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Use and limitations of three TRPV-1 receptor antagonists on smooth muscles of animals and man: A vote for BCTC

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ABSTRACT

Specificity of the effect is a crucial factor in using antagonists for detecting the physiological/pathophysiological roles of receptors. Here we examined the capsaicin receptor antagonist effects of three commercially-available substances, capsazepine, iodo-resiniferatoxin (I-RTX) and BCTC, on isolated smooth muscle preparations, including the human intestine. Care was taken to observe possible non-specific effects, to find out safe and effective concentrations. Capsazepine appeared to have a low margin of safety. I-RTX (up to 1 μM) specifically inhibited capsaicin-induced contractions in the guinea-pig ileum and urinary bladder. I-RTX showed agonist activity on the rat urinary bladder. BCTC (1 μM) abolished the contractile effects of capsaicin (1 or 2 μM) on all preparations tested (guinea-pig ileum, bladder, trachea, as well as rat and mouse bladder), and on the guinea-pig renal pelvis, where it failed to influence capsaicin-sensitive, sensory neuron-mediated positive inotropy in response to field stimulation. On human intestinal preparations BCTC prevented the relaxant effect of capsaicin. It is concluded that of the three antagonists tested BCTC seems the safest one for inhibiting TRPV-1 receptors. The effect of capsazepine may be complicated by non-specific inhibition of smooth muscle contractility and that of I-RTX by agonist activity. The "local efferent" function of capsaicin-sensitive sensory neurons is not influenced by BCTC, as shown by the results obtained in the renal pelvis. In conclusion, of the TRPV-1 receptor antagonists studied, BCTC (1 μM) seems the most reliable in isolated organ experiments. This substance is also effective in the human intestine.

1. Introduction

Capsaicin, the pungent substance in red peppers elicits hot or painful sensation in the skin and mucous membranes. Moreover, by releasing biologically active substances from peripheral endings of sensory neurons, capsaicin gives rise to "local efferent" responses, such as vasodilatation and plasma extravasation (Jancsó et al., 1967, 1968). It has also been shown that capsaicin has motor effects on smooth muscle organs and the heart in vitro (Barthó and Szolcsányi, 1978, 1980; Franco-Cereceda and Lundberg, 1988; Lundberg and Saria, 1982; Lundberg et al., 1984; Maggi et al., 1985, 1987, 1989; Molnár et al., 1969a,b; Patacchini et al., 1999; Robotham et al., 1985; Szolcsányi and Barthó, 1978, 1982). Indirect evidence for the existence of a capsaicin receptor (Szolcsányi and Jancsó-Gábor, 1976) has been followed by a molecular identification of capsaicin-sensitive structures (Caterina and Julius, 2001, Caterina et al., 1997). Specific antagonists acting on these receptors are crucial pharmacological tools for the elucidation their physiological/pathophysiological roles, whereas capsaicin or resiniferatoxin pretreatment (systemic or perineural) probably impairs the function of the whole nerve terminal or the entire neuron (see Bevan and Szolcsányi, 1990), thus allowing an assessment of the physiological/pathophysiological roles played by these neurons. Until recently, studies dealing with capsaicin-sensitive TRPV-1 receptors utilised capsazepine (Bevan et al., 1992; Franco-Cereceda and Lundberg, 1992; Urban and Dray, 1991) as specific receptor antagonist. The relatively low affinity of this compound, however, often made a differentiation between specific and non-specific effects uneasy (see among others Docherty et al., 1997; De Man et al., 2008); non-specific inhibition can occur at the level of neurons and/or smooth muscles. Yet, many studies still utilise capsazepine. Iodo-resiniferatoxin, first described as a receptor antagonist in 2001 (McDonnell et al., 2002; Undem and Kollarik, 2002; Wahl et al., 2001) has a higher affinity, as shown by binding data obtained with TRPV-1 receptors occurring naturally or expressed in a cell culture (Wahl et al., 2001) and is also widely used. An agonist effect of this compound on the rat urinary bladder has been described by Patacchini et al. (2005). Even more recently, several TRPV-1 receptor antagonists have been developed. A commercially-available one, *N*-(4-*tert*-butylphenyl)-4-(3-chloropyridin-2-yl)tetrahydropyrazine-1(2*H*)-carboxamide monohydrochloride (BCTC) has been found effective but to also inhibit TRPM-8 receptors (see among others Valenzano et al. 2003; Behrendt et al., 2004; Correll et al., 2004).

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The aim of the present study was to assess the TRPV-1capsaicin receptor antagonist effect of the above three substances in vitro, in several smooth muscle preparations taken from different laboratory animals. We have not extended our study to other types of TRP receptors. Special attention was paid to possible excitatory (capsaicin-mimicking) effects of the TRPV-1 receptor antagonists, as well as to possible non-specific actions of the compounds on cholinergic and other types of neuroeffector transmission. On this basis, the "safety margin" of antagonist concentrations can be determined. The guinea-pig renal pelvis preparation enabled us to study the effect of BCTC on responses evoked by electrical (most probably antidromic) stimulation of capsaicin-sensitive afferents (Maggi et al. 1992). In a limited number of experiments, human intestinal preparations were also used for checking the capsaicin receptor-antagonist action of BCTC. For what we know, this substance has not been tested in any human smooth muscle preparation.

