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Effects of nitric oxide and hydrogen peroxide on histamine release from RBL-2H3 cells

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Abstract

We have studied the effect of nitric oxide (NO) and hydrogen peroxide (H_2O_2), two reactive oxygen species (ROS) on histamine release (HR) from RBL-2H3 cells, a rat mucosal-type mast cell line. Marked HR was elicited by antigen (DNP-HSA), calcium ionophore A23187, sodium fluoride or phospholipase C, but not with compound 48/80 or 1,2-dioctanoyl-*sn*-glycerol. The NO-synthase substrate L-arginine and its inactive enantiomer (D-arginine), each on its own, induced a small but significant increase in HR above the basal level. However, the NO-donors (sodium nitroprusside or $NaNO_3$) or the NO-synthase inducer lipopolysaccharide did not induce HR. Moreover, methylene blue (MB), which inhibits guanylate cyclase and N^{ω}-nitro-L-arginine (L-NA), an inhibitor of NO synthase, were also without effect on either the basal HR or the L-arginine-induced HR. HR induced by A23187, DNP-HSA, sodium fluoride or phospholipase C was markedly reduced by MB, but mildly by L-NA (both at 1–100 μM). H_2O_2 (0.01–1.0 mM) on its own did not induce HR, but it had a potent inhibitory effect on DNP-HSA- or A23187-induced HR, which was not reversed by L-NA (1–100 μM). Taken together, it seems that neither the stimulatory nor the inhibitory effects of the NO-related compounds on HR can be attributed to NO, but rather to other mechanisms. The inhibition of HR by H_2O_2 also does not involve NO and suggests a negative feedback regulatory role for the peroxide in the allergic inflammation.

Keywords: RBL-2H3 cells; Mast cells; Histamine release; Nitric oxide; Hydrogen peroxide

1. Introduction

Nitric oxide (NO), biologically generated from L-arginine, is a simple and diffusable molecule of free-radical nature. By acting as an intracellular and intercellular messenger, NO is involved in the modulation of various cellular functions such as inhibition of platelet aggregation, relaxation of vascular smooth muscle, activation of K^+-channels, modulation of immune response, and others [1–4]. H_2O_2 stimulated NO synthase activity in cultured endothelial cells [5], and at low concentrations, it strongly enhanced the inhibitory effect of NO on platelet aggregation [6]. H_2O_2-induced leukocyte rolling (acute inflammatory response) was inhibited by NO, which functioned as an endogenous antioxidant in this case [7]. H_2O_2 and sodium nitroprusside (a NO donor) synergistically induced neutrophil adherence to cultured human endothelial cells [8]. These findings provide evidence that NO may interact with H_2O_2 to modulate cellular activity.

H_2O_2 belongs to the reactive oxygen species (ROS) and is produced as a byproduct of many cellular reactions. It is considered as an inter- and intracellular signaling molecule [9], and it can modify signal transduction in a number of cell systems [10]. H_2O_2 modulates protein phosphorylation, potassium channel function, and possibly G-protein activity [11,12]. It also stimulates the production of prostaglandins and leukotrienes [13,14] as well as NO formation and activity [5,6]. It causes intracellular adenosine 5′-triphosphate

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Abbreviations: ATP, adenosine 5′-triphosphate;BSA, bovine serum albumin;Con A, concanavalin A; DAG, diacylglycerol; DiC8, 1,2-dioctanoyl-*sn*-glycerol; DMSO, dimethyl sulphoxide; DNP-HSA, dinitrophenol-human serum albumin conjugate; FCS, fetal calf serum; H_2O_2, hydrogen peroxide; HR, histamine release; IP_3, inositol-1,4,5-triphosphate; L-NA, N^{ω}-nitro-L-arginine; L-NAME, N^{ω}-nitro-L-arginine- methyl ester; LPS, lipopolysaccharide; MB, methylene blue; MC, mast cell; MEM, minimal essential medium; NaF, sodium fluoride; $NaNO_3$, sodium nitrate; NO, nitric oxide; PBS, phosphate buffered saline; PKC, protein kinase C; PLC, phospholipase C; ROS, reactive oxygen species; RPMC, rat peritoneal mast cells; and SNP, sodium nitroprusside

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(ATP) depletion and increases in intracellular free calcium concentration $[Ca^{2+}]_i$, which may underlie its action [12].

Ohmori *et al.* [15] reported that H_2O_2 (0.05–0.1 mM) induced histamine release (HR) from rat peritoneal mast cells (RPMC) without causing cell lysis. Ogasawara *et al.* [16] showed that H_2O_2 was a weak stimulant of HR from human basophils. Another group of workers [17] found that at 0.02–0.2 mM, H_2O_2 did not cause HR from rat basophilic leukemia cells [RBL-2H3, a tumor analog of rat mucosal mast cells (MC)], but at 2 mM it inhibited serotonin release induced by oligomerized IgE or A23187, without affecting cell membrane integrity. In another study, it was found that H_2O_2 (0.01–1.0 mM, non-cytotoxic) produced marked inhibition of HR induced by anti-IgE, compound 48/80, A23187, or sodium fluoride from RPMC [18].

As in the case of H_2O_2 discussed above, recent attempts to clarify the role of NO in HR from MCs have produced contradictory results. Some researchers showed that NO had an inhibitory action on spontaneous and stimulated HR from rat and mouse peritoneal MCs [19,20], whereas others found that NO did not have any effect [21,22]. It is also not clear whether NO is involved in the action of H_2O_2 as shown in other cell systems.

