Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 9285–9289, October 1993
Medical Sciences

Endothelial-cell heme uptake from heme proteins: Induction of sensitization and desensitization to oxidant damage

(endothelium/heme oxygenase/ferritin)

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Communicated by L. Lorand, June 14, 1993

ABSTRACT **Iron-derived reactive oxygen species are implicated in the pathogenesis of various vascular disorders including atherosclerosis, vasculitis, and reperfusion injury. The present studies examine whether heme, when liganded to physiologically relevant proteins as in hemoglobin, can provide potentially damaging iron to intact endothelium. We demonstrate that reduced ferrohemoglobin, while relatively innocuous to cultured endothelial cells, when oxidized to ferrihemoglobin (methemoglobin), greatly amplifies oxidant (H_2O_2)-mediated endothelial-cell injury. Drawing upon our previous observation that free heme similarly primes endothelium for oxidant damage, we posited that methemoglobin, but not ferrohemoglobin, releases its hemes that can then be incorporated into endothelial cells. In support, cultured endothelial cells exposed to methemoglobin—in contrast to exposure to ferrohemoglobin, cytochrome *c*, or metmyoglobin—rapidly increased their heme oxygenase mRNA and enzyme activity, thereby supporting heme uptake; ferritin production was also markedly increased after such exposure, thus attesting to eventual incorporation of Fe. These cellular methemoglobin effects were inhibited by the heme-scavenging protein hemopexin and by haptoglobin or cyanide, agents that strengthen the liganding between heme and globin. If the endothelium is exposed to methemoglobin for a more prolonged period (16 hr), it accumulates large amounts of ferritin; concomitantly, and presumably associated with iron sequestration by this protein, the endothelium converts from hypersusceptible to hyperresistant to oxidative damage. We conclude that when oxidation of hemoglobin facilitates release of its heme groups, catalytically active iron is provided to neighboring tissue environments. The effect of this relinquished heme on the vasculature is determined both by extracellular factors—i.e., plasma proteins, such as haptoglobin and hemopexin—as well as intracellular factors, including heme oxygenase and ferritin. Acutely, if both extra- and intracellular defenses are overwhelmed, cellular toxicity arises; chronically, when ferritin is induced, resistance to oxidative injury may supervene.**

Increased body iron, reflected by elevated serum ferritin levels, may intimate an increased risk for myocardial infarction as suggested by a Finnish study (1). The risk was particularly pronounced in individuals harboring both high serum low density lipoprotein (LDL) and ferritin levels, suggesting possible linkage of *in vivo* iron, the production of potently atherogenic oxidized LDL, and vascular disease (2–7). Others have shown that iron accumulates in atherosclerotic lesions (8) in a catalytically active form (9). The proximity of vascular lining cells to circulating erythrocytes (RBCs) led us to hypothesize that one source of this potentially toxic iron may be heme released from damaged RBCs at sites of vascular turbulence (10) or in hemorrhagic atheromatous plaques (11). Indeed, we have shown (12) that hydrophobic heme rapidly intercalates into the plasma membrane of endothelial cells, releases its iron, and thereby catalyzes endothelial damage by oxidants such as H_2O_2 or those derived from activated inflammatory cells. Moreover, heme also acts as a promoter of LDL oxidation, generating moieties toxic to endothelium in less than an hour of coincubation (7). In contrast, more prolonged contact of endothelial cells with heme (e.g., 16 hr) renders them remarkably resistant to oxidant challenge. That is, we have shown (13) endothelium responds to heme by induction of the heme-degrading enzyme, heme oxygenase, and the concomitant production of large amounts of the iron-binding protein, ferritin. In fact, the ferritin content of endothelial cells was shown to be inversely proportional to their susceptibility to oxidant injury under a wide range of experimental conditions (13).

In the present studies, reported in preliminary form elsewhere (14), we have examined whether heme liganded to protein, as in hemoglobin, can actually serve as an eventual source of endothelial iron. We demonstrate that ferrohemoglobin is rapidly oxidized to methemoglobin, for instance by polymorphonuclear leukocytes (PMNs); methemoglobin can then release free heme to cultured endothelium, initially sensitizing it to oxidant damage but later inducing the cytoprotectants, heme oxygenase and ferritin. Metmyoglobin and cytochrome *c*, two other ferrihemeproteins, do not provide free heme (or iron) to endothelium and do not aggravate vascular damage.

