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REVIEW

Regulation of haeme oxygenase-1 for treatment of neuroinflammation and brain disorders

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Injury to the CNS elicits a host defense reaction that utilizes astrocytes, microglia, neurons and oligodendrocytes. Neuroinflammation is a major host defense mechanism designed to restore normal structure and function after CNS insult, but like other forms of inflammation, chronic neuroinflammation may contribute to pathogenesis. The inducible haeme oxygenase isoform, haeme oxygenase-1 (HO-1), is a phase 2 enzyme upregulated in response to electrophilic xenobiotics, oxidative stress, cellular injury and disease. There is emerging evidence that HO-1 expression helps mediate the resolution of inflammation, including neuroinflammation. Whether this is solely because of the catabolism of haeme or includes additional mechanisms is unclear. This review provides a brief background on the molecular biology and biochemistry of haeme oxygenases and the actions of haeme, bilirubin, iron and carbon monoxide in the CNS. It then presents our current state of knowledge regarding HO-1 expression in the CNS, regulation of HO-1 induction in neural cells and discusses the prospect of pharmacological manipulation of HO-1 as therapy for CNS disorders. Because of recognized species and cellular differences in HO-1 regulation, a major objective of this review is to draw attention to areas where gaps exist in the experimental record regarding regulation of HO-1 in neural cells. The results indicate the HO-1 system to be an important therapeutic target in CNS disorders, but our understanding of HO-1 expression in human neural cells is severely lacking.
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Abbreviations: AD, Alzheimer's disease; bZip, basic leucine zipper; HO, haeme oxygenase; iNOS, inducible NOS; Keap1, Kelch-like ECH-associated protein 1; Nrf2, nuclear factor erythroid 2-related factor 2; siRNA, small-interfering RNA

Introduction

It was first reported by Tenhunen *et al*. (1968) that the conversion of haeme *b* into biliverdin IXα occurred through a unique microsomal enzyme. The enzyme responsible was subsequently shown to be a specific haeme oxygenase (decycling); EC 1.14.99.3 (haeme, hydrogen-donor:oxygen oxidoreductase (α-methene-oxidizing, hydroxylating). Since its discovery, and because of the medical importance of both haeme and bilirubin to human disease states, there have been well over 400 reviews written about the structure, function, regulation and physiological functions of this enzyme, its substrate and products. However, novel discoveries on the functions and properties of haeme oxygenases continue to be made. The purpose of this review is twofold. The first is to review findings on the haeme oxygenase 1 (HO-1) isoform that relate to, or may relate to, a function of HO-1 in the pathogenesis of CNS damage and in the regulation of neuroinflammation. Although issues relating to specific upstream and downstream mediators of HO-1 expression are mentioned, it is not the intent herein to comprehensively review that literature. The second purpose is to highlight areas where further research is required to better understand the function of HO-1 in the CNS and to discuss issues regarding HO-1 as a therapeutic target in the CNS.

The mammalian CNS (brain and spinal cord) comprises several major cell types with specialized functions: neurons, astrocytes (also called astroglia), oligodendrocytes (or oligodendroglia) and microglia. In addition, the brain is highly vascularized with blood vessels composed of specialized endothelial cells that form tight junctions and contribute to a blood–brain barrier. Briefly, neurons are distinguished as electrically active cells that communicate both locally and across long distances through transmitter-mediated synapses. They are terminally differentiated cells that can be slowly renewed in at least some brain regions. Neuronal axons are often bundled together and insulated one from another to form myelinated nerves. Myelin is a specialized plasma membrane extension produced by the oligodendro-

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cyte that wraps around axons and helps increase the rate of neurotransmission. Mature oligodendrocytes are electrically silent and found primarily in white matter regions of the CNS. Astrocytes are ubiquitous cells of the CNS that serve many functions. They are trophic for neuronal survival and communicate with neurons to regulate activity. Although electrically silent, they communicate with each other through intercellular calcium waves and extracellular ATP signals. Particularly relevant to this review, astrocytes respond to CNS injury and disease by participating in neuroinflammatory and neuroimmunological reactions. As such, they proliferate, hypertrophy and communicate chemically with microglia. In contrast to neurons, astrocytes and oligodendrocytes, microglial cells are derived from the myeloid lineage. Although there is some disagreement in the literature, it appears that microglia precursors colonize the embryonic CNS and subsequently differentiate into the ramified resident microglia found throughout the CNS parenchyma. Microglia are often referred to as the brain's macrophage. In fact, as the resident microglia become activated during neuroinflammation, it becomes difficult to differentiate them from peripheral macrophages that have invaded the CNS. As will be described, all the major cell types in the CNS can express HO-1 in particular circumstances.

Molecular biology and biochemistry of haeme oxygenases

The genome of mammals contains two distinct genes for proteins that function as a haeme oxygenase. In humans, HMOX1 encodes for HO-1 and HMOX2 encodes for haeme oxygenase 2 (HO-2) (Kutty *et al*., 1994). The HO-1 isozyme is inducible, whereas the HO-2 isozyme is constitutively expressed. The cDNA for these genes was first cloned in the mid-1980s (Shibahara *et al*., 1985; Trakshel *et al*., 1986). HO-1 and HO-2 share little sequence homology, except for a small stretch of conserved amino acids that form a haeme-binding pocket. However, homology across mammalian species is high; 80% amino acid identity between rat and human HO-1 cDNA and 82% identity between murine and human HO-1 (Yoshida *et al*., 1988).

Generation of mutant *Hmox1* null mice (Poss and Tonegawa, 1997) and the discovery of a human lacking HO-1 protein expression due to mutations in maternal and paternal HMOX1 alleles (Yachie *et al*., 1999) demonstrated conclusively that the ability to express HO-1 is important for foetal development and iron regulation. Furthermore, HO-1 deficiency in patients seemed to cause a greater disruption of endothelial cells and the reticuloendothelial system (Kawashima *et al*., 2002) as compared with what is seen in *Hmox1*$^{-/-}$ mice. These results also indicate that HO-2 expression does not compensate for a lack of HO-1 and suggest that the low constitutive expression of HO-1 found mainly in the spleen and liver, but also elsewhere, (Braggins *et al*., 1986) is highly important for homoeostasis.

HO-1 is capable of degrading several haeme species (Kutty and Maines, 1982) but the preferred substrate is haeme *b*. The degradation of haeme *b* also requires NADPH–haemoprotein reductase (EC 1.6.2.4) and used 3 mol each of molecular oxygen and NADPH to produce carbon monoxide (CO), biliverdin IXα and ferrous iron (Fe^{2+}). Biliverdin is reduced to bilirubin by the enzyme biliverdin reductase (EC 1.3.1.24). An important feature of HO-1 regulation is the capacity for extensive and rapid induction of protein levels. This occurs not only in response to elevated levels of haeme or haemoproteins, such as haemoglobin, but also in response to a large number of endogenous chemicals and xenobiotics (Ryter *et al*., 2006). Although there are many different stimuli for the induction of HO-1, a common feature for many is their electrophilic chemistry and the generation of reactive oxygen and/or nitrogen species, which causes the activation of the transcription factor nuclear factor erythroid 2-related factor 2 (Nrf2). Nrf2 belongs to a subset of the basic leucine zipper (bZip) family members that share a Cap 'n' collar domain and is maintained in the cytoplasm through binding to the cytoskeletal-associated protein Kelch-like ECH-associated protein 1 (Keap1) (Kensler *et al*., 2007). Initial studies on Nrf2 activation (Itoh *et al*., 1999) led to the conclusion that oxidative stress resulted in Nrf2 release from Keap1, allowing Nrf2 to shuttle to the nucleus to initiate transcription by binding to the antioxidant-response element (Venugopal and Jaiswal, 1996) as a heterodimer with a member of the small Maf family (Itoh *et al*., 1997; Katsuoka *et al*., 2005). However, more recent studies suggest a more complex interaction, with Keap1 involved in regulating the shuttling of Nrf2 into and out of the nucleus (Nguyen *et al*., 2005; Velichkova and Hasson, 2005) and the promotion of Nrf2 proteosomal degradation (Kobayashi *et al*., 2004). Irrespective of the molecular mechanisms involved, which have yet to be studied in neural cells, Nrf2 can activate an antioxidant response in brain tissue (Shih *et al*., 2005a; Satoh *et al*., 2006) and has been shown to bind to the antioxidant-response element in cultured astrocytes (Shih *et al*., 2007), and shows increased nuclear localization under conditions that increase HO-1 expression in cultured astrocytes (Calabrese *et al*., 2005b; Chen and Regan, 2005; Vargas *et al*., 2005) and neuronal cells (Satoh *et al*., 2006). Furthermore, a lack of Nrf2 in mice makes them more susceptible to neurotoxic insults (Shih *et al*., 2005b; Wang *et al*., 2007a) and results in a vacuolar leukoencephalopathy in mice over 10 months of age (Hubbs *et al*., 2007).

