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Upregulation of tachykinin NK-1 and NK-3 receptor binding sites in the spinal cord of spontaneously hypertensive rat: impact on the autonomic control of blood pressure

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1 Effects of intrathecally (i.t.) injected tachykinin NK-1 and -3 receptor agonists and antagonists were measured on mean arterial blood pressure (MAP) and heart rate (HR) in awake unrestrained spontaneously hypertensive rats (SHR,15-week-old) and age-matched Wistar Kyoto rats (WKY). Quantitative *in vitro* autoradiography was also performed on the lower thoracic spinal cord of both strains and Wistar rats using specific radioligands for NK-1 receptor ([^{125}I]HPP[Arg^3,Sar^9,Met$(O_2)^{11}$]SP (3–11)) and NK-3 receptor ([^{125}I]HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH_2).

2 The NK-1 agonist [Sar^9,Met$(O_2)^{11}$]SP (650 and 6500 pmol) decreased MAP and increased HR in WKY. The fall in MAP was blunted in SHR and substituted by increases in MAP (65–6500 pmol) and more sustained tachycardia. The NK-3 agonist senktide (6.5–65 pmol) evoked marked increases in MAP and HR (SHR >>> WKY), yet this response was rapidly desensitized. Cardiovascular effects of [Sar^9,Met$(O_2)^{11}$]SP (650 pmol) and senktide (6.5 pmol) were selectively blocked by the prior i.t. injection of LY303870 (NK-1 antagonist, 65 nmol) and SB235375 (NK-3 antagonist, 6.5 nmol), respectively. Antagonists had no direct effect on MAP and HR in both strains.

3 Densities of NK-1 and -3 receptor binding sites were significantly increased in all laminae of the spinal cord in SHR when compared to control WKY and Wistar rats. The dissociation constant was however not affected in SHR for both NK-1 ($K_d = 2.5$ nM) and NK-3 ($K_d = 5$ nM) receptors.

4 Data highlight an upregulation of NK-1 and -3 receptor binding sites in the thoracic spinal cord of SHR that may contribute to the hypersensitivity of the pressor response to agonists and to the greater sympathetic activity seen in this model of arterial hypertension.
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Abbreviations: aCSF, artificial cerebrospinal fluid; HR, heart rate; i.c.v., intracerebroventricular; IML, intermediolateral cell column; i.t., intrathecal; MAP, mean arterial blood pressure; SHR, spontaneously hypertensive rat; SP, substance P; WKY, Wistar Kyoto rat

Introduction

Tachykinins belong to a family of neuropeptides including substance P (SP), neurokinin A and neurokinin B whose biological effects are mediated *via* the activation of three transmembrane G-protein-coupled receptors denoted as NK-1, -2 and -3. These receptors are widely distributed in the central nervous system (for a review see Ribeiro-Da-Silva *et al*., 2000) and thought to play a role in the autonomic control of blood pressure (Unger *et al*., 1988; Couture *et al*., 1995; Culman *et al*., 1995; 1997; Cellier *et al*., 1997; 1999; Lessard & Couture, 2001; Brouillette & Couture, 2002; Lessard *et al*., 2003; 2004; Deschamps & Couture, 2005). Cerebral activation of tachykinin receptors with various natural and selective agonists leads to increases in mean arterial blood pressure (MAP) and heart rate (HR) through the activation of the sympathetic nervous system (Unger *et al*., 1981; Takano *et al*., 1990; Deschamps & Couture, 2005) and the release of vasopressin (NK-3 receptors) (Polidori *et al*., 1989; Massi *et al*., 1991; Nakayama *et al*., 1992; Yuan & Couture, 1997). Cardiovascular effects evoked by intrathecally (i.t.) injected tachykinin agonists were mediated by NK-1 receptors and sympatho-adrenal activation (Hasséssian & Couture, 1989; Hasséssian *et al*., 1988; 1990; Couture *et al*., 1995). In normotensive rats, intracerebral or i.t. injection of tachykinin receptor antagonists failed to affect baseline arterial blood pressure and HR, suggesting that these peptides do not exert a tonic control of cardiovascular function although they could play a role as neuromodulators.

Pharmacological and anatomical findings suggest that the function and expression of central tachykinins and their

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receptors are altered during arterial hypertension. For instance, hypersensitivity of the pressor response to intracerebroventricular (i.c.v.) SP was reported in SHR (Unger *et al*., 1980) and both SP binding sites and SP-like immunoreactivity were higher in several brain nuclei of adult SHR (Shigematsu *et al*., 1987; Chen *et al*., 1990). More recently, injection of tachykinin NK-3 receptor antagonists in the substantia nigra or i.c.v. decreased systemic arterial blood pressure in SHR but not in WKY (Lessard *et al*., 2003; 2004). In the latter studies, NK-1 and -2 receptor antagonists failed to affect systemic blood pressure in both strains. These observations suggest a role for brain tachykinin NK-3 receptors in the maintenance of arterial blood pressure in SHR.

At the spinal cord level, previous studies have reported significant differences between SHR and WKY with regard to NK-1 receptors. Takano *et al*. (1985) have shown increased number of [^{3}H]SP binding sites and SP-like immunoreactivity in the intermediolateral cell column (IML) in the upper thoracic spinal cord of 16-week-old SHR. In the same study, the i.t. injection of the SP antagonist [D-Pro^4, D-Trp^{7,9}]SP (4–11), caused similar dose-dependent decreases in MAP and HR in anaesthetized SHR and WKY. However, the latter results are misleading as the first generation of SP antagonists was found nonspecific for SP receptors and toxic in the rat spinal cord (Couture *et al*., 1987a, b). Although Solomon *et al*. (1999) reported that the nonpeptide NK-1 receptor antagonist (RP 67580) also caused the same fall in blood pressure in both strains, this antagonist did not affect the biphasic MAP response induced by the selective NK-1 receptor agonist, GR 73632, in SHR. All these studies were performed with the use of anaesthetics which interfere with sympathetic nervous activity and baroreceptors function. Further systematic studies with selective NK-1 and -3 receptor agonists and antagonists, particularly in un-anaesthetized animals, are needed to define the putative role of tachykinin receptors in the autonomic control of blood pressure and HR at the level of the spinal cord in arterial hypertension.

The present study was undertaken to re-evaluate the role of spinal NK-1 receptors and to test the hypothesis that NK-3 receptors might also participate to the spinal autonomic control of MAP and HR by tachykinins in a model of hypertension. Two experimental approaches were used: (i) *in vivo* pharmacological experiments in freely behaving SHR (15-weeks-old) and age-matched WKY to determine the cardiovascular effects of selective NK-1 and -3 receptor agonists and antagonists i.t. injected to the ninth thoracic vertebra; (ii) quantitative autoradiography on lower thoracic spinal cord sections with newly developed selective radioligands to compare the density and distribution of NK-1 and -3 receptor binding sites in both strains and Wistar rats.

Methods

Drugs and materials

HPP-[Arg^3, Sar^9,Met(O$_2$)^{11}]SP (3–11) and HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH$_2$ were developed from the selective NK-1 ([Sar^9,Met(O$_2$)^{11}]SP) and NK-3 (senktide) receptor agonists, respectively, and were synthesized by Dr W. Neugebauer (Institute of Pharmacology, Sherbrooke University, Sherbrooke, Que, Canada). To enable the labelling of the molecule with ^{125}I, HPP (3-(4 hydroxyphenyl) propyl) was attached at the N-terminus. SP, the NK-3 receptor agonist senktide (Succinyl-[Asp^6, MePhe^8]SP (6-11)) and NK-1 receptor agonist [Sar^9,Met(O$_2$)^{11}]SP (Regoli *et al*., 1988) were purchased from Bachem Bioscience Inc. (King of Prussia, PA, U.S.A.). The NK-3 receptor agonist [MePhe^7]Neurokinin B (Regoli *et al*., 1988) was obtained from Dr D. Regoli (Institute of Pharmacology, Sherbrooke University, Sherbrooke, Que, Canada). The kinin B$_2$ receptor antagonist D-Arg^0[Hyp^3, Thi^5, D-Tic^7, Oic^8]Bradykinin also named Hoe 140 (Hock *et al*., 1991) was purchased from Peninsula Laboratories (San Carlos, CA, U.S.A.). The nonpeptide NK-1 receptor antagonist 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 and its opposite (−)-enantiomer, LY 306155, (*S*)-1-[*N*-(2-methoxybenzyl)acetylamino]-3-(1H-indol-3-yl)-2-[*N*-(2-(4-(piperidin-1-yl)piperidin-1-yl)acetyl)amino]propane (Gitter *et al*., 1995; Iyengar *et al*., 1997) were obtained from Dr S. Iyengar (Eli Lilly and Co., Indianapolis, IN, U.S.A.). The lipophilic nonpeptide NK-3 receptor antagonist SB 222200, (*S*)-(−)-*N*-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide) (Sarau *et al*., 2000) and the hydrophilic NK-3 receptor antagonist SB 235375, (*S*)-(−)-*N*-(α-ethylbenzyl)-3-(carboxymethoxy)-2-phenylquinoline-4-carboxamide (Hay *et al*., 2002) were obtained from Dr H.M. Sarau (GlaxoSmithKline, King of Prussia PA, U.S.A.).