2. Materials and methods

All experiments have been approved by the Regional Research Ethics Committee, National Research Council Ethics Committee (ETT-TUKEB) and the Regional Committee for Animal Research.

2.1. Animals and preparations

Guinea-pigs (short-haired, coloured) of either sex, weighing 350–450 g, Wistar rats of either sex and 220–280 g or adult CD1 mice were used. They were killed by a massive blow to the occiput, and then decapitated. The urinary bladder (all three species), a 15-cm piece of the pre-terminal ileum, the middle portion of the trachea, the kidneys (guinea-pigs) were removed, gently rinsed if necessary and put in Krebs' solution. Two longitudinally-oriented detrusor preparations were made out of each bladder after dividing the viscus into two halves along the sagittal axis and removing the lateral quarters, also along the sagittal axis. Mouse bladders were made up as one single strip (after opening the lateral parts). Guinea-pig tracheal zig-zag strips were prepared as described earlier (Szolcsányi and Barthó, 1982). Whole segments of the guinea-pig ileum (approx. 3 cm in length) were made up as longitudinally-oriented preparations (threads were attached at both ends to identical points of circumference, whereby the mesenteric border was used for orientation). Circularly-oriented preparations of the guinea-pig renal pelvis were made as described by Maggi et al. 1992. Briefly, the renal pelvis was prepared free so that it formed a funnel-shaped tissue. This was then cut through to form a strip. Care was taken to prepare strips of renal pelvis as soon as possible after killing the animal, since delay tended to reduce the proportion of preparations that responed to 2-Hz electrical field stimulation of their nerves.

Preparations were put into jacketed organ baths containing 3 ml (renal pelvis) or 5 ml of Krebs' solution (for the rest of the preparations), kept at 37 °C with the aid of a circulating thermostat (Experimetria, Budapest, Hungary). The bathing fluid was bubbled with a mixture of 5% CO_2 and 95% O_2. The preparations were connected to isotonic lever transducers (Hugo Sachs Elektronik/Harvard Instruments, March-Hugstetten, FRG), except the trachea, renal pelvis and the mouse bladder, where isometric transducers were used (Experimetria, Budapest, Hungary). Movements or tension of the tissues were recorded on compensographic ink writers or stored online on a personal computer. The load on the tissues were 5 mN for the rat urinary bladder, 7 mN for the guinea-pig ileum and 10 mN for the guinea-pig urinary bladder or human intestinal preparations. Preparations under isometric recording were allowed to equilibrate for 30 min under a resting tension of 1 mN. Then the baseline tension was carefully adjusted to 2.5 mN for the mouse urinary bladder and guinea-pig trachea and 4 mN for the guinea-pig renal pelvis. No further adjustments of baseline tension were made.

2.2. Human gut preparations

Human jejunal, ileal or sigmoid colon tissue was obtained from gut segments surgically removed from patients suffering from pancreatic or colorectal carcinoma. Circularly-oriented, mucosa-free strips (approximately 2 mm wide and 20 mm long) of the tissues were prepared as described earlier (Barthó et al., 2002; Benkó et al., 2007; Undi et al., 2009). There was no difference in responses of jejunal or ileal preparations, hence the results were assessed together.

2.3. Electrical field stimulation

Electrical field stimulation was delivered by a high-performance stimulator (Experimetria, Budapest, Hungary) through platinum wire electrodes placed in the bathing fluid above and below the preparation, at a distance of 4 cm from each other. Parameters of electrical field stimulation were as follows: *guinea-pig ileum* — 80 V amplitude, 0.1 ms pulse width, single shocks at a frequency of 0.05 Hz or trains of 1 Hz for 15 s; *bladders* — 100 V, 0.1 ms, single shocks or trains of impulses (1 Hz or 5 Hz for 20 s); *renal pelvis* — 90 V amplitude, 0.1 ms pulse width, 15-s trains at 2 Hz. The rest of the preparations was not stimulated electrically.

