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The role of the capsaicin-sensitive innervation of the rat urinary bladder in the activation of micturition reflex

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Summary. 1. Capsaicin applied on the serosal surface of the urinary bladder in urethane-anaesthetized rats produces two distinct types of motor effects: a) a tetrodotoxin-, hexamethonium- and lidocaine-insensitive 'tonic' contraction and b) a series of tetrodotoxin-, hexamethonium- and lidocaine-sensitive rhythmic contractions.

2. Both 'tonic' and rhythmic contractions are abolished by bladder denervation indicating their neurogenic origin. The rhythmic but not the 'tonic' component of the contractile effect of capsaicin is abolished by spinal cord transection indicating activation of a supraspinal micturition reflex.

3. The motor effects of topical capsaicin are unaffected by pretreatment with indomethacin or diphenhydramine plus cimetidine.

4. Pretreatment with a large dose of subcutaneous (SC) capsaicin increases both volume and pressure threshold for micturition while amplitude of micturition contraction is unaffected. Moreover the spinal somatovesical reflex elicited by pinching of the perineal skin is unaffected by capsaicin-desensitization.

5. The intracerebroventricular (ICV) administration of capsaicin reproduces the effects of SC capsaicin on the bladder response to saline filling. Rats pretreated with ICV capsaicin are as sensitive as controls in reacting to noxious heat (hot plate test) while the wiping response to instillation of capsaicin into one eye was abolished.

6. These findings provide functional evidence for the presence in the rat urinary bladder of a capsaicin-sensitive innervation which subserves a sensory function in relaying volume/pressure information from detrusor muscle to central nervous system. Information carried through these capsaicin-sensitive fibers appears to be relevant for initiation of a supraspinal vesico-vesical micturition reflex. Functional evidence indicates that these fibers may terminate at supraspinal level.

Key words: Capsaicin – Micturition reflex – Rat urinary bladder – Somatovesical reflex – Chemogenic efferent responses

Introduction

The urinary bladder is innervated by parasympathetic and sympathetic nerve fibers whose activation provides the efferent input (excitatory and inhibitory) to detrusor muscle (Alm and Elmer 1975). Afferent impulses generated by activation of mechanoreceptors in the bladder wall provide the information for a series of reflexes regulating micturition (Mahony et al. 1977). However, until recently, little attention has been paid to investigate the nature of the neurotransmitter system(s) involved in the afferent branch of micturition reflex.

Capsaicin, the pungent ingredient of red peppers, affects certain primary afferent nerve fibers which provide sensory information for various types of reflex responses (Coleridge and Coleridge 1977; Cervero and McRitchie 1982; Lembeck and Skofitsch 1982; Szolcsányi 1984). Although the intimate mechanism(s) of action of capsaicin is undefined, some evidence indicates that release of neuropeptide(s) stored in the sensory fibers (Gamse et al. 1980, 1981a; Saria et al. 1983, 1984) could be involved in the acute excitatory effects of this substance on reflex responses (Maggi et al. 1984a, 1985a, f).

Although at present time there is not a neurophysiological, biochemical or anatomical marker which specifically labels the capsaicin-sensitive fibers the action of this substance is, at least in certain systems, sufficiently selective to allow its use as a tool for exploring the sensory neuron function (Szolcsányi 1984a). In fact the selectivity of neurotoxic effect of capsaicin on visceral autonomic innervation was proved by the observation that capsaicin-desensitization does not modify either cholinergic, sympathetic or non-adrenergic non-cholinergic neurotransmission (Szolcsányi 1984a).

Experimental evidence indicates that, in rats, a capsaicin-sensitive mechanism regulates micturition threshold by relaying to the central nervous system information on the volume of fluid present in the bladder (Sharkey et al. 1983; Maggi et al. 1984a, 1985a; Holzer-Petsche and Lembeck 1984; Santicioli et al. 1985). In particular topical application of capsaicin on the bladder dome activates a series of neurogenic rhythmic contractions which were assumed to correspond to micturition reflex (Maggi et al. 1984a) while systemic capsaicin-desensitization increases bladder capacity (Sharkey et al. 1983; Holzer-Petsche and Lembeck 1984; Santicioli et al. 1985).

In the present study we investigated in further detail the effects of acute administration of capsaicin and of systemic capsaicin desensitization on bladder response to reflex activation to establish the potential relationship between these effects of capsaicin and the supraspinal vesico-vesical micturition reflex which subserves bladder voiding in rats (Sato et al. 1975; Satoh et al. 1978; Maggi et al. 1985b).

