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European Journal of Pharmacology 470 (2003) 45–55
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An in vitro study of histamine on the pulmonary artery of the Wistar–Kyoto and spontaneously hypertensive rats

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Received 2 December 2002; received in revised form 16 April 2003; accepted 23 April 2003

Abstract

The vascular response to most neurotransmitters of different vascular beds is altered under hypertensive condition. The modulatory effect of genetic pulmonary arterial hypertension on histamine responses is not known. The present study was undertaken to evaluate the modulatory effect of enzymatic degradation (via histamine *N*-methyl-transferase and diamine oxidase) on the vascular response of histamine, and the subtype(s) of histamine receptor present in the pulmonary artery (first branch, O.D. ~ 800 μm) of the normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) (male, 22–26 weeks old). In phenylephrine (1 μM) pre-contracted preparations, histamine and 6-[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl) heptanecarboxamide (HTMT, a histamine H_1 receptor agonist) elicited a concentration-dependent relaxation, with a smaller magnitude recorded in SHR. Application of 10 μM *S*-[4-(*N*,*N*-dimethylamino)-butyl]isothiourea (SKF 91488, a selective histamine *N*-methyl-transferase inhibitor), but not aminoguanidine (100 μM, a diamine oxidase inhibitor), significantly attenuated histamine-induced relaxation. Clobenpropit (1 nM, a potent histamine H_3 receptor antagonist) "antagonised" the suppressive effect of SKF 91488 and histamine-evoked relaxation was restored. Endothelial denudation reduced histamine- and abolished HTMT-elicited relaxation. Dimaprit (a histamine H_2 receptor agonist) caused an endothelium-independent, *cis*-*N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine (MDL 12330A, 10 μM, an adenylate cyclase inhibitor)-sensitive, concentration-dependent relaxation, with a similar magnitude in both strains of rat. Histamine-evoked relaxation was reversed into a further contraction (clobenpropit (10 nM)-sensitive) (with a greater magnitude occurred in the WKY rat) after blocking the histamine H_1 and H_2 receptors with diphenhydramine plus cimetidine (30 μM each). A similar further contraction (clobenpropit-sensitive) was observed with imetit (a histamine H_3/H_4 receptor agonist) (≥ 3 μM). Under resting tension, imetit (≥ 0.3 μM) caused a clobenpropit (10 nM)- and prazosin (1 μM)-sensitive, concentration-dependent contraction, with a greater contraction in the WKY rats. Our results suggest that inhibition of histamine catabolism using SKF 91488 (histamine *N*-methyl-transferase inhibitor) resulted in a reduction of histamine-mediated relaxation that was due to the activation of the clobenpropit-sensitive, histamine H_3/H_4 receptor and the release of catecholamine. In addition, activation of histamine H_1 and H_2 receptors resulted in relaxation whereas histamine H_3/H_4 receptor activation by imetit yielded a prazosin-sensitive contraction of the pulmonary artery.

Keywords: Histamine; Pulmonary artery; Nitric oxide (NO); (Wistar–Kyoto rat); Spontaneously hypertensive, rat

1. Introduction

In addition to the autonomic nervous system, other biogenic amine such as histamine also participated in modifying pulmonary blood flow under physiological conditions and may be involved in the pathophysiology of pulmonary vascular diseases (Russell et al., 1994; Hamada et al., 1999). Similar to other endogenous substances (acetylcholine and noradrenaline), actions of the released histamine are mainly terminated by enzymatic degradation. The existence of histamine catabolic enzymes: diamine oxidase and histamine-*N*-methyl-transferase (Schayer, 1959) has been demonstrated in plasma and various internal organs (Futo et al., 1990; Kitanaka et al., 2002). Diamine oxidase catalyses the oxidative deamination of histamine and histamine-*N*-methyl-transferase causes methylation of histamine (Fig. 1). The modulatory effect, if any, of these degrading enzymes on histamine-evoked vascular responses is not known. Therefore, the first aim of this study was to address the modulatory effect of diamine oxidase and histamine-*N*-

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doi:10.1016/S0014-2999(03)01759-X

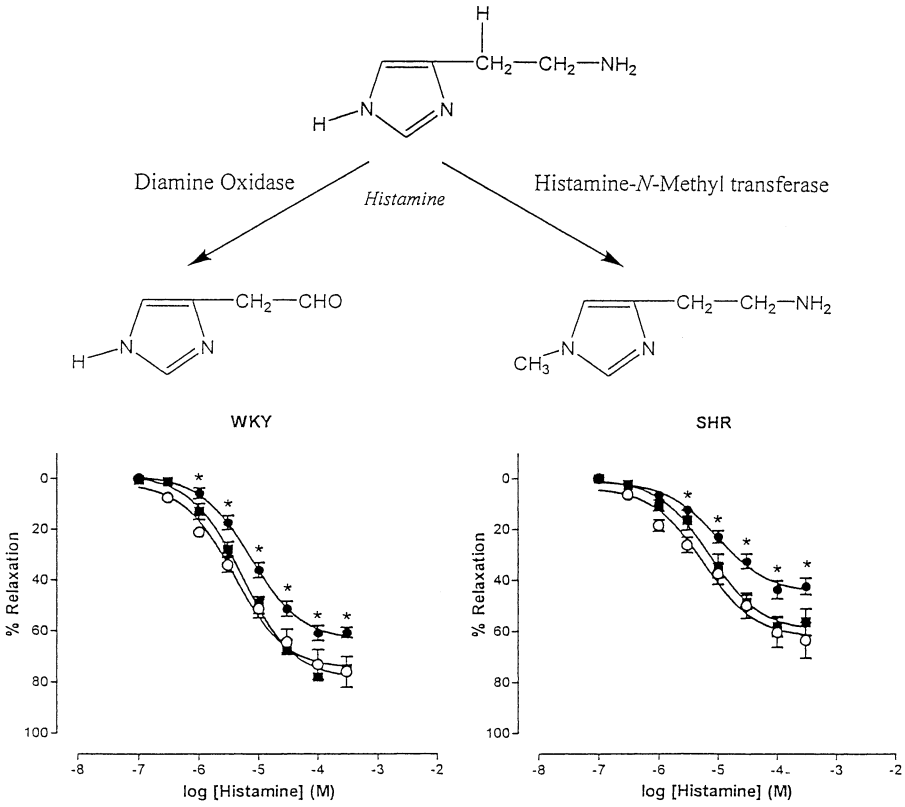

Fig. 1. Top panel: Pathways of histamine catabolism. Histamine is metabolised either by deamination with diamine oxidase or by methylation with histamine-*N*-methyl-transferase. Bottom panel: Cumulative concentration–response curves of histamine (control, ○), with SKF 91488 (10 μM, ●) alone, and in the presence of SKF 91488 (10 μM) plus clobenpropit (1 nM) (■) on phenylephrine (1 μM) pre-contracted pulmonary artery (endothelium-intact) of the normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Results are expressed as mean ± S.E.M. ($n = 6–8$). *$P < 0.05$ compared to control.

methyl-transferase on histamine-elicited responses on rat isolated pulmonary artery.

