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Negative modulation of nitric oxide production by neurotensin as a putative mechanism of the diuretic action of SR 48692 in rats

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1 We investigated the effect of the non-peptide neurotensin (NT) antagonist SR 48692 on renal function in rats and the involvement of nitric oxide (NO) in the diuretic action of this compound.

2 In fed animals, SR 48692 dose-dependently (0.5 to 12.5 mg kg^{-1}, p.o., 0.03 to 1 mg kg^{-1}, i.p. and 0.1 to 1 μg/rat, i.c.v.) increased urine output and urinary excretion of Na^+, K^+ and Cl^- and reduced urine osmolality. The diuretic activity was also evident in water-deprived, fasted animals and in fasted, water-loaded rats.

3 NT (0.1 μg/rat, i.c.v.) had no effect on urine output in fed rats, but reduced the diuretic action of SR 48692 (1 μg/rat, i.c.v.). The opposite result was obtained in fasted, water-loaded animals: NT dose-dependently (0.01 and 0.1 μg/rat, i.c.v.) inhibited diuresis and this effect was significantly inhibited by i.c.v. SR 48692. In this experimental condition, SR 48692 did not further increase the on-going diuresis.

4 The NO synthesis inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME; 30 mg kg^{-1}, i.p.) alone had no effect on urine output in fed rats but prevented the diuretic action of i.c.v. or i.p. SR 48692; L-arginine (1 g kg^{-1}, i.p.) but not D-arginine (1 g kg^{-1}, i.p.) restored the SR 48692-dependent increase in diuresis. L-NAME had no effect on furosemide-stimulated diuresis.

5 Systemically administered L-NAME or i.c.v. NT in fasted, water-loaded rats significantly reduced water diuresis but this effect was no longer seen in animals given i.p. L-arginine. Rats receiving i.c.v. NT, whose diuresis was significantly reduced, also excreted less nitrates and nitrites in urine.

6 Increased diuresis after central or systemic administration of SR 48692 to fed rats was paralleled by increased urinary excretion of nitrates and nitrites, this being consistent with peripheral enhancement of NO production after NT-receptor blockade by SR 48692. The increase in diuresis after furosemide also involved an increase of nitrates and nitrites in urine, but this effect was about half that attained with an equipotent diuretic dose of SR 48692.

7 In fed rats, the NO donor isosorbide-dinitrate, reduced systolic blood pressure (unlike SR 48692 which did not affect blood pressure) but also dose-dependently (1 and 5 mg kg^{-1}, i.p.) stimulated urine output.

8 The overall effects of SR 48692 strongly support a link between the actions of endogenous NT, AVP and peripheral NO production in the modulation of renal excretion of water, Na^+, K^+ and Cl^-.

Keywords: Neurotensin; nitric oxide; N^{ω}-nitro-L-arginine methyl ester (L-NAME); furosemide; SR 48692; diuresis; isosorbide-dinitrate; arginine-vasopressin

Introduction

Neurotensin (NT) is a tridecapeptide widely distributed in the central nervous system and intestine of several species. It acts as a paracrine and endocrine modulator of digestive functions in the gut (Ferris, 1989). It is located in specific glandular cells, called the N cells, and may be released into the blood after food ingestion, thus influencing not only the digestive system (Mashford *et al*., 1978; Blackburn *et al*., 1980; Croci *et al*., 1995a), but presumably the cardiovascular system and renal functions too (Quirion *et al*., 1982; Bloom *et al*., 1983; Unwin *et al*., 1987; Kivlighn & Jandhyala, 1990; Nisato *et al*., 1994). NT has antidiuretic action after i.v. infusion in several animal species. Arginine-vasopressin (AVP), aldosterone and atrial natriuretic peptide are all believed to take part in the inhibition of salt and urine excretion by NT (Lesniewska *et al*., 1992; Mazzocchi *et al*., 1993; Masilamani *et al*., 1994). In addition, a neuroendocrine function as a modulator of the hypothalamic-pituitary-adrenal axis has been attributed to NT, since central or systemic administration raises blood levels of adrenocorticotrophic hormone (ACTH) and corticosterone (Lesniewska *et al*., 1992; Nussdorfer *et al*., 1992; Malendowicz & Nussdorfer, 1994). Finally, NT influences brain dopaminergic neurones which are involved in body fluid homeostasis (Azzi *et al*., 1994; Brouard *et al*., 1994; Steinberg *et al*., 1995).

