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Bladder distension and activation of the efferent function of sensory fibres: similarities with the effect of capsaicin

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1 The effects of the tachykinin NK_2 receptor antagonist MEN 11420 (100 nmol kg^{-1}, i.v.) and isoprenaline (400 nmol kg^{-1}, i.v.) were compared in a model of distension-induced bladder activity in isovolumetric conditions. MEN 11420 induced a relaxation of the basal tone of the urinary bladder that was dependent on the volume of the viscus: the effect was absent at low volumes (0.2 and 0.5 ml) and it was maximal at high volumes of distension (1 and 2 ml), approaching about 60% of the isoprenaline-induced relaxation. The relaxant effect of isoprenaline was always evident at all volumes of distension.

2 Tetrodotoxin (1–100 μM, intravesically applied) abolished distension-evoked micturition contractions, but did not prevent the relaxant effect of MEN 11420- or isoprenaline on the bladder tone.

3 The cyclo-oxygenase inhibitor S-ketoprofen (0.5 μmol kg^{-1}, i.v.) produced a marked decrease of the bladder tone and a concomitant reduction of bladder motility at 1 ml volume of distension. At 2 ml of distension, S-ketoprofen still decreased the minimal pressure but had no significant effect on other parameters of vesical motility. In S-ketoprofen-pretreated rats, the relaxant effect of MEN 11420 was significant at 2 but not at 1 ml of distension, and that of isoprenaline was reduced by 50% at both 1 and 2 ml.

4 Ruthenium red (10 μmol kg^{-1}, i.v.) had no effect at a low volume of distension (0.2 ml) or at highest volume (2 ml) but decreased the basal tone and the frequency of bladder contractions at 1 ml of distension. In ruthenium red-pretreated rats, MEN 11420 failed to decrease bladder tone at 1 ml, whereas at 2 ml the effect of MEN 11420 was not different from that observed in controls (43 vs 60% of isoprenaline-induced relaxation, respectively).

5 At both 1 and 2 ml of distension, capsaicin pretreatment (164 μmol kg^{-1}, s.c. 5 days before) reduced the frequency of micturition contractions but had no effect on the bladder tone. Capsaicin pretreatment prevented the relaxant effect of MEN 11420 on the bladder tone both at 1 and at 2 ml of distension.

6 It is concluded that the release of tachykinins from capsaicin-sensitive afferent nerves induced by bladder distension is resistant to tetrodotoxin and to prostaglandin synthesis inhibition. Tachykinins modulate the vesical tone by acting through NK_2 receptors.

Keywords: Isoprenaline; MEN 11420; micturition reflex; muscle relaxation; NK_2 receptors; polymodal nociceptors; ruthenium red; S-ketoprofen; tachykinins; tetrodotoxin-resistant

Introduction

In the rat urinary bladder, neuropeptides of the tachykinin family (substance P and neurokinin A), are contained in a subset of sensory fibres which are sensitive to the stimulant and neurotoxic effect of capsaicin in adulthood (Maggi, 1993). The sensitivity of afferent fibres to capsaicin is conferred by the expression of the vanilloid (the chemical moiety of capsaicin) receptor (Szallasi, 1996), a recently cloned transmembrane protein that is able to transduce physical stimuli into electrical signals (Caterina *et al*., 1997). The unique property that characterizes tachykinin-containing fibres bearing the vanilloid receptor is their ability to exert both an afferent and an efferent response through the release of neuropeptides in the central nervous system and in the periphery (Maggi, 1993; Lecci & Maggi, 1995). Among the stimuli which are capable of activating capsaicin-sensitive afferent fibres innervating the urinary bladder, vesical distension is the one more closely related to the physiological function of the viscus. There is evidence that bladder distension stimulates the afferent function of capsaicin-sensitive nerves since: (a) pretreatment with neurotoxic doses of capsaicin increases the threshold for the activation of the distension-evoked micturition reflex in urethane-anaesthetized rats (Santicioli *et al*., 1985); (b) bladder distension (or the topical application of capsaicin) activates a sympathetic cardiovascular reflex in spinal cord-transected rats (Giuliani *et al*., 1988); (c) bladder distension (or the topical application of capsaicin) desynchronizes the electrocorticogram in urethane-anaesthetized rats (Conte *et al*., 1996). These two latter effects evoked by bladder distension (sympathetic reflex and electrocorticogram desinchronization) were absent in capsaicin-pretreated animals. Together these data indicate that the afferent function of capsaicin-sensitive fibres can be activated by stimuli of both a mechanical and chemical nature. However, the effect of bladder distension on the 'efferent' function of capsaicin-sensitive nerves is still not known. We have recently observed that MEN 11420 (Nepadutant), a potent and selective tachykinin NK_2 receptor antagonist (Catalioto *et al*., 1998), reduces the motor responses induced by the intravesical application of capsaicin in the rat bladder (Lecci *et al*., 1997). In the present study we have systematically investigated the effect of MEN 11420 on bladder motility evoked by different degrees of distension, which was taken as an indirect measure of the degree of activation of the 'efferent' function of capsaicin-sensitive nerves. Two different modalities of activation of the 'efferent'

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function of capsaicin-sensitive fibres have been described (Maggi, 1991). The 'efferent' response evoked by capsaicin in the rat urinary bladder is resistant to tetrodotoxin (Maggi *et al*., 1984), a blocker of voltage-sensitive sodium channels, and to inhibitors of prostaglandin synthesis (Maggi *et al*., 1986; Pinna *et al*., 1994), but it is abolished by ruthenium red (Maggi *et al*., 1988), a blocker of cation channels coupled to the vanilloid (capsaicin) receptors, or by neurotoxic lesions induced by pretreatment with high doses of capsaicin (Maggi *et al*., 1984). The other modality, shared by different kinds of stimuli such as electrical field stimulation or some chemical stimuli, is tetrodotoxin-sensitive but ruthenium red-resistant (Maggi *et al*., 1989b). Therefore, the interaction of these drugs (tetrodotoxin, S-ketoprofen, ruthenium red and capsaicin) with the effect of MEN 11420 was also studied in order to explore the mechanism through which bladder distension activates the 'efferent' function of capsaicin-sensitive nerves. Isoprenaline was used as an internal standard since, in the various experimental conditions, its relaxant effect on vesical tone never differed in vehicle- or MEN 11420-treated animals.

