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Histamine-Induced Relaxation in Pulmonary Artery of Normotensive and Hypertensive Rats: Relative Contribution of Prostanoids, Nitric Oxide and Hyperpolarization

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Summary

The aim of this study was to determine the relative contribution of nitric oxide (NO), endothelium-derived hyperpolarizing factor (EDHF) and prostanoids in histamine-induced relaxation of isolated pulmonary artery from normotensive and hypertensive rats. The hypertension was induced by oral administration of NO synthase inhibitor N^G-nitro-L-arginine methylester (L-NAME, 50 mg/kg/day) to normotensive rats for 8 weeks. In phenylephrine-precontracted arterial rings the histamine-induced relaxation was significantly reduced in L-NAME-treated rats compared to the controls. Indomethacin (cyclooxygenase inhibitor) and glibenclamide (ATP-sensitive K^+-channel blocker) did not inhibit the relaxation response in either control or hypertensive rats. On the other hand, tetraethylammonium (TEA), a K^+-channel blocker with a broad specificity, significantly reduced histamine-induced relaxation in the pulmonary artery from both groups examined. The TEA-resistant relaxation was completely abolished by additional administration of L-NAME to the incubation medium. The results indicate that histamine-induced relaxation of the pulmonary artery in both normotensive and hypertensive rats is mediated mainly by nitric oxide, whereas EDHF seems to play a minor role.

Key words

Pulmonary artery • Histamine • Nitric oxide • EDHF

Introduction

The endothelium plays an active role in a variety of physiological functions, including the modulation of tone of the underlying vascular smooth muscle. Endothelial cells produce and release potent vasoconstrictor and vasodilator substances. The latter include the endothelium-derived relaxing factor (EDRF), which is either nitric oxide (NO) or a nitrosocompound (Myers *et al.* 1989, Moncada *et al.* 1991), the endothelium-derived hyperpolarizing factor (EDHF) (Garland *et al.* 1995) and vasodilating prostaglandins. Their release is stimulated through endothelial receptors by various agonists, including acetylcholine and histamine (Szarek *et al.* 1992, Kyselá and Török 1996).

Endothelium-dependent relaxation of blood vessels to acetylcholine is impaired in essential hypertension as well as in different models of

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experimental hypertension (Lüscher and Vanhoutte 1990). The effect of acetylcholine is also reduced in arteries of NO-deficient hypertensive animals in comparison to arteries of normotensive controls (Holécyová *et al.* 1996, Török and Gerová 1996). Reduction in acetylcholine-induced relaxation is accompanied by morphological changes in the heart and arteries, manifested by cardiac hypertrophy and fibrosis in the myocardium (Babál *et al.* 1997), by an increase of arterial wall thickness and wall/diameter ratio (Kristek and Gerová 1996, Kristek *et al.* 1996, Bernátová *et al.* 1999). Metabolic changes in NO-deficient hypertension include an increase of the left ventricular DNA and RNA content as well as of contractile, metabolic and collagenous proteins (Pecháňová *et al.* 1997, 1999, Gerová *et al.* 1998).

In vessels of normotensive animals, the relative contribution of NO, EDHF and prostaglandins in regulating vascular tone was determined (Wallerstedt and Bodelsson 1997). In the rat pulmonary artery, relaxation to acetylcholine and histamine is strictly endothelium-dependent and seems to be mediated by both NO and EDHF (Chen and Suzuki 1989). In NO-deficient hypertension, however, it is not known whether or not the EDHF-mediated component of the endothelium-dependent relaxation is also affected. The present study was designed to evaluate the relative contribution of NO, EDHF and prostaglandins in histamine-induced relaxation of the pulmonary artery from control normotensive and NO-deficient hypertensive rats.

Material and Methods

Experiments were carried out on adult male Wistar rats divided into two groups:

1) Wistar rats receiving drinking water containing N^G-nitro-L-arginine methylester (L-NAME) with a daily intake of 50 mg/kg for 8 weeks, and

2) age-matched control normotensive Wistar rats drinking untreated water.

