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Renal effects of intrathecally injected tachykinins in the conscious saline-loaded rat: receptor and mechanism of action

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1 The effects of intrathecally (i.t.) injected substance P (SP), neurokinin A (NKA), [β-Ala^8]NKA (4–10) and [MePhe^7]neurokinin B (NKB) at T_{13} thoracic spinal cord level were investigated on renal excretion of water, sodium and potassium in the conscious saline-loaded rat. Antagonists selective for NK_1 (RP 67580), NK_2 (SR 48968) and NK_3 (R 820; 3-indolylcarbonyl-Hyp-Phg-N(Me)-Bzl) receptors were used to characterize the spinal effect of SP on renal function.

2 Saline gavage (4.5% of the body weight) enhanced renal excretion of water, sodium and potassium over the subsequent hour of measurement. Whereas these renal responses were not affected by 0.65 nmol SP, the dose of 6.5 nmol SP blocked the natriuretic response (aCSF value 3.9 ± 0.8; SP value 0.7 ± 0.3 μmol min^{-1}, $P<0.01$) as well as the renal excretion of water (aCSF value 48.9 ± 5.8; SP value 14.5 ± 4.0 μl min^{-1}, $P<0.01$) and potassium (aCSF value 4.8 ± 0.6; SP value 1.5 ± 0.6 μmol min^{-1}, $P<0.01$) at 30 min post-injection. SP had no significant effect on urinary osmolality. The SP-induced renal inhibitory effects during the first 30 min were abolished in rats subjected to bilateral renal denervation 1 week earlier or in rats injected i.t. 5 min earlier with 6.5 nmol RP 67580. In contrast, the co-injection of SR 48968 and R 820 (6.5 nmol each) did not affect the inhibitory responses to SP. On their own, these antagonists had no direct effect on renal excretion function. Since SP induced only transient changes in mean arterial blood pressure (-18.8 ± 3.8 mmHg at 1 min and $+6.3\pm2.4$ mmHg at 5 min post-injection), it is unlikely that the renal effects of SP are due to systemic haemodynamic changes.

3 NKA (6.5 nmol but not 0.65 nmol) produced a transient drop in renal excretion of water (aCSF value 31.2 ± 5.1; NKA value 11.3 ± 4.2 μl min^{-1}, $P<0.05$), sodium (aCSF value 1.7 ± 0.8; NKA value 0.4 ± 0.2 μmol min^{-1}, $P<0.05$) and potassium (aCSF value 4.1 ± 0.7; NKA value 1.5 ± 0.4 μmol min^{-1}, $P<0.05$) at 15 min post-injection. However, the same doses (6.5 nmol) of selective agonists for tachykinin NK_2 ([β-Ala^8]NKA(4-10)) and NK_3 ([MePhe^7]NKB) receptors were devoid of renal effects.

4 This study provided functional evidence that tachykinins may be involved in the renal control of water and electrolyte excretion at the level of the rat spinal cord through the activation of NK_1 receptors and the sympathetic renal nerve.

Keywords: Substance P; NK_1 receptor; tachykinin receptor antagonists; spinal cord; kidney

Introduction

The mammalian tachykinins, substance P (SP), neurokinin A (NKA), and neurokinin B (NKB), exert a variety of biological actions in the central nervous system and peripheral tissues mainly through the activation of three G protein-coupled receptors, namely neurokinin-1 (NK_1), NK_2 and NK_3 (Otsuka & Yoshioka, 1993). The rank order of potency of tachykinins is SP > NKA > NKB at the NK_1 receptor, NKA > NKB > SP at the NK_2 receptor and NKB > NKA > SP at the NK_3 receptor (Regoli *et al*., 1994).

Several lines of evidence indicate that tachykinins are implicated in the regulation of water and sodium homeostasis. SP containing fibres have been described in the rat kidney (Ferguson & Bell, 1985). Systemic infusion of low doses of SP produces diuresis and natriuresis in rats (Arendshorst *et al*., 1976; Kramer *et al*., 1983). In the central nervous system, SP, NKA, and NKB have been found in various regions of the brain associated with the presence of NK_1 and NK_3 receptors (Otsuka & Yoshioka, 1993). Intracerebroventricular (i.c.v.) administration of natural tachykinins or selective tachykinin receptor agonists to the rat inhibits angiotensin II-induced drinking, hyperosmotic NaCl-induced drinking, and sodium depletion-induced salt appetite through NK_1, NK_2 and NK_3 receptors, respectively (Massi *et al*., 1991). Recently, we showed that the i.c.v. injection of [MePhe^7]NKB (selective tachykinin NK_3 receptor agonist), but not SP or NKA, elicits dose-dependent reductions in renal excretion of water, sodium and potassium through the release of vasopressin following the activation of central NK_3 receptors in the conscious saline-loaded rat (Yuan & Couture, 1997).

Furthermore, a role for the NK_1 receptor has been suggested in cardiovascular regulation at the level of the spinal cord (Couture *et al*., 1995). SP is an excitatory transmitter in bulbospinal and intraspinal fibres projecting to the sympathetic preganglionic neurones in the intermediolateral (IML) cell column of the thoracolumbar spinal cord (Davis *et al*., 1984; Helke *et al*., 1985) where a high density of NK_1 receptors are found (Helke *et al*., 1990) as opposed to the lower density of NK_2 and NK_3 receptors (Yashpal *et al*., 1990). SP is also released in the dorsal horn from a subpopulation of capsaicin-sensitive primary sensory C-fibres in response to noxious and inflammatory cutaneous stimuli (Duggan *et al*., 1988; Kuraishi *et al*., 1989). The iontophoretic application of SP to the IML nucleus excites sympathetic preganglionic neurones in rats (Gilbey *et al*., 1983), while the intrathecal (i.t.) injection of SP at T_9 thoracic spinal cord level increases blood pressure and heart rate as well as plasma levels of catecholamines and neuropeptide Y (NPY) in the conscious rat (Hasséssian *et al*., 1990). In addition, i.t. SP enhances sympathetic renal nerve activity in the rat (Yusof & Coote, 1987), suggesting that this peptide may affect renal function through a spinal mechanism.

The objective of the present study was threefold: first, to determine the spinal action of tachykinins on renal excretion of

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Table 1 Chemical structures of tachykinin receptor antagonists

Antagonist	*Receptor*	*Structure*	*Reference*
RP 67580	NK_1	3a**R**, 7a**R**-7,7-diphenyl-2[1-imino-2-(2-methoxyphenyl)-ethyl]perhydroisoindol-4-one	Garret *et al*., 1991
SR 48968	NK_2	(S)-N-methyl-N[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]benzamide	Emonds-Alt *et al*., 1992
R 820	NK_3	3-Indolycarbonyl-Hyp-Phg-N(Me)-Bzl	Regoli *et al*., 1994

water and electrolytes in conscious saline-loaded rats; secondly, to characterize the tachykinin receptor mediating the renal effects of tachykinins administered i.t. with tachykinin antagonists selective for NK_1 (RP 67580), NK_2 (SR 48968) and NK_3 (R 820) receptors (Table 1); and, thirdly, to evaluate the contribution of the sympathetic renal nerve in the spinal action of SP on renal function. Part of this work has been presented previously (Yuan & Couture, 1996).

