Nitric oxide reacts with intracellular glutathione and activates the hexose monophosphate shunt in human neutrophils: Evidence for S-nitrosoglutathione as a bioactive intermediary

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ABSTRACT We performed experiments to determine whether nitric oxide promoted the formation of intracellular S-nitrosothiol adducts in human neutrophils. At concentrations sufficient to inhibit chemotactic-induced superoxide anion production, nitric oxide caused a depletion of measurable intracellular glutathione as determined by both the monobromobimane HPLC method and the glutathione reductase recycling assay. The depletion of glutathione could be shown to be due to the formation of intracellular S-nitrosoglutathione as indicated by the ability of sodium borohydride treatment of cytosol to result in the complete recovery of measurable glutathione. The formation of intracellular S-nitrosylated compounds was confirmed by the capacity of cytosol derived from nitric oxide-treated cells to ADP-ribosylate glyceraldehyde-3-phosphate dehydrogenase. Depletion of intracellular glutathione was accompanied by a rapid and concomitant activation of the hexose monophosphate shunt (HMPS) following exposure to nitric oxide. Kinetic studies demonstrated that nitric oxide-dependent activation of the HMPS was reversible and paralleled nitric oxide-induced glutathione depletion. Synthetic preparations of S-nitrosoglutathione shared with nitric oxide the capacity to inhibit superoxide anion production and activate the HMPS. These data suggest that nitric oxide may regulate cellular functions via the formation of intracellular S-nitrosothiol adducts and the activation of the HMPS.

Nitric oxide (NO) has been implicated as a cellular mediator which regulates stimulated responses of human neutrophils. Reported effects of NO on neutrophils include the inhibition of superoxide anion production and adhesion to endothelial cells (1-3). The biochemical mechanisms by which extracellular NO affects intracellular signaling in neutrophils are poorly understood. NO is a highly reactive (τ1/2 < 15 sec) free radical which can regulate the activity of proteins through a variety of posttranslational modifications, including the nitrosylation of transition-metal complexes and thiols (4). Potential sites of NO iron-complex targeting in cells include the heme groups or nonheme iron of enzymes such as aconitase or other enzymes of the mitochondrial respiratory pathway (4). The attack upon such enzymes may account for NO-dependent cytotoxicity (5). Nitrosylation reactions have also been implicated in signaling: the activation of guanylyl cyclase by the binding of NO to its heme iron is believed to mediate smooth muscle dilation and inhibition of platelet aggregation. Increases in cyclic GMP, however, do not appear to mediate NO-dependent inhibition of neutrophil superoxide anion production (1).

A separate reaction of potential importance involves the S-nitrosylation of free thiol groups (6). In human plasma, the predominant redox forms of NO are S-nitrosothiols, the most abundant of which is S-nitrosoalbumin (6). Such S-nitrosothiol compounds, which also include S-nitrosocysteine and S-nitrosoglutathione, assume bioactivity through their capacity to donate NO and may therefore serve as stable intermediaries. It has been speculated that this bioactive extracellular pool of S-nitroso proteins serves as a source of NO, buffering its free concentration (6). These observations have suggested that NO also exerts effects within cells by reacting with intracellular thiols. We therefore examined the effects of NO on intracellular glutathione, glucose metabolism, and oxidant production in human neutrophils. Our data indicate that extracellular NO reacts rapidly with intracellular glutathione to form a nitrosylated adduct which may regulate cellular functions.

METHODS

Preparation of Neutrophils. Neutrophils were isolated from whole blood (1). In selected experiments, neutrophils were permeabilized by using a Bio-Rad Pulser cuvette (7).

Neutrophil Function Studies. Superoxide release by activated neutrophils or the cell-free NADPH oxidase was assayed as described (1). Activation of the hexose monophosphate shunt (HMPS) was assessed with [1-14C]glucose at 1 μCi/ml (4 mM), obtained from DuPont/NEN (1 μCi = 37 kBq). HMPS activity was calculated from the production of 14CO2 from [1-14C]glucose (8).

Preparation of NO, S-Nitrosocysteine, and S-Nitrosoglutathione. NO solutions were prepared after bubbling NO gas through isotonic Hepes buffer (9). NO solutions were quantitated by using 100 μM thionitrobenzoic acid in 0.1 M Tris (pH 8.1) at 37°C for 10 min (9). S-Nitrosocysteine and S-nitrosoglutathione were prepared by reaction of reduced cysteine or glutathione with red agaro as described below and quantitated by measuring the mercuric chloride release of nitrite in the Greiss reaction (9).

