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Role of intrathecal tachykinins for micturition in unanaesthetized rats with and without bladder outlet obstruction

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1 The effects on micturition of RP 67,580, a selective NK_1 receptor antagonist, and SR 48,968, a highly potent antagonist at NK_2 receptor sites, given intrathecally (i.t.) or intra-arterially (i.a.) near the bladder, were investigated in unanaesthetized rats with and without bladder outlet obstruction.

2 In normal rats, RP 67,580, given i.t. in doses of 2 and 20 nmol per rat, decreased micturition pressure, but did not change other cystometric parameters. After 20 nmol of RP 67,580, dribbling incontinence due to retention was observed in 1 out of 7 animals. This effect was reversible. I.t. RP 67,580 in a dose of 2 nmol, had no effect on hyperactivity induced by intravesically instilled capsaicin.

3 In animals with bladder hypertrophy secondary to outflow obstruction, RP 67,580, given i.t. in a dose of 2 nmol per rat, decreased the micturiton pressure, but had no effect on other cystometric parameters. After 20 nmol, dribbling incontinence due to retention was observed in 5 out of 7 animals.

4 RP 67,580, given i.a. in a dose of 4 nmol, had little effect on the cystometric parameters investigated, both in normal animals and rats with bladder hypertrophy.

5 SR 48,968, given i.t. in doses of 2 and 20 nmol per rat, had no clear-cut effects on the micturition pattern in normal rats, or rats with bladder hypertrophy. However, the drug reduced capsaicin-induced bladder hyperactivity. When given i.a. in a dose of 4 nmol, SR 48,968 had no effect on cystometric parameters in normal rats or rats with bladder hypertrophy.

6 The effects of both RP 67,580 and SR 48,968 were stereoselective, their enantiomers (RP 68,651 and SR 48,965) being inactive.

7 These results thus suggest that at the spinal level there is a tachykinin involvement (via NK_1 receptors) in the micturition reflex induced by bladder filling, both in normal rats, and, more clearly, in animals with bladder hypertrophy secondary to outflow obstruction. The bladder response to filling was not influenced by blockade of vesical NK_1 and NK_2 receptors. On the other hand, the bladder hyperactivity evoked by intravesical capsaicin seems to involve NK_2 receptors both at the bladder and spinal levels.

Keywords: Bladder outlet obstruction; unanaesthetized rat; cystometry; tachykinins; NK_1 receptor; NK_2 receptor

Introduction

Several peptides have been found in the central and peripheral nervous systems innervating the lower urinary tract. Among them, tachykinins (substance P and neurokinin A) and calcitonin gene-related peptide (CGRP) have been implicated as mediators and/or modulators of capsaicin-sensitive primary afferents (Maggi & Meli, 1986; Maggi, 1991). It is known that both tachykinins and CGRP are released from central endings of capsaicin-sensitive primary afferent neurones in the rat and cat spinal cords (Saria *et al.*, 1986; Duggan *et al.*, 1987; Go & Yaksh, 1987), and previous functional studies support the view that the capsaicin-sensitive bladder afferents are involved in regulating the micturition reflex in the rat (Maggi *et al.*, 1984; Holzer-Petsche & Lembeck, 1984). Sharkey *et al.* (1983) demonstrated with double staining technique that 10–16% of rat dorsal root ganglionic neurones, receiving afferents from the bladder, contain substance P (SP). The authors also showed that SP-positive neurones were not observed in the dorsal root ganglia of capsaicin-pretreated animals. It has been postulated that capsaicin-sensitive fibres arising from the rat bladder may use SP as excitatory neurotransmitter to activate the second neurone in the micturition reflex pathway (Maggi *et al.*, 1984).

In the rat urinary bladder, three receptor types, classified as NK_1, NK_2, and NK_3 have been demonstrated, as evidenced by radioligand binding, autoradiographic, and functional experiments (Maggi *et al.*, 1991). Both NK_1 and NK_2 receptors seem to be involved in the contractile response of the rat isolated bladder to tachykinins (Maggi *et al.*, 1987; Hall *et al.*, 1992). This seems to be the case also *in vivo*. Thus, Palea *et al.* (1993a) showed that the selective NK_1 receptor agonist GR 73632 and the selective NK_2 receptor agonist GR 64349, given intravenously, were equipotent in activating micturition reflexes in the urethane-anaesthetized rat.

