European Journal of Pharmacology, 119 (1985) 117–120
Elsevier

Short communication

HAEMOPHILUS INFLUENZAE INDUCES A POTENTIATED INCREASE IN GUINEA-PIG PULMONARY RESISTANCE TO HISTAMINE

GERT FOLKERTS * and FRANS P. NIJKAMP

Institute for Veterinary Pharmacology, Pharmacy and Toxicology, Faculty for Veterinary Sciences, University of Utrecht, Biltstraat 172, 3572 BP Utrecht, The Netherlands

Received 3 October 1985, accepted 15 October 1985

G. FOLKERTS and F.P. NIJKAMP, *Haemophilus influenzae induces in a potentiated increase in guinea-pig pulmonary resistance to histamine*, European J. Pharmacol. 119 (1985) 117–120.

The human respiratory pathogen *Haemophilus influenzae* (H.i.) induced bronchial hyperreactivity to histamine (1.0-8.0 μg/100 g b.w. i.v.) in vivo in anaesthetized spontaneously breathing guinea-pigs. This hyperreactivity was caused by a potentiated increase in pulmonary resistance. Decreases in dynamic compliance did not differ. Atropine prevented the potentiation at 1.0 and 2.0 μg histamine/100 g b.w. These results show that H.i. induces bronchial hyperreactivity in vivo which is mediated by direct and reflex effects of histamine in the central airways.

Respiratory airway hyperreactivity *Haemophilus influenzae* Histamine Pulmonary resistance

1. Introduction

Increased bronchial irritability, or hyperreactivity to a wide variety of stimuli is a characteristic feature of patients with asthma. It is well established that respiratory airway infections in healthy subjects result in an increased bronchospasm in response to inhaled methacholine and histamine (Empey et al., 1976; Laitinen, 1974).

In our previous studies we showed that *Haemophilus influenzae* (H.i.), a Gram-negative bacterium the non-capsulated form of which can be isolated from the deeper respiratory airways of patients with chronic asthmatic bronchitis, induced in guinea-pig respiratory tissue in vitro an imbalance between autonomic bronchodilator (β-adrenergic) and bronchoconstrictor (cholinergic) mechanisms (Schreurs et al., 1980). Furthermore in vivo these animals become asphyxial in response to histamine significantly faster than the control group (Schreurs and Nijkamp, 1984).

Since bronchial hyperreactivity is one of the diagnostic features of asthmatic patients, we further investigated the hyperreactivity to histamine in H.i.-treated guinea-pigs via on-line measurement of pulmonary resistance (Rpul) and dynamic lung compliance (Cdyn).

2. Materials and methods

The animals used were male guinea-pigs weighing 350-400 g (CPB, TNO, Zeist, The Netherlands). Treatment with H.i. was performed four days prior to the experiments. The animals were injected intraperitoneally (i.p.) with 5×10^8 killed cells (colony-forming units)/100 g b.w.

The guinea-pigs were anaesthetized with urethane (2 g/kg i.p.) and allowed to breath spontaneously. The left carotid artery was cannulated for the measurement of blood pressure (B.p.) and the right jugular vein for the injection of histamine HCl and atropine sulphate (OPG, Utrecht, The Netherlands). Pulmonary resistance and dynamic lung compliance were determined breath by breath by a modified method of Amdur and Mead (1958) using a computerized respiratory

* To whom all correspondence should be addressed.

analyzer. The trachea was cannulated and connected in series with a Gould Godart pneumotachograph and airflow ($\dot{V}$) was measured using a Fleisch flowhead no. 000. This flow signal was then integrated to calculate the tidal volume (Vt). Intra Pleural Pressure (I.P.P.) was recorded with a Validine MP45-24 pressure transducer which measured the pressure difference between the tracheal cannula and a cannula filled with water inserted in the oesophagus. Rpul was calculated from the pressure and flow changes between isovolume points (50%) in the same breath (Rpul = ΔI.P.P./$\Delta\dot{V}$) and dynamic lung compliance from the volume and pressure changes between points of zero flow in each breath (Cdyn = Vt/ΔI.P.P.). B.p., I.P.P., $\dot{V}$, V, Rpul, Cdyn, were recorded on a Watanabe chart recorder.

Two dose-response curves were obtained in each animal, one before and one after the administration of atropine. Atropine was administered 15 min before the start of the second dose-response curve. Histamine injections were given at time intervals of at least 10 min. Levels of significance were calculated by using Student's t-test.