Glutathione Measurements. Neutrophils were incubated with various concentrations of NO at 37°C for 5 min. Cells were lysed by freeze–thaw after addition of an equal volume of 20 mM Tris (pH 7.4) containing lysophosphatidylcholine (50 μg/ml) and diisopropyl fluorophosphatase. After microcentrifugation at 10,000 × g for 1 min, lysate was assayed for cellular glutathione by the glutathione reductase recycling assay (10) and by the monobromobimane derivatization of glutathione and separation by C18 HPLC (11).

NaBH4 Treatment. To break an S-nitroso bond (12), one-half volume of cytosol was incubated with 0.1 M Nabh4 at

Abbreviations: GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMPS, hexose monophosphate shunt; PMA, phorbol 12-myristate 13-acetate.

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37°C for 5 min. The solution was acidified to remove unreacted NaBH₄, and NaOH was added to obtain neutral pH. The sample was analyzed for glutathione as described above.

**Treatment of Protein with Red Agarose.** Synthetic S-nitrosothiol derivatives were measured utilizing Bio-Gel A-S-nitrosothiol (red agarose) as described (12).

**Subcellular Fractionation.** Polymorphonuclear leukocytes were disrupted by N₂ cavity at 350 psi (1 psi = 6.89 kPa) for 20 min at 4°C in relaxation buffer (100 mM KCl/3 mM NaCl/3.5 mM MgCl₂/1 mM ATP/10 mM Hpes, pH 7.3) plus protease inhibitors (phenylmethylsulfonyl fluoride, leupeptin, pepstatin A, chymostatin, and aprotinin) as described (1).

**ADP-Ribosylation.** Subcellular fractions (20 μg) were incubated for 30 min at 30°C in 40 μl of 5 mM [³²P]NAD (20–40 Ci/mmol)/50 mM Tris, pH 8.0, in the presence of NO or samples. Reactions were terminated by the addition of Laemmli buffer and boiling for 5 min. ADP-ribosylated proteins were visualized by SDS/PAGE and autoradiography (13).

### RESULTS

**Effect of NO and S-Nitrosothiols on Production of Superoxide Anion.** Table 1 illustrates the effects of NO and S-nitrosoglutathione on superoxide anion production by human neutrophils. NO (10–100 μM) caused the dose-dependent inhibition of superoxide generation in response to the chemottractant fMet-Leu-Phe (0.1 μM). Preincubation of neutrophils with S-nitrosoglutathione had no effect on superoxide production. However, S-nitrosoglutathione effectively inhibited the broken-cell NADPH oxidase superoxide-generating system: the addition of S-nitrosoglutathione (80 pmol/μg of protein) 10 min before arachidonate activation (16 min before NADPH initiation), reduced superoxide release from 507 ± 75 to 272 ± 41 nmol/min per mg of protein (P = 0.0056). The potency of S-nitrosoglutathione in the cell-free system was equivalent to that of authentic NO. We hypothesized that the difference between whole-cell and broken-cell reconstitution measurements was due to the inability of intact neutrophils to transport extracellular glutathione (14). Therefore, neutrophils were permeabilized prior to incubation with NO and its derivative. Electroporation-amplified neutrophils produced significant amounts of superoxide anion in response to fMet-Leu-Phe (16 ± 3 nmol per 10⁶ electroporated neutrophil cell). Exposure of electroporation-amplified neutrophils to S-nitrosoglutathione before addition of fMet-Leu-Phe significantly reduced stimulated superoxide production (Table 1). Electroporation-amplification did not enhance the capacity of NO to inhibit superoxide. Reduced glutathione did not inhibit superoxide production in any system tested.

**Extracellular NO Depletes Intracellular Glutathione.** We next performed a series of studies to examine whether NO interacted with glutathione to form an S-nitroso intermediate. Fig. 1 illustrates the dose–response of NO-induced depletion of cellular glutathione, as measured by the glutathione reductase recycling assay (10). As shown, measurable glutathione fell to 25% of control values in the presence of 100 μM NO; half-maximal depletion was observed at 30 μM NO. This observation was confirmed with the monobromobimane HPLC method: the addition of 100 μM NO for 2 min decreased glutathione from 1.8 ± 0.5 to 0.4 ± 0.2 nmol per 10⁶ cells (P < 0.01).

