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Forum Review

Heme Oxygenase and Its Products in the Nervous System

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ABSTRACT

Heme oxygenase (HO) cleaves the tetrapyrrolic ring of cellular heme moieties liberating carbon monoxide (CO) and equimolar amounts of free iron and biliverdin (BV). BV is in turn converted into bilirubin (BR) by the cytosolic enzyme BV reductase. Three HO isoforms have been described to date: HO-1, HO-2, and HO-3. All these isoforms are present in nervous tissue with different localizations and regulation. CO, the gaseous product of HO, exerts its biological effects through the activation of soluble guanylyl cyclase, but alternative signaling pathways, such as the activation of cyclooxygenase, have also been reported in the brain. *In vitro* and *in vivo* studies showed that CO, at the hypothalamic level, plays a key role in the modulation of stress response because it inhibits the release of antiinflammatory neuropeptides, such as corticotropin-releasing hormone and arginine vasopressin, and increases body temperature in rodents exposed to psychological stressors (stress fever). In the last few years, a new role of BR as an endogenously produced antioxidant has emerged, and several reports have shown that BR contributes to prevent cell damage mediated by reactive oxygen species, as well as nitric oxide and its congeners. *Antioxid. Redox Signal.* 6, 878–887.

INTRODUCTION

In 1969, Tenhunen and colleagues described heme oxygenase (HO), a microsomal enzyme that hydrolyzes the heme moieties of heme-containing proteins, such as hemoglobin, myoglobin, and cytochromes (86). The tetrapyrrolic ring of heme is cleaved by HO at the α-methene bridge yielding equimolecular amounts of biliverdin (BV), ferrous iron and carbon monoxide (CO). In mammals, BV is in turn transformed by the cytosolic enzyme biliverdin reductase (BVR) into bilirubin (BR), which is then conjugated with glycuronic acid and excreted (34) (Fig. 1). During the following 20 years, many investigators focused their attention on HO and its products in order to understand better their biological functions and regulation. In particular, in 1987 Stocker and colleagues described the antioxidant properties of BR (81), and in 1993 Verma *et al.* proposed a role for CO as an endogenous neuromodulator (89). Following these observations, many pieces of evidence arose in literature demonstrating new and important roles for CO and BR in the central nervous system either in physiologic conditions or in pathologic situations.

The aim of this article is to review the literature on the role played by HO and its products in modulating brain functions, focusing attention on a particular aspect, such as the regulation by CO of the hypothalamic neuroendocrine system.

HEME OXYGENASE

Until 1997, two HO isoforms had been described: an inducible isoform, HO-1, and a constitutive enzyme, HO-2. These isoforms are the products of different genes and share only 43% homology. A 24-amino-acid segment, which forms a hydrophobic pocket within the tertiary structure of the protein, is common to both isoforms and is considered the active center of the enzyme (36). Metalloporphyrins such as Zn-protoporphyrin IX and Sn-protoporphyrin IX (Zn-PP-IX and Sn-PP-IX, respectively) bind to the hydrophobic pocket of HO, but do not catalyze hydrolysis (or they do so at a much lower rate than heme), thereby inhibiting HO activity (33, 41). In fact, Zn-PP-IX may be considered as an endogenous inhibitor, because it is synthesized instead of heme in the case of iron deficiency (47).

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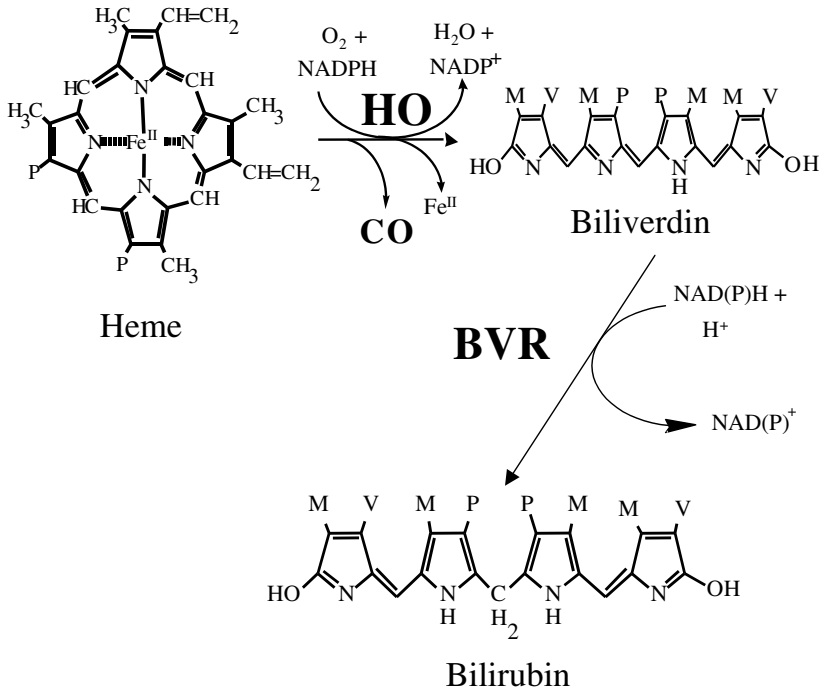

FIG. 1. The main metabolic pathway leading to CO formation. HO shunts reducing equivalents from NADPH-cytochrome P450 reductase to the α-methene bridge of cellular heme moieties. By so doing, HO cleaves the tetrapyrrolic ring of heme and liberates CO plus equimolar amounts of BV, which is then converted into BR by the NAD(P)H-requiring cytosolic enzyme BVR. The substituents on the tetrapyrroles are methyl (M), vinyl (V), and propionic (P) acid.

Apart from the identity between the active centers of the enzyme, HO-1 and HO-2 broadly differ in cell and tissue regulation and distribution.

Regulation of HO isozymes

HO-1, also referred as heat shock protein-32, is induced by various stimuli, including oxidative and nitrosative stress, ischemia, heat shock, bacterial lipopolysaccharide (LPS), hemin, and the neuroprotective agent Neotrofin (35, 36, 90). Furthermore, in cultured human cells, HO-1 expression can be repressed by hypoxia or by treatment with interferon-γ or desferrioxamine (71); interestingly, the repression of HO-1 expression seems to be specific for human cells because the same repressors of HO-1 expression could induce the expression of this isoform in cultured rodent cells (71). On the other hand, HO-2, the constitutive form, is responsive to developmental factors, opiates, adrenal glucocorticoids, and nitric oxide (NO) (35, 36). Although HO-1 and HO-2 catalyze the same reaction, they play different roles in protecting tissues against injuries. Based on several lines of evidence, the more convincing hypothesis suggests that HO-1 induction is one of the earlier cellular responses to tissue damage and is responsible for the rapid transformation of the prooxidant heme into CO and BR, two molecules with antiinflammatory and antioxidant activity. On the contrary, HO-2, constitutively expressed, is primarily involved in maintaining cell heme homeostasis and preventing NO-mediated damage (37).