The recent availability of potent, selective and biologically stable NT receptor antagonists is providing an unprecedented opportunity to clarify its multiple physiological roles. We described the biochemical and pharmacological properties of the first non-peptide NT receptor antagonist, SR 48692, (2-{[1-(7-chloroquinolin-4-yl)-5-(2,6-dimethoxyphenyl)-1H-pyrazole-3-carbonyl] amino}-adamantane-2-carboxylic acid), (Gully *et al*., 1993; Maffrand *et al*., 1993). The present study was designed to investigate the effects of SR 48692 on renal excretory functions in rats, with a view to clarifying the involvement of endogenous NT and possibly related mechanisms in the diuretic action of the drug. A preliminary account of this work has been presented previously (Experimental Biology 96, Washington DC, U.S.A., April 1996; Croci *et al*., 1996).

Methods

Male Crl:CDBR rats (200–250 g) handled according to internationally accepted principles for care of laboratory animals (E.E.C. Council Directive 86/609, OJL358, 1, Dec. 12, 1987)

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were used. The rats were kept under controlled environmental conditions (22±1°C, 55±15% relative humidity and 12 h light, 6 h 30 min to 18 h 30 min) and had free access to water and food (4RF21, standard chow, Mucedola, Italy).

One week before the experiments requiring intracerebroventricular (i.c.v.) treatment, rats were anaesthetized with pentobarbitone (40 mg kg^{-1}, i.p.), placed in a Kopf stereotaxic instrument and implanted with a polyethylene cannula in the right lateral ventricle according to a previously described method (Croci *et al*., 1995b).

Diuresis and urine assays

Rats were housed individually in metabolic cages (Tecniplast, Gazzada, Italy) without food and water, 2 h before the start of the experiment (10 h 00 min).

Diuresis was assessed under four different feeding and hydration conditions. During the 22 h before the experiment animals were: (i) allowed free access to water and food (fed); (ii) freely feeding, but given no water (water-deprived); (iii) freely drinking, but given no food (fasted); (iv) deprived of food, freely drinking and given an additional water load (fasted, water-loaded). In the last experimental condition, the animals were given 30 ml kg^{-1} bidistilled water by gavage 15 min before the urine collection was started.

Urine was always collected immediately after the dose of SR 48692 or furosemide or the drug under investigation. Urinary electrolytes (K^+, Na^+, Cl^-) were assayed by a SPOTCHEM ISE-PLATE electrolyte system (Daiichi Co. Ltd, Kyoto, Japan) after centrifugation of the urine (3000 RPM; Beckman TJ-6) and appropriate dilution (Worth, 1988; Watanabe, 1988). Urine osmolality was measured by use of freezing-point depression osmometry (OM-6030 AUTO STAT; Daiichi Co. Ltd, Kyoto, Japan). Nitrate plus nitrite concentration was measured with the Cayman's nitrate/nitrite assay kit (Alexis Co, Switzerland, purchased from Inalco S.p.A., Milan, Italy) in a simple two-step process. The first step is the conversion of nitrate to nitrite by nitrate reductase. The second step is the addition of Griess reagents which convert nitrite to a deep purple azo-compound that was read at 540 nm by a Multiskan-Bichromatic photometer (Labsystems, Helsinki, Finland).

Blood pressure, heart rate measurements

Blood pressure and heart rate of rats were recorded indirectly by an 8002 recorder (Basile, Milan, Italy), with an air sphygmomanometer, equipped with a piezoelectric ring transducer to measure pulse, connected to the tail. The lateral counterpressure value, corresponding to disappearance of the pressure signal, indicated systolic blood pressure. Pulse rate was expressed in beats per minute. The rats must be slightly warmed for monitoring the cardiovascular endpoints, so they were examined at 35°C in a thermostatic-ventilated chamber. The animals were accustomed to blood pressure recordings and manipulations for three days before the test. After baseline recordings, the rats were treated with the test drug or vehicle and changes were assessed after 1, 3 and 6 h until at least two consecutive constant values were obtained.

Treatment

Rats were treated according to the protocol described in the figures and tables. In the i.c.v. experiment, a solution containing both NT and SR 48692 was prepared immediately before injection. Isosorbide-dinitrate was given i.p. in saline 4 ml kg^{-1}. The other compounds were given in 2 ml kg^{-1} (p.o., i.p.) or 10 μl/rat (i.c.v.) of appropriate vehicles. Dexamethasone was given s.c. dissolved in 5% acetone and corn oil. NT, furosemide, N^{ω}-nitro-L-arginine methyl ester (L-NAME), L- and D-arginine were dissolved in saline; SR 48692 was given either orally suspended in low-viscosity (22 mPa s^{-1} at 25°C) 0.5% Na-carboxymethyl-cellulose (CMC) or i.p. and i.c.v., prepared as follows: 1 ml propylene glycol then 0.2 ml NaOH (1 N) were added to 2.5 mg SR 48692; after complete dissolution, bidistilled water (i.p.) or saline (i.c.v.) was added and the pH was adjusted to 7.4–7.6 with HCl (1 N). Vehicle was prepared by the same procedure, except for the absence of SR 48692. Drug-free control rats received only vehicle.