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Materials and methods

Male albino rats, Wistar-Morini strain, weighing 340–360 g were anaesthetized with subcutaneous urethane (1.2 g/kg) and the left jugular vein was cannulated for drug injection. Reasons for using urethane as an anaesthetic were detailed elsewhere (Maggi et al. 1985b).

Transurethral or transvesical recording of intravesical pressure. Through a midline incision of the abdomen the urinary bladder was exposed, emptied of urine by application of a slight manual pressure and prepared for recording of intraluminal pressure as follows: through a small urethral incision a polyethylene tubing (1.0 mm I.D., 1.5 mm O.D.) was inserted into the urinary bladder and secured in place by means of a silk ligature as described previously (Maggi and Meli 1982, 1983). The tubing was connected to a pressure transducer and the whole system filled with saline (0.9% NaCl). Pressure signals were delivered to a H.P. 8805B carrier amplifier and displayed on a H.P. four channel polygraph. Warm saline soaked cotton wool swabs were laid around the exteriorized organ to maintain its temperature and keep it moist. After a 15 min equilibration period at zero volume the bladder was rapidly filled with 0.2–0.5 ml of warm saline (37° C).

In some experiments (transvesical recording) saline was infused by means of a 20 gauge needle inserted into the bladder dome at a rate (2.8 ml/h) which simulates a maximal hourly diuresis value for this species (Maggi and Meli 1983). In these latter experiments the bladder was free to void in response to saline infusion. The needle was connected to a double lumen catheter which allowed simultaneous recording of intravesical pressure and saline infusion. The tubing was connected through a peristaltic pump to a saline reservoir (Maggi et al. 1985b).

Topical application of substances. In most experiments capsaicin was applied topically in a volume of 0.1 ml of warm (37° C) saline on the bladder dome as described previously (Maggi and Meli 1982). The suitability of this technique for testing the effects of substances on bladder response to saline filling has been established previously (Maggi et al. 1984a, b). Controls received the vehicle.

Experiments in spinal rats. In some experiments, spinal rats were obtained by severing the cord at the level of the intervertebral space T12-L1 under ether anaesthesia. The skin was closed with wound clips and the animals allowed to recover for 3 h before the induction of urethane anaesthesia. In other experiments spinalization was performed at the level of the intervertebral space C2-C3 under urethane anaesthesia. These animals were artificially ventilated by means of a Harvard respirator for small rodents. Hypogastric nerves were bilaterally cut to prevent the inhibitory effect of the sympathetic nervous system on bladder motility since intraspinal surgical procedures enhance the sympathetic outflow to the bladder (Maggi et al. 1985c).

Denervation of the rat urinary bladder. In other experiments we tried to assess whether or not the tetrodotoxin-resistant component of the capsaicin-induced contractions of the rat bladder (cf. Maggi et al. 1984a, 1985a) were of neurogenic origin. Bladder denervation was achieved under ether anaesthesia by bilateral removal of pelvic ganglia either 3 h (acute denervation) or 48–72 h (chronic denervation) before application of capsaicin. The skin was closed with wound clips and the animals received SC benzathine-benzylpenicillin 200,000 I.U. In chronic denervation experiments the animals received SC bethanechol 5 mg/kg 24–48 h before killing to facilitate bladder voiding and avoid overdistension. At the time of the experiment the rats were anaesthetized with urethane and prepared for transurethral recording of intraluminal pressure as described above.

Excitatory somato-vesical reflex. In other experiments (intraluminal pressure was recorded by the transurethral route) we assessed the effects of capsaicin desensitization on the excitatory cutaneo-vesical reflex. This was obtained as described by Sato et al. (1975) by a 5 s pinching of a localized area (about 5 × 5 mm) of the perineal skin by means of a forceps. In these experiments the bladder was filled with a small amount of saline (0.2 ml), insufficient to elicit the vesico-vesical micturition reflex. Pinching at 10–15 min intervals gave fairly reproducible bladder contractions for at least 2 h.

Systemic capsaicin desensitization. Capsaicin (50 mg/kg, dissolved in a vehicle containing 10% Tween 80, 10% ethanol and 80% saline) was administered subcutaneously 4 days before the experiment. Control rats received the vehicle. This treatment produces in adult rats a decrease of neuropeptide content in the urinary bladder (Holzer et al. 1982).

