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Role of nitric oxide in histamine release from human basophils and rat peritoneal mast cells

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Abstract

The effects of a range of nitric oxide (NO)-related compounds on histamine release from human basophils and rat peritoneal mast cells were studied. Basal and immunologic histamine releases from human basophils were not affected by N^{ω}-nitro-L-arginine, N^{ω}-nitro-L-arginine methyl ester, aminoguanidine or methylene blue (all inhibitors of NO production), sodium nitroprusside (an NO donor), L-arginine (a substrate for NO synthase) or D-arginine (the inactive enantiomer of L-arginine). In rat peritoneal mast cells, NO donors such as sodium nitroprusside, sodium nitrite and sodium nitrate, and lipopolysaccharide (an inducer of NO synthase) had little effect on basal histamine release, while 3-morpholino-sydnonimine (SIN-1, an NO donor), L-arginine and D-arginine increased this release by up to threefold. None of the inhibitors of NO production had any striking effect on histamine release induced by anti-rat immunoglobulin E (IgE), compound 48/80, sodium fluoride, phospholipase C, 1,2-dioctanoyl-*sn*-glycerol or ionophore A23187. However, haemoglobin was found to inhibit histamine release by anti-rat IgE or A23187 by ca. 40%. Alone of the NO donors, low concentrations of L-arginine produced a mild inhibition of histamine release induced by anti-IgE, compound 48/80 and A23187, but not other ligands, while sodium nitroprusside dose-dependently inhibited (by a maximum of ca. 30%) histamine release by anti-rat IgE, sodium fluoride or A23187. Stimulation with a variety of secretagogues or treatment with L-arginine, D-arginine, lipopolysaccharide, SIN-1 or sodium nitroprusside had no effect on NO production. Similarly, L-arginine, D-arginine or sodium nitroprusside did not change intracellular cGMP levels. On the basis of these results, it is suggested that NO does not play a significant role in the modulation of histamine release from human basophils or rat peritoneal mast cells. The effects of L-arginine, D-arginine and sodium nitroprusside may involve mechanisms unrelated to NO.

Keywords: Nitric oxide (NO); Histamine release; Mast cell; Basophil, human; Signal transduction

1. Introduction

Nitric oxide (NO) is a ubiquitous intracellular and intercellular second messenger that is involved in the modulation of a plethora of cellular activities (Berdeaux, 1993; Christopoulos and El-Fakahany, 1999; McDonald and Murad, 1995; Moncada et al., 1997; Muscará and Wallace, 1999; Nathan, 1992; Zidek and Mašek, 1998). The oxide is a simple and diffusible molecule of free radical nature. It does not interact with a specific receptor but rather produces its effects by activation of guanylate cyclase and the subsequent elevation in intracellular levels of guanosine-3′,5′-cyclic monophosphate (cGMP).

The release of chemical mediators from tissue mast cells and circulating basophil leukocytes has been centrally implicated in a diversity of allergic and inflammatory disorders. These mediators are typified by the autacoid histamine and lipid products of the oxidative metabolism of arachidonic acid (Metcalfe et al., 1997). In addition, the mast cell has been widely used as a model system to study the detailed biochemical events involved in stimulus–secretion coupling (Beaven and Cunha-Melo, 1988; Sagi-Eisenberg, 1993).

The pathophysiological stimulus for mast cell activation is provided by cross-linking of high affinity receptors ($Fc_{\varepsilon}RI$) for antibody immunoglobulin E (IgE) on the cell membrane. This induces a series of biochemical changes

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which are initiated by the activation of a guanine nucleotide binding protein (G-protein) and the subsequent stimulation of phospholipase C. The latter results in the generation of inositol-1,4,5-trisphosphate (IP_3) and diacylglycerol. IP_3 acts to mobilise intracellular stores of Ca^{2+} ions and diacylglycerol activates protein kinase C, both of which are thought to be essential steps in the secretory process (Beaven and Cunha-Melo, 1988; Sagi-Eisenberg, 1993). In addition to the antigen–antibody reaction, mast cells and basophils may be triggered by a range of chemical stimuli which similarly initiate some or all of the above steps (Lagunoff and Martin, 1983).

A number of workers have examined the possible role of NO in the control of mediator release from mast cells. Salvemini et al. (1991) showed that sodium nitroprusside (an NO donor) increased cGMP levels in, and inhibited histamine release from, purified rat peritoneal mast cells stimulated with compound 48/80 or ionophore A23187. They thus concluded that rat peritoneal mast cells were able to generate an NO-like factor and that NO was a regulatory mediator of mast cell reactivity. Hogaboam et al. (1993) also observed that NO was released from rat peritoneal mast cells both in the resting state and following activation with Ca^{2+} ionophore. Most recently, Brooks et al. (1999) reported that preincubation of purified rat peritoneal mast cells with N^{ω}-nitro-L-arginine methyl ester (L-NAME, an inhibitor of NO synthase) increased histamine release induced by compound 48/80 or specific antigen. Other workers have reported similar findings in mouse or human mast cells (Eastmond et al., 1997; Van Overveld et al., 1993; Bidri et al., 1995). In sharp contrast, Lau and Chow (1999) found that L-NAME and sodium nitroprusside did not affect either the spontaneous or the immunologic release of histamine from purified rat peritoneal mast cells and they concluded that NO did not have an important role in the control of mast cell function. In addition to these discrepancies, very little is known about the possible effect of NO on the signal transduction pathways leading to histamine release in the mast cell.

