Mechanisms of Histamine-Induced Coronary Vasodilatation: H₁-Receptor-Mediated Release of Endothelium-Derived Nitric Oxide

Abstract
Although the content of histamine in myocardial tissue is high, its contribution to the regulation of coronary blood flow has not been clearly defined. The aim of the present study was to investigate whether or not nitric oxide (NO), an important modulator of coronary vascular tone, is involved in histamine-induced coronary vasomotion and to characterize which histaminergic receptor subtype mediates this process. Isolated, constant-flow-perfused guinea pig hearts were challenged with histamine, the H₁-receptor agonist pyridylethyamine (PYR) and the H₂-receptor agonist dimaprit (DIM). Apart from coronary perfusion pressure (CPP), left ventricular pressure (LVP) and the development of contractile force (dp/dt), the release of NO and cyclic GMP (cGMP) were continuously measured. Histamine and DIM induced concentration dependently a coronary vasodilatation with an almost 50% decrease in CPP paralleled by an enhancement of LVP and dp/dt by more than 50%. PYR selectively reduced CPP by 47% without affecting LVP and dp/dt. Histamine- and PYR-induced coronary vasodilatation were paralleled by a more-than-twofold increase in basal cGMP release from isolated hearts, whereas DIM exerted no effects on cGMP release. Oxyhemoglobin (4 μM), an effective scavenger of NO, shifted the concentration-response curve for histamine- and PYR-induced changes in CPP significantly to the right and in parallel inhibited the increase in cGMP release. Histamine and PYR rapidly (within 2 s) decreased CPP, while the onset of DIM-induced coronary vasodilatation followed changes in LVP with a lag period of 10 s. Histamine increased basal NO release concentration dependently by a maximum of 351 ± 21 pmol/min. Amounts of NO released were within the vasodilatory effective range of exogenously applied NO. These data suggest that the rapid onset of histamine-induced coronary vasodilatation is mediated via the activation of H₁ receptors with the subsequent release of endothelium-derived NO followed by a long-lasting and profound component which is due to the H₂-receptor-mediated increase in LVP. Amounts of NO released upon stimulation of endothelial H₁ receptors are sufficient to fully account for the increase in cGMP and the observed coronary vasodilatation.

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Introduction

Despite the fact that in most animal species the concentration of histamine in myocardial tissue is relatively high, its contribution to the regulation of coronary blood flow has not been clearly defined [1,2]. Current evidence suggests the involvement of histaminergic coronary H1 receptors mediating vasodilatation, but these findings are not consistent across different studies [3]. This may be due to the action of histamine on both H1 and H2 receptors on the vascular smooth muscle and the simultaneous activation of H1 receptors on the endothelium, which possibly mediate the release of prostacyclin [4] and an endothelium-derived relaxing factor (EDRF) [5]. The apparently conflicting data reported previously are explainable by considering that the magnitude of each of these three actions of histamine is likely to differ, depending on the size and site of the coronary artery and the distribution of the histaminergic subtype of the receptors involved [6]. Nevertheless, the mechanisms responsible for the varying arterial responses need further elucidation. Recent results suggest that an increase in coronary vasomotor tone caused by this biogenic amine may be involved in the pathogenesis of coronary artery disease and vasospasm of epicardial coronary arteries [5, 7-9]. These phenomena observed in various disease states may be attributed to an alteration in the distribution of histaminergic receptors on endothelial and vascular smooth muscle cells and/or by an altered metabolic pathway coupled to the respective receptor, such as an impaired capacity of endothelial cells to synthesize nitric oxide (NO).

It has been demonstrated, that EDRF released from endothelial cells under basal and stimulated conditions is identical with NO [10, 11]. Endothelium-derived NO synthesized from L-arginine [12] relaxes vascular smooth muscle, inhibits platelet aggregation and adhesion to the vascular wall [13] and is involved in the regulation of blood pressure [14]. More recent data suggest that NO is an important modulator of coronary vascular tone [15, 16]. Apart from functional studies with sometimes conflicting results using histaminergic agonists and antagonists, no data are available on the release of NO by histamine into the coronary circulation.

