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Histamine H_1-Receptor Antagonists Do Not Prevent the Appearance of Endothelium-Dependent Relaxation to Acetylcholine in Rat Pulmonary Artery

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Summary

The purpose of this study was to determine the relaxant effects of acetylcholine after inhibition of vascular histaminergic receptors. In isolated rings of the rat pulmonary artery precontracted by phenylephrine (10^{-5} mol/l) both histamine (10^{-7} to 10^{-4} mol/l) and acetylcholine (10^{-8} to $3x10^{-5}$ mol/l) produced concentration-dependent relaxations. The arterial relaxations induced by either histamine or acetylcholine were markedly reduced or abolished by administration of NO synthase inhibitor N^G-nitro-L-arginine methyl ester (10^{-5} mol/l). Relaxant responses to histamine were not influenced by cimetidine, histamine H_2-receptor antagonist, but were significantly decreased or abolished by treatment with chlorphenyramine or diphenhydramine, histamine H_1-receptor antagonists. On the other hand, chlorphenyramine and diphenhydramine did not prevent the appearance of endothelium-dependent relaxation to acetylcholine. The results suggest that relaxation to histamine in the rat pulmonary artery is mediated by H_1-histaminergic receptors and their inactivation does not interfere with the endothelial capability to produce and/or release nitric oxide by the activation of other types of receptors.

Key words

Pulmonary artery – Acetylcholine – Histamine – Relaxation – Histaminergic receptor antagonists

Introduction

A number of agents have been shown to elicit vasodilation either directly by liberating nitric oxide (NO) from its molecule (Eichinger and Walker 1994) or indirectly through the activation of specific receptors on endothelial cells. After Furchgott and Zawadzki (1980) had shown the crucial role of endothelium-derived relaxing factor (NO) in acetylcholine-induced relaxation of the rabbit aorta, numerous studies were performed to elucidate the effect of various dilator agonists (acetylcholine, histamine, bradykinin, ATP etc.) and their respective endothelial receptors in the process of vascular relaxation (Altura and Chand 1981, Szarek *et al.* 1992, Furchgott 1993, Eichinger and Walker 1994).

Revealing the appropriate receptors in endothelium offers a suitable tool for studies on the specificity of mechanisms leading to activation of nitric oxide synthase – an enzyme of crucial role in the production of nitric oxide (Palmer *et al.* 1988). Inhibition of nitric oxide synthase by analogues of L-arginine, such as N^G-nitro-L-arginine methyl ester (L-NAME) (Rees *et al.* 1990), could prevent relaxation induced by all endothelium-dependent endogenous dilator compounds. But inhibition of specific receptors of one compound probably will not interfere with the activation of the others receptors.

The purpose of this study was to determine endothelium-dependent relaxation induced by acetylcholine during inhibition of histaminergic receptors in the rat pulmonary artery.

Materials and Methods

Male Wistar rats (350–400 g) were sacrificed by bleeding from the carotid arteries. Pulmonary arteries were isolated, cleaned of adherent connective

tissue and cut into rings (3–4 mm length). The rings were suspended vertically between two stainless steel wires in an organ bath with Krebs solution of the following composition (mmol): NaCl 118, KCl 5, $NaHCO_3$ 25, $MgSO_4$. 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11, ascorbic acid 1.1, $CaNa_2$.EDTA 0.032. The solution was bubbled with a gas mixture of 95 % O^2 + 5% CO_2 and kept at 37 °C.

The arterial rings were connected to a force transducer (Sanborn FTA 10) for recording changes in isometric tension as described earlier (Török *et al.* 1993). Resting tension was adjusted to 10 mN.

The preparations were allowed to equilibrate for 60–90 minutes before use.

The preparations were precontracted with phenylephrine (PE, 10^{-5} mol/l) and cumulative concentration-response curves for histamine and acetylcholine were obtained. The extent of relaxation was expressed as a percentage of PE-induced contraction. The concentration-response curves were also obtained in the presence of chlorphenyramine, diphenhydramine (H_1-antagonists, 10^{-6} mol/l), cimetidine (H_2-antagonist, 10^{-4} mol/l) and N^G-nitro-L-arginine methyl ester (L-NAME, an inhibitor of nitric oxide synthase, 10^{-5} mol/l). These inhibitors had been added to the organ bath 30 min before phenylephrine.

