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The effects of heavy metal cations and sulfhydryl reagents on degranulation from digitonin-permeabilized neutrophils

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Digitonin-permeabilized neutrophils were exposed to micromolar levels of a variety of heavy metal cations and sulfhydryl oxidants to gain insight into the potential biochemical mechanisms underlying neutrophil degranulation. The results from this study suggest that the oxidation of intracellular sulfhydryl groups may play a role in neutrophil signal transduction. Evidence to support this conclusion is based on the observation that cupric phenanthroline and Cu^{2+}/cysteine, agents reported to induce disulfide bond formation, evoke significant granule enzyme release when presented to permeabilized neutrophils. The stimulatory actions of these compounds occur in the absence of Ca^{2+} and are blocked by the sulfhydryl reducing agent, dithiothreitol. In addition, we observed marked potentiation of Ca^{2+}-induced secretion by potentially physiological levels of Ni^{2+}. Although we are unaware of any Ni^{2+}-requiring enzymes in eukaryotic cells that are likely to be pertinent to degranulation, the ability of this divalent metal cation to lower the Ca^{2+} requirements for granule secretion suggests that it may play an important regulatory role in Ca^{2+}-dependent processes. Finally, we observed significant granule release when permeabilized neutrophils were exposed to the heavy metal cations, Hg^{2+} and Ag^{+}. The apparent stimulatory actions of these metals were the result of lysis rather than degranulation. Thus, the ability of these metals to lyse intracellular organelles such as lysosomal granules may contribute to their toxicological properties.

Introduction

There is substantial evidence to suggest that Ca^{2+} plays an important second messenger role in neutrophil signal transduction. The calcium ionophores A23187 and ionomycin have been widely employed to activate a variety of neutrophil functional responses. In addition, receptor-mediated elevations in intracellular Ca^{2+} have been directly demonstrated using the fluorescent probes, quin2 and fura-2 [1–3]. Cell permeabilization achieved by a variety of techniques has shown that Ca^{2+} alone can serve as an effective stimulus for evoking granule enzyme release [4–6]. While the Ca^{2+}-dependent regulatory proteins, protein kinase C and calmodulin, have been implicated in mediating the actions of Ca^{2+}, more recent evidence also suggests that a Ca^{2+}-dependent neutral proteinase (calpain) may also be involved in the activation pathway [7].

A variety of metal cations are known to interfere with Ca^{2+}-dependent processes. The toxicity of several metal cations has been correlated with their ability to inhibit calmodulin [8]. Recently, it was also reported that picomolar concentrations of Pb^{2+} stimulate protein kinase C to levels observed using millimolar concentrations of Ca^{2+} [9]. The divalent cation, Mn^{2+}, can substitute for or act synergistically with Ca^{2+} in activating calpain [10]. In addition to the metal-sensitive sites proposed in the activation pathway, Ca^{2+} transport mechanisms have been shown to be inhibited by Ni^{2+} and Cd^{2+} in several different cell types [11–15]. Metal-induced alterations in Ca^{2+} distribution can lead to loss of cell responsiveness [16].

Certain neutrophil functional responses have been reported to be affected by many of the aforementioned heavy metal cations [17–20]. In addition, divalent metal cations have been reported to both stimulate and inhibit secretory events in other cell types [21,22]. Presumably, these actions could be mediated through Ca^{2+}-depen-

Abbreviations: DTT, dithiothreitol; MPO, myeloperoxidase.

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dent processes. To investigate further the role Ca^{2+} and other divalent metal cations play in neutrophil signal transduction, permeabilized neutrophils were exposed to micromolar concentrations of several heavy metal cations and their effects on azurophil and specific granule release were assessed. The results from this study suggest that intracellular sulfhydryl groups may be invovled in neutrophil degranulation. Further, in addition to disrupting normal Ca^{2+} metabolism, the ability of certain metal cations to lyse intracellular organelles such as lysosomal granules may contribute to their general toxicity.

Materials and Methods

Reagents. All heavy metal cations and sulfhydryl-reactive compounds were purchased from Sigma Chemical Company, St. Louis, MO. All other materials were of reagent grade.

Cell preparation and permeabilization. Human polymorphonuclear leukocytes were isolated from venous blood using Hypaque-Ficoll gradients [23]. Contaminating erythrocytes were removed by dextran sedimentation and hypotonic lysis [24]. Neutrophil isolation by these procedures yielded preparations which were 98 ± 2% pure.

To determine the effects of heavy metal cations on neutrophil degranulation, neutrophils were permeabilized by the method of Smolen et al. [5]. Briefly, cells were suspended at a concentration of $25 \cdot 10^6$ cells/ml in a buffer consisting of 100 mM KCl, 20 mM NaCl, 30 mM Hepes, 1 mM EGTA (pH 7.0) and warmed at 37°C for 10 min. Cell permeabilization was initiated by the addition of 10 μg/ml digitonin. After 25 min, permeabilized cells were washed and resuspended in the same buffer in the absence of EGTA to prevent chelation of metal cations.

