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HISTAMINE H_1-RECEPTOR-MEDIATED CYCLIC GMP PRODUCTION IN GUINEA-PIG LUNG TISSUE IS AN L-ARGININE-DEPENDENT PROCESS

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Abstract—Histamine produces a rapid and massive increase of the c-GMP level of guinea-pig lung tissue. The EC_{50} value for this *in vitro* response is found to be 27 μM and the c-GMP level is maximally 9-fold elevated by 100 μM histamine. The response is stereoselectively inhibited by the enantiomers of chlorpheniramine, indicating H_1-receptor involvement. Preincubation of lung tissue with 200 μM NCDC, a phospholipase C inhibitor, reduces the histamine (100 μM) responses to 16 ± 3% (N = 6) of the control c-GMP production. Inhibition of protein kinase C by 50 μM H-7 does not significantly attenuate the H_1-receptor response, whereas omittance of extracellular Ca^{2+} results in almost complete inhibition of the c-GMP production. The histamine-induced c-GMP response is inhibited by hemoglobin, methylene blue and the antioxidants butylated hydroxytoluene and nordihydroguaretic acid, indicating the involvement of a nitric oxide-dependent activation of soluble guanylate cyclase. This suggestion is supported by the concentration-dependent inhibition of the c-GMP production by N^G-monomethyl-L-arginine (NMA). At a concentration of 20 μM NMA the histamine (100 μM) response is inhibited to 34 ± 8% (N = 6) of the control response. This inhibition is reversed to 127 ± 20% (N = 6) by the exogenous addition of 1 mM L-arginine. These findings show that after an initial H_1-receptor-mediated, phospholipase C-dependent, Ca^{2+}-mobilization the enzymatic conversion of L-arginine to nitric oxide is stimulated. This nitric oxide production is finally responsible for the activation of soluble guanylate cyclase, leading to the production of c-GMP.

In allergic subjects the exposure to specific allergens causes an IgE-dependent degranulation of tissue mast cells. Such a degranulation results in the release of various biologically active agents, which are responsible for most of the allergic symptoms. One of the prominent compounds released from mast cells in allergic conditions is histamine [1]. Release of this biogenic amine has been shown to be involved in the development of various asthmatic symptoms. Interaction of histamine with the H_1-receptor in the airways is responsible for smooth muscle contraction [1, 2], increased pulmonary vascular resistance [3] and increased production of prostaglandins [4]. Histamine seems also to be responsible for the production of c-GMP in human lung tissue [4–6]. The exact role, localization and mechanism of this response is however not yet elucidated. Kaliner [7, 8] suggested that c-GMP might modulate the immunologically-induced release of mediators from mast cells through effects on the microtubular assembly. However, such a mechanism has been questioned to play any role in the anaphylactic histamine release from rat mast cells [9]. Employing monoclonal antibodies directed at c-GMP Sertl *et al.* [6] observed an absence of muscular staining in guinea-pig lung after histamine application. This indicates that the c-GMP elevation is probably not involved in the direct regulation of smooth muscle contraction/relaxation mechanism. Yet, increased staining was considerable in macrophages, endothelium and epithelium [6].

Endothelium-dependent increases in tissue c-GMP content is usually noticed in vascular preparations and associated with vascular smooth muscle relaxation [10–14]. In some vascular preparations these responses are also observed after H_1-receptor stimulation [10, 15, 16]. The c-GMP production in vascular preparations is now attributed to the formation of an endothelium-derived relaxing factor, which has been identified as nitric oxide [12–14]. This nitric oxide has been shown to be synthesized enzymatically from L-arginine in e.g. porcine aortic endothelial cells [17] and is able to activate the soluble guanylate cyclase [18]. Moreover, the production of nitric oxide has also been shown in macrophages and is probably involved in the cytotoxic effects of these cells [19, 20].

These findings led us to examine the histamine-induced c-GMP production in guinea-pig lung in more detail. Using specific agents we investigated the signal transfer pathway of the c-GMP formation after histamine H_1-receptor activation.

