J. Pharm. Pharmacol. 1986, 38: 446–451
Received November 11, 1985

Functional evidence for the existence of a capsaicin-sensitive innervation in the rat urinary bladder

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Capsaicin (0·03–3 μM) induces contractions of the rat isolated bladder which are unaffected by either atropine (3 μM) or tetrodotoxin (0·5 μM). In the presence of capsaicin (0·1 μM) an enhancement of field stimulation-induced contractions was observed. Capsaicin-desensitization did not modify the height of these. The neurogenic nature of the capsaicin-induced contractions was proved by the observation that 'chronic' (48 h) denervation prevented, while 'acute' (2 h) denervation did not modify the effect of capsaicin. Denervated bladders maintained their responsiveness to acetylcholine but not to field stimulation. Isolated bladders from rat pups (1–2 days old) did not respond to capsaicin while strong contractile responses to acetylcholine or field stimulation were obtained in these preparations. In bladders from two week old animals, capsaicin produced similar contractions to those observed in preparations from adult animals. The bladders from rats receiving a high dose of capsaicin (50 mg kg^{-1} s.c.) at birth were heavier than those of their age-matched, vehicle-treated controls. Isolated bladders from 2 month old animals pretreated with capsaicin at birth were unresponsive to capsaicin while responsiveness to acetylcholine, substance P or field stimulation was unaffected compared with that of vehicle-treated controls. These experiments provide evidence that a capsaicin-sensitive innervation exists in the rat urinary bladder which undergoes a postnatal development at end organ level.

Capsaicin, the pungent ingredient of red peppers has been shown to possess a selective neurotoxic action (Szolcsányi & Barthó 1978, 1979; Szolcsányi 1982, 1984; Nagy, 1982) on certain unmyelinated nerve fibres involved in the afferent branch of various reflex responses at cardiovascular and respiratory level (Coleridge et al 1965; Coleridge & Coleridge 1977, 1984; Dixon et al 1980).

According to Szolcsányi (1982, 1983, 1984), activation of these capsaicin-sensitive afferents also elicits a local efferent response mediated by their endings which serves a dual sensory-efferent function, visceral motility and neurogenic inflammation, in response to certain irritants being evoked in this way.

By use of capsaicin as a chemical tool for exploring the function of sensory neuron mechanisms (Nagy 1982; Szolcsányi 1984), preliminary evidence was provided indicating that, in the rat urinary bladder, a capsaicin-sensitive mechanism regulates the micturition threshold, possibly by relaying information to the central nervous system (CNS) on the degree of distension of the detrusor muscle (Maggi et al 1984a, 1985; Holzer-Petsche & Lembeck 1984; Santicioli et al 1985). Further evidence indicated that substance P (or a related tachykinin) could be released from the capsaicin-sensitive endings in this tissue (Maggi et al 1984a, 1985). This hypothesis was also supported by the observation that nerve fibres containing substance P-like immunoreactivity are present in the rat bladder and disappear following capsaicin pretreatment (Sharkey et al 1983). Moreover, the substance P content of rat bladder was strongly reduced (over 80% reduction) by pretreatment with a high dose of s.c. capsaicin (Holzer et al 1982).

In the rat isolated bladder, capsaicin produced a tetrodotoxin (TTX)-insensitive contraction (Maggi et al 1985) as did its topical application to the urinary bladder of urethane-anaesthetized animals (Maggi et al 1984a). We proposed that, by analogy with previous findings in the guinea-pig ileum (Barthó & Szolcsányi 1978; Barthó et al 1982), tracheobronchial smooth muscle (Szolcsányi & Barthó 1982; Szolcsányi 1983) and ureter (Saria et al 1983; Lundberg et al 1984), the TTX-resistant component, of capsaicin's effect could be due to release of neurotransmitter(s) from sensory nerve endings in the bladder wall (Maggi et al 1984a).

