Heme Oxygenase: Who Needs It? (44455)

RICHARD GALBRAITH[1]

Departments of Medicine, Patient Oriented Research and the General Clinical Research Center, University of Vermont, Burlington, Vermont 05405

Abstract. **Heme is a molecule that is synthesized by the sequential actions of eight enzymes and is ubiquitous throughout nature. For many years it has been known that heme is also catabolized to yield biliverdin (which is subsequently reduced to bilirubin), one atom of iron, and one molecule of carbon monoxide. There has been a recent explosion of interest in this degradative process that is catalyzed by the rate-limiting enzyme, heme oxygenase. In particular, there has been a special interest in the potential physiological and pathological roles that may be played by these breakdown products. This minireview will examine some of these potential functional correlates, with special emphasis on potential oxidant and antioxidant effects of the bilirubin, carbon monoxide, and iron that result from the activity of heme oxygenase.**

[P.S.E.B.M. 1999, Vol 222]

Heme oxygenase (HO) is an enzyme that catalyzes the degradation of heme by opening its tetrapyrrole ring structure to yield the linear tetrapyrrole biliverdin, and one atom each of iron and carbon monoxide (CO). Under almost all circumstances there is an ample quantity of biliverdin reductase to reduce biliverdin rapidly to the more familiar bilirubin. The question that has puzzled biologists for a long time is why?

Heme is a highly conserved molecule that is essential for most forms of life and is the linchpin of useful redox reactions. Its synthesis is complex, involving eight enzymes that are both mitochondrial and cytosolic. Eight molecules each of glycine and succinyl CoA are consumed to synthesize each molecule of heme. Once synthesized, heme forms the catalytic unit of numerous proteins including the respiratory chain cytochromes, numerous synthetic and degradative cytochrome P450 s, catalase, peroxidase, nitric oxide synthase, guanylate cyclase, and tryptophan pyrrolase. Heme, by virtue of its cardinal function of repetitive oxidation/reduction cycles, is extraordinarily stable and is tightly bound to its various protein partners or transport/scavenging proteins such as ligandin, albumin, or hemopexin. It is true that heme itself can act as a pro-oxidant and generate potentially toxic moieties, but if there is a free heme pool, it has evaded direct detection and must be vanishingly small. Based on these observations, the purpose of degrading heme has seemed at best obscure.

This review will present the various potential functional correlates of heme oxygenase activity and review the evidence that underlies their potential physiological significance. Special emphasis will be placed on potential oxidant or antioxidant effects of the products of heme oxygenase: bilirubin, CO, and iron. No attempts will be made to provide an exhaustive review of the literature.

Historical Perspective

It is difficult to know when the products of the action of heme oxygenase were first recognized and pondered because the chemistry of heme degradation, unlike most biochemical functions, is color-coded and readily observable in ourselves and others. Thus, the sequelae of a severe blow to the skin invariably proceeds from a bruise that is black (the color of heme), through green (the color of biliverdin) to yellow (the color of bilirubin). Doubtless, the clinical jaundice of hyperbilirubinemia, due for example to liver disease or infections, was also recognized, as it was by Hippocrates, but its significance and origin remained unknown for centuries.

Serious attempts to understand the biochemistry of heme degradation were not seen until the 19th century. In 1847, Virchow recognized the association between hemoglobin breakdown and biliverdin (1). That association was more formally established in 1926 (2) and then conclusively in 1950 by London *et al.* (3). Interestingly, endogenous formation of CO in man was reported in 1949 (4), and it was only later that its production was shown to derive from heme degradation (5–8).

[1] To whom requests for reprints should be addressed at MCHV Campus/Baird 795, University of Vermont, Burlington, VT 05405. E-mail: rgalbrai@zoo.uvm.edu

0037-9727/99/2223-0299$14.00/0

In vitro degradation of heme to biliverdin was first demonstrated in 1964 (9), and this finding was confirmed (10–12) and the enzyme activity named microsomal heme oxygenase. The activity, dependent on NADPH and molecular oxygen, attacked the α-meso bridge of heme. Activity was also shown to be inhibited by CO and reactivated by light. The activity was therefore assumed to result from the actions of cytochrome P450 (13). However, it was subsequently clearly established that this activity was separate and independent of cytochrome P450 (14–16) and further characterization of the enzyme heme oxygenase began.

Heme oxygenase (EC 1:14:99:3, heme-hydrogen donor: oxygen oxidoreductase) has now been well characterized. The salient features of its action are illustrated in Figure 1. Heme oxygenase activity has been detected in almost all species and in almost all tissues. In mammals, it is highest in spleen, brain, and testes (17). In humans, 250–400 mg of bilirubin are formed daily, and ≈ 80% is derived from the catabolism of heme in senescent erythrocytes (18), which occurs mostly in the spleen. Generally, HO activity has been measured by spectrophotometric estimation of bilirubin generated from microsomal fractions in the presence of excess concentrations of biliverdin reductase (10, 11). Alternative methods include measurement of CO release (19, 20), [C^{14}]-bilirubin elaboration from C^{14}- labeled heme (21, 22), and high pressure liquid chromotography detection of bilirubin and biliverdin (23, 24).