In the present study, we investigated the effects of NO-related compounds on spontaneous and stimulated HR from RBL-2H3 cells. The compounds we used were N^{ω}-nitro-L-arginine (L-NA), an NO synthase inhibitor; methylene blue (MB), an inhibitor of guanylyl cyclase; bacterial lipopolysaccharide (LPS), an inducer of NO synthase; sodium nitroprusside (SNP) and $NaNO_3$, NO donors; L-arginine, substrate for NO production; and D-arginine, the inactive enantiomer of L-arginine. A wide range of secretagogues, each with a different mode of action, was chosen. These included the antigen dinitrophenol-human serum albumin conjugate (DNP-HSA) and concanavalin A (Con A), as immunological stimulants, and the positively charged polyamine compound 48/80 and calcium ionophore A23187, as non-immunological stimulants. Other stimulants were sodium fluoride (NaF, a G-protein activator), phospholipase C (PLC), and 1,2-dioctanoyl-*sn*-glycerol [DiC8, a synthetic 1,2-diglyceride, which is a protein kinase C (PKC) activator], all of which are known to be involved in the signal transduction pathways leading to the HR from RBL-2H3 cells [23,24]. The effect of H_2O_2 on basal and stimulated HR in the absence and presence of NO synthase inhibitor was also studied to determine whether NO is involved in the action of H_2O_2.

2. Materials and methods

2.1. Materials and buffer solutions

Modified Tyrode solution was made up in deionized, distilled water and consisted of (mM): NaCl 137, KCl 2.7, *N*-(2-hydroxyethyl)piperazine-N′-ethanesulfonic acid (HEPES) 10, glucose 5.6, $CaCl_2$ 1.0, $MgCl_2$ 1.0, and NaH_2PO_4 0.4. Bovine serum albumin (BSA, 0.05%) was added to the Tyrode solution to prevent adsorptive loss of the antigen DNP-HSA. The solution was adjusted to pH 7.4 by the addition of NaOH. The buffer used for RBL-2H3 cell culture had the following composition: S-minimal essential medium (MEM) supplemented with 10% fetal calf serum (FCS), 2 mM glutamine, 100 U/mL penicillin, and 100 μg/mL streptomycin. Trypsin-ethylenediaminetetraacetate (EDTA) solution for trypsinization of RBL-2H3 cells contained 0.05% porcine trypsin and 0.02% EDTA in calcium- and magnesium-free phosphate buffered saline (PBS). General chemicals were from British Drug Houses/Merck Ltd., Poole, Dorset, UK. HEPES (free acid), trypsin, 0.02% EDTA solution, 200 mM L-glutamine solution, penicillin (10,000 U/mL)-streptomycin (10 mg/mL) solution, 0.4% trypan blue solution, BSA, DNP-specific monoclonal mouse IgE antibody, DNP-HSA, H_2O_2, dimethyl sulphoxide (DMSO), and *o*-phthaldialdehyde were all obtained from Sigma (Poole, Dorset, UK). FCS, PBS (without calcium and magnesium) and S-MEM were supplied by Gibco (Paisley, UK). Except for A23187 (from Calbiochem, Nottingham, UK), Con A, compound 48/80, NaF, PLC (Type I from *Clostridium perfringens*), DiC8, L-NA, MB, LPS (*Escherichia coli*), $NaNO_3$, SNP, L-arginine, and D-arginine were all from Sigma.

All NO compounds and secretagogues were made up in Tyrode solution or 0.9% saline, except L-NA, A23187, and DiC8, which were dissolved in DMSO. The final maximum concentration of DMSO ($\leq$0.1%) did not affect the cell response or histamine assay. L-arginine and D-arginine solutions were adjusted to pH 7.4 before use. Neither H_2O_2 (prepared immediately in Tyrode solution before experiments) nor NO-related compounds at the concentrations used interfered with the histamine assay.

2.2. Preparation of RBL-2H3 cells

RBL-2H3 cells were placed in a 250 mL tissue culture flask and maintained in monolayer culture in S-MEM supplemented with 10% FCS, glutamine (2 mM), penicillin (100 U/mL), and streptomycin (100 μg/mL) at 37°C in a humidified atmosphere with 5% CO_2-95% air. To harvest the adherent cells, the culture medium was discarded, and the monolayer cell culture was washed with Ca^{2+}-Mg^{2+}–free PBS; trypsinization was then carried out by adding 5 mL of trypsin-EDTA solution into the culture flask and leaving it at 37° for 5 min. After this time interval, the flask was tapped to release the cells from its base. The cell suspension was centrifuged at 225 $\times$ *g* for 3 min at 4°. The cell pellet was resuspended in fresh S-MEM. For passively sensitizing the cells, an aliquot (0.5 mL) containing 3×10^5 cells in fresh S-MEM was placed in each well of a 24-well tissue culture plate and incubated with DNP-specific monoclonal mouse IgE antibody (0.5 μg/mL) overnight. To prepare RBL-2H3 cells for future experiments, the resuspended

cells, obtained as described previously, were subcultured into a tissue culture flask containing fresh supplemented S-MEM, allowing for a doubling time of 24 hr to produce confluent culture at the time of harvesting and allowing at least 2 more days of culture before further trypsinization.