2.4. Experimental protocols

In all experiments, guanethidine (3 μM) was present in the organ bath throughout the experiments for a functional impairment of adrenergic neurons. Experiments commenced after an equilibration period of 40–60 min. Two cycles of electrical field stimulation (followed by rinsing) were then carried out in the guinea-pig ileum or rat or guinea-pig bladders, with an interval of 20 min. Renal pelvis preparations were exposed to electrical field stimulation 3 times, once in 45 min; under these circumstances the responses were reproducible. TRPV-1 receptor antagonists or their solvents were then added and electrical field stimulation was repeated after 20 min. After another 10 min, the effect of capsaicin was tested (contact time, 3 min). A similar protocol was used for those preparations where no electrical stimulation was applied. With the trachea, the contact time for capsaicin was extended to 15 min and in the renal pelvis to 10 min. Human gut preparations were allowed to equilibrate for 60–80 min and then precontracted with acetylcholine (1–3 μM) as described earlier (Barthó et al., 2002) for testing the relaxant effect of capsaicin (0.5 μM). A maximal contraction of the tissues was evoked at the end of each experiment (the agent used for this was 10 μM histamine with the guinea-pig tissues and 80 mM KCl for the rat and mouse bladder, as well as for the human intestine). With the human gut preparations, administration of KCl was preceded by an administration of 5 μM isoprenaline (for 10 min to induce maximal relaxation) followed by thorough rinsing and 40 min of rest. "Inotropic" responses of the guinea-pig renal pelvis were evaluated as % change of the amplitude of spontaneous contractions in response to capsaicin or electrical field stimulation.

In the rat urinary bladder, where I-RTX caused slow contraction (see Results section) the contact time for the drug was extended to 35 min; a rinsing was performed at 10 min and the I-RTX was readministered (for another 20 min), whereafter electrical field stimulation was repeated. This was followed by the challenge with capsaicin in the presence of I-RTX. In preparations other than the rat urinary bladder, the contact time for I-RTX was 20 min. The contact times for BCTC or capsazepine were also 20 min.

2.5. Drugs and solutions

The composition of the Krebs' solution was as follows (mM), NaCl 119, $NaHCO_3$ 25, KCl 2.5, $MgSO_4$ 1.5, $CaCl_2$ 2.5, KH_2PO_4 1.2, glucose 11. Drugs used were I-RTX and BCTC (Tocris, Bristol, UK), capsaicin, capsazepine, guanethidine sulphate, histamine dihydrochloride (Sigma, Budapest, Hungary); isoprenaline was administered from Isuprel® injection.

Capsaicin (20 mM) and capsazepine (100 mM) were dissolved in ethanol, I-RTX (3 mM) and BCTC (10 mM) in DMSO. Solutions of capsaicin were also diluted with ethanol if necessary. The drugs were added to the organ bath in a volume of 0.3 μl/ml. Ethanol (96%) or DMSO were used as solvent control and were ineffective at 0.3 μl/ml. Guanethidine (1 mM), histamine (10 mM), and acetylcholine (10 mM) were dissolved and diluted in isotonic NaCl solution.

2.6. Statistics

Data are presented as mean ± S.E.M. The following tests were used for statistical comparison, Mann–Whitney test (2 independent samples), Kruskal–Wallis test (3 independent samples), Wilcoxon's signed rank test (2 paired samples). A probability of $P<0.05$ or less was considered as significant. Number of experiments (n) refers to the number of animals used.

3. Results

3.1. Guinea-pig ileum

Capsaicin (administered only once to each preparation) caused concentration-dependent contraction (see Barthó and Szolcsányi, 1978). This response was inhibited by I-RTX (10 nM–1 μM), apparently in a non-surmountable manner with the two higher concentrations (0.1 or 1 μM) (Fig. 1A). Cholinergic "twitch" contractions evoked by single electrical shocks or tonic contractions in response to 1-Hz field stimulation were not significantly influenced by I-RTX (up to 1 μM; data not shown). A modest but significant inhibition of the twitch response was seen with 3 μM of I-RTX; numerical values were 52.4 ± 2.5% contraction before and 43.1 ± 1.6% contraction in the presence of I-RTX (3 μM; $P<0.01$, n = 11). Responses to 1-Hz stimulation were also slightly but significantly reduced by 3 μM I-RTX (from 59.0 ± 4.6 to 49.0 ± 3.1%; $P<0.05$, n = 11). The appropriate volume of the solvent (DMSO; corresponding to 3 μM I-RTX) had no inhibitory effect. I-RTX (10 nM–1 μM) showed no excitatory action on this preparation.

BCTC (10 nM–30 μM) concentration-dependently inhibited capsaicin-evoked contractions (Fig. 1B), whilst leaving cholinergic "twitch" contractions (in response to single shocks) or tonic contractions (1-Hz stimulation) uninfluenced, even at 30 μM (n = 7 for both frequencies). No excitatory effect of BCTC has been observed in any of the concentrations used.

Capsazepine, at 3 μM moderately (by approximately 50%) reduced the effect of capsaicin (2 μM). At 10 μM capsazepine caused a strong but still partial (approximately 75%) inhibition of the effect of capsaicin and a weak but consistent reduction (approximately 20%) of the electrically-induced contractions. A ten-fold higher concentration of capsazepine fully inhibited both types of responses, as well as those evoked by exogenous acetylcholine (30 nM; n = 5 for each statement; data not shown), indicating a narrow safety margin.