Rats subjected to bladder outlet obstruction show bladder hypertrophy and 'hyperactivity' (Malmgren *et al.*, 1987) or 'premicturition contractions' (Igawa *et al.*, 1994). Even if such bladder 'hyperactivity' observed in rats with outlet obstruction and bladder hypertrophy may be of myogenic origin (Igawa *et al.*, 1994), these animals show facilitation of bladder reflex mechanisms (Steers & de Groat, 1988) and several neural changes, including hypertrophy of the dorsal root ganglionic neurones innervating the bladder (Steers *et al.*, 1991). If bladder outlet obstruction leads to an increase in the afferent input from the bladder to the dorsal gangli-

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onic neurones, and the input is conveyed, at least partly, via primary capsaicin-sensitive afferents, spinal tachykinins may have an important role for micturition in rats with outlet obstruction.

Recently, several potent and selective non-peptide antagonists of SP (NK_1) and neurokinin A (NK_2) receptors have been developed (Garret *et al.*, 1991; Emonds-Alt *et al.*, 1992; Maggi *et al.*, 1993a). These drugs, given i.t., appear to be helpful tools for assessment of the functional importance of tachykinins for modulation of micturition at the spinal level. In the present study we wanted: (1) to examine the effects of RP 67,580, which is selective for NK_1 receptors (Garret *et al.*, 1991), and SR 48,968, which is a highly potent antagonist at NK_2 receptor sites (Emonds-Alt *et al.*, 1992; Maggi *et al.*, 1993a), given intrathecally or intra-arterially near the bladder, on micturition in unanaesthetized rats with and without bladder outlet obstruction; (2) to investigate whether intrathecal administration of the antagonists influenced the bladder hyperactivity induced in normal animals by intravesical capsaicin (Ishizuka *et al.*, 1994).

Methods

Animals

Female Sprague-Dawley rats (weighing 185–245 g) with and without previous outflow obstruction were used. The experimental protocol was accepted by the Animal Ethics Committee, University of Lund.

Procedures

Outlet obstruction procedure The methods used for establishing infravesical outflow obstruction and the technique of cystometry in conscious rats have been described in detail previously (Malmgren *et al.*, 1987). Six weeks after partial ligature of the urethra, the animals were subjected to cystometrical evaluation. Within two days after removal of the ligature, when the animals of this study were investigated, the bladder still exhibited a significant degree of hypertrophy and hyperactivity (Malmgren *et al.*, 1990). For simplicity, the previously obstructed rats are referred to as rats with bladder hypertrophy.

Bladder catheter implantation Rats were anaesthetized with ketamine (75 mg kg^{-1}, i.m.) and xylazine (15 mg kg^{-1}, i.m.). Thereafter, the abdomen was opened through a midline incision, and a polyethylene catheter (Clay-Adams PE-50, NJ, U.S.A.) was implanted into the bladder through the dome as described previously (Malmgren *et al.*, 1987). In the animals subjected to outlet obstruction, the urethral ligature was removed at the same time. The catheters were tunnelled subcutaneously and orifices were made on the back of the animal. After implantation of the catheters, rats were housed individually in cages on a 12 h/12 h light/dark photo cycle.

Intrathecal catheter implantation An i.t. catheter was implanted at the same time as the bladder catheter. A polyethylene catheter (Clay-Adams PE-10, NJ, U.S.A.) was inserted into the subarachnoid space at the level of L_6-S_1 spinal cord segments for i.t. administration of drugs as described in detail previously (Igawa *et al.*, 1993). The injection sites in the spinal cord and the extent of dye distribution were confirmed by injection of dye (methylene blue) in every animal at the end of the experiment.