Methods

Animal preparation and surgical procedures

The animal care and research protocols were in accordance with the principles and guidelines of the Canadian Council on Animal Care and were approved by the committee of the Université de Montréal. Male Wistar rats weighing 275–325 g (Charles River, St.-Constant, Québec, Canada) were anaesthetized with an intraperitoneal (i.p.) injection of 65 mg kg^{-1} sodium pentobarbitone (Somnotol; M.T.C. Pharmaceuticals, Cambridge, Ont., Canada) and received an intramuscular (i.m.) injection of 45,000 iu of Penicillin G procaine (Ayercilline; Laboratories Ayerst, Montréal, Qué., Canada). Two siliconized polyethylene catheters (Intramedic, Clay Adams, NJ, U.S.A.) were implanted. The first catheter (PE-60) was introduced into the bladder through a small incision in the bladder tip, and the other end of the catheter was passed through a subcutanenous tunnel and exteriorized at the back of the neck. The second catheter (PE-10; void volume of 10 μl) was inserted through an incision in the dura at the atlanto-occipital junction, directed caudally within the spinal subarachnoid space and positioned so that the inner tip reached the T_{13} thoracic spinal cord level. The catheter was anchored to the occipital bone with cyanoacrylate glue (Krazy glue, Chicago, IL, U.S.A.) and exteriorized at the back of the neck. In one group of rats, bilateral renal denervation was achieved by stripping the adventitia of renal arteries and painting blood vessels with 10% phenol in absolute alcohol as described previously (Dibona & Sawin, 1983). Control rats were sham operated (renal arteries were isolated and painted with saline). Following the operation, the rats were housed individually in a plastic cage ($40 \times 20 \times 23$ cm) with free access to chow and tap water, and maintained in a room with a 12 h light and dark cycle (lights on 06 h 00 min–18 h 00 min). The penicillin injection (45,000 iu per rat) was repeated daily for 5 days to prevent infection due to surgery. The bladder catheter was flushed with sterile distilled water every day until experiment. The rats were used at least 1 week after surgery so that the animal recovered its normal body weight and stabilized its renal excretion (Yuan & Couture, 1997). The rats which showed haematuria, motor deficit or weight loss of more than 20 g during a 1 week recovery period were excluded from experiments. The correct position of the i.t. catheter was ascertained by *postmortem* examination after laminectomy.

Measurement of renal function

Experiments were conducted on freely moving rats which had free access to chow but not to water in their resident cages. The bladder catheter was connected to an extension tube (PE-60; 65 cm long) which allowed collection of urine from outside the cage. After an equilibration period of 1 h, two urine samples were collected at intervals at 15 min into pre-weighed siliconized tubes, and a mean of data from the two samples was used to determine the baseline urinary parameters. Following this, the rats were given by gavage 13 ml isotonic saline (0.9% NaCl) equivalent to 4.5% of the body weight. After an equilibration period of 5 min, peptide was injected i.t. (10 μl). Urine was collected at intervals of 15 min for a further 60 min with a fraction collector (RediFrac, Pharmacia LKB, Uppsala, Sweden) and stored at -20°C until analysis. Urinary volume (UV) was determined gravimetrically. Urinary concentrations of sodium (UNa) and potassium (UK) were measured by flame photometry (Instrument Laboratory 943), and urinary osmolality (Uosm) was determined by freezing point depression with an osmometer (Advanced Digi Matic Osmometer model 3D2).

Measurement of blood pressure

In one group of rats, a polyethylene catheter (PE-50) was inserted into the abdominal aorta through the right femoral artery to assess the effect of i.t. SP on blood pressure in conscious saline-loaded rats. The arterial blood pressure was monitored through the intraarterial catheter with a Statham pressure transducer (P231D) connected to a Grass Polygraph (model 79D). When the animal was in a resting state and the basal blood pressure was stable, 13 ml isotonic saline was given by gavage to the rat. Rats were randomly injected i.t. with 10 μl of aCSF, 0.65 nmol SP or 6.5 nmol SP 5 min after gavage with saline on three consecutive days. The blood pressure was recorded during a period of 60 min post-injection. Mean arterial blood pressure (MAP) was calculated from systolic and diastolic blood pressures and baseline values were determined 1 min before i.t. injection.

Experimental protocol

In the first series of experiments, the effects of SP, NKA or [$MePhe^7$]NKB on renal excretion were examined following i.t. administration. On day 1, the rats received an i.t. injection of 10 μl of artificial cerebrospinal fluid (aCSF; composition in mM: NaCl 128.6, KCl 2.6, $MgCl_2$ 2.0 and $CaCl_2$ 1.4; pH adjusted to 7.2) to establish control values. On the two subsequent days, they received randomly either SP, NKA or [$MePhe^7$]NKB (0.65 or 6.5 nmol). The i.t. catheter was then flushed with 10 μl of aCSF over 15–30 s, and urinary samples were collected for 1 h. Only one peptide in a volume of 10 µl of aCSF was given to a rat, and each dose was injected at intervals of 24 h to avoid tachyphylaxis. The group of rats treated with NKA also received the i.t. injection of 6.5 nmol [β-Ala^8]NKA(4-10) (selective tachykinin NK_2 receptor agonist) two days after NKA treatment.

The second series of experiments was designed to characterize the tachykinin receptor mediating the renal effects of SP. On day 1 and day 2, the animals were randomly administered i.t. aCSF or 6.5 nmol SP five min after a prior i.t. injection of aCSF containing dimethylsulphoxide (10 μl solution containing 5–10% DMSO flushed with 10 μl of aCSF). This solution corresponded to the vehicle for antagonists. On the third day, RP 67580 (6.5 nmol) or a solution containing both SR 48968 and R 820 (6.5 nmol each) was administered i.t. in a volume of 10 μl, 5 min before 6.5 nmol SP. The doses of tachykinin receptor antagonists were selected on the basis of their efficacy to

inhibit the i.t. and i.c.v. cardiovascular effects of their respective agonists in the conscious rat (Couture *et al*., 1995; Cellier *et al*., 1995). The animals received only one antagonist solution. The intrinsic effects of the tested antagonists on renal excretion were examined in separate experiments.

The third series of experiments was aimed at examining the role of renal nerves in the renal excretory effects induced by i.t. SP in the conscious saline-loaded rat. One week after bilateral renal denervation, the i.t. effect of aCSF and 6.5 nmol SP on renal function was assessed on two consecutive days as described in the first series of experiments. The results were compared to those obtained in sham-operated rats.

Peptides and other compounds

Both SP and NKA were purchased from Hükabel Scientific Ltd (Montréal, Québec, Canada). [β-Ala^8]NKA(4-10), [Me-Phe^7]NKB and the tachykinin NK_3 receptor antagonist, R 820, were generously provided by Dr D. Regoli of Université de Sherbrooke (Sherbrooke, Québec, Canada). The tachykinin NK_1 receptor antagonist, RP 67580, was a gift from Dr C. Garret, Rhône-Poulenc (Rorer, Paris, France) and the tachykinin NK_2 receptor antagonist, SR 48968, was from Dr J.C. Brelière, Sanofi (Montpellier, France). SP, NKA, and [Me-Phe^7]NKB were dissolved directly in aCSF. The tachykinin