The following drugs were used: phenylephrine, histamine, acetylcholine, chlorphenyramine, diphenhydramine, cimetidine, L-NAME.

Experimental values shown in the text and figures are means ± S.E.M. For the comparison of statistical difference between groups, one-way analysis of variance was used. The difference of means were considered to be significant at $p<0.05$.

Results

Acetylcholine (10^{-8} to $3x10^{-5}$ mol/l) and histamine (10^{-7} to $3x10^{-4}$ mol/l) caused a concentration-dependent relaxation of rat pulmonary artery rings precontracted with phenylephrine (10^{-5} mol/l, Fig. 1A). Maximal relaxation to acetylcholine reached 90.0±3.9 % and it was not significantly different from maximal relaxation induced by histamine (89.1±3.1 %). But the minus logarithm of the IC_{50} (concentration of agent producing half-maximal inhibition of phenylephrine-contraction determined from individual concentration-response curves) for acetylcholine was significantly greater than for histamine (Table 1).

Table 1
The −log (IC_{50}) and maximal values of acetylcholine- and histamine-induced relaxation in the rat pulmonary artery.

Drug	n	−log(IC_{50})	Maximal relaxation (%)
Histamine	12	5.1±0.6	89.1±3.1
Acetylcholine	12	6.5±0.7*	90.0±3.9

*Relaxations are expressed as a percentage of steady state contraction induced by phenylephrine (10^{-5} mol/l), n indicates the number of rats * significantly different from histamine $p<0.05$).*

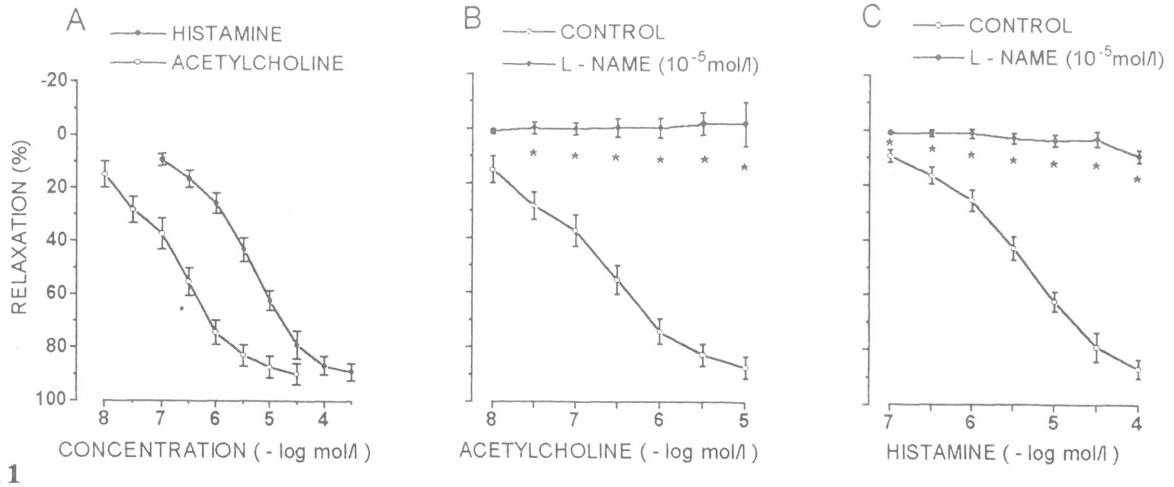

Fig. 1
Concentration-response curves for histamine (full dots) and acetylcholine (open dots) – induced relaxation in rat pulmonary artery (n=12) precontracted by 10^{-5} mol/l phenylephrine (A). Effects of L-NAME (10^{-5} mol/l) on acetylcholine-induced (B, n=4) and histamine-induced (C, n=6) relaxations in phenylephrine (10^{-5} mol/l)-precontracted rat pulmonary arteries. Segments of pulmonary artery (B,C) were either pretreated with 10^{-5} mol/l L-NAME (full dots) or untreated (open dots). The data were calculated as percentage relaxation of phenylephrine-induced tension. Each point represents the means ± S.E.M. from n preparations.