To test the hypothesis that a decrease in measurable cellular glutathione was due to the formation of intracellular S-nitrosoglutathione, we treated cytosol prepared from intact neutrophils previously exposed to NO with NaBH₄, which breaks S-nitrosothiol bonds (12). As shown by glutathione reductase recycling assay (Fig. 1) and confirmed with the monobromobimane HPLC method (data not shown), NaBH₄ treatment of such cytosol resulted in a complete recovery of measurable glutathione.

These data indicate that the apparent decrease of total measurable glutathione in NO-treated neutrophils can be accounted for by the conversion of glutathione to a nitrosylated species not reported by either the monobromobimane HPLC or glutathione reductase recycling assays.

**Effect of NO and S-Nitrosothiols on the HMPS Pathway.** We next performed studies to determine whether the decline of cellular glutathione reported by our assays was also "sensed" by the cell, as would be reflected by activation of the HMPS (14, 15). The addition of NO provoked a rapid activation of the HMPS in resting neutrophils (Fig. 2). This effect was reversed in the presence of extracellular 200 μM hemoglobin, which scavenges NO. Sodium nitroprusside and S-nitrosocysteine, but not S-nitrosoglutathione, also acti-

### Table 1. Effect of NO and S-nitrosoglutathione on fMet-Leu-Phe-stimulated superoxide release in electroporameabilized (EP) and intact neutrophils

<table>
<thead>
<tr>
<th>Agent</th>
<th>Conc., μM</th>
<th>Superoxide release, nmol/5 min per 10⁶ cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Intact</td>
</tr>
<tr>
<td>NO</td>
<td>10</td>
<td>19.2 ± 0.6*</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>13.0 ± 1.0*</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>9.6 ± 0.4*</td>
</tr>
<tr>
<td>SNO-GSH</td>
<td>10</td>
<td>24.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>25.0 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>100</td>
<td>22.0 ± 1.9</td>
</tr>
</tbody>
</table>

Intact or electroporameabilized neutrophils (1.25 × 10⁶ per ml) were incubated in the absence (control) or presence of NO, or S-nitrosoglutathione (SNO-GSH) (concentration varied, 5 min, 37°C) before exposure to 0.1 μM fMet-Leu-Phe. fMet-Leu-Phe-stimulated superoxide release (control, data not shown) was 24.0 ± 5 nmol and 16 ± 3 nmol of cytochrome c reduced in 5 min for 10⁶ intact and 10⁶ electroporameabilized neutrophils, respectively. *P < 0.003 vs. control; **P < 0.01 vs. control.

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**Fig. 1.** Effect of NO on cellular glutathione. Neutrophils (5 × 10⁶) were incubated with various concentrations of NO (time varied, 37°C). Cells were lysed by freeze-thaw after addition of an equal volume of 20 mM Tris (pH 7.4) containing lysophosphatidylcholine (50 μg/ml) and phenylmethylsulfonyl fluoride (1 μg/ml). After microcentrifugation at 10,000 × g for 1 min, lysate was processed for glutathione measurement. One-half volume of cytosol was incubated with 0.1 M NaBH₄ at 37°C for 5 min, to break S-nitroso (SNO) bonds. The solution was acidified to remove unreacted NaBH₄, and NaOH was added to obtain neutral pH. Untreated cytosol and NaN₃-treated cytosol were assayed for total glutathione by the glutathione reductase recycling assay, which measures the total glutathione (reduced and oxidized, GSH and GSSG) (10). Control neutrophil cytosol (in absence of NO exposure) contained 1.4 ± 0.7 nmol of glutathione per 10⁶ cells (equals 100% total glutathione).
products of the GAPDH, S-nitrosocysteine, S-nitrosoalbumin, red agarose-treated could cell types produces S-Nitroso Adducts with NO-Like ADP-ribosylated (GAPDH) (16). Hammock preparations product reaction deficient of NO stimulated glyceraldehyde-3-phosphate dehydrogenase (16).