3.2. Guinea-pig, rat, and mouse urinary bladders

I-RTX (1 μM) inhibited capsaicin-induced contractions in the guinea-pig bladder preparation (Fig. 2A). Its smaller inhibitory action on 1 μM of capsaicin than on 0.3 μM may indicate a certain degree of surmountability of the antagonism. The administration of I-RTX elicited a variable excitatory response in the rat urinary bladder, but not in the guinea-pig urinary bladder. The contractile response to I-RTX in the rat urinary bladder was prevented by capsaicin pretreatment (10 μM for 15 min, followed by a 45-min washout). Following its excitatory action, I-RTX (1 μM) inhibited the effect of capsaicin to a variable extent (n = 8, data not shown). Whether this was a manifestation of antagonist or sensory neuron desensitising effects was not analysed further. On bladder preparations of either species,

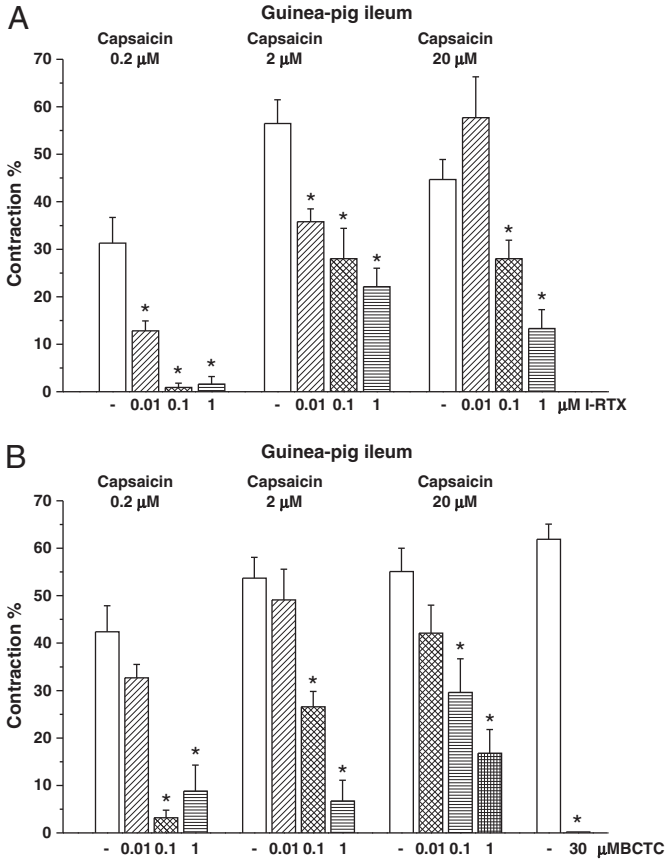


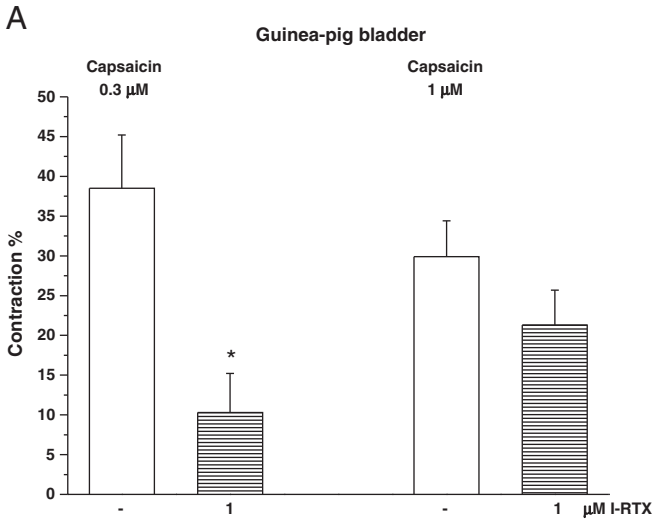
Fig. 1. Contractions of the guinea-pig ileum in response to capsaicin (0.2, 2 or 20 μM) in the absence (open columns) or in the presence of I-RTX (concentrations indicated below the columns) (A) or BCTC (B). Capsaicin was administered only once to each preparation. Note that an increase of capsaicin concentration from 2 to 20 μM did not overcome the inhibitory effect of 0.1 or 1 μM I-RTX. Mean ± S.E.M. of at least 5 experiments are shown by each column. * — significant differences ($P<0.05$ or less, Kruskal–Wallis test or, in the case of one pair of columns, Mann–Whitney test) from the untreated groups.

