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Tachykinin-independent activity of capsaicin on in-vitro lamb detrusor

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Abstract

The capsicum alkaloid capsaicin is an afferent fibre exciter. In the vesical bladder, capsaicin acts by releasing peptides stored in afferent fibres. The aim of this work was to verify the activity of capsaicin on in-vitro lamb urinary bladder and to ascertain whether this alkaloid evokes peptide release. Capsaicin relaxed about 80 % of the lamb detrusor muscle preparations tested and contracted about 20 %. Whereas neurokinin A and substance P antagonists, administered alone or together, left the contractile responses to capsaicin unchanged, atropine and tetrodotoxin totally inhibited contraction. Ruthenium red and indometacin abolished contractions and relaxation. The substance P and neurokinin A antagonists and the NO-synthesis inhibitor N^{ω}-nitro-L-arginine methyl ester (L-NAME) left relaxation unchanged; conversely, the calcitonin gene-related peptide antagonist αh-CGRP (8–37) abolished this response. These results suggest that capsaicin relaxes lamb detrusor muscle not through tachykinins but by releasing CGRP from afferent fibres. Our observation that indometacin blocks the capsaicin response in in-vitro lamb urinary bladder also suggests a role of prostanoids.

Introduction

Capsaicin, a *Capsicum* alkaloid, is an afferent fibre exciter (Holzer 1991). Numerous studies have described the actions of capsaicin on vesical bladder (Maggi 1992). Exposure of mouse isolated bladder to capsaicin (1 μM) produces a gradual rising contraction and markedly enhances the amplitude of spontaneous contractions. In hamster isolated bladder detrusor, the same concentration of capsaicin produces prompt, slight, sustained relaxation. Conversely, exposure of rabbit bladder strips to capsaicin 1–10 μM affects neither tone nor spontaneous motor activity (Maggi et al 1987).

Capsaicin acts by releasing peptides stored in afferent fibres. It chiefly releases the tachykinins, calcitonin gene-related peptide (CGRP) and somatostatin (Maggi et al 1992; Szolcsányi et al 1998). In rat vesical bladder, capsaicin produces a rapid contraction that consists of a phasic component due to substance P and a tonic component, with superimposed phasic contraction due to neurokinin A. Tissue contraction can be prevented by using substance P and neurokinin A antagonists (Maggi et al 1991a). In a previous study we found that neurokinin A strongly contracted isolated lamb detrusor muscle whereas neurokinin B induced only slight contractions and substance P induced none (Tucci et al 2001).

The aim of this work was to verify the actions of capsaicin on lamb urinary bladder and to ascertain whether this alkaloid evoked neurokinin A release.

Materials and Methods

Drugs

The drugs used (molecular weight and dilution solvent in parentheses) were: capsaicin (MW 305.42; ethanol), MEN-10376 (MW 1081.23; distilled water), sendide (MW 856.05; distilled water) and α-human-calcitonin gene-related peptide (8–37) (αh-CGRP)

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(MW 3125.62; distilled water), obtained from Neosystem (Strasbourg, France); atropine sulfate (MW 676.8; ethanol), carbachol (MW 182.6; ethanol), indometacin (MW 357.8; ethanol), N^{ω}-nitro-L-arginine methyl ester (L-NAME) (MW 269.7; ethanol) and tetrodotoxin (MW 319.3; ethanol), obtained from Sigma Chemical Co. (St Louis, MO); and ruthenium red (MW 786.35; distilled water), obtained from Research Biochemicals International (RBI).

Control experiments showed that the ethanol concentrations used in our study to dissolve the drugs left the response of the detrusor strips unchanged.

All other chemicals were analytical grade.

Tissue preparation

Lambs, 10 ± 1 kg, of both sexes were used. The lambs, killed by electroshock for alimentary purpose, were bled rapidly at the slaughterhouse (A.S.L. Rome B, Presidio Centro Carni). Immediately after killing, the urinary bladder was removed. A centrally located strip of detrusor muscle (2 mm wide, 8 mm long) was excised along the longitudinal axis and transferred to a 10-mL isolated organ bath containing Krebs solution of the following millimolar composition (Thornbury et al 1995): NaCl 120, KCl 5.9, $MgCl_2$ 1.2, $CaCl_2$ 2.5, NaH_2PO_4 1.2, $NaHCO_3$ 25.0 and glucose 11.0. The bladder was then stored at 3–4°C until use. Tissues were tested within a few hours of removal.