Three isozymes of HO have been reported and named HO1, HO2, and HO3 (25, 26). They are products of three different genes (27). HO1 is inducible in response to heme, metals, stress, UV irradiation, chemicals, hyperthermia, and drugs, and has been shown to be identical to heat shock protein 32 (28). HO2 is biochemically and structurally distinct and appears to be either little regulated or constitutive (28). HO3 is similar to HO2 and dissimilar to HO1 in structure, and, in contrast to both HO1 and HO2, is a poor heme catalyst. It has been postulated to have a regulatory role in heme-dependent processes (26).

Due to its inducibility, much has been learned concerning the regulation of HO1 gene expression and resulting HO activity. Structural motifs in the gene include an Sp1 site, a GCN4 site, and sites for heat shock and metal-dependent transcription factors (29). Induction of HO1 has also been reported to be dependent on tyrosine phosphorylation (30), AP-1 activation of regulatory elements (31, 32), c-*jun* N-terminal kinase (33), protein kinase A (34), the MAP kinases ERK and p38 (35), and nitric oxide (36). Interestingly, HO can reciprocally inhibit nitric oxide synthase by degrading heme (37). There is much work and interest in continued study of the almost bewildering array of inducers and mechanisms of induction of HO1. The recent crystallization of recombinant human HO1 (38) may accelerate studies of the mechanism of action of the protein. Meanwhile, recent studies reporting the expression of alternative transcripts of HO2 (39) and the notion that HO2 binds heme through an additional heme regulatory motif that is not involved in heme catalysis (40) open the door to further insights into HO2 regulation and function. HO3 awaits further characterization.

Products in Search of Functions

A central question alluded to earlier is, What is the purpose of activating the heme degradative pathway (illustrated in Fig. 2), which produces bilirubin, CO, and iron at the expense of heme? One answer might be that the goal is to deplete heme as a regulatory or protective maneuver, but for the reasons previously discussed this is only sometimes a satisfying explanation. Another alternative, and one which we will now examine, is that heme is degraded to obtain products that are intrinsically useful and that serve important functions, an explanation that might justify the otherwise wasteful destruction of heme.

Carbon Monoxide. The flurry of interest in and reports about the physiological role of nitric oxide (NO) in the 1980s and the similarities between NO and carbon monoxide (CO), led Marks *et al.* (41) in 1991 to pose the question, Does carbon monoxide have a physiological role? In 1993, both Verma *et al.* (42) and Maines (43) suggested that the answer to this question was in the affirmative and that CO might bind to and activate guanylate cyclase as had been previously demonstrated for NO. Literature implicating CO as a physiological regulator has continued to grow.

Relaxation of blood vessels in response to exogenous CO was reported in 1991 (44), and the existence of HO activity, as measured by CO production, was reported in arteries of rodents and humans in 1995 (45). Evidence has accrued to suggest that vascular CO is involved in the maintenance of vascular tone in small and medium-sized arteries (46) and aortas (47). CO regulation of blood pressure in response to manipulations that acutely increase blood pressure have also been reported (48), and such CO-dependent pressure regulation is modified in response to salt ingestion (49). Likewise, exogenous CO toxicity alters vascular tone, but here the effects may be compounded by activation of

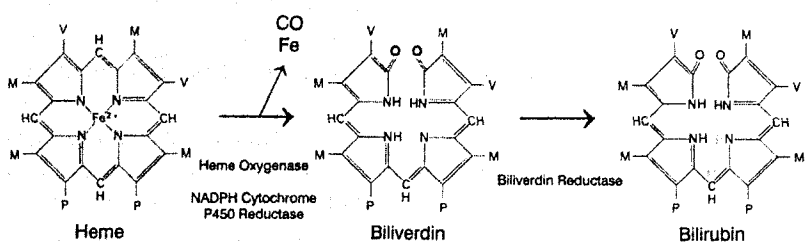

Figure 1. The heme degradative pathway. M (methyl): CH_3; V (vinyl) : CH =CH_2; P (propionate): CH_2–CH_2–COOH; Fe: iron; CO: carbon monoxide.