Intra-arterial cathether implantation The day before cystometric investigations, the rats were again anaesthetized, a femoral artery was exposed through an inguinal incision, and a polyethylene catheter (Clay-Adams PE-10, NJ, U.S.A.) filled with heparinized saline (30 iu ml^{-1}) was inserted into the vessel and advanced proximally until the tip of the catheter reached the abdominal aortic bifurcation. In order to increase the amount of drug reaching the bladder, both femoral arteries were tied. The catheter was tunnelled subcutaneously and an orifice was made on the back of the rat.

Cystometrical investigations Cystometrical investigations were performed without any anaesthesia one day and three days after the bladder catheterization in animals with bladder hypertrophy and in control animals, respectively. The bladder catheter was connected via a T-tube to a pressure transducer (P23 DC, Statham Instrument Inc., CA, U.S.A.) and an infusion pump (CMA 100, Carnegie Medicine AB, Solna, Sweden). The conscious rat was placed, without any restraint, in a metabolic cage which also enabled measurements of micturition volumes by means of a fluid collector connected to a Grass force displacement transducer (FT 03 C, Grass Instrument Co., Quincy, Mass, U.S.A.). Saline or capsaicin at room temperature were infused into the bladder at a rate of 10 ml h^{-1} and 20 ml h^{-1} in control animals and in animals with bladder hypertrophy, respectively. Intravesical pressure and micturition volumes were recorded continuously on a Grass polygraph (Model 7E, Grass Instrument Co., Quincy, Mass, U.S.A.). Three reproducible micturition cycles, corresponding to a 20 min period, were recorded before drug administration and used as baseline values. After each drug administration, recording was continued for another 60 min. In case the micturition reflex was abolished, recording was continued until it was restored. The following urodynamic parameters were investigated: micturition pressure, micturition and residual volumes, and bladder capacity (Malmgren *et al.*, 1987). Analysis was performed for a 20 min period before drug administration. Drug effects on cystometrical parameters were assessed for 60 min and the three micturition cycles showing the most pronounced changes in the parameters measured (increase or decrease) were subjected to analysis.

Administration of drugs The following drugs were used for i.t. or i.a. administration: RP 67,580 [(3a**R**, 7a**R**)-7,7-diphenyl-2-[1-imino-2(2-methoxyphenyl)ethyl] perthdroisoindol-4-one], RP68,651 (the inactive enantiomer of RP 67,580), SR 48,968 [(**S**)-N-methyl-*N*[4-(acetylamino-4-phenyl piperidino)-2-(3,4-dichlorophenyl) butyl] benzamide and SR 48,965 (the inactive enantiomer of SR 49,968). RP 67,580 and RP 68,651 were obtained from Rhone-Poulenc Rorer (Vitry, France), and SR 48,968 and SR 48,965 from Sanofi (Montpellier, France). RP 67,580 and RP 68,651 were dissolved in acidic redistilled water. SR 48,968 and SR 48,965 were dissolved in saline. The drugs were then stored at −70°C, and subsequent dilutions of the drugs were made in saline on the day of experiments.

A 100 mM stock solution of capsaicin (LabKemi, Lund, Sweden) was made in absolute ethanol and then diluted in saline, just before use, to give a concentration of 30 μM. The syringe used for saline instillation was exchanged for a syringe containing capsaicin solution, and capsaicin was instilled intravesically by a microinjection pump for a period of 28 min. With the tubing of the system used, capsaicin reached the bladder within 8 min. The doses of the drugs used were chosen on the basis of pilot experiments, and previously published data (Lecci *et al.*, 1993; Palea *et al.*, 1993a,b; Ishizuka *et al.*, 1994).

Statistical analysis

The results are given as mean values ± s.e. mean. Student's paired *t* test was used for comparison between treatments within the control group and the group with bladder hypertrophy. One way factorial ANOVA was used for comparisons between groups with regard to bladder weight, baseline

(pretreatment) cystometric parameters, and the drug effects (the difference between pre- and post-treatment in each cystometric parameter). It was followed by Scheffe's F-test. A probability level of $<5\%$ was accepted as significant.