Systolic blood pressure and heart rate were measured weekly by the indirect tail-cuff technique, using a preheated box in which the rats were heated at 36 °C for a period of 5 min.

At the end of week 18 of age, the rats were sacrificed, the heart and body weight were determined and the heart weight/body weight ratio was calculated. Extralobar branches of the main pulmonary artery were rapidly removed and placed in oxygenated (95 % O_2/5 % CO_2) physiological salt solution (PSS). The composition of the PSS was (in mmol/l): NaCl 118, KCl 5, $MgSO_4$ 1.2, $NaHCO_3$ 25, KH_2PO_4 1.2, $CaCl_2$ 2.5, glucose 11, $CaNa_2EDTA$ 0.03, ascorbic acid 1.55. The pH of the solution was 7.3-7.4.

Arteries were carefully cleaned of connective tissue and cut into rings (3-4 mm in length), with a special care to preserve the endothelium. The rings were then vertically fixed between hooks in 20 ml organ bath containing PSS at 37 °C. The hook anchoring the upper end of the ring was connected to the level of a force-displacement transducer Sanborn FT 10 and polygraphs (LABORA). An optimal load (10 mN), which ensured maximal responses to the contractile agonists used, was applied to each ring. Subsequently, preparations were allowed to equilibrate for 90 min with bath fluid exchanged every 15 min.

For relaxation studies, the preparations with an intact endothelium were precontracted by phenylephrine 10^{-6} mol/l. When the contraction reached a plateau, increasing concentrations of histamine (or acetylcholine) were applied in a cumulative fashion. The phenylephrine induced steady-state of contraction was considered to be 100 % and the relaxation responses were calculated as a percentage of this contraction. To establish the mechanisms of relaxation, some arteries were treated with indomethacin (10^{-5} mol/l), tetraethylammonium (TEA, 1-5 x 10^{-3} mol/l), glibenclamide (10^{-5} mol/l) or L-NAME (10^{-5} mol/l), before the concentration-response curves to histamine or acetylcholine were determined. Similar experiments were performed with the modified Krebs solution in which K^+ concentration was elevated to 20 mmol/l.

Drugs

The following drugs were used: phenylephrine, histamine, acetylcholine, indomethacin, sodium nitroprusside, glibenclamide, tetraethylammonium, N^G-nitro-L-arginine methyl ester (all from Sigma), noradrenaline (Léčiva, Praha, CZ) and phentolamine methane sulphonate (Regitine, Ciba-Geigy). All drugs were dissolved in distilled water, indomethacin was first solubilized in 0.2 mol/l Na_2CO_3 and diluted with distilled water.

Statistical analysis

The results are expressed as means ± S.E.M. For comparison of statistical differences between the groups, one-way analysis of variance (ANOVA) was used. Values were considered statistically significant when $P<0.05$.

Table 1. Characteristics of control and experimental group

	n	SBP (mm Hg)	Heart rate (beats/min)	Body weight (g)	Heart weight (g)	HW/BW (mg/g)
Control Wistar rats	14	126±2	386±9	448±9	1.03±0.04	2.24±0.04
L-NAME treated rats	8	181±7*	361±12*	409±11*	1.33±0.11*	3.23±0.19*

*SBP – systolic blood pressure. Data are means ± S.E.M., * Significantly different from control (P<0.05). HW/BW – relative heart weight.*

Results

General characteristics

Table 1 gives the characteristics of the control and hypertensive rats. Systolic blood pressure was significantly lower in control rats than in L-NAME treated animals. Hypertensive rats had a significantly higher heart weight and heart weight/body weight ratio than normotensive control rats (P<0.05).

