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Effects of capsazepine on human small airway responsiveness unravel a novel class of bronchorelaxants

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Abstract

Capsazepine is known as a transient receptor potential channel vanilloid subfamily 1 ($TRPV_1$) antagonist that inhibits bronchoconstriction evoked in animals by $TRPV_1$ agonists. In this study, effects of capsazepine and chemically related analogues, so called capsazepinoids, were examined in vitro on contractile effects in human small airway preparations. Repeated cycles with 1 h of LTD_4-free physiological saline solution followed by 30 min exposure to LTD_4 (10 nM) demonstrated that the contractile responsiveness of the preparations exhibited little change over time despite repeated challenges (>12 h). Capsazepine (1–100 μM) reversibly and concentration-dependently inhibited the contractile response to LTD_4 with EC_{50} ~10 μM and ~90% relaxation at 100 μM. Capsazepine (10 μM) was approximately equally effective to attenuate the contractions evoked by several different inflammatory contractile agonists (LTD_4, PGD_2, histamine), and it relaxed preparations with established tonic contraction due to LTD_4. Higher concentrations of capsazepine were needed to relax ACh-contractions. The effect of capsazepine on LTD_4-induced contractions was not significantly reduced by pre-treating the preparations with either of propranolol (10 μM) + atropine (1 μM), L-NAME (1 mM), indomethacin (1 μM), iberiotoxin (0.1 μM), capsaicin (10 μM), and nifedipine (10 μM). Although the mechanism of action of the present capsazepine-induced bronchorelaxation remains unknown it emerged here that they represent a generally effective principle exerting a functional antagonism against contractile mediators but distinct from beta receptor agonists and inhibitors of L-type calcium channels. The inhibitory effect of capsazepine is shared by chemical analogues, but not with other $TRPV_1$ antagonists, suggesting the possibility that capsazepine represents a novel class of bronchorelaxants effective in human small airways. These findings were not predicted by previous observations that have concerned quite limited effects of capsazepine on airway tone in different animal test systems. If potency can be further increased and the results translated to in vivo, compounds representing the capsazepinoid class of bronchorelaxants might become useful in the treatment of patients suffering from asthma and COPD.

Keywords: Capsazepine; Bronchorelaxant; COPD; Asthma; Human; Small airways

1. Introduction

Capsaicin, the major pungent agent isolated from chili pepper, is known to stimulate the transient receptor potential channel, vanilloid subfamily member 1 ($TRPV_1$) causing pain, cough, as well as bronchoconstriction in experimental animals [1]. Based on the structure of capsaicin, capsazepine was developed in 1992 as the first specific $TRPV_1$ antagonist [2]. A number of other $TRPV_1$-antagonists have subsequently been identified, almost exclusively with the aim to develop compounds with analgesic effect [3]. However, $TRPV_1$ antagonists are also reported to prevent bronchoconstriction evoked by more or less specific agonists acting on these receptors. For example, Satoh et al. [4] showed that capsazepine inhibits

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bronchoconstriction evoked by inhalation of citric acid in guinea-pigs, but did not prevent bronchoconstriction resulting from inhalation of histamine. More recently, Undem et al. [5] showed that the $TRPV_1$- antagonist iodo-resinferatoxin (I-RTX) antagonized broncho-constriction evoked by capsaicin or resinferatoxin in guinea pig, but had no effect on trypsin-evoked, neurokinin-mediated contractions. *N*-arachidonyl dopamine, a possible endogenous $TRPV_1$-agonist, contracts guinea-pig isolated bronchi and this effect is blocked by pre-treatment with capsazepine [6]. Rosseau et al. [7] working with guinea-pig airway smooth muscle preparations, recently reported that capsazepine selectively inhibits the tonic plateau phase contraction induced by 20-hydroxyeicosatetraenoic acid (20-HETE) without affecting the initial transient contraction by this agent.

However, the possibility that capsazepine might exhibit general bronchorelaxant properties has not been suggested previously, nor has its effects been explored in human bronchial smooth muscle preparations. The latter aspect is important since contractile and relaxant responses of airway smooth muscle may differ much between species [8]. Also, the electrophysiology of human airway smooth muscle differs from other species commonly used in airway research [9] and $TRPV_1$ generally shows striking species-related differences in biological actions [10]. Furthermore, it may be particularly important to examine effects in human small airways because they are responsible for vital resistance changes in asthma and COPD [11].

In this study, we report novel findings demonstrating that capsazepine and chemical analogues exhibit potentially important bronchorelaxation of human small airway preparations. Thus, using a methodology that provided stable preparations for more than 12 h, we could show that capsazepine and several similar conformationally restricted capsaicin derivatives produced significant inhibition of contractions of human bronchi evoked by leukotriene D_4 (LTD_4) and other mediators. The observations presented here suggest that a novel class of bronchorelaxing drugs with small airway relaxant properties distinct from the old bronchodilator principles may be developed. A minor part of these data has been preliminarily presented in a publicized patent application [12].