2.3. Histamine release from RBL-2H3 cells

The non-immunological stimulants, including compound 48/80, NaF, PLC, A23187, and DiC8 were tested on non-sensitized cells, whereas passively sensitized cells were used with DNP-HSA or Con A stimulation. On the day of the experiment, the passively sensitized cells or non-sensitized cells were washed twice with BSA (0.05%)-Tyrode buffer and then pre-incubated with the same buffer for 5 min at 37° before use. Aliquots (0.1 mL) of NO synthase inhibitor, NO donor, H_2O_2, or stimulant at 10 times the intended concentrations were added to the wells containing 0.5 mL of the cell suspension and an appropriate amount of BSA-Tyrode buffer so that the final volume in the well was 1 mL. The reaction was allowed to proceed at 37° for the designated time before it was stopped by the addition of 1 mL of ice-cold Tyrode buffer. The sample plate was immediately centrifuged (225 × *g*, 3 min, 4°). The supernatant from each well was pipetted into a fresh, correspondingly labeled test tube containing 0.2 mL of 4 M perchloric acid. To the cell pellet, 2 mL of 0.4 M perchloric acid was added. These samples, after centrifugation, were then assayed for histamine content spectrofluorometrically. Histamine release was expressed as the percentage of the total amount of histamine originally present in the cells (i.e. total amount = amount in supernatant + amount in cell pellet). In the inhibition experiments, the results were expressed as the percentage inhibition of the control release (i.e. the stimulated release in the absence of the inhibitor). All the results were corrected for the spontaneous release.

Statistical analysis was carried out by means of Student's *t*-test, and *P* values of <0.05 were considered significant.

2.4. Determination of cell viability and density

The dye-exclusion method was used to determine cell viability. Equal volumes of trypan blue solution (0.25% w/v in PBS) and cell suspension were mixed together, and a small volume of the mixture was added to a Neubauer hemocytometer. The numbers of living unstained cells and the dead stained cells were counted and the cell viability (% living cells) calculated. The cell density (number of cells/mL) of the cell suspension was calculated from the number of cells present in a known volume of the hemocytometer. The RBL-2H3 cells used for the present work had 98% viability.

3. Results

3.1. Histamine release by various secretagogs

Fig. 1 shows that the calcium ionophore A23187 (0.1–100 μM), NaF (10–40 mM), PLC (46–184 × 10^{-3} mU/ml), Con A (5–40 μg/mL), and the antigen DNP-HSA (1–1000 ng/mL) induced dose-related HR from RBL-2H3 cells. The stimulatory effect of these secretagogs was marked and highly significant, with HR showing a net increase of up to 70% above basal level (ca 10%, in the presence of buffer alone) with A23187, PLC, and NaF, and 50% with Con A and the antigen DNP-HSA. A much smaller net increase in HR (15%) was induced by DiC8 (15–30 μM). Compound 48/80, a traditional histamine releaser that activates connective tissue MCs but not mucosal MCs, did not induce any HR (results not shown).

3.2. Effect of NO releasing agents on basal HR

The results in Fig. 2A show that both L-arginine and D-arginine (0.01–2 mM) produced a dose-related increase in net HR of up to 50% (at an arginine concentration of 2 mM). The NO-synthase inhibitors, MB or L-NA (at 0.001, 0.01, and 0.1 mM), further increased HR from cells treated with L-arginine (Fig. 2B), but this increase was not significant. Similar results were obtained with D-arginine (not shown). It should be pointed out that MB or L-NA at the concentrations used had little and nonsignificant effects by themselves.

The NO donors, SNP (0.001, 0.1, 1.0 mM, 30 min incubation), $NaNO_3$ (0.01, 0.1, 1.0 mM, 30 min incubation), and the NO synthase inducer LPS (1, 10, and 100 μg/mL, 60 min incubation) did not have a significant histamine releasing effect (results not shown).

3.3. Effect of MB and L-NA on stimulated HR

Fig. 3A shows that MB (1–100 μM) inhibited HR induced by NaF, A23187, PLC, or DNP-HSA in a dose-related manner. The inhibitory effect of MB at 100 μM on stimulated HR (by each of the four stimuli) was significant ($P < 0.05$), inhibition reaching 60% ($P < 0.01$) of NaF-induced HR. It can be seen from Fig. 3B that L-NA produced a mild and dose-related inhibition of stimulated HR. The effect of L-NA was only significant at the higher concentration of 100 μM, which produced an inhibition of 22% and 27% with A23187 and NaF, respectively ($P < 0.05$). MB or L-NA by themselves had no effect on basal HR.

3.4. Effect of L-NA on H_2O_2 inhibition of stimulated HR

Basal (spontaneous) HR from cells suspended in buffer was 5.1 ± 0.6 (N = 3). When H_2O_2 was added to the buffer at 0.01, 0.1, and 1.0 mM, HR was 4.9 ± 1.0, 5.5 ± 1.2, and