Very recently, Shibahara *et al.* focused their attention on HO-1 repression as a defense strategy developed in humans (72). This idea was enforced by the concomitant discovery by Igarashi and colleagues of Bach-1/Bach-2 as heme-regulated repressors for transcription of the HO-1 gene (57). Bach-1 is a transcription factor widely expressed in mouse and human tissues, including brain, and Bach-1-deficient mice have HO-1 gene overexpressed in many tissues, including brain (82). Furthermore, Bach-1 is induced in cultured human cells by hypoxia, interferon-γ, and desferrioxamine, each of which represses HO-1 expression (57, 71). Therefore, Bach-1 has an important role in heme metabolism as a controller of the feedback regulation by sensing heme and repressing or activating transcription, depending on the heme availability. HO-1 repression may be useful in many situations because it decreases energy expenditure necessary for heme degradation and prevents local accumulation of CO, iron, and BR; in addition, HO-1 repression may decrease the iron supply to cancer cells or pathogens, such as bacteria and protozoa, which require iron as an essential cofactor for cell proliferation (71).

HO activity is regulated also by BVR because this latter reduces BV, the inhibitory product of the oxygenase activity, into BR (20). The molecular mass of BVR ranges between 41 and 42 kDa (human) and 33 and 34 kDa (rat), is dual cofactor and dual pH-dependent, and requires free-SH groups (39). Until now, BVR was considered a noninducible protein, but recent data showed that the reductase can be induced by LPS and bromobenzene at a posttranscriptional level, whereas heat shock has no effect (21, 40). In the rat brain, BVR is coexpressed in cells that display HO-1 and/or HO-2 under normal conditions, as well as in regions and cell types that have the potential to express heat shock-inducible HO-1 protein (21). Further evidence demonstrated that BVR exhibited developmental changes with the activity increasing after birth and reaching an adult level by day 28 post partum. Immunohistochemical analysis revealed an age-related pattern of expression of BVR in select rat brain areas, such as the cortex, substantia nigra, hippocampus, and cerebellum (20).

Distribution of HO isozymes

HO-1 is ubiquitous and particularly abundant in reticuloendothelial organs, such as liver and spleen, whereas HO-2 is localized in specific organs, such as brain, kidney, and testis (36). The central nervous system is endowed with very high HO activity under basal conditions, mostly accounted for by HO-2, the latter being expressed in neuronal populations in forebrain, hippocampus, hypothalamus, midbrain, basal ganglia, thalamus, cerebellum, and brainstem. The inducible isoform is instead present in very small amounts and is localized in sparse groups of neurons, including the ventromedial and paraventricular nuclei of the hypothalamus (36). This finding indicates that the activation of HO-1 and the following formation of CO can be induced by many noxious stimuli within the nuclei that are primarily involved in the central regulation of the stress response. In fact, neurons located within the parvicellular part of the paraventricular nucleus release both corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP), the neuropeptides that initiate the endocrine response to a stressor stimulating the release of pituitary adrenocorticotropin hormone (ACTH). HO-1 is also found within cells of glial lineage, where its gene expression can be induced by oxidative stress (18).

In 1997, Mahin Maines and her group described a third HO isoform called HO-3. It is a protein of ~33 kDa encoded by a single transcript of 24 kb and constitutively expressed in rat liver, spleen, kidney, and brain (49). In a very recent article, Scapagnini *et al*. investigated the regional brain expression of HO-3 and found that this isoform is expressed mainly in astrocytes of hippocampus, cerebellum, and cortex (68). The regulation of HO-3 gene expression and its synthesis is poorly understood, and its possible role in physiology and pathology remains to be further clarified.

HO and neuroprotection

The mechanism(s) responsible for neuronal death is not completely elucidated, even if many studies suggest that reactive oxygen species (ROS) are primarily involved in the genesis of neurodegenerative disorders (59, 67, 73, 75). Due to its abundance in brain tissue and prompt and robust response to oxidative stress, HO-1 has been studied for its possible activity in preventing brain injury. Panahian *et al*. using transgenic (Tg) mice overexpressing HO-1 in neurons demonstrated the neuroprotective effect of this enzyme in an experimental model of ischemic brain damage (58). When compared with non-Tg mice, Tg mice exhibited significant neuroprotection with decreased dimensions of ischemic penumbra when examined at both 6 and 24 h after induction of ischemia. The authors conclude that the neuroprotective effect of overexpressed HO-1 can be related to: (a) increase in both cyclic GMP (cGMP) and bcl-2 levels in neurons; (b) inactivation of p53, a protein involved in promoting cell death; (c) increase in antioxidant sources, as suggested by the strong reduction in the formation of lipid peroxidation products; and (d) increase in the iron-sequestering protein, ferritin (58). In addition, Takeda *et al*. explored the relationship between HO-1 and tau protein, the latter being the major component of neurofibrillary tangles, the intraneuronal lesion of Alzheimer disease (84). In transfected neuroblastoma cells overexpressing HO-1, the activity of this enzyme was increased and, conversely, the level of tau protein was significantly decreased when compared with antisense HO-1 or vector transfected cells. The suppression of tau protein expression was almost completely counteracted by zinc-deuteroporphyrin, a specific inhibitor of HO activity. The activated forms of extracellular signal-regulated kinases (ERKs) were also decreased in cells overexpressing HO-1, although no changes in the expression of total ERKs were observed (84). Taken together, all these findings do not allow a product of HO activity to be singled out as the main neuroprotective factor; rather a complex puzzle of regulatory interactions between heme degradation products and cellular pathways involved in cell death/survival is hypothesized.

CARBON MONOXIDE

Since the early 1960s, the endogenous formation of CO via HO activity became a matter of fact. For almost 30 years, scientists tried to address the following question: Why does the human body produce relatively high concentrations of such a toxic molecule? Cui prodest?. This point remained obscure until the early 1990s, when Verma *et al*. raised the hypothesis of a biological role of CO as a neuromodulator (89).

Mechanism(s) of action

It is generally agreed that endogenous CO exerts its biological effects through the activation of the cytosolic soluble guanylyl cyclase (sGC), which in turn increases intracellular cGMP levels (36). Many studies showed that CO is a weak activator of sGC; in fact, the gas increases cGMP production by a mere one- to twofold (7, 28). Therefore, CO modulation of sGC leading to significant increases in cGMP levels can only occur in those brain areas, such as the hippocampus and olfactory bulb, both endowed with high levels of sGC and HO (25, 89). Alternative signal-transduction mechanism(s) must be postulated in different brain areas, such as the hypothalamus, where a high rate of CO production by HO is associated with quite low sGC levels (5, 48). Indeed *in vitro* studies from our laboratory suggest that the activation of another hemoprotein, cyclooxygenase, plays a significant role in CO signaling in the rat hypothalamus. In these studies, we demonstrated that (a) hemin dose-dependently increases prostaglandin (PG) E_2 production from rat hypothalamus *in vitro*, and (b) this effect is specifically due to CO because it is counteracted by the HO inhibitor Sn-mesoporphyrin-IX and oxyhemoglobin, the latter being a well-known scavenger for CO (42). The direct evidence about the stimulatory role of CO on PG production was obtained by incubating hypothalami directly in CO-saturated solutions and measuring significantly increased PGE_2 levels with respect to control tissue (44). On the basis of these results and keeping in mind the chemical structure of CO, it is possible to consider all the hemoproteins as potential CO "receptors." The type of biological response ensuing from CO binding would then depend on whether or not the gas activates such hemoproteins; the effects of CO that are associated with protein activation are those mediated by sGC and, perhaps, cyclooxygenase; on the

contrary, other biological effects of endogenous CO seem to be caused by the inhibition of hemoproteins such as cytochrome P-450 (13).