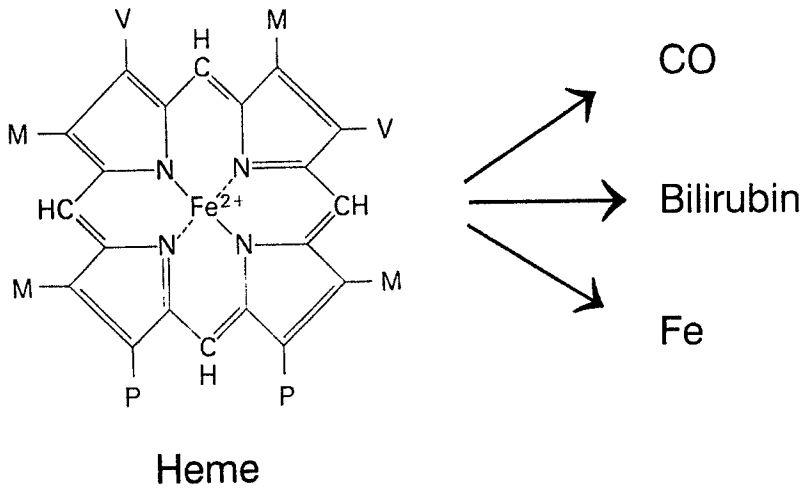

Figure 2. The products of heme degradation. Fe: Iron; CO: carbon monoxide

NO-dependent processes (50). There is also evidence to support a central role of CO in cardiovascular control by altering glutamatergic transmission in the nucleus tractus solitarius of the brain (51). Vascular resistance in the hepatic circulation is also partially controlled by CO (52) and under conditions of hemorrhagic shock, the contribution of CO appears to outweigh that of NO (53). In parenchymal cells, CO elaborated from HO2 activity appears to be the physiologic mediator of vascular tone rather than that derived from HO1 (54, 55).

CO has also been implicated in the control of cerebral blood flow in the brain, a tissue with a great capacity to generate CO from HO2 (56), and in which HO2 colocalizes with and presumably helps regulate guanylate cyclase (42, 57, 58). However, in brain there is also evidence that CO is involved in long-term potentiation (59–61), thought by some to be a surrogate for memory. Lastly, CO has been implicated in neuroendocrine control of corticotropin-releasing hormone from the hypothalamus (62, 63).

HO2 has been detected in canine jejunum (64), mouse small intestine (65), and rat stomach (66). The presumed elaboration of CO in these locales may indicate a role for CO in gut muscle activation. Both HO1 and HO2 have been detected in guinea pig placenta (67) and may play a role in the regulation of vascular tone. However, in human myometrium, expression of HO1 and HO2 were both increased greater than 15-fold during pregnancy, under which circumstances CO appeared to limit uterine contractility (68). Likewise, HO2 has been detected in rat kidney (69) where it also may play a role in vascular tone. In the pancreas, CO has been implicated in the release of insulin and glucagon, probably involving cGMP (70). However, in the lung CO release has been proposed as a mediator for oxidative stress in asthma (71) and bronchiectasis (72). CO has also been implicated as a cGMP-independent inducer of apoptosis in thymocytes (73). Moreover, in tissues with limited antioxidant reserves, CO may be associated with lipid peroxidation (74).

Bilirubin. Since the recent observations that bilirubin is an antioxidant of potential physiological importance (75) and that HO1 and heat shock protein 32 are identical, the literature has exploded with claims concerning the antioxidant effects of HO-derived bilirubin (and biliverdin, also an antioxidant). Examples have derived from studies of irradiation of the skin (76), endotoxin and ischemia in the liver (77, 78), nephrotoxic nephritis (79), oxygen toxicity in fibroblasts (80), peroxynitrite and plasma proteins (81), and hypoxia (82), atherosclerotic lesions (83), and cytokines (84) in the cardiovascular system. In the central nervous system, HO1 induction has been hypothesized or shown to be protective against hydrogen peroxide–mediated injury in astroglia (85) and transient forebrain ischemia (86–88), and HO2 protein kinase C-dependent induction was protective against hydrogen peroxide toxicity in neurons (89).

In many instances, it has been and remains difficult to establish causality for the protective effect attributed to bilirubin. After all, heme and heme-protein function must also be depleted, and CO and iron produced, in the same HO-dependent activity that creates bilirubin so which factor(s) is responsible for a given effect is unclear. More elegant evidence has accrued through the use of transgenic knockouts and overexpressers of HO, although the caveats above still apply. Examples include protective effects against hypoxia during HO1 overexpression in rat lung tissue (90, 91) and neuroprotection against ischemia from middle cerebral artery occlusion in mice with HO1 overexpression (92). Conversely, wild-type cardiac xenografts survived longer in rats than cardiac transplants from HO1 (±) and HO1 (–/–) rats (93), and HO1 knockout mice displayed right ventricular dilatation and infarction in response to hypoxia compared to wild-type mice in which these effects were not seen (94). Lastly, a 6-year-old boy with growth retardation, hemolytic anemia, an abnormal coagulation system, endothelial and renal damage, and increased iron deposition was found to have complete HO deficiency (95). *In vitro* studies of his lymphocytes revealed extreme sensitivity to hemin-induced cell injury.

Bilirubin can also be detrimental to organisms. Such detriments range from the relative inconvenience of itching in jaundice through to the profoundly disabling and irre-

versible brain damage of kernicterus in the newborn. Clearly, concentration and localization of bilirubin are of cardinal importance, but other factors such as binding and transport proteins (96) and excretion will modulate the balance between the beneficial antioxidant effects and the deleterious toxic effects of bilirubin.