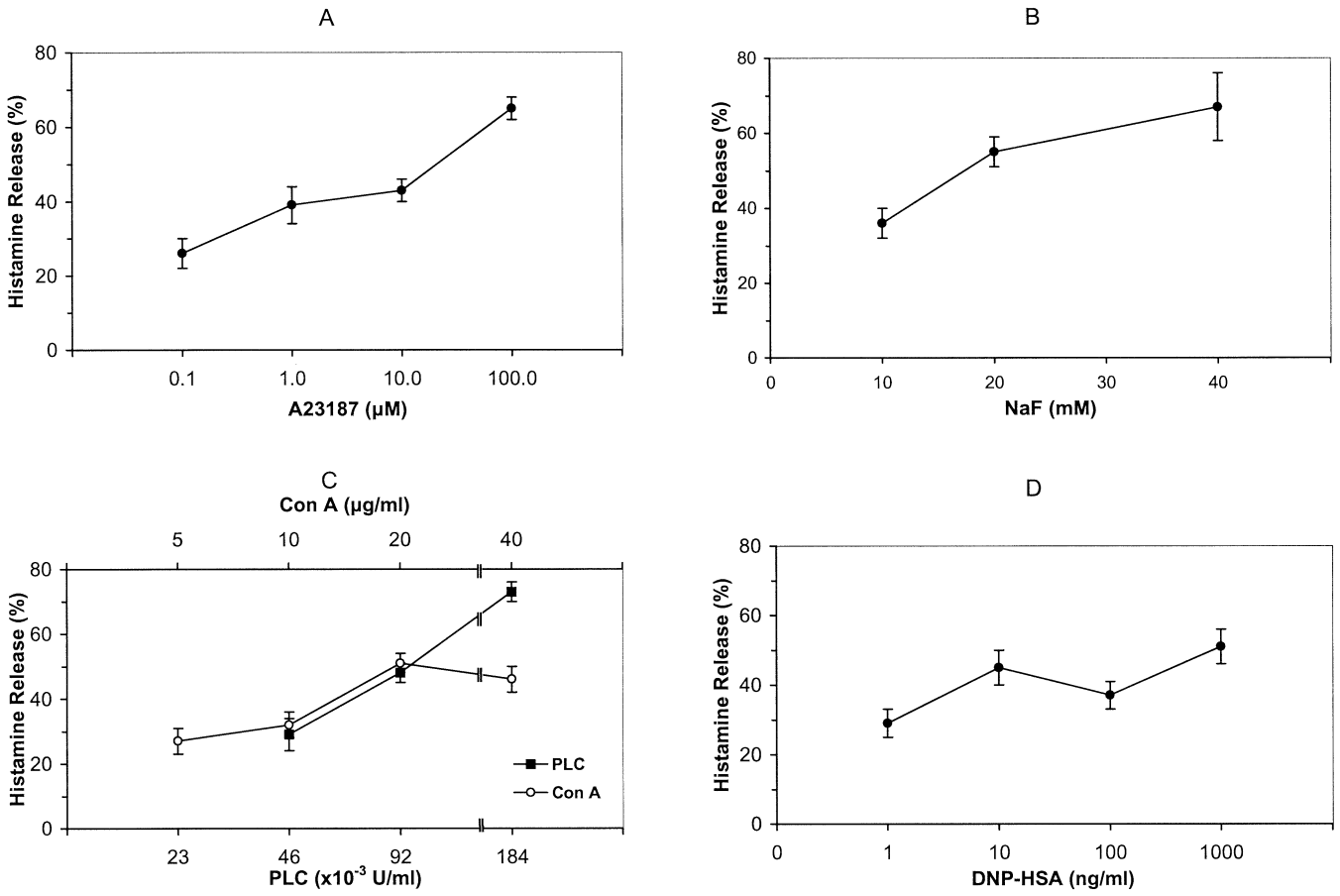

Fig. 1. Effect of various secretagogs on HR from RBL-2H3 cells. (A) A23187 (0.1–100 μM, 15 min incubation); (B) NaF (10–40 mM, 30 min incubation); (C) PLC (46×10^{-3}–184×10^{-3} mU/mL, 30 min incubation) or Con A (5–40 μg/mL, 30 min incubation); (D) DNP-HSA (1–1000 ng/mL, 15 min incubation). Each point represents mean ± SEM (N = 3–4 experiments, each with triplicate samples) of HR (%). Spontaneous HR (less than 10%) has been subtracted.

6.9 ± 1.2 (N = 3), respectively. It is thus clear that H_2O_2 did not affect unstimulated HR. H_2O_2 at those concentrations also had no effect on cell viability.

Fig. 4A shows that H_2O_2 (0.01, 0.1, and 1.0 mM) inhibited DNP-HSA or A23187-induced HR significantly and dose dependently. At 1.0 mM, H_2O_2 induced a 85% inhibition of A23187-stimulated HR compared to that of 75% with DNP-HSA as a stimulant. H_2O_2 (0.01–1.0 mM) was also a potent inhibitor of HR induced by Con A (results not shown). It can be seen from Fig. 4B that the NO synthase inhibitor, L-NA, had no significant effect on the inhibition of A23187 or DNP-HSA–stimulated HR by H_2O_2 (0.1 mM).

4. Discussion

Histamine plays a role in the generation of allergic inflammation and the immediate hypersensitivity reactions, including asthma and anaphylaxis [23]. In recent years, RBL-2H3 cells have been used extensively to study the signal transduction pathways leading to the activation of MCs and HR. Stimulation of RBL-2H3 cells with antigen (or A23187) increases PLC activity, resulting in phosphatidylinositol hydrolysis. This in turn leads to the generation of second messengers such as inositol-1,4,5-triphosphate (IP_3), which releases intracellular calcium, and diacylglycerol (DAG), which activates PKC. These second messengers initiate a cascade of biochemical activities and eventually HR. The exact mechanisms are as yet not clear, but it is thought that G proteins might be involved [24]. In the present study, we have shown that DNP-HSA, Con A, NaF, PLC, and A23187 induced marked and significant HR from RBL-2H3 cells. It is interesting to note that NaF and PLC applied exogenously can stimulate HR from RBL-2H3 cells, and this may be considered as supporting evidence that PLC and G proteins are involved in HR from RBL-2H3 cells.

