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Renal effects of intracerebroventricularly injected tachykinins in the conscious saline-loaded rat: receptor characterization

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1 The effects of intracerebroventricularly (i.c.v.) injected substance P (SP), neurokinin A (NKA) and [MePhe^7]neurokinin B (NKB) were investigated on renal excretion of water, sodium and potassium in the conscious saline-loaded rat. The central effects of [MePhe^7]NKB were characterized with selective tachykinin antagonists for NK_1 (RP 67580), NK_2 (SR 48968) and NK_3 (R 820) receptors.

2 Whereas SP or NKA (65 or 650 pmol) failed to modify the renal responses, [MePhe^7]NKB (65–6500 pmol) produced dose-dependent and long-lasting (30–45 min) decreases in renal excretion of water (maximal reduction at 65 pmol: from 66.14 ± 7.62 to 21.07 ± 3.79 μl min^{-1}), sodium (maximal reduction at 65 pmol: from 10.19 ± 2.0 to 1.75 ± 0.48 μmol min^{-1}) and potassium (maximal reduction at 65 pmol: from 4.31 ± 1.38 to 0.71 ± 0.27 μmol min^{-1}). While 650 pmol [MePhe^7]NKB elevated urinary osmolality, neither 65 pmol nor 6.5 nmol [MePhe^7]NKB altered this parameter.

3 Both the antidiuresis and antinatriuresis induced by [MePhe^7]NKB (65 pmol) were significantly blocked by the prior i.c.v. injection of R 820 (1.3 nmol, 5 min earlier), although the potassium excretion was only partially reduced. However, R 820 did not affect the antidiuresis and antinatriuresis elicited by endothelin-1 (1 pmol, i.c.v.). On its own, R 820 decreased renal potassium excretion with no effect on urinary osmolality and renal excretion of water and sodium. The i.c.v. co-injection of RP 67580 and SR 48968 (6.5 nmol each, 5 min earlier) failed to modify the renal responses to [MePhe^7]NKB in a similar study.

4 The central effects of [MePhe^7]NKB (65 pmol) on renal excretion were blocked by the prior i.v. administration of a linear peptide vasopressin V_2 receptor antagonist (50 μg kg^{-1}, 5 min earlier).

5 These results suggest that the central NK_3 receptor, probably located in the hypothalamus, is implicated in the renal control of water and electrolyte homeostasis through the release of vasopressin in the conscious saline-loaded rat.

Keywords: NK_3 receptor; tachykinin receptor antagonists; hypothalamus; renal excretion; vasopressin

Introduction

The mammalian tachykinins belong to a family of biologically active neuropeptides which include substance P (SP), neurokinin A (NKA), neuropeptide K (NPK), neuropeptide gamma (NPγ) and neurokinin B (NKB). They are widely distributed in both the central nervous system and peripheral tissues, and exert a variety of biological actions *in vivo*, the majority of which are mediated by three membrane receptors, namely neurokinin-1 ($NK_{1)}$), NK_2 and NK_3. 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 (Maggi *et al*., 1993; Otsuka & Yoshioka, 1993; Regoli *et al*., 1994).

Radioimmunoassay studies have revealed a high immunoreactivity for SP, NKA and NKB in the rat hypothalamus (Tateishi *et al*., 1989; Larsen *et al*., 1992; Merchenthaler *et al*., 1992). Autoradiographic and *in situ* hybridization studies have demonstrated that both NK_1 and NK_3 receptors are present in the rat hypothalamus (Stoessl & Hill, 1990; Dam *et al*., 1990a, b; Larsen *et al*., 1992; Maeno *et al*., 1993). Moreover, functional *in vivo* studies have shown that the intracerebroventricular (i.c.v.) injection of SP or NKA to the rat elicits pressor and chronotropic effects through the activation of the sympathetic nervous system (Unger *et al*., 1981; Takano *et al*., 1990), while the i.c.v. administration of NKB or NK_3 selective agonists, senktide and [MePhe^7]NKB, evokes a slight pressor effect and tachycardia through the release of arginine-vasopressin from the hypothalamic-hypophyseal axis (Polidori *et al*., 1989; Takano *et al*., 1990, 1993; Picard *et al*., 1994; Culman *et al*., 1995). These findings strongly suggest that tachykinins could act as neurotransmitters/neuromodulators in the central regulation of autonomic function. However, it remains to be determined whether or not the central tachykinins are involved in the regulation of renal function through the sympathetic nervous system and vasopressin, both of which play important roles in the regulation of renal function. It has been shown that the i.c.v. injection of SP induced an antidiuretic effect accompanied by an increase in plasma vasopressin level in the anaesthetized rat (Chowdrey *et al*., 1990). Similar results have been obtained with microinjection of SP into the hypothalamic supraoptic nucleus in the anaesthetized water-loaded rat (Mori *et al*., 1993). Contrary to these studies, it has been shown that the i.c.v. application of SP did not alter urinary flow rate in the conscious water-loaded rat, whilc i.c.v. injected senktide, a NK_3 selective agonist, produced an antidiuretic action by stimulating the release of vasopressin (Saigo *et al*., 1993). Furthermore, it is still uncertain as to whether or not i.c.v. tachykinins can affect the renal excretion of electrolytes.

The purpose of the present study was threefold: first to determine the i.c.v. effects of tachykinins on renal water and electrolyte excretion; second to characterize the central receptor subtype mediating these renal effects by the means of NK_1 (RP 67580), NK_2 (SR 48968) and NK_3 (R 820) receptor selective antagonists; and third to confirm the participation of vasopressin in the central effects of tachykinins on renal function.

Methods

Surgical procedures

The animal care and research protocols were in accordance with the principles and guidelines of the Canadian Council on

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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 sodium pentobarbitone, 65 mg kg^{-1} (Somnotol; M.T.C. Pharmaceuticals, Cambridge, Ont., Canada) and received an intramuscular (i.m.) injection of 45 000 iu of Penicillin G procaine (Ayercilline; Laboratoires Ayerst, Montréal, Qué., Canada). Siliconized polyethylene catheters (Intramedic, Clay Adams, NJ, U.S.A.) were implanted into the bladder, right lateral ventricle and right jugular vein. For i.c.v. implantation, the head of the rat was fixed to a stereotaxic apparatus (David Kopf Instrument, Tujunga, CA, U.S.A.), and one midline incision was made on the scalp. The angle of the head was adjusted according to the horizontal plan with respect to both bregma and lambda reference points. After a hole was drilled in the skull according to

