Journal of Neurochemistry

JOURNAL OF NEUROCHEMISTRY | 2009 | 110 | 469–485 doi: 10.1111/j.1471-4159.2009.06160.x

REVIEW

Heme oxygenase-1 and neurodegeneration: expanding frontiers of engagement

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Abstract

The heme oxygenases (HOs), responsible for the degradation of heme to biliverdin/bilirubin, free iron and CO, have been heavily implicated in mammalian CNS aging and disease. In normal brain, the expression of HO-2 is constitutive, abundant and fairly ubiquitous, whereas HO-1 mRNA and protein are confined to small populations of scattered neurons and neuroglia. In contradistinction to HO-2, the ho-1 gene (*Hmox1*) is exquisitely sensitive to induction by a wide range of pro-oxidant and other stressors. In Alzheimer disease and mild cognitive impairment, immunoreactive HO-1 protein is over-expressed in neurons and astrocytes of the cerebral cortex and hippocampus relative to age-matched, cognitively intact controls and co-localizes to senile plaques, neurofibrillary tangles, and corpora amylacea. In Parkinson disease, HO-1 is markedly over-expressed in astrocytes of the substantia nigra and decorates Lewy bodies in affected dopaminergic neurons. *HMOX1* is also up-regulated in glial cells surrounding human cerebral infarcts, hemorrhages and contusions, within multiple sclerosis plaques, and in other degenerative and inflammatory human CNS disorders. Heme-derived free ferrous iron, CO, and biliverdin/bilirubin are biologically active substances that have been shown to either ameliorate or exacerbate neural injury contingent upon specific disease models employed, the intensity and duration of HO-1 expression and the nature of the prevailing redox microenvironment. In 'stressed' astroglia, HO-1 hyperactivity promotes mitochondrial sequestration of non-transferrin iron and macroautophagy and may thereby contribute to the pathological iron deposition and bioenergetic failure amply documented in Alzheimer disease, Parkinson disease and other aging-related neurodegenerative disorders. Glial HO-1 expression may also impact cell survival and neuroplasticity in these conditions by modulating brain sterol metabolism and proteosomal degradation of neurotoxic protein aggregates.

Keywords: aging, Alzheimer disease, heme oxygenase-1, iron, oxidative stress, Parkinson disease.

J. Neurochem. (2009) **110**, 469–485.

The heme oxygenases (HOs) have been recognized as dynamic sensors of cellular oxidative stress (OS) and likely arbiters of tissue redox homeostasis across the phylogenetic spectrum. However, the HO reaction is a 'double-edged' sword! Akin to the activity of the nitric oxide (NO) synthases, superoxide dismutases, and other redox-active enzymes, the HOs may impart robust antioxidant defense in certain disease conditions while serving to amplify free radical damage in others. As illustrated in this article, the Janus faces of the HOs are particularly apparent in disorders of the mammalian CNS. The successful manipulation of tissue HO activity for clinical purposes, as considered by several laboratories (Dennery 2000; Scapagnini *et al.* 2002; Durante 2003; Otterbein *et al.* 2003; Abraham and Kappas 2008; Syapin 2008) including our own (Schipper 2004a,b;

Received March 9, 2009; revised manuscript received April 29, 2009; accepted April 30, 2009.

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Abbreviations used: AAT, α1-antitrypsin; AD, Alzheimer disease; apoE, apolipoprotein E; APP, amyloid precursor protein; CA, corpora amylacea; CO, carbon monoxide; CSH, cysteamine; DA, dopamine; DAB, diaminobenzidine; ER, endoplasmic reticulum; GFAP, glial fibrillary acidic protein; *Hmox1/HMOX1,* heme oxygenase-1 gene; HO, heme oxygenase; HSP, heat-shock protein; IL-1β, interleukin-1β; LXR, liver X activated receptor; MCI, mild cognitive impairment; Mrp1, multidrug resistance-associated protein 1; MS, multiple sclerosis; NFT, neurofibrillary tangle; NO, nitric oxide; OS, oxidative stress; PD, Parkinson disease; ROS, reactive oxygen species; SnMP, tin mesoporphyrin; TNFα, tumor necrosis factor-α; UPS, ubiquitin-proteosome system.

Schipper *et al.* 1995), pre-supposes a thorough understanding of the multiple and often disparate activities subserved by these enzymes under specific pathological conditions. This review focuses primarily on the roles of HO-1 in brain aging and the common human neurodegenerative disorders, Alzheimer disease (AD) and Parkinson disease (PD). Participation of HO-1 in other degenerative and neuroinflammatory conditions is also briefly discussed, with emphasis on pathophysiological processes shared with the more common aging-related neurodegenerations.

Heme oxygenases

Regulation and physiology

In humans and other species, degradation of cellular heme is catalyzed by the HO family of enzymes (E.C. 1:14:99:3; heme-hydrogen donor:oxygen oxidoreductase). HOs are located within the endoplasmic reticulum (ER) where they act, in association with NADPH cytochrome P450 reductase, to oxidize heme to biliverdin, free ferrous iron and carbon monoxide (CO) (Fig. 1). Biliverdin reductase further catabolizes biliverdin to the bile pigment, bilirubin (Ryter and Tyrrell 2000). Mammalian cells express at least two isoforms of HO, HO-1 [a.k.a. heat-shock protein (HSP) 32] and HO-2. A third protein, HO-3 was determined to be a retrotransposition of the HO-2 gene (pseudogene) unique to rats (Scapagnini *et al.* 2002). HO-1 and HO-2 exhibit identical substrate and cofactor specificities. Yet, the isoforms are encoded by distinct genes, share only 43% amino acid sequence homology in humans, and exhibit significant differences with regard to molecular weight, electrophoretic mobility, tissue distribution, regulation, and antigenicity (Dennery 2000; Loboda *et al.* 2008). HO-1 contains a destabilizing proline-glutamic acid-serine-threonine sequence at the carboxy terminus that renders the peptide sensitive to rapid proteolysis (Dwyer *et al.* 1992), a characteristic not exhibited by HO-2. The half-lives of HO-1 mRNA and protein have been estimated to be 3 and 15–21 h, respectively (Dennery 2000).

In humans, the *ho-1* gene (*HMOX1*) is located on chromosome 22q12 and contains four introns and five exons. A 500-bp promoter, a proximal enhancer, and two or more distal enhancers occur in the regulatory region of the mammalian *Hmox1* gene. The latter exhibits activator protein 1, activator protein 2, nuclear factor kappa B, and hypoxia-inducible factor 1 binding sites, as well as heat shock consensus sequences, metal response elements, cadmium response elements, and stress response elements. These response elements render *Hmox1* exquisitely sensitive to induction by a wide array of pro-oxidant and inflammatory stimuli including heme, β-amyloid, dopamine (DA), H_2O_2,

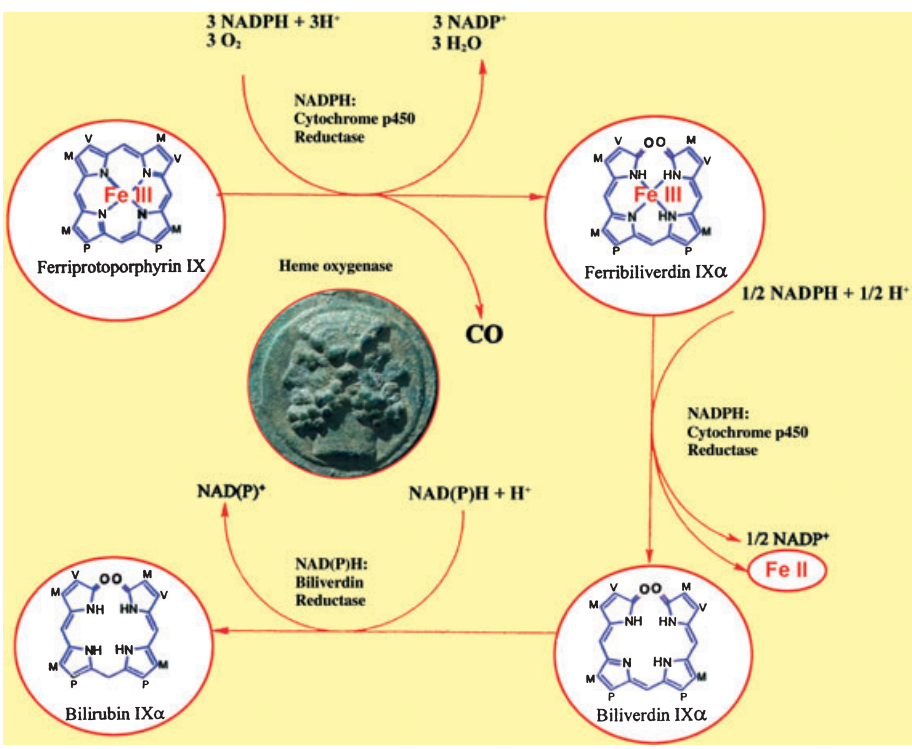

Fig. 1 The heme catabolic pathway. The heme degradation products, ferrous iron (Fe II), carbon monoxide (CO), and biliverdin/bilirubin may behave as either pro-oxidants or antioxidants accounting for the disparate influences of heme oxygenase expression on cell function and survival (symbolized by Janus faces). M = methyl; V = vinyl; P = propionate [modified from Ryter and Tyrrell (2000) with permission].

hyperoxia, UV light, heavy metals, prostaglandins, NO, peroxynitrite, Th1 cytokines, oxidized lipid products, and lipopolysaccharide, as well as certain growth factors (Dennery 2000; Schipper 2000; Kinobe *et al.* 2006; Loboda *et al.* 2008). Curiously, under hypoxic conditions, HO-1 is induced in rodent, bovine, simian, and some human (D407 retinal pigment epithelium; dermal fibroblasts; and keratinocytes) cells, whereas in other human cells, *HMOX1* is repressed (astrocytes; umbilical vein and coronary artery endothelial cells) or unaffected (ARPE19 retinal pigment epithelial cells; chorionic villus epithelium) (Loboda *et al.* 2008). In certain tissues (e.g. rat liver, lung), HO-1 expression appears to be developmentally-regulated at both transcriptional and post-transcriptional levels (Dennery 2000). Numerous transcription factors participate in the regulation of HO-1 expression, with tendencies for one or more signaling pathways to predominate in a species-specific manner. NF-E2-related factor-2 transcription factor binding to Maf response elements in the *Hmox1* promoter and its repression by the heme-regulated protein, basic leucine zipper transcription factor 1 have emerged as a key control mechanism for HO-1 induction and homeostasis in stressed brain and other organs (Ogawa 2002; Sun *et al.* 2002; Kitamuro *et al.* 2003; Suzuki *et al.* 2003). Finally, a 56-bp sequence (Signal Transducer and Activator of Transcription binding site) within the *Hmox1* promoter confers susceptibility to transcriptional suppression by glucocorticoids (Lavrovsky *et al.* 1996).

Polymorphisms in the lengths of GT repeats (11–40) within the *HMOX1* promoter appear to be an important determinant of HO-1 expression and function in humans. Long GT sequences code for relatively unstable (Z-conformational) DNA with attenuated transcriptional activity and diminished baseline and stimulated HO-1 protein expression profiles. Robust HO-1 activity associated with the short-GT polymorphisms appears protective against atherosclerosis-linked conditions (e.g. coronary artery disease) whereas the malignant behavior of various neoplasms was fairly consistently enhanced (Exner *et al.* 2004; Jozkowicz *et al.* 2007; Loboda *et al.* 2008). For further information regarding the molecular biology and regulation of *Hmox1*, several recent reviews are recommended (Jozkowicz *et al.* 2007; Loboda *et al.* 2008; Syapin 2008).