Histamine causes both endothelium-dependent (Arai and Chiba, 1999; Ortiz et al., 1992) and -independent (Krstić et al., 1996; Kishi et al., 1998; Martinez et al., 2000) relaxation of different vascular preparations. Recently, four subtypes of histamine receptors (h1 to h4) have been cloned and expressed in various cell/tissue types (Nakamura et al., 2000; Liu et al., 2001b; Coge et al., 2001; Zhu et al., 2001), and only histamine H_1, H_2 and H_3 receptors can be differentiated pharmacologically (Morisset et al., 2001). Pre-synaptic histamine H_3 receptors have been described in different vascular tissues (Ishikawa and Sperelakis, 1987; Molderings et al., 1992) that are pharmacologically distinct from histamine H_1 and H_2 receptors. In fact, histamine H_3 receptor has been found in brain (Arrang et al., 1995), rat mesenteric artery (Ishikawa and Sperelakis, 1987), guinea-pig trachea, pulmonary artery and ileum (Cardell and Edvinsson, 1994; Lee and Parsons, 2000), and porcine nasal mucosa (Varty and Hey, 2002). In pre-contracted human pulmonary artery (Ortiz et al., 1992), histamine managed to cause a further contraction, and these authors reported that histamine H_1 receptor was involved even though chlorpheniramine (a histamine H_1 receptor antagonist, 2.5 μM) was present. In addition, the histamine-evoked contraction was resistant to burimamide (a histamine H_2/H_3 receptor antagonist) and impromidine (a histamine H_2 receptor agonist/H_3 receptor antagonist) strongly suggested that other mechanism(s) may be involved. There is, however, no clear indication whether histamine H_3 receptor is present in rat pulmonary arterial tissue, and the modulatory role of histamine H_3 receptor activation is not known. Hence, the second aim of this study was to determine the effect of histamine H_3 receptor activation and the underlying mechanisms involved.

Alternation in the vascular reactivity (contraction and relaxation) has been suggested responsible for the development of hypertension. Despite the well-known participation in various mast cells-mediated responses (allergy and inflammation), the pulmonary artery vascular effects of histamine under diseased condition, e.g. hypertension, have not been addressed in detail. Therefore, the third aim of this study was to evaluate the modulatory effect of hypertensive state (Aharinejad et al., 1996), using spontaneously hypertensive rats (SHR, an animal model of human essential hypertension) (Trippodo and Frohlich, 1981), on the pulmonary arterial responses of histamine. Previous reports have shown that the pulmonary circulation of SHR developed morphological changes with an elevated blood pressure (both the pulmonary and peripheral blood pressure) that

resemble to men/women who have pulmonary hypertension (Aharinejad et al., 1996; Matsuda et al., 2000).

2. Materials and methods

2.1. Tissue preparation

The spontaneously hypertensive rats (SHR) and normotensive Wistar–Kyoto (WKY) rats were bred at The Chinese University of Hong Kong from three original pairs (3 SHR and 3 WKY rats) purchased from the Animal Resources Centre (Western Australia, Australia). Both the WKY and SHR were 22–26 weeks old (male) and weighed 325 ± 13 and 341 ± 18 g, respectively ($P>0.05$). Rats were housed under a 12:12 hour light–dark cycle and were given standard rat chow and water ad libitum before they were killed. Systolic arterial blood pressure was registered using an automatic sphygmomanometer with a tail-cuff method device. The average systolic blood pressure measured was: 132 ± 8 mmHg for the WKY ($n=7$) and 253 ± 7 mmHg for SHR ($n=9$) ($P<0.05$). They were quickly killed by cervical dislocation and the pulmonary artery (first branch; O.D. ~ 800 μm) was isolated with excess fat and connective tissue removed. Pulmonary artery was cut into ring of 1 mm, and mounted in a 5-ml organ bath containing Krebs' solution (gassed continuously with 95% O_2–5% CO_2; pH 7.4, 37 ± 1 °C) of the following composition (in mM): NaCl 118, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $NaHCO_3$ 25, glucose 11 and $CaCl_2$ 2.5. The Animal Research Ethics Committee of The Chinese University of Hong Kong has approved the experiments performed in this study (approval no. 01/002/DRG) and conformed to guidelines for the use of laboratory animals. Every effort was made to limit animal suffering as well as to limit the number of animals used in these experiments.

2.2. Measurement of isometric tension change

Two stainless steel wires (diameter ~ 50 μm) were inserted into the lumen of the blood vessel, one fixed and the other connected to a force-displacement transducer (Grass FT 03). Isometric tension measurement was performed using the MacLab Chart v 3.6 program. The pulmonary arterial ring was equilibrated under the optimum resting tension (10 ± 1 mN) in the bath solution for 90 min. During the equilibration period, the preparation was washed with drug-free Krebs' solution every 20-min and the resting tension was readjusted, if necessary, before commencing the experiments. In the preliminary experiment ($n=4$ for each strain of rat), application of indomethacin (1 μM, a cyclo-oxygenase inhibitor) resulted in an enhancement of histamine (300 μM)-elicited relaxation (~ 10% increase). These results suggest that a cyclo-oxygenase-dependent pressor substance is released (Kato et al., 1990; Ortiz et al., 1992; Török, 2000) from the pulmonary artery upon the challenge of histamine. Indomethacin was therefore included in the Krebs' solution for all experiments reported in this study.

Acetylcholine (0.3 μM) was added to induce relaxation of noradrenaline (1 μM) pre-contracted preparations (maximum relaxation of ~ 50% in WKY and ~ 40% in SHR) for the confirmation of endothelial integrity. In some tissues, endothelium was carefully removed by gently rubbing the intima of the blood vessel with a wire. Absence of the functional endothelium was confirmed, at the beginning of each experiment, by the failure of acetylcholine in producing relaxation of noradrenaline pre-contracted preparation.

In the preliminary study, high $[K^+]_o$ (50 mM, a membrane depolarising agent) caused maximum contraction, irrespective of strains of rat (WKY: 6.13 ± 1.03 mN, $n=8$; SHR: 5.88 ± 0.98 mN, $n=9$) ($P>0.05$). Hence, high $[K^+]_o$ was used as a "reference" for comparison of the arterial contraction observed under resting tension (Cardell and Edvinsson, 1994).