Chemicals

SR 48692 was synthetized at the Sanofi Research Center in Montpellier (France). The following chemicals were purchased from commercial sources as indicated: Novabiochem (Lanfelfingen, Switzerland): neurotensin; Sigma-Aldrich Corp. (St Louis, MO, U.S.A.): furosemide, N^{ω}-nitro-L-arginine methyl ester, isosorbide-dinitrate, betamethasone, dexamethasone, L- and D-arginine; Prodotti Gianni (Milan, Italy): low-viscosity Na-carboxymethyl-cellulose of alimentary grade, E 466.

Statistical analysis

Results are expressed as mean±s.e.mean. Means were compared by completely randomized one-way analysis of variance (ANOVA) followed by Duncan's (Kramer, 1956) test for multiple comparisons with RS/1 software. A probability of less than 0.05 was considered statistically significant.

Results

Effect of food and hydration on the diuretic action of systemically administered SR 48692

Table 1 shows the diuretic action of oral SR 48692 at its peak effect (3 h) under the four feeding and hydration conditions. SR 48692 increased urine output dose-dependently (0.5 to 12.5 mg kg^{-1}) in all these conditions, including in fasted, water-loaded animals. SR 48692 significantly increased loss of urine osmolytes (volume × osmolality) in fed and fasted rats and, importantly, in fasted, water-loaded rats. Similar results were obtained after 6 h cumulative urine collection (data not shown).

Table 1 Diuretic effect of oral SR 48692 under different feeding and hydration conditions[a]

	Urine							
	Fed		*Fasted*		*Water-deprived*		*Fasted water-loaded*	
	(ml 3 h^{-1}/rat)	(mosmol kg^{-1})	(ml 3 h^{-1}/rat)	(mosmol kg^{-1})	(ml 3 h^{-1}/rat)	(mosmol kg^{-1})	(ml 3 h^{-1}/rat)	(mosmol kg^{-1})
Control –	1.3±0.2	868±158	1.0±0.2	606±181	0.10±0.10	NE[b]	6.1±0.3	169±3
SR 48692 (mg kg^{-1}, p.o.)								
0.5	1.6±0.1	686±131	1.0±0.2	490±53	0±0	NE	7.8±0.6	143±17
2.5	4.0±0.3**	582±104	2.3±0.1*●	261±46**	0.67±0.17*	1280±93	9.3±0.5**	164±19
12.5	6.5±0.7**	383±45**	4.0±0.6**●	340±58*	2.50±0.30**	581±58○○	13.3±0.1**	140±19

Figures are mean±s.e.mean of 6 rats. [a]For details see Methods. [b]NE, not evaluable: two samples > 1500 mosmol kg^{-1}. *P<0.05, **P<0.01 vs control; ○○P<0.01 vs SR 48692 2.5 mg kg^{-1}, ●P<0.05 vs fed rats (Duncan's test)

Table 2 Electrolyte excretion and diuretic effect of oral furosemide and SR 48692 in fed rats

		Urine			
		Volume (ml 6 h^{-1}/rat)	Na^+	K^+ (mEq 6 h^{-1}/rat)	Cl^-
Control	–	1.7±0.2	0.10±0.01	0.30±0.02	0.44±0.02
	(mg kg^{-1}, p.o.)				
Furosemide	20	9.6±0.9**	1.77±0.19**	1.21±0.29**	2.65±0.25**
SR 48692	0.5	3.5±0.1**	0.31±0.05**	0.61±0.05**	0.88±0.06**
	2.5	5.3±0.2**	0.32±0.02**	0.81±0.08**	1.20±0.11**
	12.5	8.1±0.5**	0.53±0.05**	1.21±0.09**	1.73±0.17**

Data are mean±s.e.mean of 5 rats. **$P<0.01$ vs control (Duncan's test)

NT (0.1 to 10 $\mu g\,kg^{-1}$, i.p.) had no effect on urine output in either fed or fasted, water-loaded rats (data not shown).

Effect of SR 48692 on urinary electrolytes

The effect of SR 48692 and furosemide, used as reference compound, on urine electrolytes (Na^+, K^+, Cl^-) was investigated in fed rats (Table 2). Both drugs significantly increased the excretion of electrolytes without significantly changing urinary pH (range of mean values 7.1 to 7.4; data not shown in the table). SR 48692, unlike furosemide, had no effect on the $[Na^+]$:$[K^+]$ ratio.

