Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 486 (2004) 75–83
www.elsevier.com/locate/ejphar

Central anti-hypertensive effect of tachykinin NK_3 receptor antagonists in rat

Andrée Lessard[a], Marlène Laurin[a], Nobuharu Yamaguchi[b], Réjean Couture[a,*]

[a] *Department of Physiology, Faculty of Medicine, Université de Montréal C.P. 6128, Succursale centre-ville, Montréal, Québec, Canada H3C 3J7*
[b] *Faculty of Pharmacy, Université de Montréal C.P. 6128, Succursale centre-ville, Montréal, Québec, Canada H3C 3J7*

Received 11 September 2003; received in revised form 5 December 2003; accepted 12 December 2003

Abstract

Tachykinins are involved in the central autonomic control of blood pressure. In the present study, we examined the i.c.v. cardiovascular effects of several tachykinin receptor antagonists in awake spontaneously hypertensive rats (SHR, 15 weeks old). Results showed that two tachykinin NK_3 receptor antagonists (R-820: 3-indolylcarbonyl-Hyp-Phg-*N*(Me)-Bzl and SB 222200: (*S*)-(−)-*N*-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide) caused a sustained and dose-dependent reduction of blood pressure when injected i.c.v. but not i.v. The stereoselective anti-hypertensive effect of SB 222200 peaked at 3 h and faded at 6 h post-injection (if injected at 07:00 h) or had a slower onset and peaked at 8 h post-injection (if injected at 13:00 h). The effect of R-820 was maximal at 24 h and lasted up to 48 h post-injection. Both antagonists failed to alter blood pressure in normotensive Wistar–Kyoto rats (WKY) and heart rate was not affected in both strains. The anti-hypertensive effect of SB 222200 was not associated with changes in plasma levels of catecholamines and vasopressin and it remained unchanged in SHR subjected to acute bilateral nephrectomy. In contrast, blood pressure was not affected by tachykinin NK_1 (RP 67580: (±) 7,7-diphenyl-2[1-imino-2(2-methoxy-phenyl)-ethyl]perhydroisoindol-4-one(3*aR*,7*aR*)) and NK_2 (SR 48968: (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) receptor antagonists. Data suggest that brain tachykinin NK_3 receptors are implicated in the maintenance of hypertension in SHR. Hence, these receptors may represent promising therapeutic target in the treatment of arterial hypertension.

Keywords: Tachykinin NK_3 receptor; Arterial hypertension; Central autonomic regulation; Catecholamine; Vasopressin

1. Introduction

Substance P, neurokinin A and neurokinin B, the three main mammalian tachykinins, exert a variety of biological effects through the activation of three transmembrane G-protein-coupled receptors designated neurokinin-1 (NK_1), NK_2 and NK_3 (Otsuka and Yoshioka, 1993). Evidence suggests a role for these neuropeptides in central cardiovascular regulation (Takano et al., 1990; Culman and Unger, 1995; Culman et al., 1997; Cellier et al., 1997, 1999). In normotensive rats, intracerebroventricular (i.c.v.) injection of tachykinins or selective receptor agonists increases arterial blood pressure and heart rate (Takano et al., 1990; Couture et al., 1995; Cellier et al., 1997, 1999) and causes inhibition of water and electrolyte renal excretion (Yuan and Couture, 1997). These responses are mediated by an increased sympathetic tone (the three tachykinin receptors) (Unger et al., 1981; Takano et al., 1990; Couture et al., 1995) and the release of vasopressin (tachykinin NK_3 receptor) (Polidori et al., 1989; Massi et al., 1991; Yuan and Couture, 1997). Microinjection of the selective tachykinin NK_3 receptor agonist senktide into the rat hypothalamic paraventricular nucleus also induces antidiuretic and pressor effects, which are blocked respectively by vasopressin V_2 and V_1 receptor antagonists (Nakayama et al., 1992; Eguchi et al., 1996). These studies are consistent with autoradiographic, immunocytochemical and in situ hybridisation localization of tachykinin NK_3 receptor and its mRNA in the magnocellular part of the hypothalamic paraventricular and supraoptic nuclei (for a seminal review, see Ribeiro-Da-Silva et al., 2000). Moreover, a double fluorescence immunohistochemical study has shown that all vasopressin-immunoreactive neurons in the rat hypothalamic paraventricular and supraoptic nuclei

* Corresponding author. Tel.: +1-514-343-7060; fax: +1-514-343-2111.
E-mail address: couturer@physio.umontreal.ca (R. Couture).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2003.12.013

produce neurokinin B and co-express tachykinin NK_3 receptors suggesting that these receptors mediate an auto-regulatory mechanism for vasopressin release (Hatae et al., 2001).

Sparse information is presently available regarding the putative role of tachykinins in arterial hypertension. In the periphery, substance P may act as a partial counterregulator of vasoconstriction in models of salt-dependent hypertension (Kohlmann et al., 1997). For instance, an intravenous infusion of the tachykinin NK_1 receptor antagonist CP 96,345 increases arterial pressure in rats made hypertensive with desoxycorticosterone and salt (DOCA-salt) and in one-kidney-one clip renovascular hypertensive rats but not in salt-independent models such as spontaneously hypertensive rats and two-kidney-one clip renovascular hypertensive rats (Kohlmann et al., 1997). High plasma levels of neurokinin B are found in human pregnancy-induced hypertension and pre-eclampsia, and intravenous infusion of neurokinin B in conscious female rats which simulates late pregnancy concentrations of neurokinin B increases blood pressure (Page et al., 2000). At this time, a central site of action for the pressor response to circulating neurokinin B cannot be excluded. Recently, we have shown a significant decrease of blood pressure for over 3 h after microinjection of the selective tachykinin NK_3 receptor antagonist 3-indolylcarbonyl-Hyp-Phg-*N*(Me)-Bzl (R-820) into the *substantia nigra* of SHR (15 weeks old), yet a similar treatment had no cardiovascular effect in age-matched normotensive control WKY (Lessard and Couture, 2001a; Lessard et al., 2003).