MMPS in intact human neutrophils. Neutrophils (4 × 10^7 per ml) were incubated in the absence (control) or presence of NO S-nitrosocysteine (SNO-CYS), S-nitrosogluthathione (SNO-GSH), phorbol 12-myristate 13-acetate (PMA), sodium nitroprusside (SNP), or the prostaglandin E2 analog misoprostol (PGE2) for 60 min at 37°C. Hammock activity was tested by determining the amount of [1-14C]glucose evolved from [1-14C]glucose (8). S-Nitrosoglutathione did activate the HMPS in electrophoreseamobilized neutrophils (see text). Data represent the mean and SEM of at least three separate determinations in cells from different donors.

vated the HMPS. Interestingly, however, exposure of electrophoreseamobilized neutrophils to 0.1 mM S-nitrosoglutathione for 5 min at 37°C did activate the HMPS (1270 cpm to 7070 cpm). Activation of the HMPS by NO and its derivatives was of a magnitude comparable to that provoked by PMA (Fig. 2). The effects of NO were compared with those of misoprostol, a synthetic prostaglandin E2 analog. At concentrations sufficient to inhibit superoxide generation (70 ± 9% control, P < 0.01), 1 μM misoprostol did not activate the HMPS. Misoprostol also did not lower intracellular levels of glutathione (103 ± 7% control, n = 3).

The kinetics of NO-dependent activation of the HMPS and depletion of cellular glutathione were analyzed. Exposure of neutrophils to 100 μM NO resulted in the rapid release of 14CO2, detectable at 30 sec and complete by 5 min (Fig. 3A). Following exposure to NO, total cellular glutathione reached its nadir between 2 and 5 min and returned to baseline by 15 min. PMA exposure resulted in a gradual decrease in total cell glutathione that was first measurable at 5 min and that, unlike the response to NO, continued during the 60-min observation period (Fig. 3B).

S-Nitrosylation of Glutathione and Thiols in Cytosol Produces S-Nitroso Adducts with NO-Like Activity. S-nitrosylation of proteins in purified cytosol preparations. To determine whether thiol-containing proteins present in neutrophil cytosol could serve as targets for S-nitrosylation, we utilized the red-agarose technique to produce S-nitrosothiol species (12). We analyzed the biological activity of the red-agarose reaction product derived from a variety of substrates, including preparations of purified neutrophil cytosol. We used as a measure of NO activity the capacity to ADP-ribosylate a 37-kDa cytosolic protein which has been identified in other cell types as glyceraldehyde-3-phosphate dehydrogenase (GAPDH) (16). NO stimulated the ADP-ribosylation of GAPDH, as expected (Fig. 4). The red-agarose reaction products of the thiol-containing substrates S-nitrosothiolatation, S-nitrosocysteine, S-nitrosalbumin, including unidentified S-nitroso adducts formed in cytosol fractions, also ADP-ribosylated GAPDH. Neither oxidized glutathione nor red agarose-treated oxidized glutathione induced the ADP-ribosylation of GAPDH (data not shown). These results show that S-nitrosoglutathione can serve as an intermediary of NO activity and that stable S-nitroso adducts can be formed in neutrophil cytosol.

S-nitrosylation of proteins in intact neutrophils exposed to extracellular NO. We next utilized the ADP-ribosylation of GAPDH as an assay to determine whether extracellular NO induced the formation of intracellular S-nitroso intermediates. Utilizing the information provided by the kinetics of depletion of cellular glutathione, we examined the lysates of neutrophils in the GAPDH assay after 0, 2, or 15 min of

![Fig. 2](image-url)

**FIG. 2.** NO, S-nitrosocysteine, and sodium nitroprusside activate the HMPS in intact human neutrophils. Neutrophils (4 × 10^7 per ml) were incubated in the absence (control) or presence of NO S-nitrosocysteine (SNO-CYS), S-nitrosogluthathione (SNO-GSH), phorbol 12-myristate 13-acetate (PMA), sodium nitroprusside (SNP), or the prostaglandin E2 analog misoprostol (PGE2) for 60 min at 37°C. Hammock activity was tested by determining the amount of [1-14C]glucose evolved from [1-14C]glucose (8). S-Nitrosoglutathione did activate the HMPS in electrophoreseamobilized neutrophils (see text). Data represent the mean and SEM of at least three separate determinations in cells from different donors.

![Fig. 3](image-url)

**FIG. 3.** Kinetics of NO-dependent glutathione depletion and HMPS activation. Neutrophils (4 × 10^7 per ml) were incubated in the presence of NO (0.1 mM) A or PMA (50 ng/ml) B (time varied, 37°C). HMPS activity was tested as described (8). Total cellular glutathione was measured as described in Fig. 1. Data are representative of four experiments performed in duplicate. PMN, polymorphonuclear leukocytes (neutrophils).

