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Inhibition of Calpain Is a Component of Nitric Oxide-Induced Down-Regulation of Human Mast Cell Adhesion

Paul Forsythe and A. Dean Befus

Nitric oxide is an important messenger that regulates mast cell activity by modifications to gene expression and intracellular pathways associated with exocytosis and adhesion. Integrin interactions with extracellular matrix components modulate an array of cell activities, including mediator production and secretion. To investigate the molecular mechanisms underlying NO regulation of mast cell function, we studied its effects on adhesion of a human mast cell line (HMC-1) to fibronectin (FN). The NO donors S-nitrosoglutathione and S-nitroso-N-acetylpenicillamine strongly down-regulated the adhesion of HMC-1 to FN. Inhibitors of soluble guanylate cyclase and protein kinase G did not alter the response of cells to NO. A peroxynitrite scavenger did not affect modulation of adhesion by NO, nor could the effect of NO be mimicked by the peroxynitrite-producing compound 3-morpholinosydnonimine. NO donors inhibited the cysteine protease, calpain, while calpain inhibitors mimicked the effect of NO and led to a decrease in the ability of HMC-1 cells to adhere to FN. Thus, NO is an effective down-regulator of human mast cell adhesion.

The mechanism for this action does not involve peroxynitrite or activation of soluble guanylate cyclase. Instead, a portion of NO-induced down-regulation of adhesion may be attributed to inhibition of the cysteine protease, calpain, an enzyme that has been associated with control of integrin activation in other cell types. The inhibition of calpain is most likely mediated via nitrosylation of its active site thiol group. Calpain may represent a novel therapeutic target for the regulation of mast cell activity in inflammatory disorders. The Journal of Immunology, 2003, 170: 287–293.

Mast cells (MCs) are involved in numerous activities ranging from control of the vasculature to tissue injury and repair, allergic inflammation, and host defenses. They synthesize and secrete a wide variety of mediators, modulating the functions of nearby cells, and initiate complex physiological changes. The significant contribution of MC mediators to tissue damage and the propagation of inflammatory responses make control of MC function vital to the management of many inflammatory diseases.

MC bind to the extracellular matrix components, including fibronectin (FN), vitronectin, collagen type I, collagen type IV, and laminin (1–3). Adhesion of MC to these extracellular proteins is dependent upon the activation state of the cell. Naive bone marrow-derived cultured MC or peritoneal MC require prior stimulation to activate the integrins necessary to adhere to extracellular matrix proteins (2, 4, 5). However, the human MC line HMC-1 has been reported to constitutively express active VLA-4 and VLA-5, the integrins that mediate the interaction of these cells with FN, and adhesion of these cells to FN is integrin dependent (6). Thus, HMC-1 cells provide a model of an activated human MC, as may exist in an inflammatory state.

In many cell types, integrin interactions initially thought to play a primarily adhesive role are now known to modulate such diverse activities as cell cycle, apoptosis, differentiation, mediator production, and secretion (7, 8). For example, Ag-induced histamine secretion can be potentiated when RBL-2H3 cells are bound to FN, and this potentiation can be blocked by Abs to VLA-4, VLA-5, and vitronectin receptor integrins (1, 2). In the MC line, mast cell protease S/I stimulation with Ag at concentrations insufficient to cause significant mediator release markedly enhances cell adhesion to FN (9). Thus, interaction of MC with the extracellular matrix can produce phenotypic alterations that are important in cell regulation.

We previously demonstrated that following stimulation of RBL-2H3 cells with IFN-γ, NO plays an autocrine role in down-regulating adhesion to FN, without modifying the expression of integrins on the cell surface (10). This observation added to existing evidence that NO is an important regulator of MC activity, modulating cell survival, mediator secretion, and gene expression (11–15). The ability of NO to act as a potent down-regulator of multiple MC functions suggests that it is impinging upon one or more crucial signaling pathways in the cell.