Relaxation studies

In phenylephrine-precontracted rings of the pulmonary artery from normotensive rats, increasing concentrations of histamine induced dose-dependent relaxations. In L-NAME treated rats, the relaxations were significantly reduced (P<0.05). A qualitatively similar effect was observed when endothelial receptors were stimulated by acetylcholine. The concentration-response curves for acetylcholine were shifted to the left in both normotensive and NO-deficient hypertensive rats (Fig. 1).

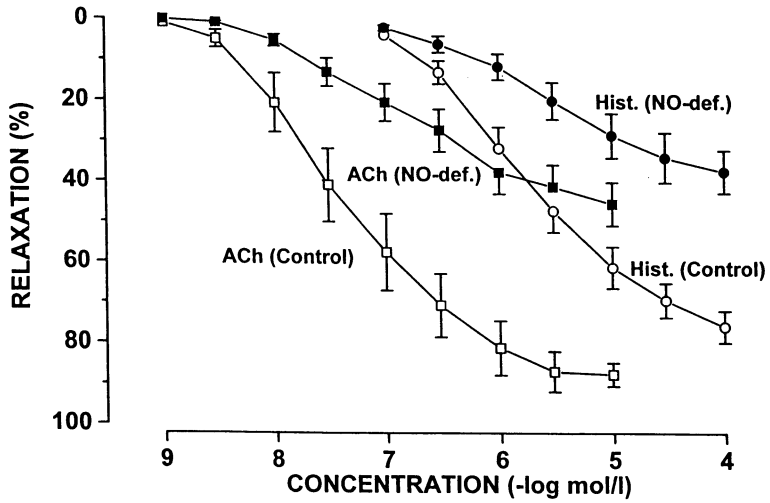


Fig. 1. *Endothelium-dependent relaxation of pulmonary artery induced by acetylcholine (ACh) and histamine (Hist.) in normotensive (Control) and NO-deficient (NO-def.) hypertensive rats. Pulmonary artery was precontracted by phenylephrine (10^{-6} mol/l). Data are means ± S.E.M.; n=8-14 animals.*

Histamine-induced relaxations were not altered, when indomethacin (10^{-5} mol/l) was used to block the cyclooxygenase (Fig. 2).

Histamine-induced relaxation of the pulmonary artery from control and NO-deficient rats (Fig. 3) was not affected by glibenclamide, an inhibitor of the ATP-sensitive K^+ channel.

Tetraethylammonium (TEA), a non-selective inhibitor of K^+ channels with some preference for the high conductance Ca^{2+}-activated K^+ channel, inhibited the histamine-induced relaxation in the pulmonary artery from normotensive and NO-deficient hypertensive rats (Fig. 4). The TEA-sensitive part of the relaxation response represented approximately 40 % in both groups.

A quantitatively similar concentration-response curve was obtained for histamine in three preparations from normotensive and three from hypertensive rats, when K^+ concentration was elevated to 20 mmol/l in the incubation medium.

