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Forum Review

Hypoxia and Heme Oxygenases: Oxygen Sensing and Regulation of Expression

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ABSTRACT

Heme is an essential molecule for life, as it is involved in sensing and using oxygen. Heme must be synthesized and degraded within an individual nucleated cell. Physiologic heme degradation is catalyzed by two functional isozymes of heme oxygenase, heme oxygenase-1 (HO-1) and HO-2, yielding carbon monoxide, iron, and biliverdin, an immediate precursor to bilirubin. HO-1 is an inducible enzyme, but the expression level of HO-2 is maintained in a narrow range. Characteristically, human HO-1 contains no Cys residue, whereas human HO-2 contains three Cys residues, each of which might be involved in heme binding. These features suggest separate physiologic roles of HO-1 and HO-2. Recent studies have shown that the expression levels of HO-1 and HO-2 are reduced under hypoxia, depending on the cell types. Moreover, we have proposed HO-2 as a potential O_2 sensor, because HO-2–deficient mice show hypoxemia and a blunted hypoxic ventilatory response with normal hypercapnic ventilatory response. HO-2–deficient mice also show hypertrophy of the pulmonary venous myocardium and enlargement of the carotid body. These morphometric changes are attributable to chronic hypoxemia. Here, we update the understanding of the regulation of HO-1 and HO-2 expression and summarize the regulatory role of HO-2 in the intercellular communication. *Antioxid. Redox Signal.* 9, 2209–2225.

INTRODUCTION

Heme is an essential molecule for life; it is involved in oxygen homeostasis by sensing and using molecular O_2. Thus, heme must be synthesized and degraded within an individual nucleated cell. Heme is synthesized through multiple steps catalyzed by eight enzymes, together with their regulators (28, 120). In contrast, heme is degraded by heme oxygenase, which catalyzes the oxidative breakdown of heme to biliverdin IXα, carbon monoxide (CO) and iron (156, 157, 178). Biliverdin IXα is rapidly reduced to bilirubin IXα by biliverdin IXα reductase (159).

Heme oxygenase consists of two structurally related isozymes, heme oxygenase-1 (HO-1) and HO-2 (73, 135, 160) (Fig. 1). Both enzymes contain the transmembrane domain at their carboxyl termini. The heme degradation products, biliverdin IXα, bilirubin IXα, CO, and ferrous iron, are important bioactive molecules, but are toxic to cells if they are present in excess. CO is bound to hemoglobin to form carboxyhemoglobin, which is transported to the lung and is excreted in exhaled air. The CO content in arterial blood reflects the overall heme degradation (84). Iron is mainly transported to the bone marrow and is reused for heme biosynthesis and erythropoiesis. Bilirubin IXα has been considered a toxic waste product, because neonatal hyperbilirubinemia could cause bilirubin encephalopathy (120). However, the beneficial role of bilirubin IXα has been established as a radical scavenger (142) and a chain-breaking antioxidant, as judged by the production of bilirubin oxidative metabolites (171).

The status of heme catabolism is also important in the pathogenesis of various infectious diseases, as evident from the fact that *Corynebacterium diphtheriae*, a gram-positive aerobic bacterium, possesses Hmu O protein, which shares 33% overall identity with human HO-1 (see Fig. 1) (128). This bacterium,

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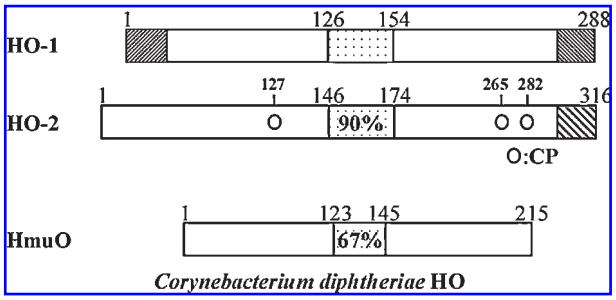

FIG. 1. Structures of human heme oxygenases. The conserved catalytic domain is shown as stippled, and the number indicates the amino acid sequence identity to human HO-1. Also highlighted are the hydrophilic N-terminus and the hydrophobic C-terminus of HO-1. Human HO-2 contains three copies of CP motif, and one of them (position 127) is not conserved in mouse and rat HO-2. HO-2 may sequester heme to maintain the intracellular level of free heme. HmuO represents heme oxygenase of *Corynebacterium diphtheriae*. Note that human HO-1 and HmuO contain no Cys residue. Reproduced with modifications from Ref. 132, with permission from Tohoku University Medical Press.

which causes diphtheria, produces a virulent diphtheria toxin under the regulation of iron. Hmu O protein lacks the hydrophobic C-terminus and thus functions as a soluble enzyme (19).

In this article, we update the regulatory network for expression of HO-1 and HO-2 and summarize the newly identified role of HO-2 in the intercellular signaling (Fig. 2). Several review articles have been published on various aspects of heme oxygenases (4, 121, 162, 175), including those on the reaction mechanisms of heme degradation (57, 179).

HO-1 AND HO-2: TWO ENZYMES ARE BETTER THAN ONE

Human HO-1 and HO-2 share 43% amino acid sequence identity (44, 79, 177). Interestingly, human HO-1 lacks a cysteine residue, whereas human HO-2 contains three cysteine residues, each of which constitutes a dipeptide of the cysteine and proline (CP motif) (see Fig. 1). Likewise, rat HO-1 lacks a cysteine residue (135), whereas rat HO-2 contains two cysteine resides as the CP motifs (78). It has been shown that these CP motifs are not involved in the heme-breakdown reaction and could bind heme (78). It is therefore conceivable that HO-2 may serve to sequester heme to maintain the intracellular free heme level or reduce heme-mediated oxidative stress. Moreover, we have shown that the selective knockdown of HO-2 expression with each of two different siRNAs is consistently associated with the increased expression of HO-1 mRNA and protein in human cell lines, suggesting that HO-2 may regulate the expression of HO-1 by modulating the cellular heme level (22).

The physiologic importance of HO-1 has been established by the clinical manifestations of a patient with HO-1 deficiency (169) and the phenotypic consequences of the HO-1–deficient mice (113, 114). Mating between heterozygous mice showed partial prenatal lethality of the HO-1–deficient ($-/-$) mice, and homozygous mating pairs did not yield viable litters. In contrast, HO-2–deficient (HO-2$^{(-/-)}$) mice survive normally for at least 1 year (112). However, subsequent studies have revealed ejaculatory abnormalities with reduced mating behavior in male HO-2$^{(-/-)}$ mice (11) (Fig. 3). Moreover, HO-2$^{(-/-)}$ mice show slower gastrointestinal transit time without bowel obstruction (182), increased susceptibility to hyperoxic lung damage (20) and cerebral ischemia (23), and exaggerated inflammatory responses in corneal injury and peritonitis (130). A recent study revealed a regulatory role of HO-2 in oxygen sensing and the maintenance of the pulmonary blood flow (2). These results suggest the roles of HO-2 in intracellular and intercellular signaling (see Fig. 2). In the latter context, calmodulin binds to HO-2 in a calcium-dependent manner, thereby enhancing HO-2 activity (9). The CO generated by HO-2 is involved in synaptic transmission.