NO has been demonstrated to inhibit adhesion to FN in a wide range of cell types, including mesangial cell, melanocytes, chondrocytes, neutrophils, and eosinophils (16–20). In the present study, we investigated the ability of NO to modulate the adhesion of human MC to FN. We also assessed whether NO exerted its effects on adhesion by activating soluble guanylate cyclase (sGC) to elevate cGMP levels, or by interaction with superoxide to generate the cytotoxic radical peroxynitrite. Finally, we investigated the involvement of the cysteine protease, calpain, in mediating the effects of NO on adhesion.
Materials and Methods

Cell culture

HMC-1 cells (kindly provided by J. H. Butterfield, Rochester, MN) were cultured in Iscove’s medium containing t-glutamine (Life Technologies, Grand Island, NY), pH 7.4, and supplemented with 10% heat-inactivated FBS and 40 U/ml penicillin/streptomycin (Life Technologies). The human basophilic leukemia cells, KU812 (kindly provided by J. S. Marshall, Dalhousie University, Halifax, Canada), were cultured in RPMI 1640 (Life Technologies) supplemented with 10% heat-inactivated FBS, 2 mM t-glutamine, 10 mM HEPES, and 40 U/ml penicillin/streptomycin (Life Technologies). To differentiate the KU812 cells, they were cultured in the above medium supplemented with 0.3 mM sodium butyrate for 3 days before use in the experiments. For further differentiation under conditions very similar to those that have previously been reported to lead to a more MC phenotype, the cells were cultured in sodium butyrate-supplemented medium for 3 days, followed by 4 days of culture in the sodium butyrate-containing medium with the addition of 50 U/ml human rIFN-γ (Genzyme, Cambridge, MA) (21). All cell cultures were at a concentration of 1 × 10^6 cells/ml before being harvested for use in experiments. Cells were plated in 12-well flat-bottom tissue culture plates (Corning Costar, Kennebunk, ME) at 5 × 10^5 cells in 1 ml to be treated with S-nitrosoglutathione (SNOG), S-nitroso-N-acetylpenicillamine (SNAP), H(3-(2,4)-xocadizolo [4,3-a:quinonxin 1,1-ec (ODQ), Rp-8-[4-chlorophenyl]thio-cGMP-triethylammonium tetrafluoroborate (cGMPs), 1,3-bromo-cGMP, 3-morpholinoisodimminone (SIN-1), peroxynitrite, 5,10,15,20-tetrakis(4-sulfonatophenyl)porphyrinato iron (III) (FeTPPS), calpeptin, calpain inhibitor I (N-acetyl-Leu-Leu-Nle-CHO), and calpain inhibitor II (Z-Val-Phe-CHO) (all obtained from Calbiochem, La Jolla, CA). Treatment time was 3 h at 37°C, unless otherwise indicated.

Cells were then washed (0.8% NaCl, 0.04% KCl, 0.035% NaHCO_3, 0.05% trypsin, 0.53 mM EDTA). The cells were washed with PBS supplemented with 0.03% BSA using a Nunc-Immuno wash. Control wells that were not washed were used to determine the washing buffer using the Nunc-Immuno wash. The plate was washed three times with PBS supplemented with 0.03% BSA using the Nunc-Immuno wash. Blocking with 3% BSA (Sigma-Aldrich, St. Louis, MO; fraction V) did not maximize such that the plate was saturated, as indicated by observations that the cell and is hydrolyzed by cell esterases. The fluorescent signal was proportional to the number of cells in each well. Cells were plated on FN coated with human plasma FN (Life Technologies) at 10^5 cells/ml before being harvested for use in experiments. Cells were then washed (0.8% NaCl, 0.04% KCl, 0.035% NaHCO_3, 0.05% trypsin, 0.53 mM EDTA), and adherent cells were removed from the plate using trypsin EDTA (0.05% trypsin, 0.53 mM EDTA). The cells were washed with medium to remove excess trypsin before use.

Adhesion assay

Flat-bottom 96-well plates (Linbro, Flow Laboratories, McLean, VA) were coated with human plasma FN (Life Technologies) at 10 μg/ml in PBS, pH 7.2, for 1 h. A control experiment was run for 1 h to establish the influence of FN. Cells (4 × 10^7 cells/ml) were treated with NO donors or calpain inhibitors (3 h, 37°C). After stimulation, cells were pelleted and lysed with 100 μl of Tris-HCl (pH 7.3) containing 0.1% Triton X-100 on ice for 10 min and microcentrifuged at 12,000 × g for 10 min at 4°C. Where stated, cell lysates were exposed to 10 mM DTT for 30 min at 4°C. The supernatant (100 μl) was transferred to a 96-well plate (Linbro), and 100 μM calpain substrate (N-succinyl-Leu-Tyr-7-amido-4-methylcoumarin; Sigma-Aldrich) was added to each well. Plates were incubated at 37°C for 30 min, and mean fluorescence was measured on a Cytofluor plate reader (excitation, 360 nm; emission, 460 nm). Calpain activity was measured in fluorescence units. Percentage of inhibition of activity was calculated as: 100 × (activity in sham-treated cells) − (activity in treated cells)/ (activity in sham-treated cells).