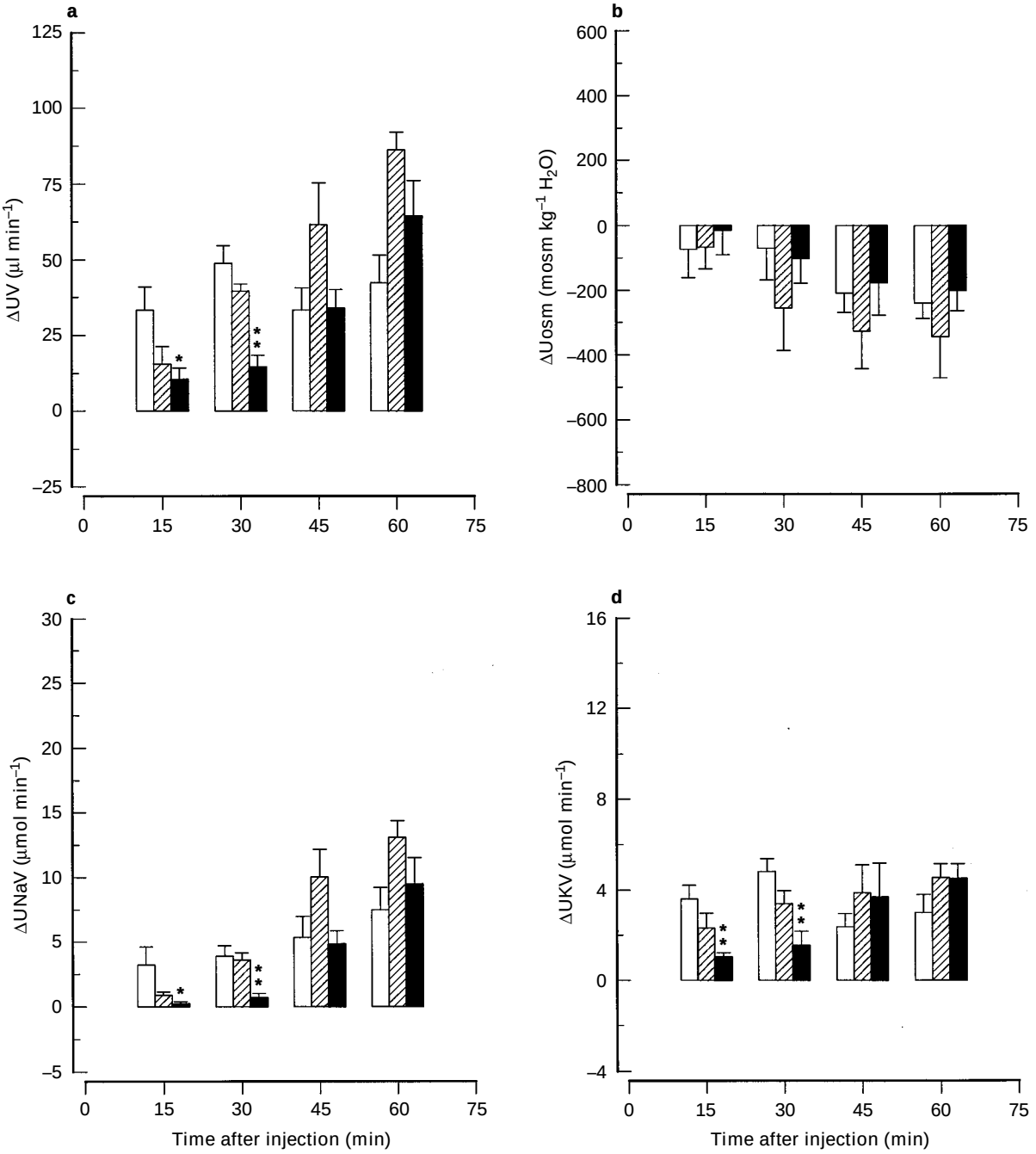

Figure 1 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.t. administration of aCSF (open columns; $n=6$) and SP at doses of 0.65 nmol (hatched columns; $n=6$) and 6.5 nmol (solid columns; $n=8$) in the conscious saline-loaded rat. Baseline values of UV (aCSF 11.8 ± 1.7 μl min^{-1}; 0.65 nmol SP 12.6 ± 1.5 μl min^{-1}; 6.5 nmol SP 10.8 ± 1.0 μl min^{-1}), UNaV (aCSF 1.0 ± 0.2 μmol min^{-1}; 0.65 nmol SP 0.8 ± 0.1 μmol min^{-1}; 6.5 nmol SP 0.8 ± 0.2 μmol min^{-1}), UKV (aCSF 1.3 ± 0.3 μmol min^{-1}; 0.65 nmol SP 1.5 ± 0.1 μmol min^{-1}; 6.5 nmol SP 1.3 ± 0.2 μmol min^{-1}) and UosM (aCSF 184.1 ± 6.6 mosm kg^{-1} H_2O; 0.65 nmol SP 216.0 ± 21.5 mosm kg^{-1} H_2O; 6.5 nmol SP 200.6 ± 76.3 mosm kg^{-1} H_2O) were not significantly different between groups. Each column represents variations ($\triangle$) from baseline values and the mean$\pm$s.e.mean of n rats. Statistical comparison to aCSF values is indicated by $^*P<0.05$; $^{**}P<0.01$.

receptor antagonists and [β-Ala^8]NKA(4-10) were dissolved in dimethylsulphoxide (DMSO, Fisher) and aCSF was added to obtain the desired solution (the final solution contained no more than 10% DMSO). Stock solutions of tachykinin receptor agonists and antagonists (6.5–65 nmol 10 μl^{-1}) were divided into aliquots of 50 μl and stored at $-20^{\circ}C$ until used. Daily dilutions were made in aCSF before each experiment.

Statistical analysis of data

All urinary parameters are expressed as changes in renal excretion of water (ΔUV), sodium (ΔUNaV) and potassium (ΔUKV), and in urinary osmolality (ΔUosm) when compared to baseline values. Results are expressed as means $\pm$ s.e.mean of (n) rats. Multiple comparisons between groups were evaluated with a one-way ANOVA in conjuction with a *post-hoc* Bonferroni test. Only probability values (P) less than 0.05 were considered to be statistically significant.

Results

Spinal actions of SP, NKA and [MePhe^7]NKB on renal excretion

When compared to baseline values, the renal excretion of water, sodium and potassium was significantly enhanced ($P<0.001$) from 15 min to 1 h in rats which received by gavage 13 ml isotonic saline (Figure 1). Although these renal responses were not significantly modified by the i.t. injection of 0.65 nmol SP, the dose of 6.5 nmol SP inhibited significantly the diuretic response during the first 30 min post-injection ($P<0.01$) when compared to aCSF values (Figure 1a). The inhibitory effect of SP had dissipated at 45 and 60 min post-injection. The antidiuretic effect evoked by 6.5 nmol SP was accompanied by decreased natriuresis and kaliuresis during the same period (Figure 1c, d). The i.t. injection of 6.5 nmol (but not 0.65 nmol) NKA also decreased renal excretion of water ($P<0.05$), sodium ($P<0.05$) and potassium ($P<0.05$) at 15 min post-injection when compared to vehicle values (Table 2; only values at 15 and 30 min are shown). These inhibitory effects of NKA were no longer observed at 30 min post-injection. In contrast to NKA, 6.5 nmol [β-Ala^8]NKA(4-10), a selective tachykinin NK_2 receptor agonist, had no effect on renal parameters (Table 2). Likewise, [MePhe^7]NKB (0.65 or 6.5 nmol) injected i.t. had no effect on renal excretion of water, sodium and potassium (Table 2). The urinary osmolality was not altered by SP (Figure 1b), NKA or [MePhe^7]NKB (data not shown) at any doses. Baseline values for UV, UNaV, UKV and Uosm were not significantly different between groups (Figure 1 and Table 2).