METHODS

Endothelial-Cell Isolation and Culture. Human umbilical vein endothelial cells (HUVECs) were removed from human umbilical veins by exposure to dispase (0.2% for 16 hr at 4°C) and cultured in medium 199 containing 15% (vol/vol) fetal calf serum (FCS), penicillin (100 units/ml), streptomycin (100 units/ml), and heparin (5 units/ml) supplemented with L-glutamine, sodium pyruvate, and EndoGro (Vec Tec, Albany, NY). Porcine aortic endothelial cells were grown as described (12).

Endothelial-Cell Cytotoxicity Assays. Confluent porcine aortic endothelial cells were radiolabeled with [^{51}Cr]Na_2CrO_4 for 8 hr in cell culture medium. The ^{51}Cr-labeled endothelial cells were exposed to hemin and heme proteins for 1 hr in appropriate medium at pH 7.4 followed by a 1-hr incubation with FCS-containing medium. The cytotoxicity (^{51}Cr release)

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Abbreviations: LDL, low density lipoprotein; RBC, erythrocyte; PMN, polymorphonuclear leukocyte; HUVEC, human umbilical vein endothelial cell; FCS, fetal calf serum; PMA, phorbol 12-myristate 13-acetate.

assays were performed with 100 μM H_2O_2 in 1 ml of Hank's balanced salt solution (HBSS) for 2 hr (12).

Heme Oxygenase Enzyme Activity and Ferritin Assays. Heme oxygenase activity was measured by bilirubin generation in HUVEC microsomes as described and modified (13, 15).

Endothelial-cell ferritin content in HUVECs was measured by the Stratus fluorometric enzyme immunoassay system (16) after cell solubilization (13) or by a cell ELISA assay after methanol permeabilization using a goat anti-human ferritin antibody as a primary antibody (ATAB, Stillwater, MN) and an alkaline phosphatase-labeled rabbit anti-goat IgG as a secondary antibody. The results were expressed as ng of ferritin per mg of endothelial-cell protein or as an ELISA index (OD_{405}). The protein content of the endothelial-cell monolayers was determined using Lowry protein assay (17).

Heme Oxygenase and Ferritin mRNA Analysis. Heme oxygenase and light and heavy chain ferritin mRNA content were analyzed in HUVECs as described (13). The cDNA probes for heme oxygenase (18) and for light and heavy chain ferritins (19) were provided by R. Tyrrell (Swiss Institute for Experimental Research, Epalinges, Switzerland) and H. N. Munro (Tufts University, Boston), respectively. Autoradiographs were quantified by computer-assisted video densitometry and expressed as arbitrary OD units.

Hemoglobin Preparation and Methemoglobin Formation by Activated PMNs. Purified hemoglobin was prepared from fresh blood drawn from volunteers by using ion-exchange chromatography on DEAE-Sepharose CL-6B column (Pharmacia) (20). Hemoglobin was assessed for purity using isoelectric focusing. Methemoglobin was prepared by incubation of hemoglobin with 1.5-fold molar excess $K_3Fe(CN)_6$ over heme, followed by dialysis. Cyanomethemoglobin was formed by the addition of 2-fold excess NaCN to methemoglobin followed by gel filtration.

Resting or phorbol 12-myristate 13-acetate (PMA; 100 ng/ml)-activated PMNs at 5×10^6 cells per ml were incubated with 20 μM ferrohemoglobin in HBSS at 37°C in a humidified atmosphere of 95% air/5% CO_2 for various times (up to 4 hr). Methemoglobin formation was calculated as described (21). To test whether hemoglobin (10 μM) exposed to PMNs for 30 min would induce endothelial heme oxygenase and ferritin, PMN–hemoglobin supernatants obtained after centrifugation at 1000 × g for 5 min were added to endothelial monolayers for 4 hr in the presence of α_1-antitrypsin (1 mg/ml)-containing medium.