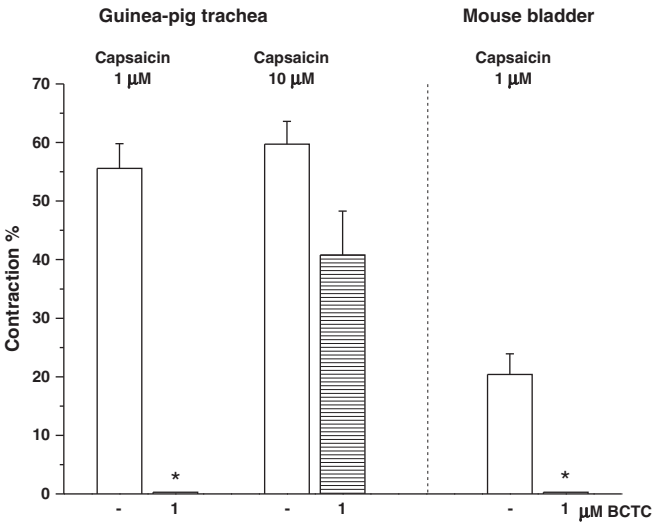
partial inhibition of the capsaicin effect by I-RTX was often characterised by an enhanced latency of capsaicin-induced contractions (1–2 min in the presence of I-RTX vs. about 10 s in untreated preparations). Contractile responses due to electrical field stimulation (single pulses, 1 Hz for 30 s or 5 Hz for 20 s) were not influenced by I-RTX (1 μM) in the guinea pig bladder preparations. In the rat bladder, contractile responses elicited by the same parameters of electrical field stimulation I-RTX (1 μM) slightly but significantly reduced the effect of stimulation with single pulses or 1 Hz, but not at 5 Hz (48.1 ± 2.9% contraction before and 38.2 ± 4.1% in the presence of I-RTX, $P<0.05$, n = 6 at 1 Hz and 97.7 ± 1.4 vs. 93.4 ± 1.9%, n = 6 at 5 Hz).

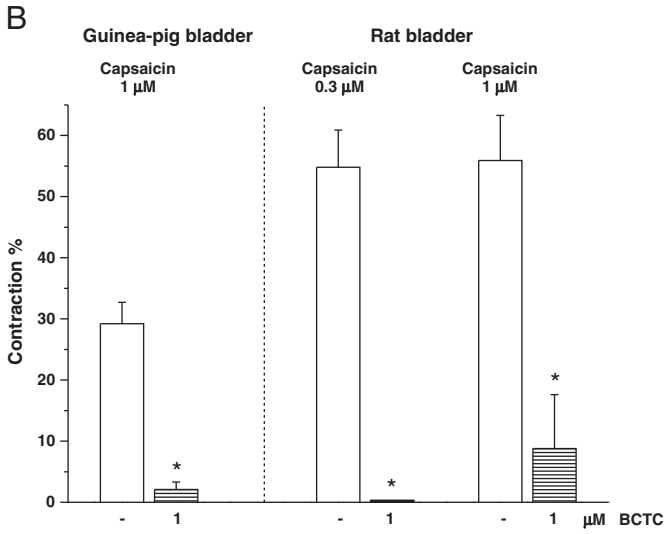
BCTC (1 μM) blocked the contractile response to capsaicin in the urinary bladders of all three species of experiental animal (Figs. 2 and 3). No inhibition of electrical field stimulation-induced moderate contractions were found in the rat or guinea-pig bladders (single pulses or 1 Hz stimulation, n = 6–9; data not shown). Likewise, BCTC did not exert any excitatory effect. On the other hand, BCTC (1 μM) inhibited the excitatory effect of I-RTX in the rat bladder preparation (Fig. 2C). In quantitative terms, the contractile effect of I-RTX (1 μM) reached 18.2 ± 2.8% and 2.9 ± 1.8% of maximal spasm in solvent-treated and BCTC-treated preparations, respectively ($P<0.001$, n = 11).

3.3. Guinea-pig trachea and renal pelvis

BCTC (1 μM) abolished the effect of 1 μM capsaicin in the guinea-pig trachea; contractions in response to 10 μM capsaicin were only weakly inhibited (Fig. 3). Capsaicin (1 μM) strongly enhanced the







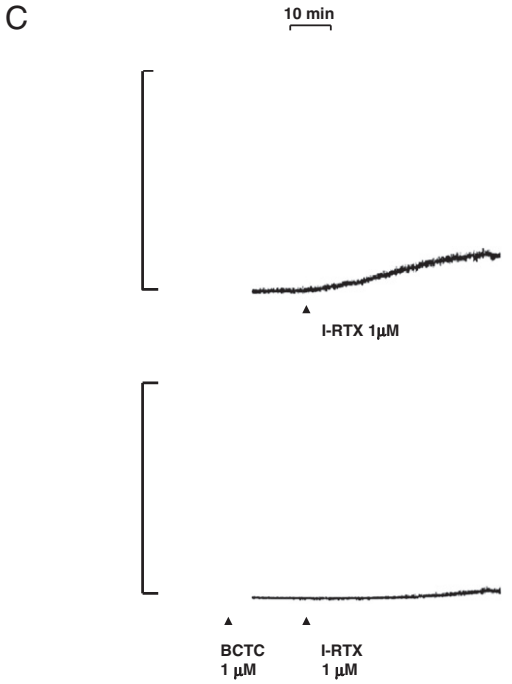


Fig. 3. The inhibitory effects of BCTC (1 μM) on capsaicin-induced contractions of guinea-pig tracheal zigzag or mouse bladder detrusor preparations. Capsaicin was administered only once to each preparation. Mean ± S.E.M. of at least 5 experiments are shown by each column. * — significant differences ($P<0.05$, Mann–Whitney test) from the untreated group.