In view of this confusion, and the fundamental importance of this issue, we have now examined the effect of a range of NO-related compounds on basal and stimulated histamine release from human basophils and rat peritoneal mast cells. To this end, we have employed a variety of stimuli that operate through different mechanisms. The compounds used included: N^{ω}-nitro-L-arginine (L-NA), L-NAME, aminoguanidine, methylene blue and haemoglobin (all of which are inhibitors of NO production); lipopolysaccharide (an inducer of NO synthase); sodium nitroprusside, sodium nitrite ($NaNO_2$), sodium nitrate ($NaNO_3$), and 3-morpholino-sydnonimine (SIN-1) (all of which are NO donors); and L-arginine (a substrate for NO production) as well as D-arginine (the inactive enantiomer of L-arginine). The secretagogues chosen for induction of histamine release were: anti-IgE (an immunological stimulus), compound 48/80 (a polyamine which is thought directly to activate a pertussis toxin sensitive G-protein), sodium fluoride (NaF, a non-specific G-protein activator), A23187 (a Ca^{2+} ionophore), phospholipase C and the synthetic diacylglycerol, 1,2-dioctanoyl-*sn*-glycerol (DiC8) (both compounds known to be involved in signal transduction pathways in histamine release) (Beaven and Cunha-Melo, 1988). In addition, the effect of selected NO-related compounds and stimulants on NO production and cGMP formation in rat peritoneal mast cells was also studied. A small part of this work has been presented to the British Pharmacological Society (Moulson et al., 1994).

2. Materials and methods

2.1. Isolation of human basophils

Venous blood (120 ml) was collected from healthy volunteers. Heparin (40 U/ml of blood) was added to each sample immediately after extraction to prevent coagulation. One part of dextran (6%) in clinical isotonic saline containing glucose (30 mg/ml) was mixed with four parts of blood. The mixture was left to stand for 60–90 min at room temperature to allow the erythrocytes to settle to the bottom of the mixture. The platelet-leukocyte-rich top layer was gently aspirated and centrifuged ($150 \times g$, 3 min, room temperature). The new platelet-rich supernatant was discarded and the cell pellet containing the basophils was washed twice with full HEPES-buffered Tyrode solution (composition described below), then resuspended in fresh prewarmed buffer and used for experiments.

The recovered cells with viability greater than 90% were used without further purification. On average, 10% of the recovered cells were basophils and each sample contained approximately $4–5 \times 10^4$ viable basophils. The means $\pm$ S.E.M. of the actual amounts of histamine released spontaneously and following anti-IgE stimulation were 0.3 ± 0.1 and 10 ± 1.5 ng per $4–5 \times 10^4$ basophils, respectively.

2.2. Isolation of rat peritoneal mast cells

Male Sprague–Dawley rats (200–400 g) were used. The animals were killed by asphyxiation under 95% CO_2, followed by cervical dislocation. The abdominal skin was removed and full HEPES-buffered Tyrode solution (20 ml per rat) containing heparin (5 U/ml) was carefully injected into the peritoneal cavity, avoiding penetration of the viscera. The abdomen was gently massaged for 2 min and a careful incision was made along the linea alba so as to avoid any blood vessels. Peritoneal lavage fluid was recovered using a plastic pipette and collected into plastic universal containers. Any sample heavily contaminated with blood was discarded. The cells were pelleted by centrifugation ($150 \times g$, 2 min, room temperature), washed

twice in full buffer, and then either resuspended in full HEPES-buffered Tyrode solution and used in functional studies directly or further purified. For experiments involving stimulation with anti-rat IgE, the rats were first sensitized with the nematode *Nippostrongylus brasiliensis* (see White and Pearce, 1982). The viability of recovered mixed cells, of which 6.2% (range 5–9%) were mast cells, was greater than 90%. The mean ± S.E.M. of the actual amount of histamine released spontaneously was 20.3 ± 2.9 ng per 2.0–3.0 × 10^4 mast cells, and the actual amounts released from stimulated cells ranged from 65.4 ± 21.0–176.8 ± 5.0 ng per 2.0–3.0 × 10^4 mast cells, depending on the secretagogue used.

Density gradient centrifugation with Percoll was used to purify rat peritoneal mast cells. Mixed rat peritoneal mast cells isolated as above were resuspended in full HEPES-buffered Tyrode solution (1 ml) containing bovine serum albumin (1 mg/ml). A Percoll solution (4 ml) consisting of nine parts of Percoll and one part of 10-times concentrated Ca^{2+} and Mg^{2+}-free HEPES-buffered Tyrode solution was mixed with the cell suspension. Bovine serum albumin-buffer (1 ml) was then carefully layered over the Percoll/cell mixture to produce an interface. The sample was centrifuged (150 × *g*, 25 min, 4 °C) and the supernatant was aspirated to leave a pure mast cell pellet at the bottom of the tube. The pellet was washed twice in bovine serum albumin-buffer and twice again in HEPES-buffered Tyrode solution before use. In this way, preparations containing ≥ 95% mast cells were obtained.

2.3. Effect of NO donors and inhibitors on histamine release

Human basophils or rat peritoneal mast cells preincubated at 37 °C were aliquoted (150 μl) into polystyrene or polypropylene test tubes already containing vehicle or test agent (50 μl) and HEPES-buffered Tyrode solution (300 μl). The reaction was allowed to proceed for a designated time before being stopped by the addition of ice-cold buffer (1500 μl). The samples were then immediately centrifuged (200 × *g*, 4 °C, 5 min). The supernatant was decanted into fresh, labelled test tubes and the residual cell pellets were resuspended in fresh buffer to the same volume as the supernatant sample. The supernatant and cell pellet samples were treated with 70% (v/v) perchloric acid (final concentration 0.4 M) for automated fluorometric assay of histamine essentially according to the procedure of Shore et al. (1959). The histamine content was determined in both the supernatant and the corresponding cell pellet samples and the release was expressed as a percentage of the total amount of histamine originally present in the cells.