3. Results

Histamine (0.5-8.0 μg/100 g b.w. i.v.), administered as a bolus injection (0.1 ml) caused a dose-dependent increase in Rpul and a dose-dependent decrease in Cdyn. Basal values for saline- and H.i.-treated guinea-pigs for Rpul were respectively 0.080 ± 0.025 and 0.073 ± 0.013 cm H_2O/ml per second, and for Cdyn 0.431 ± 0.031 and 0.539 ± 0.055 ml/cm H_2O respectively.

The increase in pulmonary resistance in H.i.-pretreated animals was significantly potentiated from 1.0-8.0 μg histamine/100 g b.w. i.v. (fig. 1). The decreases in dynamic compliance did not differ. Maximal potentiation of the histamine response was observed at 1.0 and 8.0 μg

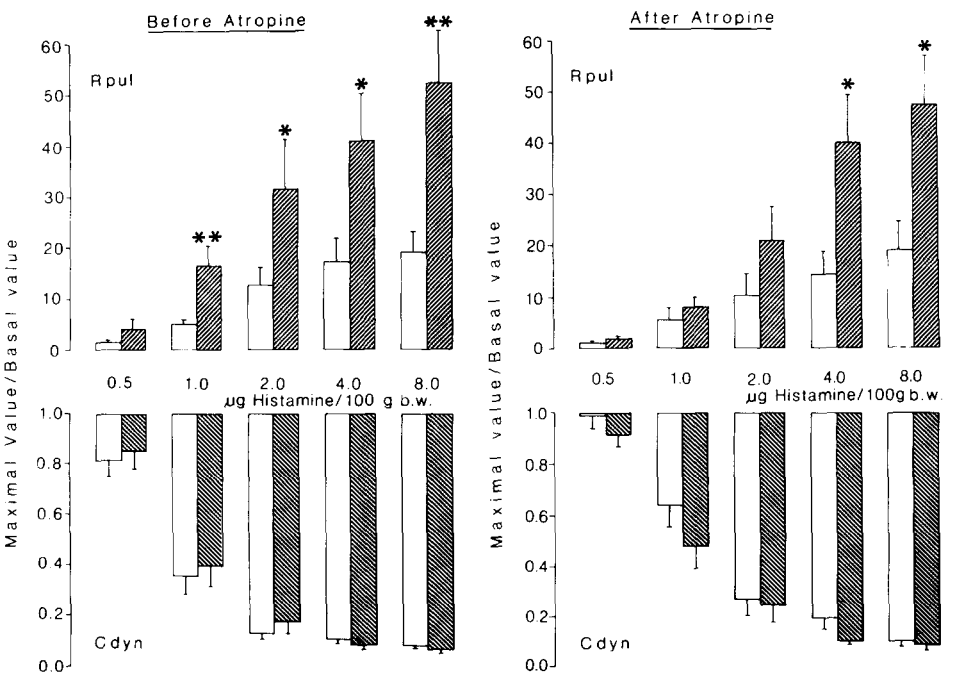

Fig. 1. Histamine-induced changes in pulmonary resistance (Rpul) and dynamic compliance (Cdyn) in *Haemophilus influenzae*-treated guinea-pigs before and after atropine (100 μg/kg b.w. i.v.). □ Controls (N = 10); ▨ *Haemophilus influenzae*-treated animals (N = 7). Values represent means ± S.E.M. * $P < 0.05$, ** $P < 0.01$ as compared to their respective control values.

histamine/100 g b.w. (+237% and +173% respectively $P < 0.01$). The parasympathetic blocking drug atropine (100 μg/kg b.w. i.v.), administered 15 min before the second dose-response curve of histamine, antagonized the potentiating effect at low doses of histamine (1.0-2.0 μg/100 g b.w. i.v.) but not at high doses (4.0-8.0 μg/100 g b.w. i.v.).

The changes in mean blood pressure did not differ significantly between the control and H.i.-treated guinea-pigs. Maximal changes in mean blood pressure after 8.0 μg/100 g b.w. histamine were -18.7 ± 0.8 and -18.6 ± 1.9 mm Hg for the control ($n = 10$) and for H.I.-treated animals ($n = 7$) respectively. The values after atropine administration were respectively -23.4 ± 1.8 and -23.4 ± 2.4 mm Hg.