Experimental procedure

Detrusor strips were stretched to a passive tension of 4 g (previously determined as the tension for optimal responses in this preparation). The Krebs solution was maintained at 37°C and gassed with oxygen (95%) and carbon dioxide (5%). Strips were connected to a Basile force displacement transducer (Type 7006, Ugo Basile) and changes in tension were recorded on a Basile 7050 Unirecord. After equilibration for 60 min, the preparations were activated for 5 min with carbachol (5.5×10^{-7} M). This solution was left in contact with the tissue for 5 min and applied twice or more at 20-min intervals until reproducible contractions were obtained.

Cumulative concentration–response curves were recorded by adding increasing quantities of capsaicin (3.3×10^{-7} to 2.6×10^{-6} M). The effect of cumulative capsaicin on the lamb detrusor strip was then observed in different experimental conditions. Atropine, 9.0×10^{-7} M, was added either 10 min before capsaicin (3.3×10^{-7} to 2.6×10^{-6} M) or at the plateau of the response to capsaicin. In other experimental sessions, the following substances were applied to the tissue before capsaicin was added: sendide (a substance P antagonist) (7.0×10^{-7} M), MEN-10376 (a neurokinin A antagonist) (5.5×10^{-7} M), ruthenium red (a selective inhibitor of sensorial neuropeptide release evoked by capsaicin) (1.3×10^{-6} M), αhCGRP(8–37) (a CGRP antagonist) (6.4×10^{-7} M), L-NAME (a nitric oxide synthesis antagonist) (3×10^{-5} M), tetrodotoxin (3.0×10^{-7} M) or indometacin (6×10^{-7} M).

Drug concentrations and contact times were chosen by preliminary experiments and in agreement with Maggi et al (1991b), Chiba et al (1989), Patacchini et al (1999) and Bolle & Tucci (1998).

Statistics

Responses were measured in grams and are presented as means ± s.d. of 10–30 experiments. The data were subjected to repeated-measures analysis of variance. All-pairs multiple comparisons were assessed by the Student–Newman–Keuls test. All differences were accepted as significant at the 0.05 level of probability. All statistical analyses were performed on a personal computer with Sigma Stat version 2.03 (Jandel Scientific Software Corporation).

Results

Effect of cumulative capsaicin on lamb detrusor

Capsaicin elicited two distinct responses in detrusor strips from lamb urinary bladder: in 20% of the preparations

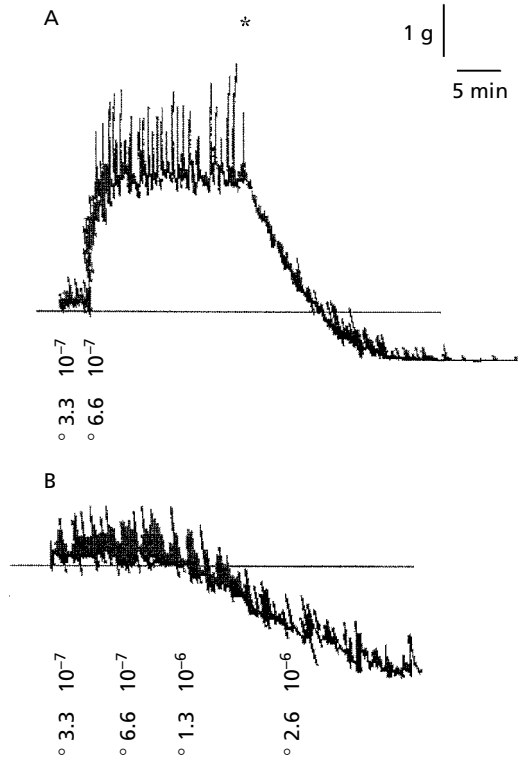

Figure 1 Representative traces of a lamb detrusor strip showing contraction (A) and relaxation (B) evoked by cumulative concentrations of capsaicin (M). °, Capsaicin. The straight line represents initial basal tone. *Washout.

Table 1 Effect of the tested substances on capsaicin-evoked contraction in lamb detrusor strips.

Substances	No. of tissues	Contraction (g)
Control	30	+3.7±2.7
Atropine (9.0×10^{-7} M)	30	Ø
MEN-10376 (5.5×10^{-7} M)	10	+3.4±0.6
Sendide (7.0×10^{-7} M)	10	+3.6±1.3
Tetrodotoxin (3.0×10^{-7} M)	30	Ø
Indometacin (6.0×10^{-7} M)	30	Ø

Values are expressed as mean±s.d. of tissues responding by contracting. Ø = abolished.