Effect of repeated treatment with SR 48692 on diuresis

Seven days of oral treatment of fed rats with SR 48692 (12.5 mg kg^{-1} per day^{-1}) produced a virtually constant diuretic action, the difference from control values (ml 6 h^{-1}/rat 2.9±0.3 (s.e.mean), $n=14$) being similar on the first and last days of treatment (10.2±1 and 8.7±0.8, $n=7$).

Effects of centrally administered NT and SR 48692

Figure 1 shows the effects of i.c.v. NT or SR 48692 in fasted water-loaded and fed rats. In the first condition (Figure 1a), NT dose-dependently (0.01 and 0.1 μg/rat) and significantly inhibited diuresis (1 and 2 h cumulative urine collection); this effect was antagonized by SR 48692 (1 μg/rat) which by itself had no real effect on urine output. In fed rats (Figure 1b), SR 48692 dose-dependently (0.1 and 1 μg/rat) and significantly increased urine output after 1, 2 and 3 h; this effect was partially reduced by NT at 1 h ($P<0.01$) (0.1 μg/rat). NT had no antidiuretic action when given alone.

Effects of SR 48692, dexamethasone, furosemide, L-NAME, D- and L-argine on urine output

As shown in Table 3, L-NAME (30 mg kg^{-1}, i.p.) significantly reduced water diuresis in fasted, water-loaded rats by 45 to 52%; this effect was inhibited by L-arginine (1 g kg^{-1}, i.p.) which significantly increased urine output in this experimental condition.

In fed rats, L-NAME (Figure 2a and b) did not affect urine output, although it prevented the diuretic action of either systemic (i.p.) or central (i.c.v.) SR 48692; L-arginine but not D-arginine fully prevented this inhibitory action. L-NAME had no effect on furosemide-induced diuresis.

Dexamethasone (2.5 mg kg^{-1}, s.c. 24 h before the test in fed rats) had no effect on diuresis induced by SR 48692 (0.3 mg kg^{-1}, i.p.) (cumulative urine output, ml 6 h^{-1}/rat, mean±s.e.mean, $n=3$, drug-free control 3.3±0.5; SR 48692 8.7±0.5; dexamethasone plus SR 48692 9.8±0.9). A similar result was obtained with betamethasone (2 mg kg^{-1}, s.c. given twice, 24 h and 12 h before the test; data not shown).

In fasted, water-loaded rats, the antidiuretic effect of centrally administered NT (0.1 μg/rat) was also completely prevented by systemic (i.p.) L-arginine (1 g kg^{-1} 120 min before NT) but not by D-arginine. However, D-arginine, like NT, significantly reduced water diuresis (cumulative urine output, ml 2 h^{-1}/rat, mean±s.e.mean, $n=6$: drug-free control 7.3±0.4; L-arginine 7.0±0.7; D-arginine 3±0.1; NT 2.1±0.1;