For relaxation experiments, phenylephrine (a receptor agonist) was used as the contractile agent to raise the active tone of the preparations. In contrast to high $[K^+]_o$, agonist-induced contraction (maximum contraction caused by 3 μM phenylephrine) was greater in the WKY (7.53 ± 0.84 mN, $n=7$; ~ 122% of 50 mM $[K^+]_o$-evoked contraction) than in SHR (5.32 ± 0.79 mN, $n=7$; ~ 92% of 50 mM $[K^+]_o$-evoked contraction). Phenylephrine 1 μM gave rise to ~ 95% of the phenylephrine-elicited maximum contractile response (3 μM phenylephrine) observed in individual strain of rat ($n=6$). Relaxation in response to histamine receptor agonist was expressed as percentage of phenylephrine (1 μM)-induced tone (as reported in guinea-pig pulmonary artery using agonist as contractile agent) (Cardell and Edvinsson, 1994), and 100% relaxation was considered when the active tone returned to baseline level. In case where there was a further contraction of the pre-contracted preparation caused by histamine agonists, the magnitude of the further contraction was expressed percentage of steady-state phenylephrine-evoked tone (Cardell and Edvinsson, 1994). Results obtained from the WKY rat were compared with SHR. To avoid the possible de-sensitisation of the preparations, only one concentration–response curve of individual agonist was constructed in each preparation. A 30-min incubation time was allowed in experiments with receptor antagonist/enzyme inhibitor, which was left in contact with the preparations before the pre-contraction of the pulmonary artery with phenylephrine and further during the construction of the concentration–response curves of histamine receptor agonist. Where stated, the concentration of receptor antagonists/blockers of intracellular messenger pathways employed in this study were the reported effective concentration of individual agent based on our previous studies (Kwan et al., 1999; Choy et al., 2002) and other groups on pulmonary artery and other vascular preparations (Jino et al., 1996; MacLean et al., 1996; Satake et al., 1996; Pulido et al., 2000; Bonnet et al., 2001). In view of the well-known non-specific effects of 1-[6-((17β-3-methoxyestra-

1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione (U 73122, a phospholipase C inhibitor) at high concentration, this agent was tested at the lowest effective concentration reported in the literature.

2.3. Chemicals

Physiological salts (GR grade) for preparing Krebs' solution were purchased from Merck (Germany). The following drugs were purchased from Sigma-Aldrich (St. Louis, MO, USA): histamine dihydrochloride, acetylcholine chloride, neostigmine bromide, L-phenylephrine hydrochloride, prazosin hydrochloride, dimaprit dihydrochloride, imetit dihydrobromide, diphenhydramine dihydrochloride, cimetidine, clobenpropit dihydrobromide, atropine sulphate, salbutamol hemisulfate, *S*-[4-(*N*,*N*-dimethylamino)-butyl]isothiourea dihydrochloride (SKF 91488), indomethacin, N^G-nitro-L-arginine methylester hydrochloride (L-NAME), N^G-nitro-D-arginine methylester hydrochloride (D-NAME) and nifedipine. 6-Anilino-5,8-quinolinedione (LY 83583), bisindolymaleimide I hydrochloride, 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione (U 73122), 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-2,5-pyrroleidinedione (U 73343) and *cis*-*N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine hydrochloride (MDL 12330A) were obtained from Calbiochem-Novabiochem (San Diego, CA, USA). 4-(*Z*)-6-(2-*o*-chlorophenyl-4-*o*-hydroxyphenyl-1,3-dioxan-*cis*-5-yl) hexenoic acid (ICI 192605), aminoguanidine hydrochloride, ketanserin tartrate, 3-[3-(dimethylamino)propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl] benzamide dihydrochloride (GR 55562) and 6-[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl) heptanecarboxamide dimaleate (HTMT) were purchased from Tocris Cookson (Bristol, UK). Indomethacin, LY 83583, U 73122, U 73343, ICI 192605 and HTMT were dissolved in dimethyl sulfoxide (DMSO). The highest concentration of DMSO in the organ bath was 0.01% (vol/vol), a concentration that does not influence vascular response (Kwan et al., 1999; Choy et al., 2002). Stock solutions of other drugs were prepared in de-ionised water. Aliquots of stock solutions were kept frozen (-30 °C) until use. Experiments using light-sensitive compounds were performed in a dimly lit room.

2.4. Statistical analysis

All concentrations reported in this study were expressed as final molar concentrations in the organ-bath. Concentration of histamine and histamine receptor agonists causing 50% of the maximal relaxation response (EC_{50}) observed in each strain of rat were calculated by non-linear regression analysis for each tissue, and the corresponding pD_2 ($-\log EC_{50}$) values were calculated for each group of experiments using GraphPad Prism (GraphPad Software, San Diego, CA, USA). pA_2 values of diphenhydramine and cimetidine were estimated using Schild analysis (Arunlakshana and Schild, 1959). Data were presented as means ± S.E.M.; when not visible, the standard error falls within the size of the symbol. Statistical analysis was performed using analysis of variance and $P<0.05$ was considered significant. Where stated, n values ($n=5-8$) represented the number of rats used in individual experiment.

3. Results

3.1. Modulatory effect of S-[4-(N,N-dimethylamino)-butyl] isothiourea (SKF 91488) and aminoguanidine on histamine-evoked relaxation

Histamine was tested in the pulmonary artery under resting tension. No apparent change in tension was observed with concentrations ≤ 0.1 mM and histamine (1 mM) caused a contraction (expressed as percentage of 50 mM $[K^+]_o$-induced contraction) only in endothelium-denuded preparation ($n=5$ for each strain of rat) (WKY: 36.2 ± 4.4%; SHR: 23.4 ± 6.9%). In phenylephrine (1 μM) pre-contracted pulmonary artery, cumulative administration of histamine caused a concentration-dependent (0.3–300 μM) relaxation of the WKY ($pD_2=5.41\pm0.09$) and SHR ($pD_2=5.25\pm0.13$) (Fig. 1). With 10 μM *S*-[4-(*N*,*N*-dimethylamino)-butyl]isothiourea (SKF 91488, a histamine *N*-methyl-transferase inhibitor), histamine-mediated relaxation was significantly attenuated (Fig. 1), with no apparent difference in the estimated pD_2 values (WKY: $pD_2=5.13\pm0.06$; SHR: $pD_2=5.01\pm0.09$, $P>0.05$ compared to respective controls). A combination of SKF 91488 (10 μM) plus aminoguanidine (100 μM, a diamine oxidase inhibitor) failed to further modify histamine relaxation, compared to when SKF 91488 was applied alone (WKY: $pD_2=4.92\pm0.15$; SHR: $pD_2=5.14\pm0.13$) ($P>0.05$ compared to respective controls, $n=5-6$ for each strain of rat). Pre-treatment with aminoguanidine (100 μM) alone did not affect histamine-mediated relaxation (WKY: $pD_2=5.25\pm0.11$; maximum relaxation at 300 μM histamine, 74.7 ± 6.2%; SHR: $pD_2=5.32\pm0.07$; maximum relaxation at 300 μM histamine, 62.4 ± 5.3%) when compared to SKF 91488-free condition ($P>0.05$, $n=5-6$). SKF 91488, aminoguanidine and a combination of SKF 91488 plus aminoguanidine did not modify the resting tension or the phenylephrine-induced tone observed in the WKY and SHR. The "inhibitory" effect of SKF 91488 on histamine-elicited relaxation was overcome in the presence of clobenpropit (1 nM, a highly selective histamine H_3 receptor antagonist) (Fig. 1). Administration of clobenpropit (1 nM) had no effect on histamine-induced relaxation (WKY: maximum relaxation at 300 μM histamine, 86.6 ± 6.1% ($P>0.05$ compared to control), $pD_2=5.38\pm0.11$; SHR: maximum relaxation at 300 μM histamine, 70.2 ± 5.1% ($P>0.05$ compared to the respective controls), $pD_2=5.19\pm0.15$).