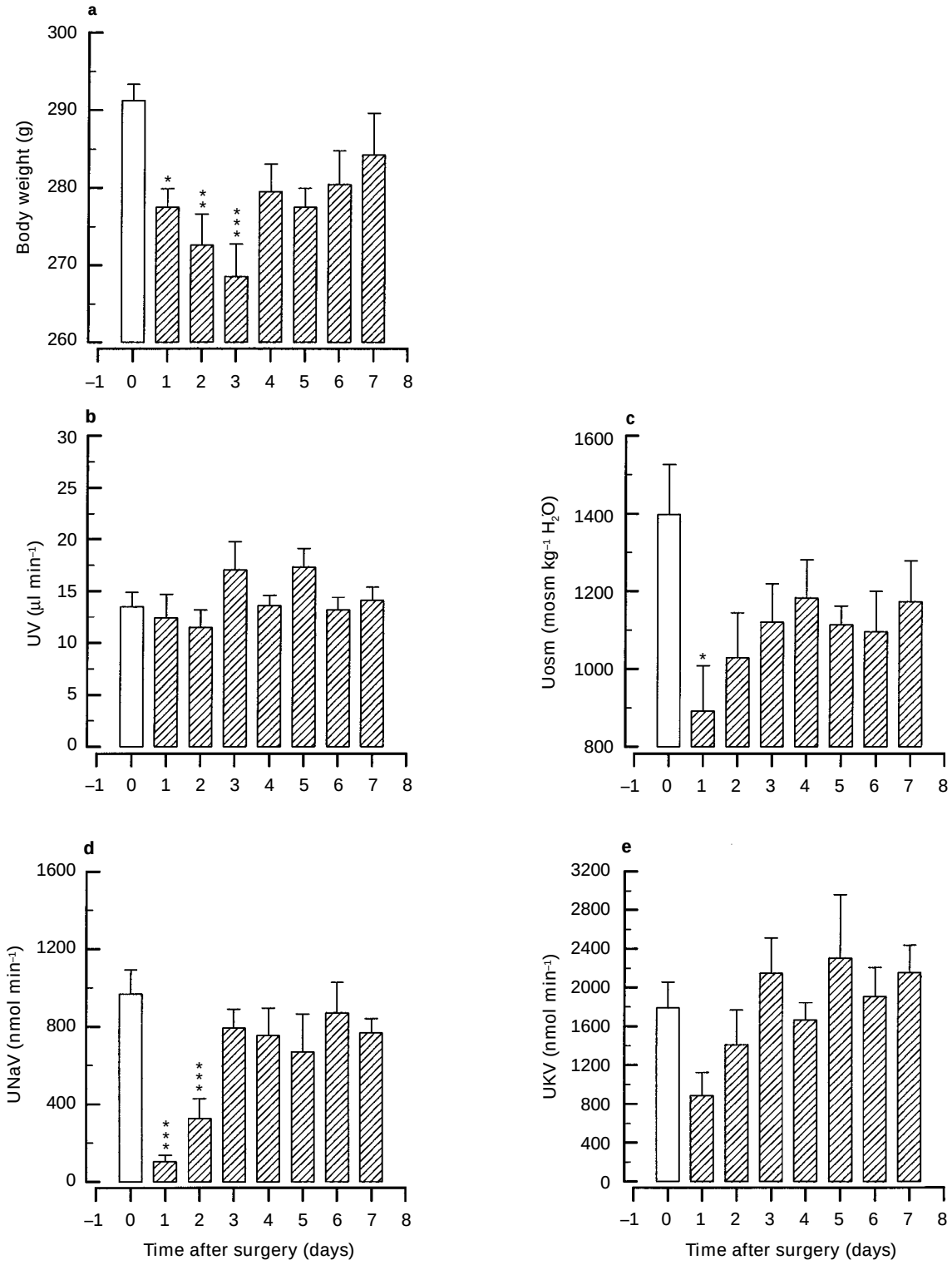

Figure 1 Effects of surgery on body weight (a), UV (b), Uosm (c), UNaV (d) and UKV (e) over a 1-week recovery period in the conscious rat. Each value represents the mean ± s.e.mean of 8 rats. Statistical comparison between values before (open columns) and after (hatched columns) surgery was evaluated with a one-way ANOVA in conjunction with Dunnett's test, and significance level is indicated by $*P<0.05$; $**P<0.01$; $***P<0.001$.

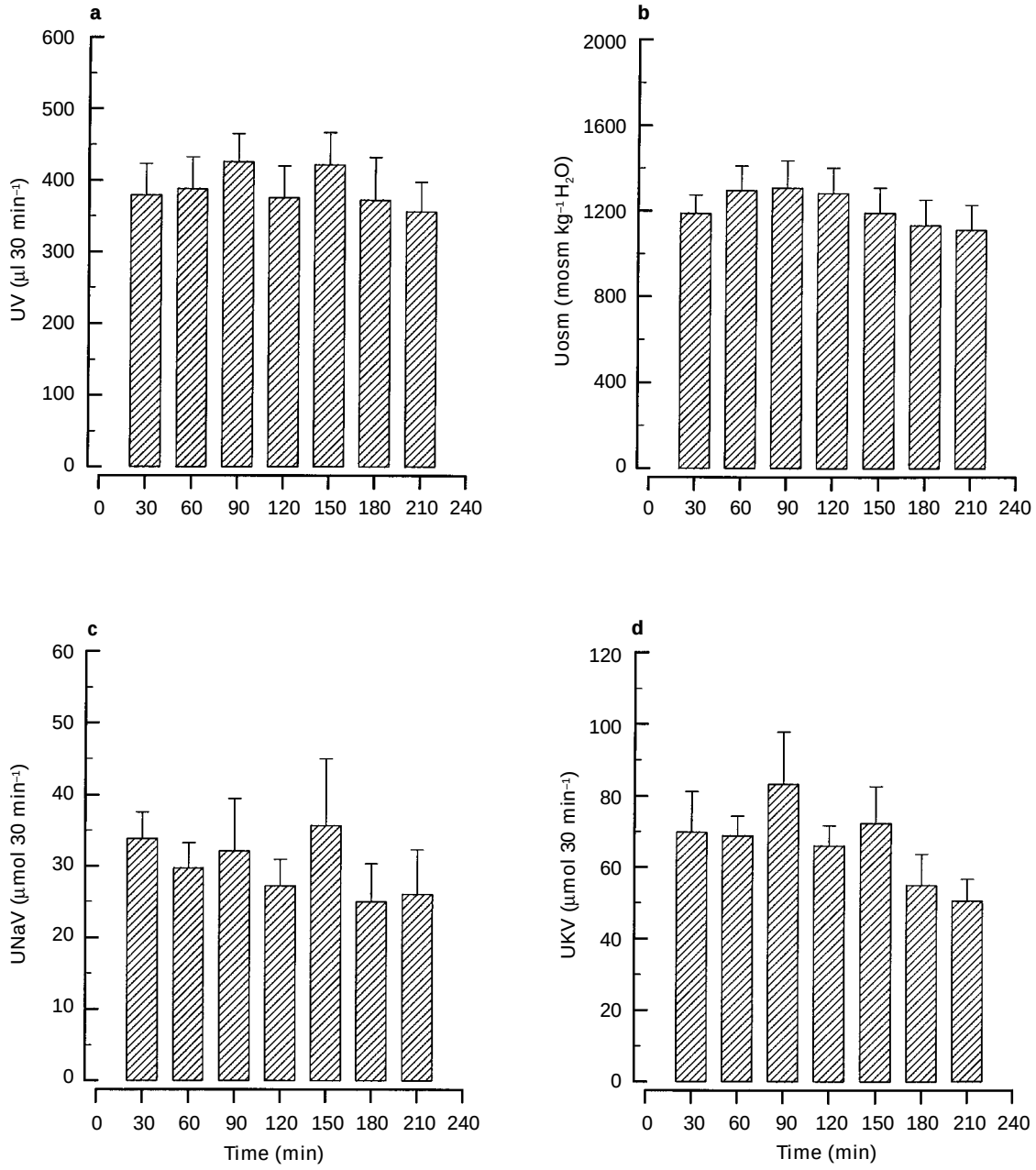

Figure 2 Stability of spontaneous renal excretion during the experimental period eight days after surgery in the conscious rat. Urinary samples were collected at intervals of 30 min for 3.5 h. Each value represents the mean±s.e.mean of 8 rats. Statistical comparison to the first urine sample was evaluated with a one-way ANOVA. No statistical difference was found between any of the parameters.

Table 1 Renal effects induced by i.c.v. SP and NKA or i.v. [MePhe^7]NKB in conscious saline-loaded rats

		Δ *UV* (μl min^{-1})		Δ *UNaV* (μmol min^{-1})		Δ *UKV* (μmol min^{-1})		Δ*Uosm* (mosm kg^{-1} H_2O)	
		Time after injection (min)							
Treatment	n	*15*	*30*	*15*	*30*	*15*	*30*	*15*	*30*
(i.c.v. injection)									
aCSF	8	24.5±11.3	68.7±9.7	3.4±1.4	8.3±2.3	5.2±2.2	2.9±1.1	−80.4±70.7	−219.6±105.3
SP 65 pmol	5	34.4±11.9	56.7±5.2	1.8±0.9	5.0±1.3	8.2±2.6	5.4±1.8	−80.5±98.6	−261.5±77.2
SP 650 pmol	7	16.8±7.4	56.7±8.7	1.9±1.1	7.0±2.0	3.0±1.3	5.3±1.5	118.2±88.6	−168.9±98.1
aCSF	9	21.9±8.4	57.8±5.8	1.2±0.4	7.9±1.7	2.1±0.6	3.5±0.7	−3.6±77.9	−60.3±77.9
NKA 65 pmol	8	28.7±12.6	64.7±10.4	3.8±1.9	11.4±4.4	5.1±2.6	5.9±1.7	−92.5±157.4	−215.6±154.8
NKA 650 pmol	10	8.5±4.5	45.1±7.0	0.9±0.4	7.1±1.8	1.3±0.7	5.2±1.1	4.5±68.0	−125.0±68.0
(i.v. injection)									
Saline	5	12.2±6.6	43.9±11.5	1.2±0.6	7.4±3.3	2.8±1.3	6.2±1.6	75.6±36.1	−65.4±43.3
[MePhe^7]NKB 4 nmol kg^{-1}	5	5.1±3.0	56.9±15.8	0.9±0.2	8.8±4.6	1.7±0.9	6.3±2.3	179.0±78.9	−138.0±75.5

Values represent the mean±s.e.mean of (*n*) rats. Statistical comparison to control values (aCSF or saline) was evaluated with a two-way ANOVA with repeated measures. No statistical difference was found between any of the parameters.

stereotaxic coordinates: 0.6 mm caudal to the bregma, 1.3 mm lateral to the midline and 5 mm vertical from the skull surface, an i.c.v. cannula (PE-20; void volume of 4 μl) was implanted with a guide into the right lateral ventricle and fixed to the skull with dental cement (Reliance Dental MFG Co., Worth, IL, U.S.A.). A second catheter (PE-60) was introduced into the bladder through a small incision in the bladder tip for collection of urine. An intravascular catheter (PE-50) filled with physiological saline containing heparin (50 iu ml^{-1}) was inserted into the right jugular vein for intravenous (i.v.) injection when needed. Both bladder and intravascular catheters were passed through a subcutaneous tunnel and exteriorized at the back of the neck. Following the operation, the rats were housed individually in a plastic cage (40 × 20 × 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 the experiment, and 0.5 ml physiological saline containing heparin (100 iu ml^{-1}) was injected into the i.v. catheter every two days to prevent blood clotting of the catheter. The rats were used at least 1 week after surgery when the animal had recovered its normal body weight and stabilized its renal excretion (Figures 1 and 2).