MATERIALS AND METHODS

Chemicals. Histamine dihydrochloride, phorbol-12,13-dibutyrate, 2-nitro-4-carboxyphenyl-*N*,*N*-diphenylcarbamate (NCDC), 1-(5-isoquinolinylsulfonyl)-2-methylpiperazine (H-7), methylene blue, L-arginine dihydrochloride, hemoglobin, nordihydroguaretic acid (NDGA) and butylated hydroxytoluene (BHT) were purchased from the Sigma Chemical Co. (St Louis, MO, U.S.A.). N^G-monomethyl-L-arginine was obtained from Calbiochem (La Jolla, U.S.A.). The stereoisomers of

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chlorpheniramine (maleate salts) were a generous gift from Dr A. J. Beld (Catholic University, Nijmegen). Oxyhemoglobin was prepared from methemoglobin as described previously [11].

c-GMP measurements. Male guinea-pigs (300–350 g) were killed by a blow on the head. The thorax was opened and the lungs were perfused via the pulmonary artery with oxygenated Krebs buffer (117.5 mM NaCl, 5.6 mM KCl, 1.18 mM $MgSO_4$, 2.5 mM $CaCl_2$, 1.28 mM NaH_2PO_4, 25 mM $NaHCO_3$ and 5.5 mM glucose). The parenchymal lung tissue was dissected into replicate fragments (1 × 5 mm) and preincubated for 30 min at 37° in 10 mL Krebs buffer. Subsequently lung tissue was preincubated with various antagonists or inhibitors for 15 min, whereafter histamine was added for the indicated time. Some experiments were performed in Ca^{2+}-free Krebs buffer. In these instances lung tissue was washed with the Ca^{2+}-free medium for 30 min before a stimulation with histamine was performed. Incubations were stopped by removal of the tissue from the medium and rapid freezing of the tissue in liquid nitrogen. Thereafter the frozen tissue was homogenized (15 sec maximal speed) in sodium acetate buffer (pH 5.8) using a Polytron homogenizer. Aliquots were deproteinized using ethanol and centrifuged for 10 min at 3000 *g*. The supernatant was evaporated under vacuum at 45° and the residue was subsequently resuspended in 50 mM sodium acetate buffer. The c-GMP content was determined using a c-GMP radioimmunoassay kit (Amersham, Buckinghamshire, U.K.).

Protein concentration was assayed by the Biorad reagent [21], using bovine serum albumin as standard. The amount of c-GMP was corrected for the amount of protein and expressed as the percentage of basal levels.

Statistics. Data shown are mean ± SE of at least three experiments. Significant differences were determined using the Student's *t*-test. A $P < 0.05$ was regarded as indicating a significant difference.

RESULTS

Exposure of guinea-pig lung tissue to 100 μM histamine leads to a rapid increase in the c-GMP content. Maximal c-GMP production is already apparent after 2 min of stimulation (Fig. 1A). At that time the c-GMP levels reach 870 ± 70% of the basal c-GMP content. Thereafter the c-GMP levels rapidly decline and reach control levels again after approximately 20 min (Fig. 1A). At a 2 min stimulation period histamine concentration dependently stimulates the production of c-GMP in lung tissue (Fig. 1B); histamine increases the c-GMP production with an EC_{50} value of 27 μM. Significant c-GMP elevation is noticed at 10 μM histamine, whereas maximal responses are observed at 100–300 μM histamine. The histamine-induced c-GMP production is stereoselectively inhibited by the two stereoisomers of chlorpheniramine (Fig. 2). Whereas both compounds do not affect basal c-GMP levels, the histamine-mediated c-GMP elevation is selectively modulated by the D-enantiomer. At a concentration of 10 nM D-chlorpheniramine the response to 100 μM histamine is inhibited to 25% of the control response. The L-enantiomer does not significantly reduce the histamine-induced c-GMP production at that concentration (Fig. 2).

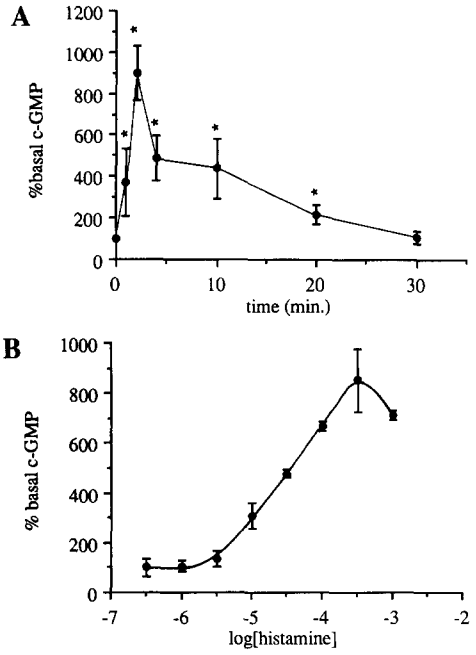

Fig. 1. Characteristics of the histamine-induced c-GMP production in guinea-pig lung tissue. Lung fragments were stimulated with 100 μM histamine for various times (A) or for 2 min with several concentrations of histamine (B). Data shown are mean ± SE (N = 6). The asterisk indicates a significant difference ($P < 0.05$) compared to control.