The addition of L-NAME (10^{-5} mol/l) to incubation medium completely abolished the acetylcholine-induced relaxation of the pulmonary artery (Fig. 1B, n=4, $p<0.05$). Similarly, relaxation of pulmonary artery to histamine was markedly reduced in the presence of L-NAME (10^{-5} mol/l). The residual relaxation represented only 9.3±2.3 % (Fig. 1C, n=6, $p<0.05$).

The administration of the potent and selective H_2-receptor antagonist cimetidine (10^{-4} mol/l) did not influence histamine-induced relaxation of the pulmonary artery. There was no significant difference in the values of maximal relaxation before and after treatment with cimetidine (Fig. 2A). Cimetidine also failed to modify the value of IC_{50}. The minus logarithm of IC_{50} for the untreated group was 5.1±0.6 (n=12) and for cimetidine-treated group 5.0±0.7 (n=6).

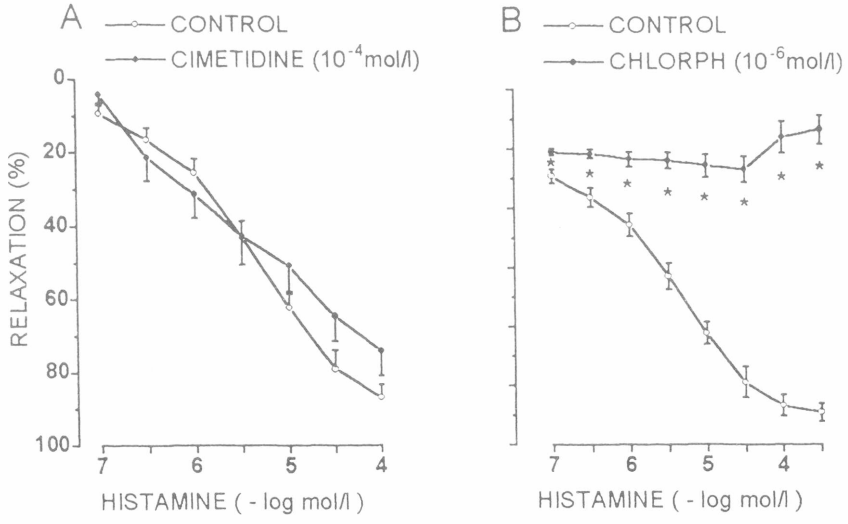

Fig. 2
Effects of cimetidine (A, n=6) and chlorphenyramine (B, n=11) in phenylephrine-precontracted rat pulmonary arteries. Segments of pulmonary artery were either pretreated with cimetidine (10^{-4} mol/l, full dot) and chlorphenyramine (10^{-6} mol/l, full dots) or untreated (open dots). The data were calculated as percentage relaxation of phenylephrine-induced tension. Each point represents means ± S.E.M. from n preparations.