HO-2 as a regulator for intercellular communication

The unique phenotypes of HO-2$^{(-/-)}$ mice indicate that HO-2 is responsible for the intercellular communication (see Fig. 3). HO-2 is involved in the function of interstitial cells of Cajal, which are pacemaker cells that play an important role in the control of gut motility (110). HO-2 immunoreactivity has been shown to be present in many interstitial cells of Cajal present around the myenteric plexus of the human colon, but absent in sparsely appearing interstitial cells of Cajal in the bowels of patients with Hirschsprung disease (110). Hirschsprung disease is characterized by the absence of ganglion cells in the gut and manifests congenital megacolon. The lack of HO-2 in the interstitial cells of Cajal may cause motility dysfunction because of impaired communication between interstitial cells of Cajal and smooth muscle cells in Hirschsprung disease. Likewise, HO-2 immunoreactivity is present in interstitial cells of Cajal in the smooth muscle layer of normal human pylorus, but is decreased in the HO-2 immunoreactivity in interstitial cells of Cajal in infantile hypertrophic pyloric stenosis (111).

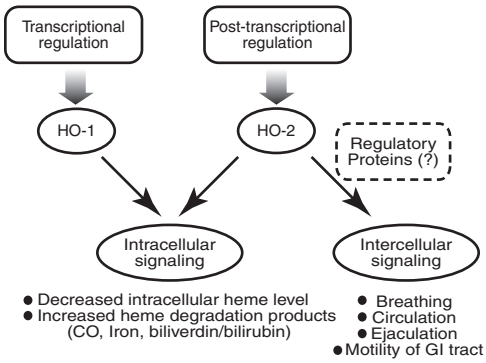

FIG. 2. Overview for the separate roles of HO-1 and HO-2. Two thick arrows highlight that the expression of HO-1 and HO-2 may be mainly regulated at the transcriptional and the posttranscriptional levels, respectively. Note that HO-2 is also involved in the intercellular signaling, as judged by the phenotypes of HO-2$^{(-/-)}$ mice (2, 11, 112, 182).

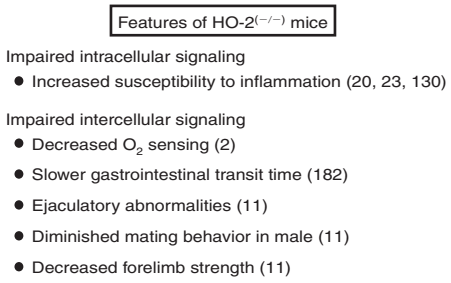

FIG. 3. Features of HO-2$^{(-/-)}$ mice. The numbers within parentheses indicate references.

FEEDBACK REGULATION OF HEME CATABOLISM

It is essential for living organisms to maintain the intracellular heme level within a narrow range, which is achieved by the appropriate balance between heme biosynthesis and heme breakdown (153). Earlier studies showed that the activity of HO, now known as HO-1, is induced by its substrate heme in animal models and primary cultures of alveolar macrophages (137, 159). Moreover, the expression levels of translatable and hybridizable HO-1 mRNA were increased by the treatment with hemin in pig alveolar macrophages and in rat liver, respectively (135, 138). Thus, HO-1 provides a good model for the substrate-mediated induction of an enzyme in mammals.

Conversely, the repression of HO-1 expression is important in the feedback regulation mediated by intracellular heme (Fig. 4) (92, 132). The reduced HO-1 expression may transiently increase the intracellular heme level, which may facilitate heme binding to Bach1, a heme-regulated transcriptional repressor (40, 42, 106), thereby derepressing transcription of the HO-1 gene. A role of Bach1 for HO-1 gene expression has been shown in Bach1-deficient mice, in which HO-1 is overexpressed in many tissues (143). Moreover, expression of Bach1 mRNA is induced in some types of cultured human cells by hypoxia, interferon-γ, or desferrioxamine, each of which reduced the expression of HO-1 (61, 161). Therefore, Bach1 is involved in the feedback regulation of HO-1 expression by sensing heme.

The expression levels of HO-1 and HO-2 affect the intracellular free heme pool, which in turn influences the availability of heme for the synthesis or function of various hemoproteins (1, 22, 188). For example, sustained overexpression of HO-1 *via* gene transfer reduced the levels of heme and cGMP in pulmonary microvessel endothelial cells (1), although acute induction of HO-1 increased cGMP through the CO-mediated activation of guanylate cyclase. Moreover, overexpression of HO-1 reduced the levels of prostaglandin E_2 (117) and cyclooxygenase activity (33, 117). These results suggest that the degree of HO-1 expression modulates the cellular heme levels, which in turn may influence the activities of heme-containing enzymes, such as soluble guanylate cyclase, nitric oxide synthase, and cyclooxygenase (74, 150). Importantly, 15-deoxy-$D^{12,14}$-PGJ_2 (15d-PGJ_2), which is spontaneously generated from prostaglandin D_2, functions as an endogenous ligand for the nuclear receptor, peroxisome proliferator–activated receptor-γ. 15d-PGJ_2 was reported to induce the expression of HO-1 (31) by activating a factor related to nuclear factor erythroid 2 (Nrf2) (see Fig. 4) (45). Thus, cyclooxygenase may contribute to the appropriate expression of HO-1 through 15d-PGJ_2, as discussed in a recent review (150).

STRUCTURAL ORGANIZATION OF THE HUMAN HO-1 AND HO-2 GENES

Human HO-1 gene

The human HO-1 and HO-2 genes are localized to chromosome 22q12 and to 16p13.3, respectively (63, 64). The human HO-1 gene contains one copy of the functional Maf recognition element (MARE) immediately downstream from the cadmium-responsive element (see Fig. 4) (61, 149). Members of the small Maf family (MafK, MafF, and MafG) are basic region leucine zipper proteins that can function as transcriptional activators or repressors (41, 87). Nrf2 functions as a transcriptional activator by forming a heterodimer with a member of the Maf family (5), whereas Bach1 heterodimerizes with MafK (42, 97, 106). Thus, various activators and repressors are involved in transcriptional control of the HO-1 gene. It has been established that the Bach1–small Maf heterodimer could repress transcription of target genes by binding to the MARE (40, 106). The reduced HO-1 expression may transiently increase the intracellular heme levels, which in turn may facilitate heme to bind to Bach1, thereby derepressing transcription of the target genes of Bach1 (97).

An enhancer internal to the human HO-1 gene has been reported (38). The entire 12.5 kb of the human HO-1 gene, including introns and exons, in conjunction with a 4.5-kb upstream region, conferred on the reporter gene significant heme-

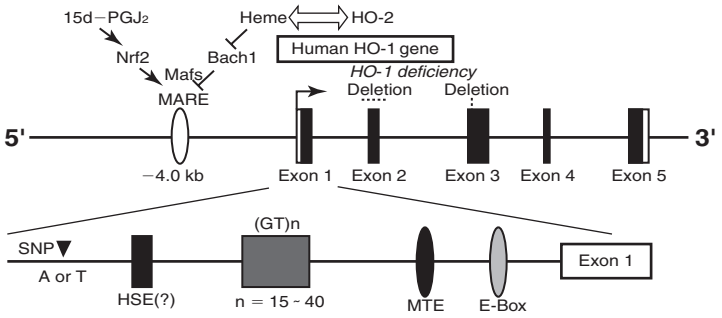

FIG. 4. Transcriptional regulation of the human HO-1 gene. The MARE is bound by heterodimers, consisting of Nrf2 or Bach1 and one of small Maf proteins. Note a feedback regulation involving HO-1, Bach1, and heme. HO-2 may sequester heme to maintain the intracellular level of free heme. Also shown are a potential heat-shock element (HSE), the polymorphic (GT)n repeat, and two E box motifs in the proximal promoter region (89, 123). The two mutations, a deletion of exon 2 and a two-base deletion in exon 3, are indicated (169).

and cadmium-mediated induction. More recently, it was reported that Nrf2 recruits a chromatin remodeling factor to the (GT)n sequence, which in turn facilitates Z-DNA formation and activates transcription of the HO-1 gene during oxidative stress (187). These results suggest that Nrf2 or other factors could be responsible for the regulation of chromatin structure, thereby allowing the collaboration between the upstream and downstream enhancers.