All mammalian cells metabolize heme. OS may transiently augment the intracellular 'free heme pool' by stimulating the conformational modification and breakdown of hemoproteins such as cytochromes, myoglobin, peroxidises and respiratory burst enzymes (Ryter and Tyrrell 2000; Loboda *et al.* 2008). Some cellular heme is also reversibly bound to proteins such as neuronal PAS domain protein 2, the DiGeorge critical region-8 protein, basic leucine zipper transcription factor 1, and proteins of the N-end rule pathway (Syapin 2008). In stressed cells, the up-regulation of HO-1 may confer protection by accelerating the degradation of pro-oxidant heme to the radical-scavenging bile pigments, biliverdin and bilirubin (Stocker *et al.* 1987; Nakagami *et al.* 1993; Llesuy and Tomaro 1994; Dore *et al.* 1999; Baranano and Snyder 2001). Co-stimulation of apoferritin synthesis, a major iron sequestration pathway, may obviate potential toxicity related to the intracellular liberation of heme-derived ferrous iron (Dennery 2000; Ryter and Tyrrell 2000). However, in some cases, heme-derived iron and CO may *exacerbate* intracellular OS and substrate damage by promoting the generation of reactive oxygen species (ROS) within the mitochondrial and other subcellular compartments (Zhang and Piantadosi 1992; Frankel *et al.* 2000; Ryter and Tyrrell 2000; Desmard *et al.* 2007). The intensity and temporal pattern of HO-1 expression and the chemistry of the local redox microenvironment may determine whether free radical damage accruing from intracellular liberation of iron/CO or the antioxidant benefits of a diminished heme : bilirubin ratio predominate (Galbraith 1999; Suttner and Dennery 1999).

Basal HO expression in mammalian CNS

Heme oxygenase-1 expression in the normal, unstressed brain is minimal and confined to scattered neuroglia and sparse populations of neurons in the cerebellum (Purkinje cells), thalamus, hypothalamus, brain stem, hippocampal dentate gyrus, and cerebral cortex (Vincent *et al.* 1994; Matz *et al.* 1996; Bergeron *et al.* 1998; Nakaso *et al.* 2000; Baranano and Snyder 2001). In contrast, HO-2 mRNA and protein are widely distributed and strongly expressed in neurons of the mammalian brain and spinal cord, with highest concentrations in hippocampal pyramidal cells and dentate gyrus, olfactory epithelium and olfactory bulb, and cerebellar granule and Purkinje cell layers (Trakshel *et al.* 1988; Verma *et al.* 1993; Dwyer *et al.* 1995b). The distribution of HO-2 expression in rat brain exhibits considerable overlap with the topography of soluble guanylate cyclase. Heme-derived CO, like NO, may modulate neuronal activity by stimulating guanylate cyclase and raising intracellular levels of the second messenger, cyclic guanosine monophosphate (Verma *et al.* 1993).

Neuroprotective roles of HO-1

There exists ample evidence implicating a neuroprotective role for HO-1 both in intact animals as well as in tissue culture. Following exposure to a variety of noxious stimuli, HO-1 induction occurs in neuronal and non-neuronal brain cells, although it has been argued that the greater capacity of astrocytes than neurons to mount a robust HO-1 (and other HSP) response may partly account for the relative preservation of the former in the face of oxidative challenge (Dwyer *et al.* 1995a; Manganaro *et al.* 1995; Snyder *et al.* 1998). Cerebellar granule cells harvested from transgenic mice designed to over-express HO-1 in neurons (Maines *et al.* 1998) appear to be relatively resistant to glutamate- and H_2O_2-mediated oxidative damage *in vitro* (Chen *et al.* 2000). Similarly, neuroblastoma cell lines transfected with HO-1

cDNA were less prone than control cells to oxidative damage resulting from exposure to H_2O_2 (Le *et al.* 1999; Takeda *et al.* 2000) or β-amyloid$_{1–40}$ (Le *et al.* 1999). Over-expression of HO-1 was also recently reported to increase brain-derived neurotrophic factor and glial cell-derived neurotrophic factor expression and protect neurons from MPP^+ toxicity (Hung *et al.* 2008). Astrocytes harvested from HO-1 knockout mice exhibit enhanced vulnerability to hemin toxicity relative to wild type cells (Chen-Roetling *et al.* 2005), although HO-1 may be inimical to astroglia under other circumstances (see below). In an *in vivo* study, HO-1 transgenic mice subjected to cerebral ischemia manifested decreased tissue staining for lipid peroxidation end-products, enhanced expression of the anti-apoptotic factor, B-cell CLL/lymphoma 2 and smaller infarct volumes relative to normal littermates (Panahian *et al.* 1999). HO-1 may also confer neuroprotection in animal models of traumatic (Fukuda *et al.* 1996; Beschorner *et al.* 2000) and excitotoxic (Huang *et al.* 2005; Ahmad *et al.* 2006) brain damage and spinal cord injury (Lin *et al.* 2007b). As described above, rapid heme/hemoprotein degradation and the intracellular accumulation of antioxidant bile pigments may be responsible, at least in part, for the observed neuroprotection associated with the induction of HO-1. CO released in the course of heme catabolism may also mediate some of the cytoprotective benefits of HO-1. For example, by engendering smooth muscle relaxation (Verma *et al.* 1993), heme-derived CO has been postulated to ameliorate cerebral vasospasm, a cause of significant morbidity in patients with subarachnoid hemorrhage (Matz *et al.* 1996; Suzuki *et al.* 1999; Tanaka *et al.* 2000).

Neurodystrophic effects of HO-1

While HO-1 induction may provide neuroprotection under certain conditions (see above), the action of this enzyme in other models of CNS injury and disease has proven detrimental. It has long been known that excessive hyperbilirubinemia of untreated neonatal jaundice may engender irreversible neurological injury (kernicterus). The latter can be prevented in these children by administration of synthetic metalloporphyrins, competitive inhibitors of HO activity, or photodegradation of circulating bilirubin (Qato and Maines 1985). Moreover, metalloporphyrin suppression of HO activity has been shown to diminish tissue necrosis and edema formation following focal cerebral ischemia in intact rats (Kadoya *et al.* 1995), confer neuroprotection in an experimental model of intracerebral hemorrhage (Koeppen and Dickson 1999; Wang and Dore 2007) and alleviate traumatic cornu ammonis 1 insults in rat hippocampal slices (Panizzon *et al.* 1996). Suppression of hippocampal HO-1 expression has also been posited as a mechanism by which nimodipine treatment ameliorates aluminum neurotoxicity in mice (Yuan *et al.* 2008). Of possible relevance to PD, HO-1 also facilitates dopaminergic cell injury following exposure to polychlorinated biphenyls (Lee *et al.* 2006). Germane to several human neurodegenerative disorders, and as discussed at length below, HO-1 over-expression in the astroglial compartment may transduce a host of noxious stimuli into altered patterns of cellular iron mobilization and attendant mitochondrial damage (Schipper 2004b). Differences in species, experimental models and therapeutic protocols may account for the disparate data regarding the roles of HO-1 induction in brain aging and disease.

Brain aging

CNS senescence has been implicated as the single most important risk factor for a host of human neurodegenerative conditions, including sporadic (non-familial) AD, idiopathic PD, and amyotrophic lateral sclerosis (Calne 1994). In accord with the prevailing ‘Free Radical-Mitochondrial’ theory of aging (Finkel and Holbrook 2000; Sohal *et al.* 2002; Hekimi and Guarente 2003), OS, altered patterns of iron mobilization and mitochondrial insufficiency have been recognized as fairly constant features of senescent and degenerating neural tissues. Based on extensive evidence adduced from neuropathological surveys, animal studies and cell culture experiments, we have argued that these ubiquitous pathological changes are causally interrelated phenomena that forge a vital link between normal brain aging and neurodegeneration (Schipper 2004a). Moreover, as discussed below, the consolidation of this pathological ‘triad’ may depend, at least in part, on the antecedent up-regulation of HO-1 by the astrocytic compartment.

HO-1 expression in normal aging brain

Heme oxygenase-1 expression in human brain has not been rigorously mapped or quantified. However, a Japanese study showed that numbers of neurons and neuroglia immunoreactive for HO-1 in the normal human cerebral cortex and hippocampus (procured from 31 autopsied brains) increased progressively between 3 and 84 years of age (Hirose *et al.* 2003). In 1995, we surveyed HO-1 immunoreactivity in post-mortem brain specimens derived from five AD (discussed below) and five neurohistologically normal human subjects aged 79.4 ± 3.4 and 79.8 ± 3.0 years, respectively (Schipper *et al.* 1995). In the non-demented subjects, neurons in the temporal cortex and hippocampus generally exhibited faint or no detectable HO-1 immunoreactivity. When present, HO-1 staining appeared as diffuse precipitates largely confined to the perikarya and apical dendrites. Stronger HO-1 immunostaining was observed in rare neurofibrillary tangles (NFT) encountered in the control specimens. The percentages of glial fibrillary acidic protein (GFAP)-positive astrocytes co-expressing immunoreactive HO-1 protein were approximately 6.8% in the normal hippocampus and 12.8% in the substantia nigra. In normal senescent human brain, robust HO-1 staining was noted in the cytoplasm of ependymocytes

and choroid plexus epithelial cells, in cerebrovascular endothelial cells, and within many glial and extracellular corpora amylacea (CA) (Schipper *et al.* 1995; Anthony *et al.* 2003). In a subsequent study (Schipper *et al.* 1998a), moderate HO-1 immunoreactivity was consistently observed in the cytoplasm of neuromelanin-containing (dopaminergic) neurons of the normal subtantia nigra pars compacta ($n = 7$; mean age = 69.6 ± 9.6 years). In contrast, HO-1 staining of other neurons within this brain region was faint or undetectable. The latter findings suggest that, during the course of normal aging, dopaminergic neurons in the human substantia nigra are subjected to increased levels of OS (and possibly additional noxious influences) relative to other neuronal populations. This formulation is consistent with the high levels of ROS generated in these cells by the enzymatic deamination and iron-mediated oxidation of DA (Stokes *et al.* 1999), and reports that aging-associated loss of nigral dopaminergic neurons may occur at an accelerated rate in comparison with other brain cell populations (Mann 1994). Other work revealed substantial HO-1 immunoreactivity in olfactory neuroepithelium of normal elderly human subjects (Perry *et al.* 2003). HO-1 expression also increases markedly in optic nerve astrocytes and in retinal ganglion cells and photoreceptors of the human eye between the second and seventh decade of life (Mydlarski *et al.* 2003). As HO-1 transcription and translation are widely accepted as sensitive and reliable reporters of tissue OS, these HO-1 expression patterns may denote regions of the human CNS that are particularly prone to OS in the course of normal aging. Moreover, as described in the following section, stress-related induction of glial HO-1 may de-regulate iron homeostasis and contribute to the decay of mitochondrial function in the senescent human CNS.

Glial senescence

The peroxidase-positive subcortical glial system

In aging rats, humans, and other vertebrates, a sub-population of subcortical astrocytes progressively accumulates cytoplasmic inclusions that exhibit an affinity for Gomori stains, orange-red autofluorescence, and non-enzymatic (pseudo-) peroxidase activity mediated by ferrous iron (Schipper *et al.* 1981, 1998b; Schipper 1996, 1999). In the rat substantia nigra, the iron-laden glial inclusions increase in abundance by a factor of four between 4 and 14 months of age (Schipper *et al.* 1998b). As described below, sustained or repeated induction of the *Hmox1* gene may be necessary and sufficient for the development of this senescent glial phenotype.