The aim of this study was to further investigate the role of central endogenous tachykinins in the maintenance of arterial hypertension in SHR. This was addressed by investigating the cardiovascular responses to intracerebral injection of selective tachykinin receptor antagonists at tachykinin NK_1 (RP 67580: (±) 7,7-diphenyl-2[1-imino-2(2-methoxy-phenyl)-ethyl]perhydroisoindol-4-one(3*aR*, 7*aR*)) (Garret et al., 1991), NK_2 (SR 48968: (*S*)-*N*-methyl-*N*[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide) (Advenier et al., 1992) and NK_3 receptors (R-820, SB 235375 [(*S*)-(−)-*N*-(α-ethylbenzyl)-3-(carboxymethyl)-2-phenylquinoline-4-carboxamide] and SB 222200 [(*S*)-(−)-*N*-(α-ethylbenzyl)-3-methyl-2-phenylquinoline-4-carboxamide]) (Regoli et al., 1994; Sarau et al., 2000; Hay et al., 2002). Since results show that tachykinin NK_3 receptor antagonists reduced high blood pressure in SHR, peripheral mechanisms underlying the anti-hypertensive effect of SB 222200 were investigated by measuring possible decreases in plasma levels of catecholamines and vasopressin, and by assessing the influence of bilateral nephrectomy. The circadian rhythm of vasopressin and catecholamines release was considered in the experimental design. Indeed, in the early morning, there is a marked rise in neuronal and hormonal sympathetic activity (Yamasaki et al., 1998; Walters et al., 2003) while vasopressin release is maximal during the day and the lowest at night (Mens et al., 1982; Windle et al., 1992; Watanabe et al., 2000). To avoid the spurious effects of anaesthesia and the stress induced by immobilisation, the monitoring of cardiovascular function was conducted in awake, unrestrained rats. A preliminary report of this work has been presented elsewhere (Lessard and Couture, 2001b).

2. Materials and methods

2.1. Animal source and care

Male spontaneously hypertensive rats (SHR, 15 weeks old, $n=116$) and age-matched Wistar–Kyoto rats (WKY, $n=35$) were purchased 3–5 weeks prior to experiments from Charles River, St. Constant, Québec, Canada, and housed under a 12-h light–dark cycle in a room with controlled temperature (22 °C), humidity (53%) with food (Charles River Rodent) and tap water 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.

2.2. Animal preparation

SHR and WKY were implanted with one 23-gauge stainless steel guide cannula into the right lateral ventricle (coordinates: 1 mm posterior to the bregma, 1.4 mm lateral to the midline, 3.0 mm ventral from the skull surface) (Cellier et al., 1997, 1999). One week after the surgery, an intravascular siliconized polyethylene tubing PE-50 catheter, filled with physiological saline containing 100 IU/ml heparin sodium salt, was inserted into the abdominal aorta via the right femoral artery for direct blood pressure recording. Some animals also were implanted with a siliconized PE-10 catheter, filled with physiological saline containing 100 IU/ml heparin sodium salt, into the right jugular vein for i.v. injection ($n=11$). The intravascular catheters were tunnelled subcutaneously to emerge at the back of the neck with the i.c.v. catheter. A separate group of rats ($n=25$) underwent bilateral nephrectomy through dorsolateral incision of the skin and muscles under halothane anesthesia.

Before each surgery, the animals received Ethacilin (5 mg/kg, i.m., rogar/S.T.B., London, Ontario, Canada) and Ketoprophen (anafen, 10 mg/kg, i.m., Merial Canada, Baie d'Urfé, Québec, 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 cages (40 × 23 × 20 cm) with a top grid and returned to their resident room. Experimental protocols were initiated 48 h after the last surgery (or 6 h after bilateral nephrectomy) in conscious and unrestrained rats (Cellier et al., 1997, 1999). Successful intravascular and

i.c.v. catheters implantation was confirmed in all rats used in the study after post-mortem examination.

2.3. Measurement of cardiovascular parameters

Blood pressure and heart rate were measured with a Statham pressure Transducer (P23ID) and a cardiac tachometer (model 7P4) coupled to a Grass polygraph (model 79; Grass Instruments, Quincy, MA, USA). 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. When resting blood pressure and heart rate were stable, i.c.v. or i.v. injections were performed on undisturbed, freely moving rats through 31-gauge stainless steel injector (i.c.v.) or a polyethylene tubing (i.v.). The injector was inserted into the guide cannula without handling the rats. All solutions for injections were freshly prepared and injected (1 μl, i.c.v.; bolus of 0.1 ml/kg, i.v.) over a period of 1 min.

2.4. Experimental protocols

2.4.1. Experiment 1: effect of i.c.v. tachykinin receptor antagonists

SHR and WKY received at random an i.c.v. injection of selective antagonists (50–500 pmol) for the tachykinin NK_1 (RP 67580), NK_2 (SR 48968) and NK_3 receptor (R-820, SB 235375 and SB 222200) or the vehicle (artificial cerebrospinal fluid (aCSF) containing less than 15% dimethyl sulphoxide (DMSO)). Each rat received only one antagonist at a single dose at 13:00 h. The inactive enantiomer of SB 222200 (SB 222201) was also injected as control. A separate group of SHR received i.c.v. SB 222200 early in the morning (07:00 h, $n=6$). Arterial blood pressure and heart rate were monitored for 8 h every day up to 4 days post-injection. Data presented at selected time points are an average of 15-min recording. Higher doses than 500 pmol SB 222200 require more than 15% of DMSO, which was not tolerated by rats (Lessard and Couture, 2001a,b). Thus, antagonists were compared at equimolar doses based on their solubility in physiological medium and on previous studies showing their effectiveness to block the effect of their respective agonists in Wistar rat (Lessard and Couture, 2001a) and to reverse hypertension (R-820) in SHR (Lessard et al., 2003) after microinjection in the *substantia nigra*.

2.4.2. Experiment 2: effect of i.v. tachykinin NK_3 receptor antagonists

To exclude peripheral leakage of i.c.v. injected tachykinin NK_3 receptor antagonists to general circulation, SHR received i.v. the tachykinin NK_3 receptor antagonists (R-820 or SB 222200, 500 pmol) or the vehicle (saline containing less than 15% DMSO). Each rat received only one antagonist at random. Arterial blood pressure and heart rate were measured during the following 3 days as described above for i.c.v. injection.

2.4.3. Experiment 3: effect of i.c.v. SB 222200 in bilaterally nephrectomized SHR

To exclude a possible effect of released vasopressin on kidneys, bilaterally nephrectomized SHR received an i.c.v. injection of SB 222200 (500 pmol) or the vehicle ($n=11$) either at 07:00 h ($n=5$) or at 13:00 h ($n=9$). Mean arterial blood pressure and heart rate were measured continuously up to 24 h post-injection.

2.4.4. Experiment 4: effect of i.c.v. SB 222200 on plasma levels of catecholamines and vasopressin

Two groups of SHR were injected i.c.v. 500 pmol SB 222200 (13:00 h) and blood samples (2 ml) were collected from the intraaortic catheter in the awake animal at the time of the maximum decrease of blood pressure and 3 days apart in the absence of SB 222200 (control hypertensive values) at the same time point of the day to prevent any circadian influence. Measurements of vasopressin (group 1, $n=8$) and catecholamines (group 2 that includes group 1 and eight additional rats) were made on the first and second ml blood sample, respectively. After collecting blood samples, rats received i.v. the same volume of ringer-lactate (273 mosM/l, pH: 6.7; Abbott Laboratories, Saint-Laurent, Québec, Canada). With this protocol, each rat had its own control value.