Granule enzyme release. Aliquots of permeabilized cell suspensions were incubated with micromolar concentrations of metal cations both in the presence and absence of 100 μM Ca^{2+}. Additionally, a variety of sulfhydryl oxidants were examined for their ability to affect granule enzyme release. Degranulation was assessed by measuring the release of azurophil and specific granule constituents in the supernatants of centrifuged cell suspensions. β-Glucuronidase, an azurophil granule enzyme, was determined using the method of Brittinger et al. [25]. Vitamin B-12 binding protein, a specific granule marker, was measured according to the procedure of Smith and Peters [26]. Lysozyme, a constituent of both granules was also routinely assayed [27]. Granule enzyme release from permeabilized cells exposed to 100 μM Ca^{2+} alone served as controls.

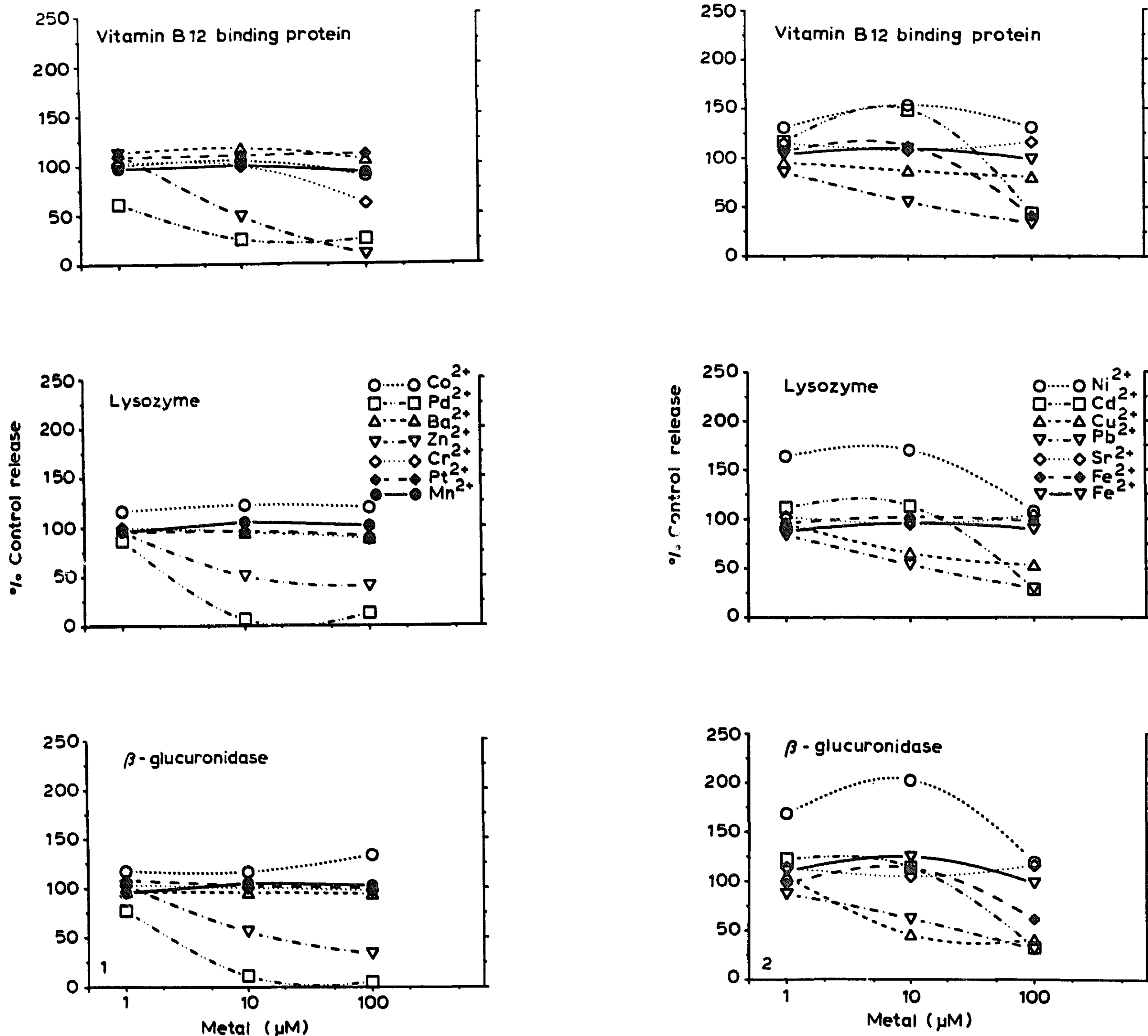
Subcellular fractionation. In addition to their effects in permeabilized cells, we examined the effects of several metal cations directly on the lysosomal granules. In these studies, neutrophils were isolated according to the method of Curnutte and Babior [28] using acid-citrate-dextrose as the anticoagulant. Specific and azurophil granules were isolated from purified neutrophils using nitrogen cavitation and density gradient centrifugation as described by Borregaard et al. [29]. Granule suspensions were incubated with the desired sulfhydryl agents for 7 min and then sedimented at $4000 \times g$ for 20 min. Granule enzyme release was determined by the methods described above except that myeloperoxidase [30] was assayed in supernatants from azurophil granules due to the interference of percoll with the β-glucuronidase assay.

