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Effect of Levocetirizine on the Contraction Induced by Histamine on Isolated Rabbit Bronchioles from Precision-Cut Lung Slices

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Key Words
Levocetirizine · Antihistamine · Lung slice, precision-cut · Bronchiole, rabbit

Abstract
The present experiments were designed to study the effects of levocetirizine (a potent selective histamine H_1 antagonist) on the contraction induced by histamine on isolated rabbit bronchioles using the precision-cut lung slice technology. Histamine induced a concentration-dependent contraction of isolated rabbit bronchioles (pD_2 value of 5.6). Mepyramine (0.01–1 μmol/l) induced a shift to the right without any decrease in the concentration-response curve to histamine (pA_2 value of 8.2). Levocetirizine (0.03–0.1 μmol/l) induced both a shift to the right and a decrease in the maximal amplitude of the concentration-response curve to histamine (pA_2 and pD'_2 values of 7.9 and 7.0, respectively). The difference between both compounds could be explained in terms of the difference in the dissociation rate from the histamine H_1 receptor coupled to a putative low receptor reserve present in the rabbit bronchioles.

Introduction

As a result of their relative inaccessibility, the role of small airways in lung diseases, such as asthma, chronic obstructive pulmonary disease, idiopathic pulmonary fibrosis, sarcoidosis or obliterative bronchiolitis, is not fully understood [for review, see 1]. For example, in asthma, originally described as a predominantly large airways complex chronic inflammatory disease characterized by bronchoconstriction and airway hyperresponsiveness as major symptoms, small airways seem to play a significant role in airway inflammation and hyperresponsiveness [1–4]. In consequence, a better understanding of the role of the small airways in lung diseases may lead to the development of new therapies. The precision-cut lung slice is a novel and unique in vitro technology developed by Martin et al. [5] in order to assess, under cell culture conditions, the integrated response of small airways down to the terminal bronchioles by quantitative video microscopy. Under these experimental conditions, it has been shown that the allergic response of sensitized rat or human airways is more pronounced in small airways than in large airways [5, 6].

Histamine is a major mediator of inflammation [for review, see 7, 8] and H_1-antihistamines are more relevant than ever in the treatment of allergic disorders [9]. Even in asthma where traditionally H_1-antihistamines have generally been considered to be ineffective, there is increasing evidence that they could have clinically beneficial effects on asthma disease control [for review, see 10, 11]. Histamine H_1 receptors [12] have been identified in small airways of the most common species (guinea pig, human, monkey, pig, dog, rabbit, cat, rat, etc.) but not in rabbit, cat or rat trachea or rhesus monkey bronchus. Moreover, the sensitivity to histamine could be higher in

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intrapulmonary airways than in tracheal strips as observed in the dog [13].

The present experiments were designed to study the reactivity to histamine of bronchioles from different levels of the respiratory tract using the method of precision-cut lung slices. The studied bronchioles were classified as follows: bronchioles with a diameter of 412–730, 730–1,000 and 1,000–1,906 μm, corresponding in the right lung lobe to airway generation 8–7, 7–6 and 6–5, respectively, based on the Weibel model of rabbit airway generation [14]. The final aim of the study was to compare the histamine H_1 antagonistic properties of levocetirizine (Xyzal™, UCB) and mepyramine on bronchioles from different levels of the respiratory tract.

Materials and Methods

Tissue Preparation

Lungs were taken from 4- to 5-week-old male New Zealand White rabbits (0.6–0.9 kg, Charles-River, Chatillon, France). Rabbits were sacrificed by intraperitoneal injection of pentobarbital (250 mg/kg) and exsanguinated by cutting the vena cava under guidelines approved by the UCB Pharma Ethical Committee. Rabbit lungs were prepared essentially as described by Martin et al. [5]. Briefly the trachea was cannulated and the lungs filled with an agarose solution (type VIIa from Sigma, 1.5% in modified Krebs' solution, 44 ml/kg, 37°C) via the trachea. At that time, ice was put on it to allow the agarose to cool and solidify. Afterwards the lungs and hearts were removed in toto from the thoracic cavity. The lung lobes were separated and the right lobe was cut into 5- to 10-mm-thick slabs from which cores along the airways were made with a coring tool. These cores were cut into ~500-μm-thick slices using a Krumdieck tissue slicer (Alabama R&D, Munford, Ala., USA). This slice thickness was determined in preliminary experiments as being the smallest one to allow the most reproducible bronchiole response with regard to the airway size used. Two slices were immediately incubated in an incubation chamber for precision-cut lung slices (Hugo Sachs Elektronik, March-Hugstetten, Germany) with modified Krebs' solution. The bathing solution was maintained at 37°C and gassed with 95% O_2-5% CO_2. The incubation chamber was placed on the stage of an inverted microscope (Hund Wilovert, Helmut Hund GmbH, Wetzlar, Germany) in order to measure the reactivity by quantitative video microscopy. The other slices were prepared under sterile conditions in a laminar flow hood and placed in a 24-well microplate containing minimal essential medium (DMEM, Cambrex Biosciences, Verviers, Belgium) supplemented with 100 U/ml penicillin and 100 μg/ml streptomycin. Slices were cultured for maximum 96 h at 37°C in a humidified atmosphere of 5% CO_2-95% air in a carbon dioxide incubator (Binder CB150, VWR, Louvain, Belgium). Two hours after slicing or at the end of the culture period, one cumulative concentration-response curve to histamine was elicited in the presence or absence of the test antagonist compound after a 60-min incubation period. Results were obtained from at least 2–3 individual experiments. Control tissues were treated with the solvent.