Effect of intracerebroventricular (ICV) capsaicin on pain sensitivity and cystometrograms. Male albino Wistar-Morini rats weighing 200–230 g were used. Capsaicin (10 mg/ml dissolved in 60% dimethylsulphoxide, control rats received the vehicle) was injected under ether anaesthesia by means of an Hamilton microsyringe at a total dose of 200 μg (50 + 50 + 100 μg at 1 h interval) (Gamse et al. 1981b). ICV injection was made through a polyethylene cannula implanted into the lateral ventricle 48–72 h before the experiment. Atropine (1 mg/kg) was administered intraperitoneally 15 min before the first two injections of capsaicin (Gamse et al. 1981b).

Twenty hours after the last capsaicin treatment the cutaneous thermal pain sensitivity was determined by the hot plate test at 55 ± 0.3° C. The time elapsed between placing the animal on the hot plate and licking of the fore or hindpaws (latency period) was determined by an observer unaware of treatment. In these animals the response to topical instillation of one drop of 0.01% capsaicin into one eye (chemogenic pain) was also investigated. Other animals were anaesthetized 20 h after the last capsaicin dose with SC urethane (1.2 g/kg) and prepared for recording of the bladder response to transvesical infusion of saline as described above. Saline infusion was made at a physiological-like filling rate (0.046 ml/min) until micturition occurred or for 60 min.

Statistical analysis. All data in the text are mean ± SE. Statistical analysis of the data was performed by means of the Student's *t*-test for paired or unpaired data when applicable. Statistical analysis of the effects of substances on the number of responders was performed by means of the chi square method (Yates correction).

Drugs. Drugs used were: capsaicin (Sigma, St. Louis, MO, USA), tetrodotoxin (Sankyo, Tokyo, Japan), atropine HCl

(Serva, Heidelberg, FRG), hexamethonium bromide (Serva), lidocaine (Sigma), acetylcholine HCl (Merck, Darmstadt, FRG), diphenhydramine HCl (Sigma), cimetidine (Sigma), and indomethacin (Merck). All substances for topical or intravenous administration were dissolved in a 0.9% NaCl solution (saline). For topical application a stock solution of capsaicin (2 mg/ml) was prepared in absolute ethanol and then diluted in saline.

Results

General

Rapid distension of the urinary bladder by infusion of 0.5 ml of saline in urethra-ligated rats activated a series of high-amplitude (16–42 mm Hg) rhythmic contractions which were suppressed by topical tetrodotoxin (20 μg in 0.1 ml, $n = 6$) or lidocaine (2 mg in 0.1 ml, $n = 6$) and by intravenous hexamethonium (20 mg/kg, $n = 6$). In acute spinal rats (C2-C3, $n = 6$; T12-L1, $n = 6$) rapid distension of the urinary bladder did not induce rhythmic contractions higher than 4–12 mm Hg ($n = 6$). Bilateral section of the pelvic but not hypogastric nerves prevented the appearance of distension-induced rhythmic contractions ($n = 6$). In some experiments when distension-induced rhythmic contractions had reached steady state, withdrawal of saline (0.3 ml) resulted in disappearance of rhythmic contractions ($n = 6$). This indicates that the repetitive feature of these contractions depends upon the failure of the bladder to void.

Taken as a whole these observations indicate that the high-amplitude rhythmic bladder contractions elicited in these experimental conditions represent a repetitive vesico-vesical micturition reflex of supraspinal origin (Maggi et al. 1984a, b).

In about 50–60% of the preparations ('non responders') rapid infusion with 0.5 ml failed to elicit neurogenic rhythmic contractions presumably because bladder activation was prevented by an inhibitory sympathetic reflex (Maggi et al. 1985d). These preparations were used to study the excitatory effects of capsaicin on bladder motility (next paragraph). The effect of topical capsaicin in 'responders' were described in detail by Maggi et al. (1984a).

Effect of topical capsaicin in 'non responders'

Capsaicin (2 μg in 0.1 ml) applied on the bladder dome of 'non responders' produced a transient 'tonic' contraction (15.3 ± 0.7 mm Hg, $n = 42$) followed in about 70% of preparations (29/42) by the appearance of a series of rhythmic contractions (see Figs. 1 and 2) whose characteristics are like those of the distension-induced rhythmic contractions (cf. Maggi et al. 1984a). Duration of these capsaicin-induced rhythmic contractions ranged 3–12 min.

Pretreatment with topical tetrodotoxin (20 μg in 0.1 ml), lidocaine (2 mg in 0.1 ml) or intravenous hexamethonium (20 mg/kg i.v. 5 min before) did not modify amplitude of the 'tonic' contraction (Table 1) but, in the majority of preparations, prevented the appearance of the capsaicin-induced rhythmic contractions (Table 1, Fig. 1).