Results

NO donors inhibit HMC-1 binding to FN in a concentration-dependent fashion

To determine whether NO could modulate adhesion of HMC-1 cells to FN, cells were pretreated for 3 h with the NO donors SNOG and SNAP (50–1000 μM). Both donors produced a concentration-dependent decrease in HMC-1 cell adhesion (Fig. 1A and B). The maximum response was observed following a 3-h preincubation with either SNAP or SNOG (500 μM) (70.7 ± 5.5% and 68.4 ± 5.8% reduction, respectively; n = 4; p < 0.01). Over the course of the entire study, there was variation in the percentage of untreated HMC-1 cells binding to FN (range 35–95% adhesion; median 67%; n = 42). However, the down-regulation of adhesion induced by NO donors did not vary greatly when taken as a percentage reduction of untreated HMC-1 binding in the individual experiments. In all subsequent experiments, the responses to both NO donors were almost identical, and thus, to avoid repetition, only results obtained with SNOG will be presented.

Investigation of the time course of this response revealed that the effect was rapid, with a statistically significant effect following preincubation of cells with an NO donor for 1 min (16.3 ± 4.2% reduction by 250 μM SNOG; n = 4; p < 0.05) (Fig. 2). Inhibition of adhesion increased with preincubation time up to 3 h. To establish the influence of enhanced integrin affinity on the ability of NO to down-regulate adhesion, cells were treated with SNOG in the presence of Mn^{2+} (10 μM). Mn^{2+} caused a significant increase in basal adhesion to FN (from 64.4 ± 6.6% to 80.1 ± 4.2%; n = 5; p < 0.05), but did not prevent the down-regulation of this adhesion by SNOG (Fig. 3). SNOG and SNAP also caused a concentration-dependent decrease in adhesion of KU812 cells treated with 37°C for 30 min, and mean fluorescence was measured on a Cytofluor plate reader (excitation, 360 nm; emission, 460 nm). Calpain activity was measured in fluorescence units. Percentage of inhibition of activity was calculated as: 100 × (activity in sham-treated cells) − (activity in treated cells)/ (activity in sham-treated cells).

![FIGURE 1. Effect of SNOG (A) and SNAP (B) on adhesion of HMC-1 cells to FN. Cells were treated with the NO donors for 3 h at 37°C, then tested for their ability to bind to FN over 1 h. Results are expressed as percentage of binding. n = 4 independent experiments; * p < 0.01 compared with untreated control.](http://www.jimmunol.org/)
with sodium butyrate and IFN-γ (Fig. 4, A and B). Interestingly, although NO donors also decreased adhesion of KU812 cells treated with sodium butyrate alone (65.7 ± 2.8% inhibition of adhesion with 500 μM SNOG; n = 4), they had no significant effect on less differentiated cells that had not been treated with sodium butyrate before the experiment (data not shown).

The effect of NO is independent of cGMP
Many effects of NO are mediated by stimulation of cGMP production through direct activation of sGC. To determine whether the regulatory effects of NO on adhesion might be through the cGMP/PKG pathway, we used the sGC inhibitor, ODQ, and the protein kinase G inhibitor, Rp-8-cGMPs. ODQ (10 μM) alone significantly reduced basal binding of HMC-1 cells to FN from 40.2 ± 1.3% to 24.0 ± 0.6% (p < 0.01, n = 4). However, ODQ did not block the decrease in adhesion produced by NO, with 250 μM SNOG causing a significant decreasing adhesion in the presence of ODQ (25.2 ± 0.6% to 16.0 ± 0.2%; p < 0.01; n = 4) (Fig. 5A). Similarly, Rp-8-cGMPs (10 μM) alone caused a decrease in basal binding (52.5 ± 4.5% to 36.0 ± 2.0%; p < 0.01; n = 4) (Fig. 5B), while 1000 μM NO caused a significant decrease in adhesion in the presence of Rp-8-cGMPs (23.1 ± 4.3%; p < 0.05; n = 4).

Furthermore, treatment of cells (3 h) with the cell-permeable analog of cGMP, 8-bromo-cGMP (10 and 100 μM), had no effect on their ability to adhere to FN (data not shown).