We now have explored in further detail the effect of capsaicin on the rat isolated urinary bladder

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(Maggi et al 1985) and attempted to ascertain, by means of denervation experiments, whether capsaicin-induced contractions are of neurogenic origin. Moreover, since a marked postnatal development occurs in the rat bladder, relative to myogenic contractile activity and postganglionic excitatory innervation (Maggi et al 1984b,c), we have also investigated the bladder response to capsaicin in bladder preparations from newborn rats.

MATERIALS AND METHODS

One to three day old albino rats (Wistar-Morini strain) of either sex received s.c. capsaicin (dissolved in 10% ethanol, 10% polysorbate 80 and 80% NaCl 0·9%) at a dose of 50 mg kg^{-1}. Experiments were performed two months after treatment and results obtained in capsaicin-treated animals were compared with those obtained in controls (vehicle-treated). Experiments in adult animals were with adult, male albino rats, Wistar Morini strain, 360–400 g.

Either newborn (1–14 days old), or adult, male rats, were killed by cervical dislocation and exsanguinated. The whole urinary bladder was rapidly removed, bisected in the sagittal plane and placed in a 5 ml organ bath containing Krebs solution at 37 °C. The composition of Krebs solution was (mM): NaCl 119, $NaHCO_3$ 25, KCl 4·7, $MgSO_4$ 1·2, $CaCl_2$ 2·5, KH_2PO_4 1·2 and glucose 11. The solution was continuously bubbled with a mixture of 96% O_2 and 4% CO_2.

Since the urinary bladder from animals treated neonatally with capsaicin was enlarged compared with controls in these experiments, a strip of the detrusor about 2 mm wide and 10 mm long was excised longitudinally from the base to the dome. At the end of the experiments the strips were blotted three times on filter paper and weighed. Contractile force developed by the detrusor strips was expressed as mg per mg of wet weight. A similar procedure was used for studying the effects of capsaicin on the isolated bladder from ganglionectomized animals.

The preparations were connected, under a constant load of 1 g (0·25–0·5 g in the case of bladders from 1–14 day old rats) to an isometric strain gauge and contractile tone was recorded by means of a Basile 7050 Unirecord Poligraph. Field stimulation was carried out by means of two platinum wire electrodes placed at the top and the bottom of the organ bath and connected to a Grass S11 stimulator.

Square wave pulses of supramaximal intensity (60 V) were delivered at a frequency of 0·1 (continuously) or 20 Hz (trains of 5 s every 60 s). Pulse width was 1 ms. Frequency response curves (FRC) (frequency range 0·1–20 Hz) were obtained, either in control or capsaicin-pretreated animals as described previously (Maggi et al 1984d). The effect of each frequency of stimulation was observed for 6 min. After a 30 min equilibration period an FRC was obtained at 15 min intervals until reproducible responses (usually the third one) were recorded, as described by Maggi et al (1984d). Concentration-response curves (CRC) for the contractile effect of capsaicin were tested after a 60 min equilibration period only once in each preparation. In some experiments a non-cumulative CRC to either acetylcholine or substance P was recorded by adding increasing concentrations of these substances at 20 min intervals until maximal responses were obtained.

Denervation of the bladder was achieved by bilateral removal of the pelvic ganglion. The rat bladder is almost devoid of intramural ganglion cells (Carpenter & Rand 1965; Elmer 1978) while postganglionic neurons are located within the pelvic ganglia which form a distinct plexus on the lateral surface of the prostate gland (Purinton et al 1973). Afferent nerve fibres having their neuronal soma in the dorsal root ganglia travel through the pelvic nerves and the pelvic ganglia (Nadelhaft & Booth 1984). In these experiments the pelvic ganglia were bilaterally removed, under ether anaesthesia, 24 or 48 h ('chronic denervation') before killing. 'Acute' denervation (2 h before excision of the bladder) was made under urethane anaesthesia (1·2 g kg^{-1} s.c.). The skin was closed with wound clips and the animals received benzathine-benzylpenicillin 200 000 iu. In 'chronic' denervation experiments the animals received bethanechol 2·5 mg kg^{-1} s.c. 24 or 48 h before killing to allow some bladder voiding and to prevent the adverse effect of overdistension on bladder contractility.