Neither tetrodotoxin, lidocaine or hexamethonium had any significant effect on amplitude of 'tonic' contractions produced by topical acetylcholine (18 μg in 0.1 ml). This dose of acetylcholine activated rhythmic bladder contractions only in 8 out of 30 preparations (27%). This value

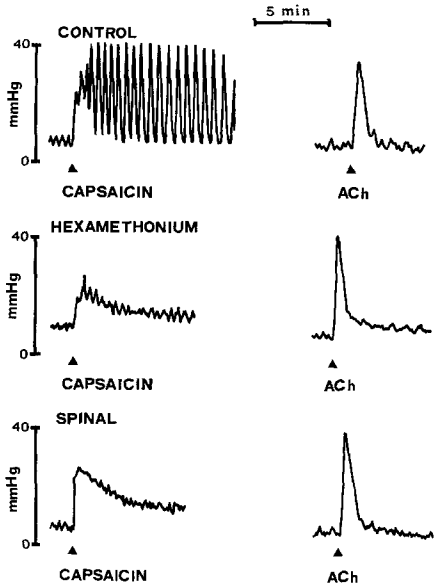

Fig. 1. Typical tracings from three different preparations illustrating the motor effect of topical application of capsaicin (2 μg in 0.1 ml) or acetylcholine (ACh, 18 μg in 0.1 ml) on the bladder dome of control (*upper panels*), hexamethonium-pretreated (20 mg/kg, 5 min before, *middle panels*) or acute spinal (C2-C3, *lower panels*) rats

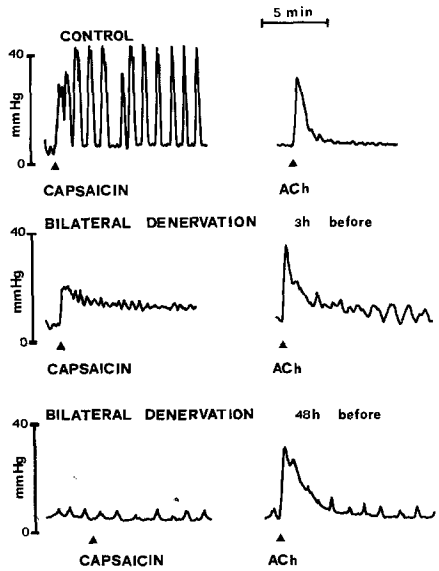

Fig. 2. Typical tracings from three different preparations illustrating the motor effects of topical application of capsaicin (2 μg in 0.1 ml) or acetylcholine (ACh, 18 μg in 0.1 ml) on the bladder dome of control rats or at various times (3 h, *middle panels*; 48 h, *lower panels*) after bilateral removal of pelvic ganglia (bladder denervation)

is significantly ($P < 0.01$) lower than that observed following capsaicin (Table 1).

Effect of topical capsaicin in acute spinal rats

Capsaicin (2–10 μg) applied on the bladder dome of acute spinal rats (C2-C3, $n = 7$; T12-L1, $n = 6$) produced a 'tonic' contraction whose morphology, amplitude and duration were similar to those observed in controls (Table 1). Amplitude of the capsaicin-induced 'tonic' contraction is

Table 1. Effect of various pretreatments on the motor effects produced by topical application of capsaicin (2 μg in 0.1 ml) or acetylcholine (18 μg in 0.1 ml) on the bladder dome of urethane-anaesthetized rats

Treatment	Dose	Route	Response to capsaicin		Response to acetylcholine	
			Amplitude of 'tonic' contraction (mm Hg)	Activation of rhythmic contractions	Amplitude of contraction (mm Hg)	Activation of rhythmic contractions
Controls	–	–	15.3 ± 0.7	29/42	30 ± 3	8/30*
Tetrodotoxin	10 μg	topical	15.9 ± 1	0/18***	26 ± 2	0/18**
Hexamethonium	20 mg/kg	intravenous	15.7 ± 2	1/12***	32 ± 2	0/12**
Lidocaine	2 mg	topical	15.4 ± 2	3/14***	27 ± 3	0/14**
Acute spinal rats	–	–	16.1 ± 2	0/13***	30 ± 3	0/13**
Bladder denervation						
Acute 3 h	–	–	16.0 ± 2	0/8***	33 ± 4	0/8
Chronic 48 h	–	–	–	0/8***	26 ± 2	0/8

* Significantly different from capsaicin group, $P < 0.01$
** Significantly different from controls, $P < 0.05$
*** Significantly different from controls, $P < 0.01$

not influenced by the level at which spinal cord is transected. The 'tonic' contractile response to topical acetylcholine (18 μg in 0.1 ml) was unaffected by spinal cord transection (Table 1). In none of the spinal rats did capsaicin or acetylcholine activate the rhythmic bladder contractions (Table 1, Fig. 1).