Under resting tension, 6-[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl) heptanecarboxamide (HTMT, a

selective histamine H_1 receptor agonist) did not alter the tone of the pulmonary artery ($n=5$ for each strain of rat). Similar to histamine, HTMT caused a concentration-dependent (0.3–300 μM) relaxation of the preparations with raised tone, with a greater magnitude of relaxation observed in the WKY rat (maximum relaxation at 300 μM HTMT, WKY: 82.4 ± 6.1%; SHR: 60.6 ± 4.2%) ($P<0.05$) (Fig. 4), with no apparent difference in the estimated pD_2 values (WKY: $pD_2 = 5.10 \pm 0.27$; SHR: $pD_2 = 5.22 \pm 0.19$) ($P>0.05$). HTMT-induced relaxation was not altered by SKF 91488 (10 μM) (maximum relaxation at 300 μM HTMT, WKY: 80.2 ± 5.2%; SHR: 63.1 ± 4.4 %) ($P>0.05$ compared to the respective controls, $n=6$), aminoguanidine (100 μM) (maximum relaxation at 300 μM HTMT, WKY: 79.1 ± 5.5%; SHR: 58.7 ± 3.9%) ($P>0.05$ compared to respective controls, $n=5$) nor a combination of SKF 91488 (10 μM) plus aminoguanidine (100 μM) (maximum relaxation at 300 μM HTMT, WKY: 81.1 ± 5.0%; SHR: 59.4 ± 5.1%) ($P>0.05$ compared to the respective controls, $n=6$).

In another set of experiments, salbutamol (a β-adrenoceptor agonist) elicited a concentration-dependent (10 nM–10 μM) relaxation of phenylephrine pre-contracted preparations, with a similar magnitude observed in both strains of rat. Salbutamol-evoked relaxation was not altered by SKF 91488 (10 μM) and aminoguanidine (100 μM) (maximum relaxation at 10 μM salbutamol, control, WKY: 88.5 ± 6.2%, SHR: 92.6 ± 5.7%; with SKF 91488, WKY: 91.3 ± 5.5%, SHR: 94.3 ± 6.1%; with aminoguanidine, WKY: 87.8 ± 4.9%, SHR: 91.2 ± 5.6%) ($P>0.05$ compared to the respective controls) ($n=5–6$).

3.2. Histamine receptor characterisation

To avoid the activation of multiple histamine receptors by histamine, two relatively selective histamine receptor agonists HTMT (a histamine H_1 receptor agonist) and dimaprit (a selective histamine H_2 receptor agonist with a moderate histamine H_3 receptor antagonistic activity) were used. Diphenhydramine (a histamine H_1 receptor antagonist) (0.1, 1 and 10 μM) caused a progressive rightward shift of the concentration–response curve (no change in maximum relaxation) of HTMT, with an estimated pA_2 value of 7.32 ($r^2=0.93$, slope of Schild plot = 0.89 ± 0.09) and 7.08 ($r^2=0.91$, slope of Schild plot = 0.91 ± 0.07) in the WKY and SHR ($P>0.05$), respectively. In the presence of either cimetidine (10 μM; a histamine H_2 receptor antagonist) or clobenpropit (10 nM; a histamine H_3 receptor antagonist with a moderate H_4 receptor agonistic property), the HTMT-induced relaxation was not altered (with cimetidine, maximum relaxation at 300 μM HTMT, WKY: 79.3 ± 6.0%, $pD_2 = 5.28 \pm 0.33$; SHR: 57.8 ± 7.1%, $pD_2 = 5.13 \pm 0.40$; with clobenpropit, maximum relaxation at 300 μM HTMT, WKY: 80.1 ± 3.8%, $pD_2 = 5.31 \pm 0.21$; SHR: 62.2 ± 3.5%, $pD_2 = 5.24 \pm 0.30$) ($n=5–6$ for each antagonist) ($P>0.05$ compared to the respective controls).

Under resting tension, dimaprit had no apparent effect ($n=5$ for each strain of rat). Unlike histamine and HTMT, dimaprit elicited a similar magnitude of relaxation (in a concentration-dependent manner), irrespective of the strain of rat (maximum relaxation at 100 μM dimaprit, WKY: 38.6 ± 6.6%, $pD_2 = 5.41 \pm 0.22$; SHR: 31.1 ± 3.3%, $pD_2 = 5.69 \pm 0.19$) ($P>0.05$) (Fig. 4). The magnitude of maximum relaxation, however, was smaller compared to that observed with histamine and HTMT. Pre-treatment with either SKF 91488 (10 μM) (maximum relaxation at 100 μM dimaprit, WKY: 35.3 ± 5.6%, $pD_2 = 5.38 \pm 0.34$; SHR: 29.8 ± 4.7%, $pD_2 = 5.51 \pm 0.42$) or aminoguanidine (100 μM) (maximum relaxation at 100 μM dimaprit, WKY: 39.0 ± 5.9%, $pD_2 = 5.21 \pm 0.30$; SHR: 30.2 ± 4.5%, $pD_2 = 5.19 \pm 0.28$) produced no apparent change on dimaprit-elicited relaxation ($n=5–6$ for each agent) ($P>0.05$ compared to controls).

Cimetidine (1, 3 and 10 μM) (a histamine H_2 receptor antagonist) caused a progressive rightward shift of the concentration–response curve (with no change in maximum relaxation) of dimaprit. pA_2 values of 6.03 ($r^2=0.91$, slope of Schild plot = 0.92 ± 0.11) and 6.04 ($r^2=0.90$, slope of Schild plot = 0.89 ± 0.13) were obtained in the WKY and SHR ($P>0.05$), respectively. A higher concentration of cimetidine 30 μM caused a further rightward shift and suppressed dimaprit-induced relaxation (maximum relaxation at 100 μM dimaprit, WKY: 22.6 ± 4.2%; SHR: 18.1 ± 4.3%) ($P<0.05$ compared to respective controls, $n=5–6$). Neither diphenhydramine (10 μM) nor clobenpropit (10 nM) modified dimaprit-evoked response (with diphenhydramine, maximum relaxation at 100 μM dimaprit, WKY: 35.8 ± 6.8%, $pD_2 = 5.40 \pm 0.30$; SHR: 30.2 ± 5.4%, $pD_2 = 5.24 \pm 0.26$; with clobenpropit, maximum relaxation at 100 μM dimaprit, WKY: 40.6 ± 3.6%, $pD_2 = 5.38 \pm 0.32$; SHR: 30.8 ± 4.7%, $pD_2 = 5.29 \pm 0.42$) ($P>0.05$ compared to the respective controls) ($n=5–6$ for each antagonist).