Statistical analysis

All data in the text are means ± s.e. Statistical analysis of the data was performed by means of Student's *t*-test for paired or unpaired data when applicable.

Drugs

Drugs used were: capsaicin (Sigma), atropine HCl (Serva), tetrodotoxin (TTX, Sankyo), hexamethonium bromide (Serva), acetylcholine HCl (Fluka), substance P (Serva), benzathine benzylpenicillin (Wyeth), bethanechol HCl (Urecholine, Merck).

A stock solution of capsaicin (10 mM) was pre-

pared with absolute ethanol and then diluted with Krebs solution.

To minimize binding of polypeptides to glassware, both the organ bath and the microsyringe used for drug administration were treated with 2·5% dichloromethylsilane in benzene for 20 min and then rinsed in water before use.

RESULTS

Contractile effect of capsaicin on the isolated urinary bladder from adult rats

Low concentrations of capsaicin (0·01–0·03 μM) enhanced the amplitude of the spontaneous phasic contractile activity of the urinary bladder and produced a delayed and slight increase in tone of the preparation (see Fig. 1).

Higher concentrations of capsaicin (0·03–3 μM) produced a contraction within a few seconds. At maximally effective concentrations (1–3 μM) the contraction was biphasic, i.e. a first rapid contraction was followed by a slow return to the baseline value. This latter 'tonic' component was characterized by superimposed phasic contractions whose amplitude was higher than that of spontaneous phasic contractile activity observed in the absence of capsaicin (Fig. 1).

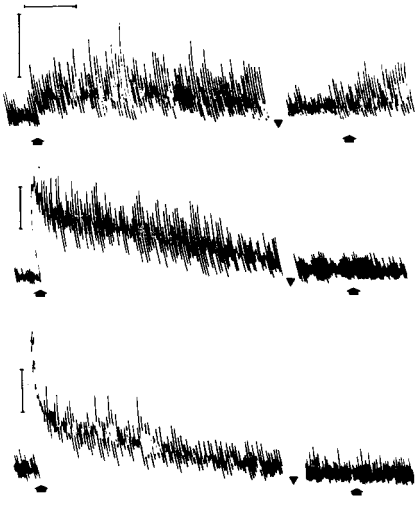

FIG. 1. Typical tracings showing the contractile effect of various concentrations of capsaicin (0·03 μM upper panel, 0·1 μM middle panel, 3 μM lower panel) on the isolated urinary bladder from adult rats. In each panel capsaicin was administered at the arrows twice in each preparation. The second challenge occurred 3 h after the first. Inverted triangles indicate washing out of capsaicin. Calibration bars: horizontal 5 min, vertical 1 g.

Capsaicin was kept in contact with the bladder tissue for 5 min or more until a maximal contractile response was obtained. In some experiments (Fig. 1) capsaicin was kept in contact for a longer time to observe the decay of its contractile effect. Tone recovery was usually complete within 20–30 min from the administration of the highest concentrations of capsaicin. At this time a phasic contractile activity having amplitude greater than that observed before capsaicin administration was still present in some preparations (Fig. 1).

Capsaicin removal by washing out resulted in a prompt recovery of resting tone and spontaneous activity of the preparation.

When the preparations had been exposed (5–30 min) to the higher capsaicin concentrations (0·1 μM or more) a second addition (up to 3 h from the first) had no further contractile effect (Fig. 1). A second exposure to lower concentrations (0·03 μM or less) still enhanced the motor activity of the bladder (Fig. 1).

The response to capsaicin was concentration-dependent in the range of 0·01–3 μM. The maximal contractile effect corresponded roughly to 20–25 and 30–40% compared with the effect produced by acetylcholine (3 mM) or field stimulation (20 Hz), respectively.

In the presence of TTX (0·5 μM) or atropine (3 μM) both amplitude and time course of capsaicin-induced contractions were similar to those observed in controls (n = 8).