2. Materials and methods

2.1. Preparation

Human lung tissue was obtained from patients undergoing lobectomy due to lung carcinoma in accordance with procedures approved by Lund ethical committee. The lung tissue was put in a dissection bowl continuously perfused with oxygenated physiological saline solution, PSS (for composition see *2.5 Solutions and Chemicals* below) at room temperature. Bronchi with a diameter between 0.5 and 1.5 mm were identified and dissected from the lung. About 2 mm long pieces from the bronchi were obtained and cut open at one side. A loop of surgical suture was made in each end of the preparation, which was then mounted in the experimental chamber to a hook connected to a force transducer in one end and to a fixed holder in the other end.

2.2. Experimental chamber

The experimental chamber had a volume of 8 ml and was continuously perfused with solutions at a rate of 3 ml/min during the experiment. The temperature was kept at 37 °C. The chamber was equipped with two separate force transducers (model AME 801, SensoNor A/S, Horten, Norway) for simultaneous registration of two parallel preparations. Each of the force transducers was connected to a micrometer screw that allowed the preparations to be stretched to the desired tone. The force development was registered on a computer. Each chamber contained one or two pieces of tissue exposed to identical conditions. If two preparations came from the same patient, the test values were regarded as a single mean value. All test values are given as arithmetic mean ± standard error of the mean.

2.3. Experimental start and termination

The preparations were mounted in the experimental chambers, and treated as described in Fig. 1.

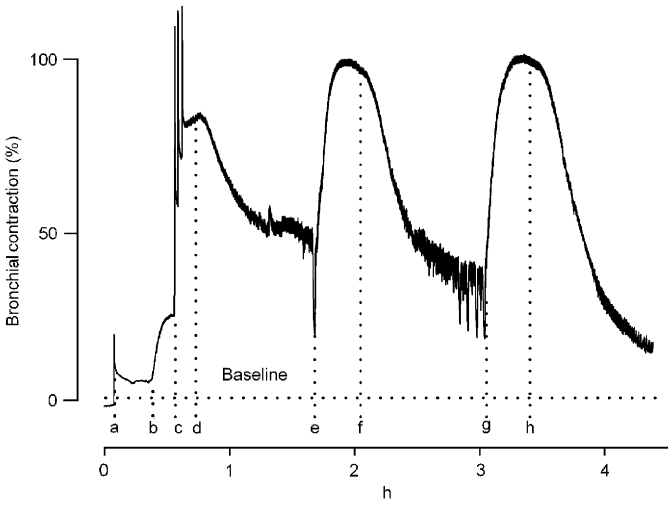


Fig. 1. An original recording of a human small bronchus showing the initial procedures to obtain a stable preparation. After mounting in the experimental chamber the preparation was stretched 0.4 mN (a). After 20 min, LTD_4 (10 nM) was added (b). When the contraction had reached a plateau, the preparation was stretched repeatedly (c) until the force had stabilized at 1.2 mN. Thereafter, the preparation was exposed to PSS (d). After 1 h in PSS, the preparation was contracted with LTD_4 to a plateau (e). This was followed by wash-out and a new cycle with 1 h of LTD_4-free PSS (f) followed by 30 min of LTD_4 (g). If two consecutive LTD_4 contractions differed less than 10%, the preparations were considered to be stable and the experiments begun. The second of the LTD_4 contractions was designated as control contraction and was used for comparison in evaluation of drug effects.

At the end of the experiments, the preparations were exposed to 0 Ca^{2+}-solution, to establish the baseline tension level.

2.4. Experimental protocols

2.4.1. Tests of the stability of LTD_4-induced contractions

In order to assess the viability and stability of preparations that had been dissected, mounted and exposed to the environmental conditions in our experimental chambers, eight preparations were exposed to repeated cycles with LTD_4-free PSS followed by LTD_4. After 13.5 h (9 cycles) the preparations displayed 71.3 ± 9.0% of the initial contraction (Fig. 2).

2.4.2. Relaxing effect of capsazepinoids

Dose–response relationship for capsazepine was determined by having different concentrations of this drug (0.1, 1, 10 and 100 μM) present during the whole cycle (1 h of LTD_4-free solution followed by 30 min of LTD_4 10 nM). The dose–response curve was obtained in a non-cumulative way. Each preparation was used for determination of the inhibitory effect of a single capsazepine concentration.

In some experiments, the preparations were contracted with histamine (10 μM), prostaglandin D_2 (PGD_2) (10 μM) or acetylcholine (ACh) (100 μM) producing contractions of a similar magnitude to 10 nM LTD_4. Inhibitory effects of a single concentration of capsazepine (10 μM) were then evaluated as described above. The effect of capsazepine 100 μM was also tested on contractions with ACh.