Results

Partial obstruction of the urethra led to a significant increase in bladder weight (from 150 ± 5 mg, $n = 12$ to 930 ± 12 mg, $n = 14$; $P < 0.001$). Repeated cystometries gave reproducible results in both control animals and animals with bladder hypertrophy. In control animals, the bladder pressure was low and almost devoid of spontaneous fluctuations during the cystometry. On the other hand, cystometry in animals with bladder hypertrophy revealed spontaneous contractile activity during filling (Figure 1).

Effects of RP 67,580

Normal animals RP 67,580, given i.t. in doses of 2 nmol ($n = 5$) and 20 nmol ($n = 6$) per rat, decreased the micturition pressure significantly from 61.4 ± 9.0 to 50.7 ± 5.6 cmH_2O ($n = 5$; $P < 0.05$) from 62.1 ± 9.8 to 43.9 ± 11.3 ($n = 6$; $P < 0.001$), respectively. There was no change in other cystometric parameters (bladder capacity, micturition volume and residual volume). After 20 nmol of RP 67,580, dribbling incontinence due to retention was observed in 1 out of 7 rats. In this rat, bladder capacity before administration of RP 67,580 was 0.80 ml, and at the time of dribbling 1.70 ml. The effect was reversible.

Intravesical capsaicin, at a concentration of 30 μM, increased micturition pressure ($P < 0.001$), and decreased bladder capacity ($P < 0.001$) and micturition volume ($P < 0.001$), (Table 1). The animals showed no signs of distress and behaved normally, except for occasional licking of the lower abdomen. Intrathecal RP 67,580, 2 nmol, had no effect on capsaicin-induced hyperactivity (Table 1).

RP 67,580, given i.a. in doses of 4 nmol ($n = 7$) had no effect on the cystometric parameters investigated, except a significant ($P < 0.001$) suppression of the micturition pressure from 63.6 ± 2.9 to 55.0 ± 2.6 cmH_2O.

Animals with bladder hypertrophy RP 67,580, given i.t. in a dose of 2 nmol ($n = 6$), decreased the micturition pressure from 149.0 ± 34.1 to 134.6 ± 33.8 cmH_2O ($P < 0.05$), but had no effect on other cystometric parameters. After 20 nmol ($n = 7$), dribbling incontinence due to retention was observed in 5 out of 7 animals (Figure 2). The effect was reversible. Before administration of RP 67,580, the mean bladder capacity was 3.04 ± 0.36 ml, and at the start of dribbling, it was 5.73 ± 0.37 ml. This difference was statistically significant ($P < 0.01$).

RP 67,580, given i.a. in doses of 4 nmol ($n = 5$) had no effect on the cystometric parameters investigated.

Effects of RP 68,651

RP 68,651, the inactive enantiomer of RP 67,580, given i.t. in a dose of 20 nmol, had no effects on the micturition pattern, in normal animals ($n = 6$), or in animals with bladder hypertrophy ($n = 6$).

Effects of SR 48,968

Normal animals SR 48,968, given i.t. in a dose of 2 nmol ($n = 5$) and 20 nmol ($n = 6$) had no clear-cut effects on the micturition pattern. After 2 nmol i.t., capsaicin-induced hyperactivity was significantly reduced, but not abolished (Table 1).

When given i.a. in doses of 4 nmol ($n = 8$), SR 48,968 had no effect on the cystometric parameters.

Animals with bladder hypertrophy SR 48,968 given i.t. in a dose of 2 nmol ($n = 5$) and 20 nmol ($n = 6$) had no clear-cut effects on the micturition pattern. When given i.a. in doses of 4 nmol ($n = 5$), SR 48,968 had no effect on the cystometric parameters.