Methods

Male Wistar rats (360–400 g, Charles River) were anaesthetized with urethane (1.2 g kg^{-1}, s.c.). The jugular vein was cannulated for drug administration, ureters were tied and intravesical pressure was recorded through a polyethylene catheter (PE 90, o.d. 1.27 mm, i.d. 0.86 mm) inserted in and tied to the proximal urethra. The other end of the catheter was connected to a pressure transducer and the intravesical pressure was recorded through a MacLab 8 S apparatus (ADInstruments, Hastings, U.K.). One hour after surgery, the bladder was rapidly (5 s) filled with 0.2, 0.5, 1 or 2 ml of saline and the preparation was left for 105 min to stabilize before MEN 11420 (100 nmol kg^{-1}, i.v.) or vehicle administration. Seventy five minutes after MEN 11420 or vehicle, rats received isoprenaline (400 nmol kg^{-1}, i.v.), the effect of which was followed for 15 min. When the effect of MEN 11420 was evaluated in animals pretreated with S-ketoprofen (0.5 μmol kg^{-1}, i.v.) or ruthenium red (10 μmol kg^{-1}, i.v.), the pretreatment was performed 45 min after the bladder had been filled (60 min before MEN 11420). Pretreatment with tetrodotoxin was performed by filling the bladder with a saline solution of tetrodotoxin at concentrations of 1, 10, 100 and 100 μM for filling volumes of 2, 1, 0.5 and 0.2 ml, respectively. These concentrations were chosen on the basis of preliminary experiments where the ability of intravesical tetrodotoxin to suppress reflex bladder activity evoked by distension was evaluated. In order to block reflex motility, higher concentrations of tetrodotoxin were required at lower bladder volumes, possibly because the vesical absorbtion of drugs augments as the degree of distension increases (Tammela *et al*., 1993). Pretreatment with capsaicin (164 μmol kg^{-1}, s.c.) or its vechicle (2 ml kg^{-1} saline containing 10% v/v ethanol and 10% v/v Tween 80) was performed 5 days before the experiments.

MEN 11420 (cyclo{[Asn(2-deossi-2-AcNH-β-D-Glc)-Asp-Trp-Phe-Dap-Leu]cyclo(2β-5β)} synthesized in the Chemistry Research Department of Menarini Ricerche, Florence, Italy) was dissolved in saline (100 nmol 100 μl^{-1} kg^{-1}), isoprenaline (Serva, Heidelberg Germany) was dissolved in saline (400 nmol 100 μl^{-1} kg^{-1}), S-ketoprofen (Lab. Menarini, Barcelona Spain) was dissolved in saline (0.5 μmol 1 ml^{-1} kg^{-1}), ruthenium red (Aldrich, Steinheim Germany) was dissolved in distilled water (10 μmol 1 ml^{-1} kg^{-1}). Tetrodotoxin citrate was purchased from Sankyo (Tokyo, Japan), capsaicin was purchased from Sigma (St. Louis, Mo U.S.A.).

Parameters evaluated for data analysis were: the minimal intravesical pressure (the minimal value of pressure recorded in the period considered, mmHg), the frequency (cycles min^{-1}) of contractions having amplitude >5 mmHg (micturition reflex) and the maximal amplitude (mmHg) of these contractions. These parameters were evaluated in periods of 15 min for 60 or 120 min. The effects of drugs on these parameters were expressed either as absolute values or as differences (Δ) between the basal values (calculated 15 min before treatments) and the values recorded in the various periods following the treatments. Data displayed in Figure 4 were calculated according to the formula: maximal relaxation (mmHg) $\times$ 100/ minimal intravesical pressure (mmHg).

Data were analysed by completely randomized or repeated measures one- (different volumes), two- (treatment $\times$ times) or three-way (treatment $\times$ times $\times$ volumes) analysis of variance; *post hoc* test (Fisher's LSD, least significant difference) was performed only if the appropriate F of the analysis of variance was significant. $P<0.05$ was regarded as significant.

Results

The filling of the urinary bladder with increasing volume (0.2, 0.5, 1 and 2 ml) of saline evoked high amplitude bladder contractions representing the supraspinal micturition reflex: the lowest volume (0.2 ml) was subthrehold, 0.5 ml was a threshold volume, whereas both 1 and 2 ml were suprathreshold for eliciting the reflex (Figure 1a). The frequency of reflex bladder contractions augmented by increasing the intravesical volume (Figure 2b), whereas the amplitude of these contrac-