Acute effect of capsaicin and capsaicin-desensitization on contractions induced by field stimulation

In the course of in-vivo experiments we observed that topical capsaicin transiently increased the amplitude of distension-induced neurogenic rhythmic contractions of the rat bladder (Maggi et al 1984a). Therefore we studied its effect on nerve-mediated contractions of the rat isolated urinary bladder. Field stimulation-induced contractions (0·1 Hz) were unaffected by hexamethonium (10 μM, n = 5), reduced (about 10%) by atropine (3 μM) and almost abolished (over 90% inhibition) by tetrodotoxin (0·5 μM, n = 6). This indicated their dependence upon neurotransmitter release from postganglionic nerve endings. Field stimulation-induced contractions were recorded before and after addition of capsaicin (0·1 μM) to the organ bath. In 6 out of 8 preparations in the presence of capsaicin (5–10 min from its administration), the amplitude of contractions evoked by field stimulation was enhanced (32 ± 6%, n = 6)

compared with controls (Fig. 2). This effect disappeared rapidly following capsaicin removal by washing out. In control experiments 5–10 min interruption of field stimulation did not affect significantly the amplitude of electrically-induced contractions.

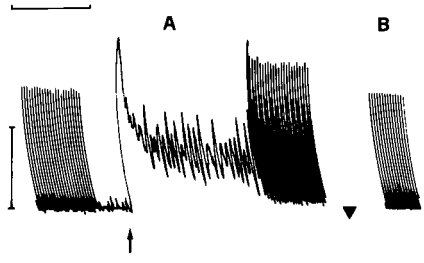

FIG. 2. Panel A is a typical tracing showing enhancement of nerve-mediated contractions of isolated urinary bladder from adult rats by capsaicin (0·1 μM). Capsaicin application is marked by an arrow. The inverted triangle indicates washing out of capsaicin. Panel B shows the nerve-mediated response of the same preparation 3 h after the challenge with capsaicin. Note that capsaicin desensitization did not alter amplitude of field stimulation-induced contractions. Calibration bars; horizontal 5 min, vertical 1 g.

In preparations desensitized to capsaicin (i.e. those receiving a high dose of capsaicin 1–3 μM, 1–3 h before) the amplitude of contractions to field stimulation (0·1–20 Hz) was similar to that observed in controls.

Effect of capsaicin following acute and chronic denervation of the rat urinary bladder

Following 'acute' denervation (2 h before, n = 6), bladder weight (121 ± 15 mg, wet weight, n = 6) did not significantly differ from that of controls (114 ± 13 mg, n = 6). On the other hand bladders excised 24 or 48 h after denervation ('chronic' denervation) were significantly ($P < 0·01$) heavier (174 ± 8 and 225 ± 15 mg, respectively, n = 6 for each group) compared with controls. The contractile response to acetylcholine (1 μM) was unaffected by denervation while the response to field stimulation (0·1–20 Hz) was significantly reduced following 'chronic' but not 'acute' denervation (Fig. 3). Responsiveness to capsaicin (3 μM) was almost abolished in bladders excised 48 h after bilateral removal of pelvic ganglia (Fig. 3). On the other hand responsiveness to capsaicin was maintained in bladders excised 2 or 24 h following bladder denervation (Fig. 3).

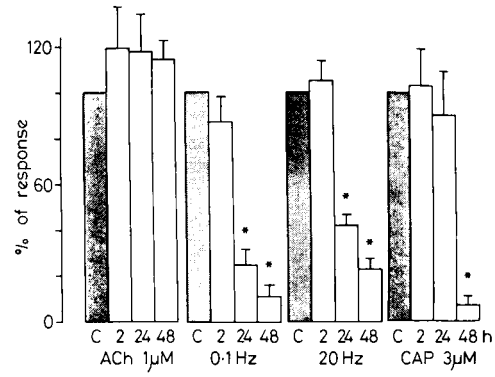

FIG. 3. Effect of denervation on response of the urinary bladder from adult rats to acetylcholine (1 μM, ACh), field stimulation (0·1–20 Hz) and capsaicin (3 μM). Each value is mean ± s.e. of 6 experiments. Denervation of the rat urinary bladder was achieved by bilateral removal of pelvic ganglia 2, 24 or 48 h before the experiment. C = Control. * Significantly different from control $P < 0·01$.