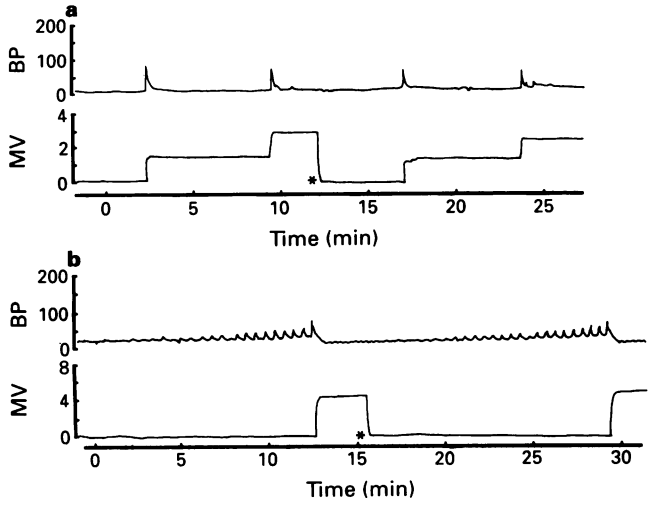

Figure 1 Micturition patterns in a normal rat (a) and in a rat with bladder outflow obstruction (b). Note the variations in bladder pressure before the micturition contractions in the rat with bladder outflow obstruction. BP = bladder pressure (cmH_2O); MV = micturition volume (ml). Asterisk (*) denotes adjustment to baseline position.

Table 1 Effect of intrathecal administration of RP 67,580 and SR 48,968 on capsaicin-induced hyperactivity in unanaesthetized, normal rats

		MP	*BC*	*MV*	*RV*
Capsaicin (30 μM)					
($n = 10$)	Before	68.0 ± 7.2	0.98 ± 0.09	0.84 ± 0.09	0.15 ± 0.01
	After	107.8 ± 10.6**	0.44 ± 0.08***	0.36 ± 0.07***	0.08 ± 0.02*
Capsaicin (30 μM) *in the presence of RP 67,580* (2 nmol) *i.t.*					
($n = 8$)	Before	68.8 ± 9.6	0.79 ± 0.04	0.72 ± 0.04	0.07 ± 0.01
	After	91.5 ± 10.6*	0.35 ± 0.06***	0.30 ± 0.06***	0.04 ± 0.01
Capsaicin (30 μM) *in the presence of SR 48,968* (2 nmol) *i.t.*					
($n = 8$)	Before	61.5 ± 8.8	0.84 ± 0.11	0.78 ± 0.11	0.10 ± 0.03
	After	75.8 ± 11.5**††	0.58 ± 0.06*†	0.53 ± 0.07*†	0.08 ± 0.03

MP: micturition pressure (cmH_2O), BC: bladder capacity (ml), MV: micturition volume (ml), RV: residual volume (ml).
Results are expressed as mean ± s.e. mean.
Before vs. after administration *$P<0.05$; **$P<0.01$; ***$P<0.001$ (Student's paired two tailed t test)
Capsaicin vs. capsaicin in the presence of RP 67,580 or SR 48,968. †$P<0.05$; ††$P<0.01$, (ANOVA followed by Scheffe's F-test).