At the end of each experiment, 25 pmol of angiotensin II was injected i.c.v. to verify the patency of the i.c.v. cannula. Most rats (90 – 100%) displayed an intense dipsogenic behaviour lasting 15 min, and then received i.c.v. injection of Evans blue to check the filling of brain ventricles. The rats which showed hematuria and lost more than 20 g of body weight during a 1-week recovery period were excluded from experiments.

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

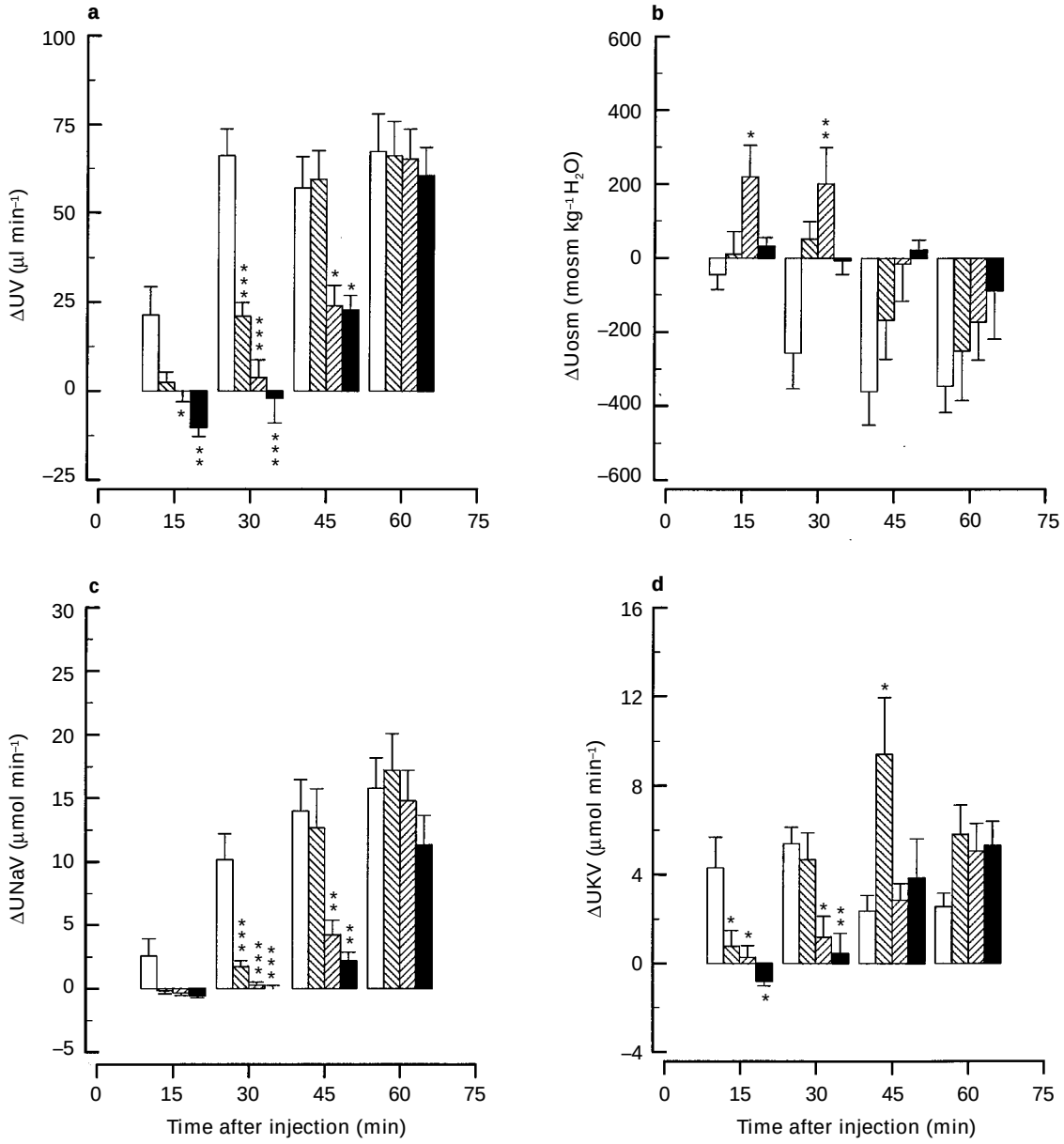

Figure 3 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.c.v. administration of [MePhe^7]NKB, at the dose of 65 pmol (first hatched columns; $n=6$), 650 pmol (second hatched columns; $n=9$) and 6.5 nmol (solid columns; $n=5$) in the conscious saline-loaded rat. Renal effects mediated by the vehicle are also shown (open columns; $n=8$). Each column represents variations (Δ) from baseline values and the mean ± s.e.mean of n rats. Statistical comparison to aCSF values was evaluated with a two-way ANOVA with repeated measures in conjunction with a *post hoc* Bonferroni test, and significance level is indicated by $^*P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$.

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 of 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 either i.c.v. (1 μl) or i.v. (50 μl 100 g^{-1} b.wt.). Urine was collected at in-