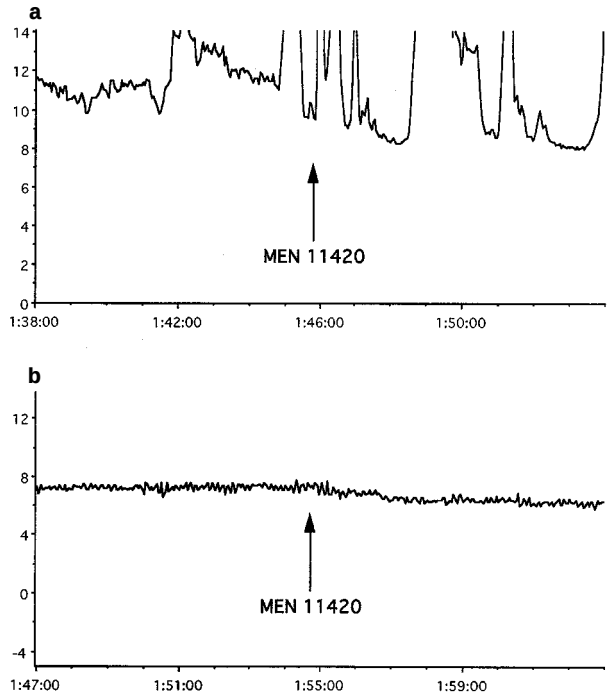

Figure 1 Typical tracing showing the effect of MEN 11420 in (a) controls or (b) tetrodotoxin (1 ml of 10 μM solution, intravesically)-pretreated rats on the pattern of bladder motility induced by 1 ml filling in urethane-anaesthetized rats. Vertical axis represents the intravesical pressure (mmHg), horizontal axis represents time (h: min: s).

tions was similar at 0.5 or 1 ml (31.4±8.0 mmHg, $n=6$ and 25.2±3.0 mmHg, $n=15$, respectively) but it decreased when the bladder was filled with 2 ml (16.0±2.1 mmHg, $n=15$, $P<0.05$ vs 0.5 or 1 ml) possibly because of the increase in the minimal pressure (Figure 2a).

MEN 11420 (100 nmol kg^{-1}, i.v.) did not significantly modify the frequency, or amplitude of bladder contractions (data not shown). MEN 11420 produced a decrease in minimal pressure that was positively related to the degree of bladder distension, its effect being absent at 0.2 ml, small at 0.5 ml and statistically significant at 1 and 2 ml (Figures 1, 3 and 4). Isoprenaline (400 nmol kg^{-1}, i.v.) significantly relaxed bladder tone at all degrees of distension (−0.8±0.2, −1.1±0.2, −2.1±0.3 and −2.2±0.1 mmHg at 0.2, 0.5, 1 and 2 ml, respectively, $n=8$ for each group), but unlike MEN 11420, its relaxant effect (measured as % of the respective value of minimal pressure) decreased as the intravesical volume increased (Figure 4). The effect of isoprenaline on minimal pressure peaked within 5 min and lasted for about 15 min.

Unlike its vehicle, capsaicin pretreatment (164 μmol kg^{-1}, s.c., 5 days before) significantly reduced the frequency of reflex bladder contractions but not the minimal pressure (Figure 2) or the amplitude of reflex contractions (data not shown). In capsaicin-pretreated rats, MEN 11420 had no effect on the minimal pressure either at 1 or at 2 ml of distension (Figure 5b and d). The relaxant effect of isoprenaline was similar in capsaicin-pretreated rats or in controls (Table 1).

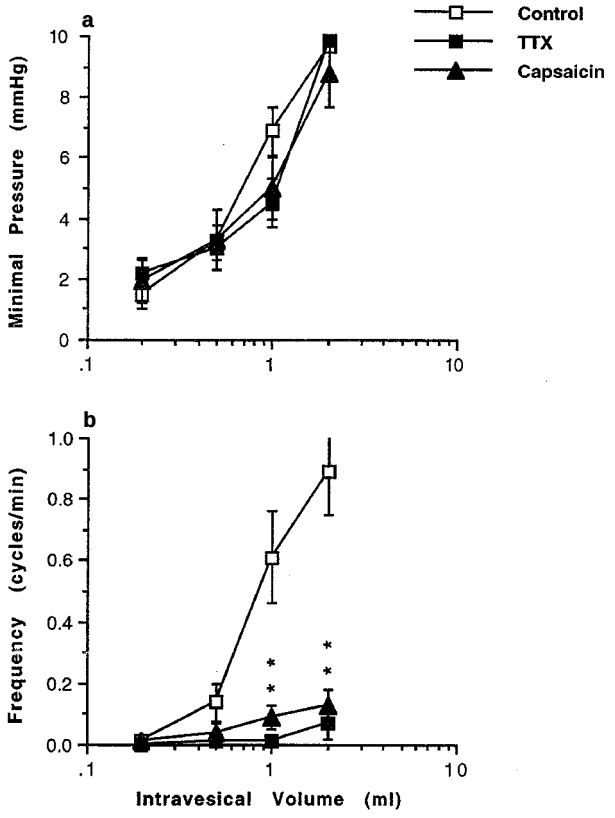

Figure 2 Effect of tetrodotoxin (TTX) or capsaicin pretreatment on (a) minimal intravesical pressure or (b) frequency of reflex contractions (>5 mmHg) at different intravesical volumes. The concentrations of TTX were 100, 100, 10 and 1 μM at 0.2, 0.5, 1 and 2 ml, respectively (see Methods). These values were recorded during the last 15 min of the stabilization period (90–105 min after bladder filling). Each point represents the mean of 16 experiments; vertical lines show s.e.mean. Fisher's LSD: $^{**}P<0.01$ *vs* control.