Small Maf family proteins also interact with other bZip members to repress activity at the antioxidant-response element of genes. These include the novel factors Bach1 and Bach2 (Oyake *et al*., 1996) and the p65 isoform of Nrf1 (Wang *et al*., 2007b). In contrast to Bach2, Bach1 contains multiple copies of a cysteine–proline motif surrounding the bZip domain that binds haeme, thus leading to a decrease in its DNA-binding activity (Ogawa *et al*., 2001). Bach2 is interesting, where its expression is restricted primarily to the brain and spleen (Oyake *et al*., 1996), and in contrast to Bach1, whose expression remained constant, Bach2 expression is induced during the differentiation of murine P19 embryonic carcinoma cells into neurons (Hoshino and Igarashi, 2002). Unfortunately, there seems to be no studies on the expression of Bach1 or Bach2 in other neural cell types, hence it is not known if Bach2 expression is neuron-

specific or not, and if these transcription factors have a function in HO-1 expression in the CNS. Curiously, in contrast to the heart, lung, thymus and liver, mice that lack Bach1 showed no difference in constitutive HO-1 expression in several brain regions (Sun *et al*., 2002), suggesting that Bach1 may not be utilized for the repression of HO-1 expression in neural cells. In addition to Nrf2 and small Maf proteins, a number of other transcription factors have been reported to participate in enhancing HO-1 expression by non-neural cells. These include AP-1 (Camhi *et al*., 1998), JunB and JunD (Hock *et al*., 2007), upstream stimulatory factors (Hock *et al*., 2004), Ets2 (Chung *et al*., 2005), SREBP-1 (Kallin *et al*., 2007) and peroxisome proliferator-activated receptors (Kronke *et al*., 2007), among others. Furthermore, Elk-3 has been shown to repress HO-1 promoter activity in a murine macrophage cell line (Chung *et al*., 2006). Thus, a large number of potential transcriptional regulators exist that may be involved in the regulation of HO-1 expression by neural cells under various conditions. Additional research is needed to identify those factors that have a significant function in HO-1 expression by neurons, astrocytes, microglia and oligodendrocytes.

Recently it was shown that rodent HO-1 protein localized to the nucleus after stimulation of cells with haeme, haeme–haemopexin or hypoxia. The localization resulted in reduced enzyme activity and transcription factor activation, yet even a totally inactive mutant protein was able to mediate activation of some transcription factors (Lin *et al*., 2007b). Although the HO-1 protein does not appear to have a nuclear localization signal sequence, not unusual for small proteins that enter the nucleus, it does contain a sequence, amino acids 207–221 in the rat protein, with >90% homology to the nuclear export sequence motif of the HIV-1 Rev protein ($LX_{1-3}LX_{2-3}LXL$). Mouse, human and monkey HO-1 proteins also share homology with this motif. These results indicate a potential additional mechanism whereby HO-1 protein may influence cell functions during exposure to stressors. It is interesting that L-glutamate has been reported to induce nuclear localization of HO-1 protein in primary cultured rat astrocytes (Li Volti *et al*., 2004), suggesting that HO-1 may activate transcription factors in these cells as well.

Regulation of HO-1 expression

The number of chemicals, drugs, procedures and conditions that are reported to result in the induction of HO-1 expression is staggering. In many cases, these exogenous (xenobiotic)-inducing agents share all or most of the following properties. They are chemically reactive, an electrophile, a substrate for glutathione *S*-transferase and capable of covalently modifying sulphhydryl groups (Talalay *et al*., 1988; Dinkova-Kostova *et al*., 2001). This group of inducers are structurally diverse, ranging from salts of transition metals, such as cobalt and cadmium used first to demonstrate *in vivo* induction of HO-1 (Maines *et al*., 1986), to complex triterpenoid derivatives of oleanolic acid (Dinkova-Kostova *et al*., 2005). Their chemical properties presumably allow them to interact directly with the Keap1–Nrf2 complex in the cytoplasm and cause the release of Nrf2 by sulphhydryl modification of Keap1, thus initiating the transcription of phase 2 enzymes, including HO-1. This mechanism shares several features with other redox-sensitive signalling pathways, such as regulation through ubiquitination of proteins that translocate to the nucleus to participate in gene transcription (reviewed in Tong *et al*., 2006). The endogenous mediator that initiates signalling to the nucleus to activate Hmox1 gene transcription under physiological and pathophysiological conditions has not been unequivocally identified, but is generally believed to be one or more reactive free radical species produced by oxidative and nitrosative stress (Naughton *et al*., 2002; Kensler *et al*., 2007). Whether these free radicals directly modify Keap1, act upstream to initiate a kinase signalling cascade that then leads to Keap1 modification and Nrf2 release, or do both, has not been fully clarified and may be cell-type selective. Furthermore, there is evidence that sulphhydryl modification of cysteine residues in the human Keap1 protein does not cause the release of human Nrf2, suggesting an evolutionary change in the regulatory mechanisms for Nrf2 activation (Eggler *et al*., 2005). Other studies have found novel inducer and enhancer regulatory sites in the human HMOX1 gene and promoter (Lavrovsky *et al*., 1993; Hill-Kapturczak *et al*., 2003), and functional genomic polymorphisms within the HMOX1 promoter region are associated as risk or protective factors for certain disease or injury outcomes (reviewed in Shibahara, 2003; Exner *et al*., 2004). These differences between induction and regulation of human HO-1 expression as compared with the regulation of rodent HO-1 expression highlight the need for species, genotype and cell type-specific studies to truly understand the mechanisms regulating HO-1 expression in organs such as the human brain and spinal cord.

In terms of suppressing HO-1 expression, besides specific inhibition of receptor-mediated HO-1 induction by a selective antagonist, such as the inhibition of *trans*-(±)-1-amino-(1*S*,3*R*)-cyclopentanedicarboxylic acid-induced HO-1 expression in rat brain astrocytes by the metabotropic receptor antagonist (±)-α-methyl-4-carboxyphenylglycine (Matsuoka *et al*., 1999a), both exogenous RNA interference (Zhang *et al*., 2004; Kaizaki *et al*., 2006; So *et al*., 2006) and antisense oligodeoxynucleotides (Lee and Chau, 2002; Chen *et al*., 2005) have been used successfully to directly reduce HO-1 expression in various cell systems. As the latter approaches still suffer from inadequacies for *in vivo* therapy, there exists a rationale to develop drugs that reduce or block HO-1 induction, particularly in view of the evidence that HO-1 activity can be either protective or detrimental to cell activity and survival, especially in the CNS (Matsuoka *et al*., 1999b). To do so, however, additional understanding of the mechanisms that regulate HO-1 induction and activity in human neuronal and glial cells is required. It is interesting that Wang *et al*. (2007c) have recently identified *all-trans* retinoic acid as an inhibitor of Nrf2. Although the study was performed on cancer cells and HO-1 expression was not specifically measured, this finding may have the relevance to the modulation of HO-1 expression in the CNS through the inhibition of Nrf2 activity.

Haeme, biliverdin/bilirubin, ferrous iron and CO in the CNS

Haeme (ferric protoporphyrin IX) is produced in the CNS by mitochondrial and cytosolic enzymes primarily for use as a prosthetic group by numerous haemoproteins. The majority of haeme is covalently bound to protein, as in the case of cytochrome *c* oxidase, but some haeme is reversibly bound, as to Bach1, the DiGeorge critical region-8 protein (Faller *et al*., 2007), neuronal PAS domain protein 2 (Kaasik and Lee, 2004) and proteins involved in the N-end rule pathway (Hu *et al*., 2008), where it can have a regulatory function. There is very little known about the presumed regulatory pool of haeme in the CNS, but it is potentially important. If free haeme gets released from neural cells, it may act upon Toll-like receptor 4 (Figueiredo *et al*., 2007) to initiate neuronal damage by microglia (Lehnardt *et al*., 2003), or if its production becomes deficient, it may lead to neuronal senescence and neurite degeneration (Chernova *et al*., 2006, 2007).