The cysteamine model

Exposure of dissociated fetal or neonatal rat brain cell cultures to the sulfhydryl agent, cysteamine (CSH; 2-mercaptoethylamine) induces a marked accumulation of peroxidase-positive astrocytic inclusions that are structurally and histochemically identical to those that naturally accumulate in subcortical astroglia of the intact aging brain (Schipper *et al.* 1990). Elemental iron is readily detected in the inclusions by electron microprobe analysis, and the presence and concentration of the metal correlates closely with the presence and intensity of diaminobenzidine (peroxidase) staining (McLaren *et al.* 1992). Within 24–72 h of CSH exposure, many astroglial mitochondria exhibit progressive swelling, rearrangement or dissolution of their cristae, subcompartmental sequestration of redox-active iron and fusion with lysosomes or cisternae of the ER (Brawer *et al.* 1994a; Chopra *et al.* 1997). In young adult rats, subcutaneous CSH injections (150–300 mg/kg twice weekly for 3 weeks) induce two- to threefold increases in numbers of peroxidase-positive astrocyte granules in the basal ganglia, hippocampus and other brain regions (Schipper *et al.* 1993). As in the case of the CSH-treated cultures, peroxidase-positive glial granules in the intact rat and human brain invariably exhibit mitochondrial epitopes (as well as identical profiles of HSP expression) in immunohistochemical preparations (Brawer *et al.* 1994b; Schipper and Cissé 1995; Schipper *et al.* 1998b). Further studies indicated that intracellular OS may be responsible for the transformation of normal astrocyte mitochondria to peroxidase-positive granules and CA, glycoproteinaceous inclusions characteristic of aging and degenerating neural (and other) tissues (Cavanagh 1999), *in vitro* and in the intact aging brain (Srebro 1971; Manganaro *et al.* 1995; Sahlas *et al.* 2002; Schipper 2004a).

Glial HO-1 expression and mitochondrial iron sequestration

Cysteamine (880 μM), β-amyloid$_{40/42}$ (3–15 μM), DA (0.1–1.0 μM), tumor necrosis factor-α (TNFα) (20 ng/mL) or interleukin-1β (IL-1β) (20 ng/mL) significantly augments the incorporation of ^{59}Fe (or^{55}Fe) into astroglial mitochondria within 3–6 days of treatment without affecting transfer of the metal into whole-cell and lysosomal compartments (Wang *et al.* 1995; Schipper *et al.* 1999; Ham and Schipper 2000; Mehindate *et al.* 2001). These effects were only discernible when inorganic $^{59}FeCl_3$, but not ^{59}Fe-diferric transferrin, served as the metal donor (ibid). Within 3–12 h of exposure to these stimuli, and preceding mitochondrial iron sequestration, augmented HO-1 mRNA, protein and/or activity levels were observed in these cells (Schipper 1999; Mehindate *et al.* 2001). Pharmacological studies revealed that OS is a likely common mechanism mediating glial *Hmox1* induction under these experimental conditions (Mydlarski *et al.* 1993; Schipper *et al.* 1999). Co-administration of tin mesoporphyrin (SnMP) (1 μM), a competitive inhibitor of HO activity, or dexamethasone (50 μg/mL), a transcriptional suppressor of *Hmox1*, significantly attenuated mitochondrial iron sequestration in cultured astrocytes exposed to DA, TNFα, or IL-1β (Schipper *et al.* 1999;

Mehindate *et al.* 2001). Similarly, administration of SnMP or dexamethasone abolished the pathological accumulation of mitochondrial ^{55}Fe observed in rat astroglia engineered to over-express human *HMOX1* by transient transfection (Schipper *et al.* 1999). These findings indicate that up-regulation of HO-1 is a key event in the cascade leading to excessive mitochondrial iron deposition in oxidatively challenged astroglia.

HO-1, intracellular oxidative stress, and the mitochondrial permeability transition pore

Treatment with SnMP or antioxidants (ascorbate, melatonin, or resveratrol) blocks the compensatory induction of the manganese superoxide dismutase (*Mnsod*) gene in astrocytes challenged with DA or transiently transfected with *HMOX1* cDNA (Frankel *et al.* 2000). Furthermore, levels of protein carbonyls (protein oxidation), 8-epi-prostaglandinF2α (lipid peroxidation), 8-hydroxy-2-deoxyguanosine (nucleic acid oxidation) and a synthetic redox reporter molecule were significantly increased in glial mitochondrial fractions after 3–4 days of *HMOX1* transfection relative to sham-transfected controls and HO-1-transfected cells receiving SnMP (Song *et al.* 2006; Vaya *et al.* 2007). These convergent data clearly indicate that HO-1 over-expression in astroglia exacerbates intracellular OS that targets the mitochondrial compartment. In 2004, Gennuso *et al.* (2004) reported that nanomolar concentrations of unconjugated bilirubin up-regulate multidrug resistance-associated protein 1 (Mrp1) expression and translocation of Mrp1 from the Golgi apparatus to the cell membrane in cultured mouse astrocytes. As Mrp1 promotes egress of bilirubin from astrocytes (ibid), resultant increments in cellular (mitochondrial?) ratios of Fe/CO : bilirubin may pre-dispose to OS in astrocytes over-expressing HO-1.

Treatment with cyclosporin A or trifluoperazine, potent inhibitors of the mitochondrial permeability transition pore, curtails mitochondrial iron trapping in hHO-1 transfected glia and cells exposed to DA, TNFα, or IL-1β (Schipper *et al.* 1999; Mehindate *et al.* 2001). Conceivably, intracellular OS accruing from HO-1 activity promotes pore opening (Petronilli *et al.* 1993; Bernardi 1996) and influx of cytosolic iron to the mitochondrial matrix.

HO-1 and glial macroautophagy

Recent ultrastructural studies in combination with dynamic secondary ion mass spectrometry disclosed profound alterations in organellar morphology and the topography of elemental iron in rat astrocytes transiently transfected with *HMOX1* cDNA (Zukor *et al.* 2009). On post-transfection day 3, transmission electron microscopic analysis revealed distended mitochondria with disrupted cristae, cytoplasmic dense inclusions and small multilamellar bodies consistent with membrane damage and possible lysosomal engagement, and spherical concretions bearing ultrastructural resemblance to human CA. The *HMOX1*-associated cytopathology was more elaborate on day 6 post-transfection and featured advanced mitochondrial degeneration, huge multilamellar bodies, multivesicular bodies, pleomorphic osmiophilic inclusions, and CA-like structures. These morphological changes were abrogated in *HMOX1*-transfected cultures exposed to SnMP, indicating that they devolve from HO enzymatic activity rather than some signaling or other non-canonical function recently attributed to the HO-1 protein (Li Volti *et al.* 2004; Lin *et al.* 2007a, 2008). The cytopathological changes represent classical ultrastructural features of macroautophagy and demonstrate that over-expression of the *HMOX1* gene is capable of driving this process in astroglia. Using dynamic secondary ion mass spectrometry, intense foci of elemental iron were observed within *HMOX1*-transfected cells which were not seen in control preparations (Zukor *et al.* 2009). Within the former, there was discrete co-localization of iron with small elliptical, vermiform, and distended organelles identified as normal and pathological mitochondria on the basis of shape, size, number, investing membranes, oxygen-labeling, and peri-nuclear deployment. Many of the large, heterogeneous autophagic vacuoles within the cytoplasm of *HMOX1*-transfected cells displayed prominent iron signals. The latter also occurred within the outer rims of the CA-like inclusions, akin to reports of histochemically detectable iron within human and experimental CA (Cisse and Schipper 1995; Schipper 1998a). *HMOX1* transfection had no significant effects on immunoreactive transferrin receptor, ferritin, ferroportin, or iron response protein 2 levels or iron response protein 1 binding, suggesting that HO-1 activity promotes mitochondrial macroautophagy and deposition of redox-active iron in cultured astroglia independently of classical iron mobilization pathways (Zukor *et al.* 2009). As HO-1 is primarily an ER enzyme, the subcellular iron pathway involved may entail (active or passive) delivery of iron from the ER to the mitochondria via iron-carrier proteins in the cytosol. Of note, ultrastructural studies of the biogenesis of iron-laden astroglial inclusions have revealed occasional close apposition and possibly membrane fusion of ER strands with osmiophilic cytoplasmic granules derived from damaged mitochondria (Brawer and Sonnenschein 1975; Brawer *et al.* 1978, 1994b). Thus, in stressed astroglia, putative organellar fusions or transient 'kiss-and-run' relationships of the ER with mitochondria may facilitate delivery of HO-1 enzyme, and hence heme-derived iron, to the mitochondrial compartment. Another intriguing possibility is that HO-1 enzyme *native* to mitochondria may contribute to the sequestration of heme-derived iron in this organelle. HO-1 protein has been detected in mitochondria of rat pulmonary endothelial cells (Kim *et al.* 2004) and liver cells (Converso *et al.* 2006) where it appears to modulate heme content and metabolism. Whether astroglial mitochondria similarly contain endogenous HO-1 remains unknown.

Alzheimer disease

AD pathology: role of iron deposition, oxidative stress and mitochondrial injury

Alzheimer disease is an aging-associated dementia featuring progressive neuronal degeneration, gliosis, and the accumulation of intracellular inclusions (NFTs) and extracellular deposits of amyloid (senile plaques) in the basal forebrain, hippocampus, and association cortices (Selkoe 1991). OS and mitochondrial deficits have been consistently implicated in the pathogenesis of this disorder (Reichmann and Riederer 1994; Beal 1995; Mattson 2002). OS in the AD brain may be secondary to ROS production by effete mitochondria, the accumulation of β-amyloid (Butterfield 2002), pro-inflammatory cytokines (TNFα, IL-1β) and NO released from activated microglia (McGeer and McGeer 1995), and pathological deposition of redox-active iron in the basal forebrain and association cortices (Schipper 1998b; Sayre *et al.* 2001). In both the AD and PD brain, enhanced expression of tissue ferritin, the major intracellular iron storage protein, parallels the topography of the excess iron and largely implicates non-neuronal (glial, endothelial) cellular compartments (Schipper 1998b). Regional concentrations of transferrin binding sites remain unchanged or vary inversely with the elevated iron stores, suggesting that the transferrin pathway of iron mobilization, important for normal iron delivery to most peripheral tissues, plays a minimal role in the pathological sequestration of this redox-active metal in AD and PD brain (Schipper 1999).

HO-1 expression in AD brain

In AD brain, HO-1 protein co-localizes to neurons, GFAP-positive astrocytes, choroid plexus epithelial cells, ependymocytes, NFTs, senile plaques, CA and some vascular smooth muscle and endothelial cells (Smith *et al.* 1994; Yan *et al.* 1994; Schipper *et al.* 1995). In one study, 86% of GFAP-positive astrocytes residing within the AD hippocampus exhibited HO-1 immunoreactivity, whereas the fraction of hippocampal astroglia expressing HO-1 in age-matched normal tissue was in the range of only 6–7%. Similarly, western blots of protein extracts derived from AD hippocampus and temporal cortex revealed strong HO-1 bands, whereas the latter were faint or undetectable in normal control preparations (Schipper *et al.* 1995). Importantly, significant augmentation of astroglial HO-1 expression was also documented in post-mortem brain specimens procured from individuals with mild cognitive impairment (MCI), a frequent harbinger of incipient AD (Schipper *et al.* 2006). In the latter, glial HO-1 immunoreactivity in temporal cortex correlated with the burden of neurofibrillary pathology. Furthermore, astroglial HO-1 expression in the temporal cortex was associated with decreased scores for global cognition, episodic memory, semantic memory, and working memory; hippocampal astroglial HO-1 expression was associated with lower scores for global cognition, semantic memory, and perceptual speed. The results of the MCI study strongly suggest that glial HO-1 induction is a relatively early event in the pathogenesis of sporadic AD (Schipper *et al.* 2006). OS provoked by the aforementioned stimuli may be responsible for the elaboration of HO-1 in the Alzheimer-diseased and MCI cerebral cortex and hippocampus. On the basis of data adduced previously (vide supra), up-regulation of HO-1 in AD/MCI-affected astroglia may perpetuate intracellular OS and contribute to pathological iron trapping, bioenergetic (mitochondrial) failure and CA formation characteristic of Alzheimer-diseased neural tissues.