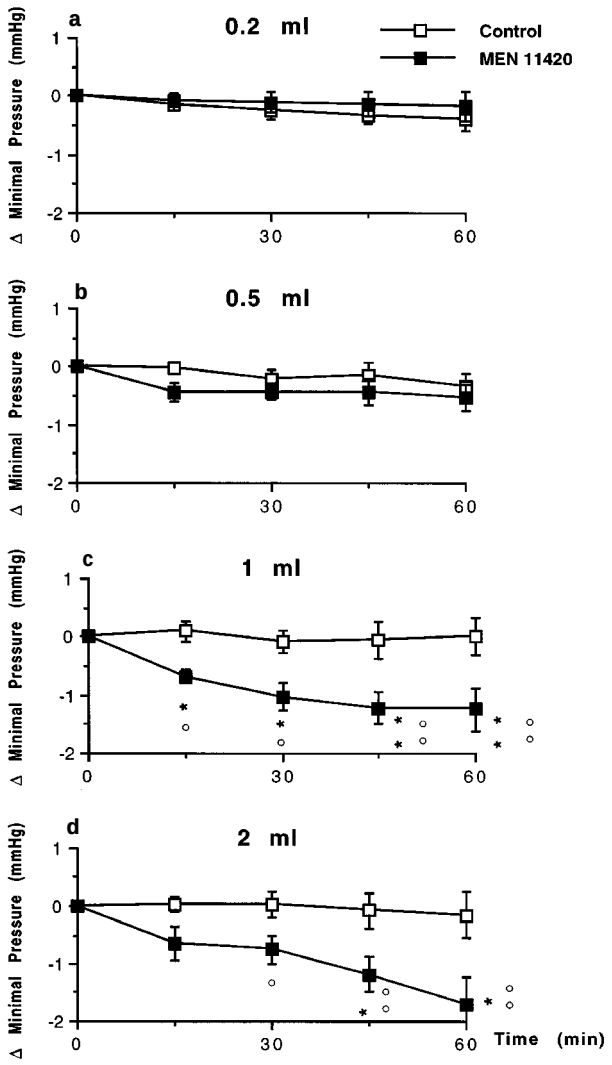

Figure 3 Effect of MEN 11420 (100 nmol kg^{-1}, i.v.) on the minimal bladder pressure with the bladder filled with (a) 0.2 ml, (b) 0.5 ml, (c) 1 ml and (d) 2 ml. Each point represents the mean of 8 animals; vertical lines show s.e.mean. Fisher LSD: $^{*}P<0.05$ and $^{**}P<0.01$ vs time-matched control, $^{\circ}P<0.05$ and $^{\circ\circ}P<0.01$ *vs* time-matched MEN 11420-treated group at 0.2 ml of distension (a).

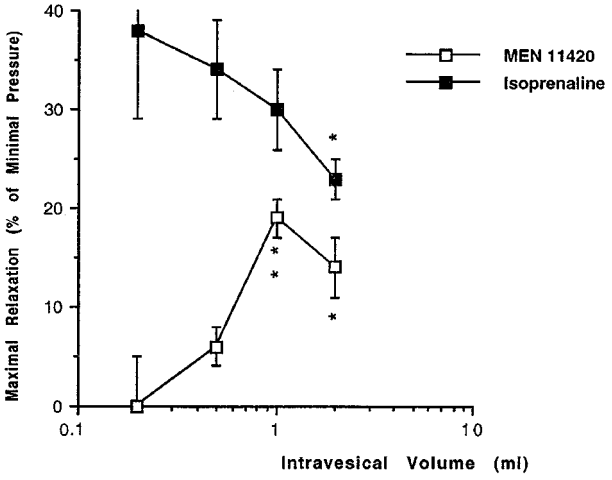

Figure 4 Maximal relaxant effect of MEN 11420 (100 nmol kg^{-1}, i.v.) or isoprenaline (400 nmol kg^{-1}, i.v.) on bladder tone at various degrees of distension, expressed as % of the absolute value of minimal pressure. Each point represents the mean and vertical lines s.e.mean of 8 animals. Fisher's LSD: $^{*}P<0.05$ and $^{**}P<0.01$ *vs* 0.2 ml.

Intravesical tetrodotoxin (10 and 1 μM) inhibited reflex activity of the bladder at 1 and 2 ml of distension (Figure 1), by reducing the frequency (Figure 2b) and the amplitude of bladder contractions (data not shown), but it did not modify the minimal pressure (Figure 2a). However, the effect of MEN 11420 on minimal pressure at the various degrees of distension was not affected by tetrodotoxin-pretreatment (Figure 1b and Figure 5a and c). In tetrodotoxin-pretreated rats, isoprenaline produced a drop in minimal pressure that was not different from control values (Table 1).

Table 1 Effect of isoprenaline (400 nmol kg^{-1}, i.v.) on the minimal intravesical pressure in the presence or in the absence of tetrodotoxin (1 ml of 10 μM or 2 ml of 1 μM, intravesically), capsaicin (164 μmol kg^{-1}, s.c.), S-ketoprofen (0.5 μmol kg^{-1}, i.v.), ruthenium red (10 μmol kg^{-1}, i.v.) at 1 and 2 ml of distension

	Δ Minimal pressure (mmHg)	
Intravesical volume (ml)	*1*	*2*
Pretreatment		
Vehicle	−2.3±0.3	−2.4±0.3
Tetrodotoxin	−1.9±0.5	−2.4±0.3
Vehicle	−2.2±0.3	−2.5±0.3
Capsaicin	−1.7±0.3	−2.7±0.5
Vehicle	−2.1±0.3	−2.5±0.3
S-ketoprofen	−1.2±0.1**	−1.2±0.2**
Vehicle	−2.4±0.5	−2.0±0.2
Ruthenium red	−2.6±0.4	−2.2±0.2

Each value represents the mean±s.e.mean of 8–10 animals. Fisher's LSD: $^{**}P<0.01$ *vs* vehicle.