Haeme catabolism, through the haeme oxygenases, results in the release of CO and ferrous iron and the production of biliverdin, which is rapidly converted into bilirubin (Ponka, 1999). It can be assumed that significant haeme catabolism occurs in the normal brain through the HO-2 isoform, which is highly expressed in the CNS (Trakshel *et al*., 1988). Although exogenous CO is a potent toxic gas (Mannaioni *et al*., 2006), when produced endogenously by cells, it can function as a signalling molecule through a cyclic GMP pathway. CO produced by neural cells also signals through cyclic GMP (Verma *et al*., 1993; Imuta *et al*., 2007), as well as through CO-sensitive brain transcription factors (Uchida *et al*., 2005). There is strong evidence from the rat that CO is involved in the regulation of blood pressure and possibly heart rate by an action in the brain nucleus tractus solitarii. Early evidence using systemic injections of non-selective haeme oxygenase inhibitors into conscious rats reported increased mean arterial pressure without changes in heart rate that was mediated by autonomic nerve activity (Johnson *et al*., 1995, 1997). Using anaesthetized rats, Lo *et al*. (2000) reported reduced blood pressure and bradycardia upon bilateral injections of haematin into the nucleus tractus solitarii. Additional studies on the same model demonstrated the interactions of CO with nitric oxide, adenosine and glutamatergic systems (Lo *et al*., 2002, 2004; Lin *et al*., 2003, 2004). It is most likely that under normal conditions, these effects of CO are produced by the actions of the constitutive isoform HO-2, as the time course for HO-1 induction after haematin injection into the nucleus tractus solitarii is much delayed relative to its cardiovascular effects (Lo *et al*., 2006). Additional areas where HO-2 produced CO mediates CNS effects include regulation of the brain circadian clock (Artinian *et al*., 2001; Kaasik and Lee, 2004) and cerebral arterial dilation (Li *et al*., 2008). For more information on the signalling actions of CO, see the general review by Maines (1997).

Ferrous iron can be toxic because of its highly reactive chemistry, thus cells have an elaborate system to bind and sequester iron, a description of which is beyond the scope of this review article. The brain uses binding and transport proteins to regulate iron, a function that appears to involve primarily glial and other non-neuronal cells (for details see Connor and Benkovic, 1992; Ke and Qian, 2007). Upon injury, iron accumulates in the brain (Lipscomb *et al*., 1998; Wagner *et al*., 2003; Gaasch *et al*., 2007) and iron dysfunction appears to be associated with neurodegenerative diseases (Lee *et al*., 2006). However, it is unclear to what extent non-haeme iron produced by haeme oxygenases is responsible for damage after brain injury and during neurodegeneration. Using both homologous and heterologous *in vitro* cell culture systems, Schipper *et al*. (1999) have shown an HO-1-dependent sequestration of iron by astrocyte mitochondria (Mehindate *et al*., 2001), which can result in death to cultured astrocyte (Song *et al*., 2006). They further speculate that the increase in HO-1 protein observed post mortem in brains of numerous patients with various neurological injuries and diseases may be causative to the brain damage. Other studies, however, have demonstrated that brain increases in HO-1-dependent non-haeme iron do not correlate with areas of focal cortical damage after experimental pneumococcal meningitis (Ren *et al*., 2007) and that HO-1-dependent haeme degradation, which releases iron, is also neuroprotective in several circumstances (Panahian *et al*., 1999b; Chen *et al*., 2000; Chen-Roetling *et al*., 2005; Chen-Roetling and Regan, 2006). Thus, it remains a quandary as to any general function of HO-1 generated iron in CNS damage.

As noted above, biliverdin is reduced to bilirubin by biliverdin reductase. Biliverdin reductase appears to be a multifaceted protein, being found in the nucleus as a transcription factor that modulates HO-1 expression (Maines *et al*., 2001; Ahmad *et al*., 2002) and shown to act as an activator of PKC (Lerner-Marmarosh *et al*., 2007; Maines *et al*., 2007). Biliverdin reductase is prominent in brain tissue (Maines, 1990; Komuro *et al*., 1996), and the protein is increased in the penumbra region of the mouse brain after ischaemic stoke (Panahian *et al*., 1999a), suggesting a need for increased conversion of biliverdin into bilirubin because of enhanced haeme catabolism and/or enhanced HO-1 expression. Both cytoprotective and cytotoxic activities of bilirubin in brain cells have been identified (Kapitulnik, 2004; Ostrow *et al*., 2004). Thus, modulation of haeme catabolism through HO-1 during brain injury or disease may significantly modify the outcome.

HO-1 expression in the CNS

The entry of drugs, proteins and other molecules into the CNS is regulated by blood–brain, blood–spinal cord, and blood–CSF barriers, comprised of neurovascular units that included specialized brain endothelial cells, astrocytes, neurons, the extracellular matrix and other cell types (recently reviewed in Hawkins and Davis, 2005; Persidsky *et al*., 2006; Banerjee and Bhat, 2007). In general, these barriers limit the entry of many molecules and drugs that could otherwise induce HO-1 expression in the CNS, but when damaged they allow the entry of blood and various molecules that initiate HO-1 expression. Furthermore, the ability to express HO-1 may impact how much damage these

barriers suffer during neurological injury. For example, disruption of and damage to the blood–spinal cord barrier was greater in mice that were heterozygous for an HO-1 null mutation than in normal mice (Lin *et al.*, 2007c). In agreement with a function of HO-1 in barrier integrity, systemic injection of haemin led to increased expression of HO-1 in the uninjured murine spinal cord vasculature 24 h after injection, which correlated with increased protection of haemin-injected mice against vasculature dysfunction and cord injury after moderate contusion injury (Yamauchi *et al.*, 2004). These results implicate HO-1 induction in cerebrovascular endothelial cells as an important protective mechanism against physical damage to the CNS. Several studies have demonstrated the presence of haeme oxygenases in brain endothelial cells (Vigne *et al.*, 1995; Zakhary *et al.*, 1996; Bergeron *et al.*, 1998; Parfenova *et al.*, 2001; Ishikawa *et al.*, 2005), including the expression of the inducible isoform (Nimura *et al.*, 1996; Ruetzler *et al.*, 2001; Yamauchi *et al.*, 2004; Nakao *et al.*, 2008). Activity of the constitutive isoform, HO-2, appears related to the direct regulation of cerebrovascular dilation and blood flow (Ishikawa *et al.*, 2005; Leffler *et al.*, 2006), which is similar to effects reported for HO-2 in peripheral vascular endothelial cells (Suematsu *et al.*, 1994; Durante and Schafer, 1998; Christova *et al.*, 2000).

Both isoforms of haeme oxygenase are differentially expressed in the parenchyma of the healthy CNS. Although initially undetectable in the rat brain by biochemical methods (Trakshel *et al.*, 1988), HO-1-immunoreactive protein was subsequently found by immunohistochemistry to be selectively expressed in rat neurons of the cerebellum, hypothalamus, brain stem and dentate gyrus of the hippocampus (Vincent *et al.*, 1994), and sparsely expressed throughout the cortex (Matz *et al.*, 1996) and other areas, such as the thalamus (Bergeron *et al.*, 1998). HO-1 protein was undetectable by western blot analysis in regions of the mouse brain, except for slight expression in the cerebellum (Calabrese *et al.*, 2002), but was present in 'vascular-like structures' (Wang and Dore, 2007), but not in the parenchyma, when examined by immunohistochemistry (Chang *et al.*, 2003). On the basis of its mRNA distribution pattern, HO-1 expression in the 'normal' human brain obtained at autopsy may be more extensive, when compared with rodent brains (Takahashi *et al.*, 1996). However, the possibility that HO-1 is induced in the human brain by non-neurological disease, ageing, dietary or drug exposures throughout the lives of the subjects studied cannot be ruled out. For instance, studies have found that the expression of HO-1 changes in the brain during ageing, but results from studies on rats and humans do not always agree. One study on aged rats found HO-1 mRNA to be increased in extracts from the hippocampus and cerebellum, but not from the cortex or striatum of 28-month-old animals (Colombrita *et al.*, 2003). Unfortunately, HO-1 protein was not measured in this study; thus it is not certain that HO-1 protein was increased as well. An increase in the rat brain HO-1 mRNA was not confirmed by a second study that reported decreased HO-1 mRNA and protein levels (and HO-2 levels) with age in the rat hippocampus and substantia nigra (Ewing and Maines, 2006). The discrepancy between studies on HO-1 mRNA expression in aged rat brain is puzzling. A study on the cortex and hippocampus of autopsied human brains from patients without traumatic brain injury or neurodegenerative disease, aged 3–84 years, found HO-1 protein levels to be increased with age by immunohistochemical estimates (Hirose *et al.*, 2003). Again, the possibility of drug, disease or environmentally induced induction of HO-1 over the course of a lifespan in this human brain study cannot be discounted.