Characterization of the tachykinin receptor mediating the renal inhibitory responses to i.t. SP

The inhibitory effects of i.t. SP (6.5 nmol) on diuretic, natriuretic and kaliuretic responses induced by administration of saline by gavage were abolished by i.t. RP 67580 (6.5 nmol, 5 min earlier) (Figure 2a,c,d). Thus, the renal excretion values in the presence of SP plus RP 67580 were not significantly different from vehicle values. Urinary osmolality was not altered by i.t. SP either in the presence or absence of RP 67580 (Figure 2b). An i.t. treatment 5 min earlier with a solution containing both SR 48968 and R 820 (6.5 nmol each) failed to alter the renal inhibitory responses induced by the i.t. injection of 6.5 nmol SP (Figure 3a,c,d), and the urinary osmolality remained unchanged (Figure 3b). RP 67580 or the co-injection of SR 48968 and R 820 (i.t., 6.5 nmol each) had no direct effects on the renal parameters (Table 2). Baseline values for UV, UNaV and UKV were not significantly different between groups (Figures 2, 3 and Table 2).

Effects of renal denervation on renal inhibitory effects elicited by i.t. SP

In agreement with the above experiments in control rats, 6.5 nmol SP administered i.t. inhibited ($P<0.05$) at 15 and 30 min post-injection the diuretic, natriuretic and kaliuretic responses evoked by saline gavage in sham-denervated rats. These renal changes evoked by i.t. SP lasted 30 min and were back to vehicle values at 45 min post-injection (Table 3). In contrast, the i.t. injection of 6.5 nmol SP failed to modify the diuretic, natriuretic and kaliuretic responses induced by saline gavage in rats which had undergone bilateral renal denervation

Table 2 Renal effects induced by i.t. injection of tachykinin receptor agonists and antagonists in conscious saline-loaded rats

		UV baseline (μl min^{-1})	ΔUV (μl min^{-1})		*UNaV baseline* (μmol min^{-1})	$\Delta UNaV$ (μmol min^{-1})		*UKV baseline* (μmol min^{-1})	ΔUKV (μmol min^{-1})	
					Time (min) *after injection*					
Treatment	n		*15*	*30*		*15*	*30*		*15*	*30*
Vehicle	12	11.0±1.4	31.2±5.1	63.0±8.0	0.8±0.2	1.7±0.8	4.6±0.8	1.8±0.4	4.1±0.7	3.3±0.8
NKA (0.65 nmol)	6	12.8±1.6	29.7±5.0	58.6±10.1	0.8±0.2	1.5±0.4	6.8±2.0	1.6±0.3	2.8±0.5	2.6±0.6
NKA (6.5 nmol)	11	13.8±1.5	11.3±4.2*	38.2±10.0	0.6±0.1	0.4±0.2*	5.2±2.0	1.4±0.2	1.5±0.4*	2.7±0.5
[β-Ala^8] NKA(4-10) (6.5 nmol)	5	11.6±2.6	32.6±4.6	42.4±4.3	0.5±0.1	0.9±0.3	3.1±0.9	2.0±0.8	4.1±0.6	2.0±0.3
Vehicle	7	18.0±4.2	22.6±12.1	40.9±6.3	1.1±0.2	1.7±0.8	5.7±1.8	2.7±0.3	3.0±1.8	3.7±1.2
[MePhe^7]NKB (0.65 nmol)	7	12.6±2.4	16.4±4.3	30.6±3.6	0.8±0.2	1.2±0.6	3.1±0.6	2.4±0.4	3.9±1.0	4.1±0.9
[MePhe^7]NKB (6.5 nmol)	6	15.5±2.8	14.9±1.5	36.5±7.1	1.1±0.2	0.6±0.1	4.0±1.2	2.8±0.6	2.9±0.8	4.2±1.4
Vehicle	5	12.6±2.4	17.0±3.3	59.5±12.0	0.8±0.3	0.6±0.2	11.0±2.6	1.6±0.5	3.1±0.4	6.9±0.7
RP 67580 (6.5 nmol)	4	9.8±1.0	16.3±9.1	59.8±11.5	0.8±0.2	1.7±1.3	16.9±6.7	1.8±0.6	3.6±2.2	9.0±1.5
SR 48968 +R820 (6.5 nmol each)	4	11.4±2.6	21.0±8.8	58.4±4.4	1.2±0.4	2.6±1.1	18.4±1.6	2.6±0.8	6.5±3.0	10.1±1.8

Values represent the mean $\pm$ s.e.mean of (n) rats. Statistical comparison to vehicle values is indicated by $^*P<0.05$.