Iron. The proverbial fly in the antioxidant ointment discussed above is iron, which is stoichiometrically released with each molecule of CO and bilirubin produced. The presence of free iron in biological systems can lead to the rapid formation of damaging reactive oxygen metabolites like the hydroxyl radical and the superoxide radical (Fenton and Haber-Weiss reactions). It has been suggested that the prooxidant tendencies of iron are rapidly neutralized by the synthesis of ferritin, a cellular storage system for iron. Ferritin is itself an acute-phase reactant. Certainly, cells pretreated with hemin have been shown to have induced levels of ferritin and increased resistance to oxidative stress (97) but the effect was seen 16–20 hr after hemin exposure. Similarly, ferritin is highly induced in skin 24 hr after ultraviolet irradiation (98). Iron regulatory proteins bind to iron-responsive elements in ferritin mRNA, and such binding is modulated by iron, nitric oxide, oxidative stress, and hypoxia/reoxygenation (99, 100). In lung tissue, hyperoxia leads to HO1 induction, but in the presence of free iron, the HO1 induction is dramatically increased (101); however, the action of induced HO would lead to the accumulation of even more iron. In HO2 knockouts, despite increased HO1 expression, there was increased sensitivity to hyperoxia and increased iron accumulation without a concomitant increase in ferritin (102). There is also evidence that reduced iron uptake can prevent oxidative stress (103) or be decreased in oxidatively imbalanced cells (104). However, it is hard to see how ferritin can be upregulated fast enough to obviate a rise in free iron which, itself, is a likely candidate for the initial signal for upregulation. Lastly, iron and hydrogen peroxide have been reported to cleave DNA by Fenton reactions in sequence-specific sites often found in telomere repeats (105). At lower concentrations of hydrogen peroxide, preferential cleavage occured in sequences often found in the promoters for normal responses of many genes to iron or oxygen stress.

Summary

What is clear, from the review of the literature, is that all the products of the activity of heme oxygenase (bilirubin, CO, and iron) are biologically active and display both beneficial and deleterious effects under different circumstances. In the case of bilirubin and iron, the majority of effects, whether beneficial or detrimental, are either mediated by or attributable to redox chemistry and the balance between oxidants and antioxidants. This is probably true for CO, but the mechanism is less direct, being mediated by the binding of CO to the heme component of a variety of cellular heme proteins. Furthermore, the bulk of the literature supporting the activity of the products of heme degradation in altering oxidant/antioxidant balance has been reported from studies in the cardiovascular and central nervous systems.

What is unclear is how heme oxygenase activity is regulated temporally and spacially, and whether or not there is specificity in promoting certain actions of individual products of heme metabolism. It is relatively straightforward to envisage that under physiological circumstances (e.g., senescent erythrocytes) where there is an unwanted increase in heme concentrations, it would be highly desirable to metabolize and neutralize any potential pro-oxidant effects of the released heme. Likewise, in pathological states (e.g., cardiovascular hemorrhage) the resulting rapid increase in heme concentrations could be neutralized by the rapid induction of HO activity. In these examples, the primary goal would appear to be to abrogate heme toxicity by its degradative removal. However, it is considerably harder to imagine how the activity of HO could be controlled and regulated so that heme is catabolized specifically to produce a biological effect secondary to an increased concentration of a specific product, either bilirubin, carbon monoxide, or iron. Is there a heme pool sequestered for just such an event or must heme be degraded at the expense of other heme-dependent processes? How would it be possible to use CO as a neurotransmitter without concomitantly exposing the brain to unwanted and potentially toxic concentrations of iron? How could beneficial antioxidant effects of bilirubin be isolated from unwanted oxidant effects of iron and CO-dependent changes in cGMP and vascular tone? Similarly, what are the relative contributions of HO1 and HO2 to these processes, and what are the as yet totally undefined roles of HO3?

Extensive use has been made in experimental systems of competitive inhibitors of HO activity, and some of them have been proposed for clinical use in hyperbilirubinemia. However, they all suffer from the same potential drawbacks described above in that inhibition of HO activity not only inhibits the production of bilirubin, but also of CO and iron, which may be required for the maintenance or regulation of other physiological processes. Further complications arise from the observation that small quantities of heme may also be degraded nonenzymatically under certain circumstances and that increased concentrations of CO have been reported secondary to the use of drugs such as volatile anesthetics (106). There is clearly no shortage of interesting questions that need to be answered in understanding the complexities of the sequelae of heme degradation in biological systems. The answer to the starting question, "Heme Oxygenase: Who needs it?" is clearly practically all of us. The questions that remain are, Under what circumstances and can detrimental effects be diminished and beneficial effects augmented? We hope that the great attention this subject has garnered in the last few years bodes well for our future understanding of the area.

