Pharmacological analysis of the local and reflex responses to bradykinin on rat urinary bladder motility *in vivo*

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1 The topical application of bradykinin (BK) (0.05–5000 pmol/rat) onto the serosal surface of the urinary bladder in urethane-anaesthetized rats, evoked low amplitude tonic contractions (not exceeding 25 mmHg) or high amplitude (about 50 mmHg), phasic reflex contractions (chemoceptive micturition reflex) which were abolished by bilateral ablation of the pelvic ganglia. In ganglionectomized rats, BK induced only a local, tonic-type contraction.

2 Systemic capsaicin pretreatment (164 $\mu\text{mol kg}^{-1}$, 4 days before) reduced the incidence of chemoceptive reflex induced by BK (500 pmol/rat) but had no effect on the magnitude of the tonic-type contraction elicited by BK in ganglionectomized rats. Indomethacin (11 $\mu\text{mol kg}^{-1}$, 20 min before) reduced the incidence but not the amplitude of the reflex contractions induced by topical application of BK (500 pmol/rat). In ganglionectomized rats, indomethacin (11 $\mu\text{mol kg}^{-1}$, 20 min before) decreased the amplitude of the tonic contraction evoked by BK. Indomethacin did not affect the chemoceptive reflex induced by topical application of capsaicin (15 nmol/rat) onto the bladder.

3 Intrathecal administration of the tachykinin NK₁ receptor antagonists, RP 67,580 (10 nmol/rat) or SR 140,333 (10 nmol/rat), abolished the chemoceptive reflex induced by BK without modifying the magnitude of the tonic contraction. SR 140,333 (10 nmol/rat) also abolished the occurrence of the chemoceptive reflex induced by capsaicin.

4 Intravenous administration of the B₂ receptor antagonist, Hoe 140 (35 nmol kg⁻¹, 10 min before) abolished the reflex and local effects induced by BK on bladder motility but failed to modify the chemoceptive reflex induced by topical application of capsaicin (15 nmol/rat).

5 Intrathecal administration of Hoe 140 (10 nmol/rat) reduced the incidence of the chemoceptive reflex induced by BK but had no effect on the amplitude of the local motor response. Likewise, Hoe 140 (10 nmol/rat, i.t.) reduced the incidence of reflex bladder contractions induced by topical application of capsaicin (15 nmol/rat) without affecting the magnitude of the tonic-type contraction.

6 These findings indicate that BK stimulates motility through B₂ receptors in the rat urinary bladder. BK activates the reflex response by stimulating capsaicin-sensitive afferent nerves with a contribution from prostanoids. At the spinal cord level, tachykinin NK₁ and BK B₂ receptors could also be involved in the chemoceptive reflex induced by BK or capsaicin.

Keywords: Micturition reflex; bradykinin; capsaicin; tachykinin; rat urinary bladder

Introduction

Bradykinin (BK) is the most potent endogenous mediator producing pain (Dray & Perkins, 1993, for review). In laboratory animals the nociceptive effect induced by BK depends on the activation of a population of afferent nerves which are sensitive to capsaicin (Dray *et al.*, 1988a; 1989). The sensory stimulant action of peripherally applied BK is accompanied by a number of local effects such as vasodilatation, smooth muscle contraction, increased vascular permeability, etc (Marceau *et al.*, 1983, for review). These peripheral effects are partly a consequence of the direct action of BK on target cells and, to varying degrees in various systems, occur indirectly, through the local release of sensory neuropeptides from capsaicin-sensitive primary afferent nerves (Geppetti, 1993, for review) together with prostanoid generation from immune and muscle cells and sensory or sympathetic nerves (Dray & Bevan, 1993).

BK is generated during inflammation or by stimuli producing tissue injury (Bhoola *et al.*, 1992). However, the urine contains significant amounts of kinins (BK-related peptides) even in physiological conditions (Oza, 1988; Bhoola *et al.*, 1992). Therefore BK could be a stimulus producing pain in the lower urinary tract whenever the urothelial barrier is disrupted. BK induces urinary bladder contraction both *in vivo* (Maggi *et al.*, 1993) and *in vitro* (Maggi *et al.*, 1989). In the latter experimental conditions, indomethacin pretreatment but not capsaicin desensitization reduced the activity of

BK. However, the role of prostanoids and of capsaicin-sensitive nerves in the sensory stimulant action of BK in the rat urinary bladder has not been investigated *in vivo*.