A novel pathway for CO signaling transduction has been recently proposed by Jaggar *et al.,* who studied the vasodilating effect of the gas in cerebral arterioles (26) (Fig. 2). These authors, in a very elegant article, demonstrated that exogenous or endogenously produced CO dilates cerebral arterioles by directly activating large-conductance Ca^{2+}-activated K^+ (K_{Ca}) channels primarily by increasing the coupling ratio and amplitude relationship between Ca^{2+} sparks and K_{Ca} channels. Although CO is a potent and effective activator of K_{Ca} channels, the gas does not dilate arterioles in the absence of Ca^{2+} sparks. Therefore, CO appears to act by priming K_{Ca} channels for activation by Ca^{2+} sparks, and this ultimately leads to arteriole dilation via membrane hyperpolarization (26).

The neuroendocrine effects of CO: in vitro *studies*

The first studies on the neuroendocrinology of CO attempted to clarify the possible role of this gas in the central control of the stress response. In 1994, Pozzoli *et al*. showed that the treatment of rat hypothalamic explants with hemin, considered the precursor of CO, inhibited the release of CRH stimulated by KCl or interleukin-1β. Such an effect was attributed to CO rather than hemin itself because it was significantly counteracted by the HO inhibitor Zn-PP-IX (62). This first report was strengthened by other studies that consistently showed that (a) hemin is effectively transformed into BV and CO in the hypothalamus and (b) this increase in CO formation is responsible for the inhibition of KCl-stimulated AVP and oxytocin release in hypothalamic explants *in vitro*. This effect on neuropeptide release is effectively due to CO formation because it is counteracted by HO inhibitors and mimicked by the incubation of the hypothalami directly in CO-saturated medium (30, 41). In addition, neither BV nor BR was able to modify basal or stimulated release of CRH, AVP, and oxytocin (30, 41, 62). Although the overall effect of CO on the hypothalamic drive controlling stress responses appears to be by the inhibition of stimulated hormone release, experiments carried out to investigate the influence of CO on the hypothalamo–pituitary–gonadal (HPG) axis showed that hemin increases basal gonadotropin-releasing hormone (GnRH) release in a dose-dependent fashion in both hypothalamic explants and immortalized cells, these effects being specifically inhibited by Zn-PP-IX (4). Although the mechanism of action of CO on GnRH release has not been fully investigated, it is possible to hypothesize that PGs are involved, because CO has been shown to increase PGE_2 production from the rat hypothalamus (42, 44) and this prostanoid is known to be a major physiological stimulus of GnRH release (43, 65).

Taken together, these results show opposing effects of CO on the control of GnRH, on the one hand, and CRH and the neurohypophyseal hormones, on the other. It is well known that an inverse relationship exists between stress and reproductive function. Interleukin-1β, a well-known mediator of inflammatory stress, shifts this balance by increasing CRH and neurohypophyseal hormone release while blunting GnRH secretion by a direct action at the hypothalamic level (65). Within this framework, the role of CO appears to be physiologically consistent, as the gas would damp down exaggerated activation of the hypothalamo–pituitary–adrenal (HPA) axis and, at the same time, enhance reproductive processes (Fig. 2).

The neuroendocrine effects of CO: in vivo *studies*

Although the results of *in vitro* studies strongly suggest a role for CO in modulating neuroendocrine function, the same encouraging results have not been obtained by *in vivo* studies,

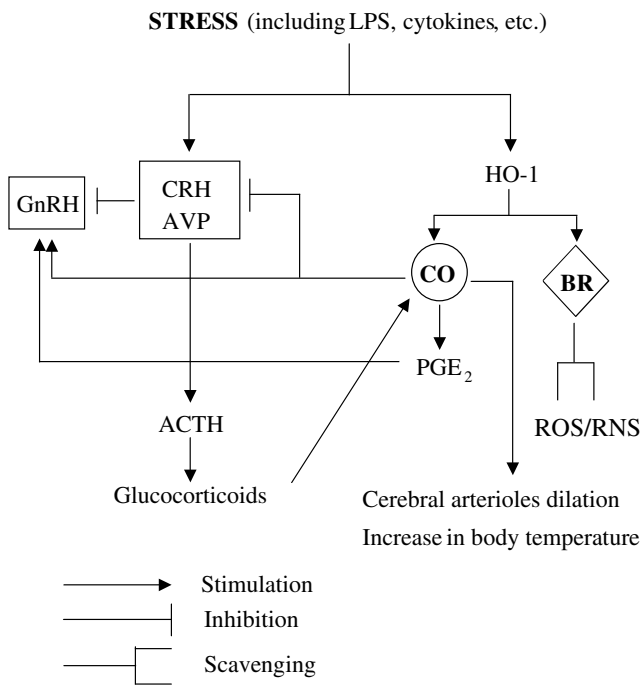

FIG. 2. Summary of the most important interactions between HO products and brain. Note that glucocorticoids may positively feedback on CO generation because they increase HO-2 expression in the brain (36). This phenomenon becomes very important in the event of sustained stress-induced adrenocortical activation. Thus, glucocorticoids may induce CO in the brain, which in turn acts as part of a negative feedback mechanism to decrease CRH and AVP release from hypothalamus, and hence eventually glucocorticoid secretion. As for the regulation of GnRH release, CO is able to increase the release of this hormone either directly (perhaps using PGE_2 as a second messenger) or by inhibiting CRH release, which is able to down-regulate GnRH synthesis (85). For further details, see text.

mainly because of the relative inability of most of HO inhibitors to cross the blood–brain barrier (43). In addition, when hemin is administered to rats subcutaneously at the sublethal dose of 25 mg/kg twice a day over 2 days, which strongly increases HO-1 activity in many organs (31), it induces only a negligible change in brain HO activity. Despite these difficulties, we performed *in vivo* studies using a different approach, and the HO inhibitor Sn-PP-IX was given to rats by the intracerebroventricular route (i.c.v.) (60). An immunoinflammatory challenge, such as LPS, was used to increase the circulating levels of stress hormones. Our results showed that the inhibition of the HO–CO pathway by i.c.v. Sn-PP-IX significantly potentiates the LPS-induced increase in AVP circulating levels while reducing the hypothalamic content of this neuropeptide (45). Collectively, these results are consistent with the notion that HO products (probably CO) exert an inhibitory influence on the AVP release from specific hypothalamic neurons. The removal of this inhibition by HO blockers enhances the release of AVP leading to the activation of the stress response (45). In 1998, Turnbull *et al.* demonstrated that the inhibition of HO activity by Sn-PP-IX is able to reduce the rise in plasma ACTH induced in rats by foot-shock stress (87). This apparent conflict between our results and those of Turnbull *et al*. in which HO inhibition reflects a blockade of the stress response, may be due to either the different stressor stimulus used (physico-emotional versus immunological) or the different routes of Sn-PP-IX administration. In fact, these authors administered by the subcutaneous route a very high dose of HO inhibitor to increase its passage through the blood–brain barrier, and this fact may have influenced the response of the HPA axis (38).