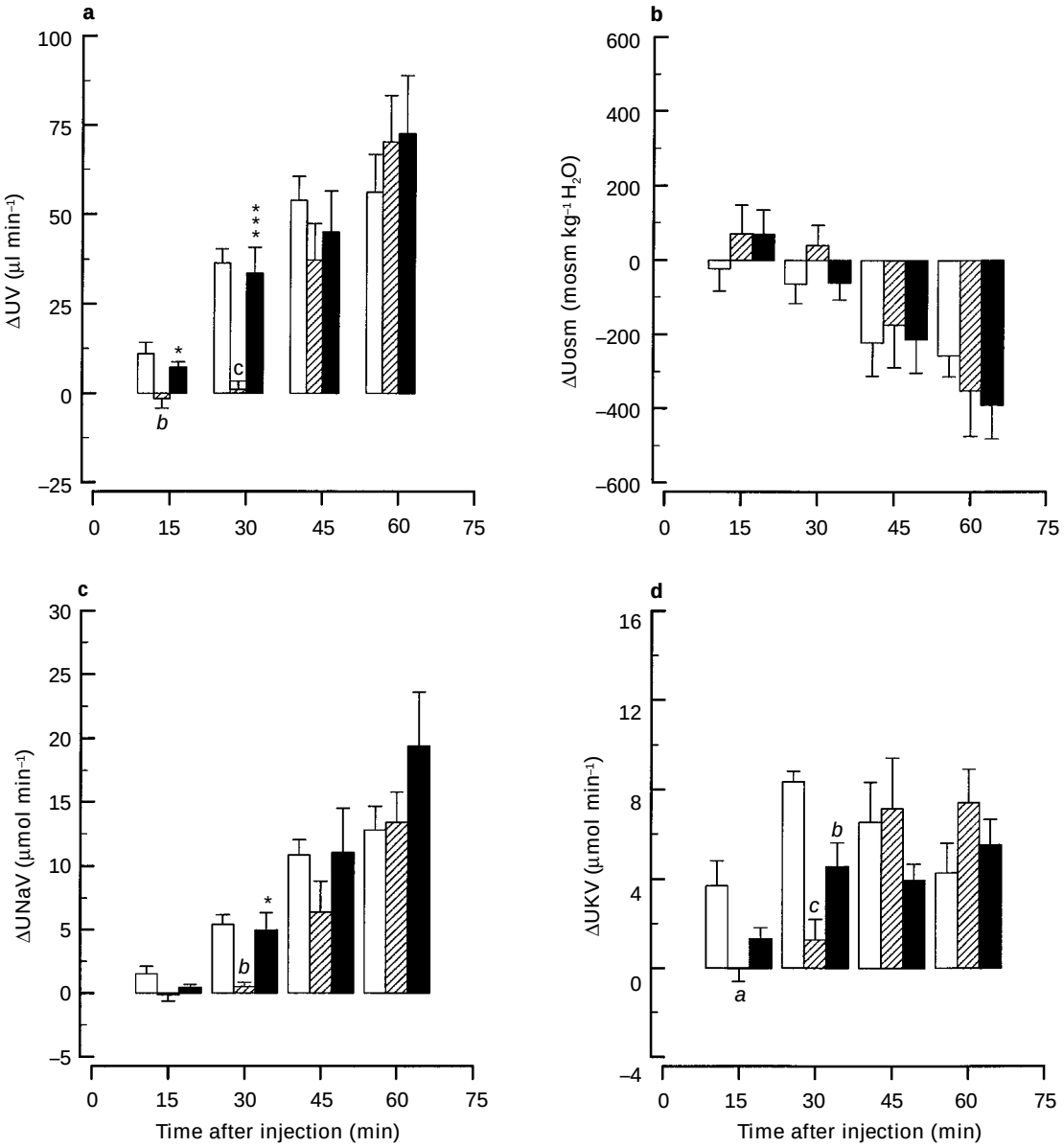

Figure 4 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.c.v. injection of 65 pmol [MePhe^7]NKB in the presence (solid columns; $n=6$) and absence (hatched columns; $n=7$) of 1.3 nmol R 820 in the conscious saline-loaded rat. Control values are also shown (open columns; $n=6$). Each column represents variations (Δ) from baseline values and the mean$\pm$s.e.mean of n rats. Statistical comparison to vehicle (letter) or to the agonist in the absence of the antagonist (asterisk) was evaluated with a two-way ANOVA with repeated measures in conjunction with a *post hoc* Bonferroni test, and significance level is indicated by ^{a},*$P<0.05$; ^{b},**$P<0.01$; ^{c},***$P<0.001$.

Table 2 Effects of R 820 on endothelin-induced renal responses in conscious saline-loaded rats

Treatment	n	Δ *UV* (μl min^{-1})	Δ *UNaV* (μmol min^{-1})	Δ *UKV* (μmol min^{-1})	Δ *Uosm* (mosm kg^{-1} H_2O)
Vehicle (i.c.v.)	4	44.0$\pm$9.2	7.1$\pm$1.8	3.5$\pm$1.0	$-$170.6$\pm$190.5
ET-1 (i.c.v., 1 pmol)	4	12.1$\pm$2.4*	0.6$\pm$0.4**	1.0$\pm$0.6	$-$18.1$\pm$102.3
R 820 (i.c.v., 1.3 nmol) + ET-1 (i.c.v., 1 pmol)	4	15.6$\pm$5.5*	1.0$\pm$0.5**	1.3$\pm$0.6	1.3$\pm$82.2

Values at 30 min post-injection represent the mean$\pm$s.e.mean of (n) rats. Statistical comparison to vehicle values was evaluated with a one-way ANOVA in conjunction with a *post-hoc* Bonferroni test, and the significance level is indicated by *$P<0.05$; **$P<0.01$.

tervals 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 urine osmolality (Uosm) was determined by freezing point depression with an osmometer (Advanced Digi Matic Osmometer model 3D2).

Experimental protocol

In the first series of experiments, the effects of SP, NKA or [MePhe^7]NKB on renal excretion were examined following i.c.v. administration. On day 1, the rats received an i.c.v. injection of 5 μl 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 subsequent days, two or three doses of SP, NKA or [MePhe^7]NKB (65–6500 pmol) were injected i.c.v. to construct the dose-response curve in conscious rats. Only one peptide in a volume of 1 μl of aCSF was given to a rat and each dose was injected at intervals of 24 h to avoid tachyphylaxis (Itoi *et al*., 1992; Picard *et al*., 1994). The i.c.v. catheter was then flushed with 4 μl of aCSF over 15–30 s and urine samples were collected for 1 h.

The second series of experiments was designed to characterize the tachykinin receptor mediating the renal effects of [MePhe^7]NKB. On the first day, aCSF (1 μl solution flushed with 4 μl of aCSF) containing dimethylsulphoxide (10–30% DMSO) in order to dissolve the tested antagonists, was administered i.c.v. 5 min before the second injection of aCSF. On the second day, the animals received a single i.c.v. injection of 65 pmol [MePhe^7]NKB flushed with 4 μl of aCSF. The aCSF containing DMSO was given i.c.v. 5 min before the agonist. On the third day, R820 (tachykinin NK_3 receptor antagonist, 1.3 nmol) or a solution containing both RP 67580 (tachykinin NK_1 receptor antagonist, 6.5 nmol) and SR 48968 (tachykinin NK_2 receptor antagonist, 6.5 nmol) was administered i.c.v. in a volume of 1 μl, 5 min before 65 pmol [MePhe^7]NKB. The

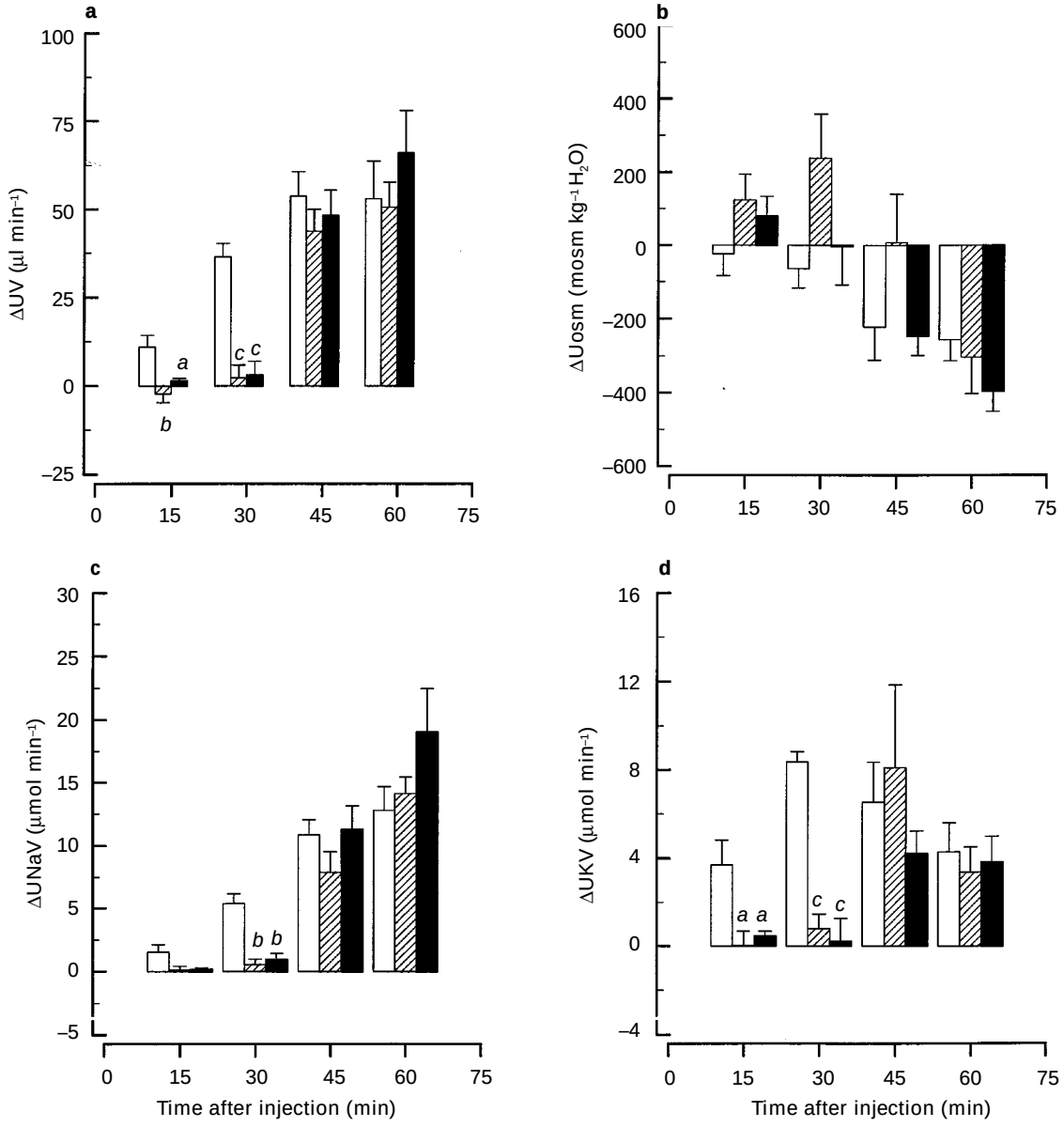

Figure 5 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.c.v. injection of 65 pmol [MePhe^7]NKB, in the presence (solid columns; $n=6$) and absence (hatched columns; $n=5$) of RP 67580 and SR 48968 (6.5 nmol each) in the conscious saline-loaded rat. Renal effects mediated by the vehicle are also shown (open columns; $n=6$). Each column represents variations (Δ) from baseline values and the mean ± s.e.mean of n rats. Statistical comparison to vehicle (letter) or to the agonist in the absence of antagonists was evaluated with a two-way ANOVA with repeated measures in conjunction with a *post hoc* Bonferroni test, and significance level is indicated by ^{a}, $P<0.05$; ^{b}, $P<0.01$; ^{c}, $P<0.001$.