In the present study the motor effects produced by topical application of BK onto the urinary bladder of urethane-anaesthetized rats were compared with the effects induced by topical application of capsaicin. Since spinally administered NK₁ receptor antagonists block the capsaicin-evoked micturition reflex (Lecci *et al.*, 1992; 1993a) we also investigated the effect of intrathecal (i.t.) administration of NK₁ receptor antagonists, such as RP 67,580 or SR 140,333 (Garret *et al.*, 1991; Jung *et al.*, 1994) on the motor effects induced by the topical application of BK. In addition, the receptor subtype involved in the responses to BK was characterized by means of a potent and selective bradykinin B₂ receptor antagonist, Hoe 140 (Wirth *et al.*, 1991).

Methods

General

Male Wistar rats weighing 340–360 g were anaesthetized with urethane (1.2 g kg⁻¹, s.c.). After laparotomy, the bladder was exposed and both ureters were tied. Intrathecal (i.t.) administration of drugs was performed via a catheter (PE 10) inserted through the atlanto-occipital membrane for 8.5 cm, in such a way that the tip of the catheter was placed just

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above the lumbosacral enlargement (L1–L2 segments), as previously described (Lecci *et al.*, 1993a). After catheter insertion, 25 μ l of saline was rapidly flushed through the catheter and all animals responding with muscle myoclonus, hindleg, or tail movements were discarded. Rats were placed on a heating pad to keep body temperature between 36.5 and 37°C. Experiments were carried out 2 h after catheterization. Drugs or vehicle were administered in a volume of 10 μ l and the cannula was rinsed with 15 μ l of saline; the flow rate for injections was 10 μ l min⁻¹. Some experiments were also performed in capsaicin pretreated animals (164 μ mol kg⁻¹, s.c., 4 days before experiments). This protocol of capsaicin pretreatment has been shown to produce a selective impairment of a limited fraction of bladder afferents which accounts for the whole content of tachykinins in the rat urinary bladder (Maggi *et al.*, 1987a,b).

Bladder contractions induced by topical application of bradykinin or capsaicin (chemoceptive vesico-vesical reflex)

The intraluminal bladder pressure was measured by a catheter (PE 90) inserted through the proximal urethra. For topical application of BK or capsaicin, the bladder was exposed, kept separated from adjoining viscera by a piece of parafilm and covered with a saline moistened cotton gauze. The urinary bladder was filled with saline until high amplitude rhythmic contractions (micturition reflex) were observed in order to check the viability of the preparation. Afterwards, a small amount of saline was withdrawn until such contractions disappeared. Therefore the urinary bladder remained filled with just a subthreshold volume for eliciting the micturition reflex (about 0.35 ml of saline in controls and 1 ml in capsaicin-pretreated rats) and, after 30 min of equilibration, BK (500 pmol/50 μ l) or capsaicin (15 nmol/50 μ l) was applied onto the serosal surface of the urinary bladder by means of a microsyringe, as previously described (Lecci *et al.*, 1993a). Indomethacin was given 20 min before, Hoe 140, SR 140,333, RP 67,580 or the vehicle were administered intravenously or intrathecally 10 min before topical application of BK or capsaicin. In these experiments the following parameters were evaluated: presence of reflex bladder contractions (two or more phasic contractions of amplitude > 16 mmHg), the maximal amplitude of reflex contractions or of tonic-type contraction, as previously described (Maggi *et al.*, 1986).

In a series of experiments the pelvic ganglia were exposed and removed by means of microscissors. In these animals, bladder filling induced only low amplitude (1–4 mmHg) myogenic contractions; a volume of 0.35 ml was used to fill the bladder of control rats whereas 1 ml of saline was infused in capsaicin-pretreated rats. These volumes are the average threshold volumes of activation of micturition reflex in animals with intact urinary bladder innervation or sensory nerve lesion induced by pretreatment with capsaicin in adult rats. The experiments were carried out 1.5 h after bilateral surgical ablation. In ganglionectomized rats the maximal value of intravesical pressure (mmHg) produced within 20 min from agonist application was recorded.