The present results show that in RBL-2H3 cells both MB and L-NA inhibited HR induced by DNP-HSA, NaF, PLC, and A23187. The effect of MB was marked, especially in the case of NaF stimulation. These results may be taken to suggest a role for NO (probably generated in the process) in the stimulation of HR by those secretagogues, a process that

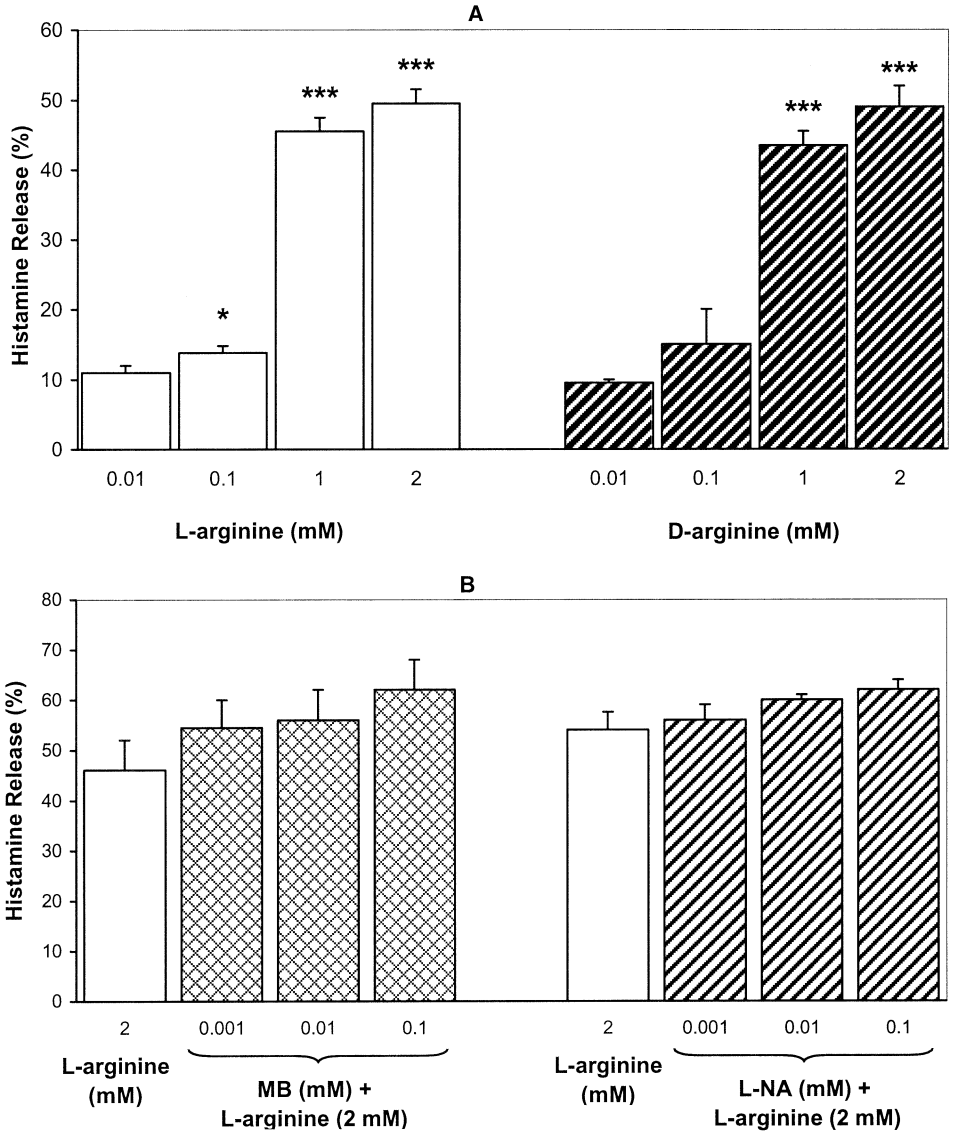

Fig. 2. Effect of L-arginine, D-arginine, MB, and L-NA on HR from RBL-2H3 cells. (A) Cells were incubated with L-arginine or D-arginine (0.01–2.0 mM for both) for 30 min. (B) Cells were incubated with L-NA or MB (0.001–0.1 mM for both) for 30 min prior to the addition of L-arginine (2.0 mM) for a further 30 min. The results are expressed as mean ± SEM (N = 3 experiments, each with triplicate samples) of HR (%), which has been corrected for the spontaneous release (<10%). Asterisks denote a significant increase in release compared to the spontaneous release (* $P < 0.05$, *** $P < 0.001$, Student's *t*-test).

appears to be abrogated by the inhibition of NO synthesis or action. However, more experimental work is needed to clarify whether inducers of HR from MCs have stimulatory effects on NO production and cyclic guanosine 3′,5′ monophosphate (cGMP) formation. MB inhibits guanylyl cyclase, which is required for cGMP formation and is generally considered as the target enzyme by which NO mediates most of its effects [25]. In the present study, L-arginine was found to stimulate HR from RBL-2H3 cells, and its effect was not antagonized by MB or L-NA. Similar results were obtained with D-arginine, the inactive enantiomer of L-arginine. This finding together with the lack of effect of the NO donors (SNP and $NaNO_3$) and NO synthase inducer (LPS) provide some evidence against NO playing a significant role in HR from RBL-2H3 cells. The inhibitory effect of MB on stimulated HR (by secretagogues) and the stimulation of HR by L-arginine may involve mechanisms unrelated to NO. NO-related compounds have other activities, e.g. MB has been shown to induce the generation of a superoxide anion that might affect HR by inactivating NO [26]. L-NA is known to act like an antagonist at muscarinic receptors [27]. Both L-arginine and D-arginine are good scavengers of hydroxyl radicals [28]. Arginine may have a non-specific pH effect that could contribute to the increase of NO release [29].