The NK-2 receptor antagonist SR 48968 (*S*)-*N*-methyl-[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]-benzamide) (Advenier *et al*., 1992) was obtained from Dr X. Emonds-Alt (Sanofi Recherche, Montpellier, France). The angiotensin AT$_1$ receptor antagonist, losartan (Dup753), 2-*n*-butyl-4-chloro-5-hydroxymethyl-1-[(2′-(1H-tetrazol-5-yl)biphenyl-4-yl)methyl]imidazole potassium salt (Siegl, 1993) was from Du Pont Merck Pharmaceutical Co. (Wilmington, DE, U.S.A.). SB 235375, SP, [Sar^9,Met(O$_2$)^{11}]SP and Hoe 140 were water soluble and therefore dissolved directly in aCSF or PIPES buffer. Otherwise, the other lipophilic agonists and antagonists were dissolved first in DMSO (Fisher Scientific, Montréal, Que, Canada) and the solution was then completed with aCSF or PIPES buffer (final concentration of DMSO $<10\%$).

Autoradiographic [^{125}I]-microscales (20 μm) and [^{3}H]-Hyperfilms (single coated, $24\times30\,cm^2$) were purchased from Amersham Pharmacia Biotech, Canada. Bacitracin, bovine serum albumin (protease free), captopril, dithiothreitol, magnesium chloride, piperazine-*N*,*N*′-bis [2-ethanesulfonic acid] (PIPES), 1,10-phenanthroline and heparin sodium salt (porcine, grade 1-A) were purchased from Sigma-Aldrich (Oakville, Ont, Canada). aCSF was purchased from Harvard Apparatus Inc. (Holliston, MA, U.S.A). All other chemicals were obtained from standard commercial sources.

Animal source and care

Male SHR ($n=48$), WKY ($n=23$) and Wistar ($n=4$) rats were purchased at least 1 week prior to experiments from Charles River, St-Constant, Que, Canada. They were housed individually in plastic cages under a 12 h light-dark cycle in a room with controlled temperature (23°C), humidity (50%) with food (Charles River Rodent) and tap water available *ad libitum*. The care of animals and research protocols were in compliance with 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. All efforts have been made to keep at a minimum the number of animals used.

Animal surgery

Rats (15-weeks-old) were anaesthetized with an i.p. injection of 65 mg kg^{-1} sodium pentobarbitone (somnotol; M.T.C. Pharmaceuticals, Cambridge, Ont, Canada) and a polyethylene catheter (PE-10; Intramedics, Clay Adams, NJ, U.S.A.) was inserted into the spinal subarachnoid space *via* an incision made in the dura at the atlanto-occipital junction and pushed to the ninth thoracic segment (T-9) as described previously (Brouillette & Couture, 2002; Cloutier *et al*., 2002). Thereafter, the rats were allowed to recover in individual plastic cages ($40 \times 23 \times 20$ cm^3) and housed in the same controlled conditions. The correct positioning of the i.t. catheter was verified by post-mortem examination at the end of experiment and the catheter was found either dorsally or laterally to the spinal cord.

After 2 days, rats were reanaesthetized with sodium pentobarbitone (65 mg kg^{-1}, i.p.) and an intravascular siliconized (Sigmacote, Sigma-Aldrich Canada) PE-50 catheter, filled with physiological saline containing 100 IU ml^{-1} heparin sodium salt (Sigma-Aldrich, Canada), was inserted into the abdominal aorta through the femoral artery for direct blood pressure recording and exteriorized at the back of the neck. Before i.t. and vascular surgery, the animals received antibiotics trimethoprime and sulphadiazine (tribissen 24%, 30 mg kg^{-1}, s.c., Schering Canada Inc., Pointe Claire, Que, Canada) and ketoprophen (anafen, 5 mg kg^{-1}, s.c., Merial Canada Inc., Baie d'Urfé, Que, Canada). Recovery from anaesthesia was monitored closely under a warming lamp to maintain the body temperature of animals. Thereafter, rats were housed individually in polyethylene cage with a top grid and returned to their resident room. Experimental protocols were initiated 24 h later, in awake and unrestrained rats.

Measurement of cardiovascular parameters

Blood pressure and HR were measured 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.). The cardiovascular response was measured 1 h after the rats were transported to the testing room. They remained in their resident cage but the top grid was removed and had no more access to the food and water for the duration of experiment. When resting blood pressure and HR were stable, rats received an i.t. injection of 20 μl artificial cerebrospinal fluid (aCSF). Only rats (99%) which did not show cardiovascular changes to aCSF for the 30 min period were selected in the study. Agonist/antagonist compounds were administered in a volume of 10 μl of vehicle followed by 10 μl volume of aCSF which corresponds to the void volume of the catheter. The compound and the aCSF flush (10 μl) were given within a total period of 60 s to avoid any compression of spinal cord. Changes in MAP and HR represent the difference between recordings obtained 30 s before the start of injection and the value at the designed time after injection. Each dose was calculated in a volume of 10 μl solution injected i.t..

Experimental protocols

Dose–response curves to i.t. [Sar^9,Met(O$_2$)^{11}]SP. Both SHR ($n=7$) and WKY ($n=7$) initially received an i.t. injection of aCSF (20 μl) followed 15 min later by four successive doses of [Sar^9,Met(O$_2$)^{11}]SP (6.5, 65, 650 and 6500 pmol) 1 h apart to construct a dose–response curve. Preliminary experiments showed the absence of desensitization to 650 pmol [Sar^9, Met(O$_2$)^{11}]SP given at 1 h intervals.

Desensitization of the cardiovascular response to senktide. Preliminary experiments showed a rapid desensitization to senktide in SHR. Thus, the conditions of desensitization to senktide were studied in SHR ($n=6$) to better establish the protocol of the dose–response curve to this agonist. Rats initially received an i.t. injection of aCSF (20 μl) followed 15 min later by senktide (65 pmol). A second i.t. injection of senktide was given 1 h apart and a third injection 24 h later.

Dose–response curves to i.t. senktide. SHR ($n=7$) and age-matched WKY ($n=7$) were used on 4 consecutive days. Rats initially received an i.t. injection of aCSF (20 μl) followed 15 min later by a single dose of senktide (0.65, 6.5, 65 and 650 pmol). Based on the protocol of desensitization established earlier, increasing doses of senktide were given at 24 h intervals.

Effects of i.t. NK-1 receptor antagonist. The effect of [Sar^9,Met(O$_2$)^{11}]SP on MAP and HR was measured in the presence and absence of LY 303870 and its opposite (−) enantiomer, LY 306155 (65 nmol). SHR ($n=5$) and age-matched WKY ($n=5$) were used on 2 consecutive days. Rats initially received an i.t. injection of aCSF (20 μl) followed 15 min later by [Sar^9,Met(O$_2$)^{11}]SP (650 pmol). After 1 h LY 303870 was injected, and 15 min later 650 pmol [Sar^9, Met(O$_2$)^{11}]SP. On the subsequent day, the same protocol was repeated with the enantiomer LY 306155. The specificity of the NK-1 antagonist was further tested by assessing its effect against senktide-induced cardiovascular changes. Senktide (6.5 pmol) was injected to SHR ($n=6$) on 2 consecutive days. On day 1, senktide was given alone while on day 2, senktide was injected 15 min after LY 303870 (6500 pmol).

Effects of i.t. NK-3 receptor antagonist. The effect of senktide on MAP and HR was measured in the presence and absence of the NK-3 antagonist SB 235375. SHR initially received a single dose of senktide (6.5 pmol) and 24 h later, was injected SB 235375 (6500 pmol; $n=7$ or 650 pmol; $n=5$) 10 min prior to 6.5 pmol senktide. Senktide was reinjected alone 24 h later to assess the reversibility of any blockade observed with the antagonist on the preceding day. To assess the specificity of the NK-3 antagonist, an additional group of SHR ($n=4$) was injected with SB 235375 (65 nmol) 10 min prior to 65 pmol [Sar^9,Met(O$_2$)^{11}]SP. The cardiovascular response induced by the NK-1 agonist was compared with that obtained 1 h earlier in the absence of SB 235375.