Table 3 Intrinsic effects of tachykinin and vasopressin receptor antagonists on renal function of conscious saline-loaded rats

Treatment	n	*Time* (min)	Δ *UV* (μl min^{-1})	Δ *UNaV* (μmol min^{-1})	Δ *UKV* (μmol min^{-1})	Δ *Uosm* (mosm kg^{-1} H_2O)
Vehicle	5	15	17.9±3.1	2.4±0.6	4.5±1.0	126.5±168.4
(i.c.v.)		30	39.9±3.5	5.3±1.9	4.5±1.0	−20.5±179.6
		45	51.1±4.7	11.1±5.1	3.8±1.0	−226.5±170.6
		60	53.2±10.1	13.2±3.5	4.3±1.0	−170.5±145.2
RP 67580+SR 48968	5	15	13.2±2.7	0.8±0.3	3.4±1.2	120.5±43.2
		30	35.0±3.1	2.8±0.5	6.7±1.8	51.5±21.8
(i.c.v.; 6.5 nmol each)		45	47.4±4.0	9.0±1.6	6.0±1.4	−26.5±67.0
		60	68.4±6.0	17.0±2.1	6.4±1.6	−158.5±70.9
R 820	5	15	7.7±3.0	0.3±0.1	0.7±0.5*	2.0±102.6
(i.c.v.; 1.3 nmol)		30	27.4±3.3	2.0±0.5	3.6±0.7	−80.0±95.0
		45	54.4±3.6	7.7±1.7	6.0±0.4	−204.0±79.7
		60	61.7±9.6	12.0±1.0	6.8±1.5	−364.0±50.0
Saline	6	15	32.1±10.1	1.9±0.7	5.2±1.4	−26.7±106.2
(i.v.)		30	43.9±4.1	4.9±1.2	3.9±1.4	−282.5±125.0
		45	73.2±12.5	15.0±2.3	4.9±1.2	−256.7±88.3
		60	60.1±11.4	13.1±2.3	2.9±0.9	−282.5±91.6
Vasopressin V_2 receptor antagonist	6	15	77.0±13.8*	2.4±0.5	8.7±1.0	−96.3±66.2
(i.v.; 50 μg kg^{-1})		30	121.7±25.1**	5.1±1.7	2.5±1.3	−372.1±64.3
		45	81.1±12.5	10.0±3.1	2.0±1.1	−343.8±102.3
		60	58.5±11.0	10.7±2.4	1.9±0.9	−229.6±105.7

Values represent the mean±s.e.mean of (n) rats. Statistical comparison to control values (vehicle or saline) was evaluated with a two-way ANOVA with repeated measures in conjunction with a *post-hoc* Bonferroni test, and significance level is indicated by $*P<0.05$; $**P<0.01$.

animals received only one antagonist solution. The doses of tachykinin NK_1, NK_2 and NK_3 receptor antagonists were selected on the basis of their effectiveness to inhibit the i.c.v. cardiovascular and behavioural effects of their respective agonists in the conscious rat (Picard *et al*., 1994; Cellier *et al*., 1995). The specificity of R 820 (1.3 nmol, 5 min earlier) was tested against endothelin-1 (i.c.v., 1 pmol) in a similar study. The intrinsic effects of the tested antagonists on renal excretion were examined in separate experiments.

The third series of experiments was primarily aimed at determining whether or not the i.c.v. effects of [MePhe^7]NKB on renal excretion result from the leakage of the peptide into systemic circulation. These animals did not have i.c.v. catheters and received randomly a bolus i.v. injection of physiological saline (50 μl 100 g^{-1} b.wt) or [MePhe^7]NKB (4 nmol kg^{-1}) dissolved in physiological saline. The i.v. catheter was flushed with 100 μl of saline. Urine samples were collected at intervals of 15 min for 1 h. At the end of the experiment, the rats were killed with an overdose of pentobarbitone.

In the fourth series of experiments, the peripheral role of vasopressin in the renal effects induced by i.c.v. [MePhe^7]NKB was evaluated with a linear peptide vasopressin V_2 receptor antagonist (propionyl-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg). The animals received a single i.c.v. injection of 65 pmol [MePhe^7]NKB. The day after, the vasopressin V_2 receptor antagonist (50 μg kg^{-1}) dissolved in saline (50 μl 100 g^{-1} b.w.) was injected i.v. 5 min before the second i.c.v. injection of 65 pmol [MePhe^7]NKB. Control animals were injected with vehicle. The dose of the vasopressin V_2 receptor antagonist used was selected according to the efficacy of similar analogues in the conscious rat (Sawyer *et al*., 1981) and to its ability to prevent the renal effects of exogenous vasopressin in the same paradigm. Saline or exogenous vasopressin (12.5 ng min^{-1}) was infused at 25 μl min^{-1} for 30 min on day 1 and 2. On day 3, the vasopressin V_2 receptor antagonist (50 μg kg^{-1}) was injected i.v. 5 min before the i.v. infusion of exogenous vasopressin (12.5 ng min^{-1}). The direct effect of the vasopressin V_2 receptor antagonist was examined in separate experiments.

Peptides and other compounds

SP, NKA and angiotensin II were purchased from Hükabel Scientific Ltd, Montréal, Québec, Canada. [MePhe^7]NKB and the tachykinin NK_3 receptor antagonist, R 820 (3-indolylcarbonyl-Hyp-Phg-N(Me)-Bzl) (Nguyen *et al*., 1994; Regoli *et al*., 1994), were generously provided by Dr D Regoli of Université de Sherbrooke (Sherbrooke, Québec, Canada). The tachykinin NK_1 receptor antagonist, RP 67580 ((3aR, 7aR)-7,7-diphenyl-2-[1-imino-2-(2-methoxyphenyl)-ethyl]perhydroisoindol-4-one), was a gift from Dr C. Garret, Rhône-Poulenc (Rorer, Paris, France) and the tachykinin NK_2 receptor antagonist, SR 48968 ((S)-N-methyl-N[4-(4-acetylamino-4-phenylpiperidino)-2-(3,4-dichlorophenyl)butyl]-benzamide), was from Dr J.C. Brelière, Sanofi (Montpellier, France). Vasopressin was obtained from Peninsula Laboratories, Inc. (Belmont, CA, U.S.A.), and the vasopressin V_2 receptor antagonist (propionyl-D-Tyr(Et)-Phe-Val-Asn-Abu-Pro-Arg-Arg; *in vivo* anti-V_2 $pA_2=7.74$ and anti-$V_1=6.71$) (Manning *et al*., 1987) and endothelin-1 were from Bachem Bioscience Inc. (King of Prussia, PA, U.S.A.). SP, NKA, [MePhe^7]NKB and angiotensin II were dissolved directly in aCSF, while vasopressin and the vasopressin V_2 receptor antagonist were made up in saline. The tachykinin receptor antagonists and endothelin-1 were dissolved in dimethylsulphoxide (DMSO, Fisher) and acetic acid (0.01%), respectively, and aCSF was added to obtain the desired solutions (the final solution contained no more than 30% of DMSO or 0.001% of acetic acid). Stock solutions of endothelin-1 (100 pmol μl^{-1}), tachykinin receptor agonists and antagonists (6.5–65 nmol μl^{-1}) were divided into aliquots of 10 μl. Stock solutions of vasopressin (2 μg ml^{-1}) and its V_2 receptor antagonist (200 μg ml^{-1}) were divided into aliquots of 500 μl. The solutions were stored at −20°C and daily dilutions were made in aCSF (i.c.v. injection) or saline (i.v. injection) 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). Results are presented as means±s.e.mean of (n) rats. Multiple comparisons between groups at different end points (effect-time) were evaluated with a two-way analysis of variance (ANOVA) with repeated measures to check overall significance. Statistical comparison to a single variable was evaluated with a one-way

ANOVA in conjunction with a *post-hoc* Bonferroni test at each period of time. Only probability values (P) smaller than 0.05 were considered to be statistically significant.