At 1 ml of distension, intravenous S-ketoprofen (0.5 μmol kg^{-1}) produced a consistent relaxation of the bladder tone (Figure 6a) and decreased the frequency of reflex bladder contractions (Figure 6b) but not their amplitude (data not shown). In S-ketoprofen-pretreated animals, MEN 11420 had no significant effect on the minimal pressure at 1 ml of bladder distension (Figure 6c). At 2 ml of distension, S-ketoprofen still decreased the minimal pressure (Figure 6d) but had no significant effect on the frequency (Figure 6e) or on the amplitude (data not shown) of reflex bladder contractions. At this degree of distension, MEN 11420 significantly decreased the minimal pressure in S-ketoprofen-pretreated rats (Figure 6f). At both volumes (1 and 2 ml) the relaxant effect of isoprenaline was decreased in S-ketoprofen-pretreated animals when compared to volume-matched controls (Table 1).

Ruthenium red (10 μmol kg^{-1}, i.v.) had no effect on the minimal intravesical pressure when the bladder was filled with 0.2 ml (data not shown). At 1 ml of distension, ruthenium red reduced the minimal pressure (Figure 7a) and the frequency of reflex contractions (Figure 7b) but not their amplitude (data not shown). At this volume, MEN 11420 had no effect on the minimal pressure in ruthenium red-pretreated animals (Figure

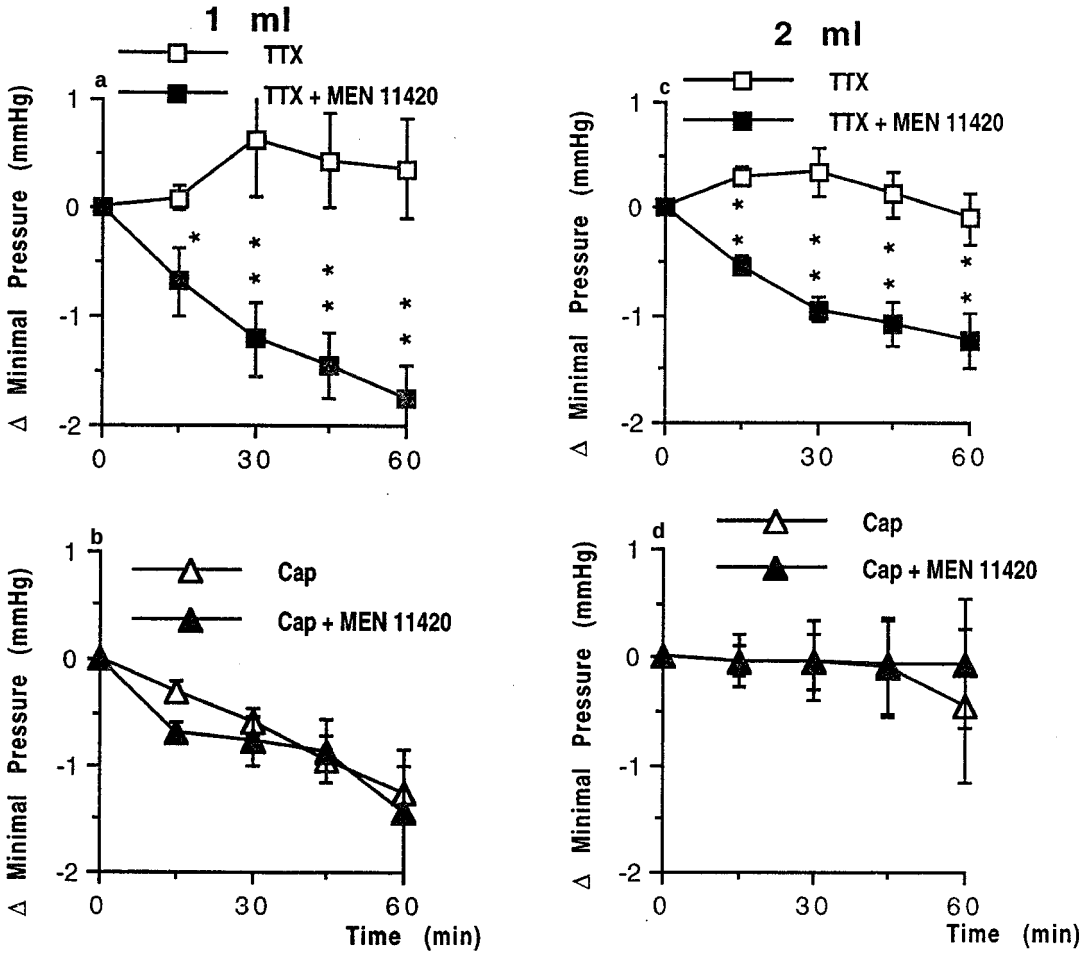

Figure 5 Effect of MEN 11420 on minimal pressure in tetrodotoxin (TTX)- or capsaicin (Cap)-pretreated rats at (a) and (b) 1 ml or (c) and (d) 2 ml of distension, respectively. The concentrations of TTX were 10 and 1 μM at 1 and 2 ml, respectively (see Methods). Each point represents the mean and vertical lines s.e.mean of 8 animals. Fisher's LSD: $^{*}P<0.05$ and $^{**}P<0.01$ *vs* TTX.

7c). When the bladders were filled with 2 ml, ruthenium red had no significant effects on bladder motility (Figure 7d and e). However, MEN 11420 reduced the minimal intravesical pressure in ruthenium red-pretreated animals (Figure 7f). The relaxant effect of isoprenaline was not changed in ruthenium red-pretreated animals as compared to controls (Table 1).