Motor effects of topical capsaicin following acute or chronic denervation of the rat urinary bladder

Following acute denervation (3 h) bladder weight did not differ significantly from that of controls (not shown). On the other hand bladders excised 48 – 72 h after denervation were heavier (240 ± 19 mg, $n = 8$, $P < 0.01$) as compared to controls (109 ± 10 mg, $n = 5$). Following acute denervation topical capsaicin (2 μg) produced a 'tonic' contraction whose characteristics (amplitude, morphology and duration) were like those of the tetrodotoxin-insensitive contractions observed in controls (Fig. 2, Table 1). In none of these preparations did topical capsaicin induce the high-amplitude rhythmic contractions observed in controls (Fig. 2, Table 1). On the other hand, following 'chronic' denervation topical capsaicin (2 – 10 μg) had almost no contractile effect (Fig. 2, Table 1).

In denervated preparations topical acetylcholine (18 μg in 0.1 ml) produced a 'tonic' contraction whose amplitude was not significantly different from that observed in controls (Fig. 2, Table 1).

Effect of indomethacin or diphenhydramine plus cimetidine on the motor effects of topical capsaicin

In certain systems capsaicin activates prostaglandin synthesis (Juan et al. 1980). Indomethacin-pretreatment (0.5 mg/kg i.v., 15 min before) did not prevent or reduce, as compared to controls, the 'tonic' (16 ± 1 mm Hg, $n = 15$) or the rhythmic bladder contractions (11 out of 15 cases) produced by topical capsaicin.

On the other hand indomethacin-pretreatment reduced significantly ($P < 0.05$) duration of the capsaicin-induced rhythmic contractions (4.2 ± 0.5 min, $n = 11$) as compared to controls (7 ± 0.6 min, $n = 26$). This may be due to the fact that indomethacin alone reduces bladder responsiveness to distension since a continuous production of arachidonic acid metabolites may be required for the maintenance of a rhythmic contractile activity (Maggi et al. 1984b).

Experimental evidence suggests that the acute motor effects of capsaicin on the rat bladder are due to release of endogenous substance *P* (Maggi et al. 1984a, 1985a) which could release endogenous histamine from mast cells (Lembeck and Gamse 1982).

As compared to controls, pretreatment with intravenous diphenhydramine (5 mg/kg) plus cimetidine (10 mg/kg) did not prevent or reduce the 'tonic' (14 ± 2 mm Hg, $n = 8$) or the rhythmic bladder contractions (7 out of 8 cases) produced by topical capsaicin (2 μg in 0.1 ml).

Effect of systemic capsaicin desensitization on micturition threshold

The transvesical infusion of saline (2.8 ml/h) in control rats ($n = 13$) elicited micturition at a threshold volume of 0.85 ± 0.1 ml. Pressure threshold and amplitude of micturition contractions were 4.0 ± 0.5 and 26 ± 3 mm Hg, respectively. In these experimental conditions micturition depends upon activation of a supraspinal vesico-vesical micturition reflex (Maggi et al. 1985b).

Systemic pretreatment with a large dose of capsaicin (50 mg/kg SC, 4 days before, $n = 13$) increased both volume (1.91 ± 0.1 ml, $P < 0.01$, $n = 9$) and pressure (5.8 ± 0.8 mm Hg, $P < 0.05$) threshold while amplitude of micturition contraction (23 ± 3 mm Hg) was unaffected.

In 4 out of 13 capsaicin-pretreated rats saline infusion did not produce micturition during a 40 min period. In these preparations, after an initial flat phase of the cystometrogram a bladder hypertonus was observed (16 – 25 mm Hg) during which drops of fluid were expelled periodically (overflow incontinence). At this time application of a noxious somatic stimulus (pinching of the perineal area, see below) induced a phasic bladder contraction (8 ± 2 mm Hg, $n = 4$) which expelled 1 or 2 drops of fluid. This was followed by a transient (1 – 3 min) and partial (2 – 6 mm Hg) reduction of intraluminal pressure. Perineal pinching did never