Analysis of the Results

Airways were imaged with a video camera (Pulnix TM 765E, Pulnix America Inc., San Jose, Calif., USA) controlled by the image analysis software Wit 7.1 Professional (Coreco Imaging Inc., Saint Laurent, Que., Canada). The airway area before addition of histamine was defined as 100%. Bronchoconstriction is expressed as μm^2 and normalized as the percentage decrease in airway area in comparison with the control value. Antagonistic activity of the test compound was estimated by calculating the pA_2 and/or pD'_2 values according to the method described by Van Rossum et al. [15] or Arunlakshana and Schild [16]. pA_2 is defined as the negative logarithm of the molar concentration of antagonist that would produce a 2-fold shift to the right in the concentration-response curve for an agonist. pD'_2 is defined as the negative logarithm of the molar concentration of antagonist that would produce a 50% reduction in the maximal effect induced by an agonist. Comparison between two means was made using Student's paired or unpaired t test [17]. Comparison between several means was made using an F test (variance analysis with one classification parameter) [17].

Drugs and Solutions

The agarose was the type VII-A from Sigma (Saint Louis, Mo., USA). The solution for cutting and incubating slices in the precision-cut lung slice chamber was a modified Krebs' solution of the following composition: 116 mmol/l NaCl, 1.8 mmol/l $CaCl_2 \cdot 2H_2O$, 0.8 mmol/l $MgSO_4 \cdot 7H_2O$, 5.4 mmol/l KCl, 0.9 mmol/l $NaH_2PO_4 \cdot 2H_2O$, 16.7 mmol/l glucose, 26.2 mmol/l $NaHCO_3$, 25.17 mmol/l HEPES (Sigma) in deionized water. Before sterile filtration, 20 ml/l minimal essential medium amino acid solution (×50), 10 ml/l minimal essential medium vitamin (×100), sodium pyruvate 1 mmol/l and glutamine 2 mmol/l (all from Gibco, Merelbeke, Belgium) were added to the solution. Lung slices were cultured in a minimal essential medium (DMEM, Cambrex Biosciences). The following drugs were used: levocetirizine (Xyzal™, UCB SA, Brussels, Belgium), mepyramine and histamine (both from Sigma, Saint Louis, Mo., USA).

Results

Histamine (fig. 1A) induced a concentration-dependent contraction of isolated bronchioles from precision-cut rabbit lung slices. Three different levels of rabbit bronchioles were tested according to the Weibel classification [14]: diameters of <730, between 730 and 1,000, and >1,000 μm. The potency and the intrinsic activity of histamine were independent of the airway generation. The calculated pD_2 values of histamine for these different levels were not statistically different: 5.41 ± 0.37 (n = 6), 5.61 ± 0.23 (n = 6) and 5.50 ± 0.32 (n = 6), respectively. Total stricture of the bronchiole was never observed and the maximal contractile effect of histamine corresponded to a decrease in the bronchiole diameter of 85.4 ± 9.9% (n = 6), 74.3 ± 14.6% (n = 6) and 83.7 ± 6.6% (n = 6) of the initial area, respectively.