Discussion

The present results provide the first evidence that bladder distension is an adequate stimulus for the activation of the 'efferent' function of capsaicin-sensitive sensory fibres and that tachykinins, released by distension, modulate the bladder tone through the activation of NK_2 receptors. Although it cannot be excluded *a priori* that the release of tachykinins represents an artifact of the surgical preparation (Houghton *et al*., 1995), there are some arguments against this possibility. First, the inhibitory effect of the tachykinin NK_2 receptor antagonist MEN 11420 on the bladder tone is evident only at high (1 and 2 ml) but not at low (0.2 and 0.5) degrees of distension. Second, the possibility that vesical distension and urethral surgery concur to release tachykinins at the bladder level (via an axon reflex) also appears unlikely, since the effect of

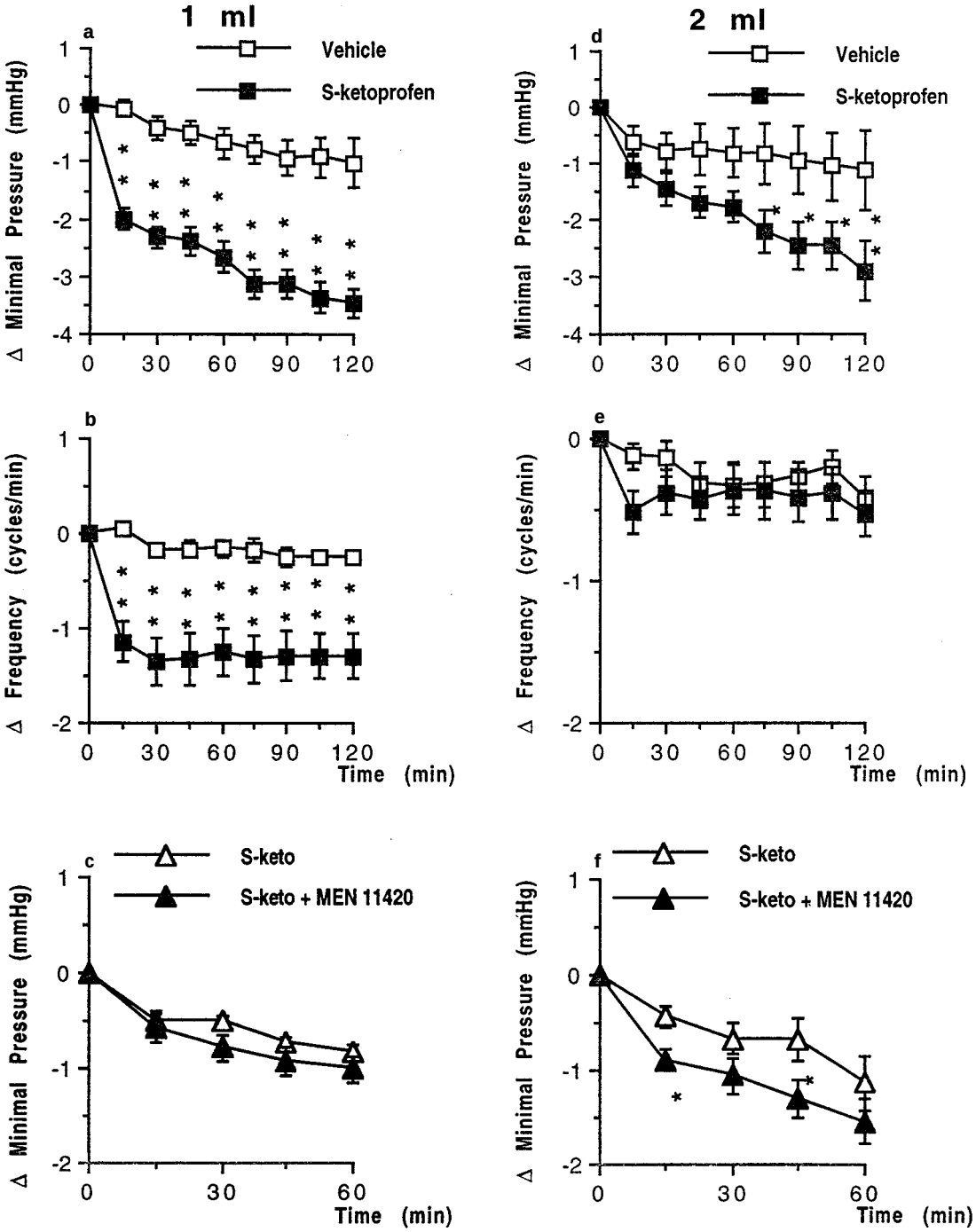

Figure 6 Effect of S-ketoprofen (S-keto, 0.5 μmol kg^{-1}, i.v.) at 1 or 2 ml of bladder distension, respectively, on: (a) and (d) minimal pressure, (b) and (e) the frequency of reflex contractions; (c) and (f) effect of MEN 11420 on minimal pressure in S-ketoprofen-pretreated rats. Each point represents the mean and vertical lines s.e.mean of 10 animals. Fisher's LSD: $^*P<0.05$ and $^{**}P<0.01$ *vs* vehicle (a, b or d) or *vs* S-keto (f).