amplitude of the spontaneous rhythmic contractions in the renal pelvis, as described by Maggi et al. (1992). BCTC (1 μM) prevented this action (Fig. 4). Some excitatory effect of capsaicin could be evoked at a higher concentration (10 μM) in the presence of BCTC (1 μM; n = 3, data not shown). Electrical field stimulation at 2 Hz (for 15 s) considerably elevated the amplitude of contractions (their frequency was also enhanced in some preparations). The effect of electrical field stimulation was abolished by in vitro capsaicin desensitisation (see Maggi et al. 1992) that was achieved by an administration of 10 μM capsaicin for 10 min, followed by a 45-min washout period (n = 4) or 1 μM of capsaicin, left in contact with the tissue until its stimulant effect faded away (30–45 min; n = 6). BCTC (1 μM) did not inhibit the effect of electrical field stimulation (Fig. 4B,C) and did not influence rhythmic (myogenic) contractions of the renal pelvis (n = 6). Moreover, capsaicin (1 μM) invariably failed to induce full inhibition of the electrically-evoked responses in the presence of BCTC (1 μM; n = 4, data not shown), i.e., the functional impairment of sensory nerves by capsaicin was attenuated by the TRPV-1 receptor antagonist.

3.4. Human gut preparations

Capsaicin-evoked relaxation in the precontracted circular muscle of the human small intestine or sigmoid colon was abolished by a pretreatment of BCTC (1 μM) (Fig. 5). No influence of BCTC on the acetylcholine-induced contraction was detected.

4. Discussion

Few data are available on the effect of BCTC on capsaicin-sensitive motor responses of viscera. Our present results indicate that BCTC is a capsaicin antagonist that can be safely used in isolated organ experiments of several species (including human tissues) due to its lack of a

Fig. 2. A Contractions of the guinea-pig urinary bladder detrusor preparation to capsaicin (0.3 or 3 μM), without (open columns) or in the presence of I-RTX (1 μM) (A) or BCTC (B). Capsaicin was administered only once to each preparation. Mean ± S.E.M. of at least 5 experiments are shown by each column. * — significant difference ($P<0.05$, Mann–Whitney test) from the untreated group. C — Original tracings showing the reaction of bladder detrusor preparations to administration of 1 μM I-RTX in the absence (upper tracing) and presence (lower tracing) of BCTC (1 μM). Vertical calibration, height of the maximal contraction in response to 80 mM KCl.