For inhibition studies, cell suspensions were aliquoted (150 μl) into test tubes containing a solution of the given inhibitor (50 μl) and HEPES-buffered Tyrode solution (250 μl). Incubation (37 °C) was allowed to take place for a defined period of time with the given inhibitor before the addition of the secretory stimulus (50 μl). After the reaction had proceeded for the designated period, it was terminated as described above. Results were expressed as the percentage inhibition of the stimulated release.

2.4. cGMP assay

A commercially available radioimmunoassay kit (Amersham) was used to determine cGMP in purified rat peritoneal mast cells. The assay is based on the competition between unlabelled cGMP and ^{125}I-labelled cGMP for binding to a limited quantity of an antibody raised with a high specificity to cGMP. The samples for cGMP assay were prepared as described below. After completing the incubation of the cells with the test compounds, the reaction was stopped with ice-cold ethanol to give a final suspension containing 65% ethanol. The supernatant was drawn off into fresh tubes and the extracts centrifuged (200 × *g*, 15 min, 4 °C). The supernatant was decanted into fresh tubes and the ethanol evaporated under a stream of nitrogen. The dried extracts were then dissolved in a suitable volume of assay buffer (500 μl) containing sodium acetate (0.05 M, pH 5.8). The rest of the assay procedure was carried out according to manufacturer's instructions.

2.5. Nitric oxide determination—diazotization reaction

Following incubation with the stimulants, the cells (purified or non-purified rat peritoneal mast cells) were sonnicated (30 s, 3 times) and the suspension clarified by centrifugation (1000 × *g*, 10 min). Sulfanilic acid (1.5% w/v in 2 M HCl, 0.5 ml) was added to an aliquot of the supernatant (0.5 ml) or to water (blank, 0.5 ml). After 5 min, *N*-(1-naphthyl) ethylenediamine dihydrochloride (0.5% w/v, 0.5 ml) was added, the mixture incubated for 30 min at 37 °C, and the absorbance determined at 540 nm. After correction for the absorbance of the blank, the concentration of nitrite ion was determined from a calibration curve prepared using sodium nitrite as a standard (Ignarro et al., 1987).

2.6. Nitric oxide formation—oxyhaemoglobin method

Purified or non-purified rat peritoneal mast cells were incubated with various stimulants or NO donors and oxyhaemoglobin (1 mM) for 15–30 min in a shaking water bath (37 °C). A control containing only cells and oxyhaemoglobin (1 mM) was used. Following incubation, the samples were centrifuged (200 × *g*, 4 °C, 5 min) and the absorbance of the supernatant was measured at 416 nm, taking into account the absorbance of the control (Kelm et al., 1988). Commercially available haemoglobin contains a mixture of oxyhaemoglobin and the oxidized derivative,

Table 1
Effect of NO releasers and inhibitors on (A) basal and (B) anti-human IgE-induced histamine release from human basophils
Human basophils were incubated with buffer or each of the test compounds for 30 min, then challenged with buffer (for effect on basal histamine release) or anti-IgE (1:1000) for 20 min. Results are expressed as mean ± S.E.M. of histamine release (%) in three (for A) or four (for B) separate experiments. Basal histamine release for (A) was 1.8 ± 0.3% and histamine release from basophils challenged with anti-IgE alone was 44.5 ± 13.8% for (B). All values for (B) were corrected for the spontaneous release (1.1–2.3%).

Treatment	Histamine release (%) in the presence of test compound at a concentration (μM) of:		
	1	10	100
(A)			
L-arginine	1.1 ± 0.5	0.8 ± 0.4[a]	0.8 ± 0.5[a]
D-arginine	1.4 ± 0.9	1.1 ± 0.7	1.2 ± 0.6
SNP[b]	1.2 ± 0.6	1.6 ± 0.8	1.6 ± 0.8
(B)			
SNP + Anti-IgE	45.3 ± 14.1	47.1 ± 13.5	47.5 ± 13.6
L-NA[c] + Anti-IgE	45.4 ± 14.6	46.6 ± 13.5	46.7 ± 13.0
L-NAME[d] + Anti-IgE	44.4 ± 14.9	42.7 ± 14.1	42.4 ± 14.2
Aminoguanidine + Anti-IgE	43.2 ± 14.0	43.9 ± 14.4	42.7 ± 13.7
Methylene blue + Anti-IgE	45.9 ± 14.8	45.7 ± 14.9	43.7 ± 13.7

[a] Denotes values which are significantly different ($P < 0.05$) from the untreated basal histamine release.
[b] SNP = Sodium nitroprusside.
[c] L-NA = L-N^{ω}-nitro-arginine.
[d] L-NAME = N^{ω}-nitro-L-arginine methyl ester.

methaemoglobin. Pure oxyhaemoglobin was prepared by adding a 10-fold molar excess of the reducing agent, sodium dithionite, to a 1 mM solution of commercial haemoglobin in distilled water. Sodium dithionite was then removed by dialysis against 100 volumes of distilled water (4 °C, 2 h). The purity of the preparation was checked by measuring the absorbance of oxyhaemoglobin at 416 nm (Kelm et al., 1988).

