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Short communication

Endothelial reactivity to the immediate hypersensitivity reaction of guinea pig pulmonary artery

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Abstract

Ovalbumin at low doses (0.1 μg/ml) caused pronounced relaxations in the precontracted pulmonary arteries of sensitized guinea pigs but, at high doses, (1–100 μg/ml) the relaxations were blunted by the contractions. The relaxations in response to ovalbumin challenge were related to histamine, which is released during the immediate hypersensitivity reaction, because they were almost blocked by mepyramine (10^{-5} M) plus cimetidine (10^{-4} M) pretreatment and never observed in unsensitized animal arteries. Additionally, the inhibition of relaxations by endothelium removal or N^G-nitro-L-arginine (L-NOARG, 10^{-4} M) treatment implies that the phenomenon requires endothelial nitric oxide synthesis. However, the contractions appear to depend on leukotriene production since they were markedly blocked in the presence of 2(*S*)-hydroxy-3(*R*)-[(2-carboxyethyl)thio]-3-[2-(8-phenyloctyl)phenyl]-propanoic acid (SKF 104353, 10^{-5} M), a leukotriene receptor antagonist. These results indicate that ovalbumin-induced nitric oxide and histamine H_2 receptor dependent relaxations in pulmonary artery may have an important role in the recovery of the increased pulmonary vascular resistance during the hypersensitivity reaction.

Keywords: Hypersensitivity; Ovalbumin; Endothelial reactivity; Nitric oxide (NO); Pulmonary artery

1. Introduction

The responsiveness of lung parenchymal and tracheal tissues from sensitized animals to ovalbumin challenge is well known, whereas few studies have been performed on pulmonary vasculature (Brocklehurst, 1960; Ogunbiyi and Eyre, 1985; Kelly et al., 1993, 1994). Ovalbumin challenge of pulmonary artery of sensitized guinea pig produces an increase in vascular smooth muscle tone due to the constrictor factors such as histamine and leukotrienes released from mast cells (Hand et al., 1982; Kelly et al., 1993). In this context, it has been shown that histamine induces both vasoconstriction, which is associated with stimulation of histamine H_1 receptors on smooth muscle, and vasodilation, which is associated with stimulation of histamine H_1 receptors on endothelium and histamine H_2 receptors on smooth muscle of guinea-pig pulmonary artery (Suzuki and Kou, 1983; Satoh and Inui, 1984; Abacioglu et al., 1987; Akar and Kanzik, 1989). On the other hand, the peptide leukotrienes, leukotriene C_4 and D_4 are potent vasoconstrictors in guinea pig main pulmonary artery (Hand et al., 1981; Berkowitz et al., 1984). However, they cause endothelium-dependent relaxations in the precontracted pulmonary artery (Sakuma et al., 1987).

The present study aimed to investigate the effect of ovalbumin challenge on pulmonary endothelial reactivity of sensitized guinea pigs.

2. Materials and methods

2.1. Sensitization procedure

Guinea pigs of either sex, weighing 250–400 g were actively sensitized by the injection of ovalbumin (10 mg/kg, i.p.) on days 1, 3 and 5 as described by Hand et al., 1982. Beginning 21 days after the last injection, the animals were killed by a sharp blow to the back of the head.

2.2. Preparation of pulmonary artery strips

A 3–4 mm wide segment of main pulmonary artery proximal to the heart was removed carefully to protect the

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endothelial lining, opened by a longitudinal cut and mounted in a 10-ml organ bath containing Krebs–Henseleit solution at 37°C, continuously aerated with 5% CO_2 and 95% O_2. The composition of the Krebs–Henseleit solution was as follows (mM): NaCl 117, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 1.2, $NaHCO_3$ 24.8, KH_2PO_4 1.2, glucose 11.