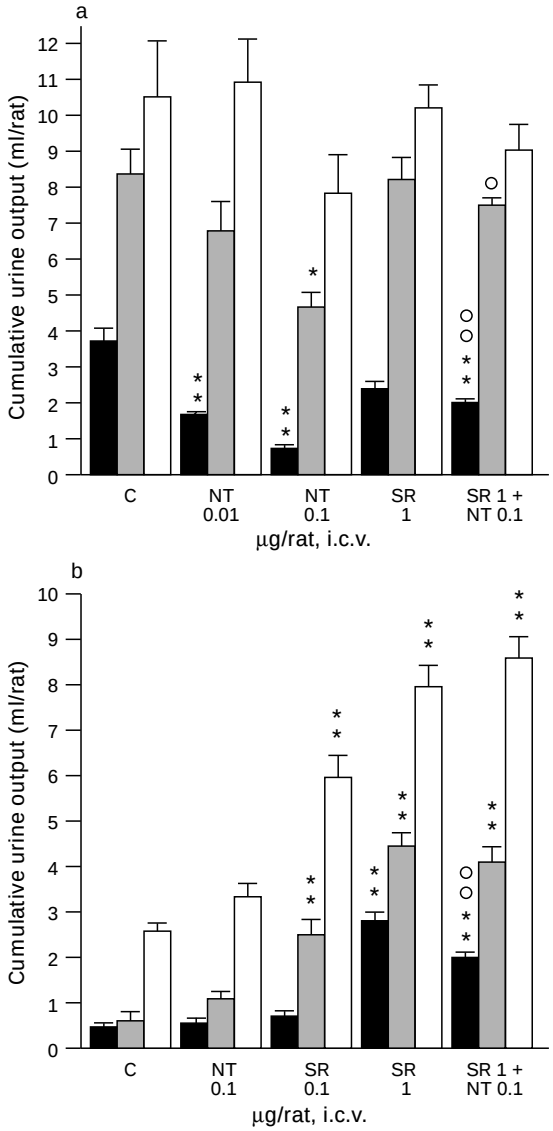

Figure 1 Effect of i.c.v. neurotensin (NT) and SR 48692 (SR) on urine output in 24 h-fasted, water-loaded (a) and fed (b) rats. Controls (C) received only vehicle (see Methods for details). Rats were placed in metabolic cages immediately after NT (0.01, 0.1 μg/rat) and/or SR 48692 (0.1, 1 μg/rat) and urine was collected cumulatively for three hours (solid columns 1 h, cross-hatched columns 2 h, open columns 3 h). Data are mean±s.e.mean of 4 to 10 rats. *$P<0.05$, **$P<0.01$ vs control; $^{\circ}P<0.05$, $^{\circ\circ}P<0.01$, vs NT (Duncan's test).

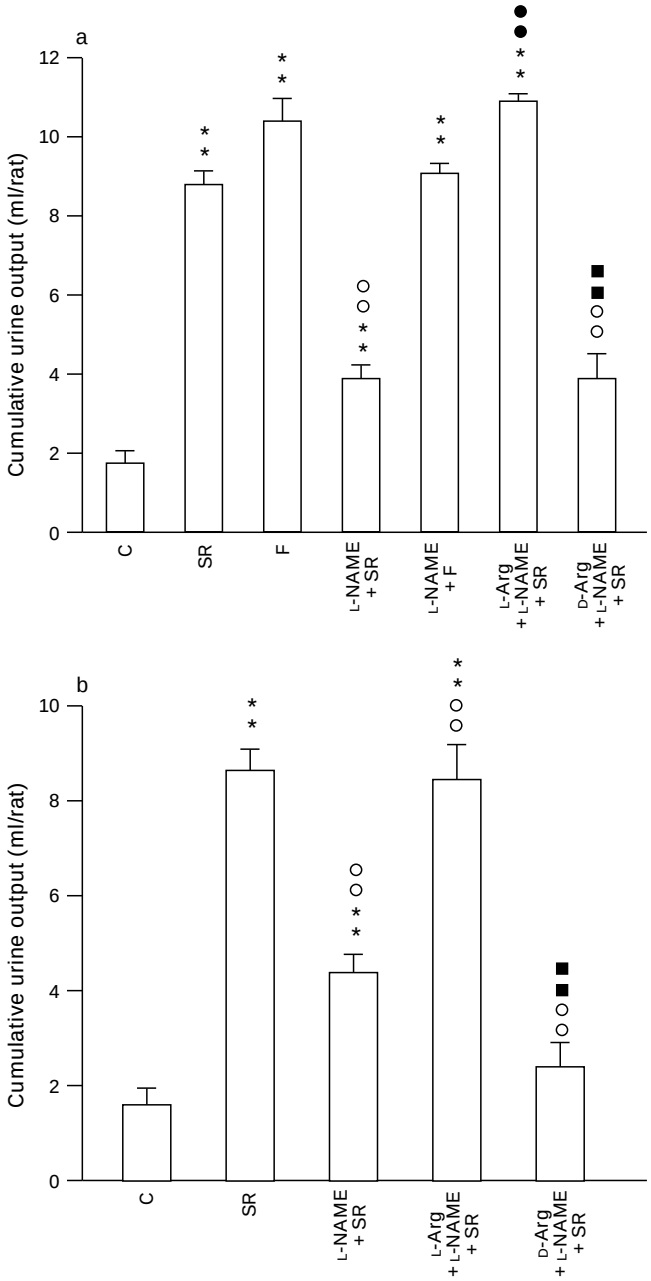

Figure 2 L-NAME (30 mg kg^{-1}, i.p.) antagonism of diuresis induced by SR 48692 (SR) (i.p. (a) or i.c.v. (b)) or furosemide (F) (i.p. (a)) in fed rats: prevention by L- but not D-arginine. (a) L-NAME was given 15 min before SR 48692 (0.3 mg kg^{-1}, i.p.) or furosemide (2 mg kg^{-1}, i.p.) and L- and D-arginine (Arg, 1 g kg^{-1}, i.p.) were given immediately before L-NAME. (b) L-NAME was given immediately before i.c.v. SR 48692 (1 μg/rat) and L- and D-arginine 15 min before L-NAME. L-NAME, L- and D-arginine alone had no significant effects on urine output (ml 3 h^{-1} rat, mean ± s.e.mean, 2.3 ± 0.3, 2.7 ± 0.3 and 2.4 ± 0.2). Data are mean ± s.e.mean of 3 to 6 rats. $^{**}P<0.01$ vs control (C); $^{\circ\circ}P<0.01$ SR 48692 or furosemide alone; $^{\bullet\bullet}P<0.01$ vs L-NAME + SR 48692, $^{\blacksquare\blacksquare}P<0.01$ vs L-arginine + L-NAME + SR 48692 (Duncan's test).