Postnatal development of contractile effect of capsaicin on the rat urinary bladder

Postganglionic excitatory (efferent) innervation of the rat or urinary bladder undergoes marked developmental changes in the early postnatal period (Maggi et al 1984b,c). For this reason it appeared worthwhile to investigate the effects of capsaicin (3 μM) on the isolated bladder from rat pups. It was found that bladders from 1–2 day old rats were almost unresponsive (n = 6) to capsaicin (Fig. 4A) although at this time strong contractile responses to acetylcholine (0·001–3 mM) or field stimulation (1–20 Hz, cf Maggi et al 1984b) could be recorded. In 3 out of 6 of these preparations capsaicin produced the

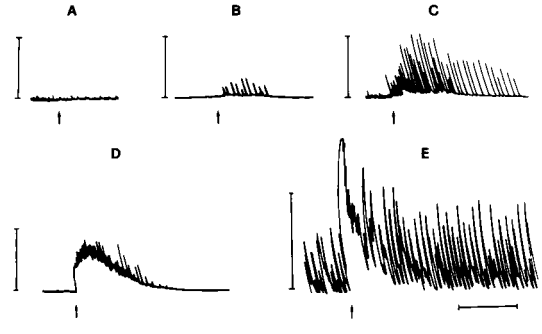

FIG. 4. Typical tracings showing postnatal development of the capsaicin-induced contraction of the isolated rat urinary bladder. Capsaicin (3 μM) was administered at the arrows. Tracings A and B were obtained from preparations of 1 day old rats, tracing C from a 3 day old rat, tracing D from a 6 day old rat and tracing E from a 14 day old rat. Calibration bars: horizontal 5 min, vertical 0·25 g for A B C and D, and 1 g for E.

appearance of a short duration (2–4 min), low-amplitude, phasic contractile activity resembling the spontaneous contractile activity exhibited by the isolated bladders from rat pups at a later stage of development (5–7 days, cf Maggi et al 1984b) but, even in these preparations, capsaicin did not increase the tone (Fig. 4B).

In preparations from 5–7 day old rats, capsaicin produced a tonic contraction which lasted for 5–10 min (Fig. 4D); in these preparations amplitude of capsaicin-induced contractions amounted to 5 or less % of response to acetylcholine (3 mM).

In preparations from rat pups aged 14–18 days capsaicin produced contractions similar to those observed in bladders from adult animals (Fig. 4E). Amplitude of these contractions amounted to 10–20% of those induced by acetylcholine (3 mM).

Capsaicin-induced contractions of the isolated bladder from rat pups desensitized in the same manner as described for bladders from adult animals.

Effect of capsaicin on isolated bladder from rats treated with capsaicin at birth

Bladders from rats receiving a high dose of capsaicin (50 mg kg^{-1} s.c.) at birth were significantly heavier compared with those of their age-matched controls (Santicioli et al 1985, cf also Sharkey et al 1983; Holzer-Petsche & Lembeck 1984). Isolated bladders from 2 month old capsaicin-treated animals (at birth) did not respond to capsaicin (3 μM). On the other hand, bladders from age-matched, vehicle-treated animals responded to capsaicin in a manner similar to adult animals. Neither the FRC (0·1–20 Hz) nor the CRC to acetylcholine (0·01–1000 μM) or substance P (0·001–3 μM) in bladders from capsaicin-pretreated animals differed from those obtained in bladders from their age-matched, vehicle-treated controls (data not shown).