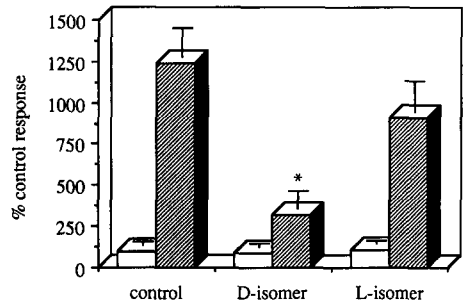

Fig. 2. Stereoselective antagonism of the histamine-mediated c-GMP production in guinea-pig lung tissue. Lung fragments were incubated for 15 min with 10 nM of the D- and L-enantiomers of chlorpheniramine. Thereafter 100 μM histamine (hatched columns) or vehicle (open columns) was added for 2 min. Data shown are mean ± SE (N = 4). The asterisk indicates a significant difference ($P < 0.05$) compared to control.

The c-GMP production induced by 100 μM histamine is potently inhibited by the phospholipase C inhibitor NCDC. At a concentration of 200 μM NCDC the c-GMP accumulation in lung tissue in response to histamine is reduced by more than 80% (Table 1). Yet, this concentration of NCDC does

Table 1. Stimulation of c-GMP accumulation in guinea-pig lung by 100 μM histamine (2 min) in the absence of extracellular Ca^{2+} or after a preincubation with the phospholipase C inhibitor NCDC (200 μM) or the protein kinase C inhibitor H-7 (50 μM)

Treatment	% Control response	N
15 min 200 μM NCDC	16 ± 3*	6
30 min Ca^{2+}-free Krebs	8 ± 4*	6
30 min Ca^{2+}-free Krebs + 2 mM EGTA	10 ± 4*	5
15 min 50 μM H-7	85 ± 15	3

Data shown are the mean ± SE.
* $P < 0.05$.

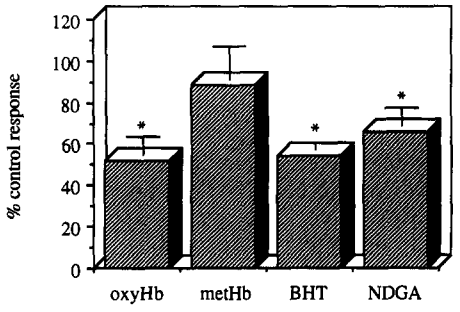

Fig. 3. Inhibitory effects of 100 μM oxyhemoglobin (oxyHb), 100 μM methemoglobin (metHb), 50 μM BHT and 50 μM NDGA on the H_1-receptor-mediated c-GMP production in guinea-pig lung. Lung fragments were incubated for 15 min with the indicated agents, whereafter 100 μM histamine was added for 2 min. Data shown are mean ± SE (N = 6). The asterisk indicates a significant difference ($P < 0.05$) compared to control.

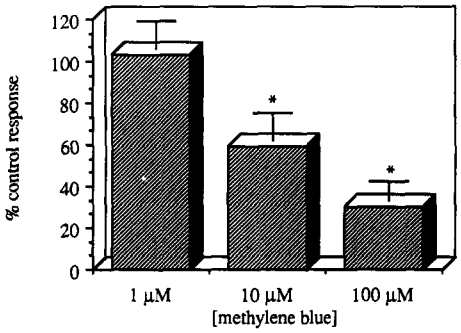

Fig. 4. Concentration-dependent reduction of the H_1-receptor-mediated c-GMP production in guinea-pig lung by methylene blue. Lung fragments were incubated for 15 min with the indicated concentrations methylene blue, whereafter 100 μM histamine was added for 2 min. Data shown are mean ± SE (n = 5 to 6). The asterisk indicates a significant difference ($P < 0.05$) compared to control.

not affect the c-GMP elevation in response to nitroprusside (data not shown). The histamine-stimulated c-GMP levels are also highly dependent upon the presence of extracellular Ca^{2+}. When Ca^{2+} is omitted from the Krebs buffer, 100 μM histamine hardly elevates tissue c-GMP levels (Table 1). This effect is noticeable without the extra addition of EGTA (Table 1). Chelation of possible additional calcium does not inhibit the histamine response further. Inhibition of protein kinase C (PKC) does not appear to modulate the histamine-induced c-GMP response. Preincubation with the PKC inhibitor H-7 (50 μM) for 15 min does not significantly reduce the c-GMP accumulation (Table 1). Exposure of lung tissue to the phorbolester phorbol-12,13-dibutyrate (1 μM) for 2–60 min does not modify the c-GMP levels (data not shown).