2.7. Materials, solutions, test compounds and statistics

All buffers were made up in glass distilled water. The full HEPES-buffered Tyrode solution used in the experiments with human basophils and rat peritoneal mast cells contained (mM): NaCl 137, KCl 2.7, HEPES 10, glucose 5.6, $CaCl_2$ 1.0, $MgCl_2$ 1.0, and NaH_2PO_4 0.4. The 10-times concentrated Ca^{2+} and Mg^{2+}-free buffered Tyrode solution had appropriately altered concentrations of solutes. The bovine serum albumin-Tyrode solution was HEPES-buffered Tyrode solution supplemented with bovine serum albumin (1 mg/ml). The buffers were adjusted to pH 7.2–7.4 by the addition of NaOH.

Test secretagogues compound 48/80, sodium fluoride, phospholipase C, 1,2-dioctanoyl-*sn*-glycerol and A23187 were from Sigma, whereas sheep anti-rat IgE and rabbit anti-human IgE were from ICN and Dako, respectively. All NO releasing compounds, NO inhibitors and haemoglobin were purchased from Sigma. For buffer solutions, the general chemicals used (including HCl, NaOH and perchloric acid) were either from BDH or Hopkins & Williams; HEPES and bovine serum albumin were from Sigma; heparin and Percoll were from CP Pharmaceuticals and May & Baker, respectively. The chemicals for the NO

Table 2
Effect of NO inhibitors on stimulated histamine release from rat peritoneal mast cells
Cells were incubated with vehicle or one of the inhibitors for 30 min before being treated with stimulants anti-rat IgE (1:250), compound 48/80 (0.5 μg/ml) or A23187 (10 μM) for a further 15 min or before being treated with sodium fluoride (NaF, 20 mM), phospholipase C (PLC, 0.05 U/ml) or 1,2-dioctanoyl-*sn*-glycerol (DiC8, 20 μM) for a further 30 min. Results are shown as mean ± S.E.M. of histamine release (%) from three or more separate experiments. All values are corrected for the spontaneous histamine release (ca. 8 %).

Treatment	Inhibitor (μM)	Anti-rat IgE	48/80	NaF	PLC	DiC8	A23187
Stimulant alone	0	21.3 ± 1.6	50.0 ± 1.4	45.4 ± 3.0	53.5 ± 2.8	25.2 ± 2.0	49.5 ± 2.8
L-NAME[a] + stimulant	1	21.0 ± 1.8	51.7 ± 3.0	38.2 ± 0.2[b]	51.1 ± 2.6	26.7 ± 3.3	52.6 ± 0.9
	10	19.9 ± 1.8	50.9 ± 2.3	36.5 ± 1.3[b]	52.6 ± 3.0	27.5 ± 2.4	53.0 ± 3.2
	100	19.2 ± 1.5	47.3 ± 0.8	37.5 ± 3.7	52.3 ± 2.6	26.3 ± 2.4	53.0 ± 3.5
L-NA [c] + stimulant	1	21.5 ± 1.9	48.1 ± 4.1	43.1 ± 2.6	50.5 ± 2.5	25.3 ± 2.3	50.2 ± 2.6
	10	22.7 ± 3.0	43.5 ± 1.1[b]	46.7 ± 5.1	50.5 ± 2.7	27.5 ± 1.8	48.2 ± 3.4
	100	20.5 ± 2.5	46.8 ± 3.5	49.4 ± 4.1	52.2 ± 3.1	26.8 ± 3.0	47.2 ± 3.7
AMG [d] + stimulant	1	22.1 ± 2.1	48.3 ± 1.9	47.8 ± 3.0	53.7 ± 2.4	26.2 ± 2.2	34.5 ± 5.7[b]
	10	22.8 ± 3.1	46.2 ± 1.7	39.5 ± 3.7	52.3 ± 2.9	27.5 ± 2.4	36.6 ± 4.5[b]
	100	23.2 ± 2.0	40.1 ± 1.6[b]	39.5 ± 2.3	52.2 ± 3.2	28.3 ± 1.2	36.6 ± 3.8[b]
MB[e] + stimulant	1	22.7 ± 2.5	48.1 ± 2.1	38.8 ± 7.8	53.2 ± 3.2	28.8 ± 2.0	48.8 ± 6.0
	10	25.1 ± 3.8	46.8 ± 3.3	40.7 ± 6.8	52.7 ± 2.8	28.6 ± 2.5	44.6 ± 5.1
	100	28.6 ± 4.1	48.2 ± 2.7	37.8 ± 7.5	58.7 ± 4.1	40.6 ± 6.5	46.2 ± 4.1

[a] L-NAME = N^{ω}-nitro-L-arginine methyl ester.
[b] Denotes values which are significantly different ($P < 0.05$) from those evoked by the stimulant alone.
[c] L-NA = N^{ω}-nitro-L-arginine.
[d] AMG = aminoguanidine.
[e] MB = methylene blue.

determinations and formation were purchased from Sigma. The cGMP assay kit was obtained from Amersham.

All NO compounds and secretagogues were made up in HEPES-buffered Tyrode solution or 0.9% saline except L-NA, A23187, and DiC8 which were dissolved in dimethyl sulfoxide (DMSO, from Sigma). 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.

Statistical analysis was carried out by means of the unpaired Student's *t*-test, Welch's *t*-test and ANOVA with Dunnett post test. A value of $P < 0.05$ was considered to be significant.