RESULTS

As shown in Fig. 1*A*, a 60-min exposure of endothelial cells to 10 μM methemoglobin resulted in their aggravated susceptibility to subsequent oxidant challenge by H_2O_2 (bar 4); a similar hypersusceptibility was provoked by exposure to free hemin (bar 2) as reported (12). In contrast, ferrohemoglobin (bar 3) and other heme proteins such as metmyoglobin (bar 5) or cytochrome *c* (bar 6) failed to sensitize endothelial monolayers to oxidant stress. Suspecting that free heme was released from methemoglobin and then incorporated into endothelial cells, we examined factors that strengthen heme–globin liganding or that scavenge free heme moieties. As shown in Fig. 1*B*, 15% human serum (bar 2) or stoichiometric amounts of two serum proteins, haptoglobin or hemopexin (bars 3 and 4), significantly inhibited methemoglobin-aggravated cytotoxicity. Sodium cyanide (NaCN; bar 5), shown to prevent release of heme groups from methemoglobin (20), also significantly reduced oxidant susceptibility.

We have demonstrated (13) that more prolonged endothelial exposure to heme induced both heme oxygenase and ferritin; concomitantly, endothelial cells became highly resistant to oxidant-mediated injury, which reflects induction of ferritin. As shown in Fig. 2 *A* and *B*, methemoglobin exposure also induced endothelial heme oxygenase mRNA (lane 4 and bar 4) in a similar, but somewhat less, degree as free heme (lane 2 and bar 2). Accompanying this mRNA induction, expression of heme oxygenase enzyme activity was also significantly enhanced (Fig. 2*D*, bars 2 and 4). Ferrohemoglobin, metmyoglobin, or cytochrome *c* did not alter heme oxygenase mRNA level and enzyme activity (Fig. 2). Similar results were observed in these experiments regarding the ferritin response; methemoglobin (10 μM) markedly increased endothelial ferritin content (from 37.4 ± 7.5 to 147.7 ± 19 ng/mg of cell protein), and in contrast, ferrohemoglobin, metmyoglobin, and cytochrome *c* (each containing 40 μM heme) failed to induce significant ferritin synthesis over 12 hr. Ferritin, light and heavy chain, mRNA levels were not affected by methemoglobin treatment (data not shown) concordant with studies (13, 19, 22, 23), demonstrating that iron-mediated regulation of ferritin synthesis occurs at the post-transcriptional/translational level.

Free heme released from methemoglobin almost certainly mediated the induction of endothelial heme oxygenase (Fig. 3), since mRNA expression and enzyme activity were markedly decreased when heme release was inhibited (20) by addition of stoichiometric amounts of haptoglobin (lanes 3 and bars 3) or cyanide (lanes 4 and bars 4). In similar experiments (Fig. 4), methemoglobin-mediated induction of ferritin (bar 1) was also significantly inhibited by serum (bar 2), hemopexin (bar 3), haptoglobin (bar 4), or cyanide (bar 5), supporting the premise that release of heme to endothelial cells is important for the induction of ferritin as well.

Although ferrohemoglobin does not itself affect endothelium, we (24) and others (25, 26) have shown it can readily be