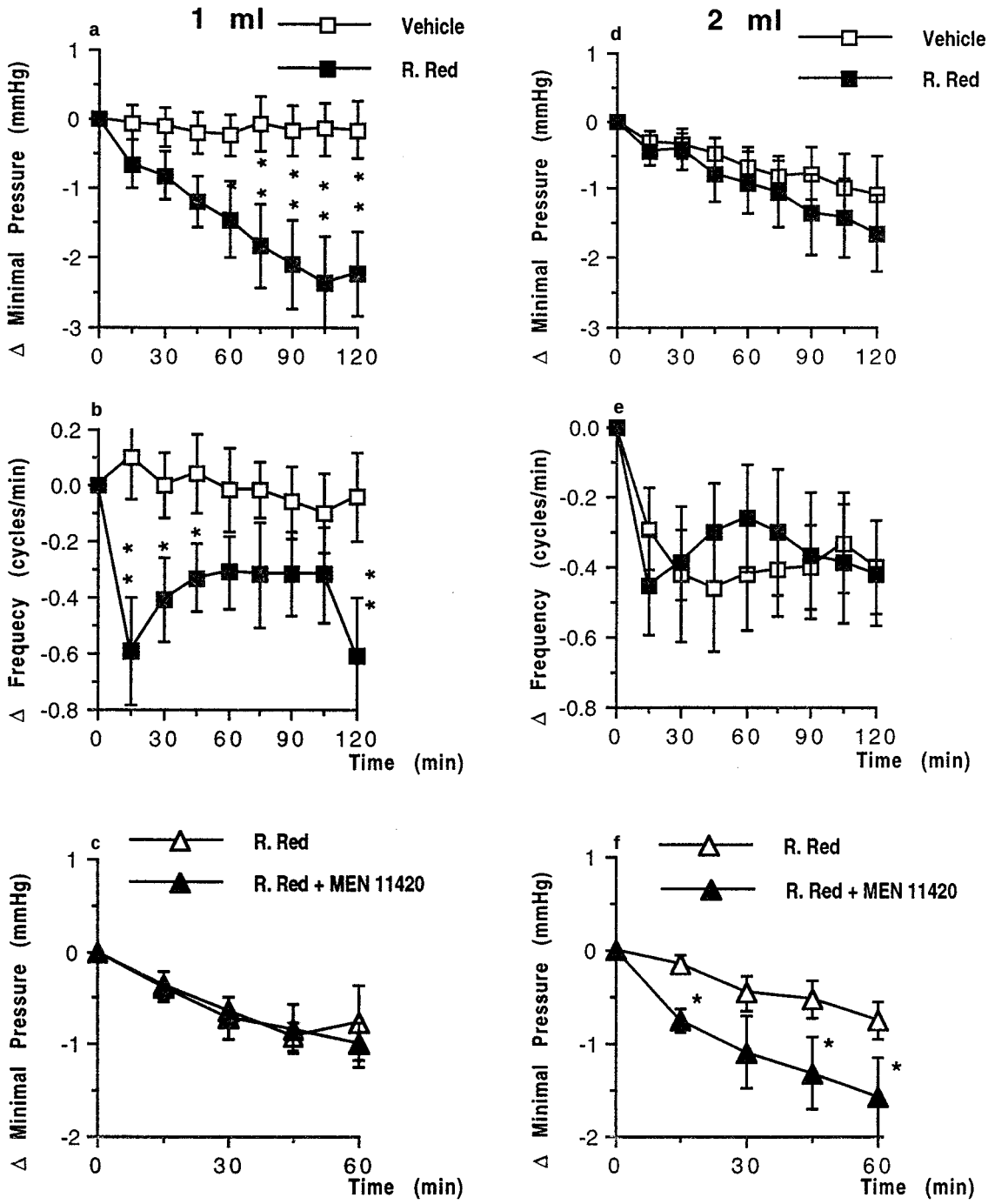

Figure 7 Effect of ruthenium red (R. Red, 10 μmol kg^{-1}, i.v.) at 1 and 2 ml of bladder distension, respectively, on : (a) and (d) minimal pressure, (b) and (e) the frequency of reflex contractions; (c) and (f) effect of MEN 11420 on minimal pressure in ruthenium red-pretreated rats. Each point represents the mean and vertical lines s.e.mean of 10 animals. Fisher's LSD: $*P<0.05$ and $**P<0.01$ *vs* vehicle (a and b) or *vs* R. Red (f).

MEN 11420 is capsaicin-sensitive but tetrodotoxin-resistant, whereas in the urinary bladder the axonal conduction of capsaicin-sensitive fibres is tetrodotoxin-sensitive (Meini & Maggi, 1994). Therefore, in the present experimental conditions, bladder distension is the main, if not the only, stimulus activating the 'efferent' function of capsaicin-sensitive nerves. It should be observed that capsaicin pretreatment had no significant effect on the minimal bladder pressure: this may be due to the fact that the tachykinergic contribution to the vesical tone is quite small (about 20% of the minimal pressure) and thus difficult to detect in experiments having unpaired groups; alternatively other mechanisms may compensate the tachykinergic modulation of the vesical resting tone in capsaicin pretreated rats.

Either capsaicin or tetrodotoxin pretreatments reduce the frequency of reflex bladder contractions. On the one hand, tetrodotoxin can abolish reflex bladder activity through the blockade of both parasympathetic efferent and sensory innervation, on the other, in the present experimental conditions (urethane anaesthesia), the impairment of the afferent function of capsaicin-sensitive nerves induced by capsaicin pretreatment is sufficient to reduce reflex activity of