With a combination of 30 μM diphenhydramine and 30 μM cimetidine, histamine (with SKF 91488 present) caused a further contraction of the pre-contracted preparations, with a greater magnitude observed in the WKY rats (Fig. 2). Moreover, a cocktail of 30 μM diphenhydramine, 30 μM cimetidine and 10 nM clobenpropit abrogated the histamine response (Fig. 2). The histamine-induced further contraction, however, was resistant to atropine (1 μM, a non-selective muscarinic receptor antagonist), ketanserin (1 μM, a 5-HT_{2A} receptor antagonist), 3-[3-(dimethylamino)-propyl]-4-hydroxy-*N*-[4-(4-pyridinyl)phenyl] benzamide (GR 55562) (1 μM, a 5-HT_{1B} receptor antagonist) and 4-(*Z*)-6-(2-*o*-chlorophenyl-4-*o*-hydroxyphenyl-1,3-dioxan-*cis*-5-yl)hexenoic acid (ICI 192605) (1 μM, a thromboxane A_2 receptor antagonist) ($n=5$ for each agent tested on individual strain of rat) (data not shown). In the preliminary study, acetylcholine (with 10 μM neostigmine), 5-hydroxytryptamine and thromboxane A_2 caused contraction of the pulmonary artery, with a greater magnitude observed in SHR (data not shown). The concentration (1 μM) of

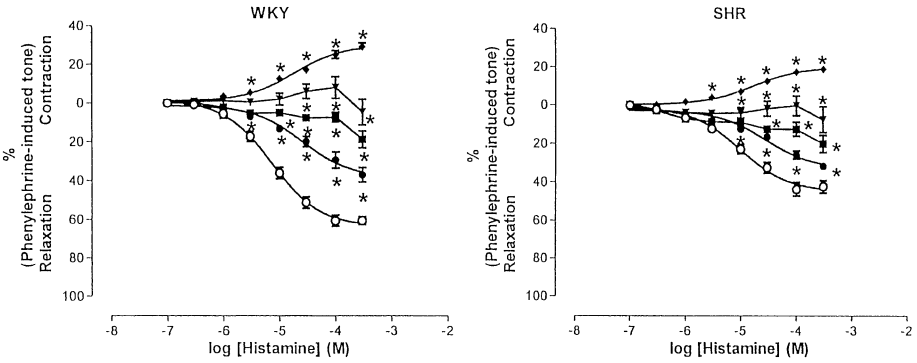

Fig. 2. Cumulative concentration–response curve of histamine (control, ○) on phenylephrine (1 μM) pre-contracted pulmonary artery (endothelium-intact) of the normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR) in the presence of 10 μM SKF 91488. The figure illustrates the effect of diphenhydramine (10 μM, ●) alone, diphenhydramine (10 μM) plus cimetidine (10 μM) (■), diphenhydramine (30 μM) plus cimetidine (30 μM) (◆) and a combination of diphenhydramine (30 μM), cimetidine (30 μM) and clobenpropit (10 nM) (▼) on histamine-induced relaxation. Abscissa: log histamine concentrations (M); ordinate: percentage of phenylephrine-induced tone; above zero indicates further contraction whereas below zero indicates relaxation.

individual receptor antagonist (atropine, ketanserin, GR 55562 and ICI 192605) tested caused a marked rightward shift (by 2.5–3 log units) of the contractile response elicited by the respective agonist ($n = 5–6$ for individual agonist). Administration of these agents had no apparent effect on resting tension and phenylephrine-induced tone.

3.3. Role of endothelium, nitric oxide and adenylate cyclase activation

Manoeuvres such as endothelial denudation, pre-treatment with N^G-nitro-L-arginine methylester (L-NAME, a nitric oxide synthase inhibitor) (20 μM) and 6-anilino-5,8-quinolinedione (LY 83583, 3 μM, a guanylate cyclase inhibitor) (in endothelium intact preparations) resulted in an increase in phenylephrine (1 μM)-induced tone (~ 27%), compared to controls. Hence, concentration of phenylephrine used was adjusted (0.3–0.6 μM) accordingly in order to have a comparable magnitude of active tone (by phenylephrine) for the determination of relaxation response. Endothelial denudation abolished HTMT-(maximum relaxation at 300 μM HTMT; WKY: 2.4 ± 1.1%; SHR: 1.5 ± 0.4%) ($P < 0.001$ compared to the respective controls, $n = 5$ for each strain of rat), and suppressed histamine-elicited relaxation (Fig. 3). Removal of endothelium had no significant effect on dimaprit-induced relaxation (maximum relaxation at 100 μM dimaprit; WKY: 32.6 ± 5.1%, $pD_2 = 5.30 \pm 0.31$; SHR: 34.1 ± 4.4%, $pD_2 = 5.44 \pm 0.29$) ($P > 0.05$ compared to the respective controls, $n = 5$ for each strain of rat).

In order to avoid the possible enzymatic degradation that may affect the interpretation of our results, SKF 91488 (10 μM) was included in all experiments when histamine was employed. Application of L-NAME (20 μM), but not N^G-nitro-D-arginine methylester (D-NAME) (20 μM, an inactive analogue of L-NAME), significantly attenuated histamine-mediated relaxation observed in both strains of rat (Fig. 3). A higher concentration of L-NAME (50 μM) failed to cause a further attenuation of histamine-elicited relaxation. The concentration–response curve of histamine constructed in endothelium-denuded preparation overlapped fairly well with that recorded when L-NAME was present (Fig. 3). L-NAME (20 μM) abolished HTMT-mediated relaxation (maximum relaxation at 300 μM HTMT; WKY: 2.1 ± 1.0%; SHR: 1.3 ± 0.2%) ($P < 0.001$ compared to the respective controls, $n = 6$ for each strain of rat). Moreover, 6-anilino-5,8-quinolinedione (LY 83583) (3 μM, a guanylate cyclase inhibitor) markedly reduced histamine- and abolished HTMT-induced relaxation (maximum relaxation at 300 μM HTMT; WKY: 1.3 ± 0.4 %; SHR: 0.9 ± 0.7%) ($P < 0.001$ compared to the respective controls, $n = 6$ for each strain of rat).