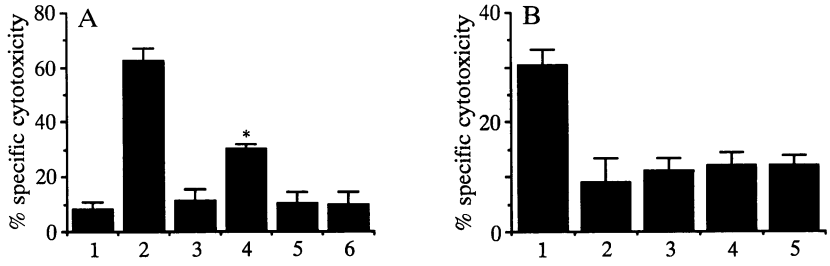

FIG. 1. (*A*) Methemoglobin, like hemin, but not ferrohemoglobin, metmyoglobin, and cytochrome *c*, sensitizes endothelial cells to H_2O_2-mediated cytolysis. Confluent ^{51}Cr-labeled porcine aortic endothelial cells were incubated with Dulbecco's modified Eagle's medium (DMEM) (bar 1), 5 μM hemin (bar 2), 10 μM ferrohemoglobin (bar 3), 10 μM methemoglobin (bar 4), 40 μM metmyoglobin (bar 5), or 40 μM cytochrome *c* (bar 6) in 500 μl of DMEM for 60 min and then exposed to 100 μM H_2O_2 for 2 hr. Results represent percent specific cytotoxicity (mean ± SEM) of at least three experiments performed in duplicate. (*B*) Endothelial-cell sensitization to H_2O_2 by methemoglobin is inhibited by serum factors and NaCN. Methemoglobin was added to endothelium alone (bar 1) or in the presence of 15% human serum (bar 2) or stoichiometric amounts of haptoglobin (bar 3) or hemopexin (bar 4). Cyanomethemoglobin (bar 5) was prepared and added to endothelium. Results represent the specific cytotoxicity (mean ± SEM) of at least three experiments performed in duplicate. *, $P < 0.0001$ vs. bar 1 (control).

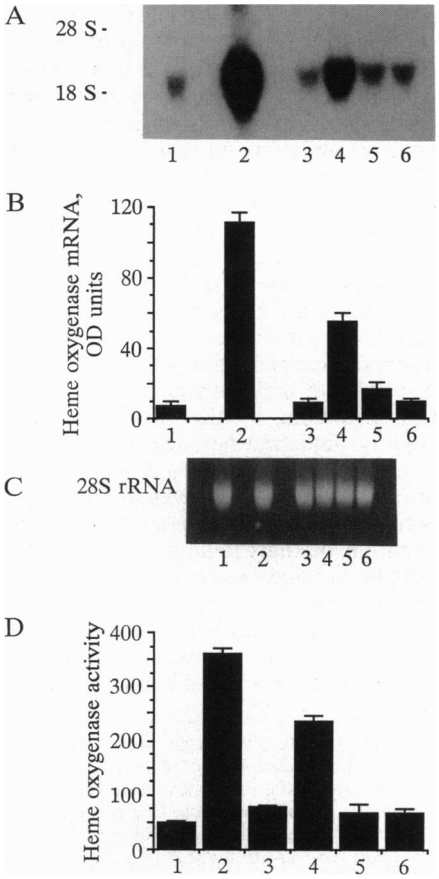

FIG. 2. Heme oxygenase induction by heme proteins. (*A*) For heme oxygenase mRNA analysis, endothelial monolayers were incubated for 4 hr with 10 μM ferrohemoglobin (lane 3), 10 μM methemoglobin (lane 4), heme-equivalent amounts of metmyoglobin (lane 5), or cytochrome *c* (lane 6). Hemin (5 μM) (lane 2) was incubated with monolayers for 1 hr plus an additional 3 hr in hemin-free medium. Lanes 1 and bars 1 represent DMEM-exposed endothelium. RNA was isolated, electrophoresed, blotted, and hybridized with a ^{32}P-labeled heme oxygenase cDNA probe. (*B*) Densitometry tracings of heme oxygenase mRNA band are expressed as arbitrary OD units. (*C*) The corresponding 28S rRNA of the Northern blot in *A*. (*D*) Heme oxygenase enzyme activity (pmol of bilirubin formed per mg of cell protein per 60 min) was measured at 8 hr after exposure of endothelium to the same heme compounds as above. Results represent the enzyme activity (mean ± SEM) of at least three experiments done in duplicate.