The aim of the present study, therefore, was to investigate whether NO is involved in histamine-induced coronary vasomotion. Furthermore, using specific H1 and H2 receptor agonists and antagonists, experiments were designed to characterize the coronary histaminergic receptor subtype that mediates the release of NO. Moreover, the question was addressed whether the extent of the histamine-induced release of NO is sufficient to explain the activation of coronary guanylate cyclase and the subsequent coronary vasodilatation.

Materials and Methods

Isolated Guinea Pig Hearts

The hearts from guinea pigs (280-320 g) were excised and perfused according to the Langendorff technique with a medium thermosterilated to 37 °C (pH 7.4), equilibrated with 100% O2 containing (in mM): NaCl 140, KCl 4.0, CaCl2 1.84, MgCl2 1.03, NaH2PO4 0.42, glucose 5.0, pyruvate 2.0, HEPES 10.0 and indomethacin 0.01. For measurement of NO, the medium was additionally supplemented with 4 μM oxyhemoglobin (HbO2). Hearts were electrically paced (285 bpm) and perfused at a constant flow (10 ml/min) by means of a roller pump. Left ventricular pressure (LVP) and the development of contractile force (dP/dtmax) were measured using a fluid-filled balloon inserted into the left ventricle through the mitral valve. Left ventricular diastolic pressure was adjusted to 0 mm Hg. Coronary perfusion pressure (CPP) was monitored by a pressure transducer connected via a needle with the aortic cannula. The hearts were accepted for further study when basal CPP was higher than 60 mm Hg.

All drugs were infused via the aortic cannula into the coronary circulation at a rate of 20-100 μl/min (Predicor pump, Infors AG, Basel, Switzerland) for 2 min. For kinetic measurements, sharp onset and offset of the application of test compounds was achieved by inserting/removing a cannula connected to a running infusion pump into/from a silicon-sealed tube inserted in the perfusion system.

Measurement of NO

NO release was continuously quantified in the effluent perfusate by a specific-difference spectrophotometric assay, which is based on the rapid oxidation of HbO2 to methemoglobin (MetHb) by NO [17]. Since HbO2 traps the entire amount of released NO in less than 100 ms [11] measurement of the extinction difference (401, 411 nm) in a flow-through cell with a double-beam, dual-wavelength spectrophotometer (UV3000 Shimadzu) permitted the continuous assay of released NO. Concentrations of NO were calculated from the extinction coefficient determined under the conditions used in this study to be 38 mM^-1 cm^-1. Histamine at concentrations between 10^-6 and 10^-4 M did not interfere with the hemoglobin assay. All substances used to challenge NO release at a concentration higher than 10^-4 M, i.e. histamine, pyridylethylamine (PYP) and dimaprit (DIM), displayed considerable interference with the difference spectrum of HbO2 and MetHb. Therefore PYR (10^-7-10^-5 M) and DIM (10^-7-10^-4 M) were excluded from experiments designed to quantify NO release from isolated guinea pig hearts.

Measurement of Cyclic GMP

To determine the release of cGMP from isolated guinea pig hearts, samples of the coronary effluent perfusate (10 ml) were passed over two SEP-PAK C18 cartridges (Waters, Eschwee, FRG) connected in line which had been conditioned with 2 ml isopropanol/water 40%/60% (vol/vol), followed by 2 ml distilled water and 2 ml phosphate buffer (pH 7.4, 0.01 M). Adsorbed cGMP was eluted with 2 ml of the propanol/water mixture and evaporated to dryness (Vor
**Fig. 1.** Concentration-response curves for the changes in CPP elicited by histamine, the H₂ receptor agonist DIM and the H₂ receptor agonist PYR infused into the coronary circulation of isolated, constant-flow-perfused guinea pig hearts. Experiments were performed in the absence (○) and presence (●) of 4 μM HbO₂. (*p < 0.05; n = 4–6).