Rat HO-1 gene

The proximal promoter region of the rat HO-1 gene contains two copies of the functional heat-shock element (HSE) (89, 100, 122, 134), and HO-1 mRNA expression and activity were increased in rat cells by heat shock (42°C) (134). Moreover, hyperthermia was shown to lead to the remarkable induction of HO-1 mRNA and protein in the rat brain (24). Thus, rat HO-1 has been established as a heat-shock protein (HSP32). Confusion has arisen from the fact that the human HO-1 gene promoter also contains an HSE (see Fig. 4), although the levels of HO-1 mRNA and protein/activity are not inducible by heat shock in human cell lines (136, 177) and in human alveolar macrophages obtained by alveolar lavage (101). Moreover, heme oxygenase activity is not induced by heat shock in cell lines of monkey, porcine, and murine origins, but is induced by hemin treatment in all cell lines examined (131). These results indicate that only rat HO-1 is a heat-shock protein. However, it should be noted that HO-1 is a stress-response protein in human cells (55, 154, 177).

Human HO-2 gene

Two noticeable features exist in the organization of the human HO-2 gene: the potential bidirectional promoter and a large intron 1 of ~30 kb (Fig. 5) (188). Because of the large size of intron 1, we determined the transcription-initiation site of the HO-2 gene by the PCR-based method for the amplification of the 5′-end of cDNA. The exon 1 of the HO-2 gene encodes the 5′-untranslated region of HO-2 mRNA. The HO-2 gene and the gene encoding HSCARG of unknown function (GenBank accession number: AAG09721) are adjacently located in the head-to-head orientation, and stand only ~1.5 kb apart. HSCARG is also known as NmrA-like family domain containing 1 (NMRAL1, Gene ID: 57407). Thus, the HO-2 gene and the HSCARG gene appear to share a common promoter region. Generally, a bidirectional promoter lacks the TATA box and contains GC-rich sequences (62), as seen in the HO-2 gene promoter. Transient expression analysis showed that hypoxia did not influence the transient expression of a reporter gene, carrying the 1.5-kb bidirectional HO-2 gene promoter in HeLa human cervical cancer cells (188), although hypoxia decreased the expression of endogenous HO-2 mRNA in HeLa cells. Moreover, the expression of HO-2 mRNA was not noticeably changed in human cells under various conditions, in which HO-1 expression was remarkably induced (139, 144, 145, 149, 188). These results suggest that the expression level of HO-2 mRNA may be regulated mainly at the posttranscriptional level (see Fig. 2).

INTERINDIVIDUAL DIFFERENCES IN THE REGULATION OF HO-1 EXPRESSION

Microsatellite polymorphism in the human HO-1 gene promoter

The human HO-1 gene promoter contains the repeat of $(GT)_{15}AT(GT)_{14}$ (136), which shows the length polymorphism (60). Analyses of Japanese people have revealed that the numbers of (GT)n repeats vary from 15 to 40, with the two common repeats of 23 and 30 (170). The (GT)n repeat may influence the basal promoter activity or the induction level of the HO-1 gene expression in response to stress, as the (GT)n repeats are likely to form Z-DNA, left-handed helix. The (GT)n sequence is involved in Z-DNA formation, which is essential for the efficient HO-1 induction during oxidative stress (187). Importantly, the polymorphic (GT)n repeats are not present at the equivalent positions of the rat HO-1 gene (88). Thus, transcription of the human HO-1 gene is under the regulation of the fine-tuning system that includes the Bach1 system and the (GT)n polymorphism (see Fig. 4).

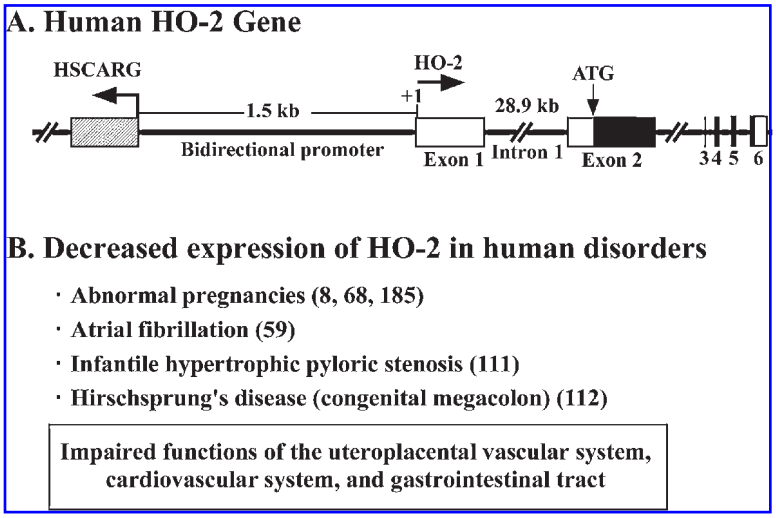

FIG. 5. Structural organization of the human HO-2 gene and the decreased expression of HO-2. (**A**) Human HO-2 gene. Exon 1 encodes the untranslated region of HO-2 mRNA, and exon 2 contains the ATG translation-initiation codon. A major transcription start site, identified by 5′-RACE, is indicated with the residue 1 (188). Note that the HO-2 gene promoter is juxtaposed to the HSCARG gene (GenBank accession number: AAG09721) in the opposite direction. (**B**) Decreased expression of HO-2 in human disorders. Note that the abnormalities listed may represent the impaired intercellular communication.

Long (GT)n alleles as a risk factor

The associations with GT repeat polymorphism have been investigated in various diseases (Table 1) (25). In most cases, long (GT)n alleles are associated with susceptibility to pathologic conditions, including pulmonary emphysema (170), restenosis of the femoropopliteal segment after transluminal angioplasty (26) and balloon angioplasty (125), restenosis after coronary stenting (16), coronary artery disease in diabetic patients (17) or patients with coronary risk factors (51), and abdominal aortic aneurysm (126). In this context, HO-1 protein is expressed in atherosclerotic lesions (165), in which bilirubin is formed (93). Moreover, the long GT alleles are associated with lung adenocarcinoma in Japanese male smokers (56), with oral squamous cell carcinoma in Chinese male areca chewers (15), with intracranial aneurysms (83), with airway obstruction in smokers (32), and with pneumonia in the older Japanese population (176).

We have shown the correlation between the length of the (GT)n repeat of the HO-1 gene promoter and susceptibility to the development of chronic pulmonary emphysema in Japanese smokers (170). The proportion of allele frequencies of a long (GT)n repeat ($\geq$33 repeats), as well as the proportion of genotypic frequencies with a long (GT)n allele, was significantly higher in the patients with chronic pulmonary emphysema. Subsequently, we established Epstein–Barr virus-transformed lymphoblastoid cell lines, derived from subjects with long or short (GT)n alleles, and the viability of the cell lines with long $(GT)_n$ alleles was lower than that with short $(GT)_n$ alleles after treatment with H_2O_2 (39). These findings suggest that large size of a $(GT)_n$ repeat may reduce HO-1 inducibility by reactive oxygen species, thereby leading to the development of chronic pulmonary emphysema (170). In contrast, other investigators (37) demonstrated no association between HO-1 gene polymorphisms and the rate of decline in lung function in Canadian smokers. Moreover, short GT alleles appear to be associated with longevity in Japanese male subjects (173) and a reduced risk of ischemic cerebrovascular events (29) (see Table 1).