2.3. Experimental protocol

At the end of a 2-h equilibration period, arterial segments were contracted with submaximal phenylephrine (10^{-6} M), which produces 60–70% of the maximal contraction. After a plateau was reached, acetylcholine (10^{-6} M), an endothelium-dependent relaxant agent, was applied to test the availability of endothelium. In some preparations, endothelium was removed by gently rubbing the luminal surface of the ring with a piece of filter paper. The effectiveness of endothelium removal was confirmed by the inability of acetylcholine to induce relaxation of phenylephrine-contracted rubbed rings.

In sensitized pulmonary artery, after a plateau was reached with 10^{-6} M phenylephrine, ovalbumin (0.1–100 μg/ml) was applied in cumulative doses. Additionally, the direct effect of ovalbumin was also tested on contracted pulmonary artery of unsensitized guinea pigs. The responses to ovalbumin challenge were evaluated in the presence of specific antagonists. The antagonists, 2(*S*)-hydroxy-3(*R*)-[(2-carboxyethyl)thio]-3-[2-(8-phenyloctyl) phenyl]-propanoic acid (SKF104353, 10^{-5} M), a peptide leukotriene antagonist, mepyramine(10^{-5} M), a histamine H_1 receptor antagonist, cimetidine(10^{-4} M), a histamine H_2 receptor antagonist and N^G-nitro-L-arginine(L-NOARG, 10^{-4} M), a nitric oxide synthase inhibitor were added to the organ bath either alone or in combination approximately 30 min before ovalbumin challenge. These concentrations of antagonists had been found to be sufficient to prevent the ovalbumin-induced contractions in quiescent sensitized pulmonary arteries in preliminary experiments. All experiments were performed in the presence of indomethacin (10^{-5} M).

2.4. Statistical analysis

The results are given as means ± S.E.M. Responses to ovalbumin were calculated as percent decreases or increases of phenylephrine-induced contractions. Statistical analysis was done with Student's unpaired *t*-test. $P < 0.05$ was considered statistically significant.

2.5. Drugs

All drugs were purchased from Sigma (St. Louis, MO) except SKF104353, a generous gift from SmithKline. Indomethacin was dissolved in 5%(w/v) $NaHCO_3$. The stock solution of ovalbumin was prepared in saline (0.9%). All other drugs were dissolved in distilled water and dilutions were made in Krebs solutions on the day of the experiment. The vehicles used as solvents had no effect on either vascular tone or response to ovalbumin.

3. Results

Ovalbumin challenge (0.1–100 μg/ ml) caused dose-dependent contractions in sensitized quiescent pulmonary artery strips (data not shown). The sensitized strips responded only once to in vitro ovalbumin challenge and the dose–response curve was not reproducible in the same preparation. The anaphylactic response was never observed in pulmonary artery from unsensitized animals.

In precontracted pulmonary artery from sensitized guinea pigs, ovalbumin challenge produced pronounced relaxation. The maximum relaxation in response to ovalbumin at the concentration of 0.1 μg/ml was $-46.75 \pm 5.8\%$ ($n = 5$). Mepyramine (10^{-5} M), a histamine H_1 receptor antagonist, markedly reduced the maximum relaxation (ovalbumin 1 μg/ml: $-13.82 \pm 2.6\%$, $n = 5$, $P < 0.05$) and converted the relaxations to contractions with high doses of ovalbumin (100 μg/ml: $+18.94 \pm 2.8\%$, $n = 5$, $P < 0.05$). SKF 104353 (10^{-5} M), a peptide leukotriene antagonist, augmented the maximum relaxation (ovalbumin 1 μg/ml: $-58.15 \pm 3.7\%$, $n = 5$). Pretreatment with L-NOARG (10^{-4} M), a nitric oxide synthase inhibitor, markedly reduced the relaxations (ovalbumin 1 μg/ml: $-9.65 \pm 2.9\%$, $n = 5$, $P < 0.05$) and turned the relaxation into contractions with high doses of ovalbumin (100 μg/ml: $+22.86 \pm 2.7\%$, $n = 5$, Fig. 1). Endothelium re-

