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The Effect of Exposure of Guinea Pig to Cigarette Smoke and their Sensitization in Tracheal Responsiveness to Histamine and Histamine Receptor (H_1) Blockade by Chlorpheniramine

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Abstract

Airway responsiveness to histamine and histamine H_1 receptor blockade by chlorpheniramine (CR-1) on guinea pig trachea were examined. Chronic obstructive pulmonary disease (COPD) and asthma were induced in guinea pigs by exposing them to cigarette smoke for 3 months and by sensitization with injection and inhalation of ovalbumin (OA). The responses of tracheal chains of COPD ($n = 8$), COPD + asthma ($n = 6$) and control animals ($n = 8$) to histamine (EC_{50} H) and (CR-1) were measured.

The in vitro histamine responses of COPD and COPD + asthmatic guinea pigs in tracheal chains were significantly higher than those of control animals ($p < 0.001$ and $p < 0.01$, respectively). The CR-1 blockade was also significantly greater in trachea of COPD and COPD + asthma compared to that of controls ($p < 0.01$ and $p < 0.05$, respectively). There were significant correlations between EC_{50} H and (CR-1) ($r = -0.542$, $p < 0.01$). The hematocrit in COPD and COPD + asthma groups was also significantly higher than in controls ($p < 0.001$ for both groups). The contractility of tracheal chains to histamine in COPD + asthma animals was significantly greater than those of control and COPD groups ($p < 0.05$ for both cases). The differences in contractility between COPD and COPD + asthmatic groups, however, suggests different basic mechanisms for AHR in COPD and asthma.

Keywords: Tracheal responsiveness; Histamine; H_1 receptors; Chlorpheniramine blockade; COPD; Asthma; Guinea pig

1. Introduction

Chronic obstructive pulmonary disease (COPD) is a global health problem, reaching almost epidemic proportions in the developing world [1]. The understanding of basic mechanisms of the disease and the development of new strategies to prevent the progression of this condition presents a major challenge.

It is believed that in cigarette smokers the airflow obstruction is caused by parenchyma disease (emphysema) and/or by smoke-induced distortion of the structure of the small airways [2–4]. A model of cigarette smoke-induced lung disease has been described in guinea pigs, which develop airflow obstruction and emphysematous lung destruction [5].

The most characteristic feature of asthma is bronchial hyperresponsiveness to a wide variety of inhaled physical, chemical, pharmacological and immunological stimuli. There are reports regarding airway hyperresponsiveness to different stimuli also in animals exposed to cigarette smoke [6–11]. However, the mechanisms of the bronchial response to pharmacological agonists such as methacholine and histamine are highly complex and inadequately understood. Therefore, little is known regarding the mechanism(s) of increased airway responsiveness (AHR).

The mechanism(s) of action of a competitive antagonist measured as the degree of rightward shift or dose ratio or concentration ratio (DR or CR) is far simpler than that of an agonist. It depends only to concentration of antagonist at the receptor sites ([*I*]) and receptor affinity (K_a) [12]. Receptor blockade by a competitive antagonist could help in revealing the mechanism(s) of airway responsiveness. In previous

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studies we have demonstrated enhanced blockade of different receptors by their antagonists both in asthmatic patients [13–15] and sensitized animals [16,17].

In the present study the tracheal responsiveness of guinea pigs exposed to cigarette smoke alone (an animal model of COPD), and exposed to cigarette smoke + sensitized to OA (an animal model of COPD and asthma) to histamine and histamine (H_1) receptor blocked by chlorpheniramine was studied.

2. Material and methods

2.1. Animals, cigarette smoke exposure and sensitization

Twenty-two adult Dunkin–Hartley guinea pigs (400–500 g) of both sexes were divided into three groups, two experimental groups including cigarette smoke exposure alone (an animal model of COPD, $n = 8$), and exposed to cigarette smoke + sensitized to OA (an animal model of COPD + asthma, $n = 6$) and one control group ($n = 8$).