Short (GT)n alleles as a risk factor

The presence of individuals with long (GT)n repeats suggests some advantage for those subjects under certain conditions, such as cancer and infection, as HO-1 is responsible for the turnover of iron that is an essential requirement for cell proliferation. The lower expression level of HO-1 could restrict iron supply to cancer cells or certain pathogens that might be carried by a host. HO may affect the properties or fate of cancer cells by modulating the iron supply, angiogenesis, and/or apoptosis. Expression levels of HO-1 and HO-2 mRNAs tended to increase in eight cases of excised primary brain tumors, including glioblastoma multiforme and anaplastic astrocytomas, compared with the control brain tissue, as judged by Northern blot analysis (36). Recently, it was reported that the short (GT)n allele is associated with the development of malignant melanoma (99). The short (GT)n allele may be beneficial for the survival and growth of melanoma cells.

Importantly, it has been reported that the short (GT)n repeats ($n < 28$) in the HO-1 gene promoter are associated with a higher incidence of cerebral malaria in Karen people, who live near the border between Myanmar and Thailand (151). Cerebral malaria represents coma associated with severe malaria caused by *Plasmodium falciparum*. The pathogenesis

Table 1. Association between GT Repeats and Disease Conditions

Disease/healthy†*	*GT repeats Long (L)/short (S)*	*References*
Pulmonary disease		
Chronic pulmonary emphysema*	L $\geq$ 33	170
Airway obstruction in smokers*	L $\geq$ 33	32
Pneumonia*	L $\geq$ 33	176
Cardiovascular disease		
Restenosis after peripheral angioplasty or coronary stenting*	L $\geq$ 26 or 25	16,26,125
Abdominal aortic aneurysm*	L $\geq$ 25	126
Coronary artery disease in patients with risk fastors*	L $\geq$ 27	51
Coronary artery disease in type 2 diabetes mellitus*	L $\geq$ 32	17
Intracranial aneurysm*	L $\geq$ 36	83
Neoplasm		
Lung adenocarcinoma*	L $\geq$ 33	56
Areca-related oral squamous cell carcinoma*	L $\geq$ 31	15
Malignant melanoma*	S $<$ 25	99
Others		
Cerebral malaria*	S $<$ 28	151
Idiopathic recurrent miscarriage*	S $\leq$ 27	21
Longevity in male subjects†	S $<$ 27	173
Reduced risk of ischemic cerebrovascular events†	S $<$ 25	29

of coma is related to the adherence of *falciparum*-infected red blood cells to vascular endothelium of the cerebral microvasculature. The shorter (GT)n repeats may be associated with efficient induction of HO-1, which results in the release of larger amounts of CO, iron, and bilirubin from vascular endothelial cells at the sites of the adherence (sequestration). Iron might be efficiently incorporated into the infected erythrocytes and used for the growth of parasites (132). In this context, we have been interested in the lack of HO-1 induction by heat shock (42°C) in human cells (101, 123, 131, 177), as fever is an evolutionarily conserved response in host defense and essentially beneficial to the host. Fever in malaria (40°C or more) is schizontocidal but contributes to synchronization of parasites' life cycle within erythrocytes, thereby generating the characteristic fever spikes (65). The potential protective role of the long (GT)n alleles in cerebral malaria provides therapeutic implications (132), as drug-resistant *falciparum* strain is common (91).

Moreover, the short (GT)n allele in the HO-1 gene promoter is associated with idiopathic recurrent miscarriage (21). Idiopathic recurrent miscarriage is defined as three or more consecutive fetal losses before 20 weeks of gestation. Idiopathic recurrent miscarriage affects 0.5–1% of women, which may be related to the impaired function of the uteroplacental vascular system.

Conversely, no significant association with any GT repeat polymorphism was found in patients with Kawasaki disease (50), neonatal unconjugated hyperbilirubinemia (49), Alzheimer's and Parkinson's diseases (60), and longevity in women (173). It should be noted, however, that the negative results do not exclude the involvement of HO-1 in the pathogenesis of the relevant diseases.

SNPs in the human HO-1 gene promoter

The two genomic clones of the human HO-1 gene, which were derived from DNA of different individuals (136, 149), allowed us to find the A/T polymorphism (−413 A → T) in the proximal promoter region (see Fig. 3) (132, 147). It should be noted that the position number for the A/T polymorphism varies depending on the number of (GT)n repeats. Importantly, the A allele promoter exhibited significantly higher activity than the T allele promoter, as judged by the luciferase reporter assay in cultured bovine cells (102, 103). The AA genotype of the HO-1 gene is associated with an increased incidence of hypertension in women but not in men (103) and with a lower incidence of ischemic heart disease (107). Moreover, Ono *et al.* (103) identified another SNP, G(-1135)A, the functional implication of which remains to be investigated. These results suggest that (GT)n repeats and the SNPs may be involved in the fine-tuning of the HO-1 expression in humans.

REGULATION OF THE EXPRESSION OF HO-1 AND HO-2 UNDER HYPOXIA

Overview of O_2 sensing

All tissues and any types of mammalian cells are able to sense O_2 (67). Hypoxia-inducible factor 1 (HIF-1) (129), which plays an important role in O_2 homeostasis, is ubiquitously expressed. HIF-1 is a heterodimeric transcription factor and comprises an O_2-regulated α-subunit and a stable β-subunit. The α-subunit (HIF-1α) is labile under normoxia (20% O_2), whereas the β-subunit is constitutively expressed. The oxygen-mediated degradation of HIF-1α depends on the tumor-suppressor protein that is related to von Hippel–Lindau (VHL) syndrome (76). VHL syndrome is characterized by a predisposition to develop tumors that are highly vascularized. Importantly, the recognition of HIF-1α by VHL protein is dependent on the hydroxylation of conserved proline residues within HIF-1α (75).

Normobaric hypoxia as a model of hypoxemia

Two types of hypoxia are known: hypobaric and normobaric hypoxia. It has been reported that hypobaric hypoxia, at an ambient pO_2 equal to 120 hPa (4,500 m), leads to a greater hypoxemia, hypocapnia, blood alkalosis, and a lower O_2 arterial saturation in healthy male subjects, compared with normobaric hypoxia (124). These physiologic differences could be the consequence of an increase in dead-space ventilation, probably related to the barometric pressure reduction (124). In the present article, the term *hypoxia* indicates normobaric hypoxia, unless otherwise specified.

Hypoxemia is a common manifestation of various diseases that affect the airways or the pulmonary parenchyma, such as chronic obstructive pulmonary disease (152) and sleep apnea syndrome. Hypoxemia is a hemodynamic stress and may cause pulmonary hypertension, which generates pressure overload to the right ventricle, eventually leading to right heart failure. It is therefore of significance to explore whether normobaric hypoxia influences the expression levels of HO-1 and HO-2 in various organs. Our earlier study in rats showed that pressure overload, generated by normobaric hypoxia (10% O_2), increases the expression level of HO-1 mRNA in the right ventricle within 6 h after hypoxia (52) (Table 2). The O_2 concentration (10% O_2) is equivalent to the value at an altitude of ~5,000 m. Under the hypoxic condition used, both the right ventricular systolic pressure and the gravimetric index for right ventricular hypertrophy were higher in rats exposed for 3 weeks to hypoxia than those of age-matched controls (53). Likewise, normobaric hypoxia (8–10% O_2) causes the remodeling of the pulmonary artery in mice, which eventually leads to pulmonary hypertension and right ventricular hypertrophy (81, 107). Consistent in part with our observations (52), Lee *et al.* (69) showed that the expression levels of HO-1 mRNA are increased in rat heart, lung, aorta, and liver within 2 h of hypoxia (7% O_2). These results indicate the important role of HO-1 in response to acute hypoxia.