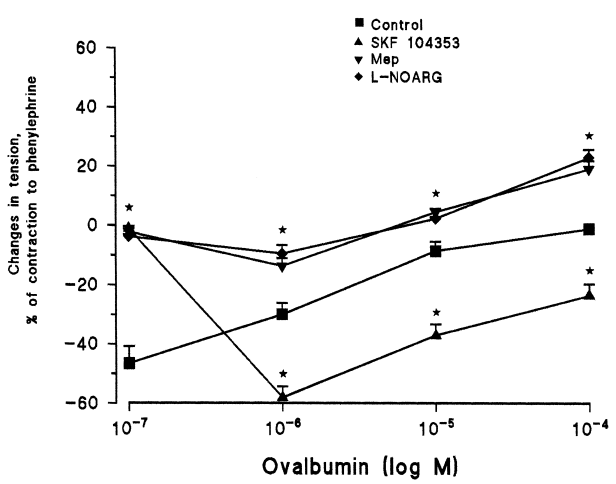

Fig. 1. Graph showing the effect of ovalbumin on phenylephrine precontracted sensitized guinea pig pulmonary artery and its modification by mepyramine (10^{-5} M), SKF 104353 (10^{-5} M) and L-NOARG (10^{-4} M) pretreatments. Sensitized guinea pig pulmonary artery was contracted with 10^{-6} M phenylephrine and then ovalbumin (0.1–100 μg/ml) was applied cumulatively. Ovalbumin challenge (0.1 μg/ml) caused apparent relaxation but this relaxation was blunted by contraction at high doses of ovalbumin (1–100 μg/ml ; Cont). Vertical bars indicate the standard error of the arithmetic mean. * Significantly different from corresponding control value ($P < 0.05$). $n = 5$ for each point.

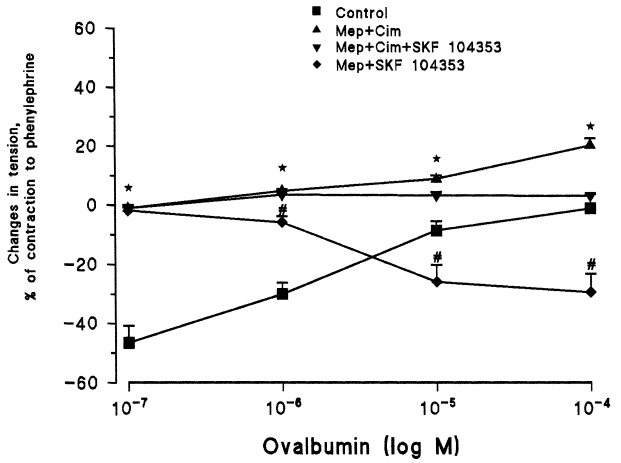

Fig. 2. Graph showing the effects of combination pretreatments with mepyramine + cimetidine; mepyramine + SKF 104353; mepyramine + SKF 104353 + cimetidine on the response to ovalbumin challenge in phenylephrine precontracted sensitized guinea pig pulmonary artery. Vertical bars indicate the standard error of the arithmetic mean. * Significantly different from corresponding control value. #Significant difference between mepyramine + SKF 104353 and mepyramine + SKF 104353 + cimetidine ($P < 0.05$). $n = 4$–5 for each point.

moval also markedly reduced ovalbumin–induced relaxations in the four experiments (data not shown). Pretreatment with mepyramine plus cimetidine (10^{-4} M) completely abolished the relaxation (ovalbumin 0.1 μg/ml: $-1.2 \pm 0.9\%$, $n = 4$, $P < 0.05$), but not the contraction (ovalbumin 100 μg/ml: $+20.13 \pm 2.4\%$, $n = 4$). With the combination of SKF 104353 and mepyramine, ovalbumin challenge still produced marked relaxations (100 μg/ml: $-29.53 \pm 6.2\%$, $n = 5$). However, the combination of the above histamine antagonists with SKF 104353 completely prevented both the relaxations and the contractions (Fig. 2).