Autoradiography

Peptide iodination Iodination of HPP-[Arg^3, Sar^9,Met(O$_2$)^{11}]SP (3–11) and HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH$_2$ was performed according to the chloramine T method

(Hunter & Greenwood, 1962). Briefly, 5 μg of peptide were incubated in 0.05 M phosphate buffer (pH 7.4) for 30 s in the presence of 0.5 mCi (18.5 MBq) of Na ^{125}I and 220 nmol of chloramine T in a total volume of 85 μl. The monoiodinated peptide was then immediately purified by high-pressure liquid chromatography on a C4 Vydac column (0.4 × 250 mm^2) (The Separations Group, Hesperia, CA, U.S.A.) with 0.1% trifluoroacetic acid and acetonitrile as mobile phases. The specific activity of the iodinated peptides corresponds to 2000 c.p.m. fmol^{-1} or 1212 Ci mmol^{-1}.

Tissue preparation for autoradiography Four rats from each strain (SHR, WKY and Wistar) not submitted to any surgery were killed by asphyxia under respiratory CO_2 inhalation and subjected to dorsal laminectomy. Spinal cords (segments T8–T11) were immediately removed after careful incision of the dura mater and frozen in 2-methylbutane cooled at −45 to −55°C with liquid nitrogen, and then stored at −80°C until use. Matched spinal cord segments (T9–T10) from 12 rats (four animals per strain × 3 strains) were mounted together in a gelatine block and serially cut into 20-μm thick coronal sections with a cryostat fixed at temperature varied between −11 and −13°C. Thus, each section of the cryostat was from 12 spinal cords. A total of three sections were made and alternatively thaw mounted on 0.2% gelatine/0.033% chromium potassium sulphate-coated slides, giving a total of 36 sections per slide (three sections × 12 spinal cords). Three slides were taken for the total binding and two slides (adjacent sections) for the nonspecific binding. A total of 20 slides (720 sections) were obtained for each receptor studied and kept at −80°C until use.

In vitro *receptor autoradiography* Sections were thawed, preincubated for 30 s in 25 mM PIPES buffer (pH 7.4; 4°C) and incubated at room temperature for 60 min in the same buffer containing: 1 mM 1,10-phenanthroline, 1 mM dithiothreitol, 0.014% bacitracin, 0.1 mM captopril, 0.2% bovine serum albumin (protease free) and 7.5 mM magnesium chloride in the presence of 10 nM of [^{125}I]HPP-[Arg^3,Sar^9,Met(O$_2$)^{11}]SP (3–11) or 15 nM of [^{125}I]HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH$_2$ for NK-1 and -3 receptors, respectively. The concentrations of radioligands chosen yielded maximal specific binding on the saturation curves in the spinal dorsal horn of Wistar, WKY and SHR. This ascertains that increases in the density of receptor binding sites in SHR are due to increases in the number of receptors and not due to changes in receptor affinity. The nonspecific binding was determined in the presence of 1 μM of unlabelled ligands (HPP-[Arg^3, Sar^9,Met(O$_2$)^{11}]SP (3–11) for NK-1 or HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH$_2$ for NK-3 receptors). At the end of the incubation period, slides were transferred sequentially through five rinses of 4 min each in 25 mM PIPES (pH 7.4; 4°C) and dipped for 15 s in distilled water (4°C) to remove the excess of salts and air dried.

Quantification of receptor binding sites [^{3}H]-Hyperfilms were juxtaposed onto the slides in the presence of [^{125}I]-microscales and exposed at room temperature for 10 h (NK-3 ligand) or 1 day (NK-1 ligand). The films were developed in D-19 (Kodak developer) and fixed in Kodak Ektaflo. Autoradiograms were quantified by densitometry using an MCID™ image analysis system (Imaging Research, St Catharines, Ont, Canada). Standard curve from [^{125}I]-microscales was used to convert density levels into fentomoles per milligram of tissue (fmol mg^{-1} tissue). Specific binding was determined by subtracting superimposed digitalized images of nonspecific labelling from total binding. The anatomical structures with the corresponding nomenclature are adapted from the Atlas of Paxinos & Watson (1998).

Competition studies In order to confirm the specificity of the radioligand for NK-1 and -3 receptors, competition studies were carried out with several compounds including selective agonists and antagonists for both receptors on spinal cord sections from SHR. Competition binding assays were carried out under the same assay conditions described above using 10 nM [^{125}I]HPP[Arg^3,Sar^9,Met(O$_2$)^{11}]SP (3–11) or 15 nM [^{125}I]HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH$_2$ in the presence of three increasing concentrations (10^{-12}, 10^{-9} and 10^{-6} M) of nonradiolabelled selective agonists (senktide, [MePhe^7]NKB) and antagonist (SB222200) for NK-3 receptors, two agonists ([Sar^9,Met(O$_2$)^{11}]SP and SP) and one antagonist (LY303870) for NK-1 receptors, one NK-2 receptor antagonist (SR48968), one kinin B$_2$ receptor antagonist (Hoe 140) and one angiotensin AT$_1$ receptor antagonist (losartan).

Statistical analysis of data

Results are expressed as means ± s.e.m. of (n) rats. Statistical significance of differences were evaluated with Student's t-test on unpaired (between groups) or paired (within the same group) samples. A one-way analysis of variance (ANOVA) followed by a *post hoc* Dunnett test was used for multiple comparisons to the same control group (maximal values and area under the curve (AUC)). For the cardiovascular effects with or without antagonist, the ANOVA was followed by a Bonferroni test. Densities of receptor binding were analysed for significance using a one-way ANOVA and a *post hoc* Tukey test, except for competition assay where a *post hoc* Dunnett test was used. $P<0.05$ was considered to be statistically significant.

Results

Baseline MAP values were significantly higher in SHR ($n=48$) (155 ± 3 mmHg, $P<0.001$) than in WKY ($n=19$) (112 ± 3 mmHg). However, baseline HR (SHR; 332 ± 6 beats min^{-1}, WKY; 322 ± 14 beats min^{-1}) values did not differ significantly between strains.

Spinal effects of [Sar^9,Met(O$_2$)^{11}]SP on MAP and HR

The effects of four increasing doses of [Sar^9,Met(O$_2$)^{11}]SP on MAP and HR in SHR and WKY are depicted in Figure 1. In WKY, 6.5 and 65 pmol of [Sar^9,Met(O$_2$)^{11}]SP failed to alter MAP when compared to aCSF values. Doses of 650 and 6500 pmol evoked dose-related hypotension that peaked at 1 and 4 min postinjection, respectively. A tachycardia with a peak effect at 2 min postinjection was elicited by the NK-1 agonist from 65 to 6500 pmol. In SHR [Sar^9,Met(O$_2$)^{11}]SP (65 and 650 pmol) caused long-lasting increases in MAP while the highest dose (6500 pmol) caused an initial drop in MAP that

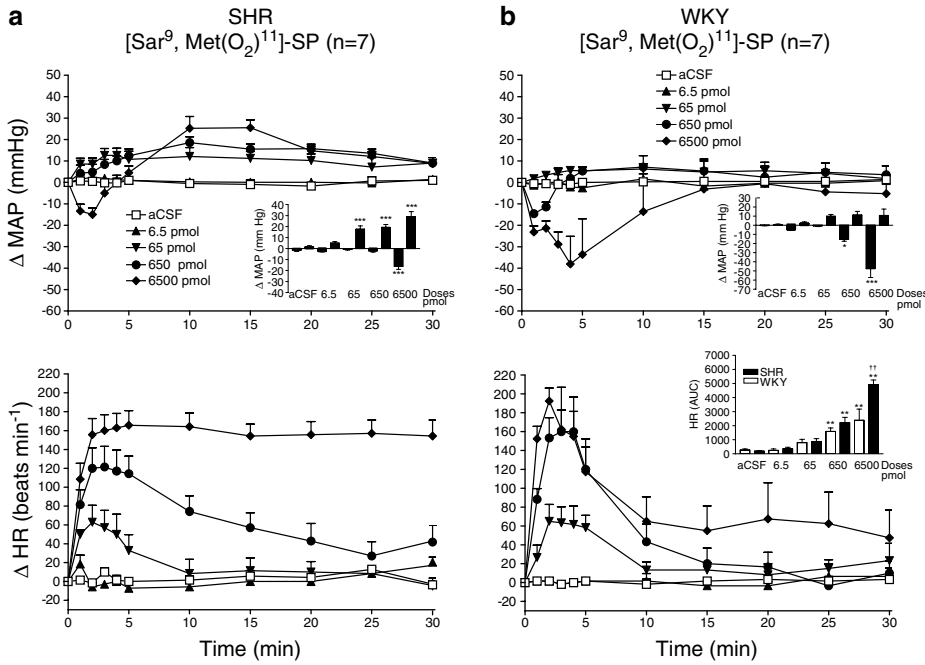

Figure 1 Time-course effects on changes in ΔMAP and ΔHR evoked by four increasing doses of [Sar^9,Met(O$_2$)^{11}]SP injected i.t. in SHR (a) and age-matched WKY (b). Upper insets represent maximal changes in MAP. The lower inset shows the area under the curve (AUC) for the response on HR (beats min^{-1} × 30 min) in both strains. Each point represents the means ± s.e.m. of seven rats. Statistical comparison to aCSF (*) or WKY (†) values is indicated by $^{*}P<0.05$, $^{**,\dagger\dagger}P<0.01$, $^{***}P<0.001$.

was followed by a prominent pressor response. Striking tachycardia occurred after injection of doses larger than 65 pmol. Thus, the hypotensive response to the NK-1 agonist was blunted in SHR and substituted by a pressor response. Increases in HR elicited by [Sar^9,Met(O$_2$)^{11}]SP were longer lasting in SHR.