1. Virchow R. Die pathologischen pigments. Arch Pathol Anat 1:379–486, 1847.

2. Mann FC, Sheard C, Bollman JL et al. The formation of bile pigment from hemoglobin. Am J Physiol **76:**306–315, 1926.
3. London IM, West K, Shemin D et al. On the origin of bile pigment in normal man. J Biol Chem **184:**351–358, 1950.
4. Sjorstrand T. Endogenous formation of carbon monoxide in man under normal and pathological conditions. Scand J Clin Lab Invest **1:**201–214, 1949.
5. Coburn RF, Williams WJ, White P et al. Production of carbon monoxide from hemoglobin *in vivo*. J Clin Invest **46:**346–356, 1967.
6. Drabkin DL. The hemophagous organ of the placenta and *in vivo* studies of endogenous carbon monoxide production. Ann N Y Acad Sci **174:**49–63, 1970.
7. Landaw SA, Callahan EW Jr., Schmid R. Catabolism of heme *in vivo*: Comparison of the simultaneous production of bilirubin and carbon monoxide. J Clin Invest **49:**914–925, 1970.
8. White P. Carbon monoxide production and heme catabolism. Ann N Y Acad Sci **174:**23–31, 1970.
9. Wise CD, Drabkin DL. Degradation of haemoglobin and hemin to biliverdin by a new cell-free system obtained from the hemophagous organ of dog placenta. Fed Proc **23:**323, 1964.
10. Tenhunen R, Marver HS, Schmid R. Microsomal heme oxygenase: Characterization of the enzyme. J Biol Chem **244:**6388–6394, 1969.
11. Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of hemoglobin to bilirubin. Proc Natl Acad Sci U S A **61:**748–755, 1968.
12. Tenhunen R, Marver HS, Schmid R. The enzymatic conversion of hemoglobin to bilirubin. Trans Assoc Am Phys **82:**363–371, 1969.
13. Tenhunen R, Marver HS, Primstone NR et al. Enzymatic degradation of heme: Oxysensitive cleavage requiring cytochrome P-450. Biochemistry **11:**1716–1720, 1972.
14. Maines MD, Kappas A. Cobalt induction of hepatic heme oxygenase, with evidence that cytochrome P-450 is not essential for this enzyme activity. Proc Natl Acad Sci U S A **71:**4293–4297, 1974.
15. Maines MD, Kappas A. Cobalt stimulation of heme degradation in the liver: Dissociation of microsomal oxidation of heme from cytochrome P-450. J Biol Chem **250:**4171–4177, 1975.
16. Yoshida T, Takahashi S, Kikuchi G. Partial purification and reconstitution of the heme oxygenase system from pig spleen microsomes. J Biochem (Tokyo) **75:**1187–1191, 1974.
17. Maines MD. Heme oxygenase: Function, multiplicity, regulatory mechanisms, and clinical applications. FASEB J **2:**2557–2568, 1988.
18. Berk PD, Howe RB, Bloomer JR et al. Studies on bilirubin kinetics in normal adults. J Clin Invest **48:**2176–2190, 1979.
19. Cavallui-Stahl E, Johnson GI, Lundh BA. A new method for the determination of microsomal heme oxygenases based on the quantitation of carbon monoxide formation. Scand J Clin Lab **38:**69–76, 1978.
20. Vreman HJ, Stevenson D. Heme oxygenase activity as measured by carbon monoxide activity. Anal Biochem **168:**31–38, 1998.
21. Tenhunen R. Method for microassay of microsomal heme oxygenase activity. Anal Biochem **45:**600–607, 1972.
22. Sierra E, Nutter LA. Microassay for heme oxygenase activity using thin-layer chromotography. Anal Biochem **200:**27–30, 1992.
23. Ryter S, Kvam E, Richman L et al. A chromatographic assay for heme oxygenase activity in cultured human cells: Application to artificial heme oxygenase overexpression. Free Radic Biol Med **24:**959–971, 1998.
24. Ryter S, Kvam E, Tyrrell RM. Heme oxygenase activity determination by high-performance liquid chromatography. Oxidants Antioxidants **300:**322–336, 1999.
25. Williams JF. Induction of tolerance in mice and rats to the effect of endotoxin to decrease the hepatic microsomal mixed-function oxidase system: Evidence for a possible macrophage-derived factor in the endotoxin effect. Int J Immunopharmacol **7:**501–509, 1985.
26. McCoubrey WK Jr., Huang TJ, Maines MD. Isolation and characterization of a cDNA from the rat brain that encodes hemoprotein heme oxygenase-3. Eur J Biochem **247:**725–732, 1997.
27. Cruse I, Maines MD. Evidence suggesting that the two forms of heme oxygenase are products of different genes. J Biol Chem **263:**3348–3353, 1988.
28. Maines MD. Characterization and regulation of heme oxygenase isozymes at the molecular level. In: Maines MD, Ed. Heme Oxygenase: Clinical Applications and Functions. Boca Raton, FL: CRC Press, pp109–144, 1999.