Increased arterial blood CO contents during acclimatization of mice to hypoxia

By using the system of normobaric hypoxia (10% O_2) to generate hypoxemia (52, 53), we assessed the changes in the total amount of heme breakdown in C57BL/6 mice during acclimatization to hypoxia by measuring arterial blood CO contents. The CO content in arterial blood reflects the overall heme degradation (84). Relative CO contents increased dy-

Table 2. Expression Levels of HO-1 and HO-2 under Hypoxia

HO-1 mRNA	*Refs*	*HO-1 protein*	*Refs*
Human cells		*Human cells*	
Dermal fibroblasts*	108	Dermal fibroblasts*	108
HaCaT keratinocytes*	47	HaCaT keratinocytes*	47
D407 retinal pigment epithelial cells*	161	D407 retinal pigment epithelial cells‡	161
KGI myeloid cells*	188	KGI myeloid cells‡	188
Microvascular endothelial cells*	70	Microvascular endothelial cells‡	70
Erythroleukemia cells (YN-1)*	188	Erythroleukemia cells (YN-1)†	188
Umbilical venous endothelial cells†	92	Umbilical venous endothelial cells†	61, 92
HeLa cervical cancer cells†	188	HeLa cervical cancer cells†	188
HepG2 hepatoma cells†	188	HepG2 hepatoma cells†	188
Coronary artery endothelial cells†	92		
Astrocytes†	92		
A549 lung cancer cells†	61		
T98G glioblastoma cells†	61		
Animals		*Animals*	
Rat heart*	52, 69	Rat lung*	13, 18
Rat lung*	18	Rat liver*	10
Rat liver*	69	Mouse heart*	35
Mouse heart*	35	Mouse liver†	35
Mouse liver*	35		
Animal cells		*Animal cells*	
Chinese hamster ovary cells*	90	Chinese hamster ovary cells*	90
Rat aortic smooth muscle cells*	85, 86	Rat aortic smooth muscle cells*	86
Rat cardiomyocytes*	27	Mouse cardiomyocytes*	168
C6 rat glioma cells*	61	Bovine aortic endothelial cells*	118
COS monkey kidney cells*	61		
Bovine brain microvascular endothelial cells*	61		
HO-2 mRNA		*HO-2 protein*	
Human cells		*Human cells*	
KGI myeloid cells‡	188	KGI myeloid cells‡	188
Erythroleukemia cells (YN-1, K562)†	188	Erythroleukemia cells (YN-1, K562)†	188
Jurkat T cells†	188	Jurkat T cells†	188
HeLa cervical cancer cells†	188	HeLa cervical cancer cells†	188
HepG2 hepatoma cells†	188	HepG2 hepatoma cells†	188
		Immortalized trophoblast cells†	6
Animals		*Animals*	
Mouse heart‡	35	Mouse heart*	35
Mouse lung‡	35	Mouse lung‡	35
Mouse liver‡	35	Mouse liver†	35

Expression levels of HO proteins were measured with antibody (Western blot analysis or ELISA) and/or enzyme assays. The numbers indicate references. Earlier reports are cited, in case of the same tissues or similar cell types. The ischemic insults are not included.

*Increase; †decrease; ‡no noticeable change.

namically in a time-dependent manner, compared with the 0-time control mice maintained under normoxia (Fig. 6) (35). The CO contents increased within 1 day of hypoxia and remained at the higher level for 21 days of hypoxia ($p < 0.05$). Unexpectedly, after 28 days of hypoxia, CO contents returned to the basal levels. The dynamic changes in the arterial CO contents suggest that the expression levels of HO-1 and HO-2 increase or decrease, depending on the organs, during acclimatization to hypoxia. Conversely, the number of red blood cells increased to reach the highest values after 3 days of hypoxia and remained at the high level during 28 days of hypoxia (35).

In a rat model of mountain sickness, rats were exposed for up to 3 weeks to hypobaric hypoxia, which is equivalent to the condition at an altitude of ~5,000 m (13). The carboxyhemoglobin level (percentage) increased within 1 day after hypobaric hypoxia, then decreased to the basal levels at 3 days, and again increased to the highest level at 3 weeks. These changes in rats under hypobaric hypoxia are similar to the changes observed in mice maintained under normobaric hypoxia for up to 3 weeks (35).

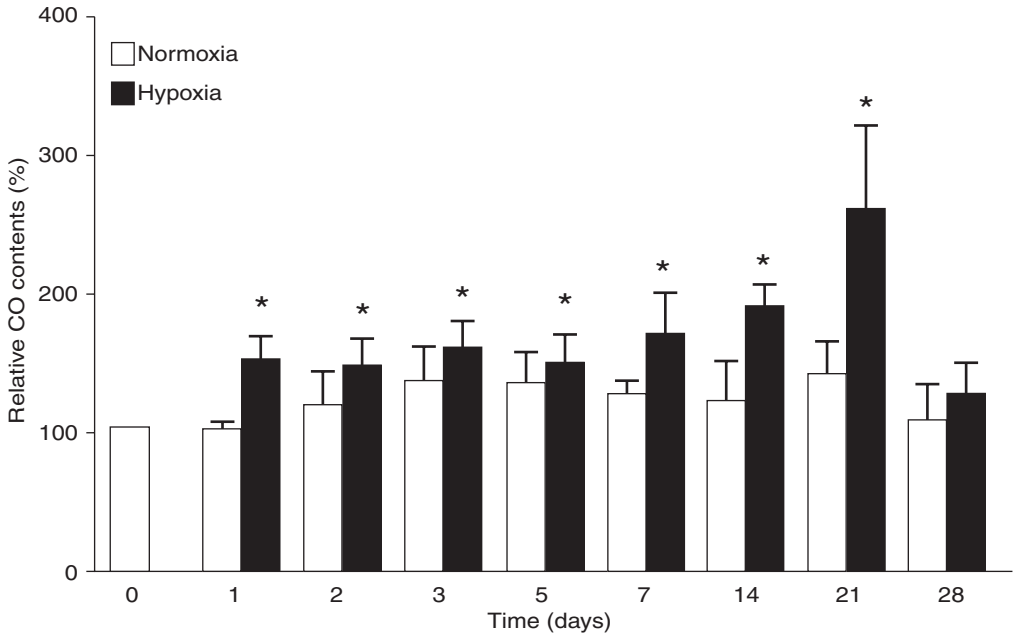

FIG. 6. Increase in the relative CO contents in arterial blood during acclimatization of mice to normobaric hypoxia. Relative CO content is the ratio to the value of the 0 time control under normoxia and shown as a percentage. Each column was calculated based on the original data of Ref. 35, expressed as mean ± SEM (three to nine animals per each time point). Statistical analyses were performed with two-way analysis of variance (factorial design) with a *post hoc* comparison test (Fisher's Protected Least Significant Difference exact test) with commercially available software (Statview 4.0, Calabasas, CA). Open and solid columns indicate the values under normoxia and hypoxia, respectively. Symbols represent statistically significant differences compared with the 0-time control ($*p < 0.05$).

Distinct expression profiles of HO-2 in mouse heart and liver during acclimatization to hypoxia

HO-2 has been considered a constitutively expressed enzyme, the expression level of which is maintained in a narrow range. Importantly, Weber and colleagues (166) reported the twofold induction of HO-2 mRNA expression in the rat brain after treatment with corticosterone. We analyzed the expression profile of HO-1 and HO-2 proteins in the heart, lung, and liver during acclimatization to normobaric hypoxia (35). HO-1 and HO-2 proteins were increased twofold and 1.3-fold, respectively, in the heart at 28 days of hypoxia, compared with the age-matched normoxia control. The increased expression of HO-1 and HO-2 may reflect the adaptation processes to hemodynamic stress, as normobaric hypoxia for 3 weeks increases pulmonary vascular resistance and induces right ventricular hypertrophy in C57BL/6 mice (107). In contrast, no statistically significant changes were detected in the expression levels of HO-1 and HO-2 mRNAs and proteins in the mouse lung (35) (see Table 2).