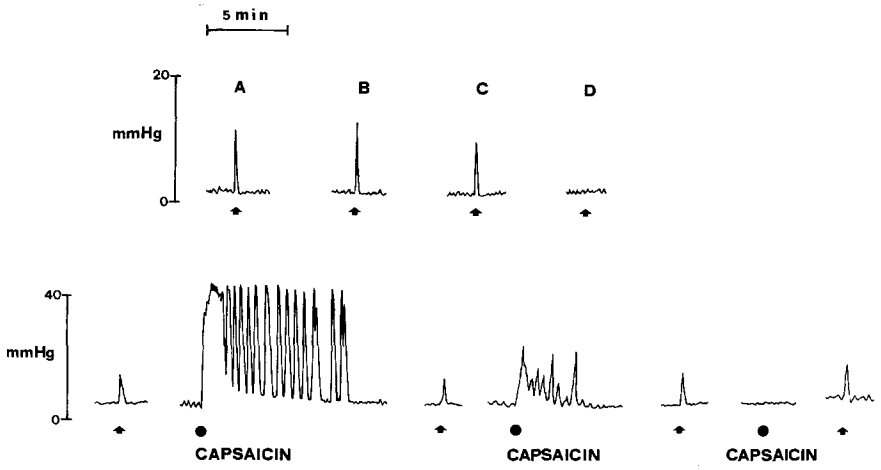

Fig. 3. *Upper panel:* typical tracings of two control responses (*A*, *B*) to perineal pinching (at the arrows, excitatory somatovesical reflex) obtained at 10 min intervals. In *C* the response was obtained in the same animal 3 min after the i.v. injection of atropine, 1 mg/kg. In *D* the somatovesical reflex is abolished by topical application of tetrodotoxin (20 μg in 0.1 ml) on the bladder dome (4 min before). *Lower panels:* typical tracings illustrating the bladder response to perineal pinching (at the arrows, excitatory somatovesical reflex) obtained at 20 min intervals. During these intervals the bladder was challenged with topical capsaicin (2 μg in 0.1 ml). Note that following capsaicin-desensitization the amplitude of the somatovesical reflex is unaffected

induce a full micturition response either in vehicle- (data not shown) or capsaicin-pretreated animals.

Effect of capsaicin-desensitization on excitatory somato-vesical reflex in adult rats

These experiments were performed in urethra-ligated rats. Pinching of the perineal skin produced phasic bladder contractions which were reproducible at 10–20 min intervals. Amplitude of the pinching-induced phasic bladder contraction (7 ± 1 mm Hg, $n = 12$) was reduced by 15–20% following i.v. atropine (1 mg/kg, 3 min before, $n = 6$, Fig. 3) and suppressed by intravenous hexamethonium (20 mg/kg, $n = 6$) or topical tetrodotoxin (20 μg in 0.1 ml) ($n = 8$, Fig. 3). It has been shown previously (Sato et al. 1975) that in adult rats the neurogenic response produced by pinching of the perineal skin represents a reflex organized at spinal level. This was confirmed in present experiments since pinching-induced phasic bladder contractions can be observed in acute spinal rats (C2-C3) within a short time (2 h) from spinal cord transection ($n = 4$).

Capsaicin desensitization (50 mg/kg SC, 4 days before) did not modify amplitude of pinching-induced phasic bladder contractions (6.5 ± 1 and 7 ± 1 mm Hg for controls and capsaicin-pretreated rats, $n = 8$ and 9, respectively). Likewise 'local' desensitization obtained by repeated topical application of capsaicin (2–10 μg in 0.1 ml) on the bladder dome did not modify amplitude of the somatovesical excitatory reflex (Fig. 3, $n = 5$).

Effect of ICV capsaicin on pain sensitivity and micturition reflex

ICV capsaicin produced an acute reaction similar to that described by Gamse et al. (1981 b). In capsaicin-pretreated animals ($n = 14$) the chemogenic pain response induced by instillation of capsaicin in one eye was abolished while vehicle-treated animals responded with wiping movements (12 ± 2 in 2 min, $n = 15$) and blepharospasm. These experiments confirm that ICV capsaicin produces desensitization

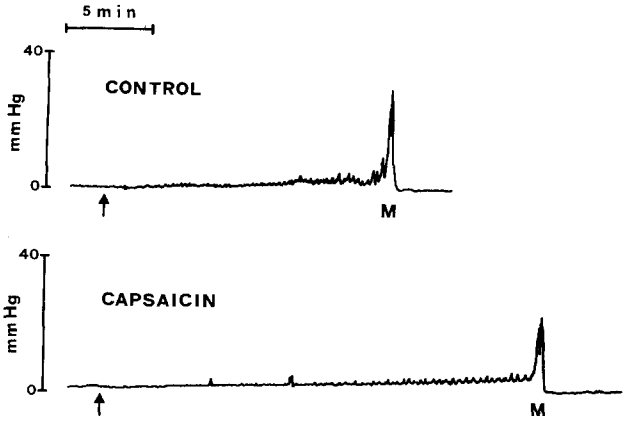

Fig. 4. Typical tracings illustrating the response (micturition at *M*) of the rat urinary bladder to transvesical infusion of fluid in a vehicle- (control, upper panel) or capsaicin-pretreated (200 μg ICV 20 h before) rat. The saline infusion (0.046 ml/min) started at the arrows

of medullary capsaicin-sensitive fibers arising from the trigeminal area (Gamse et al. 1981 b).