3. Results

3.1. Effect of NO donors and inhibitors on basal and anti-human IgE-induced histamine release from human basophils

Sodium nitroprusside, L-arginine and D-arginine (1–100 μM) had a negligible effect on basal histamine release from human basophils (Table 1). Similarly, neither L-NA, L-NAME, aminoguanidine nor methylene blue (1–100 μM) had any noticeable influence on basal histamine release (results not shown). NO inhibitors had only a small and inconsistent effect on histamine release induced by anti-human IgE. Thus, L-NAME and aminoguanidine produced a mild inhibition whereas methylene blue, L-NA and sodium nitroprusside slightly enhanced the response (Table 1).

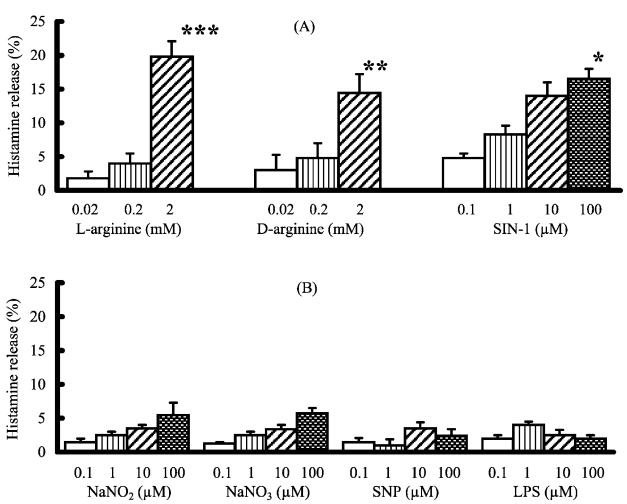

Fig. 1. Effect of NO donors on basal histamine release from rat peritoneal mast cells. (A) Effect of L-arginine, D-arginine and SIN-1. (B) Effect of $NaNO_2$, $NaNO_3$, sodium nitroprusside (SNP) and lipopolysaccharide (LPS). Cells were incubated with vehicle or one of the NO donors for 30 min. Results are shown as mean ± S.E.M. of histamine release (%) from three or more experiments. All values were corrected for the spontaneous histamine release (ca. 8%). *, **, *** Denote values which are significantly different ($^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$) from basal histamine release.

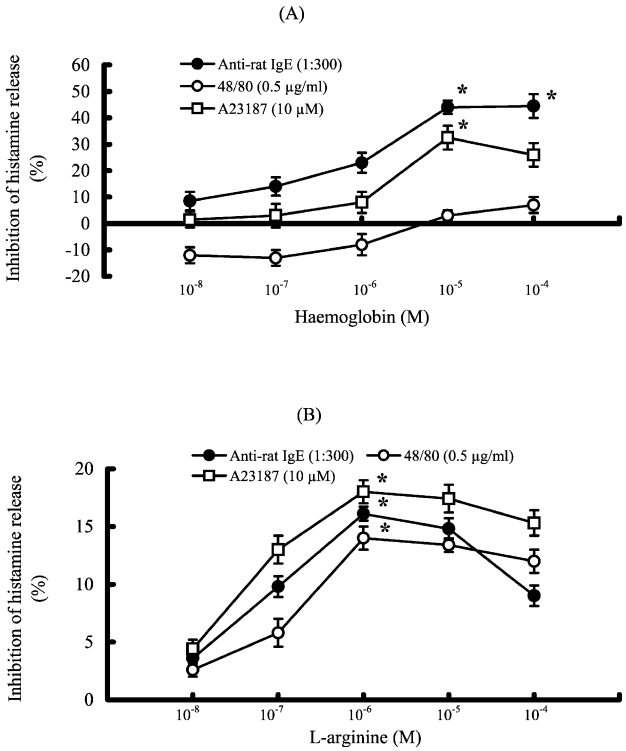

Fig. 2. Effect of (A) haemoglobin and (B) L-arginine on stimulated histamine release from rat peritoneal mast cells. Cells were incubated with vehicle, haemoglobin or L-arginine for 30 min before being stimulated with anti-rat IgE, A23187 or compound 48/80 for 15 min. Results are shown as mean ± S.E.M. of inhibition (%) of histamine release from three experiments. All values were corrected for the spontaneous histamine release (ca. 8%). Control values for stimulated histamine release were 36–49%. * Denotes values which are significantly different ($P < 0.05$) from the controls.

3.2. Effect of methylene blue and NO synthase inhibitors on basal and stimulated histamine release from rat peritoneal mast cells

L-NAME, L-NA, aminoguanidine and methylene blue (1–100 μM) had little effect on basal histamine release from rat peritoneal mast cells, or on the secretion induced by anti-rat IgE, compound 48/80, NaF, A23187, phospholipase C, or DiC8, except that aminoguanidine produced a significant inhibition in the case of A23187 (Table 2).

3.3. Effect of NO donors on basal histamine release from rat peritoneal mast cells

In non-purified rat peritoneal mast cells, $NaNO_2$, $NaNO_3$, sodium nitroprusside or lipopolysaccharide (0.1–100 μM) caused a slight stimulation of basal histamine release ($\leq 5\%$), while L-arginine (0.02–2 mM), D-arginine (0.2–2 mM) and SIN-1 (1– 100 μM) produced rather larger effects (≤ 15–20%, Fig. 1A and B). The response to the enantiomers of arginine was time-dependent and, for example, the release induced by L-arginine (2 mM) increased to ca. 30% after 60 min of incubation. The effects

of L-arginine and D-arginine were not altered by the presence of L-NA or aminoguanidine up to concentrations of 100 μM (data not shown). L- and D-arginine also released histamine from purified rat peritoneal mast cells, although the response was rather less than from unpurified cells (data not shown). In both cases, the effect was independent of added Ca^{2+} and was unchanged when the reaction was carried out in Ca^{2+} and Mg^{2+}-free buffer containing ethylenediaminetetraacetic acid (0.2 mM) as a chelating agent (data not shown).