Heme oxygenase-1 suppressor factor in AD plasma

In contradistinction to the enhanced HO-1 expression documented in AD brain parenchyma, HO-1 protein or mRNA levels are *suppressed* in AD CSF, choroid plexus epithelial cells, blood mononuclear cells, and plasma compared to control values (Schipper *et al.* 2000, 2001; Anthony *et al.* 2003). Several laboratories (Kimpara *et al.* 1997; Shibata *et al.* 2009) failed to disclose over-representation of specific *HMOX1* promoter polymorphisms in patients with sporadic AD (vs. normal elderly or PD subjects), arguing that primary genetic determinants of HO-1 expression are not responsible for the sub-normal levels of HO-1 protein or mRNA in the blood, CSF and choroid plexus of patients with sporadic AD. The diminished CSF HO-1 concentrations observed in AD may reflect decreased synthesis and release of the protein by the choroid plexus or, alternatively, curtailed delivery (transudation) from the systemic circulation. Using serial affinity chromatography and an astroglial bioassay (HO-1 mRNA response to CSH challenge), we demonstrated the presence of augmented HO-1 mRNA suppressor (HOS) activity in plasma of patients with sporadic AD relative to normal elderly and neurological controls; mean plasma HOS activity of the MCI patients was intermediate between normal elderly control and AD values (Maes *et al.* 2006; Schipper 2007). The HOS factor was determined to be a 50–100 kDa heat-labile, heparin-binding glycoprotein that was unrelated to antioxidant ingestion, plasma total antioxidant capacity, circulating cortisol levels or apolipoprotein E (apoE) ε*4* carrier status. Proteins found to be specifically enriched in AD plasma fractions by 2D gel electrophoresis were identified by matrix-assisted laser desorption time of flight mass spectrometry as isoforms of human α1-antitrypsin (AAT), hemopexin, and transferrin (Yu *et al.* 2003). Of these HOS candidates, only exogenous AAT recapitulated HOS bioactivity in our astroglial assay. Moreover, AAT levels were significantly elevated in AD plasma and correlated with HOS activity and mini-mental state exam scores. The HOS activity of AD plasma was significantly attenuated by AAT immunodepletion. In AD brain, AAT immunoreactivity was augmented relatively to control tissues

and co-distributed with HO-1. In addition, the PI*M3 allele of AAT was reported in a Polish study to occur with increased frequency in patients with sporadic AD relative to the general population (Kowalska *et al.* 1996). We conjectured that the HOS activity of AAT, derived from the circulation or elaborated within the CNS (Gollin *et al.* 1992), may limit HO-1-dependent derangement of cerebral iron homeostasis and account for diminished HO-1 expression in AD choroid plexus and peripheral tissues (Maes *et al.* 2006). Conceivably, net HO-1 mRNA and protein levels remain elevated in AD brain because the HOS activity of the AAT is overwhelmed by the pro-oxidant effects of β-amyloid, Th1 cytokines and other endogenous inducers of the *HMOX1* gene. In the absence of similar provocation outside the affected brain parenchyma, AAT suppresses *HMOX1* gene expression in AD blood and choroid plexus to sub-normal values. The biomarker potential of circulating HO-1/HOS for the diagnosis of MCI and AD is considered elsewhere (Schipper 2007). Finally, AAT may not be the only factor participating in the negative regulation of HO-1 in AD brain: Takahashi and co-workers demonstrated protein–protein interactions between amyloid precursor protein (APP) and both HO-1 and HO-2 that, in cortical neurons, attenuated enzymatic activity by 25–35% (Takahashi *et al.* 2000).

AD, HO-1, and sterol homeostasis

CNS cholesterol metabolism is pivotal for normal neurodevelopmental processes as well as for membrane repair (neuroplasticity) in AD and other neurological disorders (Bogdanovic *et al.* 2001; Refolo *et al.* 2001; Shobab *et al.* 2005). In AD, astrocytes and other neuroglia synthesize cholesterol *de novo* and recycle sterols released from degenerating neurons (Jurevics and Morell 1995; Pfrieger 2003). Pro-oxidant conditions prevailing in AD-affected neural tissues promote the formation of oxysterols and other cholesterol oxidation products. The latter are effective ligands of liver X activated receptor (LXR) nuclear receptors, major regulators of genes subserving cholesterol homeostasis. Oxysterol-mediated LXR activation may impact AD pathogenesis by (i) induction of glial apoE biosynthesis, further enhancing cholesterol re-distribution and removal; (ii) up-regulation of other genes governing key sterol elimination pathways; and (iii) impacting APP processing which may serve to attenuate β-amyloid toxicity (Vaya and Schipper 2007).

Heme oxygenase-1 over-expression in rat astroglia by transient transfection of *HMOX1* cDNA for 3 days (enhancing HO activity three- to fourfold) significantly decreases intracellular cholesterol concentrations and increases the levels of at least four oxysterol species (measured by gas chromatography/mass spectrometry) compared to untreated control cultures and *HMOX1*-transfected cells exposed to SnMP. OS was shown to be augmented in mitochondria and culture media derived from the *HMOX1*-transfected astrocytes, as evidenced by enhanced oxidation of the synthetic reporter molecules, linoleoyl tyrosine, linoleoyl tyrosine cholesterol ester, and linoleoyl tyrosine deoxyguanosyl ester (Vaya *et al.* 2007). Furthermore, *HMOX1* transfection in these cells promoted *de novo* cholesterol biosynthesis and stimulated cholesterol efflux, effects abrogated by SnMP. CO released from the synthetic CO donor molecule, CO releasing molecule-3 mimicked the effects of *HMOX1* transfection on glial cholestrerol biosynthesis; and the combination of exogenous CO and iron stimulated cholesterol efflux. Treatment with pharmacological LXR antagonists implicated LXR activation in the modulation of cholesterol homeostasis by these heme degradation products. In these cells, cholesterol efflux exceeded biosynthesis such that net glial cholesterol content was diminished (supplying further stimulus for *de novo* cholesterol biosynthesis) (Hascalovici *et al.* 2009). Thus, in diseased neural tisssues, glial HO-1 induction may transduce ambient OS and other noxious stimuli into altered patterns of cholesterol homeostasis in a manner favoring enhanced production and delivery of the sterol for neuronal membrane repair.

In a related investigation, HO-1 protein levels and various sterols were measured (by enzyme-linked immunosorbent assay and gas chromatography/mass spectrometry, respectively) in post-mortem human frontal cortex derived from subjects with sporadic AD, MCI and no cognitive impairment enrolled in the Religious Orders Study (Hascalovici *et al.* 2008). In the AD and some MCI (but not in cognitively normal) specimens, HO-1 levels correlated significantly with decreased cholesterol levels, increased cholesterol precursor levels and increased oxysterol concentrations. In addition, total cholesterol content, cholesterol precursors and specific oxysterols correlated with disease state, increasing neuropathological burden, neuropsychological deficits, age, apoE ε*4* genotype and gender. These observations, in conjunction with our *in vitro* data (vide supra), suggested a model for altered sterol homeostasis in AD brain (Hascalovici *et al.* 2008): (i) in the *normal aging brain*, sterol homeostasis is maintained by pathways governing baseline cholesterol biosynthesis, cholesterol efflux and oxysterol formation; glial HO-1 expression and its influence on sterol metabolic pathways are minimal. In *MCI* and *early* (compensated) *AD*, enhanced HO-1 expression stimulates cholesterol biosynthesis, oxysterol formation and cholesterol efflux by the astroglial compartment. Glial cholesterol efflux (to sites of neuronal repair and for egress across the blood–brain barrier) exceeds biosynthesis and total cholesterol levels in affected brain are normal or diminished. In *advanced* (de-compensated) *AD,* a massive increase in the free cholesterol pool (derived from widespread neuronal degeneration) saturates sterol efflux mechanisms resulting in increased brain cholesterol levels which, in turn, exacerbate amyloid deposition and neurodegeneration in this disease (Vaya and Schipper

2007). HO-1 may also interact with the presence of the apoE ε*4* allele, a well-established and robust genetic risk factor of sporadic AD (Saunders *et al.* 1993; Strittmatter *et al.* 1993), as Jofre-Monseny *et al.* (2007) reported greater basal and lipopolysaccharide-stimulated HO-1 expression in apoE ε4-transfected murine macrophages in comparison with apoE ε3-transfected cells.

HO-1 and tau degradation

The ubiquitin-proteosome system (UPS) mediates turnover of normal, mis-folded and chemically modified (e.g. oxidized) intracellular proteins. Tau protein is degraded by the UPS *in vitro* and *in vivo*, and ubquitinated tau is present in NFTs. Proteosomal activity is reduced in AD brain and β-amyloid peptide inhibits the UPS in cultured cells (Ciechanover and Brundin 2003; Shastry 2003). Expression of *HMOX1* by transient transfection triggers proteosomal tau degradation in M17 human neuroblastoma cells, an effect that can be blocked with SnMP or the proteosomal inhibitor, lactacystin (Song *et al.* 2009). Up-regulation of HO-1 in AD brain (Smith *et al.* 1994; Premkumar *et al.* 1995; Schipper *et al.* 1995) may therefore serve to stimulate a failing UPS in an attempt to limit the accumulation of toxic tau aggregates. Interestingly, HO-1 may keep tau levels in check by more than one mechanism as Smith and co-workers (Takeda *et al.* 2000) reported *transcriptional* suppression of tau in neuroblastoma cells transfected with human HO-1 cDNA. Finally, the impact of HO-1 may not be restricted to native tau. The P301L tau mutation, found in kindreds with heritable frontotemporal dementia-parkinsonism linked to chromosome 17, promotes tau aggregation *in vitro* and in transgenic mouse models of the disease (Lewis *et al.* 2000; Rizzu *et al.* 2000; Vogelsberg-Ragaglia *et al.* 2000). HO-1 over-expression in M17 cells elicits proteosomal catabolism of P301L tau protein to the extent observed with native tau (Song *et al.* 2009), countering the assumption that the P301L mutation fosters tau pathology by rendering the protein less susceptible to degradation (Lee *et al.* 2001). A very different situation prevails concerning HO-1-stimulated degradation of native *vs.* mutant α-synuclein, as discussed below.

Parkinson disease

Neuropathology of PD

Idiopathic PD is a late-onset movement disorder of uncertain etiology characterized pathologically by progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, formation of intraneuronal fibrillar inclusions (Lewy bodies) in this cell population, and variable depletion of noradrenaline and serotonin in other brain stem nuclei. As in the case of AD, there is abundant evidence of oxidative tissue damage, bioenergy deficits and transferrin-independant iron sequestration in PD-affected brain regions (Schipper 1998b, 1999).