Desensitization of the cardiovascular response induced by senktide

In SHR, the cardiovascular response induced by 65 pmol senktide was significant after the first injection (MAP, AUC = 144 ± 21 mmHg × 15 min, $P<0.01$; HR, AUC = 978 ± 146 beats min^{-1} × 15 min, $P<0.01$), yet no significant response was elicited when senktide was reinjected 1 h later (MAP, AUC = 38 ± 8 mmHg × 15 min, $P>0.05$; HR, AUC = 211 ± 70 beats min^{-1} × 15 min, $P>0.05$), showing that the spinal effect of senktide is subjected to marked desensitization. The cardiovascular response elicited by a third injection of senk tide 24 h later was not significantly different from that induced by the agonist at the first injection (MAP, AUC = 158 ± 29 mmHg × 15 min, $P<0.01$; HR, AUC = 1107 ± 204 beats min^{-1} × 15 min, $P<0.01$).

Spinal effects of senktide on MAP and HR

The effects on MAP and HR of four increasing doses of senktide given at 24 h apart are depicted in Figure 2. In SHR, senktide (0.65–65 pmol) evoked dose-dependent increases in MAP that peaked at 3–5 min postinjection. The dose of 65 pmol was maximal as 650 pmol did not cause further increase. Maximal increase in HR was elicited with 6.5 pmol senktide in SHR. In WKY, senktide had no significant effect on MAP, yet it caused a significant increase in HR at 65 pmol. Accompanying the cardiovascular responses, behavioural activity was increased and the following behaviours were seen with senktide (6.5–650 pmol): wet dog shake > head scratching > face washing. Maximal effects were elicited with the dose of 65 pmol.

Cardiovascular effect of [Sar^9,Met(O$_2$)^{11}]SP under receptor blockade in SHR and WKY

Effects of the NK-1 receptor antagonist LY 303870 and its enantiomer LY 306155 on the cardiovascular response to [Sar^9,Met(O$_2$)^{11}]SP in SHR and WKY are shown in Figures 3–4. The increases in MAP and HR evoked by 650 pmol [Sar^9,Met(O$_2$)^{11}]SP were completely blocked by the prior i.t. injection of LY 303870 (65 nmol, 15 min earlier) in SHR (Figure 3a). The same treatment with the antagonist abolished the hypotensive response and the tachycardia induced by 650 pmol [Sar^9,Met(O$_2$)^{11}]SP in WKY (Figure 4a). In contrast, 65 nmol LY 306155 had not significant effect on the cardiovascular response induced by the NK-1 agonist in both SHR and WKY (Figures 3b and 4b). The cardiovascular response induced by the agonist was fully recovered when it was reinjected 24 h after treatment with LY 303870 (data not shown). The NK-1 receptor antagonist LY 303870 was also tested against the cardiovascular response induced by 6.5 pmol senktide in SHR. I.t. LY 303870 (6500 pmol, 15 min earlier) did not alter the cardiovascular response induced by senktide (Figure 5a).

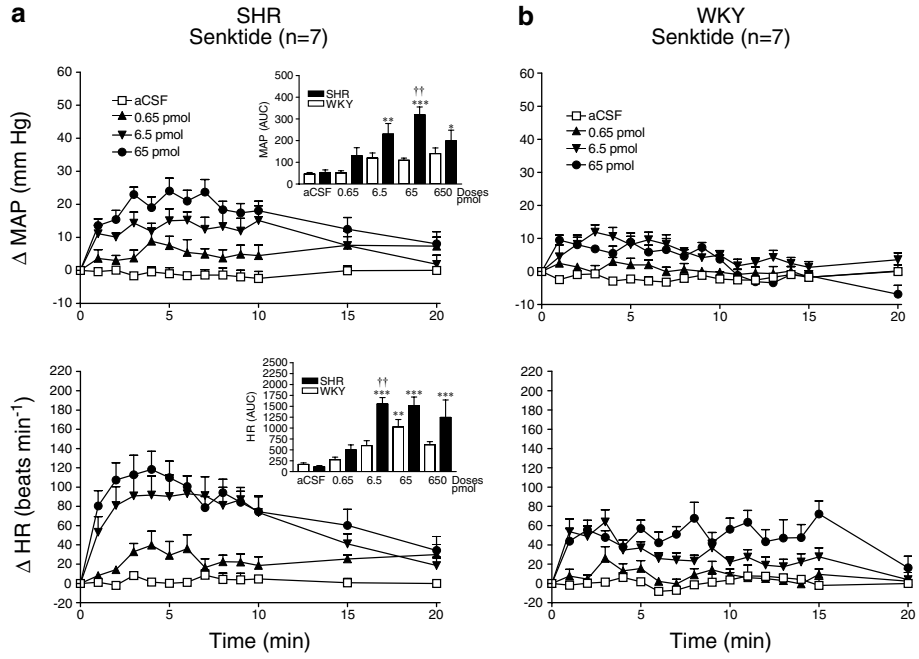

Figure 2 Time-course effects on changes in ΔMAP and ΔHR evoked by three increasing doses of senktide-injected i.t. in SHR (a) and age-matched WKY (b). Upper and lower insets represent the area under the curve (AUC) for MAP (mmHg × 20 min) and HR (beats min^{-1} × 20 min), respectively. Each point represents the means ± s.e.m. of seven rats. Statistical comparison to aCSF (*) or WKY (†) values is indicated by $*P<0.05$, $**,^{\dagger\dagger}P<0.01$, $***P<0.001$.

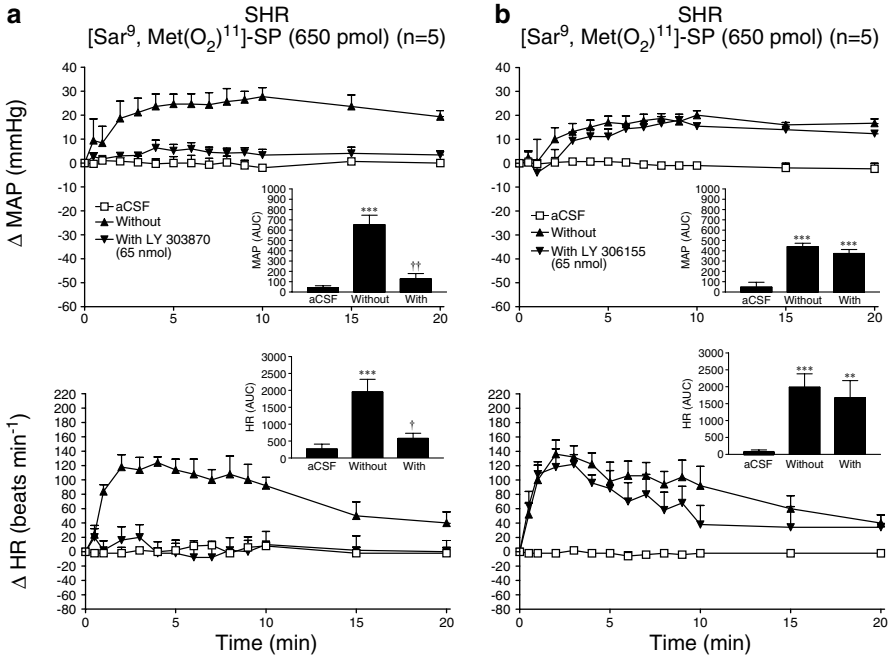

Figure 3 Time-course effects on changes in ΔMAP and ΔHR induced by 650 pmol $[Sar^9,Met(O_2)^{11}]$SP in the presence or absence of LY 303870 (a) and its enantiomer LY 306155 (b) in SHR. Upper and lower insets show the area under the curve (AUC) for MAP (mmHg × 20 min) and HR (beats min^{-1} × 20 min), respectively. Data are the means ± s.e.m. of five rats. Statistical comparison to vehicle values (*) or to the agonist in the absence of LY 303870 (†) is indicated by ($^{\dagger}P<0.05$, $**,^{\dagger\dagger}P<0.01$, $***P<0.001$).