The effect of NO is independent of peroxynitrite
When superoxide is generated in the presence of NO, these two radicals can interact to generate the toxic peroxynitrite molecule. We adopted several approaches to determine a potential role for peroxynitrite in NO-induced down-regulation of MC adhesion. SIN-1 simultaneously produces NO and superoxide, which react to form peroxynitrite. Treatment of HMC-1 cells with SIN-1 (50–1000 μM) did not significantly reduce the adhesion of HMC-1 cells to FN over the concentrations tested (Fig. 6). Cells were also treated with a bolus of peroxynitrite (50–1000 μM); this also had no effect on HMC-1 adhesion to FN (data not shown). In addition, cells were treated with the peroxynitrite scavenger FeTPPS (5–100 μM), just before addition of SNOG. Modulation of adhesion by SNOG was unaffected by the presence of the peroxynitrite scavenger (Fig. 7).
NO inhibits calpain activity

Given a potential important role for calpain in the adhesion process and previous reports of NO-induced inhibition of cysteine proteases, we measured calpain activity in cells following treatment with SNOG (3 h). A significant decrease (26.1 ± 6.3%, n = 8) in calpain activity was observed following exposure to 250 μM SNOG (Fig. 8). However, increasing the concentration of SNOG to 1000 μM did not significantly enhance this inhibition (37.5 ± 4.3%; n = 8) (Fig. 8A). The inhibition of calpain activity by SNOG could be partially reversed by washing the NO donor from the cells and exposing to DTT following lysis (Fig. 8B). The ability of SNOG-treated cells to bind to FN in the adhesion assay was also recovered when cells were exposed to DTT (10 mM, 30 min, 37°C) before the addition of calcein AM (Fig. 8C).

Calpain inhibitors mimic the effect of NO donors

Having shown that calpain activity is decreased upon treatment of HMC-1 with NO, we determined whether this decrease in calpain activity could account for the down-regulation of adhesion. HMC-1 cells were preincubated (30 min) with the calpain inhibitors N-acetyl-Leu-Leu-Nle-CHO, calpeptin, or Z-Val-Phe-CHO (10–100 μM). Each of these inhibitors mimicked the effect of NO and led to a concentration-dependent decrease in HMC-1 cell adhesion to FN (32.5 ± 0.6%, 42.3 ± 1.3%, and 64.0 ± 1.1% inhibition, respectively, at a concentration of 100 μM) (Fig. 9A).

HMC-1 cells were preincubated with SNOG (100–1000 μM) and then exposed to the calpain inhibitor, calpeptin (100 μM), for 30 min before assessment of adhesion to FN. The decrease in adhesion induced by SNOG and calpeptin was not additive (Fig. 9B), the adhesion of calpeptin-treated cells being significantly different from untreated cells only in the presence of the lowest SNOG concentration used (100 μM). This suggests that SNOG and calpeptin inhibit adhesion via the same mechanism.

Discussion

Our results demonstrate that exposure to NO decreases the adhesion of human MC to FN, supporting previous findings that NO down-regulates adhesion of RBL-2H3 cells (10).

Under conditions of oxidative stress, when both superoxide and NO can be generated simultaneously, the two radicals interact to form peroxynitrite. Peroxynitrite is thought to mediate many of the cytotoxic effects of NO (22, 23). Our investigations of the mechanism of NO-induced inhibition of MC adhesion revealed that peroxynitrite did not down-regulate adhesion to FN and a peroxynitrite scavenger did not affect the response of cells to NO. Thus, the cytotoxic reactive nitrogen oxide species (RNOS) peroxynitrite was not involved in the down-regulation of adhesion by NO, a finding supported by the fact that cell viability, determined by trypan blue exclusion and annexin V binding, was unaffected by treatment with NO donors (data not shown).

Many of the physiological processes mediated by NO, such as vascular smooth muscle relaxation, occur through interaction with the heme group on sGC, resulting in elevation of cGMP (22). Although this represents a major mode of action for NO, in the present study we found the response of HMC-1 cells to NO was...
not influenced by the permeable cGMP analog 8-bromo-cGMP, the guanylate cyclase inhibitor ODQ, nor the PKG inhibitor Rp-8-cGMPs, indicating that sGC activation does not play a role in the down-regulation of adhesion in these cells. Statistics were calculated based on enzyme activity so that SNOG-exposed cells could be compared with untreated controls. n = 8 independent experiments; *, p < 0.05 compared with untreated controls. B, SNOG induced inhibition of calpain activity in the presence (□) or absence (■) of DTT (10 mM, 30 min, 4°C). n = 3; *, p < 0.05. C, SNOG induced decrease in HMC-1 cell adhesion to FN in the presence (□) or absence (■) of DTT (10 mM, 30 min, 37°C). n = 4 independent experiments; *, p < 0.05 compared with unexposed cells.