HO-1 expression in PD brain

Moderate HO-1 immunoreactivity is observed in dopaminergic neurons of both the normal and Parkinson-diseased substantia nigra (Schipper *et al.* 1998a). In PD specimens, cytoplasmic Lewy bodies within affected dopaminergic neurons are prominently decorated with immunoreactive HO-1 (Castellani *et al.* 1996; Schipper *et al.* 1998a). The proportion of GFAP-positive astroglia that express HO-1 in the PD nigra was found to be significantly increased (77.1%) relative to age-matched control specimens (18.7%), whereas percentages of GFAP-positive astroglia co-expressing HO-1 in other subcortical nuclei, such as the caudate, putamen and globus pallidus, were relatively low in both groups (Schipper *et al.* 1998a). DA-derived pro-oxidant intermediates, MPTP-like neurotoxins generated endogenously or stemming from the environment, and microglia-derived cytokines and NO are plausible inducers of astroglial *HMOX1* gene expression in the PD nigra (Rieder *et al.* 2004; Schipper 2004a). On the basis of the aforecited *in vitro* data, the enhanced glial HO-1 activity may promote the transferrin receptor-independent accumulation of iron and mitochondrial electron transport (complex I) deficits consistently reported in the basal ganglia of PD subjects (Beal 1996; Schipper 2001).

Pro-toxin bioactivation and PD

Iron sequestered in astroglial mitochondria as a result of HO-1 activity behaves as a pseudo-peroxidase that promotes the oxidation of catechol-containing compounds (DA, 2-hydroxyestradiol) to potentially neurotoxic ortho-semiquinone radicals (Schipper *et al.* 1991; Schipper 2001). The redox-active glial iron also mediates bioactivation of the pro-toxin, MPTP, to the dopaminergic neurotoxin, MPP^+, in the face of monoamine oxidase blockade (Di Monte *et al.* 1995). Neuron-like PC12 cells grown on a substratum of astrocytes replete with mitochondrial iron induced by CSH pre-treatment (Frankel and Schipper 1999) or *HMOX1* transfection (Song *et al.* 2007) were noted to be far more susceptible to DA/H_2O_2-related killing than PC12 cells co-cultured with control, 'iron-poor' astroglia. Oxidative PC12 cytotoxicity was significantly attenuated by the administration of SnMP, iron chelators (deferroxamine, phenanthroline) or natural antioxidants (ascorbate, trans-resveratrol, and melatonin) attesting to the canonical role of HO-1 and iron-mediated redox reactions, respectively, in this paradigm (Frankel and Schipper 1999; Song *et al.* 2007). Incubation of PC12 cells in astrocyte-conditioned media resulted in similar patterns of PC12 cell death observed in the co-culture experiments except on a smaller scale (Song *et al.* 2007). Thus, in addition to direct neuronal–glial interactions, *HMOX1*-transfected astroglia exposed to DA/H_2O_2 may release neurotoxins (e.g. CO, Fe, and quinones) to the extracellular space

and/or deprive nearby PC12 cells of glia-derived trophic substances (e.g. glial-derived neurotrophic factor). The glial mitochondriopathy may also pre-dispose to neural injury by diminishing ATP production and thereby compromising important energy-dependent processes such as uptake of excitotoxic neurotransmitters (glutamate) from the synaptic cleft and *de novo* biosynthesis of glutathione destined for neuronal delivery (Aschner 2000).

In contrast to the vulnerability of the PC12 cells, DA/H_2O_2 injury to either the CSH pre-treated, *HMOX1*-transfected or control astroglia themselves was negligible. The capacity of stressed astroglia to up-regulate robust antioxidant defenses, elaborate cytoprotective HSPs, and successfully convert to anaerobic metabolism may account for the resiliency of these cells in the face of oxidative challenge (Schipper 2004a). The data summarized herein suggest that progressive, HO-1-mediated trapping of glial mitochondrial iron within subcortical brain regions may be an important mechanism predisposing the senescent nervous system to PD and other free radical-related neurodegenerative disorders (Schipper 2001, 2004a). This conjecture is consistent with reports of enhanced neurotoxicity and parkinsonism in old animals exposed to DA (Cantuti-Castelvetri *et al.* 2003), MPTP (Jarvis and Wagner 1990; Ali *et al.* 1993), amphetamines (Vasilev *et al.* 2003) and manganese (Desole *et al.* 1995) compared to respective lesions incurred in younger counterparts.

HO-1 and α-synuclein degradation

Dysfunction of the UPS contributes to the formation of toxic protein aggregates and cellular inclusion bodies in PD and other aging-related human neurodegenerations (Rubinsztein 2006). In particular, aberrant parkin activity and other identified UPS deficiencies may foster protofibrillization and aggregation of α-synuclein, a major component of the hallmark Lewy body (Ciechanover and Brundin 2003; McNaught and Olanow 2003; McNaught *et al.* 2003). Transfection of *HMOX1* in M17 cells promotes intracellular degradation of endogenous and co-transfected wild-type α-synuclein which is abrogated by treatment with the proteasomal inhibitor, lactacystin (Song *et al.* 2009). Blockade of HO-1-related α-synuclein degradation by SnMP, methylene blue or deferroxamine (but not bilirubin) provided evidence that heme-derived iron and CO are responsible for proteasomal activation and attendant α-synuclein degradation in these cells. These data also indicate that the catabolism of α-synuclein is dependant on HO enzymatic activity rather than direct HO-1/α-synuclein protein-protein interactions or some other HO-1 function. These findings suggest that up-regulation of HO-1 in the PD nigra (Schipper *et al.* 1998a) may represent an adaptive response to enhance the proteasomal degradation, and thereby pre-empt the toxic aggregation, of α-synuclein. In contrast to wild-type α-synuclein, the A30P mutant α-synuclein, implicated in some families with autosomal-dominant PD (Polymeropoulos *et al.* 1997; Kruger *et al.* 1998), exhibited marked resistance to proteosomal degradation when co-expressed with *HMOX1* in M17 cells (Song *et al.* 2009). As non-degradable, aggregated forms of α-synuclein are cytotoxic (Volles and Lansbury 2002), the resistance of the A30P protein to HO-1-mediated proteasomal catabolism may be a factor promoting Lewy body formation and parkinsonism in individuals bearing this mutation.

Other neurological disorders

In addition to AD and PD, HO-1 has been implicated in the pathobiology of numerous other degenerative and non-degenerative CNS conditions. Among the neurodegenerative disorders, the focus of this review, immunoreactive HO-1 protein localizes to diseased motor neurons in *amyotrophic lateral sclerosis* (Calingasan *et al.* 2005), Pick bodies in subjects with *frontotemporal dementia*, NFT in cases of *progressive supranuclear palsy*, and ballooned neurons in *corticobasal degeneration* (Castellani *et al.* 1995). On the basis of evidence discussed herein, it is conceivable that induction of HO-1 may contribute to the pathological brain iron deposition, oxidative substrate damage and mitochondrial lesions that have been documented in these late-onset human neurodegenerations (Reichmann and Riederer 1994; Chang *et al.* 1995; Connor 1997; Delisle *et al.* 2000). The exquisite inducibility of the *Hmox1* gene and the highly pleotropic bioactivities of all three heme degradation products have also suggested important roles for HO-1 in the pathogenesis of various neuroinflammatory [*multiple sclerosis* (MS), *falciparum malaria*], cerebrovascular (*ischemic* and *hemorrhagic stroke*), traumatic (*cerebral contusions*) and neuro-oncological (*malignant glioma*) disorders. These conditions are not primarily neurodegenerative in nature and interested readers are referred to a previous publication addressing these topics (Schipper 2004c). However, a brief consideration of MS, an autoimmune demyelinating disorder of central white matter, is germane to the current discussion given recent recognition of the significant post-inflammatory, 'degenerative' phases of the illness (Trapp and Nave 2008). In a neuropathological survey (Mehindate *et al.* 2001), the fraction of GFAP-positive astrocytes expressing HO-1 (57.3%) in spinal cord plaques derived from patients with MS was noted to be substantially higher than that computed in the spinal white matter of normal subjects (15.4%). Glial *HMOX1* induction in MS may be secondary to the enhanced release of IL-1β, TNFα (Mehindate *et al.* 2001) or myelin basic protein (Businaro *et al.* 2002) within the diseased tissues. In primary astrocyte cultures, TNFα (20 ng/mL) or IL-1β (20 ng/mL) stimulated the accumulation of non-transferrin ^{55}Fe by astroglial mitochondria, effects that were abrogated by co-administration of HO inhibitors, mitochondrial pore anatagonists or antioxidants (Mehindate *et al.*

2001). Thus, up-regulation of HO-1 in astrocytes may give rise to the aberrant iron deposits and electron transport chain defects reported in the vicinity of MS plaques (Lu *et al.* 2000; Mehindate *et al.* 2001; Levine and Chakrabarty 2004) and in experimental autoimmune encephalomyelitis, a rodent model of the disease (Levine and Chakrabarty 2004). Stahnke *et al.* (2007) reported increased levels of HO-1 protein in oligodendrocytes (myelin-producing cells) in early MS lesions and in astrocytes, microglia, and macrophages in acute disseminated encephalomyelitis. These investigators also provided evidence that acute HO-1 expression in cultured OLN-93 oligodendroglia confers cytoprotection in the face of H_2O_2 stress, whereas longer-term induction of the enzyme in these cells exacerbates oxidative injury to mitochondria and the microtubular network.

Conclusions

Delineation of the diverse roles of HO-1 in brain senescence, aging-related human neurodegenerative disorders and other CNS conditions (summarized in Fig. 2) has progressed substantially since we last reviewed this topic in 2004 (Schipper 2004a,b,c). Whereas the acute induction of this enzyme in neural and other tissues is predominantly cytoprotective in nature, protracted or repeated up-regulation of the *Hmox1* gene in astrocytes, oligodendroglia and possibly neurons may perpetuate cellular dysfunction and demise in many chronic degenerative and neuroinflammatory conditions long after provocative stimuli have dissipated. In the case of normal brain aging, AD, PD, and other late-life neurodegenerations, there is converging evidence that the associated tissue iron sequestration, intracellular OS and mitochondrial insufficiency may constitute a single cytopathological 'lesion' that forms downstream from the sustained action of HO-1 within the astrocytic compartment. The fact that induction of the *Hmox1* gene is both a cause and consequence of intra-glial OS in our models led to the prediction that iron homeostasis will invariably be disturbed in aging and diseased CNS tissues harboring mitochondrial lesions (a key source of endogenous ROS); neural tissues exhibiting primary or secondary iron overload will exhibit obligatory bioenergetic failure; and at least some portion of the sequestered metal will be confined to the mitochondrial matrix (Schipper 2004a). The fairly ubiquitous co-registration of these pathological features over a wide range of human and experimental neurological afflictions is consistent with this formulation (Schipper 2004a,b).