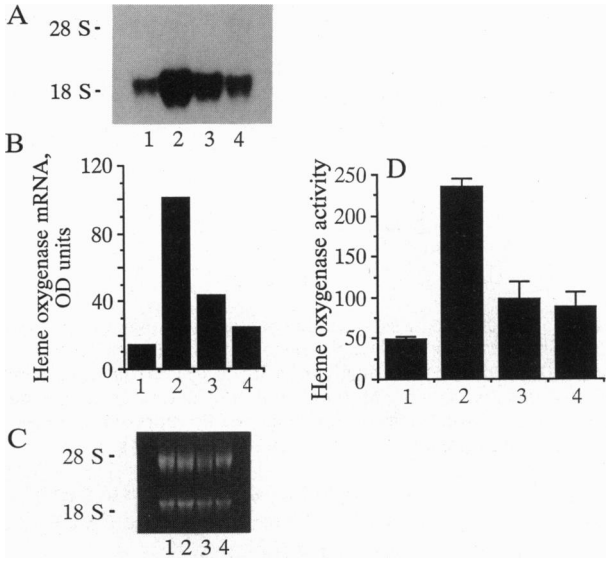

FIG. 3. Methemoglobin-induced heme oxygenase expression is inhibited by haptoglobin and NaCN. Endothelial monolayers were treated with 10 μM methemoglobin (lanes 2 and bars 2), methemoglobin in the presence of equal concentrations of haptoglobin (lanes 3 and bars 3), or cyanomethemoglobin (lanes 4 and bars 4). Lanes 1 and bars 1 represent DMEM-exposed endothelium. (*A*) After a 4-hr incubation, total cell RNA was isolated and Northern blots of endothelial cell RNA were probed with ^{32}P-labeled heme oxygenase cDNA. (*B*) Autoradiograph was quantified by video densitometry. (*C*) The corresponding 28S and 18S rRNA of the Northern blot in *A*. (*D*) Heme oxygenase enzyme activity (pmol of bilirubin formed per mg of cell protein per 60 min) was measured at 8 hr. The results represent the enzyme activity (mean ± SEM) of three experiments performed in duplicate.

oxidized to heme-releasing methemoglobin in the presence of inflammatory-cell-derived oxidants. For instance, PMNs, when activated with the phorbol ester PMA, markedly oxidized hemoglobin within 30 min (Fig. 5); unactivated PMNs (which produce little reactive oxygen species) provoked little change. Concordantly, as shown in Fig. 6, if ferrohemoglobin was exposed to PMA-activated PMNs for 30 min and the methemoglobin-containing supernatants were then added to endothelial monolayers for 4 hr, heme oxygenase mRNA was induced (Fig. 6 *A* and *B*, bar 3 and lane 3) along with increased enzyme activity (Fig. 6*C*, bar 3), and endothelial ferritin accumulated (Fig. 6*D*, lane 3). Intriguingly, the supernatants of PMA-activated PMNs, without any addition of hemoglobin, could also modestly enhance heme oxygenase mRNA (Fig. 6 *A* and *B*, lane 4 and bar 4) and enzyme activity (Fig. 6*C*, bar 4), but not ferritin (Fig. 6*D*, bar 4). Similarly, PMA alone slightly but insignificantly increased heme oxygenase mRNA and enzyme activity but had no effect on ferritin content (lane 5 and bars 5). The supernatants of hemoglobin exposed to resting PMNs (no methemoglobin present, Fig. 5) provoked no significant changes in heme oxygenase or ferritin (Fig. 6, lane 2 and bars 2).

We have discovered (13) the dichotomy that although brief exposure to free heme produced an endothelium hypersusceptible to oxidant damage, more prolonged exposure rendered it highly resistant; moreover, this resistance was shown to parallel accumulation of the newly synthesized iron-sequestering protein ferritin. Therefore, we tested whether similar chronic exposure to methemoglobin might reverse enhancement of oxidant-mediated cytotoxicity. Indeed, treatment of the endothelium with methemoglobin 16 hr prior to oxidant stress completely prevented cytolysis, supporting the likely importance of ferritin induction in attenuating oxidant damage catalyzed by endothelial exposure to heme proteins (Fig. 7). Conversely, addition of stoichiometric concentrations