In further experiments, the preparations were exposed to several capsazepine-like substances. The effects of 10 μM of a series of individual compounds (**1**–**7**; see Table 1) were determined during different cycles of LTD_4 contractions.

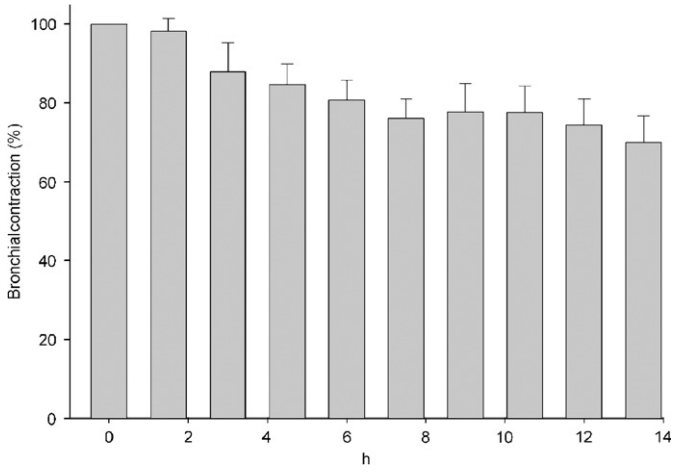


Fig. 2. Force development in small human bronchi exposed to repeated cycles with 1 h of LTD_4-free PSS followed by 30 min exposure to LTD_4 indicates that the responsiveness of the present preparations exhibit little change over time despite repeated challenges ($n = 8$). When evaluating the statistical significance of relaxations by capsazepinoids, the test contraction was compared to the third control contraction.

2.4.3. Inhibitory effect of capsazepine (10 μM) after pre-treatment with atropine+propranolol, L-name, indomethacin, capsaicin or iberiotoxin

After one control contraction by LTD_4 the preparations were exposed for 1 h to PSS containing either atropine (1 μM) + propranolol (10 μM), L-NAME (1 mM), indomethacin (1 μM) or capsaicin (10 μM). Then a further control contraction to LTD_4 was obtained. This was followed by a new contractile cycle where pre-treatment and capsazepine (10 μM) was present. The size of the LTD_4 contraction during the final, capsazepine-containing cycle was compared to the LTD_4 contraction during the cycle containing one of the pre-treatments. Only one kind of pre-treatment was examined in each preparation. The second control contraction was designated as 100%, and the contraction during the exposure to the test substance was compared to the second control contraction. In some experiments, pre-treatment with iberiotoxin (0.1 μM) was used. After one control contraction by LTD_4 the preparations were exposed for 1 h to PSS containing 0.1 μM iberiotoxin. Then a further control contraction to LTD_4 with iberiotoxin was obtained. This was followed by a new contractile cycle where pre-treatment and capsazepine (100 μM) was present.

2.4.4. Dose–response relationship of capsazepine after pre-treatment with nifedipine

After one control contraction, the preparations were exposed to one cycle of PSS + nifedipine (10 μM) for 1 h followed by LTD_4 together with nifedipine. This was followed by a new contractile cycle, now also containing capsazepine (0.1, 1, 10 or 100 μM). The size of this latter contraction was compared to the contraction after one cycle of control exposure to nifedipine.

2.4.5. Comparision with some well-established $TRPV_1$-antagonists

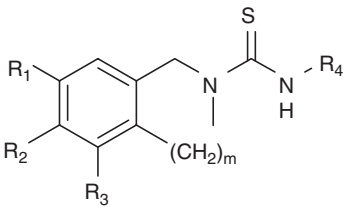
The well-known $TRPV_1$-antagonists I-RTX, Ruthenium Red, SB 366791, JYL 1421 and KJM 429 were tested in one supra-maximal concentration which in most cases is at least 100 times higher than the reported EC_{50} for its $TRPV_1$-blocking effect. The preparations were exposed to one of the substances during one cycle (1.5 h). The size of the LTD_4 contraction during the final, $TRPV_1$ antagonist containing cycle was compared to the LTD_4 contraction during the control cycle.

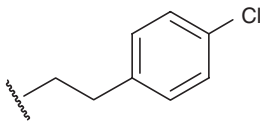
2.5. Solutions and chemicals

2.5.1. PSS

PSS contained (in mM): 117 NaCl, 4.88 KCl, 0.60 $MgSO_4$, 25.0 $NaHCO_3$, 5.23 glucose, and 1.60 $CaCl_2 \cdot 2H_2O$. The PSS during the experiments was bubbled with 94% O_2 and 6% CO_2, giving a pH of 7.40. All chemicals were purchased from Sigma Aldrich.