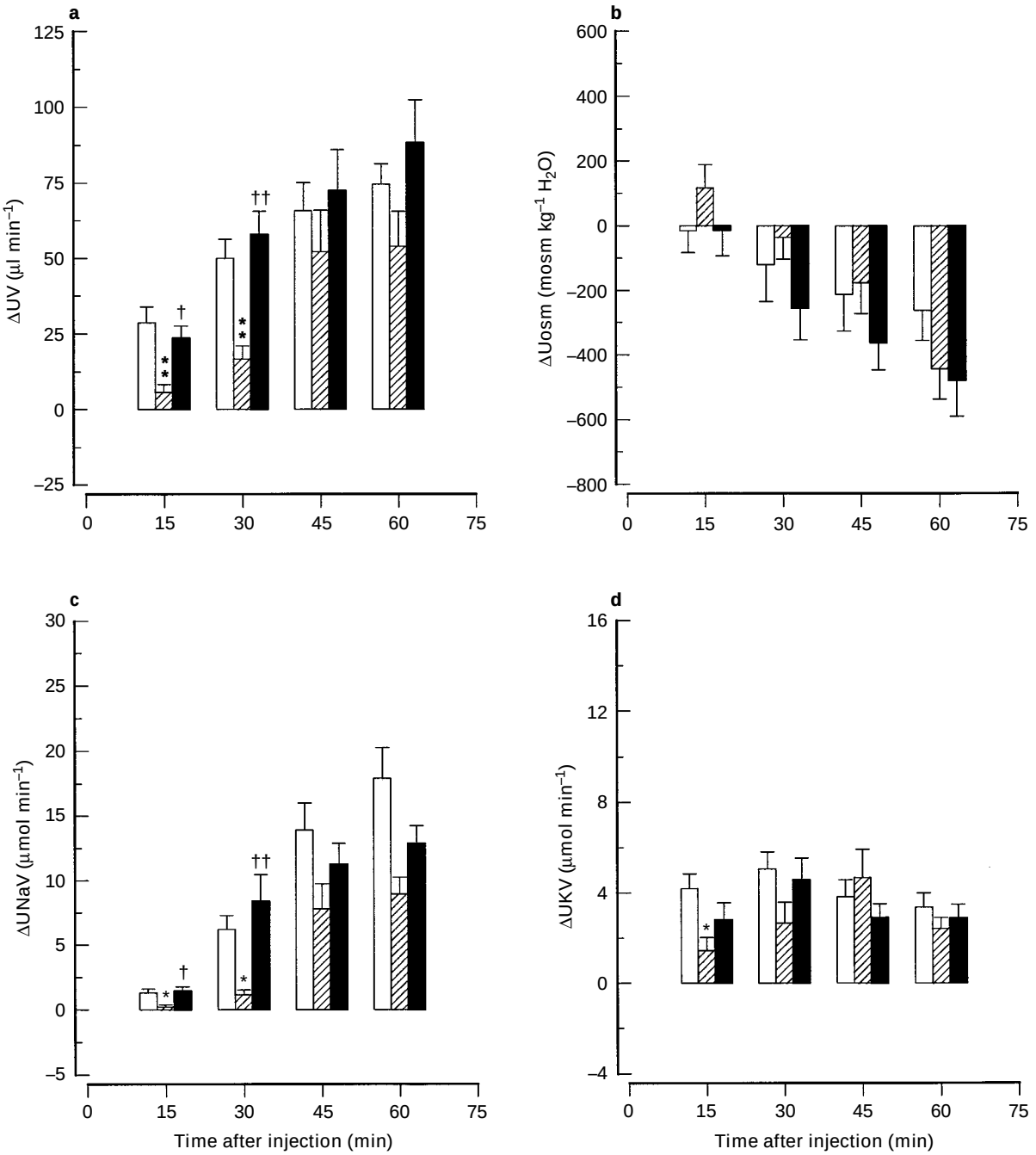

Figure 2 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.t. injection of 6.5 nmol SP in the presence (solid columns; $n=8$) and absence (hatched columns; $n=8$) of 6.5 nmol RP 67580 in the conscious saline-loaded rat. Vehicle values are also shown (open columns; $n=10$). Baseline values of UV (vehicle 16.1 ± 1.9 μl min^{-1}; 6.5 nmol SP 11.6 ± 1.4 μl min^{-1}; SP plus RP 67580 13.3 ± 2.0 μl min^{-1}), UNaV (vehicle 1.0 ± 0.2 μmol min^{-1}; 6.5 nmol SP 0.8 ± 0.1 μmol min^{-1}; 6.5 nmol SP plus RP 67580 0.7 ± 0.1 μmol min^{-1}), UKV (vehicle 2.1 ± 0.3 μmol min^{-1}; 6.5 nmol SP 1.8 ± 0.2 μmol min^{-1}; SP plus RP 67580 1.6 ± 0.4 μmol min^{-1}) and Uosm (vehicle 218.1 ± 14.0 mosm kg^{-1} H_2O; 6.5 nmol SP 243.5 ± 11.4 mosm kg^{-1} H_2O; SP plus RP 67580 224.3 ± 17.3 mosm kg^{-1} H_2O) were not significantly different between groups. Each column represents variations ($\triangle$) from baseline values and the mean$\pm$s.e.mean of n rats. Statistical comparison to vehicle (*) or to SP without the antagonist (†) is indicated by *,†$P<0.05$; **,††$P<0.01$.

1 week earlier. In that situation, the effects induced by i.t. SP were not significantly different from those evoked by i.t. vehicle (Table 3). Baseline renal values were not significantly different between sham-operated and renal-denervated rats.

Blood pressure responses to i.t. SP

While 0.65 nmol SP failed to alter MAP from 1 to 30 min post-injection when compared to aCSF values, 6.5 nmol SP produced a transient drop in MAP at 1 min post-injection ($P<0.01$) which was followed by a slight increase at 5 min post-injection ($P<0.05$). No significant changes were seen at 15 and 30 min post-injection. Baseline values of MAP were not significantly different between the three groups of rats (Table 4).

Discussion

Spinal action of tachykinins and their receptors on renal excretion

The present study demonstrated for the first time that i.t. administration of SP at T_{13} spinal cord level of conscious rats produced marked but reversible reductions in renal excretion