Drugs

Capsaicin and indomethacin were from Sigma (St Louis, U.S.A.), BK was from Peninsula Laboratories Inc. (Merseyside, UK). Hoe 140 (D-Arg[Hyp³, Thi⁵, D-Tic⁷, Oic⁸] bradykinin), RP 67,580 ((3aR, 7aR)-7,7-diphenyl-2-[1-amino-2-(2-methoxyphenyl) ethyl] perhydroisoindol-4-one) and SR 140,333 (S)-1-[2-[3-(3,4-dichlorophenyl)-1-(3-isopropoxyphenyl)-acetyl]-piperidin-3-yl] ethyl-4-phenyl-1-azoniabicyclo[2.2.2.] octane chloride) were kindly provided by Drs K. Wirth (Hoechst, Frankfurt, Germany), C. Garret (Rhone-Poulenc Rorer, Vitry, France) and by X. Exmonds-Alt (Sanofi, Montpellier, France), respectively.

Data analysis

All values represent means $\pm$ standard error of the mean (s.e.mean). Dose-response experiments for the incidence of the chemoceptive reflex were evaluated by means of Chi² test. ED₅₀ values for the activation of micturition reflex were calculated according to the Lichtfield-Wilcoxon method. The effect of antagonists on this parameter was analysed by Chi² test. The effect of antagonists on intravesical pressure was evaluated by one-way ANOVA followed by Tukey test or by Student's *t* test, when applicable.

Results

The topical application of BK onto the urinary bladder produced a dose-related increase in the incidence of the chemoceptive micturition reflex. The morphology of the intravesical pressure waves produced by BK is qualitatively similar to that observed in response to topical application of capsaicin (Lecci *et al.*, 1993a,b), i.e. a tonic-type contraction (4–25 mmHg in amplitude, lasting 2–6 min) on which a series of high amplitude (20–85 mmHg in amplitude, lasting 1–7 min) phasic contractions were superimposed. The tonic-type contraction could be seen when the reflex was not activated or was blocked by pharmacological or surgical means (Figure 1). The reflex response induced by topical application of BK is an all-or-none response, since the incidence but not the amplitude of this response is related to the dose of BK used (Table 1). In preliminary experiments (*n* = 9), we studied the response to 4 consecutive applications of BK (500 pmol/rat) at 20 min intervals: the reflex response faded on the second application of BK (9 out of 9 responders on the first challenge, 5 out of 9 on the second one, *P* < 0.05, Chi² test), with a partial recovery on the last two challenges (8 out of 9 and 7 out of 9 responders at the third and fourth challenge, respectively). To minimize these problems, the dose of BK was increased by a factor of 10 for consecutive additions of peptide (0.05–5000 pmol/rat) in either vehicle- or capsaicin-pretreated rats. The period between the administration of each BK dose was 20 min. The effect of other drug treatments (indomethacin, Hoe 140, SR 140,333 and RP 67,580) was evaluated toward a single dose of BK (500 pmol/rat).

In vehicle-pretreated rats the volume threshold for the activation of the micturition reflex (distension-induced) was 364 $\pm$ 76 μ l (*n* = 8) and the ED₅₀ for the activation of chemoceptive reflex by BK was 3.4 pmol/rat (0.3–40.8 pmol/rat, 95% confidence limits, *n* = 8). In capsaicin-pretreated rats (164 μ mol kg⁻¹, s.c., 4 days before), the volume threshold for the activation of micturition reflex was 1324 $\pm$ 364 μ l (*n* = 8, *P* < 0.05 vs vehicle). In these animals, BK was unable to activate the chemoceptive reflex, since, in a wide range of doses (0.05–5000 pmol/rat) the incidence of activation of the reflex response was never greater than 25% (Figure 1 and Table 1), although a tonic-type contraction was observed in all preparations at the highest doses used (500 and 5000 pmol/rat).

Indomethacin (5.5–11 μ mol kg⁻¹, i.v.) reduced the incidence of reflex urinary bladder contractions induced by a submaximal dose of BK (500 pmol/rat) without affecting their amplitude (Table 2A). However, in those preparations where the chemoceptive reflex was inhibited (7 out of 12 at 5.5 μ mol kg⁻¹ indomethacin), the tonic-type contraction was still present (Table 2A). In order to evaluate the effect of indomethacin on the BK-induced tonic-type contractions, a series of experiments was performed in ganglionectomized animals. Indomethacin (11 μ mol kg⁻¹, i.v.) reduced the amplitude of bladder contraction in rats subjected to the ablation of pelvic ganglia (Table 3). In these preparations, capsaicin pretreatment neither altered the effect of BK nor modified the inhibitory effect of indomethacin on the response induced by BK (Table 3). Indomethacin (11 μ mol kg⁻¹, i.v.) was

Figure 1 Representative traces showing the effects of application of bradykinin (500 pmol/rat) on urinary bladder in control rats (a), ganglionectomized rats (b), capsaicin (164 $\mu\text{mol kg}^{-1}$, s.c.) pretreated rats (c), Hoe 140 (35 nmol kg^{-1} , i.v.) pretreated rats (d).