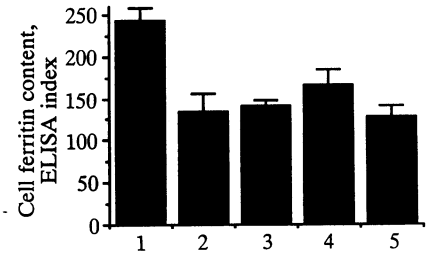

FIG. 4. Inhibition of methemoglobin-induced ferritin synthesis by both serum factors and NaCN. Endothelial cells were exposed for 4 hr to 5 μM methemoglobin (bar 1) alone or in the presence of 15% human serum (bar 2), stoichiometric amounts of hemopexin (bar 3) and haptoglobin (bar 4), or 5 μM cyanomethemoglobin (bar 5). After this exposure, the endothelial monolayers were further incubated in methemoglobin-free culture medium for 8 hr. The results represent the ELISA index (mean ± SEM) of at least five experiments done in sextuplet.

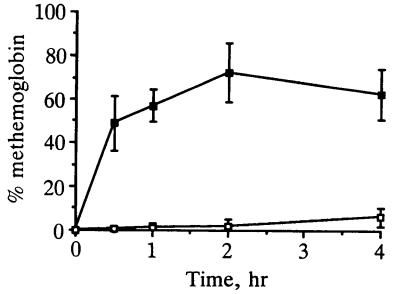

FIG. 5. Activated PMNs rapidly oxidize hemoglobin to methemoglobin. PMNs (5×10^6 cells per ml) resting (□) or activated by PMA (100 ng/ml; ■) were incubated with 20 μM ferrohemoglobin for various times up to 4 hr. Methemoglobin formation is expressed as a percentage of total hemoglobin. The results represent the percent methemoglobin (mean ± SEM) of three experiments.

of haptoglobin to methemoglobin (or hemopexin to free heme) significantly (≈70%) inhibited the benefit produced by prolonged exposure to these heme moieties.

DISCUSSION

Oxidative vascular damage is thought to underlie various human diseases, including atherosclerosis, adult respiratory distress syndrome, and reperfusion injury. We have shown (27) that increased iron content of endothelium can sensitize it to reagent oxidants such as H_2O_2 or those generated by stimulated inflammatory cells. We have reasoned that a source of endothelial iron load might be derived from heme proteins released from damaged circulating RBCs in close contact with vascular lining cells. Although we have demonstrated (12) that free heme can indeed be rapidly incorporated into hydrophobic domains of cultured endothelium and serve as a source of highly damaging Fe, the question remained as to whether intact hemoglobin would also be similarly assimilated. The present studies demonstrate that methemoglobin acutely sensitizes vascular endothelium to oxidant injury. Oxidation of ferrohemoglobin to ferrihemoglobin is essential for this deleterious effect, presumably because methemoglobin readily releases its hemes in solution (20); ferrohemoglobin or other heme proteins, such as metmyoglobin and cytochrome *c*, all of which avidly bind hemes (28, 29), do not alter endothelial integrity. Furthermore, if metheme binding to globin is strengthened by addition of haptoglobin or cyanide (20, 30, 31) or if released hemes are religanded to hemopexin (12, 32, 33), methemoglobin loses much of its capacity to sensitize endothelium.

That methemoglobin (unless treated with cyanide) readily releases free hemes that can be religanded to naked globin chains or to albumin was first demonstrated by Bunn and Jandl (20). The present studies suggest that endothelial cells, probably to their detriment, can compete with these heme-liganding proteins for free heme.§ Conversely, two plasma proteins, haptoglobin and hemopexin, may act to depress endothelial iron loading by this mechanism through their ability to tightly bind free hemoglobin and heme, respectively. We note that this depression is not absolute, however, since slight but significant increases in ferritin content continue to occur in methemoglobin-treated endothelium, despite the addition of these binding proteins (Fig. 4). This might reflect the previously reported capacity, shown with other mammalian tissues, of cells to incorporate and metabolize free iron derived from heme or heme proteins, even when they are bound to hemopexin or haptoglobin (35, 36). However, the blunted response of endothelium in the present