![Fig. 4](image-url)

**FIG. 4.** S-Nitroso adducts formed in cytosol ADP-ribosylate GAPDH. Cysteine (1 μmol), glutathione (1 μmol), albumin (1 mg), and cytosol (1 mg) were incubated for 10 min at 37°C with red agarose (1 mg) as described in Methods. A separate preparation of cytosol (10 μg) was incubated for 30 min at 30°C in ADP-ribosylation buffer [5 μM [35]P]NAD (20-40 μCi/μmol)/50 mM Tris, pH 8.0] with compounds to be tested for the capacity to ADP-ribosylate the 37-kDa GAPDH. ADP-ribosylated proteins were visualized by SDS/PAGE and autoradiography. The concentration of each compound was as follows: pertussis toxin (5 μg), sodium nitrite (NaNO2, 30 μM), NO (30 μM), glutathione (GSH, 100 μM), S-nitrosogluthathione (SNO-GSH, 100 μM), cysteine (100 μM), S-nitrosocysteine (SNO-cysteine, 100 μM), albumin (3 μg), S-nitrosocysteine (SNO-albumin, 3 μg), cytosol (3 μg), or S-nitrosylated cytosol (SNO-cytoplasm 3 μg).
In separate studies we demonstrated that thiols present in cytosol assumed bioactivity following S-nitrosylation. We used as an assay of NO activity the capacity of S-nitrosylated proteins to ADP-ribosylate GAPDH (16). There is recent controversy regarding whether the modification of GAPDH represents the covalent binding of NADP rather than ADP-ribose (18). However, in either case, the modification of GAPDH is NO-dependent. By means of the red-agarose method of S-nitrosothiol preparation, we generated compounds in purified cytosol preparations which induced the ADP-ribosylation of GAPDH. Of potentially greater interest, we demonstrated S-nitrosylation of proteins in intact neutrophils which had been exposed to extracellular NO by the capacity of such cytosol to ADP-ribosylate GAPDH. Interestingly, the kinetics of S-nitrosylation of neutrophil thiols paralleled the kinetics of glutathione depletion and replenishment. Only lysates derived from neutrophils exposed to NO for 2 min, corresponding to the nadir of measurable glutathione, were capable of promoting ADP-ribosylation of GAPDH. Since the S-nitrosothiols detected in lysates 2 min after NO exposure were stable ($t_{1/2} > 2$ hr), their absence 15 min after NO indicates an active process which reverses S-nitrosylation in vivo. The identity(ies) of the biologically active, nitrosylated species generated in cytosol following NO exposure requires investigation. However, our data suggest that intracellular S-nitrosogluthathione is among the products of the reaction between extracellularly derived NO and intracellular glutathione.

The above studies demonstrated that (i) synthetic S-nitrosglutathione could function as NO donor and (ii) that thiols present in neutrophil cytosol could serve as targets of S-nitrosylation and function as NO intermediaries. Separate studies indicated that neutrophils exposed to extracellular NO converted intracellular glutathione to an S-nitroso adduct. Using two independent methods, the monobromobimane HPLC method and the glutathione reductase recycling assay, we showed that the exposure of intact neutrophils to NO caused a dose-dependent depletion of measurable intracellular glutathione. The dose dependence for NO depletion of glutathione was similar to that observed for NO-dependent inhibition of superoxide production ($EC_{50} \approx 30 \mu M$). In both glutathione assay systems, NaBHB treatment of cytosol derived from neutrophils exposed to NO completely restored measurable glutathione to control levels. Since NaBHB is known to break S-nitrosothiol bonds (12), these data indicate that the depletion of cytosolic glutathione is due to the formation of S-nitrosogluthathione not reported by the monobromobimane HPLC method (which requires reduced sulfhydryl) or the glutathione reductase recycling assay (which measures both reduced and oxidized glutathione).

