Available online at www.sciencedirect.com
ScienceDirect
Pulmonary Pharmacology & Therapeutics 20 (2007) 273–280
PULMONARY PHARMACOLOGY & THERAPEUTICS
www.elsevier.com/locate/ypupt

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

Staffan Skogvall[a,*], Magnus Berglund[b], María F. Dalence-Guzmán[b,c], Katrin Svensson[a], Per Jönsson[d], Carl G.A. Persson[e], Olov Sterner[b]

[a]*Respiratorius AB, Magistratsvägen 10, SE-226 43 Lund, Sweden*
[b]*Department of Organic Chemistry, Lund University, P.O. Box 124, SE-221 00 Lund, Sweden*
[c]*Centro de Tecnologia Agroindustrial, Facultad de Ciencias y Tecnologia, Universidad Mayor de San Simón, Cochabamba, Bolivia*
[d]*Department of General Thoracic Surgery, Lund University Hospital, Lund, Sweden*
[e]*Department of Clinical and Experimental Pharmacology, Lund University Hospital, University of Lund, SE-221 00 Lund, Sweden*

Received 4 November 2005; received in revised form 22 March 2006; accepted 24 March 2006

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

*Corresponding author. Tel.: +46 462 863 610; fax: +46 462 863 619.
E-mail address: staffan.skogvall@respiratorius.se (S. Skogvall).

1094-5539/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.pupt.2006.03.002

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.

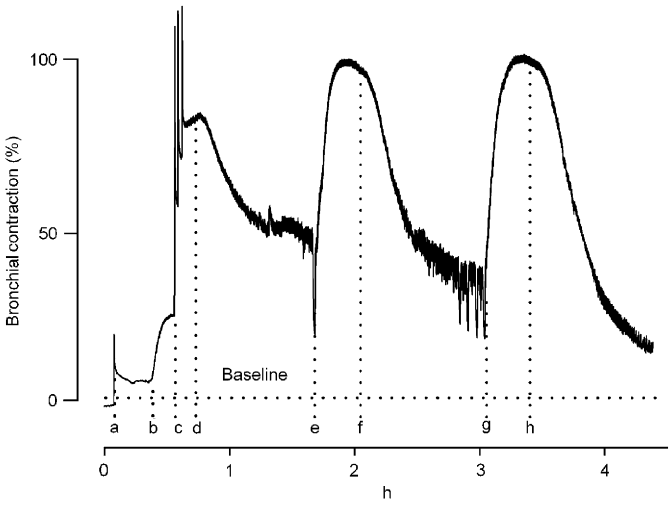

Fig. 1. An original recording of a human small bronchus showing the initial procedures to obtain a stable preparation. After mounting in the experimental chamber the preparation was stretched 0.4 mN (a). After 20 min, LTD_4 (10 nM) was added (b). When the contraction had reached a plateau, the preparation 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.