Results

Time-course of recovery of renal excretion after surgery

The effects of pre-anaesthesia and surgical stress on renal excretion in conscious rats are shown in Figure 1. Whereas daily urinary volume remained unchanged during a 1-week recovery period (Figure 1b), renal excretion of sodium was dramatically decreased during the first two days after operation, before returning to pre-surgical values. Renal potassium excretion tended to decrease the day after surgery but remained relatively stable afterwards (Figure 1e). These electrolyte changes were accompanied by a significant decrease in urinary osmolality on day 1 (Figure 1c). Rats gradually lost body weight during the first three days post-surgery and recovered within 1 week (Figure 1a). On the eighth day after surgery, spontaneous urine was collected at intervals of 30 min for 3.5 h. As shown in Figure 2, baseline renal excretion of water, sodium and potassium as well as urinary osmolality were stable over this period.

Effects of i.c.v. SP, NKA and [MePhe^7]NKB on renal excretion

The i.c.v. injection of SP or NKA (65 or 650 pmol) failed to cause significant changes in renal excretion of water, sodium and potassium and urinary osmolality during a collection period of 1 h (Table 1; only values at 15 and 30 min after injection are shown). In contrast, the i.c.v. injection of [MePhe^7]NKB produced significant effects on renal excretion in the conscious rat (Figure 3). At the dose of 65 pmol, [Me-

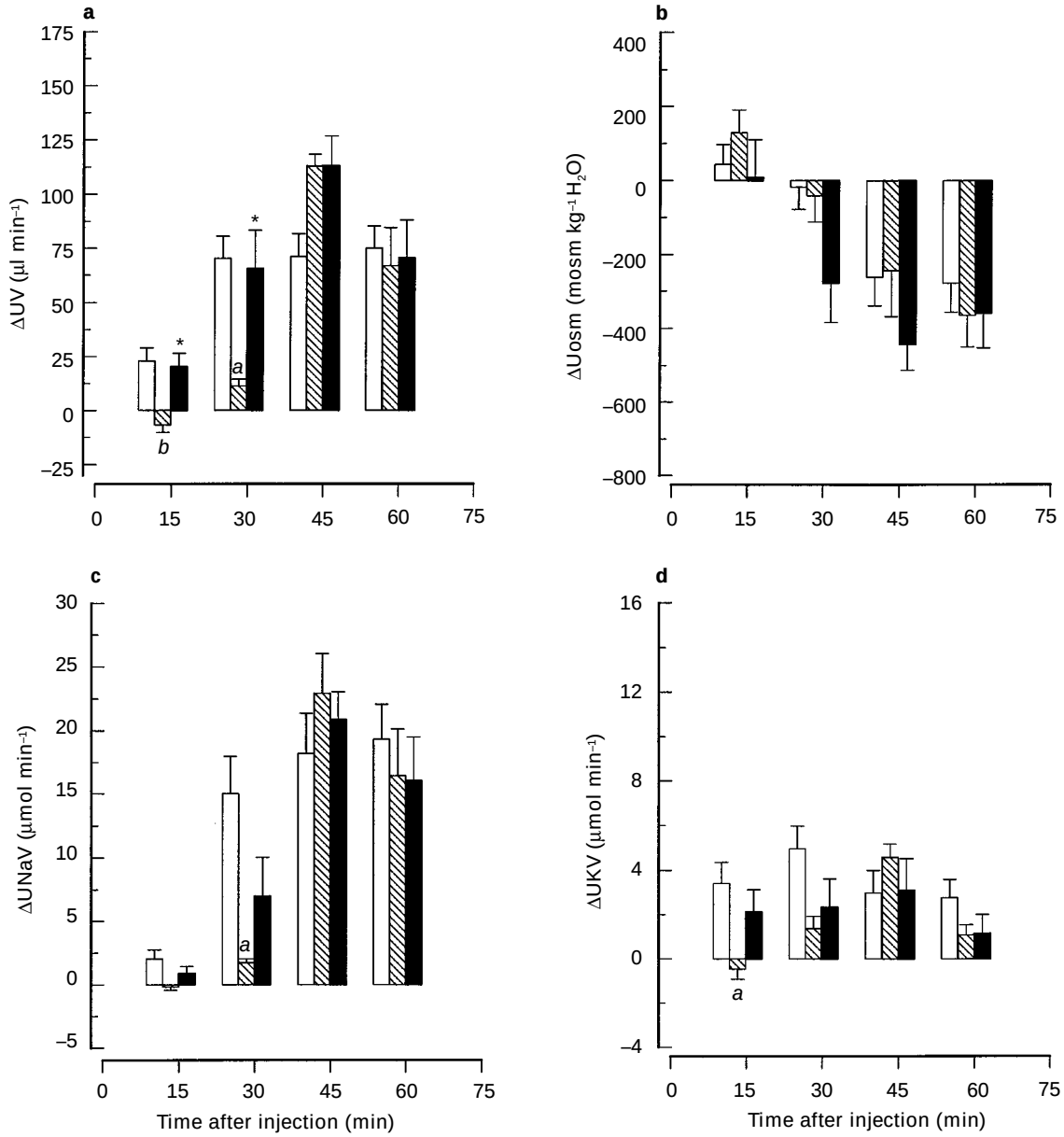

Figure 6 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.v. infusion of exogenous vasopressin (12.5 ng min^{-1}) for 30 min in the presence (solid columns; $n=4$) and absence (hatched columns; $n=4$) of a vasopressin V_2 receptor antagonist (50 μg kg^{-1}, 5 min earlier) in the conscious saline-loaded rat. Renal effects mediated by the vehicle are also shown (open columns; $n=7$). Each column represents variation (Δ) from baseline values and the mean$\pm$s.e.mean of n rats. Statistical comparison to vehicle (letter) or to the agonist in the absence of the antagonist (asterisk) was evaluated with a two-way ANOVA with repeated measures in conjunction with a *post hoc* Bonferroni test, and significance level is indicated by ^{a}, $^*P<0.05$; ^{b}, $P<0.01$.

Phe[7]]NKB elicited marked antidiuretic and antinatriuretic responses which reached a maximum at 30 min ($P<0.001$) and returned to control level at 45 min (Figure 3a, c). Renal potassium excretion was transiently decreased ($P<0.05$) at 15 min and then returned to aCSF level with a rebound response at 45 min over control value ($P<0.05$; Figure 3d). However, urinary osmolality was not significantly changed (Figure 3b). At doses of 650 pmol and 6.5 nmol, i.c.v. [MePhe[7]]NKB produced deeper and longer decreasing effects on ΔUV, ΔUNaV and ΔUKV (Figure 3a, c, d). Thus, changes in renal excretion of water, sodium and potassium induced by i.c.v. [MePhe[7]]NKB were dose-dependent. Although urinary osmolality was elevated by 650 pmol at 15 ($P<0.05$) and 30 min ($P<0.01$), changes in Uosm were not dose-dependent since neither 65 pmol nor 6.5 nmol [MePhe[7]]NKB evoked significant effects on urinary osmolality (Figure 3b).

Characterization of the tachykinin receptor underlying renal responses to [MePhe[7]]NKB

The i.c.v. administration of 65 pmol [MePhe[7]]NKB to the conscious rat reproduced the renal responses (Figure 4) observed in the previous series of experiments. R 820 (1.3 nmol), a tachykinin NK_3 receptor antagonist, when given i.c.v. 5 min before 65 pmol [MePhe[7]]NKB, completely blocked the antidiuretic and antinatriuretic effects of the agonist (Figure 4a, c). The renal responses to [MePhe[7]]NKB in the presence of R 820 were not significantly different from aCSF values. Although the reduction in renal excretion of potassium induced by [MePhe[7]]NKB was attenuated by R 820 (Figure 4d), the residual response was statistically different from aCSF values (at 30 min; $P<0.01$). Furthermore, urinary osmolality was not altered by i.c.v. [MePhe[7]]NKB either in the presence or absence of R 820 (Figure 4b). On the other hand, the i.c.v. pre-