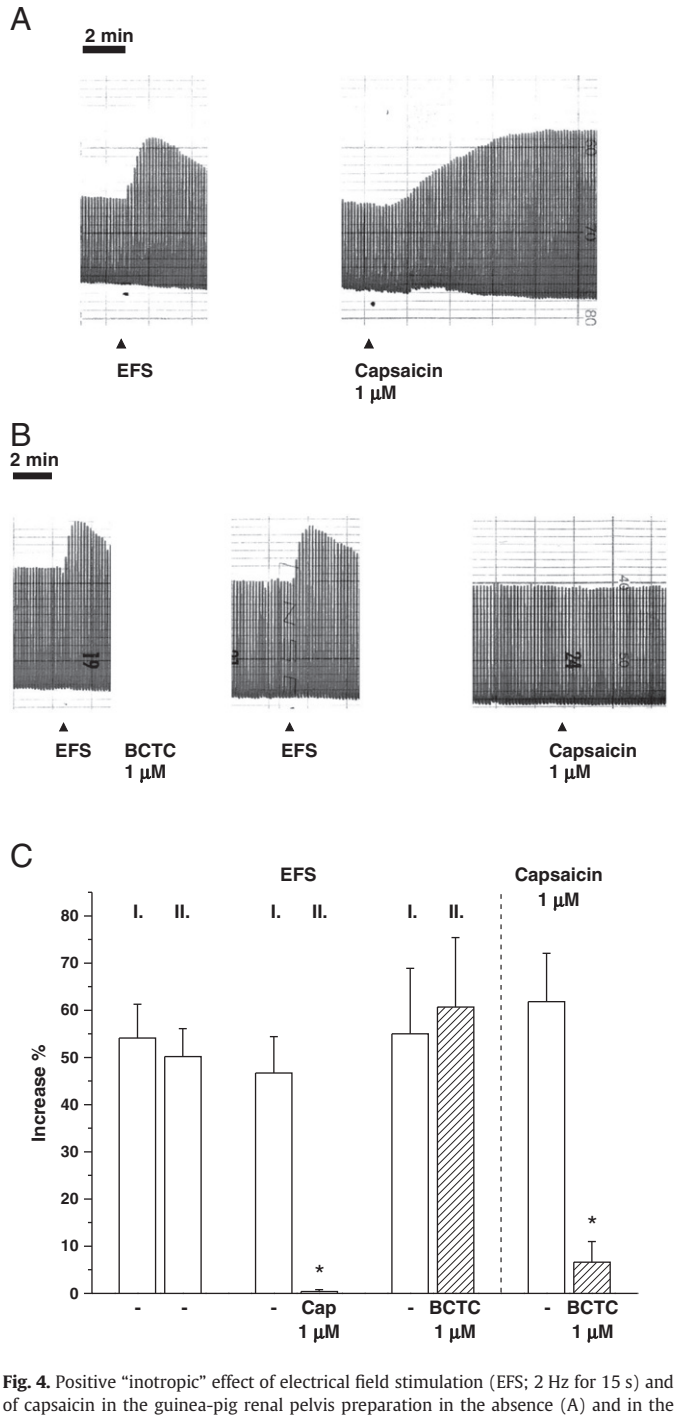


Fig. 4. Positive "inotropic" effect of electrical field stimulation (EFS; 2 Hz for 15 s) and of capsaicin in the guinea-pig renal pelvis preparation in the absence (A) and in the presence of BCTC (1 μM, added between the first and second series of pulses) (B). Quantitative data are shown in C. Two subsequent sets of field stimulation are marked as I. and II., whilst capsaicin as stimulant (last pair of columns) was administered only once. Cap denotes pretreatment with capsaicin (1 μM, left in contact with the preparation, see Materials and methods section). * — significant differences ($P<0.05$ or less, Wilcoxon's signed rank test or Mann–Whitney test) between means. Note that BCTC abolishes the action of capsaicin, whilst leaving that of field stimulation (as well as spontaneous myogenic activity) uninfluenced.

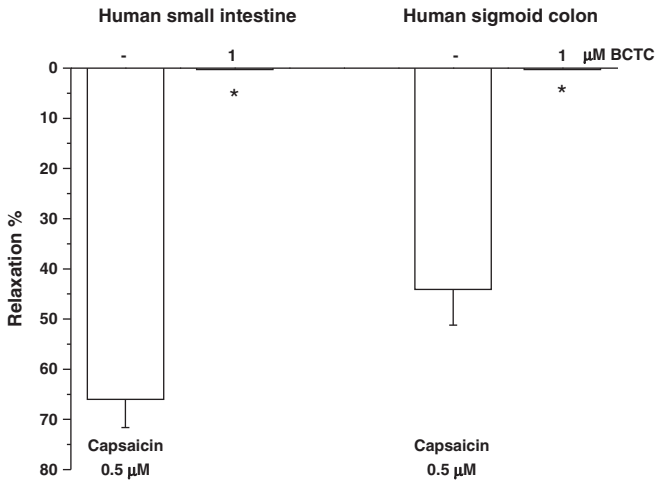


Fig. 5. Relaxant effect of capsaicin (0.5 μM, administered only once to each preparation) on the human small and large intestinal circular muscle, in the absence or in the presence of BCTC (1 μM). Intestinal strips were precontracted with a submaximal concentration of acetylcholine and capsaicin was added as soon as a constant level of tonic contraction to acetylcholine developed. Mean ± S.E.M. of 6 experiments on each type of gut segment. * — significant differences ($P<0.05$ or less).

non-specific smooth muscle depressing effect or agonist activity. I-RTX seems effective in guinea-pig tissues, but a somewhat variable effectivity, as well as some excitatory action (see below) might limit its use in the rat (where our experience, however, is limited to the urinary bladder). The current results are compatible with the reported IC_{50} values of I-RTX and BCTC in the nanomolar range (see Alexander et al., 2009). Capsazepine may be useful at 10 μM, but some smooth muscle-depressing activity cannot be ruled out even at this concentration.

For evaluating specificity of the antagonists studied, it should be remembered that a lot of drugs of different categories would affect stimulation-evoked contractile responses of the guinea-pig ileum. Besides papaverine-like non-specific smooth muscle relaxants Ca^{2+}-channel blockers, adrenergic beta-receptor stimulants or other drugs that elevate intracellular cAMP or cGMP levels (among others nitric oxide, "inhibitory" neuropeptides, e.g. VIP, PACAP, CGRP, as well as phosphodiesterase inhibitors) reduce tone and contractions of this preparation. Stimulation-evoked neurogenic contractions are selectively inhibited by Na^+ channel blockers such as tetrodotoxin and local anaesthetics, stimulants of opioid, alpha-adrenergic or adenosine receptors and some other drugs that reduce acetylcholine release. These inhibitors will not reduce the direct smooth muscle-contracting effects of agonists. On the other hand, a number of agonists that have receptors on the longitudinal muscle (among others acetylcholine itself, then histamine, serotonin, excitatory neuropeptides, lipid mediators), as well as substances that in some way activate myenteric ganglion cells (nicotine-like, cholecystokinin-like substances, serotonin, ATP — through P2X purinoceptors, NO, "inhibitory" neuropeptides that elevate cAMP, see above) contract the guinea-pig ileum. Among these substances is capsaicin that probably releases tachykinins and maybe also an ATP-like P_2 purinoceptor stimulant from primary afferent nerve endings and thereby activates myenteric neurons (Barthó et al., 2000, 2004). Furthermore, both rat and guinea-pig bladder preparations are contracted by ATP and its analogues that stimulate P_{2X}purinoceptors; on the other hand, bladder contractions in response to electrical field stimulation are inhibited by P_{2X} purinoceptor antagonists and atropine-like drugs (see below). The lack of a contractile effect of BCTC and I-RTX on the preparations studied (with the exception of rat bladder for I-RTX) probably indicates that these drugs do not activate any of the above-mentioned mechanisms.