The aforementioned, potentially dystrophic effects of HO-1 in aging and degenerating neural tissues may inform the development of neuroprotective and other disease-modifying strategies. To the extent that pathological brain iron mobilization in these conditions is HO-1-dependant, attempts to minimize iron-related neurotoxicity by targeting components of classical iron-regulatory pathways (transferrin receptor, iron response protein 1/2, and ferritin) may prove futile. On the other hand, an *enzymatic* (HO-1) basis for oxidative mitochondrial injury in degenerating or inflamed neural tissues may be considerably more amenable to pharmacological management than free radical damage accruing from unregulated chemical processes (Droge 2002). Several metalloporphyrin inhibitors of HO activity have been employed in the clinic for the control of neonatal hyperbilirubinemia (jaundice) and certain adult liver conditions (Kappas *et al.* 1993; Drummond and Kappas 2004) and could potentially be adapted for neurotherapeutic use. Along similar lines, we are currently investigating a novel class of CNS-permeable, non-peptidic small molecule inhibitors which are highly selective for the HO-1 isoenzyme. As previously demonstrated with SnMP (above), a lead compound attenuated glial HO-1 activity and mitochondrial protein carbonyl formation resulting from *HMOX1* transfection in a dose-dependent manner (Schipper *et al.* 2009). The data reviewed in this article support our earlier contention (Schipper 2004a,b) that targeted suppression of glial HO-1 expression or activity may ameliorate iron-mediated toxicity and bioenergy deficits in the brains of subjects with AD, PD, and other human neurodegenerative disorders. That HO-1 and other proteins may acquire nefarious functions in the senescent mammalian CNS should not be surprising as there is little evolutionary pressure in post-reproductive life to thwart such eventualities, a possible example of *antagonistic pleiotropy* (Williams 1957; Kirkwood and Rose 1991).

Several important caveats and further pre-clinical research demand consideration before advocating central HO-1 inhibition as a potential therapeutic modality for these neurodegenerative conditions. As described above, HO-1 exhibits Janus-faced behavior as a determinant of neuronal survival in various disease models. On theoretical grounds, there is concern that suppression of its activity in the AD or PD brain may render the latter increasingly vulnerable to the impact of intercurrent ischemic stroke or other acute injury. In the past, we viewed such risk as potentially acceptable were the intervention to mitigate substantially the chronic neurodegenerative process and inexorable clinical decline. However, the situation has since grown more complex given recent evidence suggesting that, *within a given neurodegenerative disorder*, the up-regulation of HO-1 may ameliorate certain aspects of of the disease process while concomitantly aggravating others. Thus, the effects of HO-1 on brain sterol/oxysterol homeostasis and on the proteosomal degradation of α-synuclein and tau may represent adaptive responses in AD and PD, while the accruing iron deposition and mitochondrial damage feed into the neurodegenerative cascade (Fig. 2). As such, the inhibitory effects of AAT on HO-1 transcription (Maes *et al.* 2006) and APP/HO-1 interactions on HO activity (Takahashi *et al.* 2000) may prove detrimental in AD when implicating affected neurons, but beneficial when operating within the astrocytic compart-

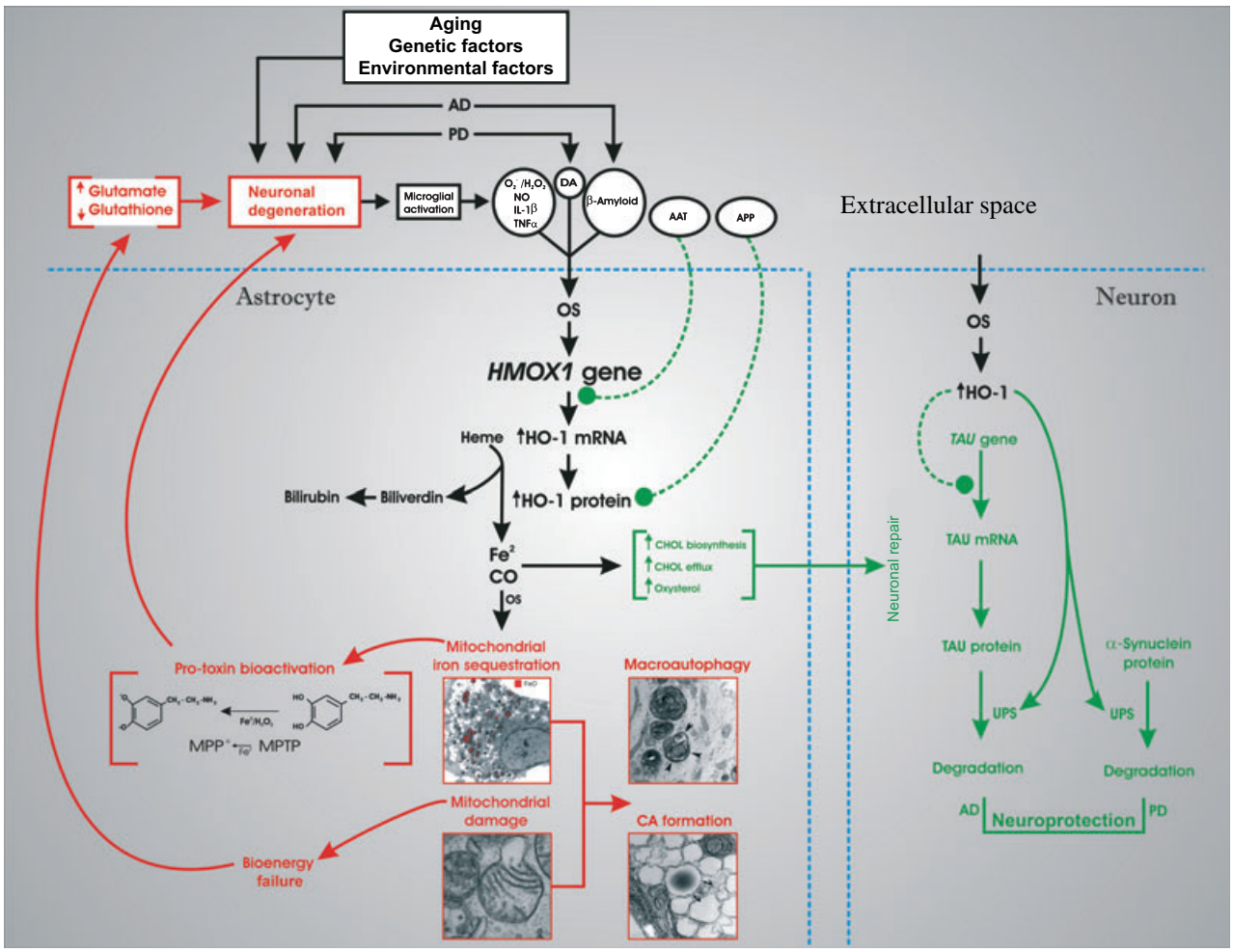

Fig. 2 Putative roles of HO-1 in aging-related human neurodegenerative disorders. Implications for AD and PD are emphasized, although neural *HMOX1* induction may exert homologous influences on brain structure and function in other degenerative and neuroinflammatory conditions. Sustained or repeated up-regulation of HO-1 may exacerbate certain degenerative processes (red) while concomitantly activating various neuroprotective responses (green), a situation possibly reflecting antagonistic pleiotropy. Dystrophic effects of HO-1 are represented by mitochondrial damage and ferrous iron sequestration in astrocytes which may, in turn, pre-dispose to bioenergetic failure, pro-toxin bioactivation, excitotoxicity, macroautophagy, and corpora amylacea formation. Neuronal degeneration stimulates microglial activation resulting in the release of ROS, NO, and proinflammatory cytokines (TNFα, IL-1β). The latter may further induce *HMOX1* in indigent astroglia, completing a self-sustaining loop of pathological cellular interactions that may perpetuate oxidative damage and mitochondrial insufficiency within senescent and degenerating neural tissues. Genetic and environmental risk factors may confer disease specificity by superimposing unique pathological signatures on this core lesion. In AD, AAT and APP may suppress glial HO-1 transcription and activity, respectively, in an attempt to restore central heme/iron homeostasis. Potential benefits conferred by the up-regulation of HO-1 include (i) stimulation of neuronal tau and α-synuclein degradation by the UPS to curtail protein aggregate toxicity (in AD and PD, respectively) and (ii) modulation of sterol homeostasis in favor of neuronal repair/neuroplasticity. See text and references for full details. Solid lines (arrows) denote causation or stimulation; dashed lines with spheres denote inhibition. AAT, α_1-antitrypsin; AD, Alzheimer disease; APP, amyloid precursor protein; CHOL, cholesterol; CO, carbon monoxide; DA, dopamine; Fe^2, ferrous iron; GSH, glutathione; HO-1, heme oxygenase-1; IL-1β, interleukin-1β; MPP^+, methyl-4-phenylpyridinium; MPTP, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; NO, nitric oxide; OS, oxidative stress; PD, Parkinson disease; TNFα, tumor necrosis factor-α; UPS, ubiquitin-proteosome system. Electron micrographs are reproduced from Zukor *et al.* (2009) with permission.

ment. Addressing this potential therapeutic conundrum, Syapin (2008) suggested that selective short interfering RNA delivery could be exploited to achieve HO-1 knockdown within specific CNS cell populations (e.g. astrocytes), guided by recent successes with targetted short interfering RNA delivery to neurons (Kumar *et al.* 2007) and hepatocytes (Rozema *et al.* 2007).

Intriguingly, reduced locomotion, exploratory behaviour (Maines *et al.* 1998), and impaired spatial navigation (Morgan *et al.* 1998) have been documented in transgenic

mice over-expressing rat HO-1 under the control of a neuron-specific enolase promoter. Studies have been initiated in our laboratory to evaluate cognitive/locomotor behavior and neuropathology in (i) transgenic mice engineered to over-express *HMOX1* selectively within the mature astroglial compartment and (ii) an APP mouse model of AD in the presence and absence of pharmacological HO-1 blockade (Schipper *et al.* 2009). The latter paradigms may help resolve some of the unsettled issues raised in this review and determine the effects of sustained glial HO-1 induction on brain structure and neurological health in intact organisms.

Acknowledgements

The author thanks Adrienne Liberman for assistance with the preparation of this manuscript. Contributions by the host laboratory were enabled by grants to HMS from the Canadian Institutes for Health Research, the Fonds de la Recherche en Santé du Québec, the Alzheimer's Association (US), the Parkinson Foundation of Canada, and Osta Biotechnologies (Montreal).

References

Abraham N. G. and Kappas A. (2008) Pharmacological and clinical aspects of heme oxygenase. *Pharmacol. Rev.* **60**, 79–127.

Ahmad A. S., Zhuang H. and Dore S. (2006) Heme oxygenase-1 protects brain from acute excitotoxicity. *Neuroscience* **141**, 1703–1708.

Ali S. F., David S. N. and Newport G. D. (1993) Age-related susceptibility to MPTP-induced neurotoxicity in mice. *Neurotoxicology* **14**, 29–34.

Anthony S. G., Schipper H. M., Tavares R., Hovanesian V., Cortez S. C., Stopa E. G. and Johanson C. E. (2003) Stress protein expression in the Alzheimer-diseased choroid plexus. *J. Alzheimers Dis.* **5**, 171–177.

Aschner M. (2000) Neuron-astrocyte interactions: implications for cellular energetics and antioxidant levels. *Neurotoxicology* **21**, 1101–1107.

Baranano D. E. and Snyder S. H. (2001) Neural roles for heme oxygenase: contrasts to nitric oxide synthase. *Proc. Natl Acad. Sci. USA* **98**, 10996–11002.

Beal M. (1995) Mitochondrial Dysfunction and Oxidative Damage in Neurodegenerative Diseases. R.G. Landes Co., Austin, TX.

Beal M. F. (1996) Mitochondria, free radicals, and neurodegeneration. *Curr. Opin. Neurobiol.* **6**, 661–666.

Bergeron M., Ferriero D. M. and Sharp F. R. (1998) Developmental expression of heme oxygenase-1 (HSP32) in rat brain: an immunocytochemical study. *Brain Res. Dev. Brain Res.* **105**, 181–194.