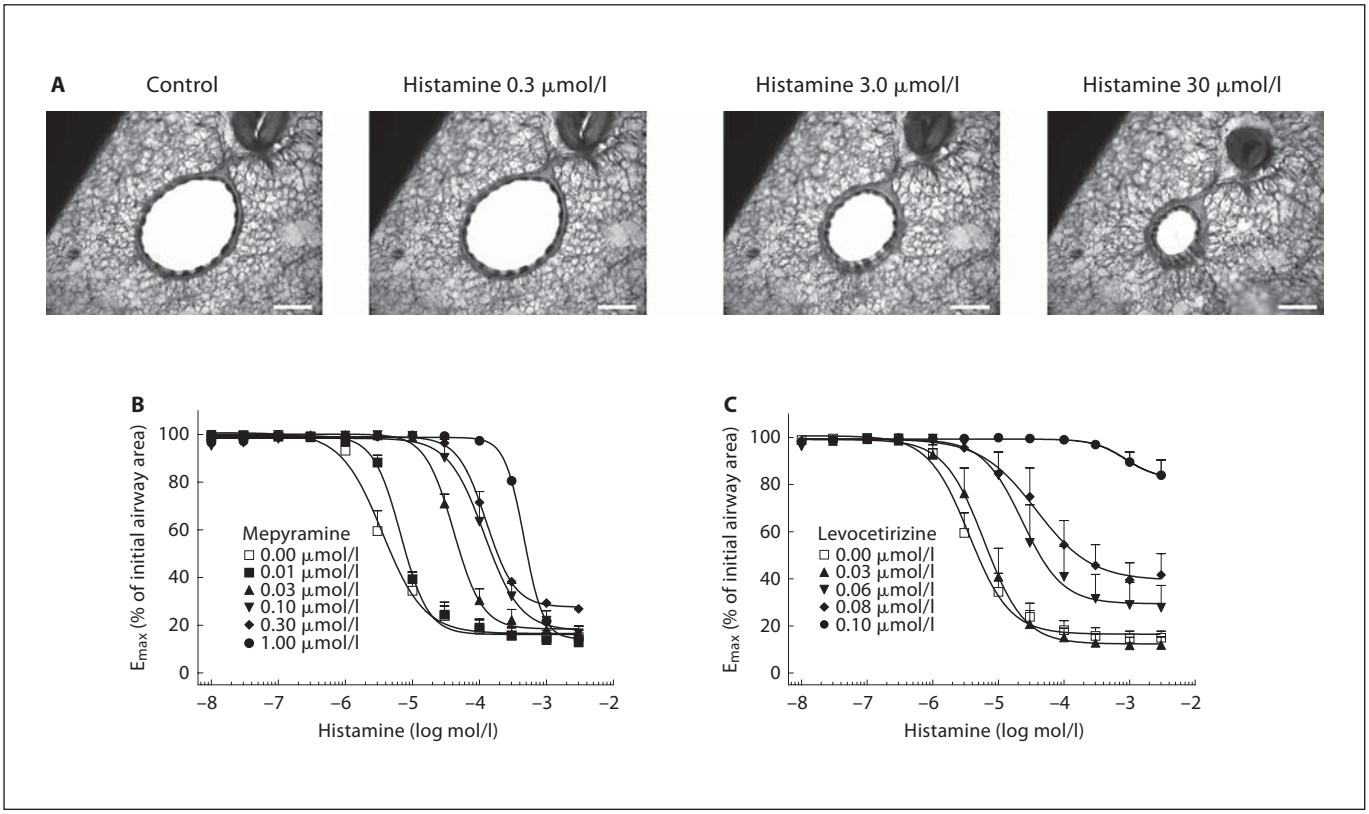

Fig. 1. Concentration-response curve to histamine elicited on the isolated rabbit bronchioles (diameter >1,000 μm) from precision-cut lung slices. **A** Typical examples of pictures obtained by video microscopy from a bronchiole in the absence or presence of different concentrations of histamine. White bar scale = 500 μm. **B** Histamine curves elicited in the absence or presence of various concentrations of mepyramine. **C** Histamine curves elicited in the absence or presence of various concentrations of levocetirizine. **B**, **C** The ordinate is the maximal amplitude (E_{max}) of the contraction induced by histamine expressed as percentage of the maximal airway area before the incubation with the compounds. The abscissa is the log molar concentration of histamine. Points represent mean values (n = 2–6). Vertical bars represent SEM.

Mepyramine (0.01–1 μmol/l) and levocetirizine (0.03–0.1 μmol/l) induced no contractile effect on the bronchioles from precision-cut rabbit lung slices whatever the size of the bronchiole.

The effect of mepyramine was tested on one set of rabbit bronchioles (diameter >1,000 μm). Increasing concentrations of mepyramine (0.01–1 μmol/l) induced a shift to the right without any decrease in the maximal amplitude of the concentration-response curve to histamine (fig. 1B). The slope of the Schild plot analysis was not different from unity (table 1), indicating the competitive nature of the antagonism existing between mepyramine and histamine on histamine H_1 receptors present on the isolated rabbit bronchioles from precision-cut lung slices. The pA_2 values calculated according the Van Rossum method [15] are presented in table 1.