FIGURE 8. A, The effect of SNOG on calpain activity in HMC-1 cells. HMC-1 cells were treated with the NO donors for 3 h at 37°C, the cells were then lysed, and calpain activity was determined. Results are expressed as percentage of inhibition of enzyme activity compared with untreated cells. Statistics were calculated based on enzyme activity so that SNOG-exposed cells could be compared with untreated controls. n = 8 independent experiments; *, p < 0.05 compared with untreated controls. B, SNOG induced inhibition of calpain activity in the presence (□) or absence (■) of DTT (10 mM, 30 min, 4°C). n = 3; *, p < 0.05. C, SNOG induced decrease in HMC-1 cell adhesion to FN in the presence (□) or absence (■) of DTT (10 mM, 30 min, 37°C). n = 4 independent experiments; *, p < 0.05 compared with unexposed cells.

FN-coated surface in a concentration-dependent manner. Treatment with exogenous NO or catabolic cytokines that induce NO synthase blocked the assembly of F-actin, focal adhesion kinase (FAK), rho A, and tyrosine-phosphorylated proteins, which make up the focal adhesion complex (24). The use of cGMP antagonists and agonists abolished or mimicked this effect, respectively, suggesting that the activation of sGC is essential to the mode of action of NO in these events. Murphy-Ullrich et al. (26) provided further evidence for the involvement of sGC activation in the down-regulation of cell adhesion. They demonstrated that cGMP-dependent protein kinase is required for thrombospondin- or tenascin-dependent focal adhesion disassembly and cytoskeletal reorganization.

Two previous studies report NO induced elevation of cGMP levels in the MC (27, 28). However, one of these studies used rat peritoneal cells with a high proportion of non-MC and used non-MC-selective activating agents (27). In addition, neither study investigated the effects of guanylate cyclase inhibitors on cGMP levels in these systems.

In support of our findings, the relatively high levels of exogenous NO used to mimic the effect of IFN-γ-induced NO on RBL-2H3 cells (10) and those used in the present study make direct signaling by NO unlikely. Furthermore, Deschoolmeester et al. (29) demonstrated that NO-induced down-regulation of serotonin

FIGURE 9. A, The effect of calpain inhibitors on HMC-1 cell adhesion to FN. HMC-1 cells were treated with calpain inhibitors for 30 min at 37°C, then tested for their ability to bind to FN over 1 h. Results are expressed as percentage of binding. n = 4 independent experiments; *, p < 0.05 compared with untreated control. B, The effect of calpeptin exposure on SNOG induced down-regulation of HMC-1 cell adhesion to FN. Cells were preincubated with SNOG for 3 h at 37°C and exposed to calpeptin for 30 min before assessment of their ability to adhere to FN. n = 4 independent experiments; *, p < 0.05, comparing cells in the presence (□) or absence (■) of calpeptin (100 µM).
release from anti-IgE-stimulated rat peritoneal MC was independent of cGMP production and peroxynitrite. Taken together with the present study, these observations suggest that NO controls MC function through an sGC-independent mechanism that involves interactions with specific proteins or factors important to both adhesion and secretion-coupling events.

At high concentrations of NO, as produced by the inducible form of NOS, and under aerobic conditions, NO is rapidly oxidized to RNS, particularly N2O3. RNS are unstable and can readily nitrosate amines and thiols (22).

Reports that NO-induced formation of nitrosothiol is important in the modulation of signal transduction pathways and gene expression of cells prompted us to examine a potential target for nitrosothiol formation that may play an important role in adhesion signaling (22, 30–34). Work on platelets has shown that the cysteine protease, calpain, is one of the signaling molecules activated following integrin-ligand interactions (35), suggesting it may modulate intracellular events required for effective adhesion. Once activated, calpain can selectively cleave a variety of substrates, many of which are membrane and cytoskeletal proteins. These substrates include talin, paxillin, ezrin, actin-binding protein, and the microtubule-associated proteins mitogen-activated protein-2 and tau (36–43). Calpain also proteolytically modifies several proteins associated with integrin-mediated focal adhesion formation, such as protein kinase C and FAK, suggesting that calpain plays an important role in cell adhesion and migration (42, 44). Inhibition of calpain has been shown to result in disassembly of stress fibers, loss of focal complexes, and focal adhesions in endothelial cells. In this system, calpain appears to mediate integrin-induced signaling at sites upstream of both Rac1 and RhoA (45). Furthermore, inhibition of calpain activity in T cells decreases the ability of the cells to adhere and spread on FN (46).