In contrast to HO-1, HO-2 activity and protein expression are markedly high in the rat brain (Trakshel *et al.*, 1988; Vincent *et al.*, 1994) and spinal cord (Dwyer *et al.*, 1995). Remarkably, it is difficult to find data on HO-2 expression in the 'normal' human brain or spinal cord. One study on autopsied brains from Alzheimer's disease (AD) patients and age-matched controls reported that HO-2-positive cortical pyramidal neurons were 'equally abundant' in control brains as in AD brains, whereas HO-2 mRNA content was only 1.3–2 times greater than HO-1 mRNA content, depending on brain region, when measured by reverse-transcriptase PCR with quantification by phosphor imaging (Premkumar *et al.*, 1995). Although additional information may be hidden within other publications that studied human brains, side-by-side comparisons are needed to more accurately determine whether the differential distribution and protein levels observed for HO-1 and HO-2 expressions in the rat brain are evolutionarily conserved and occur as such in the human brain.

Induction of HO-1 in brain cells

HO-1 expression in brain tissue and *in vitro* cultures of brain cells has been shown to be inducible by several drugs, chemicals and dietary-derived substances (Table 1), which is similar to the response of other cells and tissues (reviewed in Shibahara, 2003; Wagener *et al.*, 2003; Ogborne *et al.*, 2004; Ryter *et al.*, 2006). Athough there is an extensive literature dealing with HO-1 expression by rodent glial, particularly the microglia (Matsuoka *et al.*, 1998; Calingasan *et al.*, 1999; Mautes *et al.*, 2000; Nakaso *et al.*, 2000; Stahnke *et al.*, 2007), there is surprisingly little data available on HO-1 expression by human neural cells, being restricted to a few studies on human tumour cell lines (Takahashi *et al.*, 1996; Businaro *et al.*, 2002; Goldstein *et al.*, 2003; Rieder *et al.*, 2004). As might be expected, exposure of 118 INI human glioma cells (Yoshida *et al.*, 1988) or differentiated SH-SY5Y human neuroblastoma cells (Goldstein *et al.*, 2003) to haemin induced HO-1 protein expression and, similar to mouse primary astrocytes and rat C6 glioma cells, dopamine exposure induced HO-1 expression in the SK-N-SH human neuroblastoma cell line (Rieder *et al.*, 2004). Myelin basic protein, which is released during demyelination, was found to induce HO-1 mRNA and protein in the T67 astrocytoma cell line (Businaro *et al.*, 2002), whereas agents that release nitric oxide and cause nitrosative stress increased HO-1 expression in T98G glioblastoma cells (Takahashi *et al.*, 1996). The paucity of information on the response to human neurons, neuroglia and microglial cells to many agents known to induce HO-1 expression in rodent neural cells, and

Table 1 Substances that affect HO-1 activity and/or expression in neural cells or tissue

Substance	*Tissue source*	*Activity*	*Protein*	*mRNA*	*Reference*
Haemin	Human 118 INI glioma cell line	↑	ND	ND	Yoshida *et al.* (1988)
H_2O_2	Primary rat astrocytes and rat-transformed AT astroglia	ND	↑[a]	ND	Dwyer *et al.* (1992)
Diethyl maleate, BSO	Rat brain microsomes,[b] slices and extracts	↑	↑[a]	↑	Ewing and Maines (1993)
Cysteamine	Primary rat astrocytes	ND	↑	↑	Chopra *et al.* (1995)
Metalloporphyrin derivatives	Rat brain microsomes[b]	↓	ND	ND	Vreman *et al.* (1996)
SNP, SIN-1 and *S*-nitroso-L-glutathione	Human T98G glioblastoma cells	ND	↑	↑	Takahashi *et al.* (1996)
$PrP_{106-126}$	Primary rat astrocytes	ND	ND	↑	Rizzardini *et al.* (1997)
Phencyclidine	Rat forebrain extracts	ND	↑	↑	Rajdev *et al.* (1998)
LPS, SNAP	Rat mixed glial cultures	ND	↑	ND	Kitamura *et al.* (1998)
Dopamine, haemin	Primary mouse astrocytes and rat C6 glioma cell line	ND	ND	↑	Schmidt *et al.* (1999)
15dPG-J_2, PG-J_2, PG-D_2, ciglitazone, WY14643, NS398, indomethacin, 9-c-RA	Primary rat mix glia	ND	↑	ND	Kitamura *et al.* (1999)
H_2O_2, l-Glu, KA, QA, AMPA, ACPD, PMA	Primary rat mixed astrocytes and microglia	ND	↑	ND	Matsuoka *et al.* (1999a)
BH, AH	Mouse-transformed BV-2 microglia	ND	ND	↑	Taramelli *et al.* (2000)
L-Glu	Primary mouse cerebellar granule cells	ND	ND	↑	Chen *et al.* (2000)
15dPG-J_2, PG-J_2, PG-D_2, PG-A_2	Mouse-transformed BV-2 microglia	ND	↑	ND	Koppal *et al.* (2000)
Haemoglobin	Primary mouse astrocytes	ND	↑	ND	Regan *et al.* (2000)
$A\beta_{1-40}$, $A\beta_{1-42}$	Primary rat astrocytes	ND	↑	↑	Ham and Schipper (2000)
TNF-α, IL-1β	Primary rat astrocytes	ND	ND	↑	Mehindate *et al.* (2001)
H_2O_2, DFO, PA	Primary rat oligodendrocytes	ND	↑	ND	Goldbaum and Richter-Landsberg (2001)
PDTC	Primary rat oligodendrocytes	ND	↓[c]	ND	Goldbaum and Richter-Landsberg (2001)
Multiple CpG oligonucleotide	Rat brain astrocytes	ND	↑[a]	ND	Schluesener *et al.* (2001b)
PGG	Rat (sic) Neuro 2A neuroblastoma cell line	↑	↑	↑	Choi *et al.* (2002)
Curcumin, CAPE	Rat-transformed DI TNC1 astrocytes	↑	↑	ND	Scapagnini *et al.* (2002)
Rosmarinic acid, Resveratrol, *o*-Coumaric acid, *p*-Coumaric acid	Rat-transformed DI TNC1 astrocytes	↓	ND	ND	Scapagnini *et al.* (2002)
Myelin basic protein	Human T67 astrocytoma cell line	ND	↑	↑	Businaro *et al.* (2002)
NH_4Cl	Primary rat astrocytes and rat brain cortex[d]	ND	↑	↑	Warskulat *et al.* (2002)
EGb 761	Mouse primary neurons	ND	↑	ND	Zhuang *et al.* (2002)
Resveratrol	Mouse primary neurons	ND	↑	ND	Zhuang *et al.* (2003)
Catalposide	Mouse Neuro 2A neuroblastoma cell line	↑	↑	ND	Moon *et al.* (2003)
Haemin	Human SH-SY5Y differentiated neuroblastoma cells	ND	↑	ND	Goldstein *et al.* (2003)
Resveratrol	Rat-transformed DI TNC1 astrocytes	↓	NC	↑	Scapagnini *et al.* (2004)
Endothelin-1	Mouse spinal cord	ND	↑[a]	ND	Weinzierl *et al.* (2004)
Propofol	Primary rat astrocytes	ND	↑	ND	Acquaviva *et al.* (2004)
SIN-1	Primary rat astrocytes	ND	↑	ND	Acquaviva *et al.* (2004)
Pb^{+2}	Primary rat astrocytes	↑	↑	ND	Cabell *et al.* (2004)
Ethyl ferulate	DI TNC1 cells and rat-transformed H19-7 hippocampal neurons	↑	↑	↑	Scapagnini *et al.* (2004)
Dopamine	Human SK-N-SH neuroblastoma cells	ND	↑	↑	Rieder *et al.* (2004)
Triethyltin	Primary rat oligodendrocytes	ND	↑	ND	Stahnke and Richter-Landsberg (2004)
Oregonin	Mouse-transformed BV-2 microglia	ND	↑	ND	Lee *et al.* (2005)
Tat	Human Tat-transfected SVGA cells	ND	↑	ND	Pocernich *et al.* (2005)
Quercetin, Vitamin C, *N*-acetylcysteine	Mouse-transformed BV-2 microglia	ND	↑	↑[e]	Chen *et al.* (2005)
FGF-1	Primary rat astrocytes	ND	↑	↑	Vargas *et al.* (2005)
Acetyl-L-carnitine	Rat-transformed DI TNC1 astrocytes	↑	↑	↑	Calabrese *et al.* (2005b)
LPS + IFN-γ	Rat-transformed DI TNC1 astrocytes	ND	↑	↑	Calabrese *et al.* (2005a)
15dPG-J_2	Primary mouse neurons	ND	↑	ND	Kim *et al.* (2005)
PG-A_1	Mouse NBP_2 differentiated neuroblastoma cells	ND	↑	↑	Yan *et al.* (2005)
Imidazole-dioxolanes	Rat brain microsomes[b]	↓	ND	ND	Kinobe *et al.* (2006)
Acetyl-L-carnitine, $A\beta_{1-42}$	Primary rat neurons	ND	↑	ND	Abdul *et al.* (2006)
Lovastatin	Rat brain sonicate[b]	↑	ND	ND	Hsu *et al.* (2006)
Succinyl acetone, NMP	Primary mouse cortical neurons	ND	ND	↑	Chernova *et al.* (2006)
MG-132	Primary rat oligodendrocytes and astrocytes	ND	↑	ND	Goldbaum *et al.* (2006)
H_2O_2, 4-HNE	Mouse-transformed HT22 hippocampal neurons	ND	↑	ND	Kaizaki *et al.* (2006)
NEEP6-biotin, NEPP11	Mouse-transformed HT22 hippocampal neurons	ND	↑	ND	Satoh *et al.* (2006)
ACM	Primary rat microglia and mouse-transformed BV-2 microglia	↑	↑	↑	Min *et al.* (2006)
LPS	Mouse-transformed BV-2 microglia	ND	↑	↑	Lee and Suk (2007)