The effect of levocetirizine was tested on three sets of rabbit bronchioles (diameter <730, between 730 and 1,000, and >1,000 μm). Increasing concentrations (0.03–0.1 μmol/l) of levocetirizine (fig. 1C) induced both a shift to the right and a decrease in the maximal amplitude of the concentration-response curve to histamine whatever the size of the bronchiole. This decrease in the maximal amplitude was almost complete at 0.1 μmol/l levocetirizine. The calculated pA_2 and/or pD'_2 values are presented in table 1 and were similar whatever the size of the bronchioles.

Table 1. pA_2 and pD'_2 values for levocetirizine and mepyramine of histamine-induced contraction in rabbit bronchioles

Drug	Bronchiole diameter, μm	pK_b (slope)	pA_2	pD'_2
Levocetirizine	<730	–	8.10 ± 0.58 (n = 18)	7.03 ± 0.37 (n = 5)
	730–1,000	–	8.17 ± 0.73 (n = 27)	6.93 ± 0.29 (n = 9)
	>1,000	–	7.89 ± 0.55 (n = 13)	6.97 ± 0.38 (n = 11)
Mepyramine	>1,000	8.44 (0.82 ± 0.14, n = 17)	8.20 ± 0.32 (n = 17)	–

pK_b values were calculated according to Arunlakshana and Schild [16], and pA_2 or pD'_2 values were calculated according to Van Rossum et al. [15] as described in Materials and Methods. The results are given as mean ± SD of the number of determinations.

Discussion

The present study describes for the first time the preparation of precision-cut lung slices from rabbits and the reactivity of airways to histamine in this preparation. In contrast to guinea pigs [18], no postmortem airway contraction was observed with the rabbit during the preparation of the precision-cut lung slices, therefore avoiding the need of a relaxant agent such as isoproterenol in the initial incubation medium. The present results demonstrate that histamine is capable of contracting rabbit bronchioles of various sizes (diameter <730, between 730 and 1,000, and >1,000 μm). The pD_2 values calculated for histamine were similar not only for the three different rabbit bronchiole levels (5.41–5.61) but also for various parts of airways from various species, such as 5.7 for the guinea pig trachea [19], 5.5 for the guinea pig bronchus [20], 6.0 for the guinea pig parenchyma [21], 5.1 for the bovine trachea [22], or 5.4 for the human bronchus [23], suggesting a similar sensitivity to histamine along the whole respiratory tract whatever the species. This is also confirmed by recent data from guinea pig (6.7) and human (5.6) precision-cut lung slices [18].

The present results also demonstrate that mepyramine and levocetirizine antagonize the contractile effect of histamine on the isolated rabbit bronchiole from precision-cut lung slices. A surmountable competitive and an insurmountable profile of inhibition was observed for mepyramine and levocetirizine, respectively. The pA_2 value calculated for mepyramine (8.2) is similar to the value reported in the literature for various airway preparations from different species such as 8.54 for the guinea pig trachea [19] or 8.52 for the human bronchus [24], with the exception of 9.60 for the guinea pig lung parenchyma [21]. The pA_2/pD'_2 values calculated for levocetirizine were similar whatever the airway diameters of the bronchiole tested and were comparable to the values (7.87/7.03) already reported in the literature for the isolated guinea pig trachea [19]. The noncompliance of experimental Schild regression slope with unity or a decrease in the maximal amplitude of the concentration-response curve may be explained in a number of ways [25]: (i) the antagonism is not competitive; (ii) a drug-disposition mechanism or other non-equilibrium steady state obscures the competitive nature of the antagonism; (iii) the competitive antagonism of a heterogeneous receptor population sub-serving the same response is observed, or (iv) multiple drug properties are expressed in the concentrations used to make the measurement. The first alternative is usually considered only after elimination of the other three, because they can obscure true competitive antagonism to the point that it resembles true, noncompetitive, antagonism. In the guinea pig trachea, mixed antagonism of levocetirizine was explained by the small histamine H_1 receptor reserve present in this tissue and a very slow dissociation rate (142 min) of levocetirizine from the histamine H_1 receptor [26]. The similar mixed antagonism observed for levocetirizine with rabbit bronchioles stimulated with histamine could probably be explained with similar arguments. Nevertheless, the concentration range of levocetirizine used to obtain a maximal amplitude decrease was steeper with the rabbit bronchiole (0.03–0.1 μmol/l) than with the guinea pig trachea (0.01–0.3 μmol/l) and a nearly full inhibition of the maximal amplitude of the concentration-response curve to histamine was observed on the bronchiole. This observation suggests the existence of a smaller histamine H_1 receptor

reserve for the rabbit bronchiole in comparison to the guinea pig trachea.

The present study demonstrates the high potency inhibitory effect of levocetirizine on rabbit airways down the terminal bronchioles contracted with histamine. Comparison with normal or pathological human bronchiole using this precision-cut lung slices technology could probably help determine the role of histamine and the usefulness of antihistamine in the various diseases involving the small airways.

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