Cardiovascular effect of senktide under receptor blockade in SHR

The effect of NK-3 receptor blockade with SB 235375 was determined on the cardiovascular response induced by senktide in SHR (Figure 6). The increases in MAP and HR evoked by 6.5 pmol senktide were completely blocked by the prior i.t. injection of SB235375 (6500 pmol, 10 min earlier). The response to senktide was nearly back to preantagonist values when the NK-3 agonist was reinjected alone 24 h later. Lower

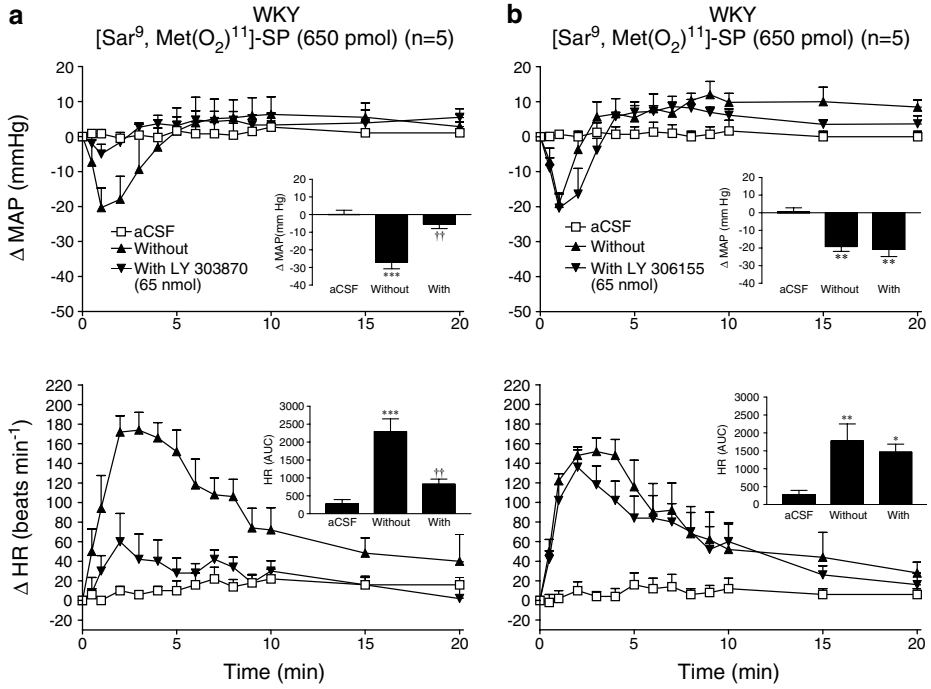

Figure 4 Time-course effects on changes in ΔMAP and ΔHR induced by 650 pmol [Sar^9,Met(O$_2$)^{11}]SP in the presence or absence of LY 303870 (a) and its enantiomer LY 306155 (b) in WKY. Upper and lower insets show the area under the curve (AUC) for MAP (mmHg × 20 min) and HR (beats min^{-1} × 20 min), respectively. Data are the means ± s.e.m. of five rats. Statistical comparison to vehicle values (*) or to the agonist in the absence of LY 303870 (†) is indicated by (*$P<0.05$, **,$^{\dagger\dagger}P<0.01$, ***$P<0.001$).

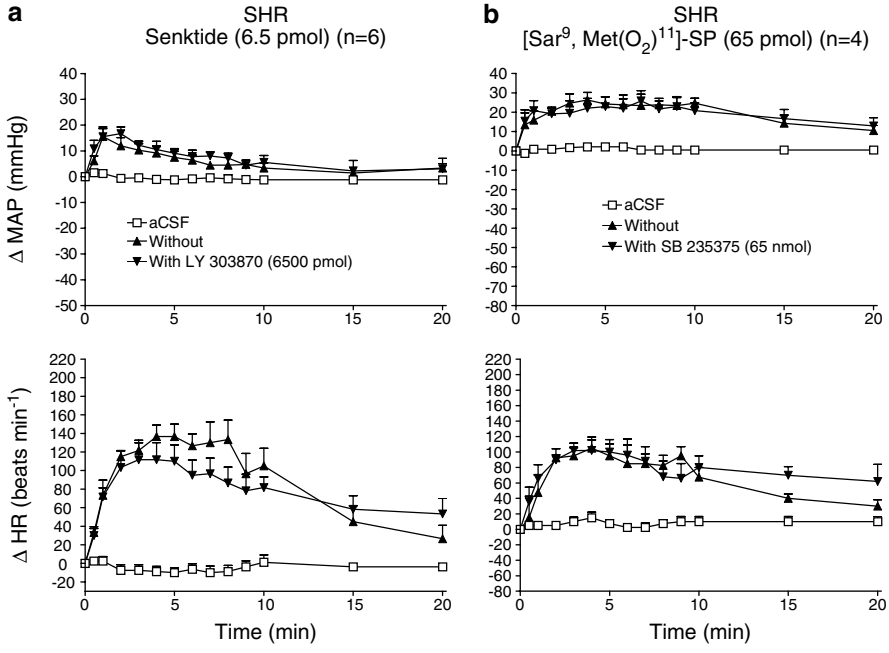

Figure 5 Time-course effects on changes in ΔMAP and ΔHR induced by 6.5 pmol senktide in the presence or absence of LY 303870 (a) and 65 pmol [Sar^9,Met(O$_2$)^{11}]SP in the presence or absence of SB 235375 (b) in SHR. Data are the means ± s.e.m. of (*n*) rats. No statistical significance of differences was found in the presence and absence of antagonist for either agonist.

doses of SB 235375 (650 pmol) had no significant effect on senktide-mediated responses (Figure 6b). On the other hand, SB 235375 (65 nmol) did not affect the cardiovascular response induced by 65 pmol [Sar^9,Met(O$_2$)^{11}]SP in SHR (Figure 5b). SB 235375 was devoid of any direct effect on MAP and HR (data not shown).

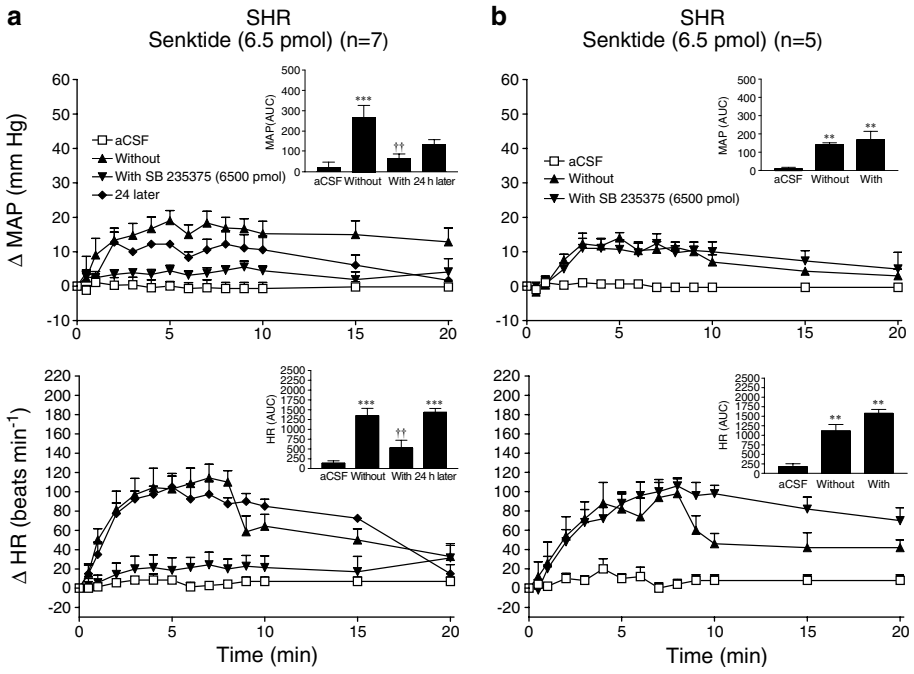

Figure 6 Time-course effects on changes in ΔMAP and ΔHR induced by 6.5 pmol senktide-injected i.t. in SHR in the presence or absence of SB 235375 (6500 pmol, (a); 650 pmol, (b)). Upper and lower insets show the area under the curve (AUC) for MAP (mmHg × 20 min) and HR (beats min^{-1} × 20 min), respectively. Data are the means ± s.e.m. of (*n*) rats. Statistical comparison to vehicle values (*) or to the agonist in the absence of SB 235375 (†) is indicated by (**,††P < 0.01, ***$P < 0.001$).