Table 2 Effect of the tested substances on capsaicin-evoked relaxation in lamb detrusor strips.

Substances	Relaxation (g)
Control	−2.0±1.1
Atropine (9.0×10^{-7} M)	−2.5±1.0
MEN-10376 (5.5×10^{-7} M)	−2.2±1.3
Sendide (7.0×10^{-7} M)	−1.9±0.9
Tetrodotoxin (3.0×10^{-7} M)	−1.8±0.9
Ruthenium red (1.3×10^{-6} M)	−0.08±0.03*
L-NAME (3.0×10^{-5} M)	−1.7±0.7
α h-CGRP (6.4×10^{-7} M)	−0.08±0.03*
Indometacin (6.0×10^{-7} M)	−0.08±0.03*

Values are expressed as mean±s.d. of 10 tissues responding by relaxing. *$P < 0.05$, vs control (analysis of variance).

tested it induced a contraction followed by relaxation and in 80% it induced relaxation alone (Figure 1). Subsequent capsaicin applications did not prevent the relaxation response (Tables 1 and 2).

Effect of the tested substances on capsaicin-evoked contraction in lamb detrusor strips

Contraction started at capsaicin concentrations from 9.9×10^{-7} M (+3.7±2.7 g). In lamb isolated detrusor strips pre-treated with atropine, capsaicin induced no contractions. Atropine added to the responsive tissues 10 min after capsaicin, when the contractile response plateaued, caused a rapid fall in the dose–response curve and markedly reduced basal tone (Figure 2C).

The antagonists sendide and MEN-10376 left the contractile responses induced by capsaicin in lamb urinary bladder unchanged. When added to the bath containing contracting tissues at the plateau phase, they again failed to alter the contractile response (Figure 2B). In detrusor strips pre-treated with tetrodotoxin, capsaicin induced no contractions (Table 1).