the urinary bladder (Lecci & Maggi, 1995). In this respect, it is worth noting that, of the drugs tested in the present study, tetrodotoxin was the only one to reduce the amplitude of bladder contractions. Although the effect of MEN 11420 is evident only when the bladder has been filled with a suprathreshold volume for the activation of the micturition reflex, the distension-induced activation of capsaicin-sensitive bladder afferents does not depend on the activity of parasympathetic postganglionic motor neurones, since tetrodotoxin abolished the reflex contractions but not the MEN 11420-evoked relaxations. Prostaglandins are possible candidates for a distension-related stimulant action on sensory nerves. In fact, although neurones are capable of synthesizing and releasing cyclo-oxygenase metabolites (Franco-Cereceda, 1989), prostanoids are also produced by non-neural cells (muscle and epithelial cells) in response to bladder distension and these substances are able to sensitize/stimulate capsaicin-sensitive afferents in the rat urinary bladder (Maggi, 1992; Ishizuka *et al*., 1995). To test the possibility that prostaglandins might be involved in the distension-induced activation of sensory fibres, we studied the effect of the cyclo-oxygenase inhibitor S-ketoprofen. Indeed, we found that S-ketoprofen greatly reduced the vesical tone, the frequency of reflex contractions and, when the bladder was filled with 1 ml, also the effect of MEN 11420. However, when we compared the data presented in Figure 2a with those displayed in Figure 6a, we observed that S-ketprofen lowers intravesical pressure at levels where the effect of MEN 11420 is no longer detected. The results obtained when the bladder was filled with 2 ml validated this hypothesis: S-ketoprofen still decreased vesical tone but not enough to lower the intravesical pressure to values similar to those observed with a moderate bladder filling (0.5 ml). In this condition, MEN 11420 caused a small but significant reduction of the minimal pressure. Although the reduction of intravesical pressure induced by MEN 11420 in S-ketoprofen-treated animals was lower, in absolute values, than that detected in similar experimental conditions (2 ml) in control animals (-0.62 vs -1.55 mmHg in S-ketoprofen-treated rats and in controls, respectively), when the effect of MEN 11420 was evaluated as a percentage of the isoprenaline-induced relaxation, these values were not different (see Table 2). This occurs because S-ketoprofen also decreased isoprenaline-induced relaxation. These results indicate that prostanoids are not involved in the peripheral activation of sensory fibres induced by distension or capsaicin (Maggi *et al*., 1986; Pinna *et al*., 1994), as they are not involved in the release of tachykinins evoked by capsaicin in sensory terminals at the spinal cord level (Vasko, 1995). The reason why S-ketoprofen reduces isoprenaline-induced bladder relaxation remains to be elucidated.

Ruthenium red is a blocker of the cation channel intrinsic in the vanilloid receptor (Caterina *et al*., 1997). This dye antagonizes the activation of the 'efferent' function induced by capsaicin in the rat isolated urinary bladder (Maggi *et al*., 1988), it selectively reduces capsaicin-evoked local and reflex responses in the rat bladder *in vivo*, but, at doses producing non specific effects, it does not antagonize the distension-induced activation of the sensory function of capsaicin-sensitive afferents (Maggi *et al*., 1989a). In our model the effect of ruthenium red on the afferent and 'efferent' function of capsaicin-sensitive nerves was not consistent. On the one hand, at the volume of 1 ml, ruthenium red mimicked the effect of MEN 11420 on the minimal intravesical pressure (efferent function), prevented the effect of the NK_2 receptor antagonist on this parameter, and shared with capsaicin an inhibitory effect on the frequency of reflex contractions (afferent function), indicating that the dye is capable of blocking the function of capsaicin-sensitive afferents when the bladder is filled with 1 ml. On the other hand, at the maximal intravesical volume (2 ml), ruthenium red did not significantly modify any parameter of bladder motility and did not prevent the effect of MEN 11420 on the minimal pressure. It could be argued that, like S-ketoprofen, at 1 ml of distension ruthenium red prevents the effect of MEN 11420 on the 'efferent' function of sensory nerves just because the dye lowers the minimal pressure by itself. However, the fall of minimal pressure induced by ruthenium red was only 65% of that caused by S-ketoprofen, but the inhibition of the effect of MEN 11420 was complete. In order to explain this complex picture of results we should postulate an interaction occurring between bladder distension and the cation channel of the vanilloid receptor, i.e., high degrees of distension act as a physiological antagonist of the action of ruthenium red on the cation channel of the vanilloid receptor and that this channel could be directly activated by distension. On the basis of these results it could be speculated that, in the urinary bladder, the cation channel of the vanilloid receptor may work as a mechanoreceptor in addition to its well-established role as chemoreceptor (Geppetti & Bevan, 1994).

In conclusion, bladder distension activates the 'efferent' function of capsaicin-sensitive afferents that participate in the regulation of bladder tone through the release of tachykinins which, in turn, stimulate NK_2 receptors located on the smooth muscle (Nimmo *et al*., 1992). However, as previously observed (Lecci *et al*., 1993; 1997), the distension-induced reflex activity is not modified by NK_2 receptor blockade indicating that the tachykininergic contribution to the vesical tone is not important for the triggering of micturition reflex. These results also suggest that the modality of tachykinin release evoked by distension resembles that induced by capsaicin, being resistant to tetrodotoxin and to prostaglandin synthesis inhibitors but relatively sensitive to ruthenium red. The bell-shaped dose-response curve obtained with this latter compound suggests that bladder distension directly activates the cation channel of the vanilloid receptor, hence this receptor could be the molecular entity characterizing, from the functional point of view, a population of polymodal nociceptors (Szolcsanyi *et al*., 1988; Szolcsanyi, 1996).

Table 2 Effect of tetrodotoxin (10 and 1 μM at intravesical volumes of 1 and 2 ml, respectively), S-ketoprofen (0.5 μmol kg^{-1}, i.v.), ruthenium red (10 μmol kg^{-1}, i.v.) and capsaicin (164 μmol kg^{-1}, s.c.) on the relaxant effect of MEN 11420 on the bladder tone in anaesthetized rats

	Maximal relaxation (% of isoprenaline)	
Intravesical volume (ml)	*1*	*2*
Pretreatment		
Vehicle	64±9	50±10
Tetrodotoxin	68±6	60±15
S-ketoprofen	15±7*	65±7
Ruthenium red	0±4**	43±13
Capsaicin	22±11*	0±4**

Values represent means±s.e.mean of 8–10 animals. Fisher's LSD: $*P<0.05$ and $**P<0.01$ *vs* vehicle.

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