2.5. Measurement of plasma catecholamines and vasopressin

Catecholamines were extracted from plasma and quantified with high-pressure liquid chromatography (HPLC) according to previous methods (Remie and Zaagsma, 1986; Musso et al., 1990) with some modifications (Yamaguchi, 1993). Briefly, 1 ml of blood was collected in a chilled glass tube containing 20 μl of preservative solution (pH 6.5) consisting of ethyleneglycol-bis (β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid (95 mg/ml) and glutathione (60 mg/ml). Tubes were then immediately centrifuged at 4 °C and 3000 rpm for 20 min. Plasma was collected and stored at −80 °C. To 400 μl of plasma in a 15-ml glass tube with a screw cap, 40 μl of an aqueous solution containing dihydroxybenzylamine (1 ng/ml), its mother solution being prepared with 0.2 M acetic acid, and 1 ml of 2 M NH_4OH–NH_4Cl buffer (pH 8.7) that contains 0.1% diphenylborate–ethanolamine and 0.5% EDTA were added. After the addition of 5 ml of *n*-heptane containing 1% *n*-octanol and 0.25% tetraoctylammonium bromide, the sample solution was mixed by a rotating mixer for 5 min and centrifuged at 2500 rpm for 5 min. Then 4 ml of the organic phase were transferred to a conic tube, mixed with 2 ml of *n*-octanol and 400 μl of 0.08 M acetic acid, mixed for 5 min and centrifuged at 2500 rpm for 5 min. The organic phase was discarded, and the aqueous phase was transferred to the amber microtube for HPLC determination.

Vasopressin was extracted according to the petro-ether method (Le Mellédo et al., 2001) and measured by radio-immunoassay (RIA) (Bichet et al., 1986). Briefly, 1 ml of blood was collected in 10-ml tubes containing EDTA. Tubes were immediately centrifuged at 4 °C and 3000 rpm for 20 min. Plasma was then collected and stored at − 80 °C. For the assay, plasma samples were extracted with the petrol-ether method, evaporated to dryness and reconstituted in 750 μl of buffer. Then 200 μl of reconstituted sample were tested in duplicate. The RIA sensitivity is 0.1 pg/tube (0.5 pg/ml), with a 50% displacement of the tracer (iodinated-vasopressin from Amersham) obtained for 1.2 pg/tube. The antiserum (AS-2849) was used at a final dilution of 1/2,500,000. The standard curve included 6 Bo and 10 concentrations in triplicate. Plasma samples were measured in duplicate. Intra- and inter-assay coefficients of variation for vasopressin plasma concentrations between 2 and 5 pg/ml were less than 10%. Non-specific binding (with the charcoal separation method) was less than 3%. The laboratory technicians performing the catecholamines and vasopressin measurements were blind to the condition.

2.6. Drugs and solutions

The composition of aCSF was, in mM: NaCl 128.6, KCl 2.6, $MgCl_2$ 2.0 and $CaCl_2$ 1.4; pH adjusted to 7.2. The non-peptide antagonists RP 67580 ((±) 7,7-diphenyl-2[1-imino-2(2-methoxy-phenyl)-ethyl] perhydroisoindol-4-one(3*aR*,7*aR*) and SR 48968 were kind gifts from Dr. C. Garret (Rhône-Poulenc Rorer, Vitry sur Seine, France) and X. Emonds-Alt (Sanofi Recherche, Montpellier, France), respectively. The pseudo-peptide antagonist R-820 was generously provided by Dr. J.L. Fauchère (Servier, Paris, France). The lipophilic non-peptide antagonist SB 222200, its inactive enantiomer SB 222201 and the hydrophilic SB 235375 were provided by Dr. H. M. Sarau (GlaxoSmithKline, PA, USA). All antagonists were solubilized in DMSO (Fisher Scientific, Montréal, Québec, Canada) except the water soluble molecule SB 235375. Other drugs were purchased from Sigma (St. Louis, MO, USA). The solution was then completed with aCSF or saline and contained 1–15% (v/v) DMSO. Vehicle (aCSF or saline containing less than 15% DMSO) was injected to all animals and failed to cause any significant cardiovascular changes.

2.7. Statistical analysis of data

Results are expressed as means ± S.E.M. of (*n*) rats. Statistical differences between means within the same group (Table 2) were evaluated with Student's *t*-test for paired values. Multiple comparisons in relation with the time course of changes in mean arterial blood pressure and heart rate were analysed with a two-way analysis of variance (ANOVA) followed by the test of Bonferroni. Multiple comparisons to pre-injection values (Table 1) were evaluated with a one-way ANOVA and a post-hoc Dunnett's test. Only probability values (*P*) less than 0.05 were considered to be statistically significant.

3. Results

3.1. Cardiovascular response induced by i.c.v. and i.v. injections of SB 222200

In SHR, the i.c.v. injection of SB 222200 at 13:00 h significantly reduced mean arterial blood pressure from 6 to 8 h (250 pmol, $n=7$) and from 3 to 8 h post-injection (500 pmol, $n=8$) when compared to vehicle values (Fig. 1A). The maximal decrease induced by 500 pmol was seen at 8 h (− 37 ± 9 mm Hg, $P<0.01$) and mean arterial blood pressure was back to hypertensive levels (167 ± 6 mm Hg) 48 h post-injection. At a lower dose (100 pmol, $n=7$), SB 222200 had no significant effect on mean arterial blood pressure when compared to vehicle values (Fig. 1A). In WKY, i.c.v. injection of SB 222200 (500 pmol, $n=12$) had no significant effect on mean arterial blood pressure when compared to vehicle values ($n=12$) (Fig. 1A). When ad-

Table 1
Heart rate values (bpm) following i.c.v. injection of tachykinin receptor antagonists in SHR

Time post-injection (h)	Vehicle ($n=8$)	SB 222200, 250 pmol ($n=7$)	SB 222200, 500 pmol ($n=8$)	SB 222201, 500 pmol ($n=6$)	RP 67580, 500 pmol ($n=6$)	SR 48968, 500 pmol ($n=5$)	SB 235375, 500 pmol ($n=8$)
0	347 ± 14	331 ± 12	363 ± 10	372 ± 19	373 ± 20	394 ± 22	301 ± 13
1	344 ± 12	347 ± 16	370 ± 11	368 ± 16	352 ± 17	378 ± 25	325 ± 29
2	351 ± 13	346 ± 19	366 ± 11	373 ± 16	382 ± 18	390 ± 20	340 ± 25
3	368 ± 16	353 ± 25	356 ± 10	387 ± 19	390 ± 24	394 ± 25	317 ± 25
4	359 ± 18	337 ± 28	374 ± 12	382 ± 15	392 ± 31	388 ± 20	305 ± 22
5	370 ± 18	337 ± 24	353 ± 12	393 ± 23	393 ± 31	398 ± 18	308 ± 20
6	361 ± 17	330 ± 22	362 ± 13	388 ± 16	387 ± 27	402 ± 23	313 ± 21
7	366 ± 17	331 ± 22	363 ± 9	388 ± 19	382 ± 26	402 ± 22	305 ± 22
8	360 ± 17	346 ± 15	359 ± 14	378 ± 16	367 ± 26	404 ± 23	304 ± 21
24	363 ± 10	373 ± 25	353 ± 11	–	–	–	296 ± 14

Values represent the mean ± S.E.M. of (*n*) rats. None of the antagonists produced statistical changes in heart rate after i.c.v. injection (comparison to pre-injection values at time 0 h).