Table 1 Cumulative dose-response of topical application of bradykinin in vehicle and capsaicin-pretreated rats (164 $\mu\text{mol kg}^{-1}$, 4 days before)

Dose/rat (pmol)	0.05	0.5	5	50	500	5000	Chi ²	ED ₅₀ (c.l.)
<i>Vehicle</i>								
Incidence of reflex contractions	2/8	3/8	3/8	5/8	7/8	8/8	$P < 0.01$	3.4 (0.3–40.8)
Amplitude of reflex contractions (mmHg)	59 ± 17 (n = 2)	46 ± 11 (n = 3)	43 ± 9 (n = 3)	48 ± 10 (n = 5)	55 ± 8 (n = 7)	56 ± 6 (n = 8)		
Amplitude of tonic contraction (mmHg)	5 ± 2 (n = 6)	4 ± 2 (n = 5)	3 ± 2 (n = 5)	6 ± 3 (n = 3)	8 (n = 1)	–		
<i>Capsaicin</i>								
Incidence of reflex contractions	0/8	2/8	2/8	2/8	2/8*	2/8**	NS	n.p.c.
Amplitude of reflex contractions (mmHg)	–	29 ± 1 (n = 2)	29 ± 1 (n = 2)	37 ± 9 (n = 2)	29 ± 1 (n = 2)	44 ± 16 (n = 2)		
Amplitude of tonic contraction (mmHg)	2 ± 2 (n = 8)	2 ± 1 (n = 6)	3 ± 1 (n = 6)	4 ± 1 (n = 6)	12 ± 4 (n = 6)	9 ± 1 (n = 6)		

Chi²: * $P < 0.05$ and ** $P < 0.01$, respectively vs vehicle-pretreated rats. NS = not significant; n.p.c. = not possible to calculate.

Table 2 Effect of i.v. indomethacin on the chemoceptive vesico-vesical reflex induced by topical application of (A) bradykinin (BK, 500 pmol/rat) in vehicle or capsaicin-pretreated rats (164 $\mu\text{mol kg}^{-1}$, 4 days before), and (B) topical capsaicin (15 nmol/rat)

Dose ($\mu\text{mol kg}^{-1}$)		Incidence of the reflex	Amplitude of reflex contractions (mmHg)	Amplitude of tonic contraction (mmHg)
A				
Vehicle	–	11/12	60 ± 5 (n = 11)	8 (n = 1)
Indomethacin	2.75	12/12	46 ± 5 (n = 12)	–
Indomethacin	5.5	7/12	46 ± 5 (n = 7)	9 ± 2 (n = 5)
Indomethacin	11	5/12 ^{oo}	49 ± 9 (n = 5)	13 ± 4 (n = 7)
Caps-Vehicle	–	2/10	30 ± 2 (n = 2)	12 ± 3 (n = 8)
Caps-Indometh	11	1/10	31 (n = 1)	5 ± 1* (n = 9)
B				
Vehicle	–	10/10	44 ± 5 (n = 10)	–
Indomethacin	11	8/10	51 ± 5 (n = 8)	10 ± 5 (n = 2)

Student's *t* test: * $P < 0.05$ vs Vehicle; Chi² test: ^{oo} $P < 0.01$ vs Vehicle.

ineffective against the chemoceptive reflex induced by topical application of capsaicin (15 nmol/rat, Table 2B).

The non-peptide tachykinin NK₁ receptor antagonists, RP 67,580 (10 nmol/rat) or SR 140,333 (10 nmol/rat) blocked the activation of the chemoceptive reflex induced by topical application of BK (500 pmol/rat) while leaving the amplitude of the tonic-type detrusor contraction unaffected (Table 4A and Figure 2c). At the dose inhibiting the reflex response activated by BK, SR 140,333 (10 nmol/rat, i.t.) also completely prevented the activation of chemoceptive vesical reflex induced by topical application of capsaicin (15 nmol/rat); even in this case the tonic-type bladder contraction was preserved (Table 2B and Figure 2).