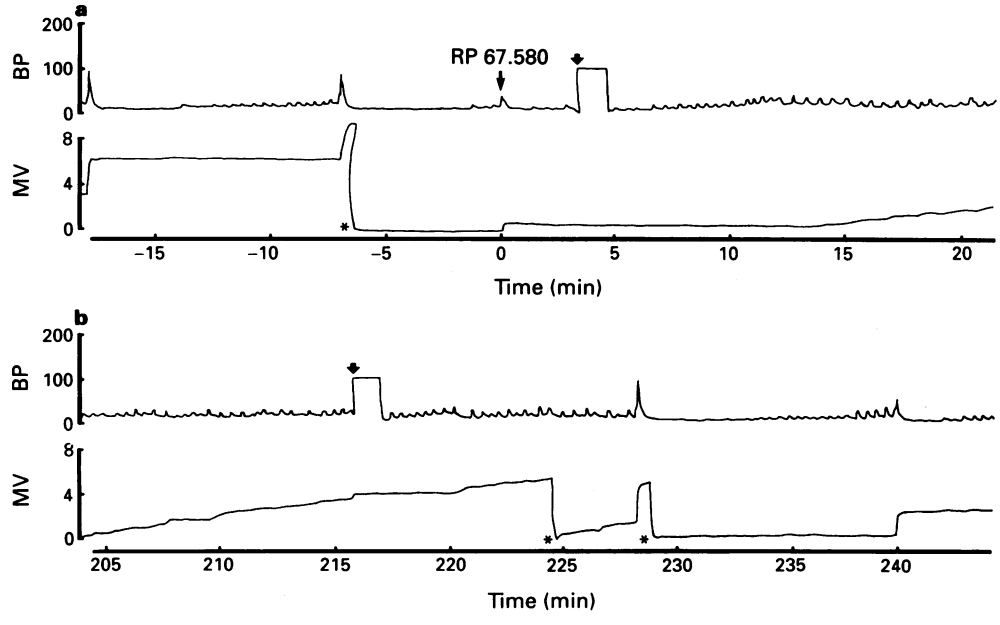

Figure 2 Effect of intrathecal RP 67,580, 20 nmol, on micturition in a rat with bladder outflow obstruction. Starting from minute 15 (a) to and lasting for more than 200 min (b), a dribbling incontinence, due to urinary retention, was induced. Micturition contractions, leading to emptying of the bladder, then reappeared. BP = bladder pressure (cmH_2O); MV = micturition volume (ml). Asterisk (*) denotes adjustment to baseline position, and short arrow (↓) change of the syringe of the infusion pump.

Effects of SR 48,965

SR 48,965, the inactive enantiomer of SR 49,968, given i.t. in a dose of 2 nmol ($n = 4$) had no effects on the capsaicin-induced hyperactivity.

Discussion

In the present investigation, we used the non-peptide tachykinin antagonists RP 67,580 (Garret *et al.*, 1991), which is a selective antagonist of NK_1 receptors, and SR 48,968, which is a potent and competitive antagonist of NK_2 receptors (Maggi *et al.*, 1993a). RP 67,580 is inactive on NK_2 and NK_3 receptors, and SR 48,968 is inactive on NK_1 and NK_3 receptors (Maggi *et al.*, 1993b). The drugs were administered i.t. to reveal whether any of these receptor subtypes at the spinal level may be involved in the micturition reflex induced by bladder filling in conscious, normal rats and/or in rats with bladder hypertrophy secondary to outflow obstruction. The doses of the drugs were selected based on our own pilot experiments, and on previously published data (Lecci *et al.*, 1993; Palea *et al.*, 1993a,b). In addition, we tested the effects of the antagonists on the bladder hyperactivity induced by intravesically administered capsaicin in normal rats (Ishizuka *et al.*, 1994).

In previous investigations performed in normal, urethane-anaesthetized animals (Lecci *et al.*, 1993), different types of micturition-related reflexes have been elicited: (1) a supraspinal chemonociceptive vesico-vesical reflex produced by topical application of capsaicin to the dome of the urinary bladder, (2) a supraspinal mechanoreceptive reflex evoked by saline filling of the urinary bladder, and (3) a spinal somatovesical mechanonociceptive reflex evoked by perineal pinching. These studies, using several NK_1 receptor selective antagonists, including RP 67,580, provided evidence for the involvement of spinal NK_1 receptors, in the activation of reflexes produced by topical application of capsaicin to the bladder, and a modulatory influence on bladder capacity elicited by distension. Magnan *et al.* (1993), studying the effects of i.t. administered NK_1 and NK_2 antagonists on xylene-induced hyperactivity in urethane-anaesthetized animals, arrived at a similar conclusion, suggesting that NK_1 receptors at a spinal level are involved in the bladder response to chemonociceptive stimuli.