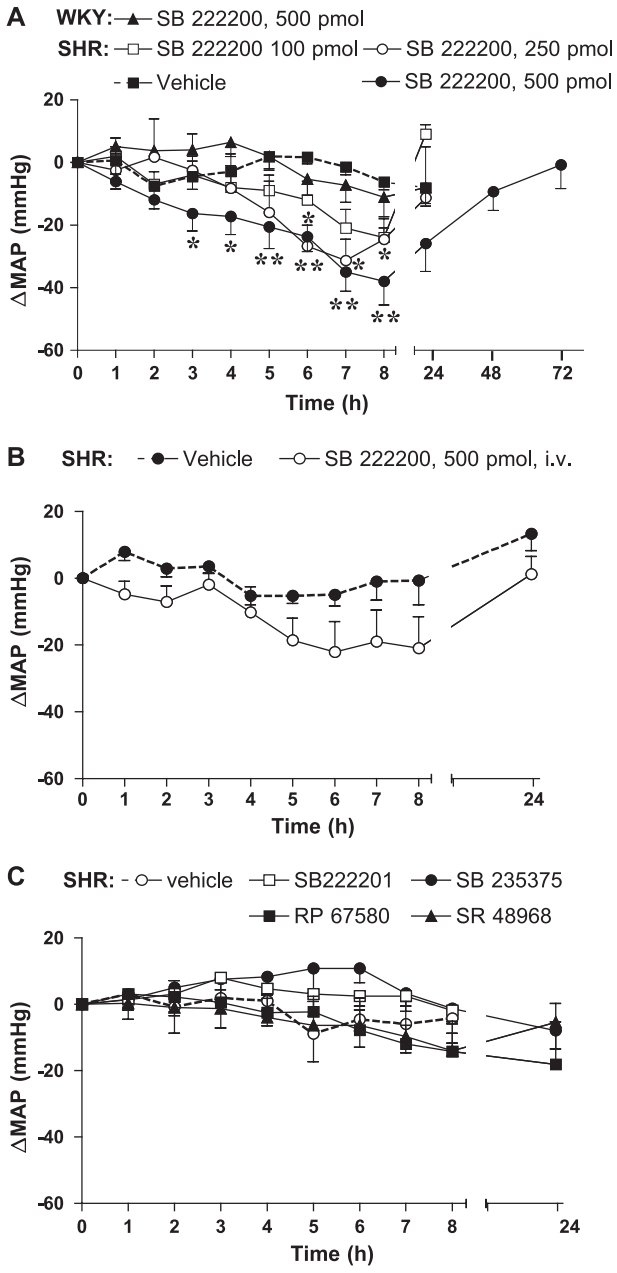

Fig. 1. Changes in mean arterial blood pressure (ΔMAP) following (A) i.c.v. injection (13:00 h) of vehicle ($n=12$) or SB 222200 at 100 pmol ($n=7$), 250 pmol ($n=7$) and 500 pmol ($n=8$) in SHR or at 500 pmol in WKY ($n=12$). Basal values were: 177 ± 3 mm Hg (vehicle), 171 ± 16 mm Hg (100 pmol), 180 ± 11 mm Hg (250 pmol), 184 ± 8 mm Hg (500 pmol) and 131 ± 3 mm Hg (500 pmol, WKY); (B) i.v. injection of vehicle ($n=12$) or 500 pmol SB 222200 ($n=7$) in SHR. Basal values were: 171 ± 4 mm Hg (vehicle) and 184 ± 8 mm Hg (500 pmol), and (C) i.c.v. injection (13:00 h) of vehicle ($n=6$) or SB 222201 ($n=6$), SB 235375 ($n=8$), RP 67580 ($n=6$) or SR 48968 ($n=5$) at the dose of 500 pmol. Basal values were 174 ± 7 mm Hg (vehicle), 172 ± 1 mm Hg (SB 222201), 195 ± 13 mm Hg (RP 67580), 182 ± 10 mm Hg (SR 48968) and 163 ± 6 mm Hg (SB 235375). Each point represents the mean ± S.E.M. of (n) rats. Comparison to vehicle values is indicated by $^{*}P<0.05$, $^{**}P<0.01$.

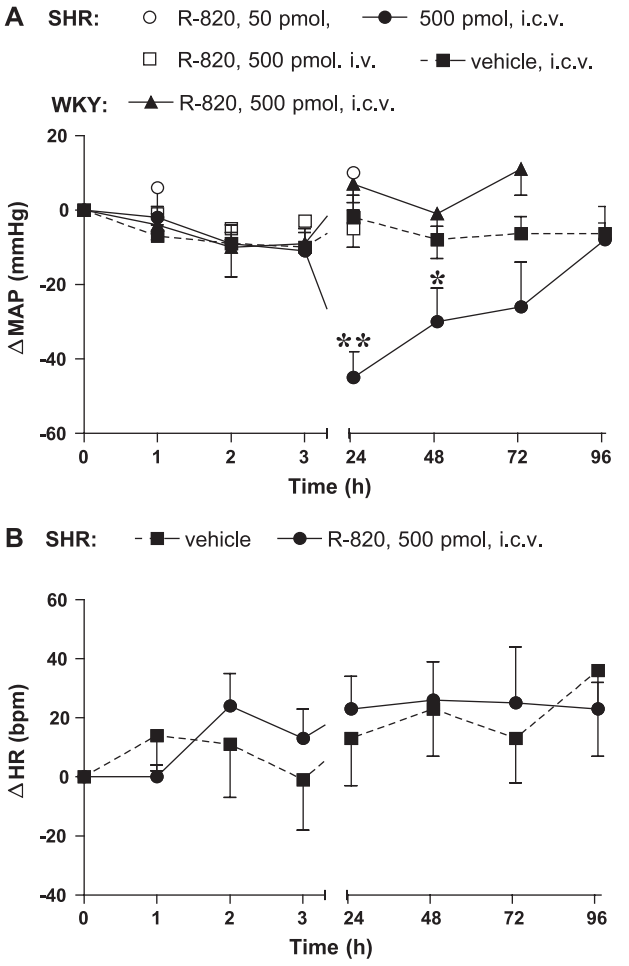

Fig. 2. Changes in mean arterial blood pressure (ΔMAP) (A) and heart rate (ΔHR) (B) following i.c.v. injection (13:00 h) of vehicle ($n=10$) or R-820 at the dose of 50 pmol ($n=6$) and 500 pmol in SHR ($n=10$) or 500 pmol in WKY ($n=4$). The dose of 500 pmol i.v. of R-820 ($n=4$) is also shown on mean arterial blood pressure in SHR. Basal values were 169 ± 5 mm Hg and 347 ± 14 bpm (vehicle), 170 ± 4 mm Hg and 344 ± 22 bpm (500 pmol R-820, i.c.v.), 153 ± 6 mm Hg (50 pmol R-820, i.c.v.), 176 ± 6 mm Hg (500 pmol R-820, i.v.) in SHR and 134 ± 5 mm Hg (R-820, 500 pmol, i.c.v.) in WKY. For the sake of clarity, only the dose of R-820 that decreased blood pressure was illustrated on heart rate. Also, the connecting lines for R-820, 50 and 500 pmol i.v. have been excluded for clarity. Each point represents the mean ± S.E.M. of (n) rats. Comparison to vehicle values is indicated by $^{*}P<0.05$, $^{**}P<0.01$.