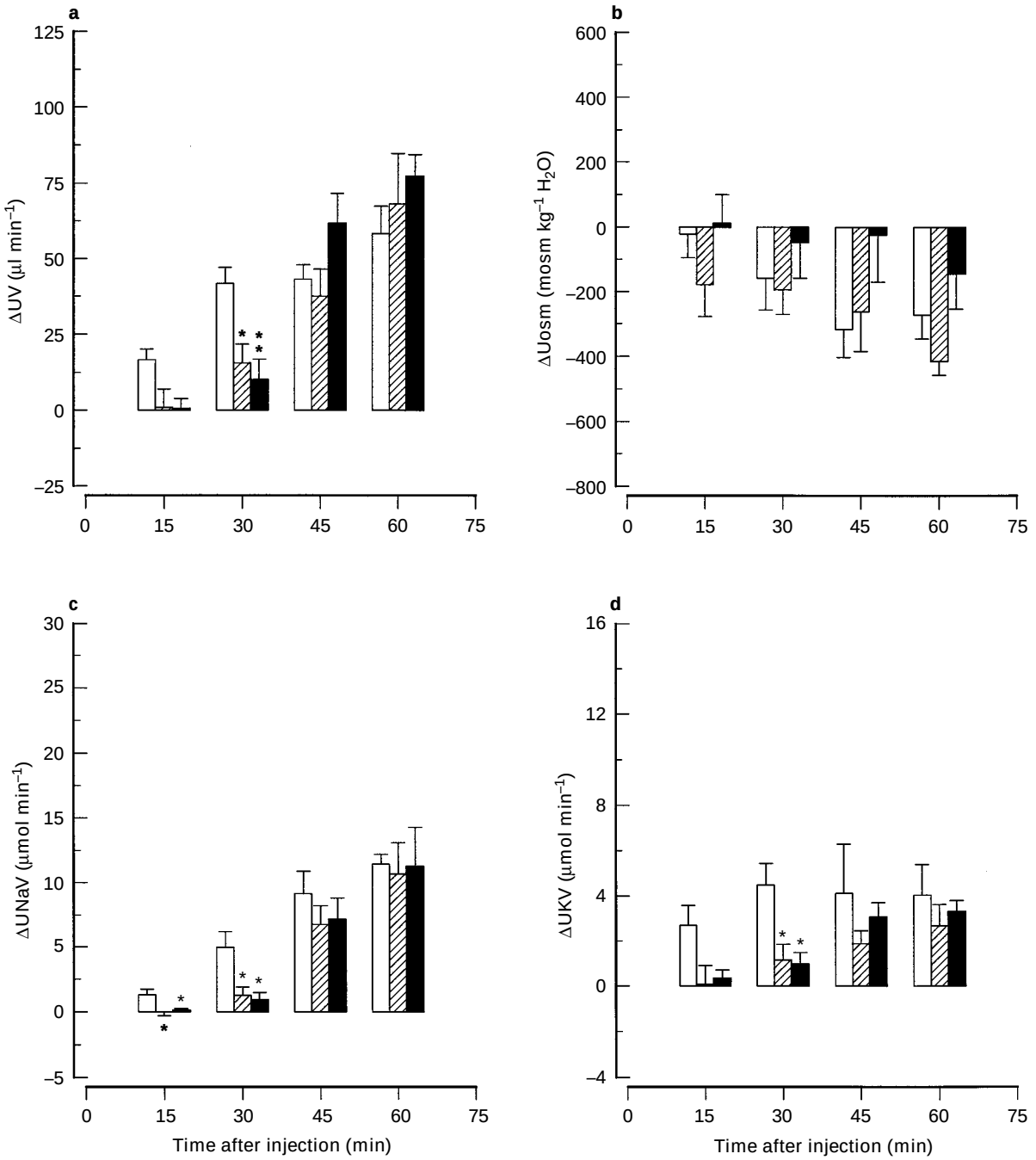

Figure 3 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.t. injection of 6.5 nmol SP in the presence (solid columns; $n=6$) and absence (hatched columns; $n=5$) of SR 48968 plus R 820 (6.5 nmol each) in the conscious saline-loaded rat. Renal effects mediated by the vehicle are also shown (open columns; $n=5$). Baseline values of UV (vehicle 14.5 ± 0.9 μl min^{-1}; 6.5 nmol SP 17.7 ± 2.7 μl min^{-1}; SP plus SR 48968 plus R 820 13.9 ± 2.1 μl min^{-1}), UNaV (vehicle 1.0 ± 0.1 μmol min^{-1}; 6.5 nmol SP 0.9 ± 0.1 μmol min^{-1}; SP plus SR 48968 plus R 820 0.4 ± 0.5 μmol min^{-1}), UKV (vehicle 1.8 ± 0.3 μmol min^{-1}; 6.5 nmol SP 2.3 ± 0.4 μmol min^{-1}; SP plus SR 48968 plus R 820 0.9 ± 0.1 μmol min^{-1}) and Uosm (vehicle 209.7 ± 17.3 mosm kg^{-1} H_2O; 6.5 nmol SP 250.4 ± 18.9 mosm kg^{-1} H_2O; SP plus SR 48968 plus R 820 159.0 ± 24.1 mosm kg^{-1} H_2O) were not significantly different between groups. Each column represents variations ($\triangle$) from baseline values and the mean$\pm$s.e.mean of n rats. Statistical comparison to aCSF values is indicated by $^*P<0.05$; $^{**}P<0.01$.

of water, sodium and potassium in response to administration of saline by gavage. These renal responses to i.t. SP were completely blocked by i.t. pretreatment with RP 67580, but not with SR 48968 and R 820. RP 67580 is a potent non-peptide tachykinin NK_1 receptor antagonist without effect at NK_2 and NK_3 receptors or at various transmitter/peptide receptors, and shows higher affinity for rat than for guinea-pig NK_1 receptors (Garret *et al*., 1991; Carruette *et al*., 1992; Rouissi *et al*., 1993). This antagonist inhibits in a competitive manner binding of labelled SP to NK_1 receptors in subcortical structures (K_i: 4.2 nM) or crude synaptosomes (K_i:2.9 nM) from the rat brain (Garret *et al*., 1991; Beaujouan *et al*., 1993). Moreover, RP 67580 is able to block the cardiovascular effects induced by i.t. and i.c.v. injection of SP in conscious rats (Picard *et al*., 1994; Couture *et al*., 1995), as well as the first and second phases of the formalin responses on dorsal horn nociceptive neurones in the rat (Chapman & Dickenson, 1993). SR 48968 is a potent nonpeptide tachykinin antagonist at NK_2 receptors in rat, guinea-pig, rabbit, hamster and man (Emonds-Alt *et al*., 1992; Advenier *et al*., 1992). The latter studies also showed that the affinity of SR 48968 for NK_2 receptors is 1000 fold higher than that for NK_1 and NK_3 receptors. SR 48968 antagonized the hyperalgesia induced by a NK_2 selective agonist in the spinal cord (Picard *et al*., 1993)

Table 3 Renal effects induced by i.t.. injection of SP in conscious saline-loaded rats 1 week after renal denervation

Treatment	n	*UV baseline* (μl min^{-1})	Δ*UV* (μl min^{-1}) *Time* (min) *after injection* 15	30	45	60
Sham-denervated						
aCSF	5	16.0±3.0	38.4±11.5	71.0±19.6	50.8±16.8	46.2±9.8
SP 6.5 nmol	4	12.5±2.7	−2.3±0.6**	−1.1±3.0**	33.4±13.4	60.6±24.5
Renal-denervated						
aCSF	4	13.1±2.0	7.6±3.0	34.8±12.3	63.6±20.1	78.8±10.3
SP 6.5 nmol	4	12.6±2.5	16.3±7.5	24.1±5.3	46.2±4.4	50.3±11.8

Treatment	n	*UNaV baseline* (μmol min^{-1})	Δ*UNaV* (μmol min^{-1}) *Time* (min) *after injection* 15	30	45	60
Sham-denervated						
aCSF	5	0.6±0.2	1.8±0.8	4.1±1.7	5.1±2.2	7.5±2.3
SP 6.5 nmol	4	0.6±0.1	−0.04±0.1*	0.2±0.1*	3.1±1.4	8.7±4.4
Renal-denervated						
aCSF	4	0.8±0.3	0.4±0.2	4.1±1.5	8.5±2.6	11.2±2.4
SP 6.5 nmol	4	0.8±0.2	1.6±0.8	2.4±1.0	6.8±1.0	7.8±1.4

Treatment	n	*UKV baseline* (μmol min^{-1})	Δ*UKV* (μmol min^{-1}) *Time* (min) *after injection* 15	30	45	60
Sham-denervated						
aCSF	5	1.2±0.4	4.1±1.3	4.8±1.5	2.0±0.7	2.1±0.4
SP 6.5 nmol	4	1.4±0.7	−0.2±0.3*	−0.3±0.7*	2.4±2.0	2.3±0.9
Renal-denervated						
aCSF	4	1.4±0.5	1.8±0.8	2.6±1.2	3.3±1.7	2.9±1.4
SP 6.5 nmol	4	1.1±0.3	1.4±0.2	2.5±0.5	2.7±1.1	2.6±1.1

Values represent the mean±s.e.mean. of (*n*) rats. Statistical comparison to aCSF is indicated by $^*P<0.05$; $^{**}P<0.01$.

Table 4 Effects of i.t. injection of SP on changes in mean arterial pressure (MAP) in conscious saline-loaded rats

Treatment	n	*MAP baseline* (mmHg)	△ *MAP* (mm Hg) *Time* (min) *after injection* 1	5	15	30
aCSF	6	116.7±2.8	0.0±0.4	−1.5±0.8	−5.3±1.4	−9.4±2.1
SP 0.65 nmol	5	120.0±7.5	0.3±3.0	3.3±1.5	−8±2.2	−13.3±2.3
SP 6.5 nmol	4	106.3±7.2	−18.8±3.8**	6.3±2.4*	0.8±0.5	−7.6±3.7

Values represent the mean±s.e.mean. of (*n*) rats. Statistical comparison with aCSF is indicated by $^*P<0.05$; $^{**}P<0.01$.

and the i.c.v. effects produced by NKA on behaviour and the cardiovascular system (Picard *et al*., 1994). R 820 is a pseudopeptide antagonist selective for NK_3 receptors in rat (Regoli *et al*., 1994) which is able to inhibit in a specific manner the cardiovascular and renal effects elicited by NK_3 receptor agonists (senktide and [MePhe^7]NKB) injected i.c.v. (Cellier *et al*., 1995; Yuan & Couture, 1997). R 820 also blocked the hypoalgesia induced by i.t. injection of senktide in the rat (Couture & Toma, 1995). Therefore, the tachykinin receptor antagonists used in the present study represent suitable pharmacological tools to characterize the central tachykinin receptors in rats.

Whereas [MePhe^7]NKB was inactive on renal function, NKA produced transient reductions in renal excretion of water, sodium and potassium which could be due to the activation of NK_1 receptors for the following reasons: (1) [β-Ala^8]NKA(4-10), a selective tachykinin NK_2 receptor agonist (Rovero *et al*., 1989), did not reproduce the renal effects of NKA; and (2) NKA is not highly selective for NK_2 receptors and can also activate NK_1 receptors (Regoli *et al*., 1994; Picard *et al*., 1994). Therefore, the present results along with those obtained with the selective antagonists strongly suggest that tachykinin-induced reductions in renal excretion of water, sodium and potassium in conscious saline-loaded rats are mediated by a NK_1 receptor in the spinal cord. This finding is consistent with our previous studies suggesting a role for NK_1 receptors in spinal cardiovascular regulation (Couture *et al*., 1995).