4. Discussion

In this study, ovalbumin at low doses (0.1 μg/ml) produced pronounced relaxation in the precontracted sensitized guinea pig pulmonary artery, but at high doses (1–100 μg/ml) the relaxation was blunted by the contractions. The ovalbumin-induced response appears to depend on the release of histamine and leukotrienes during the immediate hypersensitivity reaction which is consistent with previous findings (Hand et al., 1982; Hay et al., 1987; Kelly et al., 1993) because pretreatment with the combination of mepyramine, cimetidine and SKF 104353 completely abolished the response to ovalbumin challenge. Histamine is responsible for the relaxation in response to ovalbumin challenge since mepyramine pretreatment inhibited most of the relaxation. Besides, the combination of mepyramine with cimetidine almost completely abolished the relaxation. The incubation of sensitized pulmonary artery with SKF 104353 markedly potentiated the relaxation but inhibited the contraction elicited by ovalbumin in accordance with previous results (Kelly et al., 1993). The findings of this study indicated that leukotrienes mediated solely the contraction, which differs from the results of a previous study which showed endothelium-dependent relaxant effects of leukotrienes in guinea pig pulmonary artery (Sakuma et al., 1987). Moreover, leukotrienes masked the histamine-dependent relaxation in response to ovalbumin challenge.

On the other hand, the ovalbumin-induced relaxation was partially related to endothelial nitric oxide synthesis because the relaxation was more significantly reduced after removal of endothelium or pretreatment with L-NOARG, a nitric oxide synthase inhibitor. A similar study showed that the amplitude and duration of ovalbumin-induced contraction in sensitized guinea pig pulmonary artery was increased by pretreatment with methylene blue, an inhibitor of nitric oxide (Kelly et al., 1994). From the above and the present results it seems that ovalbumin activated the pulmonary endothelium to release nitric oxide during the immediate hypersensitivity reaction. On the other hand, we previously demonstrated that ovalbumin challenge caused abnormal endothelial reactivity in the pulmonary artery of sensitized guinea pig, possibly because of the release of destructive factors from mast cells (Uydeş-Doğan et al., 1995).

In the present study, the degree of inhibition of the relaxation by L-NOARG was nearly equal to that by mepyramine. The relaxation seems to be related to histamine H_1 receptor-mediated nitric oxide synthesis, in line with previous findings for normal tissues (Van de Voorde et al., 1983; Akar et al., 1994). The remaining relaxation after mepyramine or L-NOARG and also after endothelium removal may be mediated by the histamine H_2 receptors on vascular smooth muscle, which was shown in guinea pig pulmonary artery (Suzuki and Kou, 1983; Abacioglu et al., 1987) because this response was abolished by the addition of cimetidine. In addition, a relaxant response, sensitive to cimetidine, was observed in the presence of mepyramine plus SKF 104353. It has been proposed that an endothelium-independent relaxing factor modulates the responses to ovalbumin in sensitized guinea pig pulmonary artery (Kelly et al., 1994). In the present study, the endothelium-independent relaxant response to ovalbumin challenge was mediated by histamine H_2 receptor stimulation, and depends on the release of histamine. It has been shown that human pulmonary artery is relaxed by anti-human immunoglobulin E, which is associated with the release of histamine and prostacyclin. It has been found that the relaxation was partially related to nitric oxide production via endothelial histamine H_1 receptor stimulation, however, the participation of histamine H_2 receptor stimulation was not tested (Ortiz et al., 1993). The data now presented showed that histamine released in response to ovalbumin challenge produced marked endothelium-de-

pendent and independent relaxations in sensitized precontracted pulmonary artery of guinea pig.

It has been reported that, in anaphylactic shock pulmonary vascular resistance is increased, associated with enhancement of right ventricular pressure despite deep systemic hypotension (Brocklehurst, 1960; Halonen et al., 1976). In conclusion, the present data suggest that the endothelium-derived nitric oxide and histamine H_2 receptor mediated relaxations in pulmonary artery counteract a further increase of pulmonary vascular resistance during anaphylactic reactions and hence may have a beneficial role in the recovery of pulmonary resistance and, consequently, right ventricular pressure.

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