**Experimental Protocols, Drugs and Statistics**

Different hearts were used to obtain a concentration-response curve for the change in CPP elicited by histamine, by the H₁ receptor agonist PYR and the H₂ receptor agonist DIM (each n = 4–6). The degree of coronary vasodilatation was expressed as changes in CPP percent from control. Additional experiments were performed with a new H₁ receptor agonist thiazoylthethylamine, yielding similar results as with PYR (data not shown). In a separate series of experiments, the concentration-response curves for the above-mentioned substances were measured in the presence of 4 μM HbO₂. Hearts were initially perfused with HbO₂-free medium. When hemodynamic parameters reached stable values, the perfusion medium was switched to a buffer solution supplemented with freshly prepared HbO₂ (4 μM). In all experiments, histamine and the H₁ and H₂ agonists were applied as 2-min infusions at intervals of 10 min. This protocol was chosen in order to avoid tachyphylaxis and to provide a clearer demonstration of the on/off effects for a more precise kinetic evaluation. A stimulation period of 2 min was sufficient to reach steady-state conditions with regard to the elicited hemodynamic responses. Specificity of the observed changes induced by DIM and PYR was verified by simultaneous infusion of the respective H₁ and H₂ receptor antagonists dimetinden and cimetidine. In separate hearts, the histamine-induced release of NO into the coronary effluent was determined (n = 5).

Histamine, indomethacin and bovine hemoglobin were obtained from Sigma, Deidesheim, FRG. DIM (Σ(N,N-dimethylamino)propyl)isothiourea dihydrochloride: SK&F 91449-A2), 2-pyridylethylamine dihydrochloride (SK&F 71432-A2), 2-thiazolylethylamine dihydrochloride (SK&F 71481-A2) and cimetidine (SK&F 92234) were a gift from SmithKline Beecham Pharmaceuticals.

**Table 1.** Hemodynamic effects of intracoronary infusion of histamine

<table>
<thead>
<tr>
<th>Histamine, M</th>
<th>ΔCPP, %</th>
<th>ΔLVP, %</th>
<th>Δdp/dtₘₚₓ, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>10⁻⁸</td>
<td>0</td>
<td>2 ± 1</td>
<td>2 ± 2</td>
</tr>
<tr>
<td>3 × 10⁻⁷</td>
<td>−2 ± 1</td>
<td>11 ± 2</td>
<td>16 ± 3</td>
</tr>
<tr>
<td>10⁻⁶</td>
<td>−17 ± 5</td>
<td>29 ± 4</td>
<td>47 ± 5</td>
</tr>
<tr>
<td>3 × 10⁻⁶</td>
<td>−33 ± 6</td>
<td>61 ± 3</td>
<td>84 ± 7</td>
</tr>
<tr>
<td>10⁻⁵</td>
<td>−47 ± 5</td>
<td>81 ± 6</td>
<td>175 ± 13</td>
</tr>
</tbody>
</table>

Changes in CPP, LVP and dp/dtₘₚₓ are expressed as percent of control: CPP 72 ± 5 mm Hg, LVP 41 ± 2 mm Hg and dp/dtₘₚₓ 575 ± 40 mm Hg·s⁻¹; mean ± SEM (n = 5–6).

Betchworth, Surrey, UK. Dimeinden maleate was from Zyma, Munich, FRG.

Differences between results obtained in different hearts were evaluated using Student's t test for unpaired data, and p < 0.05 was accepted to indicate statistical significance. Results are reported as mean values ± SEM.

**Results**

Basal hemodynamic parameters in constant-flow-perfused hearts were: CPP 72 ± 5 mm Hg, LVP 41 ± 2 mm Hg and dp/dtₘₚₓ 575 ± 40 mm Hg·s⁻¹. Histamine (10⁻⁸–10⁻⁶) concentration-dependently induced a coronary vasodilatation paralleled by a significant increase in LVP and dp/dtₘₚₓ (table 1). The H₂ agonist DIM decreased CPP to a similar extent as histamine (fig. 1). This effect
was accompanied by a considerable increase in LVP up to $83 \pm 5$ mm Hg and in $\frac{dp}{dt}_{\text{max}}$ to $2,053 \pm 203$ mm Hg/s. In contrast, PYR, a specific $H_1$ antagonist, elicited a marked coronary vasodilatation (fig. 1) without significantly changing LVP or $\frac{dp}{dt}_{\text{max}}$. The simultaneous infusion of 4 $\mu$M HbO$_2$, which is an effective scavenger of NO, resulted in a significant shift of the concentration-response curve for histamine and PYR to the right (fig. 1) and only a nonsignificant leftward shift in the case of DIM.