A particular aspect of the activation of the HPA axis is the increase in body temperature. Rats exposed to a variety of stress stimuli, such as restraint, handling, and cage switching, have a rise in body temperature, and this phenomenon has been called stress fever (55). During the last few years, many studies have demonstrated that CO is a mediator of the pyrogenic response to stressors. In support of this hypothesis, the i.c.v. administration of HO inhibitors decreases LPS-induced fever in the rats (78, 79), whereas heme overload causes a rise in body temperature (77–79). In the light of these findings, Steiner *et al*. in a very recent article studied the relationship between the HO–CO system and the physico-emotional stress using an experimental system of restraint-induced stress in the rats. In this article, the authors showed that i.c.v. administration of the HO inhibitor Zn-deuteroporphyrin-bis-glycol does not affect the body temperature of euthermic rats, but markedly attenuates the restraint-induced fever (80). These results confirm that the HO–CO pathway is not involved in the tonic regulation of body temperature, but once this enzyme is activated by a specific stimulus CO becomes a pyrogenic neuromodulator.

With regard to the HPG axis, only one study using an *in vivo* approach has been performed. Alexandreanu and Lawson showed that the modulation of endogenous CO production alters the secretion of pituitary gonadal hormones. In particular, the chronic administration of the HO inibitor Cr-mesoporphyrin-IX advanced the estradiol-induced afternoon surge of plasma luteinizing hormone, this effect being effectively counteracted by the concurrent administration of hemin (1). These findings suggest that CO may be considered as a positive modulator of *in vivo* luteinizing hormone, secretion. On the contrary, CO seems not to be involved in the circadian regulation of follicle-stimulating hormone release from rat anterior pituitary.

CO and the peripheral nervous system

The effective role played by CO in the modulation of peripheral nervous system functions has been extensively studied. Most of the evidence came from studies on gastrointestinal nonadrenergic noncholinergic transmission (NANC) using several preparations, including the internal anal sphincter (64), lower esophageal sphincter (54), jejunum (22), and gastric fundus (14). Endogenous or exogenously applied CO induced smooth muscle relaxation in all the preparations tested, and this effect was related to sGC activation and cGMP formation. Due to the well-known lower potency of CO in activating sGC compared with NO (7, 28) (the main mediator of NANC inhibitory neurotransmission), many investigators questioned whether CO has a physiologic function in gastrointestinal physiology. Xue *et al.* recently tried to overcome this dualism and proposed that CO and NO are both coneurotransmitters in NANC-induced relaxation. According to this theory, CO *per se* does not have any effect on intestinal smooth muscle relaxation, but it might sensitize intestinal smooth muscle cells to the effects of NO or, alternatively, CO may increase neuronal NO synthase (NOS) catalytic activity or facilitate NO release from enteric neurons (91).

CO may also have a neurotransmitter role in the vas deferens, where it is involved in the regulation of ejaculation. Burnett *et al.* described HO-2 localization in neuronal structures regulating copulatory reflexes and showed that ejaculation is significantly reduced in mice with targeted deletion of HO-2 (6).

HO-2-derived CO is also important in blood vessel function. Immunohistochemical studies showed that HO-2 is localized to endothelial layers of blood vessels, in analogy with endothelial NOS (76). This finding, together with the evidence that specific HO inhibitors reverse the component of endothelial-derived relaxation of porcine distal pulmonary arteries, which is not reversed by an inhibitor of NOS, implies a synergistic role for CO and NO in maintaining vessel tone (76).

BILIRUBIN

BR is the final product of heme catabolism. In mammals, ~80–90% of the total BR formed originates from the degradation of heme moieties of hemoglobin, whereas 10–20% derives from the catabolism of other hemoproteins (34). As a consequence of this catabolism, at least 300 mg of BR is produced per day in normal adult humans (81). In the blood stream, BR is bound primarily to serum albumin at concentrations ranging between 5 and 15 μ*M* (50); therefore, the vascular wall is continuously in contact with the albumin–BR complex. Interestingly, at concentrations between 5 and 25 μ*M*, BR has been shown to exert a concentration-dependent cytoprotective effect against hydrogen peroxide-mediated dam-

age in aortic endothelial cells (51). The bile pigment may become toxic for many tissues if the concentration exceeds 300 μ*M*, resulting in pathological states, such as jaundice and kernicterus (50).

Mechanism of action

Whereas BR serum concentrations are high enough to provide a substantial portion of the total antioxidant capacity of serum, the low concentrations of BR in tissues (20–50 n*M*) would suggest a negligible role of this bile pigment in preventing oxidative damage (3). Nonetheless, in the last few years, several reports have shown that BR contributes to the prevention of cell damage mediated by ROS, as well as reactive nitrogen species (RNS) (10, 11, 16, 27, 81). The first hypothesis about the mechanism of action of BR as antioxidant was proposed in 1987 by Stocker *et al.,* who showed that BR is able to scavenge peroxyl radicals (81). These authors argued that the antioxidant mechanism was based on the extended system of conjugated double bonds and a reactive hydrogen atom that BR can donate, transforming itself into a carbon-centered radical ($BR^{\cdot}$) with resonance stabilization extending over the entire molecule (81). More recent studies, based on the evidence that there is not a stoichiometry between BR and molecules with prooxidant activity, proposed a novel mechanism of action for BR based on an amplification cycle whereby the bile pigment is itself oxidized to BV by ROS or RNS and recycled by BVR back to BR. This redox cycle could explain how low concentrations of BR can protect against the cytotoxicity caused by 10,000-fold higher concentrations of the oxidant (3). This hypothesis becomes more intriguing in the light of the evidence that neurons have very high levels of BR (19, 76) as compared with relatively low concentrations of glutathione (63, 74), a tripeptide involved in detoxification of ROS and very abundant in almost all mammalian tissues, suggesting a possible role of BR as an alternative endogenous antioxidant molecule in neurons (2).