Results

In order to gain a better understanding of the role of Ca^{2+} in neutrophil degranulation, preliminary studies were directed toward assessing the effects of a variety of metal cations on secretion from permeabilized cells stimulated with 100 μM Ca^{2+}. Shown in Figs. 1 and 2 are the effects of these metal cations on the Ca^{2+}-induced release of the granule markers, vitamin B-12 binding protein, lysozyme and β-glucuronidase. The majority of metals were either without effect or inhibitory in the concentration range of 10–100 μM. Granule release was most sensitive to Pd^{2+}, as almost complete inhibition of secretion was observed at concentrations as low as 10 μM (Fig. 1). In contrast, Ni^{2+} markedly potentiated the Ca^{2+}-induced release of both azurophil and specific granule constituents (Fig. 2). In the absence of Ca^{2+}, Ni^{2+} possessed no stimulatory actions on its own (vide infra). Although we observed some potentiation of Ca^{2+}-induced secretion of vitamin B-12 binding protein by Cd^{2+}, neither lysozyme nor β-glucuronidase secretion were elevated in parallel (Fig. 2).

We further examined the dose-response characteristics of Ni^{2+} to determine the concentration range over which this metal was effective (Fig. 3). We observed that Ni^{2+} enhanced Ca^{2+}-induced granule release at a concentration as low as 0.3 μM. Maximal enhancement of Ca^{2+}-induced secretion occurred at 3–10 μM Ni^{2+}, resulting in lysozyme secretion equivalent to 171 ± 19% of control values at 10 μM ($n = 4$). Stimulus-induced increases in β-glucuronidase (203 ± 6%) and vitamin B-12 binding protein (153 ± 7%) were also observed.

Following the observation that Ni^{2+} potentiated Ca^{2+}-induced secretion of both granule constituents, we wished to determine the effects of Ni^{2+} on the Ca^{2+} requirements for secretion. As shown in Fig. 4, granule secretion was achieved at significantly lower Ca^{2+} concentrations in the presence of Ni^{2+} (3 μM) than in its absence. However, the maximum amplitude of the secretory response was unaffected by Ni^{2+}. These results suggest that Ni^{2+} may play a regulatory role in Ca^{2+}-dependent processes.



Figs. 1 and 2. The effects of heavy metal cations on Ca^{2+}-induced granule secretion from digitonin-permeabilized neutrophils. Permeabilized cell suspensions ($5 \cdot 10^6$ cells/ml) were exposed to micromolar concentrations of heavy metal ions in the presence of 100 μM Ca^{2+} at 37 °C for 5 min to assess their effects on Ca^{2+}-induced degranulation. Release of azurophil (β-glucuronidase) and specific (vitamin B-12 binding protein) granule markers were assayed in the supernatants of centrifuged cell suspensions. Lysozyme, present in both granules, was also assayed. EGTA was omitted from the incubation medium to prevent chelation of metal cations. Results are expressed as percent control (100 μM Ca^{2+} alone) and represent mean values of three or more experiments.

In addition to their effects on Ca^{2+}-induced secretion, we were also interested in whether any of these metals could support secretion in the absence of Ca^{2+}. While all the metals shown in Figs. 1 and 2 were ineffective in this respect, Hg^{2+} and Ag^+ were potent Ca^{2+}-independent secretagogues (Fig. 5). Hg^{2+} (30 μM) exposure resulted in the release of 34 ± 11% ($n = 3$) of the total cellular vitamin B-12 binding protein, 29 ± 4% of the lysozyme, and 17 ± 4% of the β-glucuronidase. These values compare with a total Ca^{2+}-induced release of 21 ± 5% ($n = 3$) of the cellular vitamin B-12 binding protein, 25 ± 4% of the lysozyme and 12 ± 1% of the β-glucuronidase. Quantitatively similar results were observed following exposure to 10 and 100 μM concentrations of Ag^+, although the magnitude of the secretory response was less pronounced (see below).

In view of the reported ability of certain heavy metal cations to bind to sulfhydryl groups, we examined the ability of the sulfhydryl reducing agent, dithiothreitol (DTT), to block secetion induced by Hg^{2+} and Ag^+. As shown in Fig. 6, the stimulatory actions of these metals on granule secretion were completely reversed by 2 mM DTT. The ability of DTT to reverse the effects of these metals is consistent with the notion that their stimulatory actions are mediated through intracellular sulfhydryl groups. We also observed that the Ca^{2+}-potentiating actions of Ni^{2+} were reversed by DTT, further suggesting the involvement of sulfhydryl groups

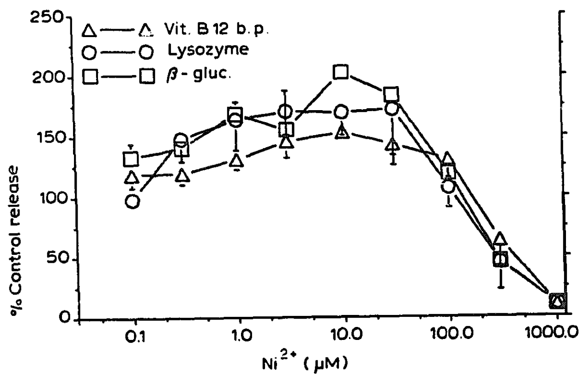


Fig. 3. Enhancement of Ca^{2+}-induced secretion by Ni^{2+}. Permeabilized neutrophils were exposed to Ni^{2+} at concentrations ranging from 0.1 to 1000 μM in the presence of 100 μM Ca^{2+} (control). Degranulation was assessed as described in Figs. 1 and 2. Data are expressed as mean ± S.E. and represent four experiments.