In our study, we have shown that NO inhibits calpain activity in HMC-1 cells, and that the use of calpain inhibitors can mimic, to some degree, the effect of NO on adhesion. This indicates that the inhibition of calpain, presumably through nitrosylation of the active site cysteine, is responsible for a significant proportion of NO-induced down-regulation of adhesion in MC. The observation that exposure of SNOG-treated cells to a calpain inhibitor does not produce an additive effect supports this conclusion. It has previously been demonstrated that NO can inhibit calpain through S-nitrosylation of the active site cysteine residue. This mechanism is supported in our study, as DTT, an agent that removes thiol-bound NO groups from proteins (34, 47), reverses the inhibition of calpain by NO. Although exposure of cells to DTT also appeared to prevent SNOG-induced down-regulation of adhesion, the possibility of DTT having cell surface effects unrelated to calpain cannot be excluded.

However, the degree of calpain inhibition produced by 250 μM SNOG is not significantly increased at higher concentrations of the NO donor, while cell adhesion continues to decrease. This suggests that, at higher concentrations, NO may interact with other, as yet unidentified, target molecules that are responsible for a further decrease in ability of human MC to adhere to FN. These additional target molecules could include other cysteine proteases, such as caspasas that are known to play a role in cleavage of FAK (48, 49). However, we have observed that Z-VAD-FMK, an inhibitor of caspasas 1, 3, 4, and 7, does not alter MC adhesion to FN (our unpublished observations).

There are two mechanisms that regulate cell adhesion to the extracellular matrix via β1 integrins. The first involves modulation of the β1 integrins, resulting in increased affinity of the receptor for extracellular matrix proteins. Activation of the cells by divalent cations (e.g., Mn2+) stimulates adhesion to FN by increasing integrin affinity. The second mechanism, which is avidity related, is initiated by biochemical events within the cell. The biochemical role of calpain in MC adhesion is undoubtedly complex. Given that calpain can cleave a number of cytoskeletal-associated proteins, such as talin, cortactin paxillin, and the β1 integrin cytoplasmic tail (39, 40, 50), the enzyme may have a role in both focal contact formation and reorganization of the actin cytoskeleton. In T cells it has been demonstrated that calpain cleaves PTP-1B, causing the relocation of the 42-kDa form of the phosphatase from the endoplasmic reticulum to sites of focal contacts via association with p130Cas (51). It is suggested this translocation allows the phosphatase to mediate inside-out signaling events involved in integrin function and cytoskeletal assembly (51). It has also been suggested that calpain may be important during the early events in focal contact formation by cleaving cytoskeletal-associated proteins tethered to integrins, thus freeing the integrins to cluster and enhance adhesion via increased avidity (50). Such a mechanism is supported by our finding that NO decreases adhesion despite treatment of the cells with Mn2+ to enhance avidity. Furthermore, we have observed that once cells have bound to FN, subsequent treatment with NO does not affect their ability to adhere (data not shown). However, given the complexity of integrin signaling and the large number of potential substrates for calpain that have a role in these signaling pathways, any number of mechanisms could be in play. We are undertaking further studies to address this issue. As with the NO-dependent decrease in adhesion of RBL-2H3 cells induced by IFN-γ, preliminary results suggest that changes in integrin expression are not involved in NO donor- or calpain inhibitor-induced down-regulation of adhesion in human MC (our unpublished observations).

In conclusion, we have determined that NO is an effective inhibitor of human MC adhesion to FN. Like NO-induced inhibition of MC degranulation, the mechanism of action does not involve direct activation of sGC or the reactive intermediate peroxynitrite. In addition, evidence presented in this work suggests that the cysteine protease calpain is specific molecular target for NO that may play an important role in the regulation of MC activity and could represent a novel therapeutic target for the regulation of MC activity in inflammatory disorders.

References