L-NAME (20 μM) and LY 83583 (3 μM) failed to modify dimaprit-mediated relaxation (with L-NAME, maximum relaxation at 100 μM dimaprit, WKY: 33.2 ± 5.4%, $pD_2 = 5.28 \pm 0.18$; SHR: 32.4 ± 4.0%, $pD_2 = 5.40 \pm 0.24$; with LY 83583, maximum relaxation at 100 μM dimaprit, WKY: 37.1 ± 6.2%, $pD_2 = 5.37 \pm 0.31$; SHR: 33.2 ± 3.4%, $pD_2 = 5.40 \pm 0.16$) ($n = 6$) ($P > 0.05$ compared to the respective controls). Pre-treatment with *cis-N*-(2-phenylcyclopentyl)azacyclotridec-1-en-2-amine (MDL 12330A) (10 μM, an adenylate cyclase inhibitor) markedly attenuated dimaprit-elicited relaxation in both strains of rat (Fig. 3). In addition, MDL 12330A (10 μM) abolished salbutamol-mediated pulmonary artery relaxation ($n = 5$ for each strain of rat) (data not shown).

In another set of experiment, acetylcholine (with 10 μM neostigmine, an acetylcholinesterase inhibitor) elicited a concentration-dependent relaxation of phenylephrine pre-contracted pulmonary artery. Maximum relaxation was observed at 1 μM in the WKY rats (52.3 ± 7.2% relaxation) whereas in SHR, maximum relaxation occurred at 0.3 μM (35.6 ± 4.1% relaxation). In contrast to histamine, there was no significant difference in the degree of relaxation (acetylcholine ≤ 0.3 μM) between the WKY and SHR. Moreover, acetylcholine (≥ 10 μM) elicited a further con-

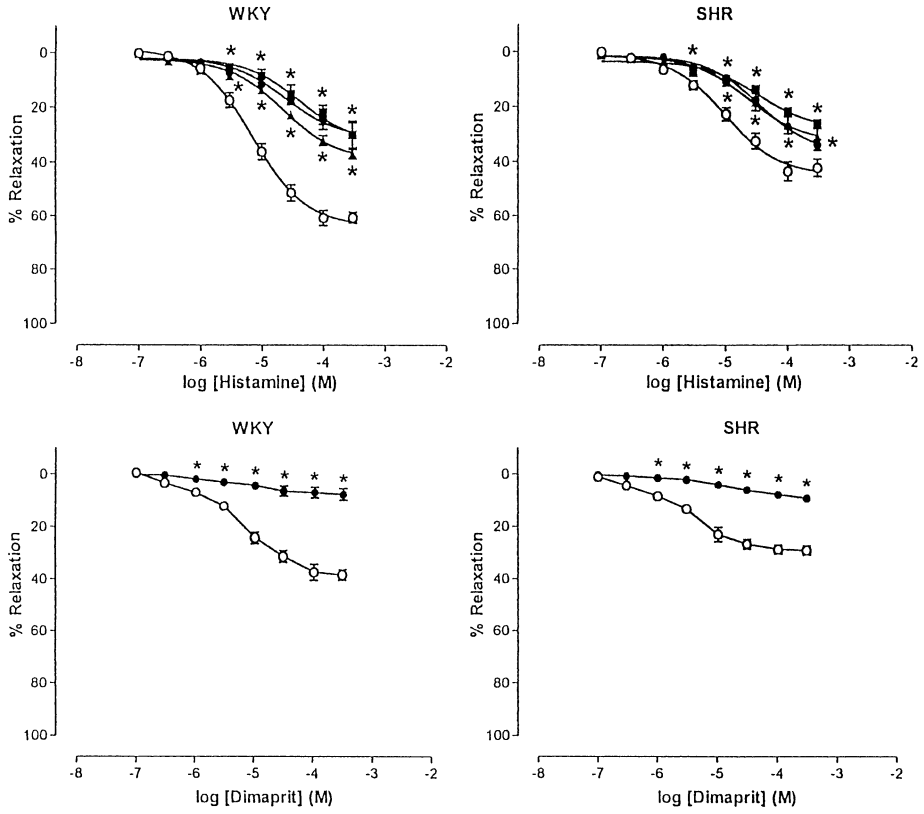

Fig. 3. Top panel: Cumulative concentration–response curve of histamine (control, ○) on phenylephrine (1 μM) pre-contracted pulmonary artery (endothelium-intact) of the normotensive Wistar–Kyoto (WKY) and Spontaneously hypertensive rats (SHR) in the presence of 10 μM SKF 91488. The figure illustrates the effect of L-NAME (20 μM, ●; 50 μM, ■) and endothelium denudation (▲) on histamine-elicited relaxation. Bottom panel: Cumulative concentration–response curve of dimaprit (control, ○; plus MDL 12330A 10 μM, ●) on phenylephrine (1 μM) pre-contracted pulmonary artery (endothelium-intact) of the normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Results are expressed as mean ± S.E.M. ($n = 6–8$). *$P < 0.05$ compared to control.

traction of the pulmonary artery (raised tone) of SHR whereas there was only a minimal change in the WKY rats.

3.4. Mechanism(s) responsible for imetit-evoked pulmonary artery contraction

Administration of imetit (1 nM–1 μM) (a histamine H_3/H_4 receptor agonist) failed to produce relaxation of the pre-contracted pulmonary artery (Fig. 4). A further contraction (in a concentration-dependent fashion) (≥ 3 μM), with a greater magnitude was observed in the WKY rats (300 μM imetit: 73.6 ± 6.6%) compared to SHR (300 μM imetit: 24.3 ± 1.3%) (Fig. 4). Endothelium denudation failed to alter imetit-mediated further contraction (300 μM imetit: WKY, 78.7 ± 5.4%; SHR, 26.7 ± 8.9%) ($P > 0.05$ compared to the respective endothelium intact controls, $n = 5$ for each strain of rat). Imetit-induced contraction recorded in the WKY rat was markedly suppressed by clobenpropit (10 nM) (the maximum contraction at 300 μM imetit: 46.9 ± 8.1%) ($P < 0.05$ compared to control), and a higher concentration of clobenpropit (30 nM) abolished imetit-evoked contraction (the maximum contraction at 300 μM imetit: 3.2 ± 4.1%) ($P < 0.001$ compared to control). In SHR, clobenpropit (10 nM) produced no effect on imetit (300 μM)-induced contraction (15.2 ± 6.6%, compared to control: 24.3 ± 11.3%) ($P > 0.05$). Similar to the WKY rat, clobenpropit (30 nM) eradicated imetit-induced further contraction (the maximum contraction at 300 μM imetit: 2.4 ± 2.1%) ($P < 0.001$ compared to control). Atropine, ketanserin, GR 55562 and ICI 192605 (each at 1 μM) did not modify imetit-mediated further contraction ($n = 5$ for each strain of rat).

Under resting tension, imetit (0.3–300 μM) elicited a concentration-dependent contraction of pulmonary artery with a greater magnitude in the WKY rats (Fig. 4). No apparent change on the resting tension by imetit at concentrations ≤ 0.1 μM. Imetit-induced contraction was partially sensitive to clobenpropit (10 nM) (Fig. 4) and prazosin (1 μM). A higher concentration of clobenpropit (30 nM) did not produce a further suppression of imetit-evoked contraction. In addition, a mixture of clobenpropit (10 nM) and prazosin (1 μM) administered failed to produce a greater magnitude of suppression of contraction in comparing to the situation in which individual agent was applied alone ($n = 5$ for each strain of rat) (Fig. 4). Similar to that observed in the raised-tone preparations, imetit-evoked contraction was resistant to atropine (1 μM), ketanserin (1 μM), GR 55562 (1 μM) and ICI 192605 (1 μM) ($n = 5–6$ for each strain of rat).