Our results and the conclusion we can draw from them are in agreement with those of Koranteng *et al.* [30], which were reported while this paper was being prepared. These

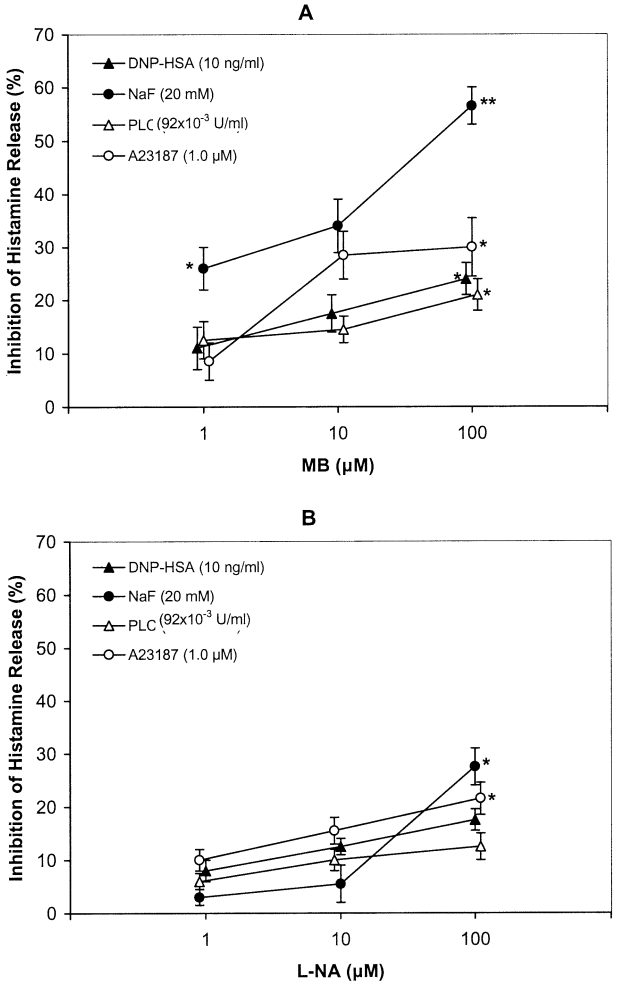

Fig. 3. Effect of MB and L-NA on stimulated HR from RBL-2H3 cells. Cells were pre-incubated with (A) MB or (B) L-NA (1–100 μM) for 30 min and then challenged with DNP-HSA (10 ng/mL, 15 min), NaF (20 mM, 30 min), PLC (92′10^{-3} U/mL, 30 min), or A23187 (1.0 μM, 15 min). The results are shown as the mean ± SEM (N = 3 experiments, each with triplicate samples) of inhibition of HR (%). Stimulated HR in the absence of MB or L-NA (%, after correction for the spontaneous HR, which was <10%) was 45 ± 5, 55 ± 4, 48 ± 3, and 39 ± 4 for the above stimulants, respectively. Asterisks denote a significant inhibition compared to the control release (induced release in the absence of MB and L-NA) (* $P < 0.05$, ** $P < 0.01$, Student's *t*-test).

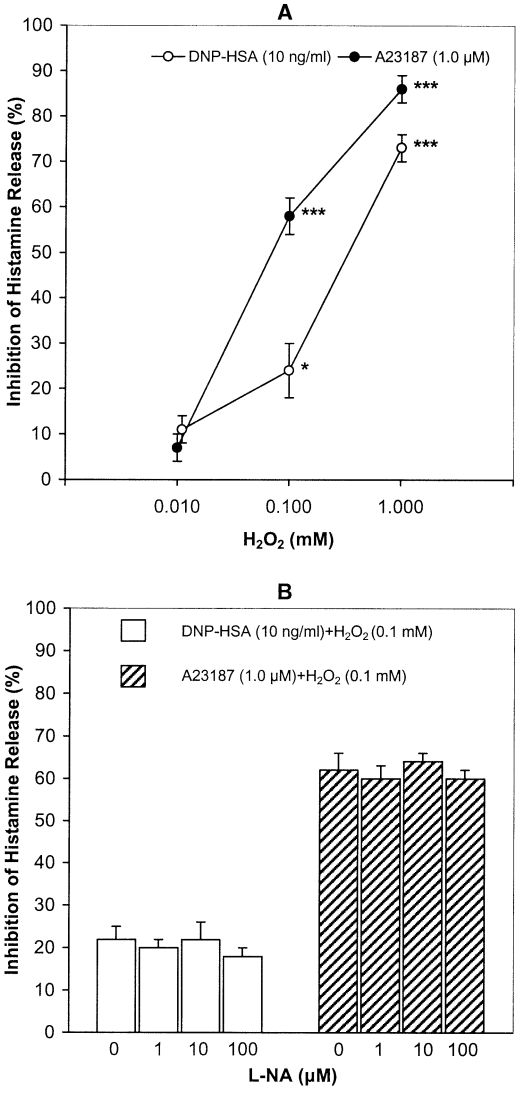

Fig. 4. Effect of L-NA on H_2O_2 inhibition of stimulated HR from RBL-2H3 cells. (A) Cells were pre-incubated with H_2O_2 (0.01–1.0 mM) for 30 min prior to the challenge with DNP-HSA (10 ng/mL, 15 min) or A23187 (1 μM, 15 min). (B) In another series of experiments, cells were pre-incubated with L-NA (0.01–1.0 mM) for 30 min, followed by a further 30 min incubation with H_2O_2 (0.1 mM), and then challenged with DNP-HSA (10 ng/mL, 15 min) or A23187 (1.0 μM, 15 min). The results are expressed as mean ± SEM (N = 3 experiments, each with triplicate samples) of inhibition of HR (%). Stimulated HR (%) in the absence of L-NA and H_2O_2 was 45 ± 5 and 39 ± 4%, with DNP-HSA and A23187, respectively. All the results have been corrected for spontaneous release (<10%). Asterisks denote a significant inhibition compared to the control HR (* $P < 0.05$, *** $P < 0.001$, Student's *t*-test).

authors found that S-nitrosoglutathione (an NO donor) did not inhibit serotonin release induced by antigen (DNP-HSA) or ionophore A23187 in RBL-2H3 cells and concluded that this cell type was resistant to NO.