Table 1 *Continued*

Substance	*Tissue source*	*Activity*	*Protein*	*mRNA*	*Reference*
CoPPIX	Mouse-transformed BV-2 microglia	ND	↑	ND	Chora *et al.* (2007)
Sodium arsenite, cobalt chloride, H_2O_2	Primary rat astrocytes	ND	↑	↑[f]	Imuta *et al.* (2007)
Pioglitazone	Rat spinal cord	ND	ND	↑	Park *et al.* (2007)
Cytomix + indomethacin or ibuprofen	Rat C6 glioma cells	ND	↑	ND	Parhizgar (2007)

Abbreviations: 15dPG-J_2, 15-deoxy-$\Delta^{12,14}$-prostaglandin J_2; 4-HNE, 4-hydroxynonenal; 9-c-RA, 9-*cis*-retinoic acid; $A\beta_{1-40}$, amino acids 1–40 of amyloid-β protein; $A\beta_{1-42}$, amino acids 1–42 of amyloid-β protein; ACM, primary rat astrocyte-conditioned media; ACPD, *trans*-(9)-1-amino-(1*S*,3*R*)-cyclopentanedicarboxylic acid; AH, α-haematin; AMPA, (9)-α-amino-3-hydroxy-5-methylisoxazole-4-propanoic acid; BH, β-haematin; CAPE, caffeic acid phenethyl ester; CoPPIX, cobalt protoporphyrin IX; Cytomix, IFN-γ + interleukin-1β + tumour necrosis factor-α; DFO, deferoxamine; EGb 761, standardized extract of Ginkgo biloba; FGF-1, fibroblast growth factor-1; IFN-γ, interferon-γ; IL-1β, interleukin-1β; KA, kainic acid; LPS, lipopolysaccharide; MG-132, carbobenzoxy-L-leucyl-L-leucyl-L-leucinal; NC, no change; ND, not determined; NEPP11, electrophilic neurite outgrowth-promoting prostaglandin derivative 11; NEPP6-biotin, biotinylated electrophilic neurite outgrowth-promoting prostaglandin derivative 6; NMP, *N*-methylprotoporphyrin IX; PA, phenanthroline; PDTC, pyrrolidine dithiocarbamate; PG, prostaglandin; PGG, 1,2,3,4,6-Penta-*O*-galloyl-β-D-glucose; PMA, phorbol 12-myristate, 13-acetate; $PrP_{106-126}$, residue 106–126 of human prion protein; QA, L-quisqualic acid; SIN-1, 3-morpholinosydnonimine; SNAP, *S*-nitroso-*N*-acetylpenicillamine; SNP, sodium nitroprusside; SVGA, a human astrocytic subclone of SVG (SV40-transformed human fetal glial cells); Tat, transactivating regulatory protein of human immunodeficiency virus-1; TNF-α, tumour necrosis factor-α.
[a]Protein estimated by immunohistochemistry.
[b]≈3:1 ratio of HO-2/HO-1 protein.
[c]Decreased both constitutive and H_2O_2-induced levels.
[d]Protein measured in rat cortex extracts only.
[e]mRNA measured after quercetin only.
[f]mRNA measured after hypoxia only.

rodent and human non-neural cells, is an impediment to understanding if human brain cells possess or have lost unique regulatory mechanisms that can help in the development of new drugs and therapeutics to better target this system in the brain and spinal cord. This lack of data is all the more important, given the evidence for evolutionary divergence in HMOX1 gene regulation mentioned above, and for differences in HMOX1 promoter regulation among human cells (Takahashi *et al.*, 1999) and between species (c.f., Pellacani *et al.*, 1998; Lin *et al.*, 2007a).

However, the induction of HO-1 expression in the CNS, or lack thereof, does have significant consequences. Typical of many enzymes and proteins induced during inflammation and the innate immune response, HO-1 appears to have both positive and negative functions in injury and disease of the CNS. The first apparent report of induced HO-1 expression in the brain was a study of hyperthermia in rats (Ewing and Maines, 1991). This observation was soon followed by similar ones, including *in vitro* induction by heat shock of primary rat brain astrocytes in culture (Dwyer *et al.*, 1992). Interestingly, HO-1 is not always induced by heat shock in human cells. Studies have found a typical induction in one hepatoma cell line, but no response in another (Mitani *et al.*, 1989), a lack of heat shock induction in U937 lymphoma and 118 INI glioma cells (Yoshida *et al.*, 1988), a suppression of HO-1 induction by hyperthermia in several other cell lines (Okinaga *et al.*, 1996) and a typical heat shock response in freshly isolated peripheral blood mononuclear cells (Sonna *et al.*, 2002). Thus, whether normal human brain cells exhibit a heat shock-induced upregulation of HO-1 expression remains unexplored. Other observations of HO-1 induction in brain tissue have been reported, including its appearance in temporal lobe neurons from patients with AD (Yan *et al.*, 1994). Subsequent studies on brains from AD patients observed colocalization of HO-1 protein in glial fibrillary acidic protein-expressing astrocytes as well as in neurons of the hippocampus (Schipper *et al.*, 1995). An increase in HO-1 mRNA in cerebral cortex tissue and cerebral vessels, but not in the cerebellum (a brain region generally spared in AD), has also been reported (Premkumar *et al.*, 1995). The HO-1 expression by astrocytes was observed to correlate with lower cognitive scores in patients with sporadic AD and mild cognitive impairment (Schipper *et al.*, 2006), suggesting that HO-1 expression is an early event in AD. *In vitro* studies provide evidence for altered haeme metabolism in AD (Atamna and Frey, 2004 and references therein) and the inhibition of HO-1 activity by amyloid precursor proteins (Takahashi *et al.*, 2000). Increased HO-1 expression has also been observed in abnormal 'balloon' neurons and glial inclusions of white matter in cases of Pick's disease (Castellani *et al.*, 1995), in the majority of glial fibrillary acidic protein-positive astrocytes of the substantia nigra of Parkinson's disease brains (Schipper *et al.*, 1998), in abnormal spinal motor neurons of patients with amyotrophic lateral sclerosis (Calingasan *et al.*, 2005) and in glial fibrillary acidic protein-positive astrocytes in the spinal cord of multiple sclerosis patients (Mehindate *et al.*, 2001). Interestingly, HO-1 expression by microglia, the immune cell of the brain often ascribed to producing neurotoxins, was not observed in these autopsy samples, although microglia robustly express HO-1 in the murine model of multiple sclerosis, experimental allergic encephalomyelitis (Emerson and LeVine, 2000; Stahnke *et al.*, 2007) and in portions of the hemisected rat spinal cord undergoing Wallerian degeneration (Mautes *et al.*, 2000). The study by Stahnke *et al.* (2007) also reported prominent HO-1 expression in microglia, macrophage and astrocytes in biopsy samples from patients with acute disseminated leukoencephalomyelitis. Reasons for this discrepancy may be the length of time between disease/injury onset and tissue sampling, or that microglia in the diseased human spinal cord are not HO-1-expressing cells. Further studies are needed to resolve this issue. HO-1 expression is also induced in rats after transient forebrain (Takeda *et al.*, 1994; Geddes *et al.*, 1996; Moreira *et al.*, 2007) and retinal (Arai-Gaun *et al.*, 2004) ischaemia, after traumatic brain injury in rats (Fukuda *et al.*, 1995; Yi and Hazell,