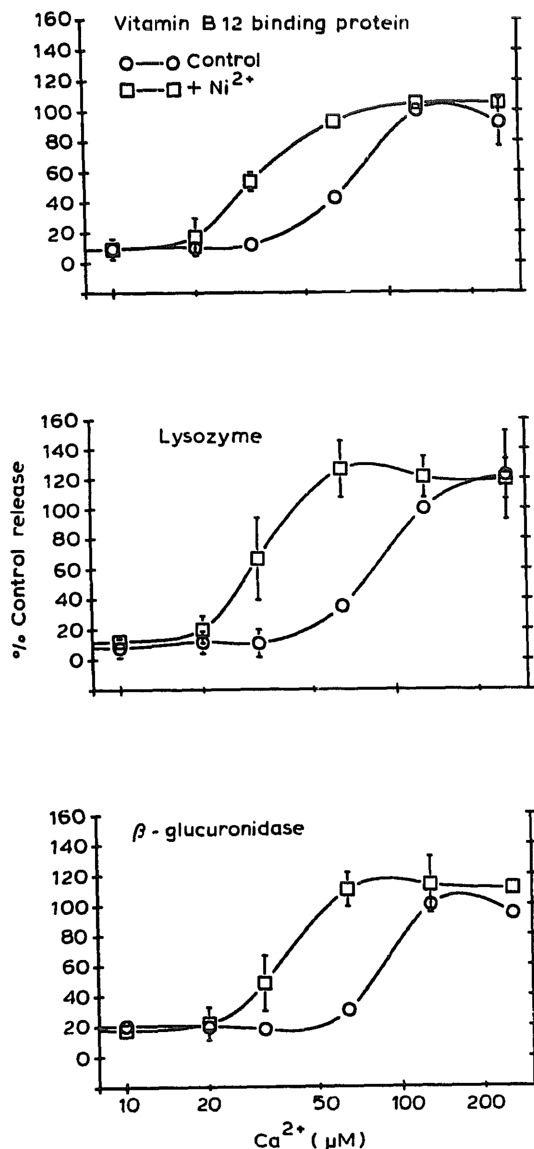


Fig. 4. Effect of Ni^{2+} on the Ca^{2+} requirements for granule enzyme secretion. Permeabilized neutrophils were exposed to 3 μM Ni^{2+} and Ca^{2+} at concentrations up to 200 μM. Data are expressed as percent control release. Values are mean ± S.E. of four experiments. In these experiments secretion in response to 100 μM Ca^{2+} alone served as the control.

in regulating neutrophil degranulation. In contrast, DTT had no effects on Ca^{2+}-induced granule secretion, suggesting that the stimulatory actions of these apparent secretagogues are mediated at different sites.

To define further the potential role intracellular sulfhydryl groups play in degranulation, we exposed permeabilized cells to a variety of putative sulfhydryl oxidants and blocking agents in the absence of Ca^{2+} (Fig. 8). *p*-Chloromercuribenzoate stimulated the release of both granule constituents, albeit at higher concentrations than of Hg^{2+}. The apparent release of β-glucuronidase, but not lysozyme or vitamin B-12 binding protein, by 100 μM plumbagin was the result of interference of this intensely colored compound with the β-glucuronidase assay. The release of vitamin B-12 binding protein was sensitive to the thiol oxidant, H_2O_2 and further enhanced by the presence of Cu^{2+}. The inability of H_2O_2 to stimulate the release of lysozyme and β-glucuronidase may be the result of a direct inhibitory effect of H_2O_2 on the enzymes themselves. No Ca^{2+}-independent granule secretion was observed for the other sulfhydryl-active compounds we tested. Since *N*-ethylmaleimide is a potent inhibitor of neutrophil degranulation in intact cells [31,32], we also examined its effects on Ca^{2+}-induced secretion. At 100 μM *N*-ethylmaleimide, vitamin B-12 binding protein secretion was 107 ± 5% ($n = 4$) of control values. Lysozyme secretion (109 ± 8%) and β-glucuronidase secretion (118 ± 6%) were also slightly enhanced by *N*-ethylmaleimide.