Autoradiographic studies

Quantitative *in vitro* autoradiography was performed to analyse the distribution and densities of NK-1 and -3 receptors in the thoracic spinal cord (T9-T10) of 15-week-old SHR and their age-matched WKY and Wistar rats. The dissociation constants calculated for NK-1 (K_d (nM) = 2.48 ± 0.06 Wistar, 2.52 ± 0.11 WKY, 2.50 ± 0.09 SHR) and NK-3 (K_d (nM) = 4.99 ± 0.04 Wistar, 4.97 ± 0.10 WKY, 5.03 ± 0.02 SHR) radioligands were not significantly different between strains (Figure 7). These data strongly suggest that the increased densities of NK-1 and -3 receptor binding sites seen in SHR are likely due to increases in the number of receptors and not to changes in receptor affinity. This is further supported by the fact that the concentrations (10 and 15 nM) of NK-1 and -3 radioligands provided complete saturation of total-specific receptor binding sites.

Autoradiographic distribution of NK-1 receptors in thoracic spinal cord of SHR, WKY and Wistar

The overall anatomical distribution of NK-1 receptor binding sites is illustrated in Figure 8. Specific binding for [^{125}I]HPP [Arg^3,Sar^9,$Met(O_2)^{11}$]SP (3–11) was found predominantly in the dorsal horn of Wistar, WKY and SHR (Figures 8 and 9). In Wistar rat, NK-1 receptor binding sites were distributed all over the grey matter with densities that varied through laminae from 1.97 ± 0.02 to 0.31 ± 0.16 fmol mg^{-1} tissue (LIII > LIV > LI + LII ≈ LX > LV > LVIII–LIX ≈ IML). The distribution and density values of NK-1 receptor binding sites in various laminae were quite similar and not significantly different between Wistar and WKY. Whereas, the pattern of distribution of NK-1 receptor binding sites was similar in SHR, density values were significantly greater in SHR when compared to age-matched WKY in all laminae of the spinal cord ($P < 0.01$): LI + LII (+75%), LIII (+35%), LIV (+45%), LV(+77%), LVIII-LIX (+132%), IML (+60%), LX (+42%).

Autoradiographic distribution of NK-3 receptors in thoracic spinal cord of SHR, WKY and Wistar

The overall anatomical distribution of NK-3 receptor binding sites is illustrated in Figure 8. The distribution and density values of NK-3 receptor binding sites were quite similar between Wistar and WKY (Figures 8 and 9). Density gradients for Wistar were as follows: LIV (1.37 ± 0.02 fmol mg^{-1} tissue) ⩾ LI + LII ≈ LIII > LX > LVIII + LIX > LV ≈ IML (0.18 ± 0.02 fmol mg^{-1} tissue), and for WKY: LIV (1.32 ± 0.08 fmol mg^{-1} tissue) ≈ LI + LII ≈ LIII ⩾ LX > LVIII + LIX > LV ≈ IML (0.3 ± 0.05 fmol mg^{-1} tissue). When compared to Wistar or WKY, densities of NK-3 receptor binding sites were significantly enhanced in LI + LII (+40%), LIII (+28%), LIV (+51%), LV (+45%), LVIII-LIX (+51%), IML (+40%) and LX (+33%).

Competition of the radioligands with various analogues

Three concentrations of various ligands (10^{-12}, 10^{-9} and 10^{-6} M) were incubated in the presence of either radioligand to determine their specificity (Figure 10). The total binding of the NK-1 radioligand [^{125}I]HPP[Arg^3,Sar^9,$Met(O_2)^{11}$]SP (3–11)

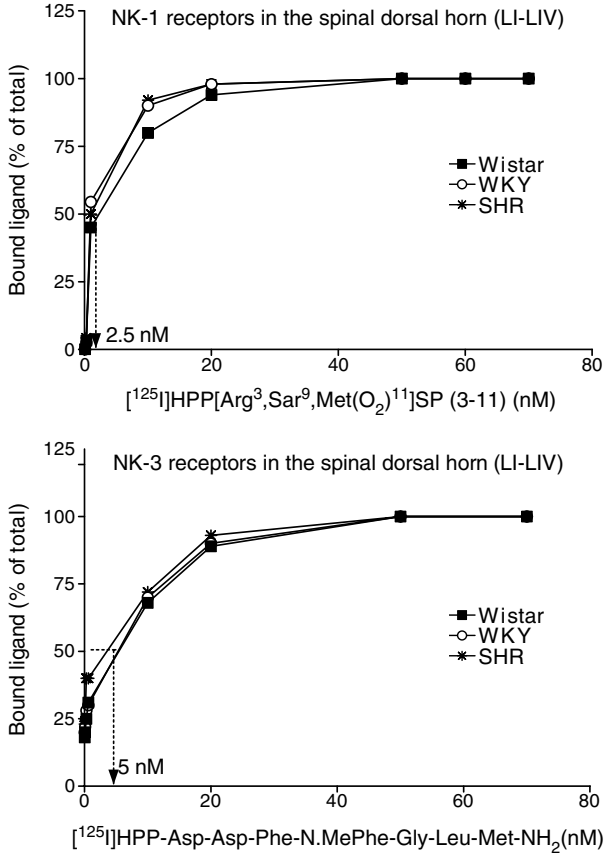

Figure 7 Affinity of binding for the NK-1 (upper panel) and NK-3 (lower panel) receptor radioligands in the thoracic spinal dorsal horn of Wistar, WKY and SHR. The dissociation constant (K_d) was calculated as the concentration of the radioligand that results in 50% of maximal specific binding. Data are from four rats in each strain. K_d values were not significantly different between strains.

(10 nM) in the spinal dorsal horn of SHR was significantly reduced in a concentration-dependent manner in the presence of the unlabelled NK-1 radioligand, [Sar^9,Met(O$_2$)^{11}]SP and SP (selective and nonselective NK-1 receptor agonist, respectively), LY 303870 (selective NK-1 receptor antagonist). However, the following ligands failed to alter the total binding measured with [^{125}I]HPP[Arg^3,Sar^9,Met(O$_2$)^{11}]SP (3–11): senktide and [MePhe^7]NKB (selective NK-3 receptor agonists), SB 222200 (selective NK-3 receptor antagonist), Hoe 140 (selective kinin B_2 receptor antagonist), SR 48968 (selective NK-2 receptor antagonist), losartan (selective angiotensin AT_1 receptor antagonist).

The total binding of the NK-3 radioligand [^{125}I]HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH$_2$ (15 nM) was significantly reduced by the unlabelled NK-3 radioligand, senktide, [MePhe^7]NKB and SB 222200 which are selective agonists and antagonist for NK-3 receptors. Total binding was also reduced by high concentration of SP, a nonselective tachykinin receptor agonist. Otherwise, the total binding was not altered by any other ligands, including those targeting NK-1 and -2 receptors. Hence, these competition studies confirmed the specificity of the radioligands used in this study for their respective receptors.

Discussion

This study provides the first quantitative laminar distribution of NK-1 and -3 receptor binding sites in the rat thoracic spinal cord using highly selective radioligands. Moreover, it provides functional and autoradiographic evidence that NK-1 and -3 receptors are upregulated in the spinal cord of SHR. The hypersensitivity of the pressor and tachycardiac responses induced by i.t. injected NK-1 and -3 receptor agonists in SHR was associated with marked increases of NK-1 and -3 receptor binding sites in the same segment of thoracic spinal cord. This may suggest that the spinal tachykininergic system contributes to the greater sympathetic activity seen in this model of arterial hypertension.

Elimination of normal supraspinal control of sympathetic activity following transection of the cervical spinal cord suggests that the generation of sympathetic hyperactivity and hyperexcitability in SHR includes a spinal component (Schramm & Chornoboy, 1982). For instance, inhibition of the expression of thyrotropin-releasing hormone (TRH) receptors following i.t. treatment with antisense oligonucleotides evidenced a role for TRH projections to spinal sympathetic preganglionic neurons in the elevation of resting arterial blood pressure in SHR (Suzuki *et al*., 1995). Receptor binding sites were increased for TRH (Bhargava & Gulati, 1988), yet they were unchanged for endothelin and delta-opiate receptors (Gulati & Rebello, 1992; Bhargava & Rahmani, 1993) and reduced for nicotinic receptors (Khan *et al*., 1994; 1996) in the spinal cord of SHR. This suggests specific upregulation in spinal cord neurotransmitters and receptors in SHR. This is further exemplified by the reduction of spinal cord contents of oxytocin, vasopressin, neurotensin and neuropeptide Y (Gaida *et al*., 1985; Maccarrone & Jarrott, 1986; Shulkes *et al*., 1987), and the lack of changes in spinal cord contents of noradrenaline and its neuronal metabolite 3, 4-dihydroxyphenylethyleneglycol in SHR (Louis *et al*., 1987). Whereas adrenergic α_{1A} receptor mRNA was greater in the spinal cord of SHR when compared to normotensive controls, adrenergic α_{2A} receptor mRNA was significantly lower (Reja *et al*. 2002). Thus, the greater content of SP-like immunoreactivity in the IML (Takano *et al*., 1985; Chen *et al*., 1990), the increased density of spinal tachykinin receptor binding sites (present study) and the exaggerated cardiovascular responses to i.t. tachykinin agonists suggest a relatively specific hyperactivity of the tachykininergic system in the spinal autonomic control of blood pressure in SHR.