2005) and humans (Beschorner *et al*., 2000; Cousar *et al*., 2006), after subarachnoid (Matz *et al*., 1996) or intracerebral haemorrhage (Matz *et al*., 1997; Wagner *et al*., 2000; Wang and Dore, 2007) in rodents, in rat brains after neurotoxin exposure (Matsuoka *et al*., 1999b; Lu and Ong, 2001; Munoz *et al*., 2005) and in infected rodent (Herden *et al*., 2005; Pamplona *et al*., 2007; Ren *et al*., 2007) and human (Schluesener *et al*., 2001a) brains. As is evident from these and other results (Tang *et al*., 2002), induction of HO-1 is a common occurrence during disease or injury of the CNS. However, it is important to understand whether the induction of HO-1 in the CNS contributes to the observed pathophysiology, all the products of haeme degradation are neurotoxic in sufficient amounts, or whether it represents a protective response through the regulation of gene expression by unbound iron and CO, or antioxidant activity of unconjugated bilirubin. A variety of approaches have been used to investigate this dilemma.

CNS effects of genetic manipulation of HO-1

HO-1 deficiency does not appear to cause any specific neurological or behavioural disorders, although the only human case died at age 6 years (Yachie *et al*., 1999), and it is not clear if *Hmox1*$^{-/-}$ mice have been extensively examined in this regard. On the other hand, *Hmox1*$^{-/-}$ null mice have been used for *in vivo* and *in vitro* studies to further understand the contribution of HO-1 to CNS pathophysiology. The inability to express HO-1 was found to make spinal motor neurons and glia susceptible to the apoptotic effect of a high dose of nitric oxide after prior exposure to a non-toxic nitric oxide dose (Bishop *et al*., 2004). This indicates a need for HO-1 expression for the development of adaptive NO resistance, a protective mechanism that may come into play at times of high output NO production during inducible NOS (iNOS) expression in the CNS, such as in AD (Vodovotz *et al*., 1996; Nathan *et al*., 2005), multiple sclerosis (Oleszak *et al*., 1998), ischaemic stroke (Forster *et al*., 1999; Askalan *et al*., 2006), brain haemorrhage (Berra *et al*., 2007) and brain infections (Koprowski *et al*., 1993) (Figure 1). Astrocytes from *Hmox1*$^{-/-}$ mice are also more susceptible to haeme-mediated oxidative damage (Chen-Roetling *et al*., 2005), as are *Hmox1*$^{-/-}$ neurons and astrocytes to haemoglobin-induced cell death (Chen-Roetling and Regan, 2006), indicating that being able to express HO-1 protein is protective under these conditions. *In vivo*, however, a lack of HO-1 protein was found to be protective after intracerebral haemorrhage, with significantly less area of damage in *Hmox1*$^{-/-}$ mice as compared with the extensive expression in microglia/macrophages and endothelial cells observed in the perihematomal region of wild-type mice (Wang and Dore, 2007). This finding appears to contradict the *in vitro* data of Chen-Roetling and Regan (2006), but could be because of the differences in the amount of haemoglobin that the cells were exposed to, or because of the reductions in infiltrating leukocytes, production of reactive oxygen species and activated microglia/macrophage also observed in the brains of *Hmox1*$^{-/-}$ mice after intracerebral haemorrhage. In the case of experimental allergic encephalomyelitis, the inability to express HO-1 protein is clearly detrimental, leading to greater mortality, whereas treatment with the HO-1 inducer cobalt protoporphyrin IX is protective (Chora *et al*., 2007). *Hmox1* is also beneficial in protection from experimental cerebral malaria (Pamplona *et al*., 2007). The BALB/c mouse strain is normally resistant to experimental cerebral malaria, and *Plasmodium* infection causes a robust induction of HO-1 in the brain of BALB/c mice $\geqslant$6 days after infection. However, *Hmox1*$^{-/-}$ BALB/c mice become susceptible to cerebral malaria, as do normal BALB/c mice treated with the HO-1 enzyme inhibitor zinc protoporphyrin IX. A *Plasmodium* susceptible mouse strain, the C57BL/6, does not show induction of HO-1 mRNA 6 days after infection, but is protected if treated with cobalt protoporphyrin IX or allowed to breathe CO (250 p.p.m.). Neither treatment changed the parasitemia after infection, but both treatments prevented breakdown of the blood–brain barrier and neuroinflammation (Pamplona *et al*., 2007).

Transgenic animals and cells that have been genetically engineered to overexpress HO-1 have been used to help delineate the function of HO-1 expression in the CNS. Mice overexpressing the rat HO-1 protein under the control of a neuron-specific enolase promoter (approximately 6–8 copies per genome) were found to be fertile and normal in gross anatomical appearance, except for an enlarged spleen in many animals. However, when females were tested upon initial exposure to a novel environment, they showed significantly reduced locomotion and exploration, an effect not seen in male transgenic mice (Maines *et al*., 1998). Further testing indicated a lack of sensorimotor deficits in the transgenic mice, but impaired spatial navigation learning was evident (Morgan *et al*., 1998). HO-1-overexpressing mice were also found to have a higher constitutive expression of the proto-oncogene bcl-2 in selected neuronal populations, and male transgenic mice were observed to have reduced stroke volumes and areas of brain infarct at 6 and 24 h after occlusion of the middle cerebral artery (Panahian *et al*., 1999b). Greater sparing of HO-1-positive neurons in the peri-ischaemic regions was also observed. Cerebellar granule cells grown in primary culture from transgenic mice were found to be resistant to the neurotoxic effects of L-glutamate and H_2O_2 and to produce lower levels of reactive oxygen species after L-glutamate exposure (Chen *et al*., 2000). Exposure to L-glutamate also induced the expression of HO-1 mRNA in cells from both non-transgenic and transgenic mice. When the murine HT22 hippocampal neuronal cell line was transfected to overexpress rat HO-1 protein, they were also protected from L-glutamate toxicity (Satoh *et al*., 2003). Overexpression of human HO-1 in human neuroblastoma cells reduced cell death from H_2O_2 exposure (Takeda *et al*., 2000). There was also reduced expressions of tau mRNA and protein in HO-1-overexpressing cells, which was reversible with zinc deuteroporphyrin treatment. A protective function of HO-1 induction against oxidant-induced cell death was also demonstrated by Kaizaki *et al*. (2006), using small-interfering RNA (siRNA) to reduce endogenous HO-1 protein expression in the mouse HT22 cell line. In contrast to the studies above, neuron-like rat PC12 pheochromocytoma cells co-cultured on rat astrocytes overexpressing human HO-1 protein were susceptible to cell