It seems clear from the present study that the NO synthase inhibitor L-NA did not reverse the inhibitory effect of H_2O_2 on HR induced by DNP-HSA or A23187, suggesting that the inhibition of stimulated HR by H_2O_2 is not mediated by NO. Very little is known about the interaction of ROS, such as H_2O_2 and NO, in relation to HR from MCs. In one study it was shown that N^{ω}-nitro-L-arginine-methyl ester (L-NAME, a NO synthase inhibitor) caused a significant enhancement of both HR and generation of intracellular ROS in RPMC stimulated with antigen or compound 48/80, suggesting that NO and possibly other ROS generated during MC activation may cause feedback inhibition of HR [31].

The present results show that in RBL-2H3 cells H_2O_2 (0.01–1.0 mM), which did not cause HR on its own, produced a dose-related inhibition of HR induced by antigen (DNP-HSA) or the calcium ionophore A23187. Our findings are in agreement with those of Peden *et al.* [17], who found that H_2O_2 inhibited serotonin release from RBL-2H3

cells. However, our results in RBL-2H3 cells (this paper) and RPMC [18] did not agree with those of Ohmori *et al.* [15], who claimed that H_2O_2 stimulated HR from RPMC. It is possible that the difference in experimental conditions may account for the discrepancy. The concentrations of H_2O_2 producing inhibition of HR were not cytoxic and did not compromise cell viability. It is thought that H_2O_2 might exert its effect intracellularly by affecting inositol phosphorylation, ATP levels, and calcium homeostasis [12,17,18].

H_2O_2 is a small, uncharged molecule that is able to cross the cell membrane to act intracellularly and intercellularly. It is produced during normal cellular metabolism, and mitochondria are considered the major cellular site for its production. Although H_2O_2 is considered as a cytotoxic agent, particularly at high concentrations, it is not very reactive. The reported concentrations of H_2O_2 in human plasma varied from close to zero to up to 35 μM [9], and high levels seemed to be associated with inflammatory conditions, vascular disease, and oxidative stress. Increased levels of H_2O_2 in exhaled air have been found in inflammatory lung disease, smoking, and asthma [9,32]. High levels of H_2O_2 (reaching up to $\sim$110 μM) were also detected in urine and ocular fluids [9]. Thus, in tissue, blood, or other body fluids H_2O_2 may reach levels close to those that produce significant inhibition of HR from RBL-2H3 cells. Furthermore, it has been shown that during stimulation of RPMC by anti-IgE, compound 48/80, or the ionophore A23187, ROS (presumably including H_2O_2) are released alongside histamine [33]. H_2O_2 thus generated might inhibit further MC activation, HR, and ROS generation, i.e. exert a negative feedback inhibitory effect and limit the inflammatory process. The feedback regulatory role of H_2O_2 in inflammation may take a more subtle and indirect form and may involve cells other than MCs and mediators other than histamine. This setting is exemplified by the ability of H_2O_2 to inhibit the production of platelet activating factor, a potent inflammatory mediator, by endothelial cells [10,34].

Although there is some evidence against NO playing a significant role in the induction or modulation HR from RBL-2H3 cells and RPMC, it may have such a role in the release of other mediator groups, such as leukotrienes and cytokines. Furthermore, cytokines and leukotrienes that are released from a wide variety of cells, including MCs, may modulate MC function through their influence on NO or H_2O_2 generation. It has been shown that interferon-γ generated from mouse spleen cells inhibits serotonin release from mouse peritoneal MCs [35]. It was later found that this inhibition is mediated via the synthesis of NO, with non-MCs in the peritoneal cell population being the major source [20]. It is also interesting to note that in RPMC, IL-1β stimulates NO synthesis along with inhibition of HR, suggesting the possible involvement of NO in the inhibition of HR [36].

References

[1] Moncada S, Palmer RMJ, Higgs EA. Nitric oxide, pathophysiology and pharmacology. Pharmacol Rev 1991;43:109–42.

[2] Berdeaux A. Nitric oxide: an ubiquitous messenger. Fundam Clin Pharmacol 1993;7:401–11.

[3] Miyoshi H, Nakaya Y. Endotoxin-induced nonendothelial nitric oxide activates the Ca^{2+}-activated K^+ channel in cultured vascular smooth muscle cells. J Mol Cell Cardiol 1994;26:1487–95.

[4] Zidek Z, Mašek K. Erratic behaviour of nitric oxide within the immune system: illustrative review of conflicting data and their immunopharmacological aspects. Int J Immunopharmacol 1998;20: 319–43.

[5] Shimizu S, Nomoto M, Ishii M, Naito S, Ymamoto T, Momose K. Changes in nitric oxide synthase activity during exposure to hydrogen peroxide in cultured endothelial cells. Res Commun Mol Pathol Pharmacol 1997;97:279–89.

[6] Naseem KM, Bruckdorfer KR. Hydrogen peroxide at low concentrations strongly enhances the inhibitory effect of nitric oxide on platelets. Biochem J 1995;310:149–53.

[7] Johnston B, Kanwar S, Kubes P. Hydrogen peroxide induces leukocyte rolling: modulation by endogenous antioxidant mechanisms including NO. Am J Physiol 1996;271:H614–21.

[8] Okayama N, Coe L, Itoh M, Alexander JS. Intracellular mechanisms of nitric oxide plus hydrogen peroxide-mediated neutrophil adherence to cultured human endothelial cells. Inflamm Res 1998;47:428–33.

[9] Halliwell B, Clement MV, Long LH. Hydrogen peroxide in the human body. FEBS Lett 2000;486:10–13.