29. Muller RM, Taguchi H, Shibahara S. Nucleotide sequence and organization of the rat heme oxygenase gene. J Biol Chem **262:**6795–6802, 1987.
30. Masuya Y, Hioki K, Tokunaga R et al. Involvement of the tyrosine phosphorylation pathway in induction of human heme oxygenase-1 by hemin, sodium arsenite, and cadmium chloride. J Biochem (Tokyo) **124:**628–633, 1998.
31. Hartsfield CL, Alam J, Choi AM. Transcriptional regulation of the heme oxygenase 1 gene by pyrrolidine dithiocarbamate. FASEB J **12:**1675–1682, 1998.
32. Camhi SL, Alam J, Wiegand GW et al. Transcriptional activation of the HO-1 gene by lipopolysaccharide is mediated by 5′ distal enhancers: Role of reactive oxygen intermediates and AP-1. Am J Respir Cell Mol Biol **18:**226–234, 1998.
33. Oguro T, Hayashi M, Nakajo S, Numazawa S, Yoshida T. The expression of heme oxygenase-1 gene responded to oxidative stress produced by phorone, a glutathione depletor, in the rat liver; the relevance to activation of c-jun n-terminal kinase. J Pharmacol Exp Ther **287:**773–778, 1998.
34. Immenschuh S, Kietzmann T, Hinke V et al. The rat heme oxygenase-1 gene is transcriptionally induced *via* the protein kinase a signaling pathway in rat hepatocyte cultures. Mol Pharmacol **53:**483–491, 1998.
35. Elbirt KK, Whitmarsh AJ, Davis RJ et al. Mechanism of sodium arsenite-mediated induction of heme oxygenase-1 in hepatoma cells. J Biol Chem **273:**8922–8931, 1998.
36. Immenschuh S, Tan M, Ramadori G. Nitric oxide mediates the lipopolysaccharide-dependent upregulation of the heme oxygenase-1 gene expression in cultured rat Kupffer cells. J Hepatol **30:**61–69, 1999.
37. Turcanu V, Dhouib M, Poindron P. Heme oxygenase inhibits nitric oxide synthase by degrading heme: A negative feedback regulation mechanism for nitric oxide production. Transplant Proc **30:**4184–4185, 1998.
38. Schuller DJ, Wilks A, de Montellano PR et al. Crystallization of recombinant human heme oxygenase-1. Protein Sci **7:**1836–1838, 1998.
39. Gibbs L, Willis D, Morgan MJ. The identification and expression of heme oxygenase-2 alternative transcripts in the mouse. Gene **221:**171–177, 1998.
40. McCoubrey W, Huang TJ, Maines MD. Heme oxygenase-2 is a hemoprotein and binds heme through heme regulatory motifs that are not involved in heme catalysis. J Biol Chem **272:**12568–12574, 1997.
41. Marks GS, Brian JF, Nakatsu K et al. Does carbon monoxide have a physiological function? Trends Pharmacol Sci **12:**185–188, 1991.
42. Verma A, Hirsch DJ, Glatt CE et al. Carbon monoxide: A putative neural messenger. Science **259:**381–384, 1993.
43. Maines MD. Carbon monoxide: An emerging regulator of cGMP in the brain. Mol Cell Neurosci **4:**389–397, 1993.
44. Furchgott RF, Jothianandin S. Endothelium-dependent and -independent vasodilation involving cGMP: Relaxation induced by nitric oxide, carbon monoxide, and light. Blood Vessels **28:**52–61, 1991.
45. Grundemar L, Johansson M-B, Ekelund M et al. Haem oxygenase activity in blood vessel homogenates as measured by carbon monoxide production. Acta Physiol Scand **153:**203–204, 1995.
46. Kozma F, Johnson R, Chang F, et al. Contribution of endogenous carbon monoxide to regulation of diameter in resistance vessels. Am J Physiol **276:**R1087–R1094, 1999.
47. Sammut IA, Foresti R, Clark JE et al. Carbon monoxide is a major contributor to the regulation of vascular tone in aortas expressing high levels of haeme oxygenase-1. Br J Pharmacol **125:**1437–1444, 1998.
48. Motterlini R, Gonzales A, Foresti R et al. Heme oxygenase-1-derived carbon monoxide contributes to the suppression of acute hypertensive responses *in vivo*. Circ Res **83:**568–577, 1998.
49. dos Santos EA, Yamaguishi GA, Heimann JC. Effect of the heme/heme oxygenase pathway on the relationship between salt consumption and blood pressure. J Hypertens **16:**1965–1969, 1998.
50. Thom SR, Fisher D, Xu YA et al. Role of nitric oxide-derived oxidants in vascular injury from carbon monoxide in the rat. Am J Physiol **276:**H984–H992, 1999.
51. Silva CCS, Almeida VA, Haibara AS et al. Role of carbon monoxide in l-glutamate-induced cardiovascular responses in nucleus tractus solitarius of conscious rats. Brain Res **824:**147–152, 1999.
52. Pannen BH, Bauer M. Differential regulation of hepatic arterial and