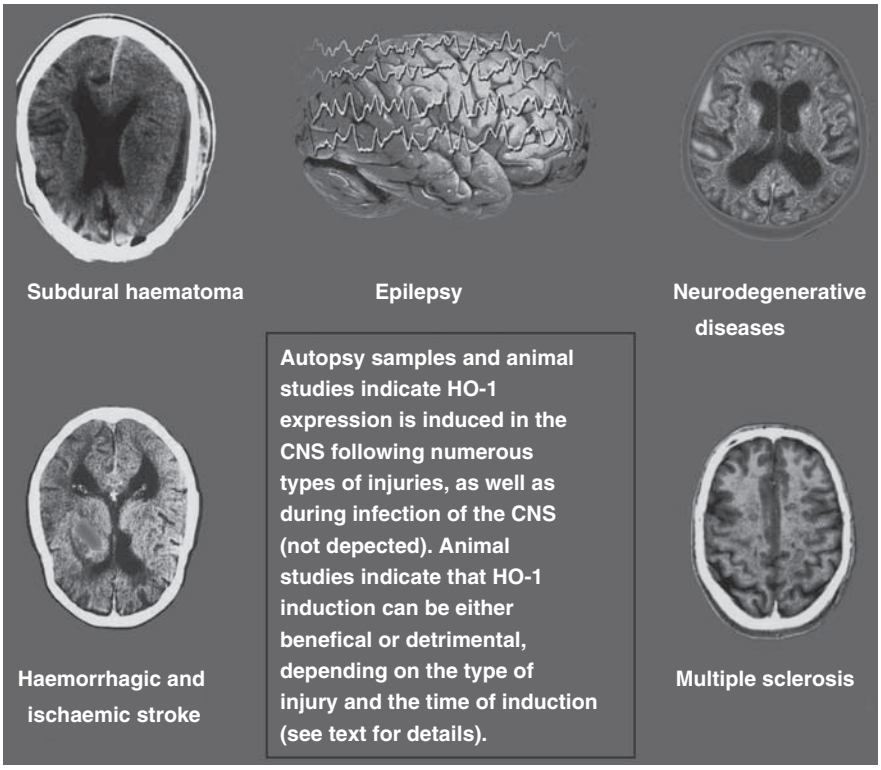

Figure 1 Physical and chemical injuries that induce HO-1 expression in the CNS. HO-1, haeme oxygenase-1. Brain images reproduced with permission of S. Camazine (photos © Scott Camazine; http://www.scottcamazine.com).

death induced by exposure to dopamine + H_2O_2 (Song *et al.*, 2007). This effect was blocked by exposure to tin mesoporphyrin, ascorbate, deferoxamine or phenanthroline. Furthermore, conditioned media from the overexpressing astrocytes induced a similar toxic response in isolated cultures of PC12 cells, indicating a function of a soluble mediator, possibly released iron, in the enhancement of cytotoxicity from HO-1-overexpressing astrocytes. These studies demonstrate the yin and yang of HO-1 expression in regard to neurotoxicity or neuroprotection. It is possible that differences in the overexpression studies described above can be explained by the relative degree of overexpression. A dependence of the outcome (cytoprotection or cytotoxicity) on the degree of HO-1 overexpression was elegantly demonstrated in hamster fibroblasts using a tetracycline-inducible HO-1 expression vector, in which a distinct cytoprotective threshold was observed for hyperoxia-induced cell death (Suttner and Dennery, 1999). It is not likely, however, that the same cytoprotective threshold observed in hamster fibroblast (< 5-fold overexpression) will apply in all cases and cell types. Other variables, such as the availability of substrate, oxygen and NADPH levels and NADPH–haemoprotein reductase activity, may all come into play for specific cell types or tissues. Another explanation for the disparate findings may be that they are dependent on the cell type expressing the HO-1 protein. For example, in addition to the overexpression studies described above, the induction of HO-1 in neurons or neuroblastoma cells imparts *in vitro* self-protection against a number of neurotoxins (Choi *et al.*, 2002; Moon *et al.*, 2003; Scapagnini *et al.*, 2004; Yan *et al.*, 2005; Park *et al.*, 2007). A similar effect is seen for agents that induce HO-1 expression in isolated astrocytes (Regan *et al.*, 2000; Acquaviva *et al.*, 2004) or BV-2 microglia cells (Lee and Suk, 2007). Yet, treating rats with 50 mg kg^{-1} phencyclidine not only induced HO-1 expression primarily in brain astrocytes and some microglia but also caused a neurodegeneration that was diminished by pretreatment with 1,3-dimethylthiourea, which also reduced the glial HO-1 expression (Rajdev *et al.*, 1998). One interpretation of these data is that, similar to HO-1-overexpressing astrocytes in co-culture with PC12 cells, the glial HO-1 activity is causing the release of a soluble neurotoxic mediator. A second interpretation is that the reduced neurodegeneration led to less neuroinflammation and hence a need for less HO-1 expression.

HO-1 in neuroinflammation

In addition to its well-known function as an indicator of oxidative and nitrosative stress, a function of HO-1 expression in the regulation of inflammation has emerged over the past decade (reviewed in Wagener *et al.*, 2003; Camara and Soares, 2005). HO-1 appears to be involved with the resolution of inflammation (Gilroy *et al.*, 2004) and that may include the resolution of neuroinflammation (Figure 2). Inflammation in the CNS, that is, neuroinflammation, shares several similarities with inflammation in peripheral tissues, particularly regarding innate host defense, but has distinct differences as well (reviewed in Mennicken *et al.*, 1999; Piehl and Lidman, 2001; Polazzi and Contestabile,

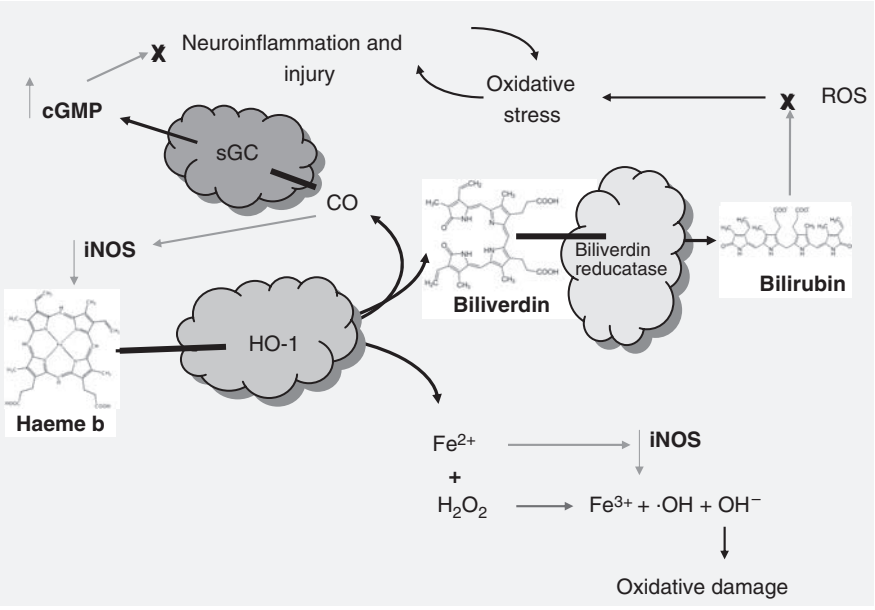

Figure 2 Potential pathways for HO-1-dependent injury or resolution of neuroinflammation. Products formed by the degradation of haeme by haeme oxygenase-1 (HO-1) can be either beneficial or detrimental. Thin arrows: grey pathways indicate beneficial actions of HO-1 products. Black pathways indicate detrimental actions. The tetrapyrroles biliverdin and bilirubin possess antioxidant activity and help reduce oxidative stress. Ferrous iron can react with hydrogen peroxide by the Fenton reaction to generate damaging hydroxyl radicals, whereas evidence suggests that iron may also mediates an HO-1-dependent decrease in iNOS expression through a transcriptional inhibition. Carbon monoxide-releasing compounds also decrease iNOS expression, suggesting that HO-1-derived CO may act similarly. ROS, reactive oxygen species; sGC, soluble GC; iNOS, inducible NOS.