[10] Vercellotti GM, Severson SP, Duane P, Moldow CF. Hydrogen peroxide alters signal transduction in human endothelial cells. J Lab Clin Med 1991;117:15–24.

[11] Carbajal JM, Schaeffer RC. H_2O_2 and genistein differentially modulate protein tyrosine phosphorylation, endothelial morphology, and monolayer barrier function. Biochem Biophys Res Commun 1998; 249:461–6.

[12] Krippeit-Drews P, Krämer C, Welker S, Lang F. Ammon HPT, Drews G. Interference of H_2O_2 with stimulus-secretion coupling in mouse pancreatic β-cells. J Physiol 1999;514(Part 2):471–81.

[13] Harlan JM, Callahan KS. Role of hydrogen peroxide in the neutrophil-mediated release of prostacyclin from cultured endothelial cells. J Clin Invest 1984;74:442–8.

[14] Dent G, Rabe KF, Magnussen H. Augmentation of human neutrophil and alveolar macrophage LTB_4 production by *N*-acetylcysteine: role of hydrogen peroxide. Br J Pharmacol 1997;122:758–64.

[15] Ohmori H, Yamamoto I, Akagi M, Tasaka K. Properties of hydrogen peroxide- induced histamine release from rat mast cells. Biochem Pharmacol 1980;29:741–5.

[16] Ogasawara H, Fujitani T, Drzewieki G, Middleton E. The role of hydrogen peroxide in basophil histamine release and the effect of selected flavonoids. J Allergy Clin Immunol 1986;78:321–8.

[17] Peden DB, Dailey L, DeGraff W, Mitchell JB, Lee J-G, Kaliner MA, Hohman RJ. Hydrogen peroxide effects on rat mast cell function. Am J Physiol 1994;267:L85–93.

[18] Moulson A, Wan BYC, Pearce FL. Calcium, ATP and H_2O_2 inhibition of stimulated histamine release from rat peritoneal mast cells. Br J Pharmacol 1994;112:88P.

[19] Salvemini D, Masini E, Pistelli A, Mannaioni PF, Vane J. Nitric oxide: a regulatory mediator of mast cell reactivity. J Cardiovasc Pharmacol 1991;17:S258–64.

[20] Eastmond NC, Banks EMS, Coleman JW. Nitric oxide inhibits IgE-mediated degranulation of mast cells and is the principal intermediate in IFN-γ-induced suppression of exocytosis. J Immunol 1997;159:1444–50.

[21] Moulson A, Wan BYC, Ho MNK, Assem ESK, Pearce FL. Evaluation of the role of nitric oxide in histamine release from rat peritoneal mast cells. Br J Pharmacol 1994;112:147P.
[22] Lau HYA, Chow SM. Nitric oxide does not affect histamine release from rat peritoneal mast cells. Inflamm Res 1999;48:S27–8.
[23] Metcalfe DD, Baram D, Mekori YA. Mast cells. Physiol Rev 1977; 77:1033–79.
[24] Oliver JM, Seagrave J, Stump RF, Pfeiffer JR, Deanin GG. Signal transduction and cellular response in RBL-2H3 mast cells. Prog Allergy 1988;42:185–245.
[25] McDonald LJ, Murad F. Nitric oxide and cGMP signalling. Adv Pharmacol 1995;34:263–75.
[26] Marczin N, Ryan US, Catravas JD. Methylene blue inhibits nitrovasodilator- and endothelium-derived relaxing factor-induced cyclic GMP accumulation in cultured pulmonary arterial smooth muscle cells *via* generation of superoxide anion. J Pharmacol Exp Ther 1992;263:170–9.
[27] Chang H-Y, Chen C-W, Hsiue T-R. Comparative effect of L-NOARG and L-NAME on basal blood flow and Ach-induced vasodilatation in rat diaphragmatic microcirculation. Br J Pharmacol 1997;120:326–32.
[28] Rehman AM, Whiteman M, Halliwell B. Scavenging of hydroxyl radical but not of peroxynitrite by inhibitors and substrates of nitric oxide synthase. Br J Pharmacol 1997;122:1702–6.
[29] Jacob M. L-arginine is endogenous source of NO. *TIPS* 1988;9:312–13.
[30] Koranteng RD, Dearman RJ, Kimber I, Coleman JW. Phenotypic variations in mast cell responsiveness to the inhibitory action of nitric oxide. Inflam Res 2000;49:240–6.
[31] Brooks AC, Whelan CJ, Purcell WM. Reactive oxygen species generation and histamine release by activated mast cells: modulation by nitric oxide synthase inhibition. Br J Pharmacol 1999;128:585–90.
[32] Jobsis Q, Raatgeep HC, Hermans PW, de Jongste JC. Hydrogen peroxide in exhaled air is increased in stable asthmatic children. Eur Resp J 1997;10:519–21.
[33] Fukuishi N, Sakaguchi M, Matsuura S, Nakagawa C, Akagi R, Akagi M. The mechanisms of compound 48/80-induced superoxide generation mediated by A-kinase in rat peritoneal mast cells. Biochem Mol Med 1997;61:107–13.
[34] Cronstein BN. Oxidative insults: sublethal injury to the endothelium by H_2O_2. Lab Clin Med 1991;117:6–7.
[35] Coleman JW, Buckley MG, Holliday MR, Morris AG. Interferon-γ inhibits serotonin release from mouse peritoneal mast cells. Eur J Immunol 1991;21:2559–64.
[36] Hogaboam CM, Befus AD, Wallace JL. Modulation of rat mast cell reactivity by IL-1β: divergent effects on nitric oxide and platelet activating factor release. J Immunol 1993;151:3767–74.