The contractile effect of I-RTX on the rat bladder most probably reflects a TRPV-1 receptor agonist action in this tissue (see Patacchini et al., 2005), all the more because we found that it was sensitive to capsaicin pretreatment. Moreover, since capsaicin pretreatment most probably impairs the function of the entire sensory nerve ending, we also tested the effect of TRPV-1 receptor blockade by BCTC and found that this pretreatment also inhibited the contracting effect of I-RTX on the rat bladder. The finding of Shimizu et al. (2005) that

I-RTX causes a capsaicin-like hypothermic effect in the mouse, as well as Ca^{2+} signals in HEK293 cells expressing rat TRPV1 receptors would also indicate a stimulant effect on capsaicin-sensitive neurons. A structural difference berween rat and guinea-pig TRPV-1 (see Phillips et al., 2004) may account for the divergent actions of I-RTX on the rat and guinea-pig urinary bladders.

The slight inhibitory action of I-RTX on the effect of electrical nerve stimulation in the rat bladder might indicate some kind of prejunctional action; a postjunctional antagonism seems less probable, since responses at the highest frequency used (5 Hz) were not inhibited. For the same reason, an inhibition of axonal conduction or an overall inhibition of neurotransmitter release is an unlikely cause of the inhibitory effect. A postjunctional inhibition of the effect of ATP cannot be completely ruled out, since data of the literature indicate that ATP and acetylcholine co-mediate nerve-mediated bladder contractions in the rat and guinea-pig (see among others Krell et al., 1981; Kasakov and Burnstock, 1982; Westfall et al., 1983; Mackenzie and Burnstock, 1984; Hourani, 1984; Hoyle et al., 1990; Creed et al., 1994; Hashimoto and Kokubun, 1995; Hashitani and Suzuki, 1995; Tong et al., 1997), and the role of the co-transmitter ATP may be more important at lower, whilst that of acetylcholine at higher frequencies of stimulation.

These studies present BCTC as the most promising TRPV-1 receptor antagonist of the three commercially available drugs tested. Furtermore, its inhibitory action on the capsaicin-induced excitatory responses was confirmed on the guinea-pig trachea and renal pelvis, as well as in the mouse bladder. The TRPV-1 antagonist action of BCTC was confirmed on human intestinal circular muscle preparations, where capsaicin causes only relaxation (Barthó et al., 2002; Maggi et al., 1990). To the best of our knowledge the effect of BCTC vs. capsaicin has not yet been studied on any human smooth muscle preparation, the guinea-pig and mouse bladder and the guinea-pig renal pelvis, ileal and tracheobronchial tissue in vitro. We are not aware of any study concerning the effect of BCTC on the "local efferent" function of capsaicin-sensitive nerves activated by (most probably antidromic) electrical stimulation. There has been one study to show a specific capsaicin antagonist action of BCTC on movements of the rat bladder (Saitoh et al., 2007); as far as the mouse is concerned, the only available study has been performed on the small intestine and also indicates specific capsaicin antagonist action (De Man et al., 2008).

The nature of the capsaicin-antagonist effect of I-RTX in the guinea-pig ileum cannot be precisely assessed on the basis of the present results. I-RTX displaces the concentration-response curve for capsaicin with a concomitant depression of the maximum, which is suggestive of non-competitive antagonism, whilst Undem and Kollarik (2002) did not observe a depression of the maximum response to TRPV-1 receptor agonists in the guinea-pig airway smooth muscle. A desensitising action of I-RTX (without visible excitation) could not be fully ruled out in the present study, but electrophysiological experiments failed to detect any stimulant effect of I-RTX in a system expressing the VR-1 (TRPV-1) receptor (Wahl et al., 2001). Preliminary experiments in our laboratory show that I-RTX, unlike capsaicin tachyphylaxis (see Barthó et al., 2004; Szolcsányi and Barthó, 1978) fails to inhibit the motor response elicited by mesenteric nerve stimulation, i.e. antidromic activation of capsaicin-sensitive neurons (Bartho et al., unpublished observations).

In summary, our findings indicate a specific capsaicin-antagonist effect of BCTC and I-RTX in smooth muscle preparations in vitro. I-RTX seems to have agonist effect in the rat bladder. Any new preparation should, however, be tested for a possible capsaicin (resiniferatoxin)-like, as well as smooth muscle relaxant effects of I-RTX. BCTC is probably devoid of such activities. Capsazepine, the only TRPV-1 receptor antagonist for a long time, might no more be recommended for this purpose.

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