2002), including additional functions for chemokines beyond their functions as classical chemical attractants (Asensio and Campbell, 1999; Adler *et al.*, 2005). As mentioned previously, iNOS is expressed in several neurodegenerative diseases and various brain pathologies (Loihl and Murphy, 1998), and the high-output production of NO appears to have a dual functions as either a harmful or beneficial modulator in the CNS. For example, inhibition of iNOS activity or genetic disruption of the gene (*Nos2*) that encodes the inducible NOS reduces cerebral damage due to ischaemic stroke (Iadecola *et al.*, 1995, 1997) and protects mice from pathology in an animal model of AD (Nathan *et al.*, 2005). On the other hand, iNOS expression has been found to be neuroprotective after traumatic brain injury in rodents (Sinz *et al.*, 1999) and in experimental meningitis (Leib *et al.*, 1998). The mechanisms that regulate iNOS expression by neural cells are complex and incompletely understood, but studies have found an apparent inverse relationship between HO-1 and iNOS expression. Kitamura *et al.* (1999) have reported that treatment of rat brain mixed glial cultures with substances characterized as peroxisome proliferator-activated receptor-γ agonists, including thiazolidinediones, nonsteroidal anti-inflammatory drugs and cyclopentenone prostaglandins, caused a reduction in lipopolysaccharide + interferon-γ-induced iNOS expression and a concomitant enhancement of HO-1 expression. The plant diarylheptanoid derivative oregonin was reported to reduce iNOS expression in the BV-2 microglia cell line while also inducing HO-1 expression (Lee *et al.*, 2005). Treatment with the CO-releasing compound $[Ru(CO)_3Cl_2]_2$ also reduced iNOS expression in BV-2 cells, suggesting a mechanistic link between HO-1 activity and reduction in iNOS expression. The flavonoid quercetin was also reported to induce HO-1 expression and to inhibit iNOS expression in BV-2 cells (Chen *et al.*, 2005), an effect that was partially reversed by treatment with an HO-1 antisense oligodeoxynucleotide and replicated by haemin. Induction of HO-1 in the DI TNC1 rat astrocyte cell line with acetyl-L-carnitine was found to reduce subsequent iNOS expression induced by lipopolysaccharide + interferon-γ (Calabrese *et al.*, 2005b). Interestingly, treatment of DI TNC1 cells with carnosine reduced both iNOS and HO-1 expressions (Calabrese *et al.*, 2005a). Conditioned media from rat primary astrocyte cultures were reported to induce HO-1 expression and to reduce iNOS expression in rat primary microglial cultures (Min *et al.*, 2006). Treatment with bilirubin, CO or iron (as $FeSO_4$) was also effective in reducing interferon-γ-induced iNOS expression in the microglia cultures.

Our laboratory has recently examined the relationship between nonsteroidal anti-inflammatory drug-induced HO-1 expression and suppression of iNOS expression in the C6 glioma cell line (Parhizgar, 2007). Both indomethacin and ibuprofen inhibited cytokine-stimulated iNOS protein levels and Nos2 promoter activity in C6 cells, while concurrently inducing HO-1 protein. Neither drug induced HO-1 in non-stimulated cells. The effects of indomethacin were reversed by the iron chelator deferoxamine, and the induction of HO-1 protein by cobalt protoporphyrin IX mimicked the effect of indomethacin on iNOS protein expression. Furthermore, the CO releaser $[Ru(CO)_3Cl_2]_2$ was shown to inhibit nitrite production and Nos2 promoter activity. Interestingly, deferoxamine did not reverse the effect of ibuprofen on

iNOS protein levels or promoter activity. Furthermore, pretreatment with tin protoporphyrin IX, an inhibitor of HO-1 enzymatic activity, failed to reverse the indomethacin-induced inhibition of Nos2 promoter activity in C6 cells. However, reducing indomethacin-induced HO-1 protein levels by RNA interference significantly decreased indomethacin inhibition of iNOS protein and Nos2 promoter activity. Curiously, RNA interference did not alter ibuprofen-induced inhibition of Nos2 promoter activity or iNOS protein expression (Parhizgar, 2007). These results suggest a mechanistic link between HO-1 protein expression and a reduction in iNOS expression at the promoter level. One mechanism consistent with the findings from our laboratory might involve nuclear localization of HO-1 protein and activation of transcription factors (Lin *et al*., 2007b). Although we did not examine whether HO-1 protein was present in the nucleus of C6 glioma cells, a previous study has reported a nuclear localization in primary astrocytes (Li Volti *et al*., 2004).

It is most likely that HO-1 expression may affect other mediators of neuroinflammation in addition to an interaction with iNOS expression. For example, the level of prostaglandin E_2 production in rat cortical astrocytes appears to be linked to HO-1 activity (Vairano *et al*., 2001). A stronger rationale for this speculation comes from studies on non-neural cells, in which HO-1 induction or overexpression has been found to influence several cytokines and other inflammatory mediators (Willis *et al*., 1996; Belcher *et al*., 2006; Tamion *et al*., 2006; Yasui *et al*., 2007).

HO-1 as a therapeutic target in the CNS

From the studies reviewed herein and relevant information in the scientific literature, it appears that both activation and suppression of the HO-1 system are promising approaches for the development of new therapeutics and drugs to treat or combat-specific disorders of the CNS. Suppression of HO-1 protein expression or enzymatic activity would appear to be beneficial in conditions such as brain haemorrhage, where the massive amount of haemoglobin in the tissue acts as a source of haeme, which induces HO-1 expression and then serves as the precursor for the production of toxic levels of iron, unconjugated bilirubin and perhaps CO. Two approaches can be envisioned for suppressing HO-1 protein expression; inhibition of HMOX1 gene transactivation, perhaps by the development of small molecules that inhibit the dissociation of the Keap1–Cullin3–Nrf2 complex (Tong *et al*., 2006) or interrupt Nrf2 interactions with Brahma-related gene 1 (Zhang *et al*., 2006, 2007), and delivery of HO-1-specific (Kaizaki *et al*., 2006) or biliverdin IXα reductase-specific (Miralem *et al*., 2005) siRNA. To use the former approach, it will be necessary, of course, to show that these complexes and nuclear interactions are involved in HO-1 induction by human astrocytes, neurons or microglia. In the latter case, the problem will be to efficiently and selectively deliver the siRNA to the correct cells in the CNS. Recent advances in selective siRNA delivery to hepatocytes (Rozema *et al*., 2007) and neurons (Kumar *et al*., 2007) may be exploited to develop selective delivery to astrocytes and microglia. Alternatively, the use of non-toxic (c.f., An *et al*., 2007) anti-HO-1 siRNA-expressing stem cells that engraft the CNS and differentiate long-term into neurons, astrocytes or microglia may be an appropriate approach for chronic conditions.

On the surface, the activation of HO-1 in CNS cells would seem very straightforward. Numerous substances have been reported to induce HO-1 in astrocytes, neurons and immortalized microglial cells (see Table 1); thus, the main issue might be the ability of the substance to cross the blood–brain barrier, and its safety. It could even be worthwhile to initiate a 'drug repurposing' programme to identify novel inducers, which is similar to the approach taken to identify non-steroidal antagonists against the human androgen receptor (Bisson *et al*., 2007). However, indiscriminant HO-1 induction may not be as beneficial as targeted induction given the diverse functions of HO-1 throughout the body. Ideally, agents that induce HO-1 expression selectively in neurons, astrocytes, oligodendrocytes or microglia would appear to have advantages in treating CNS disease. For example, iNOS expression in AD is localized primarily to astrocytes, thus selective induction of HO-1 in astrocytes could downregulate the iNOS expression. It is not clear that HO-1 induction in microglia or neurons would affect iNOS expression in astrocytes, and a pan induction in all CNS cells might not be beneficial either. The issues facing drug developers in this regard are to better understand differences in surface receptors and uptake or storage mechanisms between neurons, microglia, astrocytes and oligodendrocytes that might be used to selectively deliver molecules to specific cell types. Another option, given the possibility that CNS cells may use different pathways for HO-1 induction, might be to develop drugs that target cell-specific transcriptional regulators of HO-1 expression such as Bach1 in neurons. As is hopefully clear from this review, there is a potential to derive great benefit by selective manipulation of HO-1 activity and expression in neural cells. What is currently lacking is sufficient knowledge of the regulatory mechanisms used by neural cells, especially human cells, to control HO-1 expression.

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Conflict of interest

The author states no conflict of interest.

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