ministered i.v. in SHR, SB 222200 (500 pmol, $n=7$) slightly reduced mean arterial blood pressure when compared to vehicle values, yet changes did not reach significant level (Fig. 1B). SB 222200 (250 or 500 pmol, i.c.v.) had no significant effect on heart rate in SHR (Table 1) and WKY (data not shown).

3.2. Cardiovascular response induced by i.c.v. and i.v. injections of R-820

In SHR, the i.c.v. injection of R-820 (500 pmol, $n=10$) at 13:00 h significantly decreased mean arterial blood pressure at 24 and 48 h post-injection when compared to

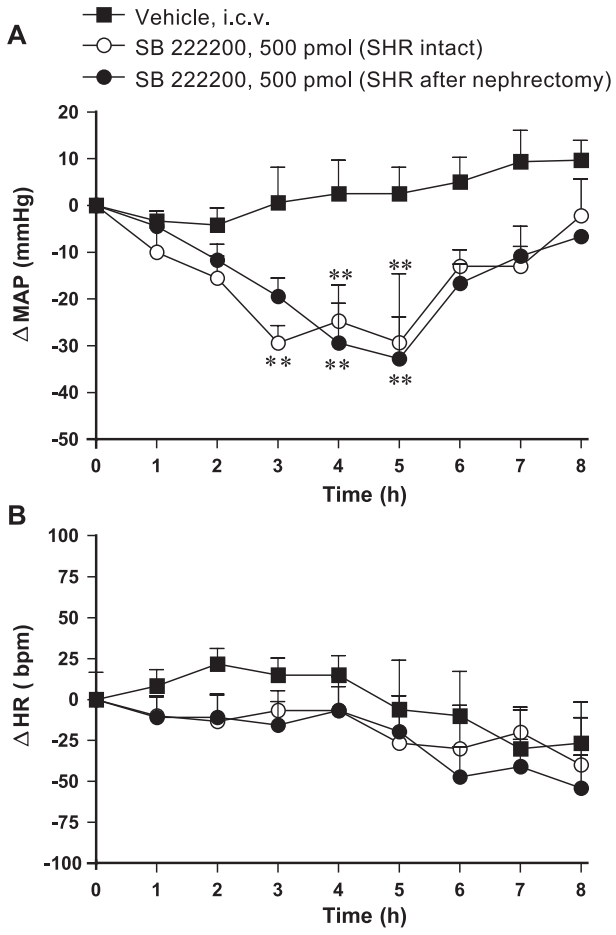

Fig. 3. Changes in mean arterial pressure (ΔMAP) (A) and heart rate (ΔHR) (B) following i.c.v. injection (07:00 h) of 500 pmol SB 222200 in intact SHR ($n=6$) and bilaterally nephrectomized SHR ($n=5$) or vehicle in bilaterally nephrectomized SHR ($n=6$). Basal values were 178 ± 8 mm Hg and 336 ± 16 bpm in intact SHR, 175 ± 5 mm Hg and 330 ± 43 bpm in nephrectomized SHR (vehicle), and 166 ± 10 mm Hg and 344 ± 38 bpm in nephrectomized SHR (SB 222200). Each point represents the mean ± S.E.M. of (n) rats. Comparison to vehicle values is indicated by $^{**}P<0.01$. No statistical difference was found between intact and bilaterally nephrectomized SHR.

vehicle values ($n=10$) (Fig. 2A). A lower dose (50 pmol, $n=6$) of R-820 had no effect on mean arterial blood pressure. The i.v. injection of 500 pmol R-820 was also inactive (basal: 176 ± 6 mm Hg; 24 h post R-820: 172 ± 6 mm Hg, $n=4$, $P>0$ .05). In WKY, 500 pmol R-820 (i.c.v., $n=4$) had no significant effect on mean arterial blood pressure (Fig. 2A). Whatever the doses tested i.c.v. or i.v., R-820 had no significant effect on heart rate in both SHR (Fig. 2B) and WKY (data not shown).

3.3. Lack of effects of i.c.v. SB 235375, RP 67580, SR 48968 and SB 222201 in SHR and WKY

When compared to vehicle values, none of the following antagonists injected i.c.v. at the dose of 500 pmol had a significant effect on mean arterial blood pressure and heart rate in SHR for a period of 1–24 h post-injection: SB 235375 ($n=8$), the selective tachykinin NK_1 (RP 67580, $n=6$) and NK_2 receptor antagonists (SR 48968, $n=5$) and the inactive enantiomer of SB 222200 (SB 222201, $n=6$) (Fig. 1C, Table 1). In WKY, blood pressure was not significantly altered for a period up to 8 h after i.c.v. injection of the same antagonists (500 pmol): maximal changes in mean arterial blood pressure were −14 ± 3 mm Hg (RP 67580, $n=5$), −6 ± 6 mm Hg (SR 48968, $n=5$), −5 ± 4 mm Hg (SB 222201, $n=8$) and −11 ± 4 mm Hg (vehicle, $n=6$). All these drugs were injected in the afternoon.

3.4. Cardiovascular response induced by i.c.v. SB 222200 in bilaterally nephrectomized SHR

In contrast to data presented at 13:00 h (Fig. 1A), there was a quicker recovery toward baseline when 500 pmol of SB 222200 was administered i.c.v. at 07:00 h (Fig. 3A). The maximal decrease in mean arterial blood pressure peaked at 3 h (−29 ± 5 mm Hg, $n=6$, $P<0.01$) and the response was no more significant 6 h post-injection in SHR.

The time-course of the anti-hypertensive response induced by i.c.v. injection of 500 pmol SB 222200 at 07:00 h was quite similar and not significantly different in intact and bilaterally nephrectomized SHR (Fig. 3A). The same treatment with SB 222200 had no significant effect on heart rate in intact and bilaterally nephrectomized SHR (Fig. 3B). When the antagonist was injected at 13:00 h, the anti-hypertensive response was not significantly different in intact (maximal changes in mean arterial bloodpressure = −37 ± 9 mm Hg, $n=8$) and bilaterally nephrectomized SHR (maximal changes in mean arterial bloodpressure = −40 ± 16 mm Hg, $n=9$).

