Comparison to vehicle values is indicated by $*P<0.05$. Other values did not reach statistical significance.

potentiated by peripheral administration of D-amphetamine and blocked by pretreatment with haloperidol (Eison *et al*., 1982). These two mechanisms are supported by neuroanatomical and electrophysiological data showing that dopamine cells in the VTA receive important serotonergic innervation from the dorsal raphe nucleus (Oades & Halliday, 1987; Gervais & Rouillard, 2000).

The injection of [Sar^9, Met $(O_2)^{11}$]SP in the VTA induced face washing and sniffing, whereas [β-Ala^8]NKA (4–10) produced head scratching. In addition, both agonists induced wet dog shake, but at lower intensity than senktide. These data confirm earlier studies showing similar behavioural effects with increased locomotor activity following the injection of SP, NKA, DiMe-C7, GR 73632 (NK_1 agonist) and senktide in the rat VTA (Elliott & Iversen, 1986; Elliott *et al*., 1991) or in the rat cerebral ventricles (Tschöpe *et al*., 1992; Cellier *et al*., 1997; 1999). According to several studies, the behavioural manifestations and locomotor activity induced by injection of SP or NK_1 agonist in the VTA are mediated by dopamine since they can be blocked by antagonist of DA receptors in the nucleus accumbens or by 6-hydroxydopamine lesions of the ascending dopaminergic A10 neurons (Kelley *et al*., 1979; Elliott *et al*., 1992). The present study confirms that increases of behavioural activity induced by the activation of NK_1 receptors are mediated by dopamine and the subsequent activation of D_1 dopamine receptors.

Whereas data show that both the cardiovascular and behavioural changes elicited by tachykinin agonists are dopamine mediated, it was possible to dissociate the HR response from the behaviour. At low doses (1 pmol), NK_1 and NK_3 agonists evoked marked HR increases without blood pressure and behavioural changes. One cannot exclude, however, that behavioural changes contribute to the cardiovascular response at higher doses of tachykinin agonists because they occurred simultaneously. This may contribute to the lack of dose-dependent cardiovascular effects mediated by agonists. In addition, maximal increases of HR responses were achieved at low doses of agonists, particularly with NK_2 and NK_3 agonists, explaining why higher doses did not elicit greater responses.

Results with atenolol indicate that the tachycardia induced by selective activation of NK_1, NK_2 and NK_3 receptors in the VTA is due to the peripheral release of catecholamines and the subsequent activation of cardiac β_1-adrenoceptors. The pressor response was either reduced or abolished (senktide) in the presence of atenolol. This suggests that the pressor response to tachykinin agonists depends on the increased cardiac output. This finding reduces the likelihood that vasopressin release is the primary mechanism underlying the pressor response mediated by NK_3 receptor activation as previously suggested with high doses of DiMe-C7 (Cornish & van den Buuse, 1995). As expected, behavioural effects mediated by tachykinin agonists were not affected by atenolol, demonstrating that behavioural responses are not attributable to cardiovascular changes.

It is worth mentioning that the mesolimbic dopaminergic pathway projects to the amygdala, a nucleus involved in the cardiovascular defense reaction to physiological stress (van den Buuse & Catanzariti, 2000). Likewise, the mesocortical dopaminergic pathway is involved in the cardiovascular component of the defense reaction (Al Maskati & Zbrozyna, 1989; Kawahara *et al*., 2002). SP was endogenously released in the VTA following the application of a peripheral noxious stimulus (Bannon *et al*., 1986), and the NK_1 receptors activated by endogenous SP in the VTA participate to stress-induced analgesia in a tonic pain model (Altier & Stewart, 1999). As the time course of the cardiovascular responses seems too slow to be related to a defense type of reaction, the tachykinins may exert, however, a neuromodulatory function in the coordination of the behavioural and cardiovascular response to stress and noxious stimulation.

Conclusion

The present *in vivo* pharmacological study identifies the VTA as a potential site for the autonomic control of blood pressure

and HR in rat brain by tachykinin receptors that can increase midbrain dopaminergic transmission and the subsequent activation of D_1 dopamine receptors.

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