BR and brain: in vitro *and* in vivo *studies*

The potential neuroprotective role of BR was hypothesized in a series of articles by Solomon Snyder and his group. In 1999, Doré *et al.* reported that (a) hydrogen peroxide toxicity is much higher in hippocampal neurons from HO-2-knockout mice (which have decreased BR production in neurons) compared with wild-type mice and (b) the exogenous administration of free BR (25–50 n*M*) or albumin-conjugated BR (10–250 n*M*) improves neuronal survival (16). The same authors later showed that following ischemia–reperfusion or excitotoxic injuries, the extent of the cerebral damage was greater in HO-2-knockout mice than in wild-type mice, which is consistent with a protective role of HO products in brain damage (15). In the follow-up study, it was demonstrated that cultured cerebellar granule neurons from both wild-type and HO-2-knockout mice exposed to serum withdrawal or potassium deprivation undergo apoptotic death; however, apoptosis was about twofold greater in wild-type compared with HO-2-knockout mice, giving further evidence of a protective role of HO products (17). In line with these findings are the studies of Takahashi *et al.,* who found that cortical neurons cultured from mice expressing the Swedish mutation of Alzheimer's disease had defects in BR production with subsequent increase of hydrogen peroxide toxicity (83). Taken together, these studies corroborate the notion that BR is an efficient scavenger of oxygen free radicals and suggest that the protective role of this pigment might be extended to other reactive species originated within the cellular milieu (Fig. 2).

IRON

Iron is a very important element in mammals because it is cofactor for several enzymes. Particularly important is heme iron, which is involved in the regulation of many cellular functions, such as respiration, proliferation, and differentiation (61). Iron plays a key role in modulating specific brain functions because it increases the release and the turnover rate of dopamine and other neurotransmitters (9). However, when present in excess, iron triggers the formation of very toxic oxygen radicals that ultimately cause lipid peroxidation and cell death (9, 24, 88). Many studies reported that excessive sequestration of redox-active iron is a characteristic feature of many neurodegenerative disorders, such as Alzheimer disease and Parkinson disease (for details, see 69), but the mechanisms responsible for this pathological iron sequestration need to be further addressed. Although much evidence considers HO-1 an enzyme with cytoprotective function (58, 84), other studies suggest that this enzyme can be involved in neurodegenerative disorders (29, 70). This opinion is corroborated by the findings that HO-1 is up-regulated in astrocytes of the Parkinson disease substantia nigra, as well as in Alzheimer disease brain (70). In this light, free iron liberated during heme catabolism may exacerbate intracellular oxidative stress and mediate brain injury.

CONCLUSIONS AND PERSPECTIVES

Several lines of evidence developed in the last 10 years demonstrated the importance of HO and its products, including BR, in the regulation of brain functions. The role of CO in modulating the release of hypothalamic neuropeptides clearly emerged from *in vitro* and *in vivo* studies. In particular, the ability of this gas to blunt the increase of CRH and AVP in response to different stimuli puts CO in the "proinflammatory" molecules arena as the final outcome is a reduction in glucocorticoid production by the adrenal gland (43) (Fig. 2). This proinflammatory action of CO seems to be rather specific for the brain because recent progress indicates that, in other organs and tissues, CO exerts antiinflammatory and antiapoptotic effects dependent on the modulation of the p38 mitogen-activated protein kinase signaling pathway (56, 92). By virtue of these effects, CO confers protection in oxidative lung injury models, and likely plays a role in HO-1-mediated tissue protection (66). The recent discovery by Motterlini and his group of a new class of substances that release CO [CO-releasing molecules (CORMs)] will open new frontiers in the study of CO actions (12, 52). In particular, CORMs will be very useful tools for *in vivo* studies because they allow the

direct administration of this gas to animals, thus overcoming all the problems related to the apparent inability of hemin to cross the blood–brain barrier, as well as the toxicity of hemin when administered *in vivo* to animals (43).

Very interesting also is the role played by BR in the prevention of oxidative damage in the brain. In particular, Minetti *et al*. (50) have shown that BR is able to reduce the propagation of cell damage triggered by the prooxidant peroxynitrite, suggesting that the bile pigment could also prevent cellular injury elicited by RNS. In addition, Kaur *et al*. and our group demonstrated that BR also interacts with other RNS and *S*-nitrosothiols (27, 46). The ability of BR to scavenge not only ROS, but also NO and its congeners, is quite important in the brain, because brain cells are very sensitive to damage elicited by NO and RNS (Fig. 2). In fact, many studies show that excessive production of NO in the brain, as a consequence of NOS induction in activated glia, participates in neurodegeneration (8, 32), and it is in the light of these findings that BR could exert its role as a neuroprotective agent. The full characterization of nitrosated BR is now required, and its possible physiologic role needs to be clarified. However, the consistent evidence showing that the HO-1/BR pathway is very sensitive to activation by nitrosative reactions involving NO and its redox-activated forms (23, 53) suggests a role for bile pigments as important intracellular metabolites with antinitrosative capacities.

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ABBREVIATIONS

ACTH, adrenocorticotropin hormone; AVP, arginine vasopressin; BR, bilirubin; BV, biliverdin; BVR, biliverdin reductase; cGMP, cyclic GMP; CO, carbon monoxide; CORMs, carbon monoxide-releasing molecules; CRH, corticotropin-releasing hormone; ERK, extracellular signal-regulated kinase; GnRH, gonadotropin-releasing hormone; HO, heme oxygenase; HPA, hypothalamo–pituitary–adrenal; HPG, hypothalamo–pituitary–gonadal; i.c.v., intracerebroventricular; K_{Ca}, Ca^{+}-activated K^{+}; LPS, bacterial lipopolysaccharide; NANC, nonadrenergic noncholinergic transmission; NO, nitric oxide; NOS, nitric oxide synthase; PG(s), prostaglandin(s); RNS, reactive nitrogen species; ROS, reactive oxygen species; sGC, soluble guanylyl cyclase; Sn-PP-IX, Sn-protoporphyrin IX; Tg, transgenic; Zn-PP-IX, Zn-protoporphyrin IX.