§Evaluation of the possible physiologic significance of this competition requires some consideration of free heme concentrations extant in patients and of the efficiency of its scavenging by plasma proteins, especially albumin. We note that plasma free heme levels of >20 μM (much higher than the concentrations used in these studies) have been reported in diverse hemolytic diseases, such as sickle cell anemia or thalassemia major (34). Moreover, in separate studies (not shown), we have found that albumin must be present in ≈5 times heme stoichiometric amounts to inhibit significantly heme-catalyzed endothelial cytotoxicity or in roughly equal stoichiometric amounts to inhibit methemoglobin-catalyzed damage; both of these amounts are far greater than the ratio found in whole blood in which serum albumin (≈300 μM) is present at 1:25 molar ratio of heme (≈8 mM). Thus, it seems reasonable to suggest that hemorrhage, for instance, into an atherosclerotic plaque could provide sufficient free heme to sensitize vascular cells to oxidant damage.

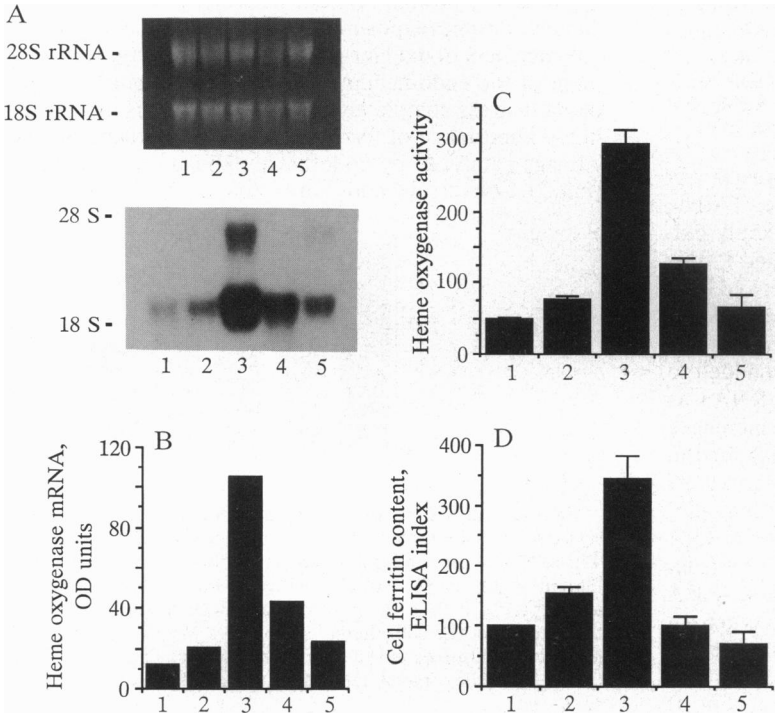

FIG. 6. Activated PMNs induce endothelial-cell heme oxygenase and ferritin via hemoglobin oxidation. Endothelial monolayers were incubated with control medium (lanes 1 and bars 1), with the supernatant of resting (lanes 2 and bars 2) or PMA-activated (lanes 3 and bars 3) PMNs preincubated with ferrohemoglobin for 30 min, supernatant of PMA-activated PMNs (lanes 4 and bars 4), or PMA alone (lanes 5 and bars 5). Supernates from activated PMN mixtures (lanes 3 and bars 3) contained 85% methemoglobin and 15% hemichrome. (*A*) Northern blot analysis of mRNA for heme oxygenase after a 4-hr induction of endothelial cells. (*Upper*) Equal quantities of RNA are shown in the ethidium bromide-stained agarose gel. (*Lower*) After transfer and hybridization, the heme oxygenase mRNA is recognized by ^{32}P-labeled cDNA probe. (*B*) Labeling intensity as determined by densitometry of the bands. (*C*) Heme oxygenase enzyme activity (pmol of bilirubin formed per mg of cell protein per 60 min) at 8 hr. (*D*) Ferritin content at 12 hr after treatment of endothelium was measured for the same groups as in *A*. Results are the ELISA index (mean ± SEM) of at least three experiments.