Table 3 Effect of i.v. indomethacin (11 $\mu\text{mol kg}^{-1}$, i.v.) and of capsaicin pretreatment (164 $\mu\text{mol kg}^{-1}$, 4 days before) on the tonic contraction induced by topical application of bradykinin (500 pmol/rat) in rats subjected to bilateral ablation of pelvic ganglia (1.5 h before)

Pretreatment-treatment	Incidence of reflex	Amplitude of tonic contraction (mmHg)
Vehicle-vehicle	0/8	7.7 $\pm$ 1.4 (n = 8)
Capsaicin-vehicle	0/8	6.2 $\pm$ 0.8 (n = 8)
Vehicle-indomethacin	0/8	3.4 $\pm$ 0.7** (n = 8)
Capsaicin-indomethacin	0/8	1.9 $\pm$ 0.5** (n = 8)

Tukey test: ** $P < 0.01$ vs respective vehicle.

Table 4 Effect of i.t. SR 140,333 and RP 67,580 on the chemoceptive vesico-vesical reflex induced by topical application of (A) BK (500 pmol/rat) or (B) capsaicin (15 nmol/rat)

Dose (nmol/rat)		Incidence of the reflex	Amplitude of reflex contractions (mmHg)	Amplitude of tonic contraction (mmHg)
A				
Vehicle	–	7/8	53 $\pm$ 4 (n = 7)	12 (n = 1)
SR 140,333	1	6/7	43 $\pm$ 7 (n = 6)	14 (n = 1)
SR 140,333	10	0/8**	–	18 $\pm$ 2 (n = 8)
RP 67,580	10	0/8**	–	14 $\pm$ 3 (n = 8)
B				
Vehicle	–	8/9	57 $\pm$ 5 (n = 8)	10 (n = 1)
SR 140,333	10	0/9**	–	20 $\pm$ 3 (n = 9)

Chi² test: ** $P < 0.01$ vs Vehicle.

I.v. administration of the selective B₂ receptor antagonist, Hoe 140 (10 and 35 pmol kg⁻¹) inhibited all the responses produced by topical application of BK (500 pmol/rat) (Table 5A) in a dose-dependent manner but at the dose of 35 nmol kg⁻¹, i.v. Hoe 140 did not alter the response to capsaicin (15 nmol/rat, Table 5B).

As a negative control, the effect of i.t. administration of Hoe 140 was also tested on BK-induced chemoceptive reflex. Surprisingly, Hoe 140 (10 nmol/rat, i.t.) reduced the incidence of the reflex activated by local application of BK onto the urinary bladder (Table 6A and Figure 2b). In order to exclude the possibility that the inhibitory effect of Hoe 140 was due to leakage out of the central nervous system and blockade of peripheral B₂ receptors, some experiments were performed after bilateral removal of pelvic ganglia. In these animals, only the tonic-type detrusor contraction to BK was evident; however, Hoe 140 (10 nmol/rat, i.t.) did not affect this response (Table 6B).

The specificity of the effect of Hoe 140 after i.t. administration was verified on the chemoceptive reflex induced by local application of capsaicin (15 nmol/rat). Hoe 140 blocked the reflex response without modifying the tonic-type contraction (Table 6C and Figure 2e).

Discussion

BK is a potent stimulant of urinary bladder motility both *in vitro* (Maggi et al., 1989) and *in vivo* (Maggi et al., 1993).

Figure 2 Representative traces showing the effects of application of (upper panel) bradykinin (500 pmol/rat) or (lower panel) capsaicin (15 nmol/rat) on urinary bladder in control rats (a and d), in rats pretreated with Hoe 140, 10 nmol/rat, i.t. (b and e), and in rats pretreated with SR 140,333, 10 nmol/rat, i.t. (c and f).

Table 5 Effect of i.v. Hoe 140 on the chemoceptive vesico-vesical reflex induced by (A) topical application of BK (500 pmol/rat) or (B) capsaicin (15 nmol/rat)

Dose (nmol kg ⁻¹)		Incidence of the reflex	Amplitude of reflex contractions (mmHg)	Amplitude of tonic contraction (mmHg)
A				
Vehicle	—	8/10	60 ± 5 (n = 8)	9 ± 5 (n = 2)
Hoe 140	10	3/10	44 ± 18 (n = 3)	3 ± 1 (n = 7)
Hoe 140	35	0/8**	—	1 ± 1 (n = 8)
B				
Vehicle	—	7/8	46 ± 7 (n = 7)	15 (n = 1)
Hoe 140	35	7/8	40 ± 8 (n = 7)	20 (n = 1)