DISCUSSION

Our data provide functional evidence for the existence of a capsaicin-sensitive innervation in the rat urinary bladder whose activation produces motor responses which do not require an intact neural connection between the detrusor muscle and the central nervous system. We previously reported that application of capsaicin on the outer surface of the urinary bladder of urethane-anaesthetized rats produces a TTX-resistant 'tonic' contraction followed by a series of TTX-sensitive rhythmic contractions. The latter were ascribed to activation of a repetitive micturition reflex (Maggi et al 1984a). Therefore the capsaicin-induced TTX-insensitive contractions of the rat isolated bladder (cf also Maggi et al 1985) appear to be similar to the TTX-resistant component of the effect observed following topical application of capsaicin to the bladder dome of urethane-anaesthetized rats (Maggi et al 1984a).

As described for the guinea-pig isolated ileum (Barthó & Szolcsányi 1978; Barthó et al 1982) and trachea (Szolcsányi & Barthó 1982; Szolcsányi 1983), the capsaicin-induced TTX-resistant contractions of the rat urinary bladder may be due to the release of neurotransmitter(s) from sensory nerve endings in the bladder wall (Maggi et al 1984a).

The present experiments show that chronic, but not acute, denervation made the rat bladder unresponsive to capsaicin although the response to acetylcholine was unchanged. Since the ability of the bladder muscle to respond to direct activation is preserved, the loss of the responsiveness to capsaicin may be attributed to degeneration of the capsaicin-sensitive sensory nerve endings in the bladder wall. Thus, in spite of their insensitivity to TTX, the capsaicin-induced contractions of the rat isolated bladder are neurogenic.

It appears conceivable that the capsaicin-sensitive innervation of the rat urinary bladder, in the sense proposed by Szolcsányi (1982, 1983, 1984), plays a dual sensory-efferent function in regulating the micturition threshold. Capsaicin, in low concentrations, produces a marked increase in amplitude of the spontaneous contractile activity of the rat bladder and similar effects could be observed in response to low concentrations of tachykinins (unpublished data). Since the capsaicin-induced TTX-insensitive neurogenic contractions of the rat bladder are antagonized by a substance P-antagonist (Maggi et al 1985) it is conceivable that they are produced through the release of small amounts of endogenous tachykinins.

Amplitude of the myogenic contractile activity of the rat bladder is a major determinant for triggering the micturition reflex (Maggi et al 1984b,d). Therefore the TTX-insensitive local release of mediator(s) from the capsaicin-sensitive nerve endings might be involved in modulating afferent discharge from the detrusor to CNS through an amplifying effect on spontaneous contractile activity of the bladder muscle.

Moreover, as suggested by present experiments, a local release of mediators from the capsaicin-sensitive nerve endings might ensure a more efficient bladder emptying by increasing the contractions due to activation of postganglionic elements in the bladder wall.

We have recently shown that rat urinary bladder undergoes marked developmental changes in the early postnatal period as far as myogenic contractile activity and postganglionic excitatory innervation are concerned (Maggi et al 1984b,c). Taking the capsaicin-induced TTX-resistant contraction of the rat isolated bladder as an indirect measure for the presence of a capsaicin-sensitive sensory innervation, we now have evidence indicating that, at birth, such innervation is not developed at end organ level.

The capsaicin-sensitive innervation of the rat bladder appears to be involved in relaying to the CNS information relative to bladder volume (Sharkey et al 1983; Maggi et al 1984a; Holzer-Petsche & Lembeck 1984; Santicioli et al 1985). Afferent information from the detrusor muscle provides the basis for the vesico-vesical excitatory micturition reflex subserving bladder voiding in adult animals (De Groat & Ryall 1969; De Groat 1975). Absence of a capsaicin-sensitive innervation in the early postnatal period has a functional counterpart represented, in rat pups, by the cutaneo-vesical excitatory reflex which subserves bladder voiding (cf Maggi et al 1984b).

Acknowledgement

We wish to thank Prof. Janos Szolcsányi, Department of Pharmacology, University of Pecs, Hungary, for his helpful advice and suggestions.

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