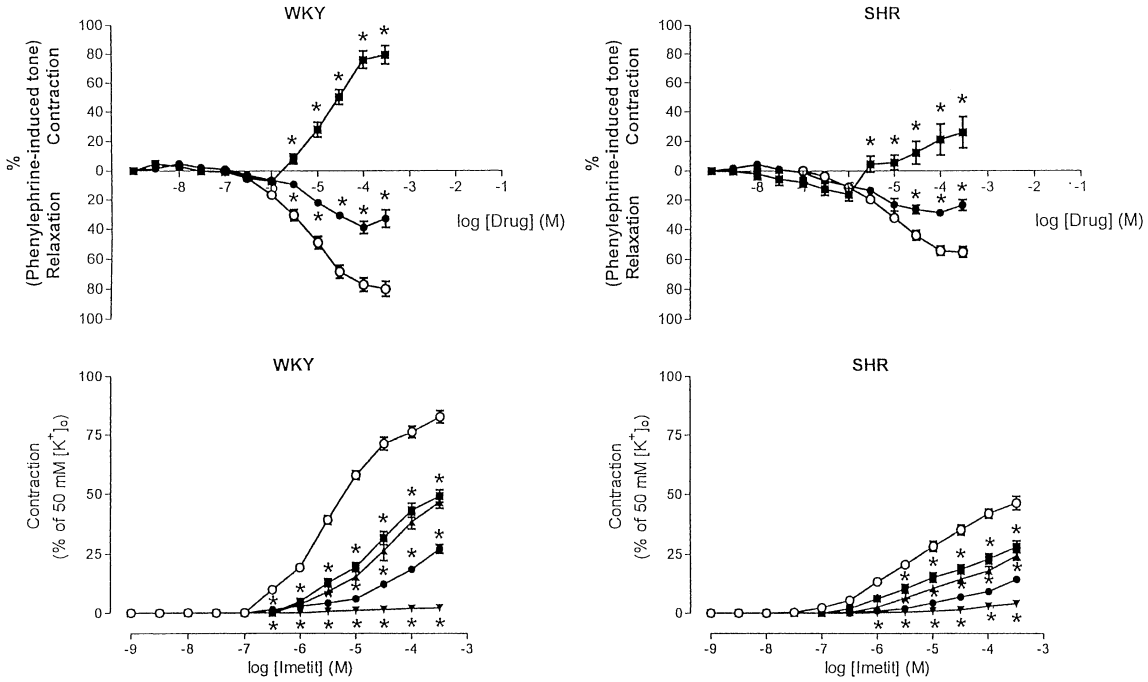

Fig. 4. Top panel: Cumulative concentration–response curve of HTMT (○), dimaprit (●) and imetit (■) on phenylephrine (1 μM) pre-contracted pulmonary artery (endothelium-intact) of the normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). Abscissa illustrates log concentrations of drug (M) and ordinate represents percentage of phenylephrine-induced tone. Above zero indicates further contraction whereas below zero indicates relaxation. Results are expressed as mean ± S.E.M. ($n=6-8$). *$P<0.05$ compared to control (○). Bottom panel: Cumulative concentration–response curve of imetit (control, ○) on the pulmonary artery (endothelium-intact, under resting tension) of the normotensive Wistar–Kyoto (WKY) and spontaneously hypertensive rats (SHR). The figure illustrates the effect of U 73122 (3 μM, ●), bisindolymaleimide I (30 nM, ▼), clobenpropit (10 nM, ■) and a combination of clobenpropit (10 nM) plus prazosin (1 μM) (▲) on imetit-induced contraction. Results are expressed as mean ± S.E.M. ($n=6-8$). *$P<0.05$ compared to control.

Application of 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-1H-pyrrole-2,5-dione (U 73122, 3 μM) but not the inactive analogue 1-[6-((17β-3-methoxyestra-1,3,5(10)-trien-17-yl)amino)hexyl]-2,5-pyrroleidinedione (U 73343, 3 μM) ($n=6$ for each strain of rat) markedly suppressed imetit-evoked contractile response (Fig. 4). Moreover, bisindolymaleimide I (30 nM) (a highly potent protein kinase C inhibitor, $K_i=10$ nM) (Fig. 4) and nifedipine (100 nM) administered alone, abolished imetit-induced contraction (with nifedipine, maximum contraction at 300 μM imetit, WKY: 3.4 ± 1.2%; SHR: 1.2 ± 0.7%) ($P<0.001$ compared to respective controls, $n=6$ for each strain of rat).

4. Discussion

We have evaluated the in vitro effect of histamine receptor activation on the pulmonary artery of both the normotensive Wistar–Kyoto (WKY) rats and age-matched (22–26 weeks old, male) spontaneously hypertensive rats (SHR). In this study, histamine consistently causes a concentration-dependent relaxation in preparations with raised tone. The rat pulmonary artery to histamine is about 1000 times less responsive than that was observed in human pulmonary artery (rat: pD_2, ~ 5; human: pD_2, ~ 8) (Ortiz et al., 1992). In contrast to the resistant mesenteric artery of SHR (Hendriks et al., 1993; Kam et al., 1994), the magnitude of histamine-induced pulmonary artery relaxation was significantly smaller in SHR. Removal of endothelium only attenuated, but not abolished, histamine-evoked relaxation in both strains of rat. The diminished relaxation observed in SHR seems to correlate fairly well with a reduced NO/endothelium-dependent (van de Voorde et al., 1998), histamine H_1 receptor-mediated relaxation through guanylate cyclase activation. However, we could not rule out the possibility of a change in the density of histamine receptor present in the endothelium of SHR and warrants a further investigation. In addition, there was no apparent difference in the estimated pA_2 of diphenhydramine (a histamine H_1 receptor antagonist) (Douglas et al., 1984; Hill et al., 1997) on 6-[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl) heptanecarboxamide (HTMT)-induced relaxation observed in the WKY and SHR, suggesting that the reduced relaxation observed in SHR was not due to a change in the affinity of diphenhydramine-sensitive, endothelial histamine H_1 receptors.

In contrast to histamine and HTMT, application of dimaprit (Hill, 1990; Leurs et al., 1995) produced a similar degree of adenylate cyclase-dependent, endothelium-independent relaxation (in both strains of rat) that involved the activation of adenylate cyclase pathway.