REFERENCES

1. Alexandreanu IC and Lawson DM. Heme oxygenase in the rat anterior pituitary: immunohistochemical localization and possible role in gonadotropin and prolactin secretion. *Exp Biol Med (Maywood)* 228: 64–69, 2003.
2. Baranano DE and Snyder SH. Neural roles for heme oxygenase: contrasts to nitric oxide synthase. *Proc Natl Acad Sci U S A* 98: 10996–11002, 2001.
3. Baranano DE, Rao M, Ferris CD, and Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci U S A* 99: 16093–16098, 2002.
4. Brann DW, Bhat GK, Lamar CA, and Mahesh VB. Gaseous transmitters and neuroendocrine regulation. *Neuroendocrinology* 65: 385–395, 1997.
5. Burgunder JM and Cheung PT. Expression of soluble guanylyl cyclase gene in adult rat brain. *Eur J Neurosci* 6: 211–217, 1994.
6. Burnett AL, Johns DG, Kriegsfeld LJ, Klein SL, Calvin DC, Demas GE, Schramm LP, Tonegawa S, Nelson RJ, Snyder SH, and Poss KD. Ejaculatory abnormalities in mice with targeted disruption of the gene for heme oxygenase-2. *Nat Med* 4: 84–87, 1998.
7. Burstyn JN, Yu AE, Dierks EA, Hawkins BK, and Dawson JH. Studies of the heme coordination and ligand binding properties of soluble guanylyl cyclase (sGC): characterization of Fe(II)sGC and Fe(II)sGC(CO) by electronic absorption and magnetic circular dichroism spectroscopies and failure of CO to activate the enzyme. *Biochemistry* 34: 5896–5903, 1995.
8. Calabrese V, Bates TE, and Stella AM. NO synthase and NO-dependent signal pathways in brain aging and neurodegenerative disorders: the role of oxidant/antioxidant balance. *Neurochem Res* 25: 1315–1341, 2000.
9. Chiueh CC. Iron overload, oxidative stress, and axonal dystrophy in brain disorders. *Pediatr Neurol* 25: 138–147, 2001.
10. Clark JE, Foresti R, Green CJ, and Motterlini R. Dynamics of heme oxygenase-1 expression and bilirubin production in cellular protection against oxidative stress. *Biochem J* 348: 615–619, 2000.
11. Clark JE, Foresti R, Sarathchandra P, Kaur H, Green CJ, and Motterlini R. Heme oxygenase-1-derived bilirubin ameliorates post-ischemic myocardial dysfunction. *Am J Physiol* 278: H643–H651, 2000.
12. Clark JE, Naughton P, Shurey S, Green CJ, Johnson TR, Mann BE, Foresti R, and Motterlini R. Cardioprotective actions by a water-soluble carbon monoxide-releasing molecule. *Circ Res* 93: E2–E8, 2003.
13. Coceani F, Kelsey L, and Seidlitz E. Carbon monoxide-induced relaxation of the ductus arteriosus in the lamb: evidence against the prime role of guanylyl cyclase. *Br J Pharmacol* 118: 1689–1696, 1996.
14. Colpaert EE, Timmermans JP, and Lefebvre RA. Investigation of the potential modulatory effect of biliverdin, carbon monoxide and bilirubin on nitrergic neurotransmission in the pig gastric fundus. *Eur J Pharmacol* 457: 177–186, 2002.
15. Dore S, Sampei K, Goto S, Alkayed NJ, Guastella D, Blackshaw S, Gallagher M, Traystman RJ, Hurn PD, Koehler RC, and Snyder SH. Heme oxygenase-2 is neuroprotective in cerebral ischemia. *Mol Med* 5: 656–663, 1999.
16. Doré S, Takahashi M, Ferris CD, Zakhary R, Hester LD, Guastella D, and Snyder SH. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxida-

tive stress injury. *Proc Natl Acad Sci U S A* 96: 2445–2450, 1999.
17. Doré S, Goto S, Sampei K, Blackshaw S, Hester LD, Ingi T, Sawa A, Traystman RJ, Koehler RC, and Snyder SH. Heme oxygenase-2 acts to prevent neuronal death in brain cultures and following transient cerebral ischemia. *Neuroscience* 99: 587–592, 2000.
18. Dwyer BE, Nishimura RN, and Lu SY. Differential expression of heme oxygenase-1 in cultured cortical neurons and astrocytes determined by the aid of a new heme oxygenase antibody. Response to oxidative stress. *Mol Brain Res* 30: 37–47, 1995.
19. Ewing JF and Maines MD. *In situ* hybridization and immunohistochemical localization of heme oxygenase-2 mRNA and protein in normal rat brain: differential distribution of isozyme 1 and 2. *Mol Cell Neurosci* 3: 559–570, 1992.
20. Ewing JF and Maines MD. Immunohistochemical localization of biliverdin reductase in rat brain: age related expression of protein and transcript. *Brain Res* 672: 29–41, 1995.
21. Ewing JF, Weber CM, and Maines MD. Biliverdin reductase is heat resistant and coexpressed with constitutive and heat shock forms of heme oxygenase in brain. *J Neurochem* 61: 1015–1023, 1993.
22. Farrugia G, Miller SM, Rich A, Liu X, Maines MD, Rae JL, and Szurszewski JH. Distribution of heme oxygenase and effects of exogenous carbon monoxide in canine jejunum. *Am J Physiol* 274: G350–G358, 1998.
23. Foresti R and Motterlini R. The heme oxygenase pathway and its interaction with nitric oxide in the control of cellular homeostasis. *Free Radic Res* 31: 459–475, 1999.
24. Goldstein L, Teng ZP, Zeserson E, Patel M, and Regan RF. Hemin induces an iron-dependent, oxidative injury to human neuron-like cells. *J Neurosci Res* 73: 113–121, 2003.
25. Ingi T and Ronnett GV. Direct demonstration of a physiological role for carbon monoxide in olfactory receptor neurons. *J Neurosci* 15: 8214–8222, 1995.
26. Jaggar JH, Leffler CW, Cheranov SY, Tcheranova DES, and Cheng X. Carbon monoxide dilates cerebral arterioles by enhancing the coupling of Ca^{2+} sparks to Ca^{2+}-activated K^+ channels. *Circ Res* 91: 610–617, 2002.
27. Kaur H, Hughes MN, Green CJ, Naughton P, Foresti R, and Motterlini R. Interaction of bilirubin and biliverdin with reactive nitrogen species. *FEBS Lett* 543: 113–119, 2003.
28. Kharitonov VG, Sharma VS, Pilz RB, Magde D, and Koesling D. Basis of guanylate cyclase activation by carbon monoxide. *Proc Natl Acad Sci U S A* 92: 2568–2571, 1995.
29. Koeppen AH and Dickson AC. Neuroprotection in intracerebral hemorrhage with tin-protoporphyrin. *Ann Neurol* 46: 938, 1999.
30. Kostoglou-Athanassiou I, Forsling ML, Navarra P, and Grossman AB. Oxytocin release is inhibited by the generation of carbon monoxide from the rat hypothalamus—further evidence for carbon monoxide as a neuromodulator. *Mol Brain Res* 42: 301–306, 1996.
31. Kutty RK and Maines MD. Hepatic heme metabolism: possible role of biliverdin in the regulation of heme oxygenase activity. *Biochem Biophys Res Commun* 122: 40–46, 1984.
32. Liu B, Gao HM, Wang JY, Jeohn GH, Cooper CL, and Hong JS. Role of nitric oxide in inflammation-mediated neurodegeneration. *Ann N Y Acad Sci* 962: 318–331, 2002.
33. Maines MD. Zinc-protoporphyrin is a selective inhibitor of heme oxygenase activity in the neonatal rat. *Biochim Biophys Acta* 673: 339–350, 1981.
34. Maines MD. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J* 2: 2557–2568, 1988.
35. Maines MD. *Heme Oxygenase in Clinical Applications and Functions*. Boca Raton, FL: CRC Press, 1992.
36. Maines MD. The heme oxygenase system: a regulator of second messenger gases. *Annu Rev Pharmacol Toxicol* 37: 517–554, 1997.
37. Maines MD and Panahian N. The heme oxygenase system and cellular defense mechanisms. Do HO-1 and HO-2 have different functions? *Adv Exp Med Biol* 502: 249–272, 2001.
38. Maines MD and Trakshel GM. Tin-protoporphyrin: a potent inhibitor of hemoprotein-dependent steroidogenesis in rat adrenals and testes. *J Pharmacol Exp Ther* 260: 909–916, 1992.
39. Maines MD and Trakshel GM. Purification and characterization of human biliverdin reductase. *Arch Biochem Biophys* 300: 320–326, 1993.
40. Maines MD, Ewing JF, Huang TJ, and Panahian N. Nuclear localization of biliverdin reductase in the rat kidney: response to nephrotoxins that induce heme oxygenase-1. *J Pharmacol Exp Ther* 296:1091–1097, 2001.
41. Mancuso C, Kostoglou-Athanassiou I, Forsling ML, Grossman AB, Preziosi P, Navarra P, and Minotti G. Activation of heme oxygenase and consequent carbon monoxide formation inhibits the release of arginine vasopressin from rat hypothalamic explants. Molecular linkage between heme catabolism and neuroendocrine function. *Mol Brain Res* 50: 267–276, 1997.
42. Mancuso C, Pistritto G, Tringali G, Grossman AB, Preziosi P, and Navarra P. Evidence that carbon monoxide stimulates prostaglandin endoperoxide synthase activity in rat hypothalamic explants and in primary cultures of rat hypothalamic astrocytes. *Mol Brain Res* 45: 294–300, 1997.
43. Mancuso C, Preziosi P, Grossman AB, and Navarra P. The role of carbon monoxide in the regulation of neuroendocrine function. *Neuroimmunomodulation* 4: 225–229, 1997.
44. Mancuso C, Tringali G, Grossman A, Preziosi P, and Navarra P. The generation of nitric oxide and carbon monoxide produces opposite effects on the release of immunoreactive interleukin-1beta from the rat hypothalamus *in vitro*: evidence for the involvement of different signaling pathways. *Endocrinology* 139: 1031–1037, 1998.
45. Mancuso C, Ragazzoni E, Tringali G, Liberale I, Preziosi P, Grossman A, and Navarra P. Inhibition of heme oxygenase in the central nervous system potentiates endotoxin-induced vasopressin release in the rat. *J Neuroimmunol* 99: 189–194, 1999.
46. Mancuso C, Bonsignore A, Di Stasio E, Mordente A, and Motterlini R. Bilirubin and *S*-nitrosothiols interaction: evidence for a possible role of bilirubin as a scavenger of nitric oxide. *Biochem Pharmacol* 66: 2355–2363, 2003.