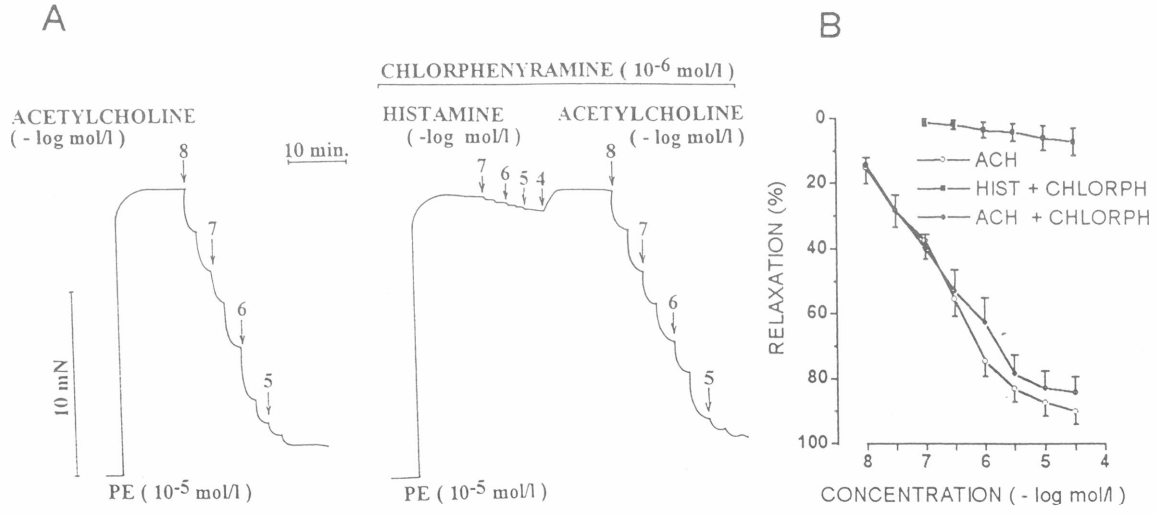

Fig. 3
(A) Recording of response to histamine (10^{-7} to 10^{-4} mol/l) and acetylcholine (10^{-8} to $3x10^{-5}$ mol/l) in phenylephrine (PE, 10^{-5} mol/l)-precontracted rat pulmonary artery after treatment with chlorphenyramine (10^{-6} mol/l). (B) Concentration-response curve for histamine-induced relaxation (full squares, n=12) and the following acetylcholine-induced relaxation (full dots, n=12) after treatment with chlorphenyramine (10^{-6} mol/l). The data were calculated as percentage relaxation of phenylephrine-induced tension. Each point represents means ± S.E.M. from n preparations.

When the H_1-receptor antagonist chlorphenyramine (10^{-6} mol/l) was present in the incubation bath, the histamine-induced relaxation of the pulmonary artery was abolished (Fig. 2B, n=11, $p<0.05$). The maximal relaxation in the control group was attained at $3x10^{-4}$ mol/l. In the chlorphenyramine-treated group, lower concentrations of histamine did not induce significant relaxations but higher concentrations of histamine (10^{-4} to $3x10^{-4}$ mol/l) elicited slight contractions (Fig. 2B). Treatment with diphenhydramine completely blocked relaxation to histamine in the whole range of concentrations used (10^{-7} to $3x10^{-4}$ mol/l, not shown).

Figure 3a shows the recordings of response to acetylcholine and histamine in phenylephrine-precontracted arterial rings. The relaxant response to histamine (10^{-7} to 10^{-4} mol/l) was completely inhibited in the presence of chlorphenyramine (10^{-6} mol/l). Following the addition of cumulative concentrations of acetylcholine (without washing histamine out from the bath) the pulmonary artery is relaxed similarly as under control conditions. These findings are summarized in Figure 3B. In the presence of chlorphenyramine, histamine did not cause significant relaxation of the pulmonary artery. However, acetylcholine induced dose-dependent relaxation with a maximal value of 84.1±4.8 % at $3x10^{-5}$ mol/l, which was not significantly different from that before chlorphenyramine treatment (90.0±3.9 %, n=12). Also, there was no significant difference in IC_{50} values for control (6.5±0.7, n=12) and chlorphenyramine-treated groups (6.7±0.8, n=11). Similarly, after diphenhydramine blockade of histamine-induced relaxation, acetylcholine-induced maximal relaxation (75 %) was not different from that under control conditions (80 %).

Discussion

In the present study, the acetylcholine-induced maximum relaxation of the rat pulmonary artery represented a 90 % decrease in tension of contraction in response to phenylephrine (10^{-5} mol/l). This value is comparable with those obtained in the rat pulmonary artery (Chen *et al.* 1988, Holécyová *et al.* 1996) and in other species (Chand *et al.* 1987). Maximum relaxation to histamine was of the same magnitude as that obtained with acetylcholine but the sensitivity of vascular smooth muscle to this agent was significantly weaker than to acetylcholine.