It is worth noting that the sympathetically mediated pressor response to i.c.v.-injected SP (Unger *et al*., 1981; Takano *et al*., 1990) is two- to three-fold increased in SHR (Unger *et al*., 1980). Likewise, the sympathetically mediated pressor responses to i.t. or i.c.v. injection of bradykinin that involves kinin B_2 receptors (Lopes & Couture, 1992; Qadri *et al*., 1999) were significantly enhanced in SHR (Buñag & Takahashi, 1981; Lindsey, 1995; Cloutier *et al*., 2002; 2004). The hypersensitivity of the pressor response to bradykinin in SHR was associated with significant increases of kinin B_2 receptor binding sites in the spinal cord and in various cardiovascular brain centres; these abnormalities preceded the development of hypertension (Cloutier *et al*., 2002; 2004).

Putative role of tachykinin NK-1 receptors in spinal autonomic control of blood pressure in SHR

The stereo selective and reversible inhibition achieved with LY 303870 (Gitter *et al*., 1995; Iyengar *et al*., 1997) confirms that

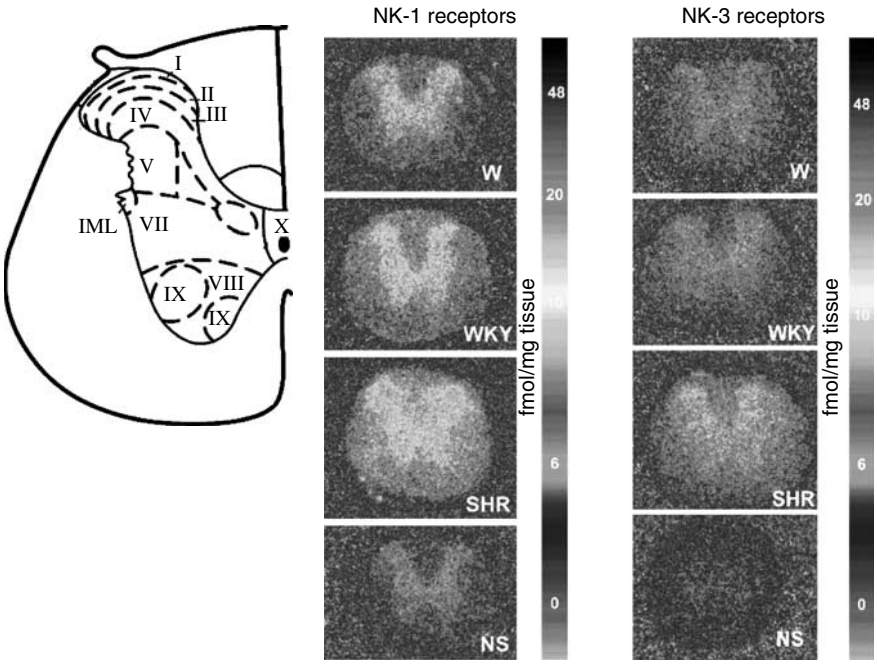

Figure 8 Autoradiographic distribution of the total binding of [^{125}I]HPP[Arg^3,Sar^9,Met(O$_2$)^{11}]SP (3–11) on NK-1 receptors (left) and of [^{125}I]HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH$_2$ on NK-3 receptors (right) in thoracic spinal cords from Wistar (W), WKY and SHR. Nonspecific (NS) binding was obtained in the presence of 1 μM of unlabelled radioligand. Anatomical identification of spinal cord laminae (L1–L10) is also represented schematically as reference areas. IML = intermediolateral horn.

the cardiovascular response mediated by [Sar^9,Met(O$_2$)^{11}]SP in the spinal cord of SHR and WKY is mediated by NK-1 receptors as reported earlier in Wistar rats (Couture *et al*., 1995; Brouillette & Couture, 2002). Previous studies have reported that i.t. injection of SP or [Sar^9,Met(O$_2$)^{11}]SP evoked a biphasic response characterized by a transient drop in MAP followed by increases in MAP and HR in awake Wistar rats (Couture *et al*., 1995; Brouillette & Couture, 2002). The pressor and tachycardiac responses evoked by SP were attributable to the peripheral release of catecholamines from sympathetic fibres and the adrenal medulla (Hasséssian *et al*., 1990). The transient hypotension produced by [Sar^9,Met(O$_2$)^{11}]SP in Wistar rats was ascribed to the inhibition of sympathetic preganglionic fibres following the release of GABA and the subsequent activation of GABA$_B$ receptors in the spinal cord (Brouillette & Couture, 2002). This latter mechanism could play a physiological role in the spinal autonomic control of blood pressure by preventing excessive sympathetic activation during cardiovascular reflexes or after the release of NK-1 receptor agonists (SP) in the spinal cord in response to peripheral nociceptors activation. Since no pressor response occurs in WKY in contrast to Wistar rats upon the spinal activation of NK-1 receptors, it appears that the GABAergic inhibitory component is more effective in WKY. More importantly, this offset mechanism appears dysfunctional in SHR because the amplitude of the initial hypotensive response to i.t. injection of the NK-1 agonist was markedly reduced and occurred only at the highest dose. Thus, the conversion of the initial drop in blood pressure to a sustained pressor response in the presence of the NK-1 agonist points to a greater activation of the sympathetic nervous system in SHR.

In addition to an augmented density of NK-1 receptors, the smaller contribution of the GABAergic component may therefore account for the greater pressor response induced by the NK-1 agonist in SHR. It is worth noting that a downregulation of the GABAergic system and GABA$_B$ receptors was reported in the brain of SHR, preceding blood pressure elevation (Czyzewska-Szafran *et al*., 1989; Sasaki *et al*., 1992; Ichida *et al*., 1995). It is unknown whether such abnormalities of the GABAergic system also occur in the spinal cord of SHR. Whereas, the proportion of GABA-containing neurons in the brainstem with projections to sympathetic preganglionic neurons in the IML of the upper thoracic spinal cord is similar in SHR and WKY (Miura *et al*., 1994), another group of GABAergic interneurons that impinge directly onto sympathetic preganglionic neurons to inhibit their activity was recently identified in the spinal central autonomic area (Deuchars *et al*., 2005).

Sympathetic preganglionic neurons could be modulated by bulbospinal SP-containing neurons which originate in the ventral medulla but also by intraspinal and primary sensory C-fibres which can release SP in the vicinity of sympathetic preganglionic neurons where positive synaptic contacts were shown in the IML (Couture *et al*., 1995; Tan *et al*., 1996). A majority of sympathetic preganglionic neurons in the IML projecting to the adrenal medulla are NK-1 receptor immunoreactive (Ribeiro-da-Silva *et al*., 2000; Burman *et al*., 2001). The finding that the NK-1 antagonist injected in the spinal cord failed to alter resting blood pressure and HR in SHR may however indicate that the tachykininergic system is not tonically hyperactive in SHR and does not contribute to the sympathetic hyperactivity consistently demonstrated in this model of arterial hypertension (Laflamme *et al*., 1997; de Champlain, 2001). However, one should keep in mind that injection of NK-1 antagonist to T-9 spinal cord level may not achieve optimal blockade of tachykinin receptors throughout