Table 1
The inhibitory effect of capsazepine and related derivatives on LTD_4 evoked bronchoconstriction





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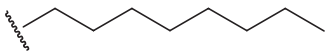

II

Compound	R_1	R_2	R_3	R_4	m	Remaining contraction[a]	Significance[b]	*n*
Capsazepine	OH	OH	H	**I**	3	55 ± 3.0	***	24
1	OMe	OMe	H	**I**	3	65 ± 8.0	*	6
2	OH	OH	H	**II**	3	60 ± 11.2	**	6
3	OH	OH	H	**I**	2	36 ± 6.5	***	5
4	OMe	OMe	H	**I**	2	79 ± 3.3	$p = 0.32$	6
5	H	OH	OH	**I**	2	63 ± 7.5	*	4
6	H	OH	OH	**II**	2	75 ± 7.0	$p = 0.18$	5
7	OH	OH	H	**I**	1	56 ± 10.0	**	5

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$.
[a]Arithmetic mean ± the standard error of the mean.
[b]Statistical significance compared top LTD_4 control contraction.

2.5.2. Ca^{2+}-free solution

In the end of all experiments, the preparations were exposed to Ca^{2+}-free PSS in order to find the level of passive tension. Ca^{2+}-free solution contains all of the chemicals in PSS except for $CaCl_2 \cdot 2H_2O$. Also, 2 mM EGTA was added to bind any remaining Ca^{2+}.

2.5.3. Chemicals

All substances are prepared as stock solution dissolved in the vehicles water, ethanol or DMSO. Leukotriene D_4 (*LTD_4*; *Cayman Chemical*): 1.0e−4 M in ethanol, Capsazepine (*Tocris Bioscience*): 0.1 M in ethanol, Histamine (*Sigma Aldrich*) 0.1 M in water, PGD_2 (*Sigma Aldrich*) 0.1 M in ethanol, ACh (*Sigma Aldrich*) 1 M in water, Atropine (*Sigma Aldrich*) 1.0e−2 M in water, Propranolol (*Sigma Aldrich*) 0.1 M in water, *Nω*-nitro-L-arginine methyl ester (L-NAME) (*Sigma Aldrich*) 1 M in water, Capsaicin (*Sigma Aldrich*) 0.1 M in ethanol, Indomethacin (*Sigma Aldrich*) 1.0e−2 M in EtOH, Iberiotoxin (*Tocris Bioscience*) 1.0e−3 M in water, Nifedipine (*Tocris Bioscience*) 0.1 M in DMSO, I-RTX (*Tocris Bioscience*) 1.0e−2 M in EtOH, Ruthenium Red (*Sigma Aldrich*) 0.1 M in water, SB 366791 (*Tocris Bioscience*) 0.1 M in DMSO. JYL 1421: 3.0e-2 M in EtOH and KJM 429: 0.1 M in EtOH, synthesized as previously described [13]. *Capsazepinoids*, **1**–**7** synthesized as previously described [14]. All spectral data was in accordance with published data. Spectral data of the two new derivatives, **2** and **4** is given. Stock solutions for capsazepinoids: **1**: 0.1 M DMSO, **2**: 0.1 M ethanol, **3**: 0.1 M ethanol, **4**: 0.1 M ethanol, **5**: 0.1 M ethanol, **6**: 0.1 M ethanol, **7**: 0.033 M ethanol.

HRESIMS spectra were recorded with a Micromass Q-TOF Micro spectrometer. NMR spectra (in CD_3OD) were recorded with a Bruker DRX 400 spectrometer at 400 MHz (^1H) and at 100 MHz (^{13}C). Chemical shifts are given in ppm relative to TMS using the residual CD_2HOD peak in CD_3OD solution as internal standard (3.32 and 49.0 ppm, respectively relative to TMS).

7,8-Dihydroxy-N-octyl-1,3,4,5-tetrahydro-2H-2-benzazepine-2-carbothioamide (2): ^{1}H-NMR δ 0.89 (t, $J = 6.9$ Hz, 3H), 1.27 (bm, 10H), 1.55 (m, 2H), 1.79 (m, 2H), 2.79 (m, 2H), 3.54 (t, $J = 7.3$ Hz, 2H), 4.09 (bs, 2H), 4.68 (s, 2H), 6.59 (s, 1H), 6.82 (s, 2H) ^{13}C-NMR δ 14.5, 23.7, 27.9, 29.0, 30.4, 30.4, 30.5, 33.0, 34.9, 47.0, 54.5, 54.8, 118. 2, 118.2, 128.8, 134.2, 143.8, 145.3, 181.0. HRESI-MS calculated for $C_{19}H_{31}N_2O_2S$ (M + H) 351.2106, found 351.2103.