Endothelium-dependent relaxations either to acetylcholine or histamine were inhibited by L-NAME, an inhibitor of nitric oxide synthase. Concentration of L-NAME (10^{-5} mol/l) was sufficient for complete abolishing of relaxation. The results are in agreement with that of Rees *et al.* (1990) and they suggest that the endothelium-dependent relaxation to both acetylcholine and histamine is mediated by nitric oxide produced in endothelial cells.

The release of dilator prostaglandins is associated with activation of muscarinic and histaminergic receptors in various blood vessels (Toda *et al.* 1982, Okamura *et al.* 1994). In other series of experiments we showed that indomethacin, a cyclooxygenase inhibitor, did not affect the relaxant responses of the pulmonary artery to acetylcholine and histamine in both control and L-NAME-treated rats (Török *et al.* 1995). This means that prostaglandins are probably not involved in histamine- and acetylcholine-induced relaxations of the rat pulmonary artery.

Whether histamine-induced relaxation is mediated by H_1- or H_2-histaminergic receptors is currently somewhat controversial. The potent and selective H_2-receptor antagonist cimetidine did not influence a histamine-induced relaxation of rat pulmonary artery rings (Fig. 2A). This finding indicates that H_2-receptors are probably not involved in histamine-induced relaxation of pulmonary artery. On the other hand, after blockade of H_1-histaminergic receptors by chlorphenyramine or diphenhydramine, the histamine-induced relaxation of the pulmonary artery was abolished. These results suggest that histamine-induced relaxant responses in rat pulmonary artery are mediated by activation of H_1-histaminergic receptors. These results are in agreement with the results of other authors obtained in the pulmonary artery of the rat (Chen and Suzuki 1989, Szarek *et al.* 1992) and guinea-pig (Satoh and Inui 1984) and on other vessels (rat thoracic aorta – Van de Voorde and Leusen 1983, rat aorta – Carrier *et al.* 1984, rat renal artery – Krstič *et al.* 1989). On the other hand, other authors demonstrated that histamine-induced relaxation is mediated by activation of H_2-histaminergic receptors. These results were obtained under different conditions compared to conditions in our experiment (Chand and Altura 1980) and/or in other species (dog – Chand and Altura 1980) and vessels (canine spinal artery – Kawai and Ohhashi 1995, cerebral arteries – Kitamura *et al.* 1995).

Acetylcholine activates muscarinic receptors of endothelial cells and promotes the production of a relaxing factor (Furchgott 1993). Atropine, a muscarinic-receptor antagonist, blocks the activation of muscarinic receptors and prevents acetylcholine-induced relaxation of vascular smooth muscles (Chen and Suzuki 1989).

We hypothesized that inhibition of receptors of one dilator compound (histamine) probably will not prevent the appearance of endothelium-dependent relaxation to the other one (acetylcholine). After treatment with the H_1-receptor antagonist chlorphenyramine the relaxant response to histamine was abolished. But the following relaxation to

chlorphenyramine the relaxant response to histamine was abolished. But the following relaxation to acetylcholine was unchanged compared to the control response. Similar results have been observed after treatment with diphenhydramine. This means that after blockade of histamine-induced relaxation mediated through H_1-receptors the production of nitric oxide may be stimulated from endothelial cells by activation of muscarinic receptors. On the other hand, the atropine – antagonist of muscarinic receptors – did not suppress endothelium-dependent relaxation induced by histamine (Krstič *et al.* 1989).

In summary, the results obtained in the present study indicate that endothelium-dependent relaxations of the rat pulmonary artery induced by the activation of endothelial muscarinic and histaminergic receptors are mediated by nitric oxide released from endothelium cells. After inhibition of H_1-histaminergic receptors the activation of muscarinic receptors by acetylcholine still induces relaxations comparable to the control ones. These data show that inactivation of one type of endothelial receptors does not interfere with the endothelial capability to produce and/or release nitric oxide by the activation of other types of receptors.

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