47. Marks GS, Nakatsu K, and Brien JF. Does endogenous zinc protoporphyrin modulate carbon monoxide formation from heme? Implications for long-term potentiation, memory, and cognitive function. *Can J Physiol Pharmacol* 71: 753–754, 1993.
48. Matsuoka I, Giuili G, Poyard M, Stengel D, Parma J, Guellaen G, and Hanoune J. Localization of adenylyl and guanylyl cyclase in rat brain by in situ hybridization: comparison with calmodulin mRNA distribution. *J Neurosci* 12: 3350–3360, 1992.
49. McCoubrey WK Jr, Huang TJ, and 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.
50. Minetti M, Mallozzi C, Di Stasi AM, and Pietraforte D. Bilirubin is an effective antioxidant of peroxynitrite-mediated protein oxidation in human blood plasma. *Arch Biochem Biophys* 352: 165–174, 1998.
51. Motterlini R, Foresti R, Intaglietta M, and Winslow RM. NO-mediated activation of heme oxygenase: endogenous cytoprotection against oxidative stress to endothelium. *Am J Physiol* 270: H107–H114, 1996.
52. Motterlini R, Clark JE, Foresti R, Sarathchandra P, Mann BE, and Green CJ. Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities. *Circ Res* 90: E17–E24, 2002.
53. Motterlini R, Green CJ, and Foresti R. Regulation of heme oxygenase-1 induction by redox signals involving nitric oxide. *Antioxid Redox Signal* 4: 615–624, 2002.
54. Ny L, Alm P, Ekstrom P, Larsson B, Grundemar L, and Andersson KE. Localization and activity of haem oxygenase and functional effects of carbon monoxide in the feline lower oesophageal sphincter. *Br J Pharmacol* 118: 392–399, 1996.
55. Oka T, Oka K, and Hori T. Mechanisms and mediators of psychological stress-induced rise in core temperature. *Psychosom Med* 63: 476–486, 2001.
56. Otterbein LE, Bach FH, Alam J, Soares M, Tao Lu H, Wysk M, Davis RJ, Flavell RA, and Choi AM. Carbon monoxide has anti-inflammatory effects involving the mitogen-activated protein kinase pathway. *Nat Med* 6: 422–428, 2000.
57. Oyake T, Itoh K, Motohashi H, Hayashi N, Hoshino H, Nishizawa M, Yamamoto M, and Igarashi K. Bach proteins belong to a novel family of BTB-basic leucine zipper transcription factors that interact with MafK and regulate transcription through the NF-E2 site. *Mol Cell Biol* 16: 6083–6095, 1996.
58. Panahian N, Yoshiura M, and 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.
59. Pappolla MA, Chyan YJ, Omar RA, Hsiao K, Perry G, Smith MA, and Bozner P. Evidence of oxidative stress and in vivo neurotoxicity of beta-amyloid in a transgenic mouse model of Alzheimer's disease: a chronic oxidative paradigm for testing antioxidant therapies *in vivo*. *Am J Pathol* 152: 871–877, 1998.
60. Percy VA and Shanley BC. Factors affecting haem degradation in rat brain. *Biochem Pharmacol* 29: 1590–1592, 1980.
61. Ponka P. Rare causes of hereditary iron overload. *Semin Hematol* 39: 249–262, 2002.
62. Pozzoli G, Mancuso C, Mirtella A, Preziosi P, Grossman AB, and Navarra P. 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. Raps SP, Lai JC, Hertz L, and Cooper AJ. Glutathione is present in high concentrations in cultured astrocytes but not in cultured neurons. *Brain Res* 493: 398–401, 1989.
64. Rattan S and Chakder S. Inhibitory effect of CO on internal anal sphincter: heme oxygenase inhibitor inhibits NANC relaxation. *Am J Physiol* 265: G799–G804, 1993.
65. Rivest S and Rivier C. The role of corticotropin-releasing factor and interleukin-1 in the regulation of neurons controlling reproductive functions. *Endocr Rev* 16: 177–199, 1995.
66. Ryter SW, Otterbein LE, Morse D, and Choi AM. Heme oxygenase/carbon monoxide signaling pathways: regulation and functional significance. *Mol Cell Biochem* 234–235: 249–263, 2002.
67. Sayre LM, Smith MA, and Perry G. Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Curr Med Chem* 8: 721–738, 2001.
68. Scapagnini G, D'Agata V, Calabrese V, Pascale A, Colombrita C, Alkon D, and Cavallaro S. Gene expression profiles of heme oxygenase isoforms in the rat brain. *Brain Res* 954: 51–59, 2002.
69. Schipper HM. Heme oxygenase-1: role in brain aging and neurodegeneration. *Exp Gerontol* 35: 821–830, 2000.
70. Schipper HM, Bernier L, Mehindate K, and Frankel D. Mitochondrial iron sequestration in dopamine-challenged astroglia: role of heme oxygenase-1 and the permeability transition pore. *J Neurochem* 72: 1802–1811, 1999.
71. Shibahara S. The heme oxygenase dilemma in cellular homeostasis: new insights for the feedback regulation of heme catabolism. *Tohoku J Exp Med* 200: 167–186, 2003.
72. Shibahara S, Nakayama M, Kitamuro T, Udono-Fujimori R, and Takahashi K. Repression of heme oxygenase-1 expression as a defense strategy in humans. *Exp Biol Med (Maywood)* 228: 472–473, 2003.
73. Simonian NA and Coyle JT. Oxidative stress in neurodegenerative diseases. *Annu Rev Pharmacol Toxicol* 36: 83–106, 1996.
74. Slivka A, Mytilineou C, and Cohen G. Histochemical evaluation of glutathione in brain. *Brain Res* 409: 275–284, 1987.
75. Smith MA, Hirai K, Hsiao K, Pappolla MA, Harris PL, Siedlak SL, Tabaton M, and Perry G. Amyloid-beta deposition in Alzheimer transgenic mice is associated with oxidative stress. *J Neurochem* 70: 2212–2215, 1998.
76. Snyder SH and Baranano DE. Heme oxygenase: a font of multiple messengers. *Neuropsychopharmacology* 25: 294–298, 2001.
77. Steiner AA and Branco LG. Central CO–heme oxygenase pathway raises body temperature by a prostaglandin-independent way. *J Appl Physiol* 88: 1607–1613, 2000.
78. Steiner AA and Branco LG. Carbon monoxide is the heme oxygenase product with a pyretic action: evidence for a cGMP signaling pathway. *Am J Physiol* 280: R448–R457, 2001.