4. Discussion

The present study showed that following pretreatment with H.i. there was increased reactivity to histamine as measured by the potentiated increase in pulmonary resistance. Since the decreases in dynamic lung compliance were not different between the two groups, these data point to hyperreactivity of the central respiratory airways.

Van der Zwan (1976) showed that comparable pathophysiological mechanisms are involved in the human. Forty-eight hours following inhalation of H.i. vaccine a decreased histamine threshold was measured in patients with chronic non-specific lung diseases and the effect lasted for several days.

Histamine induces airway constriction in guinea-pigs mainly by activation of H_1-receptors. Besides direct stimulation of H_1-receptors of respiratory smooth muscle, bronchoconstriction due to histamine in vivo is caused by stimulation of sensory 'irritant' receptors which are mainly localized in the major conducting airways (Mortela et al., 1975). This reflex bronchonconstriction is mediated through afferent and efferent pathways in the vagus nerve. Since atropine antagonizes the potentiating effect at low doses of histamine in H.i.-treated guinea-pigs, hyperreactivity is at least partly mediated by this reflex arc. The hyperreactivity may be due to increased sensitivity of the muscarinic receptor system since it was shown in in vitro experiments that carbachol induced an increased contractile response of isolated guinea-pig tracheal spirals in the H.i.-pretreated animals (Schreurs et al., 1980). However, no changes in cholinergic receptor number in peripheral lung tissue were observed after H.i. administration (C. Loesberg and F.P. Nijkamp, manuscript in preparation).

The hyperreactivity at higher doses of histamine is likely to be due to direct stimulation of histamine receptors because atropine does not influence this increased sensitivity. Changes in the H_1-receptor-effector system however seem unlikely since changes in mean blood pressure after histamine were not different between the control and H.i.-treated animals.

Douglas et al. (1976) showed in experiments with β-adrenergic blocking drugs that an increased histamine sensitivity is a function of β-adrenergic hyporesponsiveness. We have shown earlier that vaccination with H.i. resulted in impaired β-adrenergic function accompanied by a decrease in radioligand-detectable number of β-adrenoceptor binding sites in guinea-pig respiratory airways (Schreurs et al., 1980; Schreurs and Nijkamp, 1982). A reasonable suggestion for the hyperreactivity at low and high doses histamine in vivo may therefore be β-receptor hyporesponsiveness. This suggestion needs to be further substantiated.

Acknowledgements

This study was subsidized by a grant of the Dutch Asthma Foundation. We are grateful to the Electronic Department/Laboratory for Pharmacology of the National Institute of Public Health and Environmental Hygiene for the technical realization of the respiratory analyzer device (R.I.V.M. No. SE 503).

References

Amdur, M.O. and J. Mead, 1958, Mechanics of respiration in unanaesthetized guinea pigs, Am. J. Physiol. 192, 364.

Douglas, J.S., C. Brink and A. Bouhuys, 1976, Action of histamine and other drugs on guinea pig airways smooth muscle, in: Lung Cells in disease, ed. A. Bouhuys (Elsevier, North-Holland Biomedical Press) p. 245.

Empey, D.W., L.A. Laitinen, L. Jacobs, W.M. Gold and J.A. Nadel, 1976, Mechanisms of bronchial hyperreactivity in

normal subjects after upper respiratory tract infection, Am. Rev. Respir. Dis. 113, 131.

Laitinen, L.A., 1974, Histamine and methacholine challenge in the testing of bronchial reactivity, Scand. J. Respir. Dis. Suppl. 86.

Mortela, M., G. Sant Amrogio and M.G. Clements, 1975, Localisation of irritant receptors in the airways of dogs, Respir. Physiol. 24, 107.

Schreurs, A.J.M. and F.P. Nijkamp, 1982, *Haemophilus influenzae* induced loss of lung β-adrenoceptor binding sites and modulation by changes in peripheral catecholaminergic input, European J. Pharmacol. 77, 95.

Schreurs, A.J.M. and F.P. Nijkamp, 1984, Bronchial hyperreactivity to histamine induced by *Haemophilus influenzae* vaccination, Agents Actions 15, 211.

Schreurs, A.J.M., G.K. Terpstra, J.A.M. Raaijmakers and F.P. Nijkamp, 1980, Effects of vaccination with *Haemophilus influenzae* on adrenoceptor function of tracheal and parenchymal strips, J. Pharmacol. Exp. Ther. 215, 691.

Van der Zwan, J.C., 1976, Bronchial obstructive reactions and *Haemophilus influenzae*, Thesis, Groningen.