Unexpectedly, normobaric hypoxia transiently reduced the expression levels of HO-1 and HO-2 proteins in the mouse liver; HO-1 and HO-2 proteins were decreased by 20% and 40%, respectively, at 7 days of hypoxia, which returned to the basal levels at 14 days (35). Thereafter, HO-1 and HO-2 proteins remained at the basal levels for up to 28 days. Importantly, unlike HO-2 protein, the expression level of HO-2 mRNA was unchanged in the liver. In contrast, it has been reported that the HO activity was increased in the rat liver by 20% after exposure to hypobaric hypoxia for 28–30 days (10).

Differential effects of hypoxia in cultured cells

HO-1 expression was repressed in cultured human cells under hypoxia (61, 92) (see Table 2) or thermal stress (101), or by the treatment with interferon-γ (61, 146) or an iron chelator, desferrioxamine (61, 92). Hypoxia (1% O_2) decreased the expression levels of HO-1 mRNA and protein in human umbilical vascular endothelial cells, despite the functional activation of HIF-1 (92). Moreover, 15-deoxy-$D^{12,14}$-PGJ_2 (15d-PGJ_2) induced HO-1 protein in human microvascular endothelial cells under hypoxia (2% O_2), whereas it inhibited the activity of HIF-1 under hypoxia (48). Hypoxic repression of HO-1 mRNA was also observed in cultured human astrocytes and coronary artery endothelial cells (92). These results suggest that HIF-1 may be dispensable for the regulation of HO-1 expression under hypoxia, at least in some cell types. Recently, we showed that hypoxia reduces the expression levels of HO-1 and HO-2 in human cell lines, including HepG2 hepatoma and HeLa cervical cancer cells (188) (see Table 2). Hypoxia also reduced HO-2 protein in immortalized human trophoblast cells (118). In this context, expression levels of HO-1 and HO-2 proteins were transiently decreased in the liver during acclimatization of mice to hypoxia (35).

Conversely, hypoxia induced expression of HO-1 mRNA and/or protein in human dermal fibroblasts (108), HaCaT human keratinocytes (47), and D407 human retinal pigment ep-

ithelial cells (161). Hypoxia also exerted differential effects on the expression levels of HO-1 mRNA and protein (see Table 2). Hypoxia induced HO-1 mRNA expression in human KG1 myeloid cells (188), YN-1 erythroleukemia cells (188), and microvascular endothelial cells (70), but rather reduced or did not change the HO-1 protein level (70, 188). Likewise, hypoxia does not affect the expression of HO-1 in explants of normal human chorionic villi from term placentas (6) and in ARPE19 human retinal pigment epithelium (161). In contrast, hypoxia induces expression of HO-1 mRNA and/or protein in Chinese hamster ovary cells (90), rat aortic smooth muscle cells (85, 86), rat and mouse cardiomyocytes (27, 168), and bovine aortic endothelial cells (118). Moreover, under hypoxia, HO-1 mRNA expression is remarkably induced in rat, bovine, and monkey cell lines (61) (see Table 2). Thus, a species and cell-type difference appears to exist in the mechanism sensing hypoxia or the response to hypoxia.

Taken together, we hypothesize that a certain degree of the repression of HO-1 or HO-2 expression or both may represent an important defense strategy. For example, the reduced expression of HO-1 and HO-2 decreases energy expenditure consumed for oxidative heme breakdown and prevents the local accumulation of CO, iron, and bilirubin IXα beyond certain threshold levels in the cells expressing HO-1 and HO-2. Moreover, the repression could restrict iron supply to cancer cells or certain pathogens, such as bacteria and protozoa, in a host. Consistent in part with our hypothesis, we showed the (GT)n repeat polymorphism in the human HO-1 gene promoter (60) and its functional consequences (170).

HO-2 AS A POTENTIAL OXYGEN SENSOR IN THE CAROTID BODY

HO-2 is localized to glomus cells in the cat and rat carotid bodies, as judged by immunocytochemistry (116). Moreover, zinc protoporphyrin IX, a potent HO inhibitor, increased carotid body sensory activity, and exogenous CO reversed the stimulatory effects of zinc protoporphyrin IX (116). These results suggest that endogenous CO appears to be a physiologic regulator of carotid body sensory activity. Recently, it was reported that HO-2 interacts with the α-subunit of a large-conductance, calcium-sensitive potassium channel (the BK channel) and may function as an oxygen sensor for the BK channel (167). Furthermore, carotid body cells demonstrated HO-2–dependent hypoxic calcium-sensitive potassium channel inhibition, indicating that HO-2 is an oxygen sensor that controls channel activity during oxygen deprivation (167).

Ortega-Saenz *et al*. (105) reported the enlargement of the carotid body in HO-2$^{(-/-)}$ mice, which is associated with the altered expression of stress-dependent genes, including the maxi-K^+ channel α-subunit. The enlargement of the carotid bodies has been shown to result from chronic hypoxemia in patients with cystic fibrosis or cyanotic heart disease (66) and in rats after prolonged exposure to hypoxia (10% O_2) (109). However, the sensitivity to hypoxia of the sliced carotid body is similar in HO-2$^{(-/-)}$ mice and their control littermates (105). Thus, HO-2 deficiency does not alter the O_2 sensitivity of the carotid body, which is consistent in part with our study that the immediate hypoxic ventilatory response is retained in HO-2$^{(-/-)}$ mice (2). HO-2$^{(-/-)}$ mice were able to respond to hypoxia (10% O_2) by increasing respiratory frequency, the degree of which is similar to that in wild-type mice (Fig. 7).

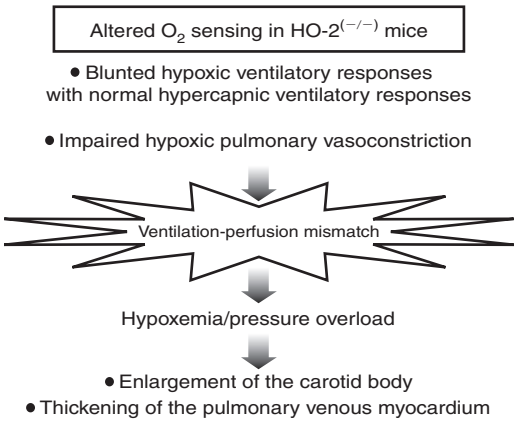

FIG. 7. Altered O_2 sensing in HO-2$^{(-/-)}$ mice.

IMPAIRED VENTILATORY RESPONSES TO HYPOXIA IN HO-2$^{(-/-)}$ MICE

The appropriate ventilatory responses to hypoxia and hypercapnia are essential for survival. Various biochemical processes in the brain are responsible for generation of respiratory rhythm and respiratory pattern (12). Hypoxia stimulates the peripheral chemoreceptors to increase ventilation (71), whereas CO_2 sensing depends mainly on chemoreceptors in the brainstem (12). HO-2$^{(-/-)}$ mice on the C57BL/6 background present mild hypoxemia (low arterial O_2 tension and low O_2 content) and blunted ventilatory responses to hypoxia (10% O_2) with normal ventilatory responses to hypercapnia (10% CO_2) (see Fig. 7). Importantly, HO-2$^{(-/-)}$ mice show normal breathing patterns under basal conditions and retain the intact alveolar architecture (2). Moreover, no significant differences in major hematologic parameters were noted between HO-2$^{(-/-)}$ mice and wild-type mice (112).