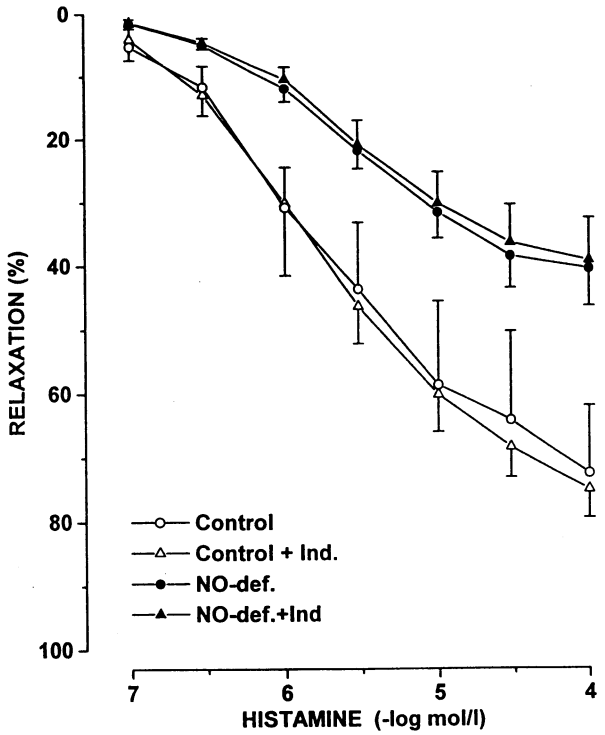


Fig. 2. *Effect of indomethacin (Ind. 10^{-5} mol/l) on endothelium-dependent relaxation induced by histamine in normotensive (Control) and NO-deficient (NO-def.) hypertensive rats. Data are means ± S.E.M; n=8 animals.*

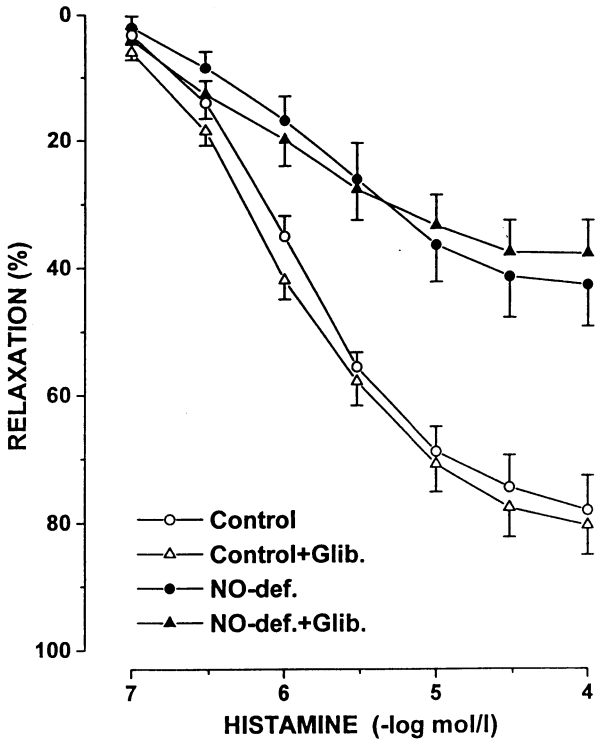
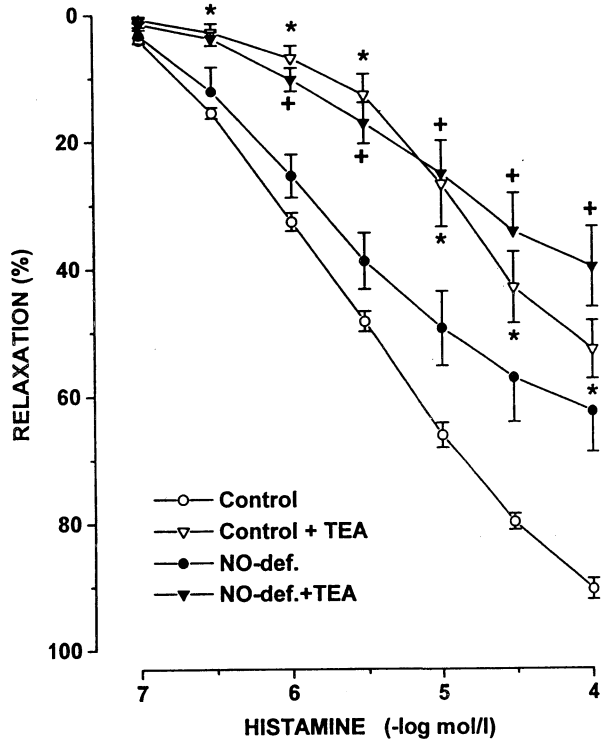


Fig. 3. *Influence of glibenclamide (Glib., 10^{-5} mol/l) on relaxation response induced by histamine in normotensive (Control) and NO-deficient (NO-def.) hypertensive rats. Data are means ± S.E.M.; n=7 animals.*



Histamine-induced relaxations of the pulmonary artery in normotensive rats as well as residual relaxations in NO-deficient hypertensive rats were abolished by additional administration of L-NAME (10^{-5} mol/l) to the incubation medium (Fig. 5).