3.4. Effect of haemoglobin, L-arginine, D-arginine and NO donors on stimulated histamine release from rat peritoneal mast cells

As shown in Fig. 2A, haemoglobin (0.1–100 μM) produced a significant and dose-dependent inhibition of histamine release induced by anti-rat IgE ($\leq 45\%$) and A23187 ($\leq 35\%$) but not compound 48/80. L-arginine showed a mild inhibitory effect (Fig. 2B) whereas D-arginine was essentially inactive (results not shown). At the highest test concentration (100 μM), the two enantiomers had no significant effect on histamine release induced by NaF (20 mM), phospholipase C (0.05 U/ml) or DiC8 (20 μM) (results not shown). Other NO donors, with the exception of sodium nitroprusside (see below), such as lipopolysaccharide, $NaNO_2$, $NaNO_3$, and SIN-1 (all at 100 μM) also had no effect on histamine release evoked by compound 48/80 (0.5 μg/ml), A23187 (10 μM), anti-rat IgE (1:300 dilution), phospholipase C (0.05 U/ml), DiC8 (20 μM) or NaF (20 mM) (results not shown).

3.5. Effect of sodium nitroprusside on stimulated histamine release from rat peritoneal mast cells

Sodium nitroprusside (1–100 μM) had differential effects on histamine release induced by various secretagogues (Fig. 3). Thus, the compound enhanced the stimulatory effect of compound 48/80 and DiC8, caused a weak inhibition of the secretion induced by phospholipase C and produced a dose-dependent inhibition of the histamine release evoked by anti-rat IgE, NaF and A23187. At the highest test concentration (100 μM) of sodium nitroprusside, the corresponding percentage inhibitions for the latter stimuli were $31.5 \pm 2.8\%$, $15.9 \pm 0.7\%$, and $17.6 \pm 2.2\%$ (all with $P < 0.05$).

3.6. Effect of NO donors and stimulants on nitric oxide production from rat peritoneal mast cells

Incubation of purified rat peritoneal mast cells with various stimulants (anti-rat IgE, compound 48/80, NaF

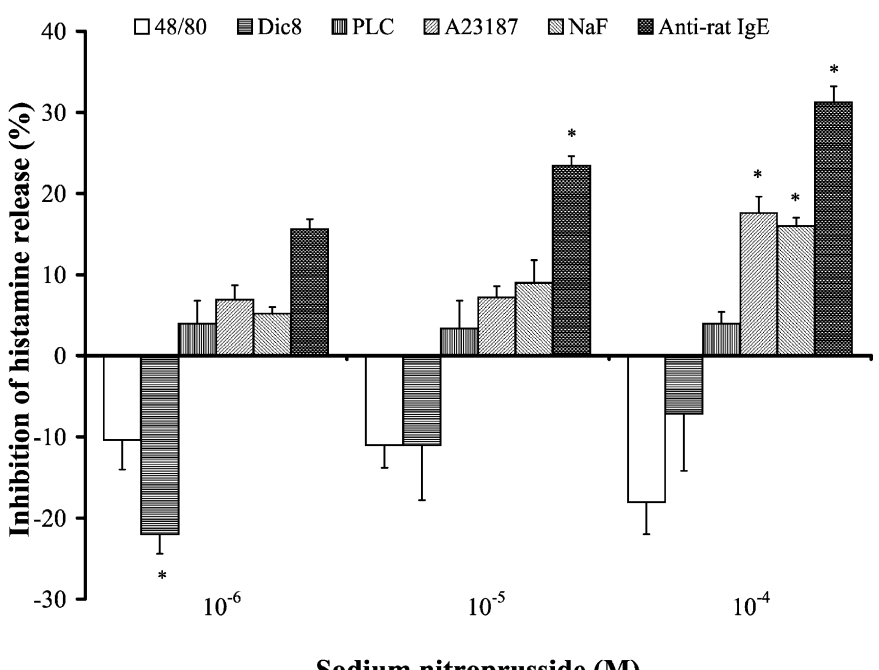

Fig. 3. Effect of sodium nitroprusside (SNP) on stimulated histamine release from rat peritoneal mast cells. Cells were incubated with sodium nitroprusside for 30 min. They were then stimulated with anti-rat IgE (1:250), compound 48/80 (0.5 μg/ml) or A23187 (10 μM) for a further 15 min or stimulated with NaF (20 mM), phospholipase C (PLC, 0.05 U/ml) or DiC8 (20 μM) for a further 30 min. Control samples received vehicle instead of sodium nitroprusside. The results are expressed as mean inhibition (%) of histamine release ± S.E.M. for three or more experiments. The corresponding stimulated histamine releases for anti-rat IgE, compound 48/80, NaF, phospholipase C, DiC8, and A23187 were 31%, 44%, 41%, 57%, 21%, and 45%. All values were corrected for the spontaneous release (ca. 8%). * Denotes values which are significantly different ($P < 0.05$) from the controls.