N-[2-(4-chlorophenyl)ethyl]-6,7-dimethoxy-3,4-dihydroisoquinoline-2(1H)-carbothioamide (4): ^{1}H-NMR δ 2.83 (t, $J = 5.8$ Hz, 2H), 2.95 (t, $J = 7.4$ Hz, 2H), 3.82 (s, 3H), 3.82 (s, 3H), 3.84 (t, $J = 7.4$ Hz, 2H), 3.96 (t, $J = 5.8$ Hz, 2H), 4.79 (s, 2H), 6.73 (s, 1H), 6.79 (s, 1H), 7.23 (m, 4H). ^{13}C-NMR δ 29.1, 35.7, 47.0, 47.9, 50.3, 56.5, 56.6, 111.0, 112.8, 126.6, 128.7, 129.4, 129.4, 131.6, 131.6, 133.0, 139.7, 149.2, 149.5, 182.1. HRESI-MS calculated for $C_{20}H_{24}ClN_2O_2S$ (M + H) 391.1247, found 391.1251.

Vehicle test: During experiments with substances that were dissolved in ethanol or DMSO, vehicle was added when the preparations were not exposed to the test substance, in order to exclude any influence by the vehicle.

2.6. Statistics

All test values are given as mean value ± standard error of the mean. Tests of statistical significance were performed using the ANOVA test. When evaluating the statistical significance of relaxations by capsazepinoids, the test contraction was compared to the third contraction in test experiments (Fig. 2).

3. Results

3.1. Effect of capsazepine on LTD_4-induced contractions

Human small bronchial preparations exposed to capsazepine showed a reversible, dose-dependent inhibition of LTD_4 contractions (Figs. 3 and 4). Capsazepine appeared equally effective in preventing LTD_4-contractions as in relaxing preparations with an established LTD_4-contraction (Fig. 5).

3.2. Effects of capsazepine on different contractile agonists

The reversible inhibitory effect of capsazepine (10 μM) was of a similar magnitude whether the bronchi were contracted with LTD_4 (10 nM), histamine (10 μM) or PGD_2 (10 μM) (Fig. 6), but significantly smaller when the bronchi were contracted with 100 μM ACh ($p = 0.04$,*). However, when ACh contracted preparations were instead exposed to 100 μM capsazepine the preparations relaxed strongly ($62 \pm 14\%$, $n = 3$).

3.3. Inhibitory effects of capsazepine analogues

Interestingly, several substances with capsazepine-like chemical structure (capsazepinoids) also produced distinct relaxations (Table 1) suggesting that the inhibitory effect of capsazepine is a class effect. The relaxation amounted to 20–70% for this set of different but structurally related compounds.

3.4. Interaction between capsazepine and pharmacological antagonists and capsaicin

The anti-cholinerigic substance atropine (1 μM) and the β-adrenergic receptor antagonist propranolol (10 μM) were present during one contractile cycle (1.5 h) before and during the capsazepine treatment. However, the inhibitory effect of capsazepine on LTD_4 contractions was not significantly reduced by these antagonists (Table 2).

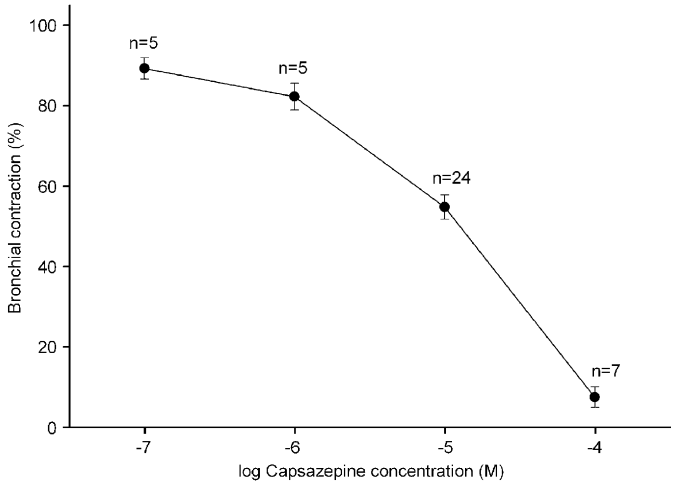

Fig. 3. Dose–response relationship for capsazepine-induced inhibition of LTD_4-induced contractions.