Fig. 4. *Effect of TEA (10^{-3} mol/l) on relaxation response induced by histamine in normotensive (Control) and NO-deficient (NO-def.) hypertensive rats. Data are means ± S.E.M; n=8-9 animals. * P<0.05 significantly different from controls; $^+$ P<0.05 significantly different from NO-deficient rats.*

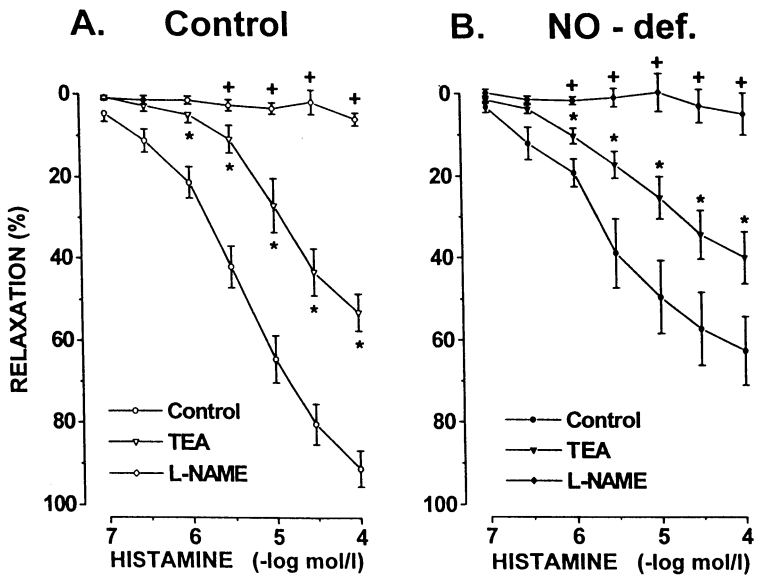


Fig. 5. *Concentration-response curves for histamine-induced relaxation of pulmonary artery rings of normotensive control* ***[A]*** *and NO-deficient hypertensive rats* ***[B]****. Responses to histamine were compared before and after administration of TEA (10^{-3} mol/l) and subsequently after addition of L-NAME (10^{-5} mol/l) to the incubation medium. Data are means ± S.E.M; n=8-9 animals. * $P<0.05$ significantly different from controls; $^{+}P<0.05$ significantly different from TEA-treated preparations.*

Discussion

The results illustrated in Figure 1 are in accordance with our previous studies in which acetylcholine- and histamine-induced relaxation was impaired in arteries isolated from L-NAME-treated rats with chronic NO-deficient hypertension compared to those obtained from normotensive controls (Holécyová *et al.* 1996, Török *et al.* 1998). These findings were interpreted to indicate that attenuated relaxation responses of L-NAME-treated rats resulted from the reduced production and/or release of NO from endothelial cells. In our study, the heart weight and heart weight/body weight ratio were significantly elevated in NO-deficient hypertensive rats, indicating the presence of cardiac hypertrophy in hypertensive animals.

The ability of vascular preparations to exhibit endothelium-dependent responses varies widely with the stimulus and the blood vessel under study. Van de Voorde *et al.* (1987) showed that endothelial cells of human umbilical blood vessels released a relaxation factor in response to histamine, acting *via* H_1-receptors, but not in response to acetylcholine. The present study as well as our previous report (Kyselá and Török 1996), have documented that in normotensive rats the course of relaxation to histamine was of the same magnitude as that observed with acetylcholine, but the sensitivity of vascular smooth muscle to histamine was weaker by about two orders of magnitude than that to acetylcholine. A similar pattern of relaxation to these two agonists was also obtained after long-term inhibition of NOS, suggesting that in the pulmonary artery, receptor sensitivity to these two agonists is not changed after chronic inhibition of NOS.