Responses to 100 μM histamine are markedly reduced by NDGA and BHT. Preincubation of lung tissue with 50 μM BHT or 50 μM NDGA for 15 min results in an inhibition of the histamine-induced c-GMP accumulation to approximately 60% of the control response (Fig. 3). Using a similar preincubation 100 μM oxyhemoglobin also appears to attenuate the histamine response. In contrast, the oxidized hemoglobin, methemoglobin, does not significantly affect the c-GMP accumulation (Fig. 3).

Using the guanylate cyclase inhibitor methylene blue, it is also possible to block the effects of histamine application on c-GMP levels of lung tissue. Preincubation with methylene blue for 15 min leads to a concentration-dependent inhibition of the c-GMP production (Fig. 4). At a concentration of 10 μM methylene blue the response to 100 μM histamine is inhibited for 41 ± 13% (N = 6), whereas 100 μM methylene blue can reduce the histamine response further.

Finally the possible contribution of an L-arginine-dependent pathway was evaluated. The increase in c-GMP content after stimulation with 100 μM histamine can be potently inhibited by N^G-monomethyl-L-arginine (NMA, Fig. 5). Whereas the response to histamine is hardly affected at a concentration of 2 μM, inhibition to 34 ± 8% (N = 6) of the control response is observed after preincubation with 20 μM NMA. Higher concentrations of NMA almost completely abolish the c-GMP response to histamine (Fig. 5). The inhibition by NMA can be reversed by the simultaneous addition of L-arginine (Fig. 5). Moreover, preincubation of lung tissue for 15 min with 1 mM L-arginine significantly enhanced the histamine-induced c-GMP production after stimulation with 100 μM histamine for 2 min under control conditions (Fig. 5). Yet,

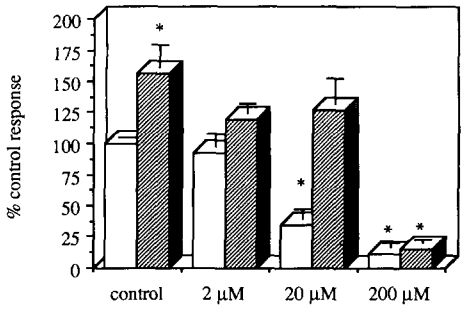

Fig. 5. Concentration-dependent reduction of the H_1-receptor-mediated c-GMP production in guinea-pig lung by NMA in the absence (open columns) or presence of 1 mM L-arginine (hatched columns). Lung fragments were incubated for 15 min with the indicated concentrations NMA, whereafter 100 μM histamine was added for 2 min. Data shown are mean ± SE (N = 3–6). The asterisk indicates a significant difference ($P < 0.05$) compared to control.

stimulation of the c-GMP production by 100 μM histamine for 20 min is not altered by the addition of L-arginine. In the presence of 1 mM L-arginine histamine-induced c-GMP production after 20 min of incubation is 10 ± 2% (N = 3) of the peak response after 2 min of stimulation. This is not significantly different from the response in the absence of extracellular L-arginine. In this case the response at 20 min is 15 ± 5% of the peak response after 2 min (Fig. 1).

DISCUSSION

To explain the endothelium-dependent vasodilator action of acetylcholine in isolated rabbit arterial strips, only one decade ago Furchgott and Zawadski [22] described a new vasoactive agent. An "endothelium-derived relaxing factor" (EDRF) was suggested to be released from the endothelial lining of the vasculature [22]. It took several years of extensive research to reach the final conclusion that nitric oxide [18, 22, 23] was identical to the EDRF, first described by Furchgott and Zawadski [22].