Bernardi P. (1996) The permeability transition pore. Control points of a cyclosporin A-sensitive mitochondrial channel involved in cell death. *Biochim. Biophys. Acta* **1275**, 5–9.

Beschorner R., Adjodah D., Schwab J. M., Mittelbronn M., Pedal I., Mattern R., Schluesener H. J. and Meyermann R. (2000) Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. *Acta Neuropathol. (Berl)* **100**, 377–384.

Bogdanovic N., Bretillon L., Lund E. G., Diczfalusy U., Lannfelt L., Winblad B., Russell D. W. and Bjorkhem I. (2001) On the turnover of brain cholesterol in patients with Alzheimer's disease. Abnormal induction of the cholesterol-catabolic enzyme CYP46 in glial cells. *Neurosci. Lett.* **314**, 45–48.

Brawer J. R. and Sonnenschein C. (1975) Cytopathological effects of estradiol on the arcuate nucleus of the female rat. A possible mechanism for pituitary tumorigenesis. *Am. J. Anat.* **144**, 57–88.

Brawer J. R., Naftolin F., Martin J. and Sonnenschein C. (1978) Effects of a single injection of estradiol valerate on the hypothalamic arcuate nucleus and on reproductive function in the female rat. *Endocrinology* **103**, 501–512.

Brawer J. R., Reichard G., Small L. and Schipper H. M. (1994a) The origin and composition of peroxidase-positive granules in cysteamine-treated astrocytes in culture. *Brain Res.* **633**, 9–20.

Brawer J. R., Stein R., Small L., Cisse S. and Schipper H. M. (1994b) Composition of Gomori-positive inclusions in astrocytes of the hypothalamic arcuate nucleus. *Anat. Rec.* **240**, 407–415.

Businaro R., Fabrizi C., Caronti B., Calderaro C., Fumagalli L. and Lauro G. M. (2002) Myelin basic protein induces heme oxygenase-1 in human astroglial cells. *Glia* **37**, 83–88.

Butterfield D. A. (2002) Amyloid beta-peptide (1-42)-induced oxidative stress and neurotoxicity: implications for neurodegeneration in Alzheimer's disease brain. A review. *Free Radic. Res.* **36**, 1307–1313.

Calingasan N. Y., Chen J., Kiaei M. and Beal M. F. (2005) Beta-amyloid 42 accumulation in the lumbar spinal cord motor neurons of amyotrophic lateral sclerosis patients. *Neurobiol. Dis.* **19**, 340–347.

Calne D. B. (1994) Neurodegenerative Diseases. W.B. Saunders Co., Philadelphia, PA.

Cantuti-Castelvetri I., Shukitt-Hale B. and Joseph J. A. (2003) Dopamine neurotoxicity: age-dependent behavioral and histological effects. *Neurobiol. Aging* **24**, 697–706.

Castellani R., Smith M. A., Richey P. L., Kalaria R., Gambetti P. and Perry G. (1995) Evidence for oxidative stress in Pick disease and corticobasal degeneration. *Brain Res.* **696**, 268–271.

Castellani R., Smith M. A., Richey P. L. and Perry G. (1996) Glycoxidation and oxidative stress in Parkinson disease and diffuse Lewy body disease. *Brain Res.* **737**, 195–200.

Cavanagh J. B. (1999) Corpora-amylacea and the family of polyglucosan diseases. *Brain Res. Brain Res. Rev.* **29**, 265–295.

Chang L., Cornford M., Miller B. L., Itabashi H. and Mena I. (1995) Neuronal ultrastructural abnormalities in a patient with frontotemporal dementia and motor neuron disease. *Dementia* **6**, 1–8.

Chen K., Gunter K. and Maines M. D. (2000) Neurons overexpressing heme oxygenase-1 resist oxidative stress-mediated cell death. *J. Neurochem.* **75**, 304–313.

Chen-Roetling J., Benvenisti-Zarom L. and Regan R. F. (2005) Cultured astrocytes from heme oxygenase-1 knockout mice are more vulnerable to heme-mediated oxidative injury. *J. Neurosci. Res.* **82**, 802–810.

Chopra V. S., Moozar K. L., Mehindate K. and Schipper H. M. (1997) A cellular stress model for the differential expression of glial lysosomal cathepsins in the aging nervous system. *Exp. Neurol.* **147**, 221–228.

Ciechanover A. and Brundin P. (2003) The ubiquitin proteasome system in neurodegenerative diseases: sometimes the chicken, sometimes the egg. *Neuron* **40**, 427–446.

Cisse S. and Schipper H. M. (1995) Experimental induction of corpora amylacea-like inclusions in rat astroglia. *Neuropathol. Appl. Neurobiol.* **21**, 423–431.

Connor J. R. (1997) Metals and Oxidative Damage in Neurological Disorders. Plenum Press, New York.

Converso D. P., Taille C., Carreras M. C., Jaitovich A., Poderoso J. J. and Boczkowski J. (2006) HO-1 is located in liver mitochondria and modulates mitochondrial heme content and metabolism. *FASEB J.* **20**, 1236–1238.

Delisle M. B., Uro-Coste E., Murrell J. R., Rascol O. and Ghetti B. (2000) Neurodegenerative disease associated with a mutation of

codon 279 (N279K) in exon 10 of Tau protein. *Bull. Acad. Natl Med.* **184**, 799–809; discussion 809-711.

Dennery P. A. (2000) Regulation and role of heme oxygenase in oxidative injury. *Curr. Top. Cell. Regul.* **36**, 181–199.

Desmard M., Boczkowski J., Poderoso J. and Motterlini R. (2007) Mitochondrial and cellular heme-dependent proteins as targets for the bioactive function of the heme oxygenase/carbon monoxide system. *Antioxid. Redox Signal.* **9**, 2139–2155.

Desole M. S., Esposito G., Migheli R., Fresu L., Sircana S., Zangani D., Miele M. and Miele E. (1995) Cellular defence mechanisms in the striatum of young and aged rats subchronically exposed to manganese. *Neuropharmacology* **34**, 289–295.

Di Monte D. A., Schipper H. M., Hetts S. and Langston J. W. (1995) Iron-mediated bioactivation of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in glial cultures. *Glia* **15**, 203–206.

Dore S., Takahashi M., Ferris C. D., Zakhary R., Hester L. D., Guastella D. and Snyder S. H. (1999) Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. *Proc. Natl Acad. Sci. USA* **96**, 2445–2450.

Droge W. (2002) Free radicals in the physiological control of cell function. *Physiol. Rev.* **82**, 47–95.

Drummond G. S. and Kappas A. (2004) Chemoprevention of severe neonatal hyperbilirubinemia. *Semin. Perinatol.* **28**, 365–368.

Durante W. (2003) Heme oxygenase-1 in growth control and its clinical application to vascular disease. *J. Cell. Physiol.* **195**, 373–382.

Dwyer B. E., Nishimura R. N., De Vellis J. and Yoshida T. (1992) Heme oxygenase is a heat shock protein and PEST protein in rat astroglial cells. *Glia* **5**, 300–305.

Dwyer B. E., Nishimura R. N. and Lu S. Y. (1995a) 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. *Brain Res. Mol. Brain Res.* **30**, 37–47.

Dwyer B. E., Nishimura R. N. and Lu S. Y. (1995b) Differential localization of heme oxygenase and NADPH-diaphorase in spinal cord neurons. *Neuroreport* **6**, 973–976.

Exner M., Minar E., Wagner O. and Schillinger M. (2004) The role of heme oxygenase-1 promoter polymorphisms in human disease. *Free Radic. Biol. Med.* **37**, 1097–1104.

Finkel T. and Holbrook N. J. (2000) Oxidants, oxidative stress and the biology of ageing. *Nature* **408**, 239–247.

Frankel D. and Schipper H. M. (1999) Cysteamine pretreatment of the astroglial substratum (mitochondrial iron sequestration) enhances PC12 cell vulnerability to oxidative injury. *Exp. Neurol.* **160**, 376–385.

Frankel D., Mehindate K. and Schipper H. M. (2000) Role of heme oxygenase-1 in the regulation of manganese superoxide dismutase gene expression in oxidatively-challenged astroglia. *J. Cell. Physiol.* **185**, 80–86.

Fukuda K., Richmon J. D., Sato M., Sharp F. R., Panter S. S. and Noble L. J. (1996) Induction of heme oxygenase-1 (HO-1) in glia after traumatic brain injury. *Brain Res.* **736**, 68–75.

Galbraith R. (1999) Heme oxygenase: who needs it? *Proc. Soc. Exp. Biol. Med.* **222**, 299–305.

Gennuso F., Fernetti C., Tirolo C. *et al.* (2004) Bilirubin protects astrocytes from its own toxicity by inducing up-regulation and translocation of multidrug resistance-associated protein 1 (Mrp1). *Proc. Natl Acad. Sci. USA* **101**, 2470–2475.

Gollin P. A., Kalaria R. N., Eikelenboom P., Rozemuller A. and Perry G. (1992) Alpha 1-antitrypsin and alpha 1-antichymotrypsin are in the lesions of Alzheimer's disease. *Neuroreport* **3**, 201–203.

Ham D. and Schipper H. M. (2000) Heme oxygenase-1 induction and mitochondrial iron sequestration in astroglia exposed to amyloid peptides. *Cell. Mol. Biol. (Noisy-le-grand)* **46**, 587–596.

Hascalovici J. R., Vaya J., Khatib S., Holcroft C., Zukor H. Y., Song W., Arvanatakis Z., Bennett D. and Schipper H. M. (2008) A model for sterol dys-regulation in AD brain. *Alzheimers Demen.* **4** (Suppl. 1), T718.

Hascalovici J. R., Song W., Vaya J., Khatib S., Fuhrman B., Aviram M. and Schipper H. M. (2009) Impact of heme oxygenase-1 on cholesterol synthesis, cholesterol efflux and oxysterol formation in cultured astroglia. *J. Neurochem.* **108**, 72–81.

Hekimi S. and Guarente L. (2003) Genetics and the specificity of the aging process. *Science* **299**, 1351–1354.

Hirose W., Ikematsu K. and Tsuda R. (2003) Age-associated increases in heme oxygenase-1 and ferritin immunoreactivity in the autopsied brain. *Leg. Med. (Tokyo)* **5** (Suppl.), S360–S366.

Huang E., Ong W. Y., Go M. L. and Garey L. J. (2005) Heme oxygenase-1 activity after excitotoxic injury: immunohistochemical localization of bilirubin in neurons and astrocytes and deleterious effects of heme oxygenase inhibition on neuronal survival after kainate treatment. *J. Neurosci. Res.* **80**, 268–278.

Hung S. Y., Liou H. C., Kang K. H., Wu R. M., Wen C. C. and Fu W. M. (2008) Overexpression of heme oxygenase-1 protects dopaminergic neurons against 1-methyl-4-phenylpyridinium-induced neurotoxicity. *Mol. Pharmacol.* **74**, 1564–1575.

Jarvis M. F. and Wagner G. C. (1990) 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine-induced neurotoxicity in the rat: characterization and age-dependent effects. *Synapse* **5**, 104–112.

Jofre-Monseny L., Loboda A., Wagner A. E., Huebbe P., Boesch-Saadatmandi C., Jozkowicz A., Minihane A. M., Dulak J. and Rimbach G. (2007) Effects of apoE genotype on macrophage inflammation and heme oxygenase-1 expression. *Biochem. iophys. Res. Commun.* **357**, 319–324.

Jozkowicz A., Was H. and Dulak J. (2007) Heme oxygenase-1 in tumors: is it a false friend? *Antioxid. Redox Signal.* **9**, 2099–2117.