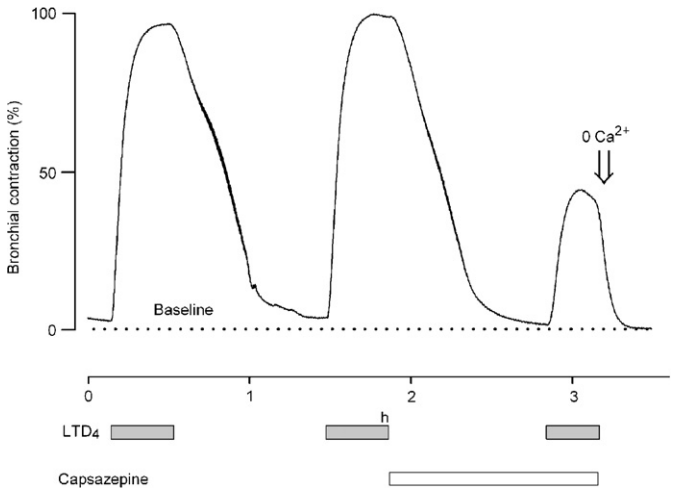

Fig. 4. Original recording of a preparation exposed to capsazepine (10 μM) for 1 h. Two control contractions were followed by an LTD_4 (10 nM) contraction in the presence of capsazepine. The experiment was concluded with Ca^{2+}-free solution to illustrate the passive tension level.

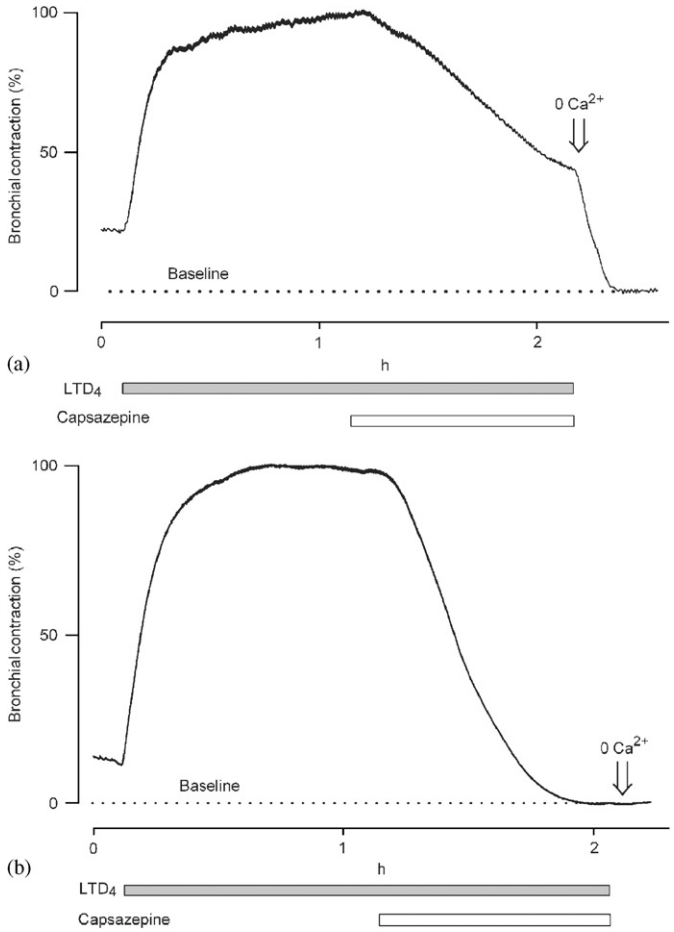

Fig. 5. Relaxing effect by capsazepine 10 μM (a) and 100 μM (b) in the continuous presence of LTD_4. Capsazepine gives strong relaxations of the already established LTD_4-contractions.

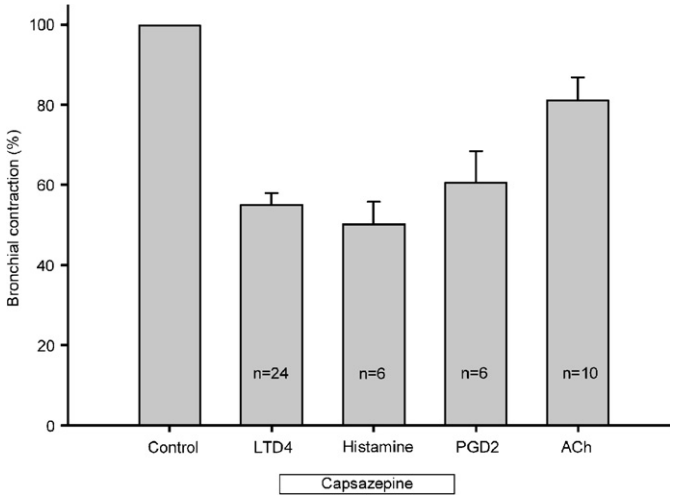

Fig. 6. Inhibitory effect of capsazepine (10 μM) on preparations contracted with different agonists.

Table 2
Inhibitory effect of capsazepine (10 μM) in control conditions and after different pre-treatments

Pre-treatment	Mean relaxation	*n*	Significance[a]
Control	45±3.0	23	
Atropine + propranolol	41±5.3	10	n.s.
L-NAME	39±5.0	11	n.s.
Indomethacin	35±4.1	13	n.s.
Capsaicin	43±7.8	9	n.s.

[a]None of the pre-treatments significantly reduced the effect of capsazepine.