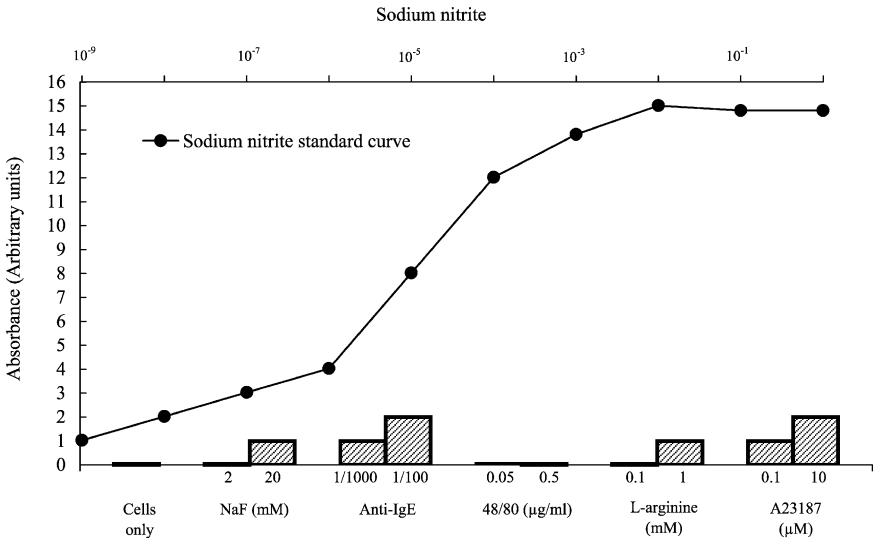

Fig. 4. Effect of various stimulants and NO donors on nitric oxide production, as measured by the diazotization reaction, in purified rat peritoneal mast cells. Cells were incubated with anti-rat IgE, compound 48/80, NaF, A23187 or L-arginine for 30 min. Inorganic nitrite was measured by the diazotization reaction and the change in absorbance expressed per 50,000 cells. The standard curve for sodium nitrite has been superimposed. The results shown are representative of four separate experiments.

and A23187) or the NO donor, L-arginine, had little or no effect on NO production as determined by the diazotization reaction (Fig. 4). In four further experiments, oxyhaemoglobin absorbance was determined following incubation of rat peritoneal mast cells with oxyhaemoglobin and various stimulants: A23187 (0.1 and 10 μM), NaF (2 and 20 mM), anti-IgE (1:1000 and 1:100 dilution), compound 48/80 (0.05 and 0.5 μg/ml), L-arginine (100 μM), D-arginine (100 μM), sodium nitroprusside (100 μM), SIN-1 (100 μM) and lipopolysaccharide (1 mg/ml). In no case did the absorbance values of the treated samples differ from that of the control samples (12 arbitrary units of absorbance per 50,000 cells, data not shown).

Essentially the same results were obtained with non-purified rat peritoneal mast cells (results not shown).

3.7. Effect of NO donors on cGMP levels in rat peritoneal mast cells

The basal level of cGMP in resting, purified rat peritoneal mast cells was 2.8 ± 1.2 pmol/10^6 cells ($n = 4$) and this value was unchanged following incubation with L-arginine, D-arginine or sodium nitroprusside (100 μM) for up to 60 min.

4. Discussion

Mast cells and basophils are the major repositories of histamine and the signal transduction pathways are thought to be similar in both cell types (Beaven and Cunha-Melo, 1988; Razin et al., 1995; Metcalfe et al., 1997). However, little is known about the effect of NO on histamine secretion from basophils. In the present work, it was found that the spontaneous release of the amine or that induced by anti-IgE was not significantly affected by the NO donors sodium nitroprusside, L-arginine or D-arginine, or the NO inhibitors L-NA, L-NAME, aminoguanidine or methylene blue. Iikura et al. (1998) have recently also reported that sodium nitroprusside had no effect on anti-IgE-induced histamine release from basophils in the absence of *N*-acetylcysteine (which increased the biovailability of NO). It would be useful to study further the effect of NO-related compounds on the signal transduction pathways involved in basophil histamine release and to assess the relationship between NO synthase, cGMP levels, NO formation and NO activity in these cells.

L-NAME, L-NA, aminoguanidine and methylene blue were similarly inactive in rat peritoneal mast cells and had little effect on either the basal histamine release or the release induced by anti-rat IgE, compound 40/80, NaF, phospholipase C, DiC8 or A23187, except that aminoguanidine produced a small but significant inhibition of A23187-induced histamine release. In contrast, haemoglobin inhibited histamine release induced by anti-rat IgE and A23187 but not compound 48/80. Haemoglobin and methylene blue are thought to act by binding NO and destroying its biological activity while the remaining compounds inhibit NO synthase. Methylene blue also inhibits the soluble isoform of guanylate cyclase and thus prevents cGMP formation by this route. In interpreting the present results, however, it is important to bear in mind that all of these agents may have other pharmacological effects unrelated to NO. For example, it is known that haemoglobin and methylene blue may generate hydroxyl radicals or superoxide anion (Marczin et al., 1992; Van Dyke and

Saltman, 1996). Also, most of the NO synthase inhibitors are not strictly specific, although it is considered that L-NA has a relative selectivity for the constitutive NO synthase whereas aminoguanidine is more specific than L-NAME for the inducible NO synthase (Nathan, 1992; Griffiths et al., 1993). In addition, L-NAME has antimuscarinic properties (Chang et al., 1997) and aminoguanidine has direct scavenging activities against hydrogen peroxide and peroxynitrite and also inhibits diamine oxidase, which has a role in the metabolism of histamine (Yildiz et al., 1998).