Table 2
Values of mean arterial pressure (MAP), heart rate (HR) and plasma levels of vasopressin (group 1) and catecholamines (group 2) in the presence and absence (control) of SB 222200 (500 pmol, i.c.v.) in SHR

	Basal	SB 222200 7–8 h post-injection	Control
Group 1			
MAP (mm Hg)	169 ± 4	133 ± 3[a]	170 ± 5
HR (bpm)	308 ± 22	290 ± 17	311 ± 16
Vasopressin (pg/ml)	–	4.9 ± 2.0	6.9 ± 4.2
Group 2			
MAP (mm Hg)	174 ± 3	136 ± 2[a]	172 ± 3
HR (bpm)	363 ± 10	353 ± 11	370 ± 19
Epinephrine (pg/ml)	–	158 ± 52	187 ± 54
Norepinephrine (pg/ml)	–	239 ± 31	253 ± 29
Dopamine (pg/ml)	–	59 ± 7	49 ± 6

Values represent the mean ± S.E.M. of 8 SHR (group 1) and 16 SHR (group 2).
[a] $P<0.01$ when compared to control values (Student's t-test).

3.5. Effect of SB 222200 on plasma levels of catecholamines and vasopressin in SHR

Plasma levels of vasopressin, epinephrine, norepinephrine and dopamine measured in SHR at the peak of the maximal decrease in mean arterial blood pressure (7–8 h) induced by i.c.v. injection of 500 pmol SB 222200 were not significantly different from control values measured in the same animals (Table 2). Values of vasopressin were in the same range than those reported previously in SHR (Möhring et al., 1979).

4. Discussion

The present study provides the first pharmacological evidence that tachykinin NK_3 receptor antagonists exert dose-dependent and reversible anti-hypertensive effects when injected intracerebrally in SHR. This reduction of systemic blood pressure likely derives from specific blockade of brain tachykinin NK_3 receptors because it was reproduced with two structurally unrelated molecules: a non-peptide (SB 222200) and a pseudo-peptide antagonist (R-820), while the inactive enantiomer SB 222201 was inactive. In addition to excluding non-specific central effects, the latter result with SB 222201 rules out the possibility that the decline in blood pressure over the recording period is merely due to the conditioning of the rats to recording apparatus. The dissimilar time course of the anti-hypertensive effect of R-820 and SB 222201 may be explained by their different pharmacological profiles: R-820 is more hydrophilic than SB 222200, a highly lipophilic molecule, which can rapidly diffuse into the brain after i.c.v. injection. The readily water soluble SB 235375 (Hay et al., 2002) was found inactive most likely because it does not penetrate into the nervous tissue deeply enough to reach its target tachykinin NK_3 receptors located beyond the circumventricular organs. While SB 222200 penetrates the blood brain barrier, R-820 does not (Regoli et al., 1994; Sarau et al., 2000). Thus, the differential physico-chemical features of these molecules could explain why SB 222200 displays a quicker but shorter lasting effect than R-820. The site of action of these molecules is most likely located in the brain because both antagonists were ineffective when injected at similar doses in the systemic blood circulation. Even if SB 222200 crosses the blood brain barrier, the dose administered i.v. was insufficient to cause significant central effects. Our preliminary experiments have shown that i.v. injection of SB 222200 requires doses greater than 1 mg/kg to decrease systemic blood pressure. Recent results from our laboratory showed partial but significant decrease (about −20 mm Hg) of blood pressure during the first 3 h following microinjection of R-820 into the *substantia nigra* in SHR (Lessard et al., 2003). Thus, the inhibition of the nigro-striatal dopaminergic pathway may represent a putative target for i.c.v. tachykinin NK_3 receptor antagonists in SHR, whereas other sites may also be involved.

Although heart rate was not affected by central administration of tachykinin NK_3 receptor antagonists, it is possible that the anti-hypertensive effect of these molecules is attributable to a decrease of the cardiac stroke volume or to a decrease of peripheral resistance. We tested the hypothesis that the withdrawal of the sympathetic tone could be involved since it is well known that the activity and reactivity of this system are increased in experimental models of hypertension (De Champlain, 1990). I.c.v. injection of the tachykinin NK_3 receptor selective agonist senktide increased heart rate and blood pressure in normotensive rat (Cellier et al., 1997) and in guinea-pig (Roccon et al., 1996). This cardiovascular response was blocked by the prior i.c.v. injection of R-820 in rat (Cellier et al., 1997) and was ascribed to the activation of the sympathetic nervous system in guinea-pig (Roccon et al., 1996). Also, we recently suggested that the activation of tachykinin NK_3 receptors in the rat *substantia nigra* evoked a tachycardia by enhancing the sympatho-adrenal drive to the heart (Lessard and Couture, 2001a). However, the present study failed to show a significant reduction of plasma catecholamines at the peak of the anti-hypertensive effect of SB 222200. Although this finding provides an argument against a primary role for the sympatho-adrenal system, one cannot completely rule out that central tachykinin NK_3 receptor antagonists may cause inhibitory action on sympathetic neurotransmission to specific organ beds (e.g., the vasculature). Differential effects of central tachykinin NK_3 receptor activation and/or inhibition on sympathetic outflow to the periphery may afford a reasonable explanation for the lack of changes on heart rate and the apparent discrepancy between the effects of tachykinin NK_3 receptor agonists and antagonists on the sympatho-adrenal system. I.c.v. agonists and antagonists can interact with different brain sites as consequence of dissimilarities in the physico-chemical properties of these molecules. Further studies will be necessary to explore these hypotheses.

Because the anti-hypertensive response displayed a slow onset and was subjected to a circadian cycle, a hormonal mechanism can take place. Incidentally, it was suggested that the hypothalamic tachykinin NK_3 receptor is associated with the regulation of blood volume through a vasopressinergic-dependent mechanism. Indeed, tachykinin NK_3 receptor agonists injected i.c.v. or directly into the rat paraventricular nucleus of the hypothalamus increased plasma vasopressin levels (Polidori et al., 1989; Massi et al., 1991) and caused anti-diuretic, anti-natriuretic and pressor effects which were blocked by the prior i.c.v. injection of R-820 or by i.v. treatment with vasopressin V_2 and V_1 receptor antagonists (Nakayama et al., 1992; Eguchi et al., 1996; Yuan and Couture, 1997). However, the present results do not support the hypothesis that the

anti-hypertensive effect of SB 222200 derived from an inhibition of the vasopressin release and the subsequent loss of its renal anti-diuretic action because plasma levels of vasopressin were not modified by SB 222200 and the anti-hypertensive effect of the drug persisted in bilaterally nephrectomized SHR. In addition to excluding a diuretic mechanism, results in bilaterally nephrectomized rats also exclude the participation of other renal influence such as the renin–angiotensin system.

Although highly speculative at this time, the contribution of other putative mediators such as melatonin and atrial natriuretic peptide in the anti-hypertensive effects of the tachykinin NK_3 receptor antagonists remain to be addressed.