We analyzed the immediate ventilatory responses of unanesthetized HO-2$^{(-/-)}$ mice to acute hypoxia (10% O_2) or acute hypercapnia (10% CO_2) by whole-body plethysmography (82, 96, 155). The degree of the increase in respiratory frequency in response to acute hypoxia was similar in HO-2$^{(-/-)}$ mice, compared with wild-type mice, suggesting that the oxygen chemosensors in the carotid body are functional (2). However, the degrees of the increases in the tidal volume (TV/g) and minute ventilation (VE/g) to acute hypoxia were significantly lower in HO-2$^{(-/-)}$ mice. These results suggest the impaired function of the oxygen chemosensors in the lung or in the brainstem or both of HO-2$^{(-/-)}$ mice, distributed from the thalamus to the medulla (94). HO-2 is expressed in the oxygen-sensing regions of the rat rostral ventrolateral medulla (77).

Inbred mice differ in their abilities to control air breathing and respond to hypoxia (3, 82, 148, 155). In general, basal respiratory variables and hypoxic ventilatory responses differed

among the nine inbred mouse strains (3), whereas the hypercapnic ventilatory response did not differ among these mice. Likewise, the hypercapnic ventilatory responses are indistinguishable in HO-2$^{(-/-)}$ mice from those in wild-type mice (2), suggesting the normal function of the central chemoreceptor for hypercapnia in the HO-2$^{(-/-)}$ brain. Thus, genetic factors may have differentially influenced the ventilatory responses to hypoxia and hypercapnea. Indeed, altered functions of the central chemosensors may be involved in the pathophysiology of various diseases, including bronchial asthma (58, 172), chronic obstructive pulmonary disease (152), and Parkinson's disease (104).

Patients with Parkinson's disease showed blunted ventilatory responses to hypoxia but normal responses to hypercapnia (104), which is similar to the altered ventilatory responses seen in HO-2$^{(-/-)}$ mice. It is therefore conceivable that HO-2 may be involved in the survival or function of the dopaminergic neurons in the substantia nigra. Oxidative stress has been assumed as an important pathogenic factor in Parkinson's disease; HO-1 immunoreactivity was detected in the substantia nigra of Parkinson disease (14, 127). In addition, asthmatic patients with a high risk of a fatal attack showed the blunted hypoxic ventilatory response that was accompanied by the decrease in perception of dyspnea (58). Likewise, HO-2$^{(-/-)}$ mice might be free of perception of dyspnea under basal conditions. In this context, HO-2$^{(-/-)}$ mice appear to avoid exercise, as they diminished mating behavior and decreased forelimb strength (11).

PULMONARY VENOUS MYOCARDIUM

The pulmonary veins have been largely ignored until recently, as they are simply regarded as passive conduits (30). The pulmonary venous myocardium represents the extension of atrial myocardium into the vascular walls of the pulmonary veins (46, 163). Moreover, the thickening of the venous myocardium has been shown to represent the adaptation to hypobaric hypoxia in mice (46) and humans (163). Interestingly, HO-2$^{(-/-)}$ mice have hypertrophy of the pulmonary venous myocardium (2) and the carotid body (105). These morphometric changes are attributable to chronic hypoxemia (66). Moreover, in the hypertrophied pulmonary venous myocardium, expression level of HO-1 protein was increased, as judged by immunochemical analyses (2).

It has been reported that the carboxyhemoglobin level is significantly higher in the arterial blood than that in the central venous blood, taken from the right atrium, in critically ill patients and healthy individuals before orthopedic surgery (80), indicating that a substantial amount of CO is produced and released from the lung or the pulmonary vasculature. It is therefore conceivable that HO-1 and HO-2 in the pulmonary venous myocardium are actively involved in the production and release of CO, which accounts for the arteriovenous CO difference.

It is also noteworthy that atrial fibrillation is a common cardiac arrhythmia, and frequently originates from the pulmonary venous myocardium (34, 119). It has been reported that HO-2 protein is expressed in human atrial appendages, and its expression level is reduced in those from patients with atrial fibrillation (59) (see Fig. 5). Taken together with the thickening of the pulmonary venous myocardium of HO-2$^{(-/-)}$ mice (2), these results suggest a regulatory role of HO-2 in the cardiac function.

VENTILATION–PERFUSION MISMATCH IN HO-2$^{(-/-)}$ MICE

At least four basic mechanisms could cause hypoxemia: decreased O_2 pressure in inspired gas, hypoventilation, shunting, and ventilation–perfusion mismatching. Among these, ventilation–perfusion mismatch is the most common cause of hypoxemia in clinics. Ventilation–perfusion matching is regulated by the lung chemoreceptors that initiate vasoconstriction of the pulmonary arterioles by sensing hypoxia. The chemoreceptors in the lung consist of airway neuroepithelial bodies that are clusters of amine- and peptide-producing cells distributed throughout the airway mucosa (54, 181) and pulmonary artery smooth muscle cells (7). Neuroepithelial bodies secrete vasoactive substances in response to hypoxia, thereby leading to vasoconstriction through pulmonary artery smooth muscle cells (54). Accordingly, in contrast to the systemic arteries that exhibit hypoxic vasodilatation, the pulmonary arteries constrict in response to hypoxia, which is an essential mechanism that optimizes the oxygenation of pulmonary blood at alveoli.

The hypoxic pulmonary vasoconstriction is initiated by the cooperation of airway neuroepithelial bodies and pulmonary artery smooth muscle cells, both of which share the function of an oxygen sensor with glomus cells in the carotid body (181). In this context, hypoxia-sensitive K^+ channels have been implicated as a potential mechanism for oxygen sensing in pulmonary artery smooth muscle cells, neuroepithelial bodies, and glomus cells (115). For instance, K^+ channels in the pulmonary artery smooth muscle cells are inhibited by hypoxia, which causes membrane depolarization and increases cytosolic calcium, ultimately leading to hypoxic pulmonary vasoconstriction. It is therefore conceivable that the lung chemoreceptors initiate hypoxic pulmonary vasoconstriction through the HO-2–mediated mechanism (Fig. 8). HO-2 may be involved in O_2 sensing, which is achieved by the intercellular communication between certain chemoreceptors in the lung and target cells.

Ventilation–perfusion mismatch is enhanced with aging and in various inflammatory diseases, such as pneumonia, bronchial asthma, and chronic obstructive pulmonary disease. It is of particular significance to prevent the onset of pneumonia in the elderly, which is the fourth leading cause of death, despite the availability of potent new antimicrobials in Japan (98). Bronchial asthma is characterized by chronic and allergic airway inflammation, which could induce cytologic and histologic changes in the airway structure, termed *airway remodeling* (172). Airway remodeling, which includes goblet cell hyperplasia, subepithelial fibrosis, and hyperplasia and hypertrophy of airway smooth muscle cells, can cause irreversible airflow limitation (172). Various cytokines and mediators produced in chronic allergic airway inflammation are responsible for airway remodeling. Thus, early intervention with inhaled corticosteroids may prevent progress of airway remodeling by suppressing allergic airway inflammation. The elevated level of exhaled CO was decreased in asthmatic patients after treatment with inhaled glucocorticoids (183). Likewise, a decrease in peak expiratory flow rate and an increase in exhaled CO in patients with bronchial asthma were returned to baseline levels after oral glucocorticoids (174).

We have proposed that the ventilation–perfusion mismatch is a likely cause of hypoxemia in HO-2$^{(-/-)}$ mice (2) (Fig. 7).