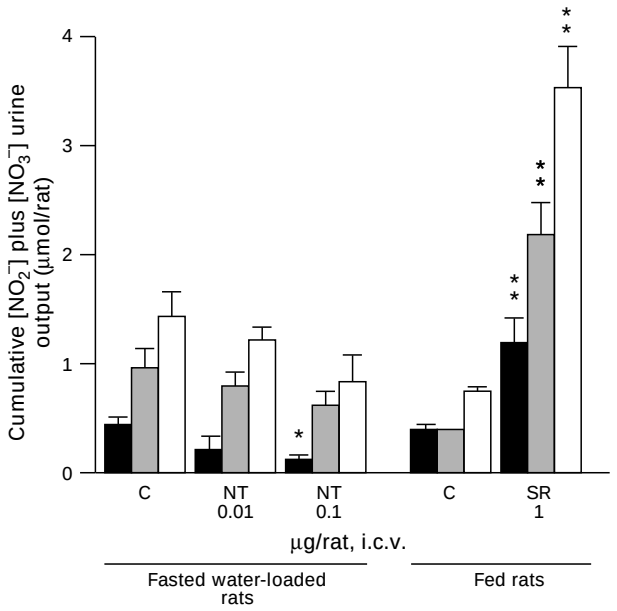

Figure 3 Effects of i.c.v. neurotensin (NT) and SR 48692 (SR) on total nitrates and nitrites recovered in urine of 24 h-fasted, water-loaded and fed rats (same experiment as Figure 1). Rats were placed in metabolic cages immediately after NT (0.01, 0.1 μg/rat) or SR 48692 (1 μg/rat) and urine was collected cumulatively for three hours (solid columns 1 h, cross-hatched columns 2 h, open columns 3 h). Data are mean ± s.e.mean of 4 to 10 rats. $^{*}P<0.05$, $^{**}P<0.01$ vs control (C) (Duncan's test).

Table 3 L-NAME antagonism of water diuresis and its prevention by L-arginine in fasted water-loaded rats[a]

	Urine output (ml 2 h^{-1}/rat)	(ml 4 h^{-1}/rat)
Control	6.43 ± 0.24	7.08 ± 0.23
L-Arginine	8.57 ± 1.23	12.8 ± 2.26**
L-NAME	3.12 ± 0.23**	3.90 ± 0.38**
L-Arginine + L-NAME	6.57 ± 0.29$^{\bullet\bullet}$	10.7 ± 0.60**$^{\bullet\bullet}$

Data are mean ± s.e.mean of 3 to 6 rats. [a]For details see Methods. L-Arginine (1 g kg^{-1}, i.p.) was given immediately before L-NAME (30 mg kg^{-1}, i.p.). $^{**}P<0.01$ vs control; $^{\bullet\bullet}P<0.01$ vs L-NAME alone (Duncan's test).

L-arginine plus NT 6.3 ± 0.1; D-arginine plus NT 3.1 ± 0.1; $P<0.01$ vs drug-free control, D-arginine, NT and D-arginine plus NT).

Effects of SR 48692 and NT on urinary nitrate and nitrite excretion

In fed rats, SR 48692 (1 μg/rat, i.c.v.) increased urinary excretion of nitrates and nitrites concurrently with its diuretic action (Figure 3). In fasted, water-loaded rats centrally administered NT (0.1 μg/rat), produced antidiuresis and significantly ($P<0.05$) reduced the amount of nitrates and nitrites recovered in urine (Figure 3).

As shown in Figure 4, in fed animals SR 48692 (0.1, 0.3 mg kg^{-1}, i.p.) dose-dependently increased the urinary excretion of nitrates and nitrites, whilst also increasing urine volumes. However, furosemide at an equipotent diuretic dose (3 mg kg^{-1}, i.p.), increased the excretion of nitrates and nitrites to levels less than SR 48692.

Diuretic and cardiovascular effects of SR 48692 and isosorbide-dinitrate in fed animals

The diuretic and cardiovascular effects of the NO donor isosorbide dinitrate (1 and 5 mg kg^{-1}) were compared with SR 48692 (0.03 to 1 mg kg^{-1}). Both dose-dependently increased urine output in fed rats after i.p. administration: cumulative urine output, ml 6 h^{-1}/rat, mean ± s.e.mean, $n=4$ to 6: drug-free control 2.5 ± 0.1; isosorbide-dinitrate 1 and 5 mg kg^{-1} 6.8 ± 0.25 and 11.2 ± 0.05, respectively; SR 48692 0.03, 0.1, 0.3 and 1 mg kg^{-1}, 4.4 ± 0.1, 6.5 ± 0.2, 8.3 ± 0.1 and 12.7 ± 0.16, respectively; $P<0.01$ vs drug-free control, all doses.