To examine if production of nitric oxide is responsible for the capsazepine-induced relaxation, preparations were pretreated with the nitric oxide synthase inhibitor L-NAME (1 mM) during one contractile cycle (1.5 h) before and during the capsazepine treatment. No significant interaction occurred (Table 2). To clarify if prostaglandins are involved in the capsazepine induced relaxation, preparations were pretreated with the COX inhibitor indomethacin (1 μM) during one contractile cycle (1.5 h) before and during the capsazepine treatment. This did not significantly reduce the capsazepine relaxation (Table 2). Similarly, pre-treatment with the $TRPV_1$ agonist capsaicin (10 μM), did not reduce the effect of capsazepine (Table 2). A similar pre-treatment with the Ca^{2+} activated K^+ channel inhibitor iberiotoxin (0.1 μM, $n = 2$) did not reduce the effect of a subsequent 100 μM capsazepine exposure, which caused a full relaxation of 93±0.5%.

3.5. Interaction between capsazepine and nifedipine

It has previously been shown [15] that capsazepine can relax isolated rat ileum, and that this relaxation is abolished by pre-treatment with the voltage operated L-type calcium channel antagonist nifedipine (1 μM). Therefore, dose–response relationships for capsazepine-induced inhibition of LTD_4 contractions were examined with nifedipine present during one contractile cycle (1.5 h) before and during the capsazepine treatment. However, the capsazepine relaxation was not appreciably affected by pre-treatment even with the relatively high nifedipine concentration of 10 μM (Fig. 7). The LTD_4 contraction after the pre-treatment with nifedipine was 47±8.7% of the control contraction.

3.6. Comparision with known $TRPV_1$-antagonists

In order to clarify if $TRPV_1$ receptor antagonism is responsible for the capsazepine-induced relaxation, preparations were exposed to several well-established $TRPV_1$

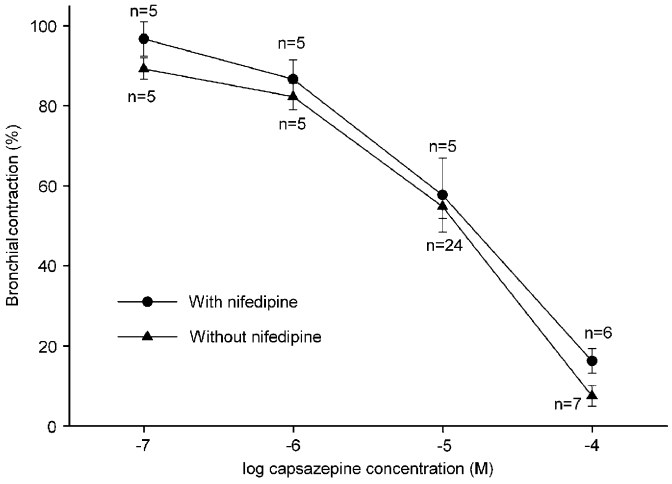

Fig. 7. Dose–response relationship of capsazepine in control situation and after exposure to nifedipine (10 μM) during one contractile cycle (1.5 h) before and during the capsazepine treatment. The capsazepine curve was not changed by nifedipine pre-treatment. One preparation exhibiting exceedingly poor response (~25%) to capsazepine (100 μM) in the presence of nifedipine was considered an outlier and was excluded from the calculations for dose response.

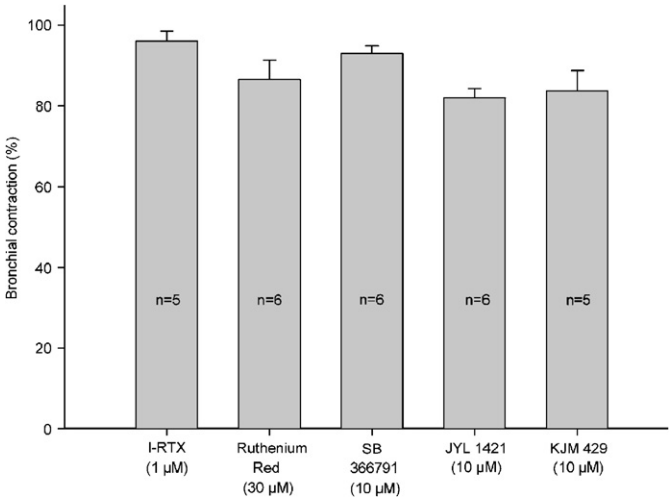

Fig. 8. Poor bronchorelaxing effect by known $TRPV_1$-antagonists ($p = 0.3$, 0.8, 0.5, 0.4, 0.5, respectively).

receptor antagonists. Most of them were given in concentrations more than 100 times higher than the substances reported EC_{50} for their $TRPV_1$ antagonistic effect. In spite of the high concentrations, none of the $TRPV_1$-antagonists caused any significant effect on the contraction (Fig. 8).