Indomethacin did not significantly alter the residual histamine-induced relaxation in pulmonary rings from L-NAME treated rats. This appears to exclude the possibility that the attenuation of histamine-induced relaxation would be related to the production of endothelium-dependent contracting factors (prostaglandin H_2 or thromboxane A_2) (Kato *et al.* 1990).

To examine the involvement of NO in residual histamine-induced relaxation of the pulmonary artery, we used L-NAME in acute experiments after obtaining control responses to histamine. In hypertensive animals, the acute addition of L-NAME completely abolished residual relaxations (Török and Ježek 1997). This suggests that there was still a capacity of endothelial cells to produce or release NO.

Most endothelium-dependent vasodilators which release NO, such as histamine, also cause hyperpolarization of endothelial and smooth muscle cells *via* opening ATP-sensitive K^+ channels (Garland and McPherson 1992). If a K^+ channel mechanism were present in the large pulmonary artery, then its contribution to endothelium-dependent relaxation should become more apparent during NO-deficient hypertension. This study, however, showed that glibenclamide, an inhibitor of the ATP-sensitive K^+ channel, did not affect the histamine-induced relaxation of the pulmonary artery. in either control or NO-deficient hypertensive rats (Fig. 3).

On the other hand, the residual relaxation induced by histamine in arteries from NO-deficient hypertensive rats was significantly attenuated by TEA. Inhibition of the histamine-induced relaxation by TEA suggests that EDHF may act through high conductance Ca^{2+}-activated K^+ channels in this artery. Furthermore, when the extracellular K^+ concentration was raised to about 20 mmol/l, inhibition of histamine-induced relaxation was quantitatively similar to that induced by TEA, and subsequent addition of the NOS inhibitor L-NAME abolished the residual relaxation. Our observations indicate that approximately 40 % of histamine-induced relaxation in large pulmonary arteries (TEA-sensitive part of relaxation) can be explained by an increase in the smooth muscle membrane potential. These results are similar to those found in arteries from SHR, where acetylcholine was used as stimulant (Fujii *et al.* 1993, Li *et al.* 1994, Mantelli *et al.* 1995, Sunano *et al.* 1999), suggesting that the residual relaxation observed in our hypertensive rats was caused by hyperpolarization of the smooth muscle membrane due to increased K^+ conductance. A similar attenuation of histamine-induced relaxation by TEA was observed in a variety of arteries of control normotensive animals (Eckman et al. 1992, Li *et al.* 1994, Chen and Cheung 1997). This means that the attenuation of histamine relaxation in pulmonary arteries from L-NAME-treated rats is not inevitably caused by impaired release of EDHF. In support of this statement, it has been reported that hyperpolarization by cromakalin, a K^+ channel opener, did not differ in mesenteric arteries from normotensive and spontaneously hypertensive rats (Fujii *et al.* 1992), indicating that the K^+ channel itself was not different in these preparations. A close correlation between hyperpolarization and relaxation was also observed in other arteries (Zygmunt *et al.* 1994). Moreover, the relationship between the membrane potential and KCl-induced tension was not altered in mesenteric arteries from age-matched normotensive and hypertensive rats. The tension increased steeply with small increases in the membrane potential (Cheung *et al.* 1999).

Endothelium-dependent smooth muscle relaxation of pulmonary arteries in normotensive rats and the residual relaxation in NO-deficient hypertensive rats appears to reflect predominantly the action of NO. In addition to NO, the release of EDHF evoked by the receptor-dependent agonist histamine may also, at least in part, contribute to histamine-induced relaxation, representing an important factor in the maintenance of vascular tone in pulmonary circulation.

In conclusion, both NO and EDHF were found to contribute to the relaxant effect of histamine in large pulmonary arteries from normotensive as well as from hypertensive rats, NO being more important than EDHF.

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