Isosorbide-dinitrate, but not SR 48692, significantly (at 3 and 6 h, $P<0.05$) lowered systolic blood pressure (mmHg,

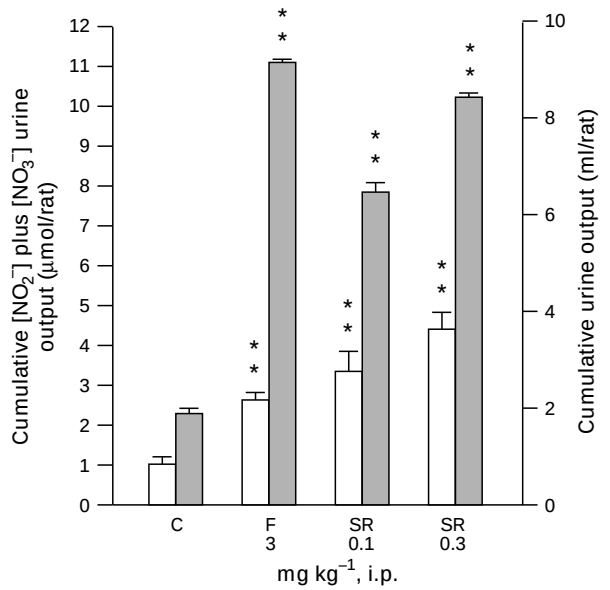

Figure 4 Effects of i.p. SR 48692 (SR) and furosemide (F) on total nitrates and nitrites recovered in urine of fed rats. Animals were placed in metabolic cages immediately after SR 48692 (0.1, 0.3 mg kg^{-1}) or furosemide (3 mg kg^{-1}) and urine was collected cumulatively for six hours. Nitrate plus nitrite concentration (open columns) and corresponding urine volumes (cross-hatched columns). Data are mean ± s.e.mean of 4 to 9 animals. $^{**}P<0.01$ vs control (C) (Duncan's test).

mean ± s.e.mean, $n=6$ to 12): basal, 142 ± 1; at 1, 3 and 6 h after 5 mg kg^{-1} isosorbide-dinitrate, 142 ± 4, 128 ± 6 and 125 ± 7; after 1 mg kg^{-1} SR 48692, 139 ± 2, 145 ± 3 and 141 ± 2. Neither compound affected heart rate (data not shown).

Discussion

In the present study we showed that a selective non-peptide NT-receptor antagonist, SR 48692, potently and dose-dependently induced diuresis in rats. SR 48692 had similar diuretic action after systemic (i.p., p.o.) and central (i.c.v.) administration; it also increased sodium, potassium and chloride excretion. The increase in urine volume after SR 48692 (i.p., p.o.) was comparable to that induced by furosemide which, like the NT-antagonist, also significantly increased electrolyte excretion. Our study suggests that endogenous NT is involved in the diuretic action of SR 48692. This was seen at doses within the range of those effective in other *in vivo* functional animal tests (Gully *et al*., 1993; Vita *et al*., 1993; Dubuc *et al*., 1994; Nisato *et al*., 1994; Brun *et al*., 1995) in which selective NT-receptor antagonism was used.

NT has an antidiuretic action after i.v. infusion in several animal species (Unwin *et al*., 1987; Kivlighn & Jandhyala, 1990) and our findings showing inhibition of diuresis in fasted, water-loaded rats given i.c.v. NT further support this.

We tested the diuretic action of SR 48692 in rats under different feeding and hydration conditions. SR 48692 (p.o.) substantially increased urine output, whilst lowering urine osmolality, regardless of whether animals were fed or fasted, or had been water-deprived. Since water deprivation is a natural stimulus that raises arginine vasopressin (AVP) levels (Leander *et al*., 1987), AVP may be involved in the mechanism of SR 48692 diuretic action. Current views also suggest that NT and AVP have a marked aldosterone secretagogue effect, and that NT is antidiuretic through stimulation of AVP release (Mazzocchi *et al*., 1993). In fasted, water-loaded animals in which AVP levels are presumably very low (Leander *et al*., 1987), systemically administered SR 48692 had a significant diuretic effect. In this experimental condition, total osmolar urinary excretion increased, so an AVP-independent mechanism is likely to contribute to the increased salt and water loss seen with SR 48692.