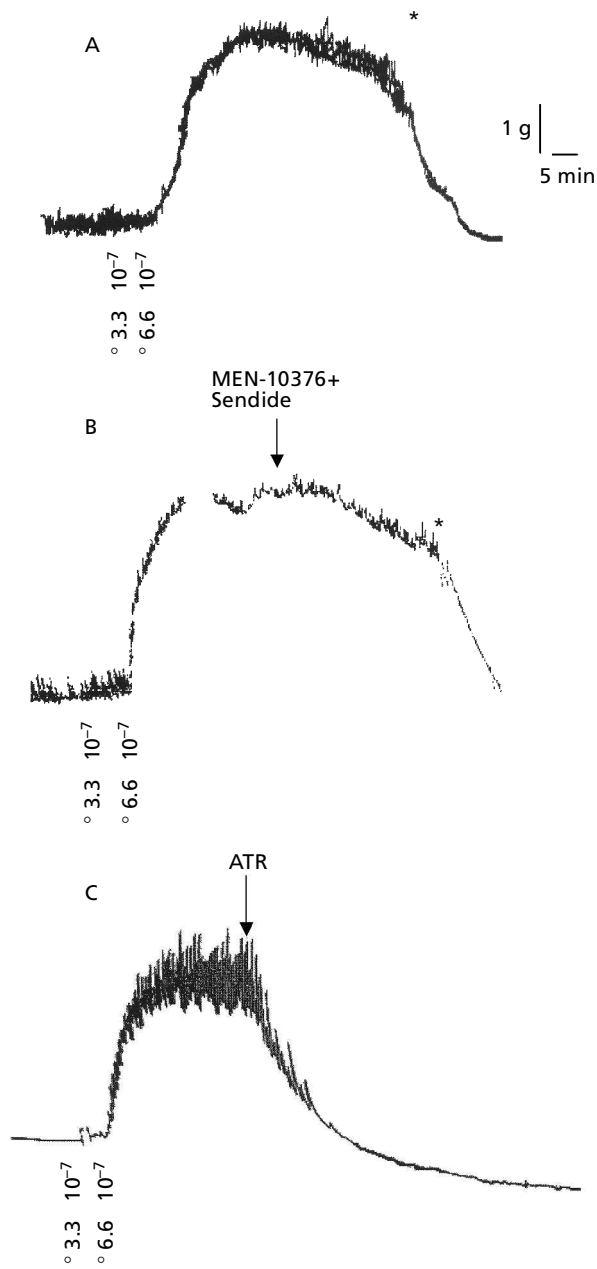

Figure 2 Representative traces of a lamb detrusor strip showing contraction evoked by cumulative concentrations of capsaicin (3.3×10^{-7} to 6.6×10^{-7} M) (A), effect of MEN-10376 (5.5×10^{-7} M) and sendide (7.0×10^{-7} M) on capsaicin-evoked contraction (B) and effect of atropine (ATR) 9.0×10^{-7} M on capsaicin-evoked contraction (C). °, Capsaicin. The straight line represents initial basal tone. *Washout.

Effect of the tested substances on capsaicin-evoked relaxation in lamb detrusor strips

At concentrations starting from 9.9×10^{-7} M, and in most tissue preparations tested (80%), capsaicin induced relaxation (−2.0±1.1 g). Whereas pre-treatment with αh-CGRP completely prevented capsaicin relaxation, pre-treatment with atropine, sendide, MEN-10376, tetrodotoxin, and L-NAME had no effect (Table 2).

Effect of ruthenium red and indometacin on capsaicin-evoked response in lamb detrusor strips

In detrusor strips pre-treated with ruthenium red, capsaicin induced neither contraction nor relaxation.

The anti-inflammatory agent indometacin added to the medium 60 min before capsaicin invariably prevented contraction and strongly inhibited capsaicin-induced relaxation (Tables 1 and 2).

Discussion

In the lamb urinary bladder detrusor tissue tested in this study, capsaicin induced two distinct responses: contraction followed by relaxation or relaxation alone. Unexpectedly, neurokinin A and substance P antagonists given either alone or together left the capsaicin-induced contractions unchanged. By contrast, atropine relaxed the capsaicin-contracted tissues and pre-treatment with this muscarinic antagonist prevented the contractions, thus suggesting a cholinergic mechanism.

Ruthenium red effectively inhibited the capsaicin-induced relaxation, whereas tetrodotoxin did not. Because substance P and neurokinin A antagonists had no influence on relaxation, this effect could depend on a factor other than tachykinins released by capsaicin-sensitive fibres. CGRP relaxes the detrusor muscle of numerous species (guinea-pig and dog) but not every (rat, man) species (Maggi 1995). In lamb detrusor muscle, capsaicin-induced relaxation is most probably mediated by CGRP released after capsaicin treatment. Accordingly, capsaicin-induced relaxation is completely inhibited by αh-CGRP (8–37). Relaxation does not depend on nitric oxide (NO) since an inhibitor of NO synthesis, L-NAME, leaves capsaicin-induced relaxation unchanged.

A receptor-mediated interaction in capsaicin-induced relaxation and contraction is indicated by the antagonistic effect of the selective inhibitor ruthenium red. Tissue pre-treatment with tetrodotoxin prevented capsaicin-induced contractions, confirming that capsaicin contracts the tissue indirectly, by stimulating pre-synaptic release of acetylcholine. Prostanoids appear to sensitise afferent fibres (especially prostaglandin E_2) and to enhance the effect of capsaicin (Lundberg 1996; Lopshire & Nicol 1997). In our study, indometacin inhibited capsaicin-induced relaxation and contraction, indicating that, on lamb detrusor muscle, prostanoids are essential for capsaicin activity. CGRP (Franco-Cereceda 1989; Geppetti et al 1991; Herbert & Holzer 1994; Holzer et al 1995) and acetylcholine (Das & Ganguly 1984; Zhao et al 1994) are released following prostaglandins. Capsaicin could directly stimulate production of prostanoids that then release CGRP and acetylcholine from the nerves. Prostanoid production probably differs in tissues that respond to capsaicin by contracting and those that do not; in fact, only high prostaglandin concentrations can eventually release acetylcholine in equine trachea (Zhao et al 1994).

Capsaicin relaxes lamb bladder as well as hamster detrusor and horse trachea (Maggi et al 1987; Zhu et al 1997). Neurokinin A produces a strong contraction of hamster urinary bladder whereas capsaicin evokes a relaxation (Tramontana et al 2000). Most probably, under the reported experimental conditions, the capsaicin concentration was too small to release neurokinin A. Precisely which stimuli can elicit release of tachykinins in lamb bladder is an interesting question for future study.

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