Comparing the kinetics of the hemodynamic changes following stimulation with histamine and the $H_1$ and $H_2$ agonists revealed the following features (fig. 2): the $H_2$ agonist DIM rapidly increased LVP, whereas the onset of coronary vasodilatation occurred after a considerable latent period of almost 15 s presumably as a consequence of the marked enhancement of LVP. In contrast, the $H_1$-induced decrease of CPP occurred much earlier without accompanying changes in LVP. Histamine-induced coronary vasodilatation revealed a biphasic pattern comprising a rapid, phasic component and a slower, long-lasting component (see original tracing, fig. 2). Similarly to DIM coronary vasodilatation preceded the increase in LVP by 10 s (fig. 2), indicating that the pattern of hemodynamic changes elicited by histamine represents the sum of the net effect observed upon selective stimulation of the $H_1$ and $H_2$ receptor subtypes alone.

It is well-documented that coronary vasodilatation elicited by endothelium-derived NO is mediated via an activation of guanylate cyclase. Unstimulated, isolated, guinea pig hearts continuously release cGMP as a consequence of basal NO release into the coronary effluent perfusate [16]. Histamine and PYR in a maximally vasoactive concentration increased basal cGMP release (372 ± 41 fmol/min) severalfold (fig. 3). As observed with the changes in CPP (fig. 1), 4 $\mu$M HbO$_2$ significantly decreased cGMP release into coronary effluent perfusate under basal conditions and upon stimulation with histamine and PYR. This effect is presumably mediated via an inactivation of NO by HbO$_2$. In contrast, the $H_2$ agonist DIM did not exert any effect on basal cGMP release from isolated guinea pig hearts (fig. 3).

To evaluate the specificity of the hemodynamic changes observed with DIM and PYR in a separate series of control experiments half maximally effective doses of DIM and PYR were infused into hearts during the simultaneous infusion of the respective antagonists. The $H_1$ receptor antagonist dimetindene completely abolished the PYR-induced coronary vasodilatation (table 2). $H_2$-mediated changes of CPP and LVP were effectively prevented by simultaneous infusion of cimetidine (5 $\times$ 10$^{-5}$ M). Correspondingly, cimetidine did not affect PYR-induced coronary vasodilatation. The $H_1$ receptor antagonist dimetindene, however, appears to exert some unspe-

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Fig. 2. Kinetics of the hemodynamic changes elicited by intracoronary infusion of histamine (HIS; 10$^{-6}$ M), DIM (10$^{-5}$ M) and PYR (3 $\times$ 10$^{-5}$ M). In the upper part, representative tracings of the changes in CPP are depicted. The arrow indicates start of intracoronary infusion of the respective compound. The lower part describes the kinetics of the changes in CPP after start of infusion (lag period), the time interval to reach the maximal response and the lag period for the onset of increases in LVP after start of infusion (n = 6).
specific effects on $H_2$ receptors, as indicated by the slight reduction of the DIM-induced coronary vasodilatation.

The basal release of NO into the coronary circulation of isolated guinea pig hearts amounted to $216 \pm 27$ pmol/min. Histamine, in the same concentration range used as before and in a concentration-dependent manner, increased the release of NO by $351 \pm 21$ pmol/min at a maximally effective concentration. Comparing the extent of histamine-induced NO release and changes in CPP with the dose-response curve for exogenously applied NO previously determined under the same conditions (fig. 4, inset) revealed that the amounts of released NO fell within the vasoactive range, and can thus explain the observed coronary vasodilatation.

**Discussion**

This is the first demonstration of the release of NO into the coronary circulation of isolated hearts by histamine. In the present model, histamine-induced coronary vasodilatation comprised two components: an $H_1$-receptor-mediated rapid decrease of CPP and a slower $H_2$-receptor-mediated component of vasodilatation, which most likely is explained by the enhanced oxygen demand following an increase in LVP elicited by cardiac $H_2$ receptors. The amounts of released NO mediated by an activation of $H_1$ receptors are sufficient to account for the increase in cGMP release and are within the vasodilatory effective range for intracoronary applied authentic NO, indicating that the rapid phase of the histamine-induced coronary vasodilatation can be solely explained by an activation of $H_2$ receptors with the subsequent increase in endothelial NO release.
Recent studies revealed that NO is an important modulator of coronary vascular tone [16]. Basal release of NO from cultured macrovascular and coronary microvascular endothelial cells can be enhanced severalfold by the classical stimuli of EDRF such as bradykinin, serotonin and acetycholines as well as by increases in flow, while it is not affected by changes in partial oxygen pressure or perfusion pressure [18–20]. However, up to now, no data were available concerning the role of NO in histamine-induced coronary vasomotion.