Nowadays, it has become clear that several hormones, including histamine, can stimulate the production of nitric oxide from the endothelium. Histamine H_1-receptor-mediated production of EDRF has, for example, been documented in a variety of vascular preparations [10, 15, 16]. Moreover, mass spectroscopy studies, using porcine aortic endothelial cells in culture and ^{15}N-labelled L-arginine, led Palmer *et al.* [17] to conclude that L-arginine was the precursor for an enzymatic nitric oxide synthesis in endothelial cells. Finally, it was noticed that the production of EDRF/nitric oxide was not confined to the vascular endothelium [12, 19]. Nitric oxide has, for example, been shown to be involved in the cellular regulation of macrophages [19, 20], hepatocytes [24], mast cells [25], platelets [25] and several central and peripheral neuronal tissues [26–28]. Nowadays, it seems that the production of nitric oxide might be a new and widespread second messenger system.

The histamine H_1-receptor stimulates the production of EDRF in several vascular preparations [10, 15, 16]. The histamine-induced production of c-GMP is also not confined to the vasculature. Histamine-induced c-GMP production has, for example, been reported in isolated rabbit cardiac preparations [29], N1E-115 neuroblastoma cells [30], guinea-pig tracheal and bronchial preparations [2, 31] and human and guinea-pig lung tissue [5, 6]. Nevertheless, only limited information is available about the exact signal transduction pathway, responsible for the production of c-GMP after H_1-receptor activation, although a dependency on calcium is apparent [30].

In the present study we tried to characterize the signal transfer in guinea-pig lung tissue, leading to c-GMP production after H_1-receptor stimulation. Histamine increases the c-GMP content of guinea-pig lung tissue very rapidly. Within 2 min of exposure to 100 μM histamine, an increase in the c-GMP levels of approximately 900% is noticed. Thereafter the c-GMP levels drop quickly and reach control levels again after approximately 20 min. These responses are apparent in micromolar concentrations of histamine and are in good correspondence with previous findings of Sertl *et al.* [6]. The histamine-induced responses are mediated by an interaction with the H_1-receptor, since the c-GMP response is stereoselectively antagonized by the enantiomers of the H_1-antagonist chlorpheniramine. Previously the two chlorpheniramine stereoisomers proved to be highly potent H_1-antagonists with the H_1-antagonistic activity mainly residing in the D-isomer [32]. This pair of enantiomers proved therefore to be highly useful tools for H_1-receptor identification [32, 33].

The H_1-receptor is coupled to the phosphatidylinositol turnover in a variety of tissues [32] and in several airway preparations histamine-induced inositolphosphate production has been shown [34]. Also the H_1-receptor-mediated c-GMP production in guinea-pig lung appears to be dependent upon a phospholipase C-catalysed hydrolysis of phosphatidylinositol-4,5-bisphosphate. After application of 200 μM of the phospholipase C inhibitor NCDC the H_1-receptor-mediated c-GMP response is highly reduced, whereas the nitroprusside-induced c-GMP production is hardly affected. Previously it was shown that NCDC was able to inhibit phospholipase C activity in platelets [35]. Moreover, H_1-receptor mediated inositolphosphate production in guinea-pig left atria was reported to be markedly inhibited after NCDC administration [36]. De Nucci *et al.* [37] showed a similar dependency for the bradykinin-mediated endothelium-dependent relaxation of bovine aorta. The c-GMP elevation does not appear to be dependent upon PKC activation. The protein kinase C inhibitor H-7 does hardly attenuate histamine-induced c-GMP production, whereas the protein kinase C-activating phorbolester phorbol-12,13-dibutyrate does not affect the c-GMP level of guinea-pig lung tissue at all.

Besides protein kinase C activation, stimulation of receptors, coupled to the phosphatidylinositol turnover, also results in an elevation of the

cytoplasmatic Ca^{2+} concentration [32]. In the present study the c-GMP production appears to be highly dependent upon the presence of extracellular Ca^{2+}. Even without the extra addition of the Ca^{2+}-chelator EGTA the H_1-receptor-mediated c-GMP production is reduced to approximately 10% in a Ca^{2+}-free buffer. The influx of extracellular Ca^{2+} is therefore responsible for the observed c-GMP response. This is in contrast to the recent reported findings with N1E-115 neuroblastoma cells [30]. In the latter study H_1-receptor-mediated c-GMP production was suggested to be mainly due to IP_3-induced Ca^{2+}-release from intracellular stores [30]. This discrepancy might indicate cell type-dependent regulation of the Ca^{2+}-mobilization after H_1-receptor stimulation. The dependency of the c-GMP response upon phospholipase C activation and Ca^{2+}-influx is in good correspondence with the suggested role of the IP_3-induced Ca^{2+}-release in the regulation of Ca^{2+}-influx [38].