On the other hand cutaneous thermal sensitivity, which is carried through capsaicin-sensitive fibers terminating at spinal level, is unaffected by ICV capsaicin (licking latency was 14 ± 3 and 15 ± 3 s, $n = 15$ and 14 in vehicle- and capsaicin-pretreated rats, respectively). The number of capsaicin-pretreated rats whose first response consisted in licking of forepaws (9/14) does not differ from that of controls (7/15). In the remainders (both vehicle- and capsaicin-pretreated) licking of the hindlimbs was the first response observed.

These findings are consistent with the observation that ICV capsaicin reduces substance *P* content in the medulla oblongata but not in the lumbosacral dorsal spinal cord (Gamse et al. 1981 b), that is, the level at which the afferent fibers from the bladder enter the CNS (Nadelhaft and Booth 1984).

The transvesical infusion of saline (Fig. 4) in vehicle-treated rats ($n = 19$) elicited a micturition response hav-

ing the following characteristics: pressure threshold 4.1 ± 0.3 mm Hg, volume threshold 0.736 ± 0.1 ml, amplitude of micturition contraction 30 ± 2 mm Hg. Pretreatment with ICV capsaicin ($n = 16$) increased significantly both pressure (9.7 ± 2 mm Hg, $P < 0.01$) and volume threshold (1.268 ± 0.2 ml, $P < 0.02$). On the other hand amplitude of micturition contractions (29 ± 2 mm Hg) was unaffected by pretreatment with ICV capsaicin. A flat volume-pressure curve preceded micturition both in vehicle- and capsaicin-pretreated animals (Fig. 4). In two capsaicin-pretreated rats no micturition response was obtained following a 60 min infusion period (volume threshold > 2.76 ml). These findings indicate that ICV capsaicin produces a selective impairment of the afferent branch of the micturition reflex similar to that described following its systemic administration.

Discussion

Neurogenic nature of the motor effects of capsaicin: functional evidence for the presence of a capsaicin-sensitive innervation in the rat urinary bladder

Topical application of capsaicin on the outer surface of the urinary bladder produced: a) a tetrodotoxin-resistant 'tonic' contraction followed by b) a series of tetrodotoxin-sensitive rhythmic contractions. Since the rat bladder is almost devoid of intramural ganglion cells it could be assumed that the motor effects observed following topical application of substances on its dome depend upon an action on smooth muscle cells or nerve endings (cf. Maggi et al. 1984b). As shown to occur in the isolated guinea pig ileum and trachea (Szolcsányi 1984) it is possible that capsaicin-induced tetrodotoxin-resistant contraction of the rat bladder is due to release of neurotransmitter(s) from sensory nerve endings in the bladder wall (Maggi et al. 1984a). This hypothesis, strengthened by the observation that capsaicin-induced contractions of the isolated rat bladder are selectively antagonized by a substance *P* antagonist (Maggi et al. 1985a), is substantiated by present experiments indicating that capsaicin does not produce contractions in denervated bladders.

The 'tonic' contraction produced by topical capsaicin is unaffected by lidocaine or tetrodotoxin in concentrations which prevent both the distension- and the capsaicin-induced rhythmic bladder contractions. Likewise, chemical activation of the sensory nerve endings by capsaicin induces a neurogenic inflammatory response which is resistant to tetrodotoxin or local anaesthetics (Szolcsányi 1984b). It appears therefore that the tetrodotoxin-resistant component of the motor effect of capsaicin in the rat bladder represents the ability of certain bladder mechanoreceptors to release the stored neuropeptide(s) in response to environmental (chemogenic) stimuli, i.e. an 'efferent' function in the sense proposed by Szolcsányi (1984a). The capsaicin-induced neuropeptide(s) release from sensory nerve endings in the bladder wall might produce a series of biological effects ranging from activation of micturition reflex (Maggi et al. 1984a) smooth muscle contraction (Maggi et al. 1984a, 1985b) and induction of changes in vascular tone and permeability (Saria et al. 1983, 1984). Further studies are needed to establish the natural stimuli for triggering the 'efferent' function of the capsaicin-sensitive fibers of the urinary bladder and, consequently, define their potential pathophysiological role.