As shown in Fig. 7, we were also able to evoke significant Ca^{2+}-independent degranulation of vitamin B-12 binding protein and lysozyme by exposing cells to cysteine or phenanthroline in the presence of 100 μM Cu^{2+}. However, very little β-glucuronidase was released by Cu^{2+} and cysteine. Cu^{2+} has been reported to catalyze the oxidation of cysteine to cystine [33]. Likewise, cupric phenanthroline also induces disulfide bond for-

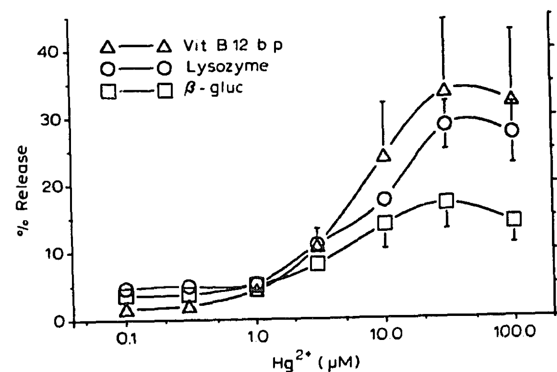


Fig. 5. Effect of Hg^{2+} on Ca^{2+}-independent neutrophil degranulation. Permeabilized neutrophils were incubated with the indicated concentrations of Hg^{2+} in the absence of added Ca^{2+} and its effects on degranulation were assessed as described in Figs. 1 and 2. Data are expressed as the percent release of total cellular content. The total content was determined in samples treated with 0.2% Triton X-100. Values represent means ± S.E.

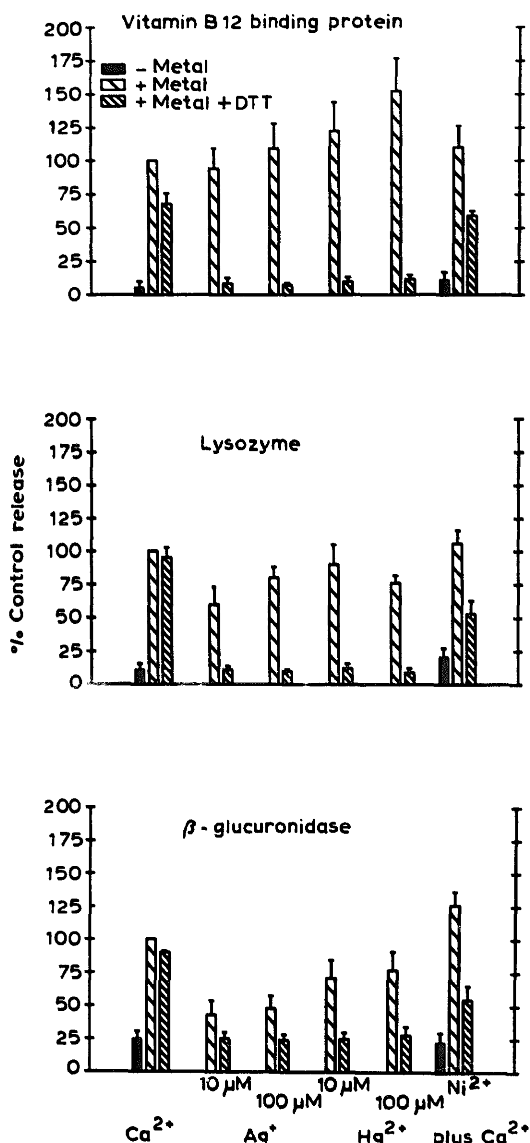


Fig. 6. Blocking of metal effects by DTT. Permeabilized neutrophils were exposed to stimulatory concentrations of Hg^{2+} and Ag^{+} in the presence or absence of 2 mM DTT. DTT was also included in the incubation medium of cells either exposed to 100 μM Ca^{2+} alone or 100 μM Ca^{2+} and 3 μM Ni^{2+}. Data are expressed as percent control release and represent mean ± S.E. of three experiments.

mation [34]. These sulfhydryl oxidants have been used to demonstrate the importance of sulfhydryl redox state in regulating Ca^{2+} permeability of the sarcoplasmic reticulum [35]. The mechanism by which Cu^{2+} and cysteine induce degranulation appears to involve the formation of a mixed disulfide bond between cysteine and an intracellular protein sulfhydryl. Evidence to support this conclusion is based on the inability of cysteine or Cu^{2+} alone to affect granule secretion. In addition, cystine, the oxidized form of cysteine, was also inert in this respect.

Subsequent studies were carried out in an attempt to determine the intracellular target mediating the actions of these sulfhydryl oxidants. Diamide, which is believed to preferentially oxidize cellular glutathione and influence microtubule assambly [36] had no effect on neutrophil degranulation in either the presence or absence of Ca^{2+} in the concentration range of 1–100 μM (not shown). In addition, oxidized glutathione possessed no stimulatory actions on its own, as shown in Fig. 4. These results argue against a role for cellular glutathione and microtubules in neutrophil degranulation.