The biochemical and HPLC evidence that NO decreased intracellular glutathione was supported by physiological evidence: the rapid and concomitant activation of the HMPS in resting neutrophils following exposure to nitric oxide. Activation of the HMPS in intact cells was shared by the NO donors sodium nitroprusside and S-nitroso cysteine, but not by S-nitroso glutathione or a prostaglandin analog. However, consistent with its effects on superoxide production, S-nitroso glutathione did activate the HMPS when introduced into the cytosol after electroporation. The very rapid (second) decline of glutathione and activation of the HMPS following exposure to NO contrasted with the more gradual (minutes) changes observed following exposure to PMA. The kinetics of the PMA effect are consistent with its capacity to provoke oxidant production by neutrophils, an effect which also requires a lag phase of 2–3 min. The activation of the respiratory burst in response to PMA would be expected to oxidize glutathione, decrease intracellular NADPH, and thereby trigger the activity of the HMPS pathway. In contrast to PMA, NO does not activate the respiratory burst in resting

**DISCUSSION**

In the extracellular compartment there is evidence that the reaction between NO and extracellular thiol-containing proteins results in the formation of stable S-nitroso adducts which have the properties of endothelium-derived relaxing factor (6). It has been suggested that extracellular S-nitroso proteins, present in human plasma at micromolar concentrations, prolong the half-life of NO in the blood and tissues (4, 6). The studies reported here provide evidence that NO reacts with thiols in human neutrophils, including glutathione, to form stable, bioactive intermediaries which exert effects on oxidant production and glucose metabolism. NO inhibited superoxide generation by intact neutrophils exposed to the chemoattractant fMet-Leu-Phe. S-Nitrosogluthathione, which had no effect on the intact cell, did inhibit superoxide release in the reconstituted broken-cell NADPH oxidase system. Since it is known that glutathione is not readily taken up by cells (14), the absence of an S-nitrosoglutathione effect on intact neutrophils was most likely due to an inability to gain access to the intracellular compartment. Indeed, we demonstrated that the introduction of S-nitrosoglutathione into the cytosol by means of electroporation resulted in an effective inhibition of the respiratory burst. The capacity of S-nitrosoglutathione to serve as an NO intermediary in the intracellular compartment was further supported by its ability to activate the HMPS in electroporated neutrophils, to ADP-ribosylate GAPDH in purified cytosol preparations, and, as previously reported, to activate the guanylyl cyclase of human lymphocytes (17) and inhibit platelet aggregation (12).
neutrophils (1), and therefore the mechanism by which it depletes reduced glutathione and activates the HMPS requires an alternative explanation. Concomitant with the conversion of S-nitrosogluthathione to oxidized glutathione is the utilization of NADPH and activation of the HMPS which rapidly restores reduced glutathione levels to baseline levels.

Our observations are consistent with those of Albina and Mastrofrancesco (19), who demonstrated that treatment of elicited rat macrophages with \( \text{N}^\text{G} \)-monomethyl-L-arginine inhibited basal activity of the HMPS. Mauel and Corradin (20) have demonstrated in cytokine activated macrophages an increase in the production of nitrite that is accompanied by activation of the HMPS. We suggest that the observations reported by those authors can be explained by the capacity of NO, in either an autocrine or paracrine fashion, to react with and deplete intracellular glutathione.

Our data have implications for both signaling and for susceptibility to NO-dependent cytotoxicity. With regard to signaling, S-nitrosogluthathione may serve as a stable \((t_{1/2} > 2 \text{ hr})\) source of NO, able to exert effects on cellular function (e.g., superoxide production, activation of guanylyl cyclase, and ADP-ribosylation). The formation of S-nitrosothiols could also protect the cell against injury. NO in this form is less reactive with oxygen and superoxide anion, reducing the likelihood of toxic peroxynitrite formation (4). In addition, the reaction of NO with glutathione, the latter present in millimolar concentrations within the cell, could compete with the nitrosation of iron-containing proteins, the inactivation of which is implicated in cytotoxicity (4, 21).

The kinetics of glutathione depletion and HMPS activation may also have implications regarding cell injury. In neutrophils, rapid activation of the HMPS would be expected to protect the cell from the susceptibility to oxidant injury which would otherwise result from glutathione depletion. Therefore, the capacity of different cell types to replenish reduced glutathione stores via activation of the HMPS could be an important determinant of susceptibility to the cytotoxic effects of NO. This hypothesis is supported by the observation that macrophages and neutrophils, which utilize NO for microbial killing, have high HMPS activity and are resistant to attack by NO (22, 23).

In summary, our data indicate that NO reacts with intracellular glutathione and activates the HMPS. As has been suggested for the extracellular compartment, S-nitrosothiol compounds such as S-nitrosogluthathione may function as stable intracellular intermediates of NO activity and, perhaps, protect against NO-dependent cytotoxicity.

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