It is worthy of note that the renal effects evoked by i.t. SP depend on the experimental conditions. In an earlier study, we showed that 6.5 nmol SP injected i.t. produced a prolonged natriuresis in the pentobarbitone-anaesthetized rat infused i.v. with a diuretic solution (0.7% w/v NaCl, 5 mM glucose and 5.0% w/v mannitol) (Hu *et al*., 1990). However, the same dose of SP had no effect on renal excretion in conscious normal hydrated rats (Yuan, 1995). This indicates that the involvement of the NK_1 receptor in spinal regulation of renal excretion depends on physiological conditions and can be markedly altered in anaesthetized animals.

Site of action of SP

The possibility that the renal responses induced by i.t. SP are due to the leakage of the peptide into the systemic circulation

is unlikely as intravenous or intrarenal infusion of low doses of SP increased renal excretion of water, sodium and potassium in rats (Arendshorst *et al*., 1976; Kramer *et al*., 1983). Moreover, it has been shown that at 1, 6, 11 and 16 min after i.t. injection of [^{125}I]-SP, the venous blood samples contain only 0.8 to 3.5% of the total labelled peptide injected. In the same study, the peptide dose injected i.t. diffused over a distance of 0.5 cm rostrally and caudally from the site of injection as demonstrated in radioautographs of longitudinal sections of rat spinal cord (Cridland *et al*., 1987). Further support for the limited spread of peptide within the i.t. space comes from a study with [^{3}H]-angiotensin II which demonstrated that 12 min after i.t. injection of labelled angiotensin II in the rat, 81% of the recovered radioactivity was limited to one segment on either side of the catheter tip. Only 0.37% was detected in the most rostral segments of the spinal cord (4–6 cm distant from cannula tip) (Suter & Coote, 1987). A supraspinal site of action is also unlikely since the i.c.v. administration of SP (65 or 650 pmol) did not affect renal responses in the same experiment model (Yuan & Couture, 1997). Our earlier cardiovascular studies with complete dose-response curves have shown that 650 pmol SP administered i.c.v. produced maximal cardiovascular and behavioural changes via the activation of an NK_1 receptor (Couture *et al*., 1995).

In spinal cord, SP and NK_1 receptors are widely distributed in dorsal and lateral horns, and moderately in ventral horn (Helke *et al*., 1986; 1990; Yashpal *et al*., 1990). NK_1 binding sites in the dorsal horn are located postsynaptically to sensory neurones, whereas NK_1 sites in the intermediolateral cell column are present on preganglionic sympathetic neurones (Helke *et al*., 1986). Since the majority of renal sympathetic postganglionic neurones originate in the paravertebral chain ganglia from T_{12} to L_1 segments in the rat (Ferguson *et al*., 1986), the intermediolateral cell column is the most probable site of action of i.t. SP (T_{13} spinal cord level) on renal function. However, it would be premature to reach any definitive conclusion regarding the exact cellular site of action of SP in the spinal cord with regard to the renal changes induced by i.t. injection of SP.

Possible mechanism of action of i.t. SP

The present results show that i.t. injection of 6.5 nmol SP at T_{13} thoracic spinal cord level produced a biphasic effect on blood pressure. Previous studies from this group have shown that i.t. administration of SP at T_9 spinal cord level produced dose-dependent pressor responses which are sometimes preceded by a short lasting vasodepressor effect in the conscious rat. Although the origin of the initial vasodepressor component is still unknown, the subsequent pressor response to i.t. SP was ascribed to the peripheral release of noradrenaline from sympathetic fibres (Hasséssian *et al*., 1990; Couture *et al*., 1995). The increase in MAP induced by 6.5 nmol SP at T_9 level (Hasséssian *et al*., 1990) was more prolonged (20–30 min) than that induced at T_{13} level (present study) although the magnitude of the response was quite similar at both levels. These differences might be due to the site of injection (T_9 versus T_{13}) and/or to the experimental condition (saline-loaded or saline-unloaded rat). The tachykinin receptor agonists (NKA, [β-Ala^8]NKA(4-10) and [MePhe^7]NKB) and antagonists (RP 67580, SR 48968 and R 820) used in the present study did not cause any cardiovascular changes when injected i.t. at 6.5 nmol in conscious rats (Couture *et al*., 1995).

According to the above evidence, it is unlikely that the changes in systemic blood pressure contribute to the inhibitory action of tachykinin on renal function. Whereas vascular changes induced by i.t. SP were transient (5 min), renal effects induced by i.t. SP lasted 30 min. It has been shown that i.t. SP at T_9 spinal cord level enhances sympathetic renal nerve activity in anaesthetized rats (Yusof & Coote, 1987). That action could reduce renal excretion of water and electrolytes through direct renal vasoconstriction and tubular sodium reabsorption via α-adrenoceptors or alternatively via the release of renin from the juxtaglomerular granular cells through β_1-adrenoceptors. The possibility that the spinal inhibitory action of SP on renal function is attributable to an increase of sympathetic renal nerve activity is supported by the absence of renal effect after i.t. injection of SP in renal denervated rats.

Functional implication

At the site of i.t. injection of SP, three sources of endogenous SP release have been identified: primary sensory C-fibres, intrinsic spinal cord neurones and bulbospinal descending fibres (see Introduction). The i.t. injection of SP would mimic the release of SP or a related peptide from any of these terminals in the spinal cord. Moreover, the presence of SP-immunoreactive fibres in renal sensory innervation (Ferguson & Bell, 1985) suggests a functional role for SP as sensory transmitter conveying information from chemoreceptors, mechanoreceptors or nociceptors within the kidney to the spinal cord.

In conclusion, the present study demonstrates that the renal excretion of water, sodium and potassium measured in conscious saline-loaded rats is blunted after intrathecal administration of SP or NKA at T_{13} spinal cord level. These renal modulatory effects appear to be mediated by the activation of an NK_1 receptor in the spinal cord and the sympathetic renal nerve.

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References

ADVENIER, C., ROUISSI, N., NGUYEN, Q.T., EMONDS-ALT, X., BRELIÈRE, 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.

ARENDSHORST, W.J., COOK, M.A. & MILLS, I.H. (1976). Effect of substance P on proximal tubular reabsorption in the rat. *Am. J. Physiol*., **230,** 1662–1667.

BEAUJOUAN, J-C., HEUILLET, E., PETITET, F., SAFFROY, M., TORRENS, Y. & GLOWINSKI, J. (1993). Higher potency of RP 67580, in the mouse and rat compared with other nonpeptide and peptide tachykinin NK_1 antagonists. *Br. J. Pharmacol*., **108,** 793–800.

CARRUETTE, A., MOUSSAOUI, S.M., CHAMPION, A., COTTEZ, D., GONIOT, P. & GARRET, C. (1992). Comparison in different tissue preparations of the in vitro pharmacological profile of RP 67580, a new non-peptide substance P antagonist. *Neuropeptide*., **23,** 245–250.

CELLIER, E., PICARD, P., BARBOT, L. & COUTURE, R. (1995). The non peptide SR 142801 behaves as a full agonist in the rat central nervous system. In *International Symposium on Tachykinins*, Florence, Italy, October 16-18, 1995, Abstract p. 91. Menarini: Fondazione Internazionale.

CHAPMAN, V. & DICKENSON, A.H. (1993). The effect of intrathecal administration of RP 67580, a potent neurokinin 1 antagonist on nociceptive transmission in the rat spinal cord. *Neurosci. Lett*., **157,** 149 – 152.