We also examined the direct effects of stimulatory metal cations and sulfhydryl oxidants on isolated granule fractions, to determine whether granule release occurred by mechanisms involving exocytosis or granule lysis. As shown in Table I, direct exposure of granules

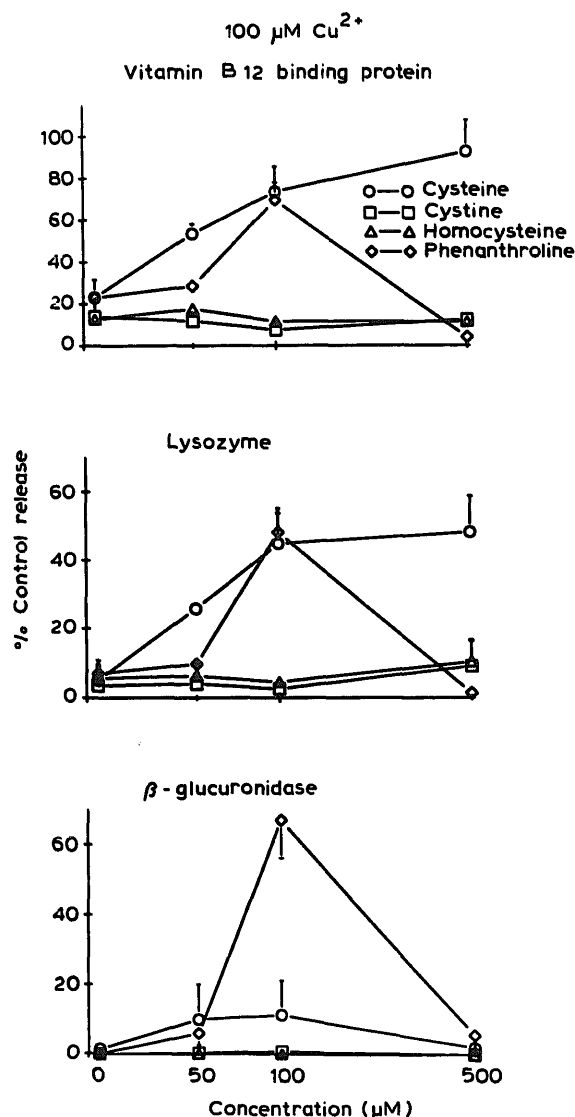


Fig. 7. Effect of sulfhydryl oxidants on neutrophil degranulation. Permeabilized neutrophils were exposed to 50, 100 and 500 μM concentrations of cysteine, homocysteine, cystine and phenanthroline in the presence of 100 μM Ca^{2+}. Specific and azurophil granule release under these conditions was compared to release obtained using 200 μM Ca^{2+} (control). Secretion obtained in the absence of thiol represents background release due to 100 μM Ca^{2+} alone. Data are expressed as percent control release and represent means ± S.E. of three of more experiments.

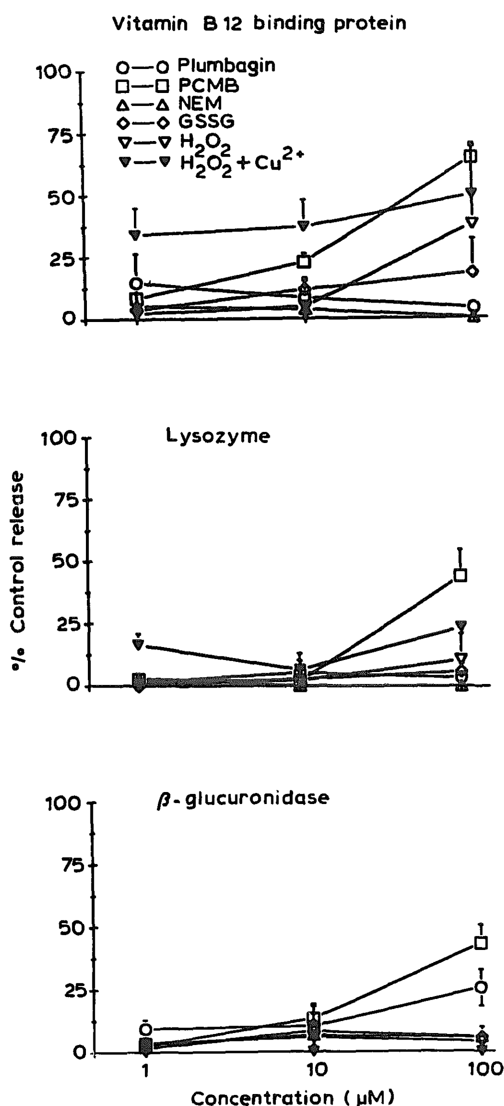


Fig. 8. The effects of other sulfhydryl reagents on neutrophil degranulation. Permeabilized neutrophils were incubated with a variety of other sulfhydryl compounds at concentrations of 1–100 μM Ca^{2+} (control). Results are expressed as percent control release and represent mean ± S.E. of three experiments.

to stimulatory concentrations of Hg^{2+} and Ag^{+} resulted in the release of both specific and azurophil granule constituents. No significant release above background levels was observed under the other conditions employed. These results suggest that Hg^{2+}- and Ag^{+}-induced release occur as a result of granule lysis. However, these results do not rule out the importance of sulfhydryl groups in neutrophil degranulation, since other sulfhydryl oxidants did not appear to induce granule lysis directly.