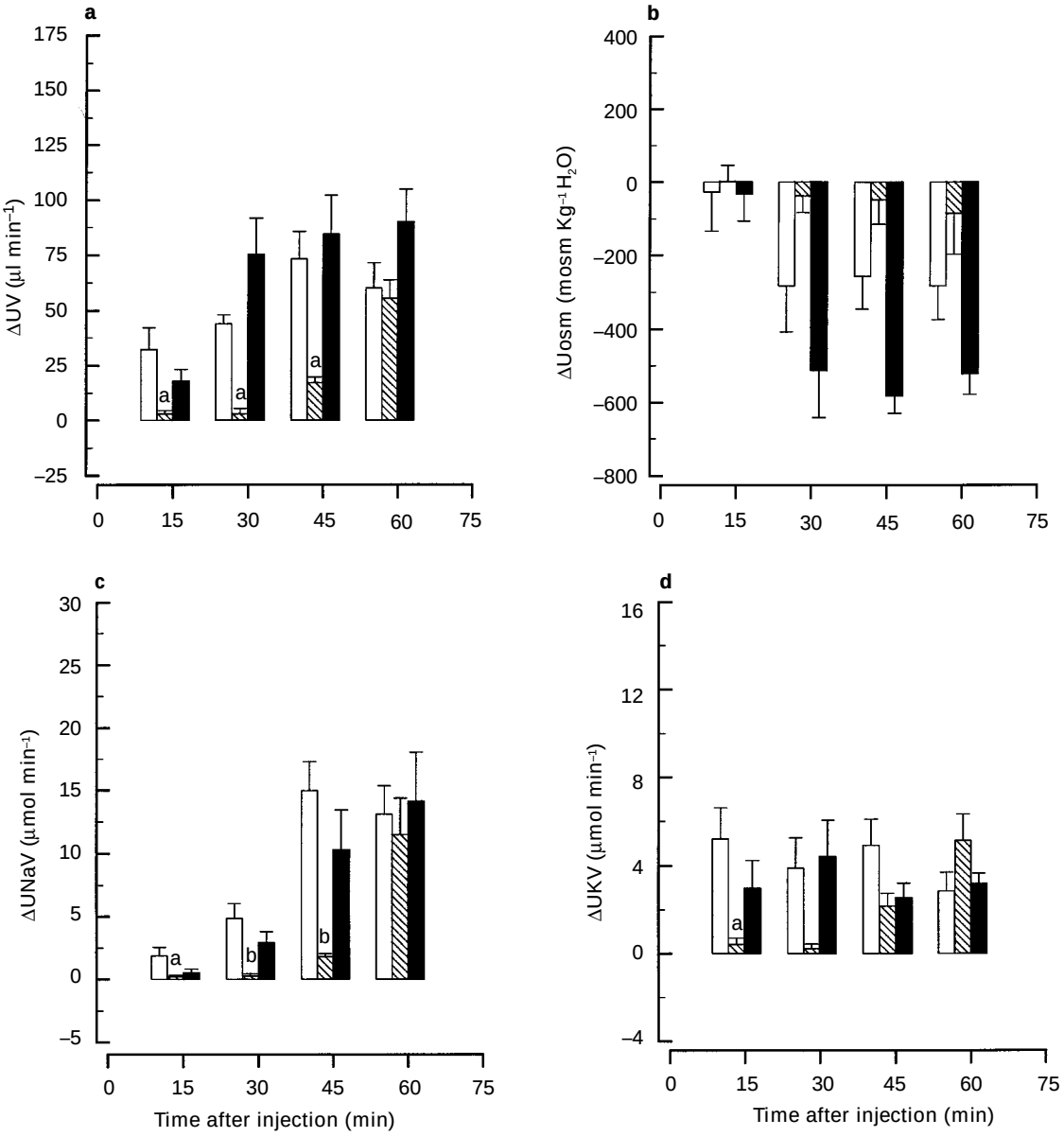

Figure 7 Changes in UV (a), Uosm (b), UNaV (c) and UKV (d) elicited by the i.c.v. injection of 65 pmol [MePhe[7]]NKB in the presence (solid columns; $n=6$) and absence (hatched columns; $n=6$) of a vasopressin V_2 receptor antagonist (50 μg kg^{-1}, 5 min earlier) in the conscious saline-loaded rat. Renal effects mediated by the vehicle are also shown (open columns; $n=6$). Each column represents variation (Δ) from baseline values and the mean ± s.e.mean of n rats. Statistical comparison to vehicle (letter) or to the agonist in the absence of the antagonist (asterisk) was evaluated with a two-way ANOVA with repeated measures in conjunction with a *post hoc* Bonferroni test, and significance level is indicated by [a], $*P<0.05$; [b], $**P<0.01$; $***P<0.001$.

treatment (5 min earlier) with 1.3 nmol R 820 did not alter the antidiuresis and antinatriuresis induced by the i.c.v. injection of 1 pmol endothelin-1 (Table 2; only values at 30 min post-injection which were significant from those of the vehicle are shown).

Pre-administration (i.c.v.; 5 min earlier) of a solution containing both RP 67580 and SR 48968 (6.5 nmol each) failed to alter renal responses induced by 65 pmol [MePhe^7]NKB (Figure 5a, c, d). Urinary osmolality remained unchanged by 65 pmol [MePhe^7]NKB in the presence and absence of tachykinin NK_1 and NK_2 receptor antagonists (Figure 5b). Intrinsic effects of tachykinin receptor antagonists on renal excretion are illustrated in Table 3. While co-injection of RP 67580 and SR 48968 (i.c.v.; 6.5 nmol each) had no direct effects on renal excretion, R 820 (1.3 nmol) caused a transient decrease in renal excretion of potassium (at 15 min, $P<0.05$) without causing changes in the other urinary parameters.

Peripheral effect of [MePhe^7]NKB on renal excretion

As shown in Table 1, a bolus injection of [MePhe^7]NKB (4 nmol kg^{-1}) did not produce significant changes in ΔUV, ΔUNaV, ΔUKV or ΔUosm when compared to saline values.

Effects of a vasopressin V_2 receptor antagonist versus the renal responses to [MePhe^7]NKB

The intravenous infusion of exogenous vasopressin (12.5 ng min^{-1}) produced marked antidiuresis during the infusion period of 30 min (at 15 min: $P<0.01$ and at 30 min: $P<0.05$) and returned to control value within 45 min (Figure 6a). The antidiuresis elicited by exogenous vasopressin was accompanied by an antinatriuresis which reached a significant level ($P<0.05$) at 30 min (Figure 6c), and by a decrease in renal excretion of potassium at 15 min ($P<0.05$; Figure 6d). The renal responses to i.v. infusion of exogenous vasopressin were blocked by the prior administration of a vasopressin V_2 receptor antagonist (50 μg kg^{-1}) (Figure 6a, c, d). Urinary osmolality was not significantly altered by the i.v. infusion of exogenous vasopressin either in the presence or absence of vasopressin V_2 receptor antagonist (Figure 6b). The i.v. pretreatment with the vasopressin V_2 receptor antagonist (50 μg kg^{-1}) blocked also the decreases in renal excretion of water, sodium and potassium induced by the i.c.v. injection of 65 pmol [MePhe^7]NKB (Figure 7a, c, d). The renal responses to [MePhe^7]NKB in the presence of the vasopressin V_2 receptor antagonist were not significantly different from control values. Whereas urinary osmolality was not significantly altered by [MePhe^7]NKB compared to control value, the vasopressin V_2 receptor antagonist significantly decreased urinary osmolality in response to [MePhe^7]NKB at 30 and 45 min post-injection (Figure 7b). The vasopressin V_2 receptor antagonist had no direct effects on ΔUNaV, ΔUKV and ΔUosm, but baseline diuresis was significantly enhanced at 15 ($P<0.05$) and 30 min ($P<0.01$) after i.v. injection of the antagonist (Table 3).

Discussion

Central effects of tachykinins and their receptors on renal excretion

In the present study, we found that the i.c.v. administration of SP (65 or 650 pmol) failed to alter renal excretion of water, sodium and potassium as well as urinary osmolality in conscious saline-loaded rats. This finding is in agreement with data from a previous study showing that i.c.v. SP was without effect on urinary volume in conscious water-loaded rats (Saigo *et al*., 1993) or in conscious rats receiving an i.v. infusion of an hydrating solution (Cantalamessa *et al*., 1984). However, at relatively higher doses (20 nmol), i.c.v. SP caused an antidiuresis accompanied by an increase in plasma vasopressin level in ethanol-anaesthetized rats infused with an isotonic glucose/saline solution (Chowdrey *et al*., 1990). These latter conflicting results may be due to the higher doses of SP used. A recent study demonstrated that the microinjection of SP (1 or 5 nmol) into the supraoptic nucleus of the hypothalamus in water-loaded and ethanol-anaesthetized rat produced a long-lasting antidiuresis which was blocked by the intravenous injection of a vasopressin V_1/V_2 receptor anatagonist and was slightly reduced by spantide, a tachykinin receptor antagonist (Mori *et al*., 1993). However, spantide also induced marked decreases in urinary outflow which casts some doubt as to whether or not the antidiuresis induced by central SP is mediated by NK_1 receptors. It should also be noted that high doses of SP can activate the three tachykinin receptors (Regoli *et al*., 1994). In agreement with our findings, relatively lower doses of i.c.v. SP (in contrast to i.c.v. senktide) did not elevate the plasma vasopressin level in conscious rats (Unger *et al*., 1981; Polidori *et al*., 1989; Saigo *et al*., 1993). Our results also reveal that i.c.v. NKA (65 or 650 pmol) did not alter renal responses in the conscious saline-loaded rat. Central SP and NKA can activate the sympathetic nervous system, which is believed to be involved in the control of renal function in the conscious animal (Kopp & DiBona, 1992; DiBona, 1994). Thus, one would expect an alteration in renal excretion following the i.c.v. injection of SP or NKA. However, our results are in favour of the interpretation that endogenous SP and NKA in the brain are unlikely to be involved in the regulation of renal excretion in this model of saline-loaded rat.