Experimental animals were exposed to cigarette smoke as previously described [18–20]. The animals were exposed to cigarette smoke in an awake, restrained state and spontaneously breathing in a smoking chamber which was a modification of that described by Simani et al. [18]. Animals were placed in a Plexiglas box with their heads secured in a compartment (15 cm × 12 cm × 7 cm). Twenty-milliliter puffs of cigarette smoke was drawn out of cigarettes with syringes and then exhausted at a rate of two puffs per minute into the animal's head chamber. Exposure of animals to each cigarette lasted for 8–9 min, with a 10 min resting period between cigarettes. The animals were initially exposed to one commercial non-filter cigarette per day, and this dose was increased to a maximum of 5 cigarettes per day over a 2-week period. In a pilot study it was observed that animals could not tolerate the exposure of cigarette smoke of more than 5 cigarettes per day. The exposure to the smoke of 5 cigarettes per day, 6 days per week, continued for 3 months. The guinea pigs exposed to cigarette smoke were called COPD animals. The control animals were not exposed to cigarette smoke, and they were kept in the animal house under normal conditions for the same period of time.

Sensitisation of animals to ovalbumin (OA) was performed using the method described by McCaig [21–23] during their exposure period to cigarette smoke. Briefly, 6 guinea pigs were sensitised to ovalbumin (Sigma Chemical Ltd., UK) by injecting 100 mg i.p. and 100 mg s.c. on day 1 and a further 10 mg i.p. on day 8. From day 14 sensitised animals were exposed to an aerosol of 4% OA for 18 ± 1 days, 4 min daily. The aerosol was administered in a closed chamber, dimensions 30 cm × 20 cm × 20 cm.

The study plan was approved by the Ethical Committee of the Mashhad University of Medical Sciences.

2.2. Tissue preparation

Guinea pigs were killed by a blow on the neck. Trachea was removed and cut into 10 rings (each containing 2–3 cartilaginous rings). All the rings were then cut open opposite the trachealis muscle, and sutured together to form a tracheal chain [21,22] and then suspended in a 10 ml organ bath (organ bath 61300, BioScience Palmer-Washington, Sheerness, Kent, UK) containing Krebs–Henseleit solution of the following composition (mmol/l): NaCl 120, $NaHCO_3$ 25, $MgSO_4$ 0.5, KH_2PO_4 1.2, KCl 4.72, $CaCl_2$ 2.5 and dextrose 11 maintained at 37 °C and gassed with 95% O_2 and 5% CO_2. Tissue was suspended under isotonic tension of 1 g and allowed to equilibrate for at least 1 h while it was washed with Krebs solution every 15 min.

2.3. Measurement of tracheal responses to histamine and histamine (H_1) receptor blockade

In each experiment two cumulative log concentration–response curves (LCRC) of histamine phosphate (BDH Chemical Co. Ltd., UK) induced contraction of tracheal chain were obtained, one 10 min after producing 10 nM concentration of chlorpheniramine maleate (Sigma Chemical Ltd., UK) in organ bath (adding 0.1 ml of 1 μM chlorpheniramine solution to organ bath = post-chlorpheniramine histamine response curve), and the other 10 min after adding the same volume of saline (baseline histamine response curve).

Cumulative log concentration–response curve of tracheal chain to increasing concentrations of histamine (0.1 μM–10 mM) was obtained with additions of consecutive concentrations every 2 min. To obtain the curve the percentage of contraction of the tracheal smooth muscle due to each concentration of histamine in proportion to the maximum contraction obtained, in baseline histamine response curve, was calculated and plotted against log concentration of histamine.

The effective concentration of histamine causing 50% of maximum response (EC_{50} H) of baseline and post-chlorpheniramine histamine response curve in each experiment was measured (expressed as EC_{50} H and post-chlorpheniramine EC_{50} H, respectively). The tracheal response to histamine was considered as EC_{50} H. The histamine H_1 receptor blockade by chlorpheniramine was assessed as concentration ratio minus one (CR-1) which was calculated by: (post-chlorpheniramine EC_{50} H/EC_{50} H) − 1.

The experiments to measure post-chlorpheniramine histamine response curve and baseline histamine response curve in each tracheal chain were performed randomly with 1 h resting period between each of the two experiments while washing the tissues every 10 min. Tracheal responses to histamine were tested on incubated tissues with 1.4 μM indomethacin 30 min prior and during obtaining LCRC in the presence of both saline and chlorpheniramine. In all experiments responses were recorded on a kymograph (ET8 G-Boulitt, Paris, France) and measured after fixation.