Our findings in unanaesthetized rats, showed that RP 67,580, when given intrathecally to normal animals, reduced micturition pressure, and in one animal out of seven produced dribbling incontinence due to urinary retention. In this animal, the effect was reversible. Since dribbling incontinence was not produced by i.t. saline, or by RP 68,651, the inactive enantiomer of RP 67,580, the finding may be interpreted as an effect of the NK_1 antagonist. The failure of the highly potent non-peptide NK_2 receptor antagonist, SR 48,968, to affect normal micturition when given i.t., seems to exclude spinal involvement of NK_2 receptors in the micturition reflexes induced by filling. This interpretation is in line with the findings of Lecci *et al.* (1993), suggesting that spinal NK_1 receptors, at least to some extent, may be involved in the micturition reflex induced by bladder filling in normal animals, but at variance with those of Magnan *et al.* (1993), who found no evidence in support of involvement of spinal NK_1 (or NK_2) receptors in regulation of normal micturition.

In animals with bladder hypertrophy, the effect of RP 67,580, given i.t., was more convincing, producing not only a decrease in micturition pressure at a low dose (2 nmol), but also dribbling incontinence, due to urinary retention, in five out of seven animals at a high dose (20 nmol). That this in fact was due to urinary retention and not to relaxation of the outflow region, making filling of the bladder impossible, was indicated by the finding that there was an increase of the bladder volume (twice bladder capacity before drug administration) before leakage started. No effect was obtained with the inactive isomer RP 68,651, supporting the view that the urinary retention was caused by NK_1 receptor blockade. It may therefore be speculated that the changes of obstruction were associated with an increased afferent activity involving spinal NK_1 receptors. However, the present results do not exclude an additional supraspinal action of the NK_1 antagonist.

I.a. administration of the NK_1 receptor antagonists near

the bladder had no effect either in normal animals (except for a decrease in micturition pressure), or in rats with bladder hypertrophy. This suggests that afferent activity induced by bladder filling, also in rats with bladder hypertrophy, does not involve release of bladder tachykinins.

I.t. RP 67,580 had no effect on the hyperactivity induced by intravesical capsaicin. However, this activity was reduced, but not abolished by i.t. SR 48,968. It has previously been shown (Ishizuka *et al.*, 1994) that intravesical capsaicin evokes a concentration-dependent, reversible and repeatable bladder hyperactivity. This hyperactivity could be abolished by intravenous hexamethonium or i.t. administered morphine, suggesting that it was reflex-mediated and not secondary to a direct contractile effect on bladder smooth muscle. I.a. administration of SR 48,968, and the nonselective NK receptor antagonist spantide, which by themselves did not affect cystometric parameters, both counteracted this capsaicin-induced hyperactivity, whereas RP 67,580 failed to do so. Thus, it seemed that intravesical capsaicin released tachykinins from sensory nerves, and that these tachykinins via stimulation of NK_2 receptors provoked bladder hyperactivity.

Maggi *et al.* (1991) and Pietra *et al.* (1992) have previously shown that NK_2 receptors can be involved at the peripheral level in detrusor hyperactivity induced by irritants. Thus, selective antagonists of NK_2, but not antagonists of NK_1 receptors, counteracted hyperactivity associated with xylene-induced cystitis in the rat (Pietra *et al.*, 1992; Magnan *et al.*, 1993). The findings of the present study suggest that the bladder hyperactivity induced by intravesical capsaicin may involve spinal NK_2 receptors. Such receptors have been demonstrated in the dorsal horns of the rat spinal cord (Yashpal *et al.*, 1990), and have been implicated in the processing of nociceptive inputs in rats (Fleetwood-Walker *et al.*, 1990; 1991; Xu *et al.*, 1991). However, as in the case of the effects of RP 67,580, the present results do not exclude an additional supraspinal action of the NK_2 antagonist.

These results thus suggest that at the spinal level, there is a tachykinin-mediated (via NK_1 receptors) involvement in the micturition reflex induced by bladder filling, both in normal rats, and, more clearly, in animals with bladder hypertrophy secondary to outflow obstruction. The bladder response to filling was not influenced by blockage of vesical NK_1 and NK_2 receptors. The bladder hyperactivity evoked by intravesical capsaicin in normal rats, however, seems to involve NK_2 receptors both at the bladder and spinal levels.

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