Discussion

The major impetus behind this study was to gain further insight into the role of Ca^{2+} in neutrophil degranulation. Previous studies in this laboratory have shown that Ca^{2+} alone is an effective stimulus for evoking both azurophil and specific granule release from digitonin-permeabilized cells [5,37]. However, the subsequent biochemical events mediating the actions of Ca^{2+} are not completely understood. In view of the multiplicity of Ca^{2+}-dependent processes affected by heavy metal cations, we examined their ability to substitute for or interfere with Ca^{2+}-induced secretion from permeabilized neutrophils.

The vast majority of metals employed in this study were either without effect or inhibited Ca^{2+}-induced secretion at high concentrations. The inhibitory effect of these metals may be related to their reputed actions as Ca^{2+} antagonists [8,11–13]. In contrast, Hg^{2+} and Ag^{+}, metals widely known to bind to sulfhydryl groups, were potent secretagogues in the absence of Ca^{2+}. Secretion was also observed following exposure of permeabilized cells to either cupric phenanthroline or Cu^{2+} and cysteine. Both of these agents are known to act as thiol oxidants [33,34]. The apparent stimulatory actions of both metals and sulfhydryl oxidants were reversed by dithiothreitol, suggesting that the oxidation of intracellular sulfhydryl groups may play a role in neutrophil degranulation. However, we observed significant enzyme release when granule-enriched fractions were exposed directly to stimulatory concentrations of Hg^{2+} or Ag^{+}. In contrast, Ca^{2+} had no direct effects on isolated granules, supporting morphological and biochemical data which suggest that secretion from permeabilized cells occurs through processes involving exocytosis [5,38]. These results suggest that sulfhydryl groups are critical for maintaining granule integrity and that the interaction of Hg^{2+} and Ag^{+} with those sulfhydryl groups results in granule lysis rather than granule exocytosis. In intact cells, Hg^{2+} inhibits neutrophil chemotaxis, adherence, phagocytosis and motility even at concentrations as low as 10^{-17} M [20,32]. The intracellular release of lysosomal enzymes may constitute one mechanism underlying inhibition of neutrophil function by Hg^{2+}. Thus, Hg^{2+}- and Ag^{+}-induced granule release have implications with regard to the toxicity of these metals and probably have no mechanistic significance in neutrophil degranulation.

Although Hg^{2+} and Ag^{+} appeared to lyse neutrophil granules, granule stability was not markedly altered by the sulfhydryl oxidants, cupric phenanthroline or Cu^{2+}/cysteine. These observations tend to support a role for sulfhydryl oxidation in neutrophil degranulation. Although vitamin B-12 binding protein release appeared to be markedly enhanced by cupric phenanthroline, MPO release from azurophil granules was not. Secretion of the azurophil granule marker, β-glucuronidase was, however, stimulated in permeabilized cells. In addition, cupric phenanthroline has also been shown to induce the release of histamine from digitonin-permeabilized platelets [39].

TABLE I

The effects of sulfhydryl reagents on specific and azurophil granules

Neutrophil specific and azurophil granules were isolated by nitrogen cavitation and density gradient centrifugation [29]. Granules were incubated with the desired agents at 37 °C for 7 min. Enzyme release into supernatants was determined following sedimentation of granules at 4000 × *g* for 20 min. Data are expressed as percent of total released by 0.2% Triton X-100. Values are mean ± S.E.

	Specific granules		Azurophil granules	
	vitamin B-12 binding protein	lysozyme	MPO	lysozyme
$-Ca^{2+}$	8.3 ± 6.5	11.0 ± 3.6	2.6 ± 1.4	18.4 ± 4.4
100 μM Ca^{2+}	8.7 ± 6.5	10.7 ± 4.7	2.8 ± 1.5	20.3 ± 4.9
3 μM Ni^{2+}	9.3 ± 4.9	9.3 ± 3.5	2.5 ± 1.3	17.7 ± 11.7
Ni^{2+} + Ca^{2+}	11.0 ± 3.6	10.3 ± 2.3	2.6 ± 1.4	14.0 ± 5.6
10 μM Hg^{2+}	15.6 ± 0.6	18.7 ± 10.7	6.6 ± 2.5	35.3 ± 9.3 [a]
100 μM Hg^{2+}	32.3 ± 9.5 [a]	29.0 ± 13.2	18.9 ± 5.5	40.0 ± 11.5 [a]
10 μM Ag^{+}	17.3 ± 1.5	18.7 ± 4.6	3.7 ± 1.5	27.7 ± 9.0
100 μM Ag^{+}	19.0 ± 2.6	20.7 ± 6.4	5.2 ± 3.2	33.0 ± 11.4
100 μM Cu^{2+}	8.7 ± 4.6	8.7 ± 4.2	2.7 ± 1.6	15.9 ± 9.8
100 μM cysteine	9.0 ± 3.6	8.0 ± 2.6	1.4 ± 1.6	15.0 ± 4.6
100 μM phenanthroline	8.3 ± 3.2	9.0 ± 4.0	2.8 ± 1.6	14.7 ± 1.5
Cu^{2+}/cysteine	11.0 ± 3.6	10.3 ± 4.9	4.3 ± 2.3	22.7 ± 2.3
Cu^{2+}/phenanthroline	24.0 ± 17.5	13.3 ± 3.8	3.6 ± 2.3	18.0 ± 4.4

[a] Release is significantly greater than control (100 μM Ca^{2+}) at $P < 0.05$.