COUTURE, R. & TOMA, N. (1995). Antinociceptive effects of SR 142801 and SR 142806 in the rat tail flick test. In *International Symposium on Tachykinins*, Florence, Italy, October, 16-18, 1995, Abstract p. 154. Menarini: Fondazione Internazionale.

COUTURE, R., PICARD, P., POULAT, P. & PRAT, A. (1995). Characterization of the tachykinin receptors involved in spinal and supraspinal cardiovascular regulation. *Can. J. Physiol. Pharmacol*., **73,** 892 – 902.

CRIDLAND, R.A., YASHPAL, K., ROMITA, V.V., GAUTHIER, S. & HENRY, J.L. (1987). Distribution of label after intrathecal administration of ^{125}I-substance P in the rat. *Peptides*, **8,** 213 – 221.

DAVIS, B.M., KRAUSE, J.E., MCKELVY, J.F. & CABOT, J.B. (1984). Effects of spinal lesions on substance P levels in the rat sympathetic preganglionic cell column: evidence for local spinal regulation. *Neuroscience*, **13,** 1311 – 1326.

DIBONA, G.F. & SAWIN, L.L. (1983). Renal nerves in renal adaptation to dietary sodium restriction. *Am. J. Physiol*., **245,** F322 – F328.

DUGGAN, A.W., HENDRY, I.A., MORTON, C.R., HUTCHISON, W.D. & ZHAO, Z.Q. (1988). Cutaneous stimuli releasing immunoreactive substance P in the dorsal horn of the cat. *Brain Res*., **451,** 261 – 273.

EMONDS-ALT, X., VILAIN, P., GOULAOUIC, P., PROIETTO, V., VAN BROECK, D., ADVENIER, C., NALINE, E., NELIAT, G., LE FUR, G. & BRELIÈRE, J.C. (1992). A potent and selective non-peptide antagonist of the neurokinin A (NK_2) receptor. *Life Sci*., **50,** PL101 – PL106.

FERGUSON M. & BELL, C. (1985). Substance P-immunoreactive nerves in the rat kidney. *Neurosci. Lett*., **60,** 183 – 188.

FERGUSON, M., RYAN, G.B. & BELL, C. (1986). Localization of sympathetic and sensory neurons innervating the rat kidney. *J. Auton. Nerv. Syst*., **16,** 279 – 288.

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,** 10280 – 10212.

GILBEY, M.P., MCKENNA, K.E. & SCHRAMM, L.P. (1983). Effects of substance P on sympathetic preganglionic neurones. *Neurosci. Lett*., **41,** 157 – 159.

HASSÉSSIAN, H., COUTURE, R. & DE CHAMPLAIN, J. (1990). Sympathoadrenal mechanisms underlying cardiovascular responses to intrathecal substance P in conscious rats. *J. Cardiovasc. Pharmacol*., **15,** 736 – 744.

HELKE, C.J., CHARLTON, C.G. & KEELER, J.R. (1985). Bulbospinal substance P and sympathetic regulation of the cardiovascular system: a review. *Peptides* (Ankho International Inc.) **6**, (suppl. 2) 69 – 74.

HELKE, C.J., CHARLTON, C.G. & WILEY, R.G. (1986). Studies on the cellular localization of spinal cord substance P receptors. *Neuroscience*, **19,** 523 – 533.

HELKE, C.J., KRAUSE, J.E., MANTYH, P.W., COUTURE, R. & BANNON, M.J. (1990). Diversity in mammalian tachykinin peptidergic neurons: multiple peptides, receptors, and regulatory mechanisms. *FASEB J*., **4**, 1606 – 1615.

HU, F.Y., DENIS, G. & COUTURE, R. (1990). Spinal substance P (SP) enhances sodium excretion through a sensory vagal mechanism in the rat. *International Symposium on Substance P and Related Peptides: Cellular and Molecular Physiology*, Worcester, Mass., U.S.A., July 18-21, 1990, abstract, No. 51.

KRAMER, H.J., KLINGMULLER, D., FLACHSKAMPF, F.A. & DUSING, R. (1983). Substance P-induced changes in kidney function in the conscious rat: relation to the renal prostaglandin system. *Renal Physiol*., **6,** 10 – 18.

KURAISHI, Y., HIROTA, N., SATO, Y., HANASHIMA, N., TAKAGI, H. & SATOH, M. (1989). Stimulus specificity of peripherally evoked substance P release from the rabbit dorsal horn in situ. *Neuroscience*, **30,** 241 – 250.

MASSI, M., POLIDORI, C., PERFUMI, M., GENTILI, L. & DE CARO, G. (1991). Tachykinin receptor subtypes involved in the central effects of tachykinins on water and salt intake. *Brain. Res. Bull*., **26,** 155 – 160.

OTSUKA, M. & YOSHIOKA, K. (1993). Neurotransmitter functions of mammalian tachykinins. *Physiol. Rev*., **73,** 229 – 308.

PICARD, P., BOUCHER, S., REGOLI, D., GITTER, B.D., HOWBERT, J.J. & COUTURE, R. (1993). Use of non peptide tachykinin receptor antagonists to substantiate the involvement of NK_1 and NK_2 receptors in a spinal nociceptive reflex in the rat. *Eur. J. Pharmacol*., **232,** 255 – 261.

PICARD, P., REGOLI, D. & COUTURE, R. (1994). Cardiovascular and behavioural effects of centrally administered tachykinins in the rat: Characterization of receptors with selective antagonists. *Br. J. Pharmacol*., **112,** 240 – 249.

REGOLI D., BOUDON, A. & FAUCHÈRE, J-L. (1994). Receptors and Antagonists for Substance P and Related Peptides. *Pharmacol. Rev*., **46,** 551 – 599.

ROUISSI, N., CLAING, A., NICOLAU, M., JUKIC, D., D'ORLÉANS-JUSTE, P. & REGOLI, D. (1993). Substance P (NK-1 receptor) antagonists: *in vivo* and *in vitro* activities in rats and guinea pigs. *Life Sci*., **52,** 1141 – 1147.

ROVERO, P., PESTELLINI, V., PATACCHINI, R., GIULIANI, S., SANTICIOLI, P., MAGGI, C.A., MELI, A. & GIACHETTI, A. (1989). A potent and selective agonist for NK-2 tachykinin receptor. *Peptides*, **10,** 593 – 595.

SUTER, C. & COOTE, J.H. (1987). Intrathecally administered angiotensin II increases sympathetic activity in the rat. *J. Auton. Nerv. Syst*. **19,** 31 – 37.

YASHPAL, K., DAM, T.V. & QUIRION, R. (1990). Quantitative autoradiographic distribution of multiple neurokinin binding sites in rat spinal cord. *Brain Res*., **506,** 259 – 266.

YUAN, Y.D. (1995). Central actions of neurokinins and their receptors on renal function in the conscious rat. *Master Thesis in Physiology, Université de Montréal.*

YUAN, Y.D. & COUTURE, R. (1996). Spinal action of tachykinins on renal function in the conscious saline-loaded rat. *An International and Multidisciplinary Symposium on Peptide Receptors*, Montreal, Canada, July 28-August 1, 1996, Abstract, P 3.16. Montreal: Physiology Université de Montreal.

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.

YUSOF, A.P.M. & COOTE, J.H. (1987). The action of a substance P antagonist on sympathetic nerve activity in the rat. *Neurosci. Lett*., **75,** 329 – 333.

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