Because i.c.v. tachykinin NK_3 antagonists did not reduce blood pressure in normotensive WKY as already reported in Wistar rats (Couture et al., 1995; Cellier et al., 1997), it appears that central tachykinin NK_3 receptors are unlikely to be involved in the tonic autonomic control of blood pressure in normotensive rats. On the other hand, they are likely activated by a greater basal release of tachykinin NK_3 receptor agonists in the brain of SHR. Interestingly, NKB-like immunoreactive contents were reported to be increased in discrete brain nuclei in SHR when compared to age-matched WKY (Nagashima et al., 1989).

While the role of central tachykinin NK_2 receptors in brain functions remains elusive, central tachykinin NK_1 receptors were reported to be associated with cardiovascular and behavioural responses occurring during stress and defence reaction (Culman and Unger, 1995; Culman et al., 1997). It seems quite unlikely that the anti-hypertensive effects of SB 222200 and R 820 in SHR are mediated by central tachykinin NK_1 and NK_2 receptors mechanisms on the basis of the negative results obtained with antagonists at these receptors. It is also unlikely that the lack of effect of SR 48968 and RP 67580 is due to a problem of solubility because they block the cardiovascular and behavioural effects induced by i.c.v. injection of NK_2 and NK_1 agonists, respectively (Couture et al., 1995; Cellier et al., 1999). Moreover, i.c.v. RP 67580 prevents the cardiovascular and behavioural responses mediated by the endogenous release of substance P in response to stress (Culman et al., 1997). Finally, SR 48968, RP 67580 and SB 222200 are lipophilic molecules and should all diffuse through brain tissues to a similar degree.

In conclusion, the present study provides the first pharmacological evidence that endogenous tachykinins exert a greater tonic activation of brain tachykinin NK_3 receptors in SHR thereby contributing to the state of high blood pressure. The anti-hypertensive effect measured after the intracerebral injection of tachykinin NK_3 receptor antagonists appears to be unrelated to the inhibition of catecholamines and vasopressin release. Also, data rule out a diuretic effect and a renal endocrine component as putative mechanisms.

Acknowledgements

The authors thank Dr. Daniel Bichet (Centre de recherche, Hôpital du Sacré-Coeur de Montréal) for the measurements of plasma vasopressin. The donations of the following compounds are also gratefully acknowledged: SB 222200/SB222201/SB 235375 from Dr. Henry M. Sarau (GlaxoSmithKline, PA, USA), RP 67580 from Dr. Claude Garret (Rhône-Poulenc Rorer, Vitry sur Seine, France), SR 48968 from Dr. Xavier Emonds-Alt (Sanofi Recherche, Montpellier, France) and R-820 from late Dr. Jean-Luc Fauchère (Institut de recherches Servier, Paris, France). Andrée Lessard held Studentships Awards from the Heart and Stroke Foundation of Canada and the FRSQ-FCAR program. This work was supported by a Grant-in-Aid from the Canadian Institutes of Health Research (MOP-14379).

References

Advenier, C., Rouissi, N., Nguyen, Q.T., Emonds-Alt, X., Breliere, J.C., Neliat, G., Naline, E., Regoli, D., 1992. Neurokinin A (NK_2) receptor revisited with SR 48968, a potent non-peptide antagonist. Biochem. Biophys. Res. Commun. 184, 1418–1424.

Bichet, D.G., Kortas, C., Mettauer, B., Manzini, C., Marc-Aurele, J., Rouleau, J.L., Schrier, R.W., 1986. Modulation of plasma and platelet vasopressin by cardiac function in patients with heart failure. Kidney Int. 29, 1188–1196.

Cellier, E., Barbot, L., Regoli, D., Couture, R., 1997. Cardiovascular and behavioural effects of intracerebroventricularly administered tachykinin NK_3 receptor antagonists in the conscious rat. Br. J. Pharmacol. 122, 643–654.

Cellier, E., Barbot, L., Iyengar, S., Couture, R., 1999. Characterization of central and peripheral effects of septide with the use of five tachykinin NK_1 receptor antagonists in the rat. Br. J. Pharmacol. 127, 717–728.

Couture, R., Picard, P., Poulat, P., Prat, A., 1995. Characterization of the tachykinin receptors involved in spinal and supraspinal cardiovascular regulation. Can. J. Physiol. Pharm. 73, 892–902.

Culman, J., Unger, Th., 1995. Central tachykinins: mediators of defence reaction and stress reactions. Can. J. Physiol. Pharm. 73, 885–891.

Culman, J., Klee, S., Ohlendorf, C., Unger, Th., 1997. Effect of tachykinin receptor inhibition in the brain on cardiovascular and behavioral responses to stress. J. Pharmacol. Exp. Ther. 280, 238–246.

De Champlain, J., 1990. Pre- and postsynaptic adrenergic dysfunctions in hypertension. J. Hypertens. 8 (suppl. 7), S77–S85.

Eguchi, T., Takano, Y., Hatae, T., Saito, R., Nakayama, Y., Shigeyoshi, Y., Okamura, H., Krause, J.E., Kamiya, H.-O., 1996. Antidiuretic action of tachykinin NK-3 receptor in the rat paraventricular nucleus. Brain Res. 743, 49–55.

Garret, C., Carruette, A., Fardin, V., Moussaoui, S., Peyronel, J.F., Blanchard, J.C., Laduron, P.M., 1991. Pharmacological properties of a potent and selective nonpeptide substance P antagonist. Proc. Natl. Acad. Sci. U. S. A. 88, 10208–10212.

Hatae, T., Kawano, H., Karpitskiy, V., Krause, J.E., Masuko, S., 2001. Arginine–vasopressin neurons in the rat hypothalamus produce neurokinin B and co-express the tachykinin NK-3 receptor and angiotensin II type 1 receptor. Arch. Histol. Cytol. 64, 37–44.