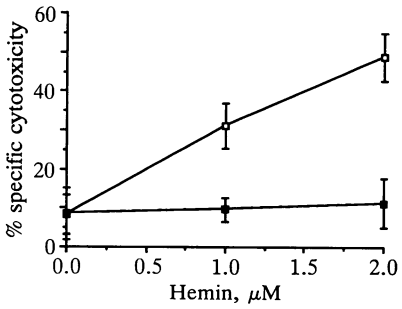

FIG. 7. Methemoglobin pretreatment of endothelium provides cell protection against hemin/H_2O_2-mediated lysis. Porcine endothelial monolayers were pretreated with methemoglobin (80 μM; ■) or DMEM (control; □) for 60 min and the culture medium was then replaced with methemoglobin-free solution for 15 hr. Endothelial-cell oxidant stress was then provided by addition of various concentrations of hemin for 60 min followed by H_2O_2 (100 μM) for 2 hr. Results are the specific cytotoxicity (mean ± SEM) of two experiments performed in duplicate.

studies suggests these cells may have relatively few haptoglobin or hemopexin receptors.

Likewise, in diverse other cells, studies have shown that uptake of heme induces heme oxygenase (15, 18, 37). This 32-kDa heat shock protein degrades heme to biliverdin, iron, and carbon monoxide. We believe the rapid and marked induction by methemoglobin of endothelial heme oxygenase mRNA in the present studies (Fig. 2) serves as a sensitive assay for the release of free heme from methemoglobin and its subsequent incorporation into the endothelium. This conclusion is supported by the finding that heme oxygenase induction was significantly reduced if heme release was inhibited by cyanide or haptoglobin (Fig. 3) or if heme was scavenged by simultaneously added hemopexin (data not shown).

Since oxidation of hemoglobin to methemoglobin is essential for endothelial perturbation, we sought to model oxidant conditions that might be relevant to vascular pathophysiology. Previous studies by us (24) and others (25, 26) have demonstrated that activated inflammatory cells (PMNs and monocytes) can efficiently oxidize hemoglobin contained in RBCs to methemoglobin. In the present studies, we demonstrate that soluble hemoglobin is even more rapidly oxidized to methemoglobin when exposed to activated, but not resting, PMNs (Fig. 5); moreover, supernatants of this mixture when added to endothelial cells rapidly induce heme oxygenase and ferritin formation. Although we believe released heme is the critical component in these inductions, some caveats are acknowledged: for instance, we have shown (12) that activated PMNs can directly release inorganic Fe from heme, so that free iron may itself contribute to the demonstrated ferritin accumulation. In addition, the supernate of PMA-activated PMNs alone (with no hemoglobin present) was also shown to induce small amounts of endothelial heme oxygenase (Fig. 6 *A–C*, lane 4 and bars 4). This might reflect the presence of reactive oxidant species, such as H_2O_2, since heme oxygenase has been shown to be induced in other cells by reagent oxidants (18, 37) or instead might be due to other heme-containing PMN constituents, such as myeloperoxidase.

As in our studies with heme itself (13), the effects of methemoglobin on endothelial susceptibility to oxidant damage were found to be dichotomous. Acute exposure markedly sensitized endothelial cells to oxidants, whereas more chronic exhibition rendered them virtually completely resistant. This resistance parallels and, we believe, results from the synthesis and accumulation of large amounts of the iron-chelator protein ferritin. This, coupled with our observation (38) that human atherosclerotic plaques are remarkably enriched for immunoreactive ferritin, suggests that the *in vitro* results presented herein may have relevance to atherogenesis *in vivo*. In addition, more recent evidence from epidemiologic studies of Salonen and coworkers (1) in Finland also suggests a critical role of iron in atherogenesis. They report an impressive 5-fold increased risk of myocardial infarction in persons with high serum LDL (>5 mM) who also manifest elevated serum ferritin levels (>200 ng/ml). It is tempting to speculate that elevated serum ferritin might, at least in part, reflect increased ferritin stores in endothelial cells, which in turn, might suggest their prior exposure to heme proteins in the circulation.

This work was supported in part by National Institutes of Health Grants 4R37-HL28935, 5R01-HL33793, R29-DH38767, and R01-A125625.

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