portal venous vascular resistance by nitric oxide and carbon monoxide in rats. Life Sci **62**:2025–2033, 1998.
53. Pannen BH, Kohler N, Bauer M et al. Protective role of endogenous carbon monoxide in hepatic microcirculatory dysfunction after hemorrhagic shock in rats. J Clin Invest **102**:1220–1228, 1998.
54. Goda N, Suzuki K, Naito M et al. Distribution of heme oxygenase isoforms in rat liver. J Clin Invest **101**:604–612, 1998.
55. Durante W, Schafer A. Carbon monoxide and vascular cell function (review). Int J Mol Med **2**:255–262, 1998.
56. Montecot C, Seylaz J, Pinard E. Carbon monoxide regulates cerebral blood flow in epileptic seizures but not in hypercapnia. Neuroreport **9**:2341–2346, 1998.
57. Rich A, Farrugia G, Rae JL. Stimulation of a potassium current in rabbit corneal epithelium by carbon monoxide. Am J Physiol **267**:C435–C442, 1994.
58. Maines MD, Eke BC, Weber CM et al. Corticosterone has a permissive effect on expression of heme oxygenase-1 in CA1-CA3 neurons of hippocampus in thermal-stressed rats. J Neurochem **64**:1769–1779, 1995.
59. Stevens C, Wang Y. Reversal of long-term potentiation by inhibitors of haem oxygenase. Nature **364**:147–149, 1993.
60. Zhuo M, Laitinen JT, Li X-C et al. On the respective roles of nitric oxide and carbon monoxide in long-term potentiation in the hippocampus. Learning Memory **5**:467–480, 1998.
61. Zhuo M, Hu Y, Schulz C et al. Role of guanyl cyclase and cGMP-dependent protein kinase in long-term potentiation. Nature **368**:635–639, 1994.
62. Pozzoli G, Mancuso C, Mirtella A et al. Carbon monoxide as a novel neuroendocrine modulator: Inhibition of stimulated corticotropin-releasing hormone release from acute rat hypothalamic explants. Endocrinology **135**:2314–2317, 1994.
63. Snyder SH, Jaffrey SR, Zakhary R. Nitric oxide and carbon monoxide: Parallel roles as neural messengers. Brain Res Rev **26**:167–175, 1998.
64. Farrugia G, Miller SM, Rich A et al. Distribution of heme oxygenase and effects of exogenous carbon monoxide in canine jejunum. Am J Physiol **274**:G350–G358, 1998.
65. Miller SM, Farrugia G, Schmalz PF et al. Heme oxygenase-2 is present in interstitial cell networks of the mouse small intestine. Gastroenterology **114**:239–244, 1998.
66. Hu Y, Yang M, Ma N et al. Contribution of carbon monoxide-producing cells in the gastric mucosa of rat and monkey. Histochem Cell Biol **109**:369–373, 1998.
67. Odrcich MJ, Graham CH, Kimura KA et al. Heme oxygenase and nitric oxide synthase in the placenta of the guinea pig during gestation. Placenta **19**:509–516, 1998.
68. Acevedo CH, Ahmed A. Hemeoxygenase-1 inhibits human myometrial contractility *via* carbon monoxide and is upregulated by progesterone during pregnancy. J Clin Invest **101**:949–955, 1998.
69. Hu Y, Ma N, Yang M et al. Expression and distribution of heme oxygenase-2 mRNA and protein in rat kidney. J Histochem Cytochem **46**:249–256, 1998.
70. Henningsson R, Alm P, Ekstrom P et al. Heme oxygenase and carbon monoxide: Regulatory roles in islet hormone release. A biochemical, immunohistochemical, and confocal microscopic study. Diabetes **48**:66–76, 1999.
71. Horvath I, Donnelly LE, Kiss A et al. Raised levels of exhaled carbon monoxide are associated with an increased expression of heme oxygenase-1 in airway macrophages in asthma: A new marker of oxidative stress. Thorax **53**:668–672, 1998.
72. Horvath I, Loukides S, Wodehouse T et al. Increased levels of exhaled carbon monoxide in bronchiectasis. Thorax **53**:867–870, 1998.
73. Turcanu V, Dhouib M, Gendrault J-L et al. Carbon monoxide induces murine thymocyte apoptosis by a free radical–mediated mechanism. Cell Biol Toxicol **14**:47–54, 1998.
74. Vreman HJ, Wong RJ, Sanesi CA et al. Simultaneous production of carbon monoxide and thiobarbituric acid reactive substances in rat tissue preparations by an iron-ascorbate system. Can J Physiol Pharmacol **76**:1057–1065, 1999.
75. Stocker R, Yamamoto Y, McDonagh AF et al. Bilirubin is an antioxidant of possible physiologic importance. Science **235**:1043–1046, 1987.
76. Applegate LA, Noel A, Vile G et al. Two genes contribute to different extents to the heme oxygenase enzyme activity measured in cultured human skin fibroblasts and keratinocytes: Implications for protection against oxidant stress. Photochem Photobiol **61**:285–291, 1995.
77. Rizzardini M, Carelli M, Cabello Porras MR et al. Mechanisms of endotoxin-induced haem oxygenase mRNA accumulation in mouse liver: Synergism by glutathione depletion and protection by N-acetylcysteine. Biochem J **304**:477–483, 1994.
78. Ito K, Ikeda S, Shibata T et al. Immunohistochemical analysis of heme oxygenase-1 in rat liver after ischemia. Biochem Mol Biol Int **43**:551–556, 1997.