79. Steiner AA, Colombari E, and Branco LG. Carbon monoxide as a novel mediator of the febrile response in the central nervous system. *Am J Physiol* 277: R499–R507, 1999.
80. Steiner AA, Reste G, and Branco LG. Role of the brain heme oxygenase–carbon monoxide pathway in stress fever in rats. *Neurosci Lett* 341: 193–196, 2003.
81. Stocker R, Yamamoto Y, McDonagh AF, Glazer AN, and Ames BN. Bilirubin is an antioxidant of possible physiological importance. *Science* 235: 1043–1046, 1987.
82. Sun J, Hoshino H, Takaku K, Nakajima O, Muto A, Suzuki H, Tashiro S, Takahashi S, Shibahara S, Alam J, Taketo MM, Yamamoto M, and Igarashi K. Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. *EMBO J* 21: 5216–5224, 2002.
83. Takahashi M, Dore S, Ferris CD, Tomita T, Sawa A, Wolosker H, Borchelt DR, Iwatsubo T, Kim SH, Thinakaran G, Sisodia SS, and Snyder SH. Amyloid precursor proteins inhibit heme oxygenase activity and augment neurotoxicity in Alzheimer's disease. *Neuron* 28: 461–473, 2000.
84. Takeda A, Perry G, Abraham NG, Dwyer BE, Kutty RK, Laitinen JT, Petersen RB, and Smith MA. Overexpression of heme oxygenase in neuronal cells, the possible interaction with Tau. *J Biol Chem* 275: 5395–5399, 2000.
85. Tellam DJ, Mohammad YN, and Lovejoy DA. Molecular integration of hypothalamo-pituitary-adrenal axis-related neurohormones on the GnRH neuron. *Biochem Cell Biol* 78: 205–216, 2000.
86. Tenhunen R, Marver HS, and Schmid R. Microsomal heme oxygenase. Characterization of the enzyme. *J Biol Chem* 244: 6388–6394, 1969.
87. Turnbull AV, Kim CK, Lee S, and Rivier CL. Influence of carbon monoxide, and its interaction with nitric oxide, on the adrenocorticotropin hormone response of the normal rat to a physico-emotional stress. *J Neuroendocrinol* 10: 793–802, 1998.
88. Van Bergen P, Rauhala P, Spooner CM, and Chiueh CC. Hemoglobin and iron-evoked oxidative stress in the brain: protection by bile pigments, manganese and *S*-nitrosoglutathione. *Free Radic Res* 31: 631–640, 1999.
89. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, and Snyder SH. Carbon monoxide: a putative neural messenger. *Science* 259: 381–384, 1993.
90. Wang X, Hauptmann N, Taylor E, Foreman M, Khawli LA, and Maines MD. Neotrofin increases heme oxygenase-1 selectively in neurons. *Brain Res* 962: 1–14, 2003.
91. Xue L, Farrugia G, Miller SM, Ferris CD, Snyder SH, and Szurszewski JH. Carbon monoxide and nitric oxide as coneurotransmitters in the enteric nervous system: evidence from genomic deletion of biosynthetic enzymes. *Proc Natl Acad Sci U S A* 97: 1851–1855, 2000.
92. Zhang X, Shan P, Otterbein LE, Alam J, Flavell RA, Davis RJ, Choi AM, and Lee PJ. Carbon monoxide inhibition of apoptosis during ischemia–reperfusion lung injury is dependent on the p38 mitogen-activated protein kinase pathway and involves caspase 3. *J Biol Chem* 278: 1248–1258, 2003.

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2. Rao Muralikrishna Adibhatla , James Franklin Hatcher . 2010. Lipid Oxidation and Peroxidation in CNS Health and Disease: From Molecular Mechanisms to Therapeutic Opportunities. *Antioxidants & Redox Signaling* **12**:1, 125-169. [Abstract] [Full Text] [PDF] [PDF Plus]
3. Naohiko Imuta , Osamu Hori , Yasuko Kitao , Yoshiyuki Tabata , Tanihiro Yoshimoto , Tomohiro Matsuyama , Satoshi Ogawa . 2007. Hypoxia-Mediated Induction of Heme Oxygenase Type I and Carbon Monoxide Release from Astrocytes Protects Nearby Cerebral Neurons from Hypoxia-Mediated Apoptosis. *Antioxidants & Redox Signaling* **9**:5, 543-552. [Abstract] [PDF] [PDF Plus]
4. Jian-Li Ma, Peng-Yuan Yang, Yao-Cheng Rui, Ling Lu, Hui Kang, Jing Zhang. 2007. Hemin Modulates Cytokine Expressions in Macrophage-Derived Foam Cells via Heme Oxygenase-1 Induction. *Journal of Pharmacological Sciences* **103**:3, 261-266. [CrossRef]
5. Vittorio Calabrese , Rukhsana Sultana , Giovanni Scapagnini , Eleonora Guagliano , Maria Sapienza , Rita Bella , Jaroslaw Kanski , Giovanni Pennisi , Cesare Mancuso , Anna Maria Giuffrida Stella , D. A. Butterfield . 2006. Nitrosative Stress, Cellular Stress Response, and Thiol Homeostasis in Patients with Alzheimer's Disease. *Antioxidants & Redox Signaling* **8**:11-12, 1975-1986. [Abstract] [PDF] [PDF Plus]
6. Prof. Mahin D. Maines . 2005. The Heme Oxygenase System: Update 2005. *Antioxidants & Redox Signaling* **7**:11-12, 1761-1766. [Citation] [PDF] [PDF Plus]