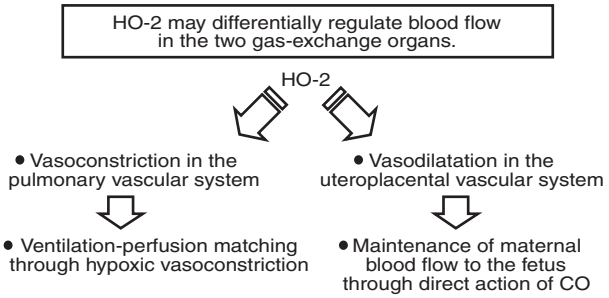

FIG. 8. Proposed roles of HO-2 in the two gas-exchange organs, lung and placenta. HO-2 may be involved in hypoxic pulmonary vasoconstriction, which is mediated by vasoactive substances released from airway neuroepithelial bodies. In contrast, HO-2 may direct vasodilatation of the uteroplacental vascular system through locally produced CO.

HO-2 may be involved in oxygen sensing, probably at the pulmonary artery smooth muscle or the airway neuroepithelial body or both, both of which are responsible for the ventilation–perfusion matching that optimizes oxygenation of pulmonary blood. Thus, HO-2 functions as a regulator for the maintenance of the pulmonary blood flow. HO-1 and HO-2 are expressed in human pulmonary artery smooth muscle cells (2, 140). Moreover, HO-2 is expressed predominantly in H146 small cell lung cancer cells, compared with the relatively low expression level of HO-1 protein (22). Small cell lung cancer is derived from the airway neuroepithelial body (164).

During transition from placental to air breathing, HO-1 and HO-2 showed the differences in their expression levels in the porcine and murine lung (141). HO-2 is constitutively expressed in the porcine and murine lung after birth in vascular and airway structures (141). In contrast, HO-1 protein is induced after birth in vascular and airway structures and HO-1 mRNA declines after birth in the mouse lung (141).

PREGNANCY AND PLACENTA

An essential function of the placenta is gas exchange for the fetus through villi of large surface areas: the chorionic villi of the placenta ensure efficient supply of maternal blood to the fetus (see Fig. 8). The placental blood flow is directly regulated by vasoactive substances, such as CO, which are locally produced (7). Intrauterine growth restriction is associated with increased perinatal morbidity and mortality as well as with lifelong cardiovascular and metabolic complications. An earlier study (43) showed the expression of HO-1 mRNA and nonspecific δ-aminolevulinate synthase (ALAS-N) mRNA in the rat placenta at the terminal stage of pregnancy. Both HO-1 and ALAS-N are exclusively expressed in the trophoblast, as judged by immunohistochemical analyses. During gestation, ALAS-N mRNA increased, whereas HO-1 mRNA significantly decreased. Notably, acute fetal hypoxia, induced by ligation of the uterine vessels, resulted in an increase in ALAS-N mRNA and in a decrease in HO-1 mRNA (43). These findings indicate that placental heme metabolism is influenced by the oxygen supply. Moreover, deficiency of HO-1 is associated with growth restriction in mice (113). Zenclussen *et al.* (184) reported the diminished expression of HO-1 and HO-2 in decidua and placenta from mice undergoing Th1-mediated abortion. More recently, overexpression of HO-1 with adenoviral vector was shown to reduce the abortion rate in a murine model of abortion (186). These results suggest that HO-1 and HO-2 are required for pregnancy maintenance in rodents.

Immunoreactive HO-1 protein is expressed in human villous trophoblasts, whereas HO-2 is localized to endothelial cells and smooth muscle cells of blood vessels of placental villi (180). HO-2 immunostaining was prominent in syncytiotrophoblasts in the first trimester and reduced toward term, whereas HO-2 endothelial immunostaining was increased by term (72). Moreover, the placental perfusion pressure significantly increased in the presence of zinc protoporphyrin, an inhibitor of HO (72). These results suggest a regulatory role for HO-2 in the maintenance of the placental blood flow and pregnancy (see Fig. 8).

Decreased expression of HO-2 in abnormal pregnancies in humans

The reduced expression of HO-2 has been reported in abnormal pregnancies (8, 68, 185), such as in preeclampsia and spontaneous abortion. Preeclampsia is characterized by maternal hypertension and proteinuria and is also associated with an inadequately perfused placenta and areas of tissue damage (7). Barber *et al.* (8) reported the decreased activity of HO in infarcted chorionic villi and the reduced expression of immunoreactive HO-2 protein in endothelial cells of the placental bed of pregnancies complicated by preeclampsia and fetal growth restriction. HO-1 was undetectable in the placenta by Western blotting in control and abnormal pregnancies (8). Moreover, HO-2 protein levels were decreased in periinfarct regions and infarcted chorionic villi of mildly preeclamptic pregnancies, whereas no significant difference in HO-1 protein levels was found in uncomplicated placentas and those of mildly preeclamptic pregnancies (68). Likewise, the decreased expression of HO-2 has been observed in invasive trophoblast cells, endothelial cells, and syncytiotrophoblasts in placental and decidual first-trimester tissues from patients with spontaneous abortion (185). Hydatidiform mole samples showed the diminished expression of HO-2 in invasive trophoblast cells and endothelial cells in comparison with normally progressing pregnancies, whereas choriocarcinoma samples showed no significant differences. It has been suggested that the reduced expression of HO-2 represents a risk factor for abnormal pregnancies. In this context, Newby *et al.* (95) reported the decreased HO-2 protein levels in placentas of women who reside at high altitude. The implication of the reduced expression of HO-2 remains to be investigated.

CONCLUDING REMARKS

Why are HO-2$^{(-/-)}$ mice seemingly healthy?

Generally, the phenotypes of the gene-deficient mice reflect the consequences of various compensatory adaptations during embryogenesis to assure their survival. The normal fetal growth and birth of HO-2$^{(-/-)}$ mice (112) indicate that the placental blood flow is maintained during fetal life by the mechanism in-

dependent of HO-2, which ensures sufficient blood supply to the fetus through the placenta. HO-$2^{(-/-)}$ mice are able to compensate for the loss of HO-2 in part by increasing the expression of HO-1, which is supported by the fact that HO-$2^{(-/-)}$ mice show no noticeable changes or only marginal decrease in the arterial carboxyhemoglobin, a marker for overall heme degradation (2, 112). In addition, HO-1 protein is overexpressed in the lung (20) and the pulmonary venous myocardium of HO-$2^{(-/-)}$ mice (2). These results suggest that the amount of overall heme degradation is maintained in HO-$2^{(-/-)}$ mice through proper resetting of HO-1 expression levels.

HO-$2^{(-/-)}$ mice manifest mild hypoxemia and may provide a good model for hypoxemia. Importantly, HO-$2^{(-/-)}$ mice do not exhibit the apparent remodeling of the small pulmonary arteries (2), despite the persistent hypoxemia. We therefore suggest that an inhibitor specific to HO-2 or a reagent that downregulates HO-2 expression may be helpful for the treatment of pulmonary hypertension. Conversely, induction of HO-2 expression by pharmacologic means will be a promising strategy for the treatment of various disorders associated with ventilation–perfusion mismatch. Considering the well-known significance of HO-1, we appreciate the presence of two great isozymes in heme catabolism.

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ABBREVIATIONS

CO, carbon monoxide; HSE, heat-shock element; HO, heme oxygenase; HIF, hypoxia-inducible factor; MARE, Maf recognition element, a factor related to nuclear factor erythroid 2, Nrf2; ALAS-N, nonspecific Δ-aminolevulinate synthase; PG, prostaglandin; 15d-PGJ_2, 15-deoxy-$D^{12,14}$-PGJ_2; VHL, von Hippel–Lindau.

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