79. Mosley K, Wembridge DE, Cattell V et al. Heme oxygenase is induced in nephrotoxic nephritis and hemin, a stimulator of heme oxygenase synthesis, ameliorates disease. Kidney Int **53**:672–678, 1998.
80. Dennery PA, Sridhar KJ, Lee SC et al. Heme oxygenase-mediated resistance to oxygen toxicity in hamster fibroblasts. J Biol Chem **272**:14937–14942, 1997.
81. Minetti M, Mallozz C, Di Stasi AM et al. Bilirubin is an effective antioxidant of peroxynitrite-mediated protein oxidation in human blood plasma. Arch Biochem Biophys **352**:165–174, 1998.
82. Borger DR, Essig DA. Induction of HSP 32 gene in hypoxic cardiomyocytes is attenuated by treatment in N-acetyl-l-cysteine. Am J Physiol **274**:H965–H973, 1998.
83. Wang L, Lee T-S, Lee F-Y et al. Expression of heme oxygenase-1 in atherosclerotic lesions. Am J Pathol **152**:711–720, 1998.
84. Terry CM, Clikeman JA, Hoidan JR et al. Effect of tumor necrosis factor-α and interleukin-1α on heme oxygenase-1 expression in human endothelial cells. Am J Physiol **274**:H883–H891, 1998.
85. Dwyer BE, Lu S-Y, Laitinen JT et al. Protective properties of tin- and manganese-centered porphyrins against hydrogen peroxide–mediated injury in rat astroglial cells. J Neurochem **71**:2497–2504, 1998.
86. Matsuoka Y, Kitamura Y, Okazaki M et al. Induction of heme oxygenase-1 and major histocompatibility complex antigens in transient forebrain ischemia. J Cereb Blood Flow Metab **18**:824–832, 1998.
87. Takeda A, Onodera H, Sugimoto A et al. Increased expression of heme oxygenase mRNA in rat brain following transient forebrain ischemia. Brain Res **666**:120–124, 1994.
88. Takizawa S, Hirabayashi H, Matsushima K et al. Induction of heme oxygenase protein protects neurons in cortex and striatum, but not in hippocampus, against transient forebrain ischemia. J Cereb Blood Flow Metab **18**:559–569, 1998.
89. Dore S, Takahashi M, Ferris C et al. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. Proc Natl Acad Sci U S A **96**:2445–2450, 1999.
90. Suttner DM, Sridhar K, Lee CS et al. Protective effects of transient HO-1 overexpression on susceptibility to oxygen toxicity in lung cells. Am J Physiol **276**:L443–L451, 1999.
91. Otterbein LE, Kolls JK, Nantell LL et al. Exogenous administration of heme oxygenase-1 by gene transfer provides protection against hyperoxia-induced lung injury. J Clin Invest **103**:1047–1054, 1999.
92. Panahian N, Yoshiura M, Maines MD. Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice. J Neurochem **72**:1187–1203, 1999.
93. Soares MP, Lin Y, Anrather J et al. Expression of heme oxygenase-1 can determine cardiac xenograft survival. Nat Med **4**:1073–1077, 1998.
94. Yet S-F, Perrella MA, Layne MD et al. Hypoxia induces severe right ventricular dilatation and infarction in heme oxygenase-1 null mice. J Clin Invest **103**:R23–R29, 1999.
95. Yachie A, Niida Y, Wada T et al. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. J Clin Invest **103**:129–135, 1999.
96. Mireles LC, Lum MA, Dennery PA. Antioxidant and cytotoxic effects of bilirubin on neonatal erythrocytes. Pediatr Res **45**:355–362, 1999.
97. Lin F, Girotti A. Hemin-enhanced resistance of human leukemia cells to oxidative killing: Antisense determination of ferritin involvement. Arch Biochem Biophys **352**:51–58, 1998.
98. Applegate LA, Scaletta C, Panizzon R et al. Evidence that ferritin is UV inducible in human skin: Part of a putative defense mechanism. J Invest Dermatol **111**:159–163, 1998.
99. Eisenstein RS, Blemings KP. Iron regulatory proteins, iron responsive elements, and iron homeostasis. J Nutr **128**:2295–2298, 1998.
100. Pantopoulos K, Hentze MW. Activation of iron regulatory protein-1

by oxidative stress *in vitro*. Proc Natl Acad Sci U S A **95:**10559–10563, 1998.
101. Fogg S, Agarwal A, Nick HS et al. Iron regulates hyperoxia-dependent human heme oxygenase-1 gene expression in pulmonary endothelial cells. Am J Respir Cell Mol Biol **20:**797–804, 1999.
102. Dennery PA, Spitz DR, Yang G et al. Oxygen toxicity and iron accumulation in the lungs of mice lacking heme oxygenase-2. J Clin Invest **101:**1001–1011, 1998.
103. Braun V. Regulation of iron uptake minimizes iron-mediated oxidative stress. J Biosci **23:**483–489, 1998.
104. Malorni W, Testa U, Rainaldi G et al. Oxidative stress leads to a rapid alteration of transferrin receptor intravesicular trafficking. Exp Cell Res **241:**102–116, 1998.
105. Henle ES, Han Z, Tang N et al. Sequence-specific DNA cleavage by Fe^{2+}-mediated Fenton reactions has possible biological implications. J Biol Chem **274:**962–971, 1998.
106. Baxter PJ, Garton K, Kharasch ED. Mechanistic aspects of carbon monoxide formation from volatile anesthetics. Anesthesiology **89:**929–941, 1998.