4. Discussion

4.1. Principal findings and conclusions

This paper examines the effect of capsazepine and several capsazepinoids on human small airway preparations. The principal findings are: (1) capsazepine reversibly and concentration-dependently inhibits the contractile response to LTD_4 and is about equally effective against several different contractile agonists; (2) the effect of capsazepine is not caused by β-adrenergic receptor activation or inhibition of L-type calcium channels; (3) the inhibitory effect of capsazepine is shared by chemical analogues, but not with other classes of $TRPV_1$ antagonists, suggesting the possibility that capsazepine represents a novel class of bronchorelaxants effective in human small airways. These findings were not predicted by previous observations that have concerned quite limited effects of capsazepine on airway tone in different animal test systems.

4.2. Preparation

Since the small bronchi are thin and delicate, particular care was taken in dissecting them free from surrounding lung parenchyma. Also, other factors, including temperature, pH, pO_2 and pCO_2 and minimal stress at washing procedures were carefully controlled. The present human small airway preparations were stable and consistently responsive to contractile agonists for more than 12 h suggesting that the handling of the preparations during transport, dissection, mounting and running of the experiments was appropriate.

4.3. Bronchorelaxing effect of capsazepine

In this paper, we describe for the first time that capsazepine is a powerful and general bronchorelaxant in human small airways. Owing to a slow onset of the relaxant action of this compound (this study) the present evaluation focused on the ability of capsazepine to inhibit contractile effects. Hence, we demonstrate here that the presence of capsazepine prevents the initial rise of mediator-induced contractions. Equally, the established LTD_4-induced tonic contraction was relaxed by capsazepine in this study. This observation contrasts the findings by Rousseau et al. [7] who reported that the initial contractile effect of 20-HETE in guinea-pig trachea was unaffected by the presence of capsazepine. Other previous findings in guinea-pigs suggested that capsazepine merely would exert selective effects on $TRPV_1$ mediated effects [4]. Thus, bronchoconstriction by histamine or neurokinin A (17), as well as the basal tone [16], was unaffected by capsazepine in guinea-pigs whereas in the present human small airways the contractile effect of histamine was prevented along with that of LTD_4 and PGD_2. It is possible that both species differences and airway generation differences could have contributed to the diverging observations.

Further work is now warranted to explain the present efficacy of capsazepine as well as its mechanisms of action in human small airways. Its mode of action remains unknown although it could be ruled out (this study) that currently established bronchodilator principles likely do not contribute to the inhibitory effects of capsazepine. Some compounds in Table 1 have previously been reported [14] as $TRPV_1$-agonists (**3** and **7**) and some other as $TRPV_1$-antagonists (**2**, **5** and **6**). Clearly, the bronchorelaxing effect of our compounds does not reflect their $TRPV_1$ antagonistic effect. We further could demonstrate that a series of known $TRPV_1$ antagonists, that are chemically unrelated to capsazepine, were without the bronchorelaxing property exhibited by capsazepine. The present finding that compounds with slightly different chemical structure from capsazepine produced distinct inhibitory effects supports the notion that the present discovery may represent a novel class of bronchorelaxants. Furthermore, one derivative, **3**, out of only a small number of chemical analogues, produced a greater relaxation than capsazepine making it likely that further modifications of the structure can increase the relaxing potency of this kind of drugs even more.

Doubts have been raised about the possibility to find bronchodilators as effective and free from side-effects as the β_2-agonists [17–20]. Although the mechanism of action of the present capsazepine-type of bronchorelaxants is unknown, it emerges here that they, similar to the β_2-agonists, may represent a generally effective principle exerting a functional antagonism against contractile mediators. Thus, the present compounds differ from the pharmacologic antagonists, including antihistamines, anti-leukotrienes, and anti-cholinergics which are effective only against a single type of mediator or neurotransmitter. Furthermore, it has been reported that β_2-agonists may only produce inconsistent relaxation of human small airway preparations [21]. Hence, a novel principle such as the present capsazepinoids that reliably inhibit contractile effects may be a useful addition to the presently available drugs to treat diseases such as asthma and COPD. Since COPD and to a significant extent asthma may be considered small airways diseases [22,23] it is of particular interest that the present compounds exhibit efficacy in human small bronchi. Indeed, since previous work involving animal studies [4,16,24] has failed to identify the general bronchorelaxing properties of capsazepine the present discovery apparently required the use of human bronchial preparations as a primary study approach.

In conclusion, capsazepine and some closely related analogues have been found to inhibit human small airway responsiveness to contractile mediators. If potency can be further increased and the results translated to in vivo, compounds representing this novel class of bronchorelaxants might become useful in the treatment of patients suffering from asthma and COPD. The present results thus stress the need of structure–activity relationship studies for this class of compounds as well as further investigations into their mechanism of action.

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