The i.c.v. injection of [MePhe^7]NKB elicited dose-dependent antidiuretic effect which lasted 30-45 min in conscious saline-loaded rats. This confirms a previous study which demonstrated that the i.c.v. injection of another NK_3 selective agonist, senktide, resulted in an antidiuresis in the conscious water-loaded rat (Saigo *et al*., 1993). The novel findings of the present study are that i.c.v. [MePhe^7]NKB simultaneously decreased renal excretion of sodium and potassium in addition to its antidiuretic effect. It is unlikely that these renal responses induced by i.c.v. [MePhe^7]NKB are due to the leakage of the peptide into the peripheral circulation, as i.v. injection of [MePhe^7]NKB at a dose of 4 nmol kg^{-1}, which is about 20 times the dose of 65 pmol given i.c.v., did not alter renal excretion in conscious saline-loaded rats. The possibility of a spinal site of action is also unlikely since the intrathecal injection of [MePhe^7]NKB (0.65 or 6.5 nmol) failed to alter renal (data not shown) and cardiovascular responses (Hasséssian *et al*., 1988). The lateral ventricle is close to various hypothalamic nuclei, and aCSF containing peptide can bathe the circumventricular organs including the paraventricular and supraoptic nuclei of the hypothalamus. Moreover, the magnocellular part of the rat hypothalamic paraventricular nucleus has been identified as a site for the central action of an NK_3 receptor agonist on the release of vasopressin (Massi *et al*., 1991). Studies with autoradiography and immunocytochemistry have revealed a higher level of NKB and NK_3 receptors in the paraventricular and supraoptic nuclei (Stoessl & Hill, 1990; Dam *et al*., 1990a; Merchenthaler *et al*., 1992). These results suggest that the anterior hypothalamus is the most probable site of action of i.c.v. [MePhe^7]NKB.

Antidiuresis, antinatriuresis and antikaliuresis evoked by i.c.v. [MePhe^7]NKB were blocked by i.c.v. pretreatment with R 820. The residual decrease in renal excretion of potassium may be due to the fact that R 820 itself slightly inhibited renal excretion of potassium. So far, several tachykinin NK_3 receptor antagonists such as SR 142801, R 486 and R 820 are available. SR 142801 is a potent nonpeptide antagonist at the human NK_3 receptor ($pA_2=9.15$; Emonds-Alt *et al*., 1995). However, this antagonist acts as a full agonist in the rat central nervous system (Cellier *et al*., 1995; Couture & Toma, 1995). Although R 486, a peptide antagonist, shows a high apparent pA_2 value of 7.45 at the rat NK_3 receptor (Drapeau *et al*., 1990), it maintains some agonistic activity at NK_1 and NK_2 receptors when injected i.c.v. in the conscious rat (Picard *et al* 1994). In contrast, R 820, a dipeptide antagonist with a pA_2 value of 7.6 on the rat portal vein (Nguyen *et al*., 1994; Regoli

et al., 1994), blocks the antinociceptive effect produced by intrathecal injection of [MePhe^7]NKB (Couture & Toma, 1995) as well as the cardiovascular effects of i.c.v. senktide (Cellier *et al*., 1995) without direct effects in conscious rats. On the other hand, R 820 failed to inhibit the cardiovascular and behavioural responses induced by [Sar^9,Met(O$_2$)^{11}]SP (tachykinin NK$_1$ receptor agonist) injected i.c.v. in the conscious rat (Cellier, E. & Couture, R., unpublished data). In the present study, R 820 was inactive against the renal effects induced by i.c.v. injection of endothelin-1. Moreover, R 820 had no direct effect on renal excretion of water and sodium. Collectively, these results indicate that R 820 blocks the central effects of [MePhe^7]NKB in a specific manner.

The i.c.v. pretreatment with the combination of RP 67580 and SR 48968 (6.5 nmol each) did not affect the renal responses induced by i.c.v. administered [MePhe^7]NKB. No intrinsic effect on renal excretion was shown with the i.c.v. injection of RP 67580 and SR 48968, consistent with data from *in vitro* studies (Advenier *et al*., 1992; Carruette *et al*., 1992). Both RP 67580 and SR 48968 are potent nonpeptide tachykinin NK$_1$ and NK$_2$ receptor antagonists, respectively (Garret *et al*., 1991; Emonds-Alt *et al*., 1992). Our previous cardiovascular studies have shown that RP 67580 (6.5 nmol) and SR 48968 (6.5 nmol) inhibit, although not completely, the cardiovascular effects induced by i.c.v. SP and NKA, respectively. However, the i.c.v. co-injection of both antagonists completely abolished the cardiovascular responses induced by i.c.v. SP or NKA (Picard *et al*., 1994), suggesting that both NK$_1$ and NK$_2$ receptors are involved in the central effects of SP and NKA. Taken as a whole, our present results clearly indicate that central (i.c.v.) tachykinins produce antidiuresis and antinatriuresis through the activation of the NK$_3$ receptor most probably located in the hypothalamus. Nevertheless, it seems unlikely that the NK$_3$ receptor is involved in the tonic modulation of renal function in the present experimental paradigm, because the tachykinin NK$_3$ receptor antagonist alone was devoid of direct effects on renal excretion of water and sodium.

Mechanisms of action of [MePhe^7]NKB

It has been demonstrated that the i.c.v. injection of NKB or NK$_3$ selective agonists ([MePhe^7]NKB or senktide) increases blood pressure and heart rate in the conscious rat (Itoi *et al*., 1992; Picard *et al*., 1994; Culman *et al*., 1995) or in the anaesthetized rat (Takano *et al*., 1990). These cardiovascular effects were attributed to a rise in plasma vasopressin level since they were blocked by an intravenous vasopressin V$_1$ receptor antagonist (Takano *et al*., 1990; 1993). Moreover, central tachykinin NK$_3$ receptor agonists could directly stimulate the release of vasopressin via the activation of NK$_3$ receptors in the magnocellular part of the hypothalamus (Massi *et al*., 1991). In agreement with these studies, we also found in the present study that the prior i.v. injection of a selective vasopressin V$_2$ receptor antagonist (50 μg kg^{-1}) blocked the antidiuresis and antinatriuresis induced by i.c.v. [MePhe^7]NKB and i.v. vasopressin. The greater diuresis induced by the vasopressin V$_2$ receptor antagonist is probably due to its inhibition of the tonic action of vasopressin. Since the vasopressin V$_2$ receptor antagonist alone did not alter renal excretion of sodium, it is unlikely that the antagonist interacts with an atrial natriuretic factor (ANF) mechanism. Although the possibility that the vasopressin V$_2$ receptor antagonist crosses the blood-brain barrier cannot be completely excluded, it seems to be unlikely that the antagonist acts centrally, since there is no evidence that vasopressin V$_2$ receptors are present in the central nervous system. Moreover, it is well known that vasopressin produces renal effects through the renal V$_2$ receptor. Finally, peptide antagonists generally do not readily cross the blood-brain barrier.

Vasopressin is a well known antidiuretic hormone which can also stimulate sodium reabsorption by the thick ascending tubule and the distal tubule/collecting duct (Koeppen & Stanton, 1992; Li & Smyth, 1993). A decrease in renal excretion of potassium may reflect a greater magnitude of antidiuresis, since the action of vasopressin on renal excretion of potassium depends on water balance when vasopressin itself is able to stimulate the secretion of potassium in the distal tubule (Koeppen & Stanton, 1992). Although the involvement of the sympathetic nervous system in the central effects of [MePhe^7]NKB cannot be completely excluded, the lack of effects of SP and NKA, two potent central activators of the sympathetic nervous system via NK$_1$ and NK$_2$ receptors, makes this possibility unlikely.

Conclusion

Whereas the i.c.v. injection of SP or NKA failed to elicit changes in renal excretion of water, sodium and potassium, [MePhe^7]NKB produced dose-dependent reductions in renal excretion of water, sodium and potassium. The renal responses to [MePhe^7]NKB were abolished specifically by the prior i.c.v. administration of R 820, but not by RP 67580 and SR 48968. Moreover, the supraspinal effects of [MePhe^7]NKB on renal excretion were abolished by the prior i.v. administration of a vasopressin V$_2$ receptor antagonist. These results suggest that hypothalamic NK$_3$ receptors are involved in the renal control of water and electrolyte excretion through the release of vasopressin in the conscious saline-loaded rat.

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