The question how the initial mobilization of Ca^{2+} is "translated" into the final c-GMP production is highly intriguing. Previously it was found that due to their antioxidant capacity BHT and NDGA could inhibit the endothelium-dependent relaxation of rabbit aorta [39, 40]. As shown in the present study both compounds are also able to reduce the histamine-mediated c-GMP response in guinea-pig lung tissue. Moreover, oxyhemoglobin, which in contrast to methemoglobin can bind nitric oxide [41], has been widely used for the scavenging of nitric oxide [11, 26–28] and also shows an inhibitory effect on the H_1-receptor response in guinea-pig lung. These experiments with BHT, NDGA and hemoglobin already point to the possible involvement of nitric oxide, which in turn should activate soluble guanylate cyclase. Studies with the guanylate cyclase inhibitor methylene blue are in good agreement with such a suggestion. Histamine-induced c-GMP responses in guinea-pig lung are potently inhibited by methylene blue.

More definite evidence is found in the experiments with the N-methylated L-arginine analog NMA. This compound has been reported to be a competitive inhibitor of the enzymatic conversion of L-arginine to nitric oxide and citrulline [42]. In guinea-pig lung the H_1-receptor-mediated responses are concentration-dependently inhibited by NMA. Moreover, this inhibition can be reversed by the exogenous addition of L-arginine, indicating competitive inhibition. In the presence of exogenous added L-arginine the histamine-induced c-GMP production is markedly enhanced. This suggests that under normal conditions the L-arginine supply is not sufficient for a maximal enzymatic activity to produce nitric oxide. These findings might also explain the rapid decrease in c-GMP levels after a few minutes of histamine exposure. Yet, inclusion of 1 mM L-arginine in the incubation medium does not prevent the decrease of the c-GMP production after 20 min of incubation. Other mechanisms, like receptor desensitization might be additional explanations for this finding. Previously we showed that histamine H_1-receptor-mediated contractions of guinea-pig lung strips and ileal smooth muscle are indeed subjected to a rapid desensitization [43, 44].

The data of this study clearly show that the H_1-receptor-mediated c-GMP response is dependent upon the production of nitric oxide from its precursor L-arginine. This nitric oxide production can be considered as the direct linkage between the initial Ca^{2+}-mobilization and the final c-GMP response. Mayer and Böhme [45] recently reported a Ca^{2+}-dependent formation of an L-arginine-derived activator of soluble guanylate cyclase in bovine lung. Moreover, Ca^{2+}-dependent nitric oxide synthesis in endothelial cells and rat cerebellum was suggested to be mediated by calmodulin [46, 47]. The enzymatic conversion of L-arginine to nitric oxide is therefore under direct control of the cytoplasmatic Ca^{2+} concentration in these tissues and such a mechanism is also a good explanation for the H_1-receptor response in guinea-pig lung. The Ca^{2+}-dependency of the nitric oxide synthesis is, however, not uniformly distributed. A Ca^{2+}-independent nitric oxide generation has been reported for rat hepatocytes [24] and murine macrophages [46]. Although previously Sertl *et al.* [6] reported histamine-mediated c-GMP production in alveolar macrophages of guinea-pig lung, using immunohistochemical techniques, the results of the present study argue against the conclusion that macrophages might possess H_1-receptors, coupled to c-GMP production. This suggestion is based upon the observation that the histamine-mediated c-GMP production is highly dependent upon the presence of extracellular Ca^{2+}, indicating that histamine activates cells, which possess a Ca^{2+}-dependent enzymatic pathway for the generation of nitric oxide. Since macrophages are reported to lack such a Ca^{2+}-dependency [46], an indirect modulation of the c-GMP levels through nitric oxide diffusion from its generator cell is more likely [12]. Since Sertl *et al.* [6] previously reported that histamine does not raise the c-GMP levels in smooth muscle cells of guinea-pig lung tissue, the nitric oxide is probably generated by vascular endothelium, which is also present in the parenchymal tissue. In conclusion, the results of this study show a rapid and massive increase of the c-GMP levels of guinea-pig lung after H_1-receptor stimulation. This c-GMP production is highly dependent upon the phospholipase C-mediated Ca^{2+} mobilization, which results in the L-arginine-dependent production of nitric oxide, probably via a calmodulin-dependent enzymatic factor. This nitric oxide is finally responsible for the final c-GMP response through interaction with the soluble guanylate cyclase.

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