Histamine is present in the walls of arteries and veins and is also synthesized by endothelial cells [1]. Furthermore, histamine can be rapidly released from granulocytes and activated macrophages in amounts sufficient to induce a profound vasodilatation in peripheral resistance arteries [1, 21]. The release of vasoactive amounts of cardiac histamine has been reported to be mediated by exogenous liberators such as dextran, polymeric amines and by endogenous liberators acting via the production of free radicals, such as ATP, adenosine and catecholamines [21]. The present study describes for the first time a histamine-mediated NO release from isolated hearts. HbO2, which effectively converts the free NO radical into vasoactive nitrate, shifted the concentration-response curve for histamine and the H1-agonist PYR significantly to the right. Consistent with this finding is the observation that the increase in cGMP release from isolated hearts elicited by these two compounds was significantly reduced by HbO2. In contrast, the H2 agonist DIM neither affected basal cGMP release nor was its coronary vasodilatory effect reduced by HbO2.

The present study extends previously reported results on histaminergic subtypes in the coronary circulation and additionally characterizes the kinetics of the hemodynamic changes induced. Using specific H1 and H2 receptor antagonists in a closed-chest dog model, Miller and Bove [3] have demonstrated an H1-receptor-mediated vasodilatation and an H2-receptor-mediated vasodilatation of large epicardial arteries, while distal resistance vessels revealed H1- and H2-receptor-mediated vasodilatation. Similar conclusions were derived from studies performed with isolated human coronary artery rings [6]. In addition Christensen et al. [2] have reported that the stimulation of canine coronary H1 receptors preferentially distributes flow to the subendocardium, whereas H2 receptors mediate vasodilatation in epicardium as well as subendocardium [2]. In the present study, H1-mediated coronary vasodilatation occurred rapidly and represented the initial phasic part of the histamine-induced vasodilatation, without affecting LVP. In contrast, specific stimulation of cardiac H2 receptors first resulted in an increase in LVP followed by a decrease in CPP with a considerable delay. This tonic coronary vasodilatation may either be an effect of an enhanced metabolic demand due to the augmented LVP or be the result of a delayed histaminergic vasodilatory action on vascular smooth muscle. These data are in line with the results by Broadley [22] obtained in isolated guinea pig hearts perfused at low flow. An initial H1-receptor-mediated vasodilatation was followed by a second vasoconstriction and then by profound and prolonged vasodilatation probably associated with the increased metabolic activity of the heart.

Moreover, a comparison of the amounts of NO released upon challenge of the hearts by physiological concentrations of histamine with the concentration-response curve for authentic NO derived under the same conditions revealed that the measured NO concentrations are sufficient to fully account for the observed decrease of CPP. Although, due to interference with the hemoglobin assay, no information on the extent of NO formation could be obtained for PYR, the increase in cGMP release induced by this compound strongly suggests that histamine-induced NO release is mediated mainly via a stimulation of H1 receptors.

Increased vasoconstrictor reactivity to histamine has been demonstrated in arteriosclerotic human epicardial arteries [7, 23]. In addition, current data suggest the involvement of histamine in epicardial coronary artery spasm [8, 9, 23]. Furthermore, elevated plasma levels of histamine have been suspected to play a role in the pathophysiology of myocardial infarction [24]. Interestingly, Matsumoto and colleagues [25] have recently reported that selective stimulation of H1 receptors in cultured vascular smooth muscle cells significantly increased cytosolic calcium, which, in turn, under in vivo conditions, may result in vascular hypercontractility [25]. In view of these data
and the results from the present study, a disturbed endothelial NO release and/or impaired balance between the number of H$_2$ receptors on endothelial and vascular smooth muscle cells may trigger coronary vasoconstriction. Furthermore, an altered histaminergic NO release may affect the balance between coronary H$_2$- and H$_2$- receptor-mediated effects. In isolated human epicardial arteries, Godfraind and Miller [26] have shown that both receptors independently of each other mediate coronary vasodilatation. Especially under pathophysiological circumstances which are associated with an elevated plasma level of histamine, these changes may become an important determinant in the regulation of coronary blood flow. Therefore, the effects of histamine on coronary vascular tone in various disease states, and the potential beneficial effects of histamine-blocking agents in particular, will become a relevant issue in the near future.

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