We also studied the effect of a wide range of NO donors on histamine release from rat peritoneal mast cells. The test agents have rather different mechanisms of action. Thus, sodium nitroprusside and SIN-1 spontaneously release NO intracellularly or extracellularly, respectively, while $NaNO_2$ and $NaNO_3$ require intracellular bioactivation. Lipopolysaccharide is an inducer of NO synthase and L-arginine, in contrast to the inactive enantiomer D-arginine, is the natural precursor for NO formation (Bauer et al., 1995; Feelisch, 1998). Of the NO donors tested, only SIN-1 (0.1–100 μM) and high concentrations (2 mM) of L- and D-arginine had any direct histamine releasing effect. In this regard, it should be noted that SIN-1 may have cytotoxic properties (Law et al., 1999). In assessing the role of NO in a given cellular response, the lack of effect of D-arginine as compared to the positive action of L-arginine has often been taken as evidence in favour of the involvement of the oxide. However, in other situations both L-arginine and D-arginine may have similar effects. For example, the two enantiomers are equiactive at increasing insulin levels in isolated mouse islets of Langerhans (Panagiotidis et al., 1995) and both are good scavengers of hydroxyl radicals (Rehman et al., 1997). Arginine may also exert non-specific pH effects due to alkalinisation of the buffer medium by the basic amino acid (Jacob, 1988) or have a direct depolarizing action (Panagiotidis et al., 1995). Since the present work showed that the stimulatory effects of L-arginine and D-arginine on histamine release were not antagonized by L-NA or aminoguanidine, or by the removal of Ca^{2+} which is required for the activity of the Ca^{2+}-calmodulin-dependent constitutive form of NO synthase, the response to these amino acids may similarly involve mechanisms unrelated to NO.

With the exception of L-arginine and sodium nitroprusside, none of the NO donors had any effect on histamine release induced by a range of different stimuli. Low concentrations of L-arginine produced a weak inhibition of the release evoked by anti-IgE, compound 48/80 and A23187, but not that caused by NaF, phospholipase C or DiC8, while the response to sodium nitroprusside varied with the secretagogue. Thus, the compound potentiated the release induced by compound 48/80 and DiC8 but inhibited that evoked by anti-IgE, NaF or A23187. These effects may again be unrelated to NO production and sodium nitroprusside has been shown to induce Ca^{2+} movements and substrate phosphorylation (Huang et al., 1998; Kemalavias and Lincoln, 1996; Miyoshi and Nakaya, 1994; Volk et al., 1997; Xu et al., 1994), two events which may be centrally involved in histamine release (Botana et al., 1992; Pearce et al., 1981; Schneider et al., 1992).

Incubation of purified rat peritoneal mast cells with various secretagogues (anti-rat IgE, compound 48/80, NaF and A23187) or NO donors (L-arginine, D-arginine, sodium nitroprusside, SIN-1 and lipopolysaccharide) had negligible effects on NO production as assessed by the formation of nitrite or oxyhaemoglobin absorbance. Similarly, the enantiomers of arginine and sodium nitroprusside failed to change intracellular cGMP levels. These findings further militate against a role for NO in mast cell activation and imply that any observed effects of NO donors are independent of the oxide.

It should be pointed out that our findings are at variance with those of some other workers who, using different experimental procedures and rodent strains, have claimed that NO may modulate mast cell mediator release. For instance, Salvemini et al. (1991) found that sodium nitroprusside inhibited histamine release, produced NO and increased cGMP levels in purified rat peritoneal mast cells from male Wistar Albino rats. Eastmond et al. (1997) demonstrated that interferon-gamma induced NO production and inhibited the IgE-mediated secretory function of mouse mixed peritoneal mast cells. Hogaboam et al. (1993) reported that L-arginine released NO from purified rat peritoneal mast cells of male Sprague–Dawley rats. Bidri et al. (1995) showed that sodium nitroprusside increased cGMP levels and inhibited IgE-mediated β-hexosaminidase release from mouse bone marrow-derived mast cells. Most recently, Koranteng et al. (2000) showed that NO generated by *S*-nitrosoglutathione (an NO donor) inhibited IgE-mediated serotonin release from mouse peritoneal mast cells and rat peritoneal mast cells from Brown Norway rats. The latter effects were optimal after 24 h in culture although much shorter periods of incubation were reportedly effective in the earlier studies. However, in keeping with our results, Lau and Chow (1999) found that L-NAME and sodium nitroprusside did not affect the spontaneous or anti-rat IgE-induced histamine release from purified rat peritoneal mast cells obtained from Wistar Albino or Sprague–Dawley rats. These authors suggested that the differences between their results and the earlier studies might be due to the higher degree of purity of the mast cell preparations used and the consequent removal of contaminating cells capable of producing NO. In total, the reasons for these disparate findings are not clear but the strain of animals and the detailed experimental conditions could both be important. To this end, we have here used both mixed peritoneal cells and highly purified rat peritoneal mast cells, together with a wide range of NO-active compounds and different secretagogues. In any event, the reported differences stress the need for careful comparative work from independent laboratories.

In conclusion, the present results show that NO does not play a significant role in the signal transduction pathways leading to histamine release from human basophils and rat peritoneal mast cells. However, there is now increasing evidence that the interaction between NO, mast cells and histamine release is important in the regulation of various physiological and pathological conditions such as the modulation of the immune system (Zidek and Mašek, 1998), control of the human nasal airway response (Dear et al., 1995), endotoxin-induced damage to the rat colon (Brown et al., 1998) and inhibition of gastric acid secretion (Kato et al., 1998). It is obvious that further work is required to clarify the role of NO in these systems.

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