Chi² test: ***P* < 0.01 vs Vehicle.**Table 6** Effect of i.t. Hoe 140 on the chemoceptive vesico-vesical reflex induced by (A) topical application of BK (500 pmol/rat) in intact animals or (B) ganglionectomized animals or (C) topical application of capsaicin (15 nmol/rat) in intact rats

Dose (nmol/rat)		Incidence of the reflex	Amplitude of reflex contractions (mmHg)	Amplitude of tonic contraction (mmHg)
A				
Vehicle	—	8/8	51 ± 6 (n = 8)	—
Hoe 140	1	4/6	45 ± 7 (n = 4)	9 ± 3 (n = 2)
Hoe 140	10	1/8**	52 (n = 1)	6 ± 1 (n = 7)
B				
Vehicle	—	0/8	—	7 ± 1 (n = 8)
Hoe 140	10	0/8	—	8 ± 1 (n = 8)
C				
Vehicle	—	8/8	54 ± 7 (n = 8)	—
Hoe 140	10	2/8**	49 ± 1 (n = 2)	15 ± 3 (n = 6)

Chi² test: ***P* < 0.01 vs Vehicle.

Following topical application onto the urinary bladder, BK induced both high amplitude rhythmic contractions and a low amplitude tonic contraction. On the basis of previous studies in this model (Maggi *et al.*, 1984b; Lecci *et al.*, 1993c), it appears that the tonic contraction is a local response, while the high amplitude phasic contractions represent the activation of a chemoceptive micturition reflex. This assumption was verified in animals subjected to acute, bilateral removal of pelvic ganglia where, following topical application of BK, only the tonic contraction was evident.

In vitro, the urinary bladder contraction induced by BK is capsaicin-resistant, although a release of sensory neuropeptides is produced by BK (Maggi *et al.*, 1989; 1993). The present results show that, *in vivo*, a large component of urinary bladder response to BK is sensitive to capsaicin pretreatment. Since only the reflex response to BK was reduced in capsaicin-pretreated animals, we suggest that BK acts predominantly on the afferent rather than the efferent function of capsaicin-sensitive fibres in the rat urinary bladder. Alternatively, the motor component of the 'efferent' activation of capsaicin-sensitive fibres by BK is masked by other local motor effects induced by BK (Maggi *et al.*, 1989).

The activation of the reflex response by BK or by capsaicin is critically dependent on the integrity of the capsaicin-sensitive sensory innervation as indicated by the failure of BK (present results) or capsaicin (Maggi *et al.*, 1984b) to induce the chemoceptive reflex in capsaicin-pretreated rats. The suppression of the BK-induced chemoceptive reflex by capsaicin pretreatment does not follow a non-specific depression of the distension-induced micturition reflex, because, in similar experimental conditions, the reflexogenic effect of a

selective tachykinin NK₂ receptor agonist was not modified by capsaicin pretreatment (Lecci *et al.*, 1993b). On the other hand, the reflex response induced by BK is not a direct consequence of the BK-evoked, local, tonic-type contraction since capsaicin pretreatment reduces the former but not the latter response. Therefore these results indicate that BK stimulates reflex bladder motility by stimulating those sensory fibres which are sensitive to the neurotoxic effect of topical capsaicin.

Present and previous (Dray *et al.*, 1992) studies indicate that prostanoids play an important role in the activation of reflex responses by BK. In fact indomethacin reduced the incidence of the BK-evoked chemoceptive reflex as well as the BK-induced neuropeptide release from the urinary bladder (Maggi *et al.*, 1989) or from the spinal cord (Andreeva & Rang, 1993). Since BK receptors are present on sensory nerves (Steranka *et al.*, 1988) and BK can directly excite sensory nerves without any contribution of arachidonic acid metabolites (Fox *et al.*, 1993), the relevance of prostaglandins in the stimulatory activity of BK on sensory nerves might vary between different tissues. The present results indicate that in the rat urinary bladder, prostanoids could play a permissive role (sensitization) on BK-induced activation of capsaicin-sensitive nerves, and that a large component of the local motor response produced by BK in the rat bladder is indomethacin-sensitive, thus confirming the results of previous investigations (Maggi *et al.*, 1989; Andersson *et al.*, 1992; Pinna *et al.*, 1992).