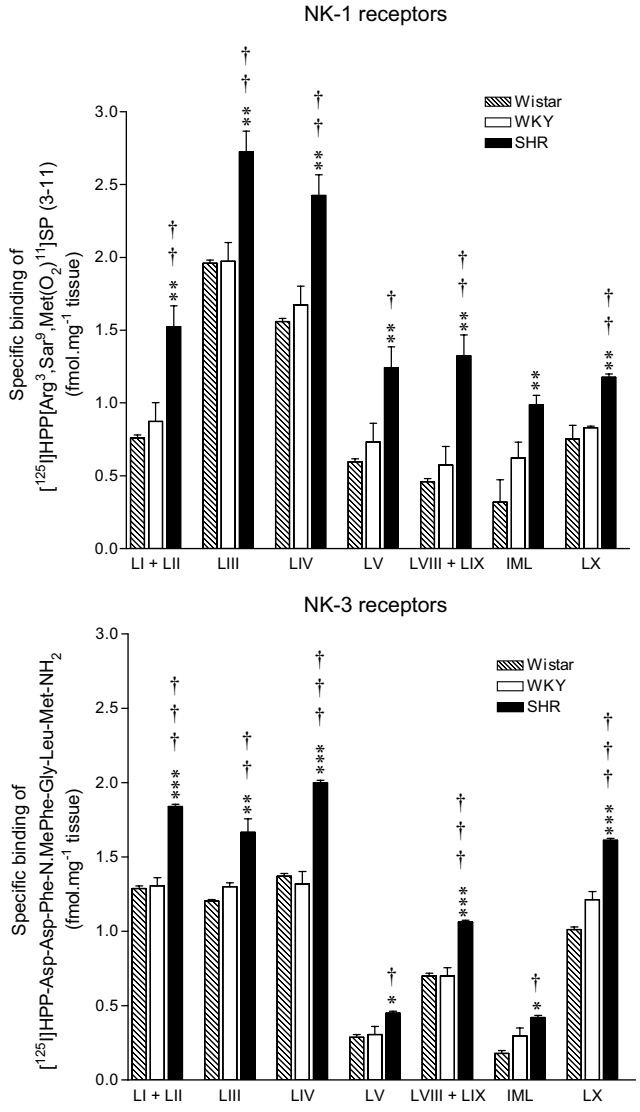

Figure 9 Specific density values of tachykinin NK-1 (upper panel) and NK-3 (lower panel) receptor binding sites in the thoracic spinal cord of Wistar, WKY and SHR. The columns represent the mean ± s.e.m. of four rats in each strain and densities are in fmol mg^{-1} of tissue. Statistical comparisons to Wistar (*) and WKY (†) are indicated by $*,^{\dagger}P<0.05$, $**,^{\dagger\dagger}P<0.01$, $***,^{\dagger\dagger\dagger}P<0.001$.

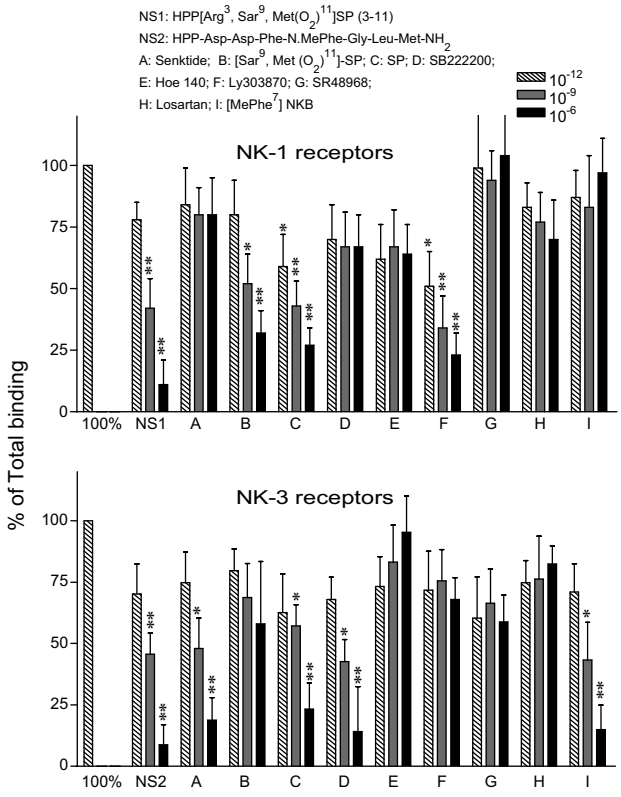

Figure 10 Competition studies were carried out with three concentrations (10^{-12}, 10^{-9} and 10^{-6} M) of various compounds in the presence of [^{125}I]HPP[Arg^3,Sar^9,Met(O$_2$)^{11}]SP (3–11) (upper panel) or [^{125}I]HPP-Asp-Asp-Phe-N-MePhe-Gly-Leu-Met-NH_2 (lower panel) in the thoracic spinal dorsal horn of SHR. The columns represent the mean ± s.e.m. of four rats. Statistical comparison to the total binding achieved with the radioligand alone (100%) is indicated by $*P<0.05$, $**P<0.01$.

the spinal cord necessary to cause significant withdrawal of the sympathetic tone. Indeed, calibration experiments using bromophenol blue dye, ^{3}H-naloxone, ^{14}C-urea or [^{125}I]SP revealed that there was little diffusion of the injectate along the spinal axis and the penetration extended about 0.5 cm rostrally and caudally from the site of i.t. injection (Yaksh & Rudy, 1976; Cridland *et al*., 1987).

A peripheral site of action of the tachykinin NK-1 agonist is also unlikely as its i.v. injection is known to cause vasodilatation and decrease blood pressure (Couture *et al*., 1989; Pompei *et al*., 1993). Moreover, LY 303870 injected i.v. at the dose which was effective i.t. did not modify the cardiovascular response induced by i.t. [Sar^9,Met(O$_2$)^{11}]SP (Brouillette & Couture, 2002). Finally, venous blood samples contain only 0.8–3.5% of the total [^{125}I]SP injected i.t. 1 and 16 min earlier in rat (Cridland *et al*., 1987).

Putative role of tachykinin NK-3 receptors in spinal autonomic control of blood pressure in SHR

One unexpected finding of this study was the high sensitivity of i.t. injected senktide to elicit cardiovascular changes in SHR. This contrast with the absence of cardiovascular changes to NK-3 receptor agonists injected in the spinal cord of freely behaving Wistar rats (Hasséssian *et al*., 1988). Senktide also failed to elicit significant changes in blood pressure in WKY while its effect on HR was marginal. In contrast, prominent and almost maximal cardiovascular changes were achieved with a physiological dose of 6.5 pmol senktide in SHR. In comparison, the NK-1 agonist achieved maximal cardiovascular changes with doses 1000-fold higher. The pressor and tachycardiac responses induced by senktide in SHR are likely due to the release of peripheral catecholamines as shown for other excitatory neuropeptides injected in the spinal cord, including angiotensin II, SP, bradykinin and neuropeptide K (Suter & Coote, 1987; Hasséssian *et al*., 1990; Lopes & Couture, 1992; Pham *et al*., 1993). The possibility that senktide diffuses into the systemic blood circulation to exert its cardiovascular effect is unlikely because its spinal pressor effect was seen only when given to SHR and also because i.v. injections of NK-3 agonists did not modify blood pressure and HR in SHR (Pompei *et al*., 1993). Senktide increased blood

pressure only when nmol doses were injected i.v. in conscious Wistar rats (Cellier *et al*., 1997).

Data with the potent and selective nonpeptide NK-3 receptor antagonist (SB 235375) (Hay *et al*., 2002) confirm that the cardiovascular response to senktide is mediated by NK-3 receptors in the spinal cord of SHR. Thus, the hypersensitivity of the cardiovascular response in SHR seems attributable to an increased number of NK-3 receptors in the spinal cord as evidenced by the increased number of receptor binding sites and the lack of changes of receptor affinity (K_d values were not significantly different between WKY, Wistar and SHR). Using the same autoradiographic approach with the same NK-3 radioligand, we reported recently that the affinity and densities of specific NK-3 receptor binding sites measured in the substantia nigra, ventral tegmental area, hippocampus and amygdala are not significantly different in SHR and WKY aged of 15 weeks (Lessard *et al*., 2003). This indicates that the upregulation of NK-3 receptors in SHR is not a widespread phenomenon in the CNS and seems to be restricted to the spinal cord.

As i.t. SB 235375 had no effect on blood pressure and HR in SHR, the NK-3 receptor does not seem to contribute under resting condition to the maintenance of arterial hypertension. Nevertheless, one cannot exclude as stated earlier for NK-1 receptors that a complete blockade of NK-3 receptors in the whole thoracic spinal cord may have a different impact by unmasking tonic activation of cardiovascular sympathetic preganglionic neurons. Indeed, intracerebral tachykinins were found to contribute to the maintenance of arterial hypertension since blockade of NK-3 receptors with antagonists injected either i.c.v. or directly into the substantia nigra led to a normalization of blood pressure in SHR (Lessard *et al*., 2003; 2004). Therefore, the upregulation of spinal cord tachykinin NK-3 receptor binding sites and the hypersensitivity of the pressor response to i.t. senktide in SHR may contribute with NK-1 receptors to the hyperactivity of the sympathetic nervous system in hypertension.

Further studies will be needed to determine the mechanism underlying the rapid desensitization of the cardiovascular response to senktide in SHR. It is uncertain whether this phenomenon also occurs upon stimulation of NK-3 receptors by endogenous tachykinins. If this occurs, short-term desensitization of NK-3 receptors may afford a protective mechanism by preventing the overactivation of cardiovascular sympathetic preganglionic neurons during arterial hypertension.

Conclusion

This study shows enhanced densities of NK-1 and -3 receptor binding sites, but no changes of receptor affinity, in the lower thoracic spinal cord of SHR. This is thought to contribute to the hypersensitivity of the pressor response to agonists and to the greater sympathetic activity seen in this model of arterial hypertension. Studies using other models of hypertension are however needed before concluding that spinal NK-1 and -3 receptors are relevant central therapeutic targets in the treatment of arterial hypertension.

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