If sulfhydryl groups play a role in neutrophil functions, the intracellular sites are unclear. In intact neutrophils, membrane-permeant sulfhydryl blocking agents have been shown to inhibit degranulation [31,40] as well as phagocytosis, adhesiveness and motility [32]. Intracellular targets proposed for mediating these inhibitory effects include the components of both the cytoskeleton and glycolytic pathway. In particular, there is evidence that microtubule assembly is regulated by the redox state of intracellular glutathione. The sulfhydryl oxidant, diamide, which preferentially oxidizes intracellular glutathione, inhibits microtubule assembly and promotes disassembly [36]. There is also evidence that oxidation increases the tyrosinolation of tubulin, which also favors disassembly [41,42]. However, we obtained no evidence to indicate that either the glutathione redox status or microtubules play a significant role in mediating the stimulatory actions of sulfhydryl oxidants. Neither diamide nor GSSG stimulated granule release in the absence of Ca^{2+}. In addition, diamide did not inhibit Ca^{2+}-induced secretion. These results are consistent with the inability of colchicine to inhibit secretion in this system [5] and minimize the importance of microtubules in signal transduction.

Although we were unable to identify an intracellular target sensitive to sulfhydryl oxidation, the mechanism by which Cu^{2+}/cysteine induces granule release appears to involve disulfide bond formation between cysteine and an intracellular protein sulfhydryl. Neither Cu^{2+} nor cysteine alone possessed stimulatory properties; both were required to induce degranulation. In addition, the oxidized form of cysteine, cystine, was also an ineffective secretagogue. These results suggest that Cu^{2+} catalyzes the formation of a disulfide bond between cysteine and an intracellular sulfhydryl species. These conditions have been shown to regulate the Ca^{2+} permeability of the sarcoplasmic reticulum in vitro [35]. Protein sulfhydryls have also been reported to be important in the Ca^{2+} release mechanism of internal platelet membranes [43]. Sulfhydryl oxidants may likewise regulate the permeability of analogous Ca^{2+} storage pools in neutrophils and induce degranulation by elevating intracellular Ca^{2+}.

One of the most interesting observations from our study was the ability of submicromolar concentrations of Ni^{2+} to markedly enhance Ca^{2+}-induced degranulation. These Ca^{2+}-potentiating concentrations of Ni^{2+} were far below toxicological levels and in the range of reported serum concentrations of 2.6 μg/l [44]. Similar enhancement of Ca^{2+}-induced secretion has been observed with guanine nucleotides, suggesting a similar mechanism of action [6,37]. Although it is tempting to speculate that these observations are of physiological significance, no Ni^{2+}-requiring enzymes have been identified in eukaryotic cells thus far. However, several Ni^{2+}-sensitive sites have been reported by other investigators which could potentially play a role in regulating neutrophil degranulation. Pallen and Wang [45] reported that Ni^{2+} stimulated the phosphoprotein phosphatase, calcineurin. Although protein dephosphorylation has been considered an important mechanism regulating exocytosis in paramecium [46], its role in neutrophil degranulation has not been established. In addition, Roychaudhury et al. [47] observed that low micromolar concentrations of Ni^{2+} stimulated the in vitro polymerization of goat brain tubulin. However, as

discussed above, we observed no evidence to suggest that microtubules are important in the signal transduction pathway in permeabilized cells.

While the biochemical mechanisms underlying the Ca^{2+}-potentiating actions of Ni^{2+} are unknown, the ability of Ni^{2+} to lower the Ca^{2+} requirements for granule secretion suggests that Ni^{2+} may regulate the affinity of a Ca^{2+}-dependent target for Ca^{2+}. Alternatively, Ni^{2+} may inhibit Ca^{2+} homeostatic mechanisms responsible for restoring stimulatory increases in intracellular Ca^{2+} to resting levels. In neutrophils, two intracellular Ca^{2+}-sequestering pools [48] as well as a plasma membrane-bound Ca^{2+}-ATPase [49] are believed to participate in the regulation of Ca^{2+} metabolism. Mitochondria, which constitute one of these intracellular pools, exhibits a high capacity to transport Ca^{2+} in the absence of ATP. Ni^{2+} has been reported to be an inhibitor of both Ca^{2+}-ATPase activity [11] and mitochondrial Ca^{2+} transport [14]. Thus, Ni^{2+} may potentiate submaximal doses of Ca^{2+} by preventing is sequestration into intracellular organelles or its extrusion by the plasma membrane. The inability of Ni^{2+} to alter the maximum response to Ca^{2+} is consistent with this hypothesis. Disruptions in Ca^{2+} metabolism could constitute one mechanism underlying the toxicity of Ni^{2+}, or represent an important physiological regulatory mechanism.

Acknowledgement

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