Although prostanoids modulate the micturition reflex (distension-induced) through an action on afferent innervation (Maggi *et al.*, 1984a; 1985; 1988), the effect of

indomethacin on the BK-evoked chemoceptive reflex does not depend upon a general depression of bladder-related reflexes. In fact when the chemoceptive reflex was triggered by capsaicin, indomethacin was ineffective, as previously observed for other kinds of capsaicin-induced reflexes (Staszewska-Woolley *et al.*, 1991). These results also indicate that the excitatory effect of BK and that of capsaicin are produced on the same population of bladder afferents through different mechanisms as established for the activation of intracellular effectors by these agents on sensory neurones in culture (Dray *et al.*, 1988a). The hypothesis that BK and capsaicin excite an overlapping population of sensory fibres in the rat urinary bladder, is also supported by the fact that the intrathecal administration of NK₁ receptor antagonists abolished the reflex response induced by either capsaicin (Lecci *et al.*, 1992; 1993a) or BK. These results would suggest that the application of capsaicin or BK onto the serosal surface of the urinary bladder causes the release of tachykinins from capsaicin-sensitive afferent neurones at the spinal cord level and that tachykinins act through NK₁ receptors to induce reflex motor response of the urinary bladder.

BK acts through two different receptors which have been termed B₁ and B₂. However, only B₂ receptors have been reported to mediate *in vivo* responses to BK in the absence of inflammation or other pathological conditions (Hall, 1993, for review). This proposal is in agreement with the finding that both reflex and local responses to topical BK were completely blocked by i.v. administration of the peptide B₂ receptor antagonist, Hoe 140.

Hoe 140 also reduced the incidence of the chemoceptive vesico-vesical reflex induced by BK or capsaicin when administered intrathecally. Despite the fact that this effect occurred at a dose of Hoe 140 which was similar to that producing full inhibition of the response to BK after systemic administration, the inhibition seen after i.t. administration of Hoe 140 does not depend on a leakage of the antagonist out of the central nervous system. On the other hand, systemic administration of Hoe 140 does not inhibit the reflex response induced by the local application of capsaicin. These results suggest that Hoe 140 may possess pharmacological

properties which are not related to the BK antagonism. However, Hoe 140 does not block tachykinin NK₁ receptors in the rat bladder (Maggi *et al.*, 1993) or in the spinal cord (Lopes *et al.*, 1993b), thus excluding an effect of Hoe 140 on tachykininergic transmission. Another possibility is that either capsaicin or BK, when applied at the peripheral level, may stimulate the synthesis of BK-like material within the central nervous system. However, this hypothesis is not supported by previous studies showing that a nociceptive reflex induced by the peripheral application of capsaicin was not blocked by BK receptor antagonists applied to the spinal cord (Dray *et al.*, 1988b).

Although BK-like immunoreactivity (Correa *et al.*, 1979; Perry & Snyder, 1984) as well as BK receptors (Fujiwara *et al.*, 1988; Sharif & Whiting, 1991; Privitera *et al.*, 1992; Lopes *et al.*, 1993a) have been identified in the spinal cord there is no evidence to support BK as a neurotransmitter (Dray *et al.*, 1988b). Despite this, i.t. administration of B₂ receptor antagonists selectively blocked the effects of administration of BK in the spinal cord (Lopes *et al.*, 1993b) and induced an antinociceptive effect (Chapman & Dickenson, 1992) in a model sensitive to i.t. administration of NK₁ receptor antagonists (Yamamoto & Yaksh, 1991; Birch *et al.*, 1992; Chapman & Dickenson, 1993). The real significance of the inhibitory action of i.t. administered Hoe 140 on the reflex response elicited by peripherally applied BK or capsaicin, remains to be established.

In conclusion, the present findings indicate that BK and capsaicin excite with a different mechanism (indomethacin-sensitive and resistant, respectively) a common population of sensory fibres which use tachykinins (and possibly BK) as neurotransmitters for activation of a chemoceptive micturition reflex. This effect of BK is mediated by B₂ receptors in the bladder wall. These findings provide further evidence that BK and tachykinin receptor antagonists may prove useful for treatment of irritative symptoms of cystitis.

This work has been supported by IMI contract No. 56665. We are grateful to Drs X. Emonds Alt, C. Garret and K. Wirth for gifts of SR 140,333, RP 67,580 and Hoe 140, respectively.

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(Received February 11, 1994
 Revised September 15, 1994
 Accepted October 4, 1994)