Two catabolic enzymes that mainly degrade the released histamine are diamine oxidase and histamine *N*-methyl-

transferase (Fig. 1). The concentration of histamine would be elevated with a prolonged action (e.g. a greater degree of relaxation) after the inhibition of the catabolic enzymes. Surprisingly, incubation with *S*-[4-(*N*,*N*-dimethylamino)-butyl]isothiourea (SKF 91488, a selective histamine *N*-methyl-transferase inhibitor; a dimaprit analogue with no reported agonist activity) (Beavan and Shaff, 1979) resulted in an attenuation of histamine-induced relaxation in both strains of rat. We speculated that the blockade of one catabolic pathway (histamine *N*-methyl-transferase) shifts histamine metabolism to the alternate pathway (diamine oxidase). Our results, however, argued against this possibility as aminoguanidine (100 μM, a diamine oxidase inhibitor) (Tamura et al., 1989; Kitanaka et al., 2002) failed to modify the histamine response, and indicated the lack of involvement of this enzyme. In addition, SKF 91488 did not alter the basal tension and the raised tone (by phenylephrine) suggesting that, in contrast to human pulmonary artery (Ortiz et al., 1992), there was no basal release of histamine. Our results suggest that there is a "SKF 91488-sensitive component" in the pulmonary artery (of both strains of rat) upon the challenge of histamine. However, the source and location of histamine *N*-methyl-transferase remain unknown.

With clobenpropit (1 nM) (a highly potent histamine H_3 receptor antagonist with reported $pA_2 \sim 10$ and $pK_i = 9.3$) (Kathmann et al., 1993; Schlicker et al., 1996; Hill et al., 1997; Valentine et al., 1999), histamine-induced relaxation was significantly enhanced, and the concentration–response curves of histamine (with 1 nM clobenpropit present) constructed virtually overlap with that observed in SKF 91488-free condition (Fig. 1). Without SKF 91488, application of clobenpropit caused a trend of, but non-significant, increase in histamine-evoked relaxation in both strains of rat. We therefore hypothesise that a higher concentration of histamine resulted when SKF 91488 was included, and this condition favours the activation of clobenpropit-sensitive, histamine H_3/H_4 receptor and contraction occurred (see below).

In most immune-related tissues (Nguyen et al., 2001; Zhu et al., 2001) and in Chinese hamster ovary cells expressing porcine and human histamine H_4 receptor (Oda et al., 2002), clobenpropit possess moderate histamine H_4 receptor agonistic property. However, the histamine H_4 receptor demonstrates a strong sequence and pharmacological similarity to the histamine H_3 receptor (Liu et al., 2001a). The possible existence and the physiological role of histamine H_4 receptor in the pulmonary vasculature are not known. In guinea-pig (Satoh and Inui, 1984) and human (Ortiz et al., 1992) pulmonary artery with raised tone, histamine evoked a further contraction through the activation of histamine H_1 receptor of the vascular smooth muscle. In contrast, our results demonstrated that activation of endothelial histamine H_1 only leaded to relaxation, and there was no evidence of histamine H_1 receptor-mediated contraction in endothelium-denuded preparations.

After blocking histamine H_1 and H_2 receptors (using a mixture of diphenhydramine plus cimetidine), histamine elicited a clobenpropit-sensitive further contraction, with a greater magnitude observed in the WKY rats. Similarly, imetit (≥ 3 μM) (a histamine H_3/H_4 receptor agonist) (Garbarg et al., 1992; Liu et al., 2001a) elicited a clobenpropit-sensitive, further contraction of the pulmonary artery (in both the pre-contracted and under resting tone), with a greater contraction occurred in the WKY rats. Our results are consistent with observations in canine isolated pial vein (Monge et al., 1997) in which imetit caused contraction (but the observed contraction was thioperamide-insensitive) in preparations with raised tone (by endothelin-1) and under resting tension. However, the underlying reason(s) responsible for a greater magnitude of contraction by imetit in the WKY rats, compared to SHR, is not known but may be related to the difference in innervation of the pulmonary vasculatures (McLean et al., 1985).

In guinea-pig isolated pulmonary artery (Rizzo et al., 1995), the histamine H_3 receptor-mediated contraction (caused by electrical field stimulation) involved the neurogenic release of noradrenaline. In our study, imetit-evoked pulmonary artery contraction (observed in preparations under resting tension) was sensitive to prazosin pre-treatment, and probably involved the activation of phospholipase C/protein kinase C pathway with an influx of Ca^{2+} through the L-type Ca^{2+} channels upon the activation of clobenpropit-sensitive histamine H_3/H_4 receptor. We, however, did not measure the release of noradrenaline upon the administration of imetit and the source of the released neurotransmitter remains unknown. In addition, the clobenpropit (30 nM)/prazosin (1 μM)-resistant, contractile component of imetit observed in preparations under resting tension deserves a further investigation.

Comparing the magnitude of clobenpropit-sensitive, imetit-evoked contractile responses (both the further contraction in raised-tone preparations and contraction observed under resting tension) revealed that there was no clear maximum response (except in preparation of the WKY rat with raised tone) at the highest concentration (300 μM) tested. Hence, an estimation of pD_2 of imetit could not be determined. In addition, a greater magnitude of contraction caused by imetit was observed in preparations under resting tension. The discrepancy may be due to the tone (the pre-existing tone) of the pulmonary artery preparations before the administration of imetit.

In conclusion, our in vitro pharmacological study demonstrates the possible existence of multiple histamine receptor subtypes (H_1, H_2 and H_3/H_4 receptors) in the pulmonary artery of the WKY and SHR. Our novel result indicate that inhibition of enzymatic (histamine *N*-methyl-transferase) degradation of histamine resulted in an attenuation of histamine-mediated relaxation probably due to the activation of the histamine H_3/H_4 receptor and the release of catecholamine. A diminished histamine H_1 receptor-mediated relaxation (a NO/endothelium-dependent, L-NAME-

sensitive pathway) is responsible for the reduced histamine (and HTMT) response observed in SHR. The histamine H_2 and H_3/H_4 receptors-mediated responses are all endothelium-independent. The histamine H_2 receptor-mediated relaxation response (through adenylate cyclase activation) is indistinguishable between the WKY and SHR. Activation of clobenpropit-sensitive, histamine H_3/H_4 receptor by imetit (and histamine) elicits a greater contraction (probably due to the release of noradrenaline) of the pulmonary artery of the WKY rats.

Despite our results suggest that histamine H_3/H_4 receptors are probably existed in rat pulmonary artery and activation of these receptor yielded contraction, there is no evidence in the literature, to the best of our knowledge, confirming the existence of these histamine receptors in rat lungs. Hence, interpretation of our results should be exercised with caution.

Acknowledgements

The authors are indebted to staff of the Multidiscipline Laboratory (The Chinese University of Hong Kong, Hong Kong) for providing technical assistance, bench space and all the necessary equipment for this study. Financial assistance (W.H. Cheung) from the Department of Pharmacology (The Chinese University of Hong Kong, Hong Kong SAR, P.R. China) is also appreciated. A generous financial support from The Chou's Foundation Fund (Student Campus Work Scheme, CUHK 2001/2002) (W.H. Lau) is also acknowledged.

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