We investigated the role of central NT-receptors in SR 48692-induced diuresis by administering it to rats with chronically implanted cannulae for intracerebroventricular (i.c.v.) injection. In fasted, water-loaded animals, SR 48692 had no effect on urine output but inhibited NT antidiuresis. The opposite effect was seen in fed rats, where NT significantly reduced the diuretic action of SR 48692 at 1 h though by itself it had no effect on diuresis.

Since i.c.v. NT is antidiuretic only in fasted, water-loaded rats both feeding and hydration may affect the responsiveness of NT receptors in the CNS. Feeding raises plasma NT levels in different animal species and in man (Blackburn *et al*., 1980). Our results suggest that central NT-receptors in fed rats, highly activated because of raised NT, may be refractory to further stimulation by exogenously administered NT that thereby fails to reduce diuresis, whereas in this condition susceptibility to the diuretic action of SR 48692 remains. Conversely, in fasted, water-loaded rats, low NT receptor activation means they are highly susceptible to antidiuresis by the agonist (NT), but not to the diuretic effect of the NT-antagonist (SR 48692).

The overall results point to NT receptors in the CNS as an important target of the diuretic action of SR 48692. SR 48692 easily crosses the blood/brain barrier after systemic administration (Brun *et al*., 1995). However, peripheral actions are likely since NT receptors have been found in the rat kidney too (Quirion *et al*., 1992). In any case the mechanism of the diuretic action of SR 48692, although most probably inherent in its ability to block NT receptors, apparently encompasses a peripheral component involving NO synthesis and release. In fed rats, the specific inhibitor of NO synthesis, L-NAME, given i.p., substantially prevented the increase in urine output induced by either i.c.v. or i.p. SR 48692, and the simultaneous administration of the NO precursor L-arginine, but not D-arginine, restored it. L-NAME had no effect on the diuretic action of furosemide and in fed rats essentially no intrinsic effect on urine output. However, in fasted, water-loaded animals it was antidiuretic. These findings suggest that NO-synthetase is also activated in water diuresis. Indeed, D-arginine, like i.c.v. NT, significantly reduced water diuresis, suggesting that this unnatural amino acid competes with the endogenous pool of L-arginine.

Further support for a link between central NT receptors, peripheral NO production and diuresis is given by the finding that: (a) in fasted, water-loaded rats the antidiuretic action of i.c.v. NT was completely prevented by systemic L-arginine but not D-arginine; (b) in the same experimental condition, less nitrates and nitrites were recovered in urine when diuresis was reduced by i.c.v. NT; (c) in fed animals both central and systemic SR 48692 increased urine output and more nitrates and nitrites were recovered in urine, whereas an equipotent diuretic dose of furosemide had less effect than SR 48692 on urinary recovery of nitrates and nitrites; (d) systemic administration of the NO donor isosorbide-dinitrate dose-dependently induced diuresis.

Altogether, our findings are consistent with the view that NT in the CNS may act as a negative modulator of the peripheral release of NO, mediated through a complex pathway in which AVP might also play a role. Should this be the case, NT neuroendocrine functions might also be involved, operating as a link between the CNS and renal excretory functions.

A mechanism different from that underlying stimulation of urine output by SR 48692 apparently applies to the diuretic action of furosemide, which was much less influenced by the production of NO, as shown by the smaller amount of nitrates and nitrites excreted in urine and the inability of L-NAME to prevent increased diuresis. However, enhancement of endothelial synthesis and release of NO is only one of the factors contributing the haemodynamic and diuretic actions of furosemide (Greger, 1985; Wiemer, 1994). We found that the NO

donor isosorbide-dinitrate induced diuresis and slightly lowered systemic blood pressure, presumably through the slow release of NO. But SR 48692, although diuretic, did not cause hypotension. This appears consistent with enhanced NO synthesis – possibly through activation of the constitutive enzyme isoform, since there was no antagonism by dexamethasone (Moncada & Higgs, 1995) – only within the kidney, not throughout the systemic vascular system. There is increasing evidence that endothelium-derived NO is tonically synthetized in the kidney and that it is vital to the regulation of renal haemodynamics and excretory function (Baylis *et al*., 1990; De Nicola *et al*., 1992; Radermacher *et al*., 1992; Bachmann & Mundel, 1994).

In summary, we have shown for the first time that SR 48692 promotes diuresis in rats presumably through a NT receptor-mediated mechanism. NO is apparently also involved both in water diuresis and in the diuretic action of SR 48692, and central NT receptors may modulate the renal production of NO. Finally our results indicate that in rats repeated daily dosing with SR 48692 does not entail any loss of its diuretic effect.

The clinical relevance of these findings in rats, and of the similar effect of SR 48692 in guinea-pigs (unpublished data, Croci *et al*.) remains to be established, but NT receptor antagonists may represent a new class of diuretics with a distinct mechanism of aciton.

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