Role of the capsaicin-sensitive innervation in the initiation of micturition: functional evidence that fibers arising from the rat bladder terminate at supraspinal level

Our past (Maggi et al. 1984a) and present findings indicate that the tetrodotoxin- and lidocaine-sensitive rhythmic bladder contractions produced by topical capsaicin represent activation of a micturition reflex of supraspinal origin which is induced through stimulation of sensory elements in the bladder wall.

In adult rats certain vesicoexcitatory reflex(es) are organized at spinal level (Sato et al. 1975; Maggi et al. 1985c). However, the amplitude of these spinal reflexes (4–16 mm Hg) is lower than that (>20 mm Hg) required for fluid emission. In rats (Sato et al. 1975; Satoh et al. 1978; Maggi et al. 1985b, e) as in cats and humans (Blaivas 1982), micturition requires an intact connection between a pontine micturition center (Satoh et al. 1978) and the sacral parasympathetic center (Nadelhaft and Booth 1984) which provides the preganglionic excitatory input to the bladder. Satoh et al. (1978) described the existence of a vesico-excitatory center in the dorsolateral tegmentum of the pons whose destruction leads to urine retention. This observation agrees well with our findings indicating that neurogenic bladder contractions in response to bladder filling at a physiological-like rate are of supraspinal origin (Maggi et al. 1985b, e). Therefore we have evidence that either mechanical (saline filling) or chemical stimulation of bladder mechanoreceptors can activate an afferent discharge of sufficient intensity to trigger the supraspinal vesico-vesical micturition reflex in bladders containing a subthreshold amount of fluid.

Systemic capsaicin desensitization increased micturition threshold without affecting amplitude of the distension-induced neurogenic contractions. Moreover amplitude of field stimulation-induced contractions was unaffected by neonatal capsaicin desensitization (Sharkey et al. 1983; Santicioli unpublished data). These findings confirm previous hypothesis (Sharkey et al. 1983; Maggi et al. 1984a) that the capsaicin-sensitive innervation of the rat bladder does not play a major role in determining the contractile response of the detrusor during micturition contraction. This conclusion is further supported by the observation that capsaicin-desensitization does not modify amplitude of the spinal excitatory somatovesical reflex.

The capsaicin-sensitive fibers carrying a somatic information appear to terminate in the spinal cord at level of lamina I and II (Fitzgerald 1983). Primary afferent fibers from the rat bladder enter the spinal cord at L6-S1 level (Nadelhaft and Booth 1984) but the exact localization of the second neuron(s) in the micturition reflex pathway has not been determined. Neurophysiological evidence indicates that some sensory fibers from cat detrusor muscle enter the spinal cord at sacral level without synapsing and then ascend at supraspinal level to complete the afferent branch of the micturition reflex (Mahony et al. 1977). Our findings provide functional evidence that the capsaicin-sensitive fibers arising from the rat bladder terminate at supraspinal level to provide the information required for initiation of the vesico-vesical reflex leading to micturition. This conclusion stems from

the observation that ICV capsaicin reproduces the effect of systemic capsaicin desensitization on the bladder response to saline filling. It is worth mentioning that Jhamandas et al. (1984) reported that intratechal capsaicin increases bladder capacity. Since capsaicin produces its neurotoxic effect even at axonal level (Jancsó et al. 1980) it appears conceivable that intrathecal capsaicin could have affected the primary afferent fibers from the rat bladder in the spinal cord even if they terminate at supraspinal level.

Application of horseradish peroxidase to the central end of the transected rat pelvic nerve revealed, among other projections, the presence of a bundle just ventral to the medullary canal ('medial afferent bundle') (Nadelhaft and Booth 1984) which contains substance *P* nerve fibers through the entire spinal cord (Ljungdahl et al. 1978; Wiesenfeld-Hallin et al. 1984). This bundle is absent in the spinal cord of capsaicin-desensitized animals (Fitzgerald 1983). Taken as a whole these observations led to the hypothesis that one function of the 'medial afferent bundle' (Nadelhaft and Booth 1984) is to carry information on bladder volume to supraspinal structures which activate micturition. Quite obviously this hypothesis does not rule out the possibility that some capsaicin-sensitive primary afferent nerve fibers provide synaptic input for the vesicovesical reflexes which, under certain experimental conditions, could be demonstrated in spinal rats (Maggi et al. 1985c).

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