Hay, D.W.P., Giardina, G.A.M., Griswold, D.E., Underwood, D.C., Kotzer, C.J., Bush, B., Potts, W., Sandhu, P., Lundberg, D., Foley, J.J., Schmidt, D.B., Martin, L.D., Kilian, D., Legos, J., Barone, F.C., Luttmann, M.A., Grugni, M., Raveglia, L.F., Sarau, H.M., 2002. Nonpeptide tachykinin receptor antagonists: III. SB 235375, a low central nervous system-penetrant, potent and selective neurokinin-3

receptor antagonist, inhibits citric acid-induced cough and airways hyper-reactivity in guinea pigs. J. Pharmacol. Exp. Ther. 300, 314–323.
Kohlmann, O., Cesaretti, M.L., Ginoza, M., Tavares, A., Zanella, M.T., Ribeiro, A.B., Ramos, O.L., Leeman, S.E., Gavras, I., Gavras, H., 1997. Role of substance P in blood pressure regulation in salt-dependent experimental hypertension. Hypertension 29 (part 2), 506–509.
Le Mellédo, J.M., Bradwejn, J., Koszycki, D., Bellavance, F., Bichet, D., 2001. Arginine–vasopressin and oxytocin response to cholecystokinin-tetrapeptide. Peptides 22, 1349–1357.
Lessard, A., Couture, R., 2001a. Modulation of cardiac activity by tachykinins in the rat substantia nigra. Br. J. Pharmacol. 134, 1749–1759.
Lessard, A., Couture, R., 2001b. Central tachykinin NK_3 receptor as a novel target for anti-hypertensive drugs. Peptide Receptors, from Gene to Therapy, An International Multidisciplinary Symposium, Montréal, Canada, July 29–August 2, 2001, p. 71. Abstract, P 62.
Lessard, A., Campos, M.M., Neugebauer, W., Couture, R., 2003. Implication of nigral tachykinin NK_3 receptors in the maintenance of hypertension in spontaneously hypertensive rats: a pharmacologic and autoradiographic study. Br. J. Pharmacol. 138, 554–563.
Massi, M., Saija, A., Polidori, C., Perfumi, M., Gentili, L., Costa, G., De Caro, G., 1991. The hypothalamic paraventricular nucleus is a site of action for the central effect of tachykinins on plasma vasopressin. Brain Res. Bull. 26, 149–154.
Mens, W.B., Andringa-Bakker, E.A., Van Wimersma Greidanus, T.B., 1982. Changes in cerebrospinal fluid levels of vasopressin and oxytocin of the rat during various light–dark regimes. Neurosci. Lett. 34 (1), 51–56.
Möhring, J., Kintz, J., Schoun, J., 1979. Studies on the role of vasopressin in blood pressure control of spontaneously hypertensive rats with established hypertension (SHR, stroke-prone strain). J. Cardiovasc. Pharmacol. 1, 593–608.
Musso, N.R., Vergassola, C., Pende, A., Lotti, G., 1990. Simultaneous measurement of plasma catecholamines (norepinephrine, epinephrine, and dopamine) and free *N*-methyl dopamine (epinine) levels, by HPLC with electrochemical detection. J. Liq. Chromatogr. 13, 2217–2228.
Nagashima, A., Takano, Y., Tateishi, K., Matsuoka, Y., Hamaoka, T., Kamiya, H.-O., 1989. Central pressor actions of neurokinin B: increases in neurokinin B contents in discrete nuclei in spontaneously hypertensive rats. Brain Res. 499, 198–203.
Nakayama, Y., Takano, Y., Saito, R., Kamiya, H.-O., 1992. Central pressor actions of tachykinin NK-3 receptor in the paraventricular nucleus of the rat hypothalamus. Brain Res. 595, 339–342.
Otsuka, M., Yoshioka, K., 1993. Neurotransmitter functions of mammalian tachykinins. Physiol. Rev. 73, 229–308.
Page, N.M., Woods, R.J., Gardiner, S.M., Lomthaisong, K., Gladwell, R.T., Butlin, D.J., Manyonda, I.T., Lowry, P.J., 2000. Excessive placental secretion of neurokinin B during the third trimester causes pre-eclampsia. Nature 405, 797–800.
Polidori, C., Saija, A., Perfumi, M., Costa, G., De Caro, G., Massi, M., 1989. Vasopressin release induced by intracranial injection of tachykinins is due to activation of central neurokinin-3 receptors. Neurosci. Lett. 103, 320–325.
Regoli, D., Boudon, A., Fauchère, J.-L., 1994. Receptors and antagonists for substance P and related peptides. Pharmacol. Rev. 46, 551–599.
Remie, R., Zaagsma, J., 1986. A new technique for the study of vascular presynaptic receptors in freely moving rats. Am. J. Physiol. 251, H463–H467 (Heart Circ. Physiol. 20).
Ribeiro-Da-Silva, A., Mc Leod, A.L., Krause, J.E., 2000. Neurokinin receptors in the CNS. In: Quirion, R., Björklund, A., Hökfelt, T. (Eds.), Handbook of Chemical Neuroanatomy. Peptide Receptors, Part I, vol. 16. Elsevier, Amsterdam, pp. 195–240.
Roccon, A., Marchionni, D., Nisato, D., 1996. Study of SR 142801, a new potent non-peptide NK_3 receptor antagonist on cardiovascular responses in conscious guinea-pig. Br. J. Pharmacol. 118, 1095–1102.
Sarau, H.M., Griswold, D.E., Bush, B., Potts, W., Sandhu, P., Lundberg, D., Foley, J.J., Schmidt, D.B., Webb, E.F., Martin, L.D., Legos, J.J., Whitmore, R.G., Barone, F.C., Medhurst, A.D., Luttmann, M.A., Giardina, G.A.M., Hay, D.W.P., 2000. Nonpeptide tachykinin receptor antagonists: II. Pharmacological and pharmacokinetic profile of SB-222200, a central nervous system penetrant, potent and selective NK-3 receptor antagonist. J. Pharmacol. Exp. Ther. 295, 373–381.
Takano, Y., Nagashima, A., Hagio, T., Tateishi, K., Kamiya, H.-O., 1990. Role of central tachykinin peptides in cardiovascular regulation in rats. Brain Res. 528, 231–237.
Unger, Th., Rascher, W., Schuster, C., Pavlovitch, R., Schömig, A., Dietz, R., Ganten, D., 1981. Central blood pressure effects of substance P and angiotensin: II. Role of the sympathetic nervous system and vasopressin. Eur. J. Pharmacol. 71, 33–42.
Walters, J.F., Skene, D.J., Hampton, S.M., Ferns, G.A.A., 2003. Biological rhythms, endothelial health and cardiovascular disease. Med. Sci. Monit. 9 (1), RA1–RA8.
Watanabe, K., Vanecek, J., Yamaoka, S., 2000. In vitro entrainment of the circadian rhythm of vasopressin-releasing cells in suprachiasmatic nucleus by vasoactive intestinal polypeptide. Brain Res. 877, 361–366.
Windle, R.J., Forsling, M.L., Guzek, J.W., 1992. Daily rhythms in the hormone content of the neurohypophysial system and release of oxytocin and vasopressin in the male rat: effect of constant light. J. Endocrinol. 133 (2), 283–290.
Yamaguchi, N., 1993. In vivo evidence for adrenal catecholamine release mediated by nonnicotinic mechanism: local medullary effect of VIP. Am. J. Physiol. 265, R766–R771 (Reg. Int. Comp. Physiol.).
Yamasaki, F., Scwartz, J.E., Gerber, L.M., 1998. Impact of shift work and race/ethnicity on the diurnal rhythm of blood pressure and catecholamines. Hypertension 32, 417–423.
Yuan, Y.-D., Couture, R., 1997. Renal effects of intracerebroventricularly injected tachykinins in the conscious saline-loaded rat: receptor characterization. Br. J. Pharmacol. 120, 785–796.