Jurevics H. and Morell P. (1995) Cholesterol for synthesis of myelin is made locally, not imported into brain. *J. Neurochem.* **64**, 895–901.

Kadoya C., Domino E. F., Yang G. Y., Stern J. D. and Betz A. L. (1995) Preischemic but not postischemic zinc protoporphyrin treatment reduces infarct size and edema accumulation after temporary focal cerebral ischemia in rats. *Stroke* **26**, 1035–1038.

Kappas A., Drummond G. S. and Galbraith R. A. (1993) Prolonged clinical use of a heme oxygenase inhibitor: hematological evidence for an inducible but reversible iron-deficiency state. *Pediatrics* **91**, 537–539.

Kim H. P., Wang X., Galbiati F., Ryter S. W. and Choi A. M. (2004) Caveolae compartmentalization of heme oxygenase-1 in endothelial cells. *FASEB J.* **18**, 1080–1089.

Kimpara T., Takeda A., Watanabe K. *et al.* (1997) Microsatellite polymorphism in the human heme oxygenase-1 gene promoter and its application in association studies with Alzheimer and Parkinson disease. *Hum. Genet.* **100**, 145–147.

Kinobe R. T., Vlahakis J. Z., Vreman H. J., Stevenson D. K., Brien J. F., Szarek W. A. and Nakatsu K. (2006) Selectivity of imidazole-dioxolane compounds for in vitro inhibition of microsomal haem oxygenase isoforms. *Br. J. Pharmacol.* **147**, 307–315.

Kirkwood T. B. and Rose M. R. (1991) Evolution of senescence: late survival sacrificed for reproduction. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **332**, 15–24.

Kitamuro T., Takahashi K., Ogawa K. *et al.* (2003) Bach1 functions as a hypoxia-inducible repressor for the heme oxygenase-1 gene in human cells. *J. Biol. Chem.* **278**, 9125–9133.

Koeppen A. and Dickson A. (1999) Neuroprotection in intracerebral hemorrhage with tin-protoporphyrin. *Ann. Neurol.* **46**, 938.

Kowalska A., Danker-Hopfe H., Wender M., Florczak J. and Walter H. (1996) Association between the PI*M3 allele of alpha 1-antitrypsin and Alzheimer's disease? A preliminary report. *Hum. Genet.* **98**, 744–746.

Kruger R., Kuhn W., Muller T. *et al.* (1998) Ala30Pro mutation in the gene encoding alpha-synuclein in Parkinson's disease. *Nat. Genet.* **18**, 106–108.

Kumar P., Wu H., McBride J. L., Jung K. E., Kim M. H., Davidson B. L., Lee S. K., Shankar P. and Manjunath N. (2007) Transvascular delivery of small interfering RNA to the central nervous system. *Nature* **448**, 39–43.

Lavrovsky Y., Drummond G. S. and Abraham N. G. (1996) Downregulation of the human heme oxygenase gene by glucocorticoids and identification of 56b regulatory elements. *Biochem. Biophys. Res. Commun.* **218**, 759–765.

Le W. D., Xie W. J. and Appel S. H. (1999) Protective role of heme oxygenase-1 in oxidative stress-induced neuronal injury. *J. Neurosci. Res.* **56**, 652–658.

Lee V. M., Goedert M. and Trojanowski J. Q. (2001) Neurodegenerative tauopathies. *Annu. Rev. Neurosci.* **24**, 1121–1159.

Lee D. W., Gelein R. M. and Opanashuk L. A. (2006) Heme-oxygenase-1 promotes polychlorinated biphenyl mixture aroclor 1254-induced oxidative stress and dopaminergic cell injury. *Toxicol. Sci.* **90**, 159–167.

Levine S. M. and Chakrabarty A. (2004) The role of iron in the pathogenesis of experimental allergic encephalomyelitis and multiple sclerosis. *Ann. NY Acad. Sci.* **1012**, 252–266.

Lewis J., McGowan E., Rockwood J. *et al.* (2000) Neurofibrillary tangles, amyotrophy and progressive motor disturbance in mice expressing mutant (P301L) tau protein. *Nat. Genet.* **25**, 402–405.

Li Volti G., Ientile R., Abraham N. G. *et al.* (2004) Immunocytochemical localization and expression of heme oxygenase-1 in primary astroglial cell cultures during differentiation: effect of glutamate. *Biochem. Biophys. Res. Commun.* **315**, 517–524.

Lin Q., Weis S., Yang G. *et al.* (2007a) Heme oxygenase-1 protein localizes to the nucleus and activates transcription factors important in oxidative stress. *J. Biol. Chem.* **282**, 20621–20633.

Lin Y., Vreman H. J., Wong R. J., Tjoa T., Yamauchi T. and Noble-Haeusslein L. J. (2007b) Heme oxygenase-1 stabilizes the blood-spinal cord barrier and limits oxidative stress and white matter damage in the acutely injured murine spinal cord. *J. Cereb. Blood Flow Metab.* **27**, 1010–1021.

Lin Q. S., Weis S., Yang G., Zhuang T., Abate A. and Dennery P. A. (2008) Catalytic inactive heme oxygenase-1 protein regulates its own expression in oxidative stress. *Free Radic. Biol. Med.* **44**, 847–855.

Llesuy S. F. and Tomaro M. L. (1994) Heme oxygenase and oxidative stress. Evidence of involvement of bilirubin as physiological protector against oxidative damage. *Biochim. Biophys. Acta* **1223**, 9–14.

Loboda A., Jazwa A., Grochot-Przeczek A., Rutkowski A. J., Cisowski J., Agarwal A., Jozkowicz A. and Dulak J. (2008) Heme oxygenase-1 and the vascular bed: from molecular mechanisms to therapeutic opportunities. *Antioxid. Redox Signal.* **10**, 1767–1812.

Lu F., Selak M., O'Connor J., Croul S., Lorenzana C., Butunoi C. and Kalman B. (2000) Oxidative damage to mitochondrial DNA and activity of mitochondrial enzymes in chronic active lesions of multiple sclerosis. *J. Neurol. Sci.* **177**, 95–103.

Maes O. C., Kravitz S., Mawal Y. *et al.* (2006) Characterization of alpha(1)-antitrypsin as a heme oxygenase-1 suppressor in Alzheimer plasma. *Neurobiol. Dis.* **24**, 89–100.

Maines M. D., Polevoda B., Coban T., Johnson K., Stoliar S., Huang T. J., Panahian N., Cory-Slechta D. A. and McCoubrey W. K. Jr (1998) Neuronal overexpression of heme oxygenase-1 correlates with an attenuated exploratory behavior and causes an increase in neuronal NADPH diaphorase staining. *J. Neurochem.* **70**, 2057–2069.

Manganaro F., Chopra V. S., Mydlarski M. B., Bernatchez G. and Schipper H. M. (1995) Redox perturbations in cysteamine-stressed astroglia: implications for inclusion formation and gliosis in the aging brain. *Free Radic. Biol. Med.* **19**, 823–835.

Mann D. H. A. (1994) Vulnerability of specific neurons to aging, in Neurodegenerative Diseases (Calne D. B., ed.), pp. 15–31. Saunders, Philadelphia, PA.

Mattson M. P. (2002) Contributions of mitochondrial alterations, resulting from bad genes and a hostile environment, to the pathogenesis of Alzheimer's disease. *Int. Rev. Neurobiol.* **53**, 387–409.

Matz P., Turner C., Weinstein P. R., Massa S. M., Panter S. S. and Sharp F. R. (1996) Heme-oxygenase-1 induction in glia throughout rat brain following experimental subarachnoid hemorrhage. *Brain Res.* **713**, 211–222.

McGeer P. L. and McGeer E. G. (1995) The inflammatory response system of brain: implications for therapy of Alzheimer and other neurodegenerative diseases. *Brain Res. Brain Res. Rev.* **21**, 195–218.

McLaren J., Brawer J. R. and Schipper H. M. (1992) Iron content correlates with peroxidase activity in cysteamine-induced astroglial organelles. *J. Histochem. Cytochem.* **40**, 1887–1897.

McNaught K. S. and Olanow C. W. (2003) Proteolytic stress: a unifying concept for the etiopathogenesis of Parkinson's disease. *Ann. Neurol.* **53** (Suppl. 3), S73–S84; discussion S84-76.

McNaught K. S., Belizaire R., Isacson O., Jenner P. and Olanow C. W. (2003) Altered proteasomal function in sporadic Parkinson's disease. *Exp. Neurol.* **179**, 38–46.

Mehindate K., Sahlas D. J., Frankel D., Mawal Y., Liberman A., Corcos J., Dion S. and Schipper H. M. (2001) Proinflammatory cytokines promote glial heme oxygenase-1 expression and mitochondrial iron deposition: implications for multiple sclerosis. *J. Neurochem.* **77**, 1386–1395.

Morgan D., Holcomb L., Saad I., Gordon M. and Mahin M. (1998) Impaired spatial navigation learning in transgenic mice overexpressing heme oxygenase-1. *Brain Res.* **808**, 110–112.

Mydlarski M. B., Liang J. J. and Schipper H. M. (1993) Role of the cellular stress response in the biogenesis of cysteamine-induced astrocytic inclusions in primary culture. *J. Neurochem.* **61**, 1755–1765.

Mydlarski M., Deschenes J. and Schipper H. (2003) Expression of heme oxygenase-1 in the human eye, in Assoc Res Vis Ophthalmo (ARVO). *Invest Ophthalmol Vis Sci* 44, pp. E-Abstract 649, Ft. Lauderdale.

Nakagami T., Toyomura K., Kinoshita T. and Morisawa S. (1993) A beneficial role of bile pigments as an endogenous tissue protector: anti-complement effects of biliverdin and conjugated bilirubin. *Biochim. Biophys. Acta* **1158**, 189–193.

Nakaso K., Kitayama M., Fukuda H., Kimura K., Yanagawa T., Ishii T., Nakashima K. and Yamada K. (2000) Oxidative stress-related proteins A170 and heme oxygenase-1 are differently induced in the rat cerebellum under kainate-mediated excitotoxicity. *Neurosci. Lett.* **282**, 57–60.

Ogawa K. (2002) Heme metabolism in stress response. *Nippon Eiseigaku Zasshi* **56**, 615–621.

Otterbein L. E., Soares M. P., Yamashita K. and Bach F. H. (2003) Heme oxygenase-1: unleashing the protective properties of heme. *Trends Immunol.* **24**, 449–455.

Panahian N., Yoshiura M. and Maines M. D. (1999) Overexpression of heme oxygenase-1 is neuroprotective in a model of permanent middle cerebral artery occlusion in transgenic mice. *J. Neurochem.* **72**, 1187–1203.

Panizzon K. L., Dwyer B. E., Nishimura R. N. and Wallis R. A. (1996) Neuroprotection against CA1 injury with metalloporphyrins. *Neuroreport* **7**, 662–666.

Perry G., Castellani R. J., Smith M. A. *et al.* (2003) Oxidative damage in the olfactory system in Alzheimer's disease. *Acta Neuropathol. (Berl)* **106**, 552–556.

Petronilli V., Cola C., Massari S., Colonna R. and Bernardi P. (1993) Physiological effectors modify voltage sensing by the cyclosporin A-sensitive permeability transition pore of mitochondria. *J. Biol. Chem.* **268**, 21939–21945.

Pfrieger F. W. (2003) Outsourcing in the brain: do neurons depend on cholesterol delivery by astrocytes? *BioEssays* **25**, 72–78.

Polymeropoulos M. H., Lavedan C., Leroy E. *et al.* (1997) Mutation in the alpha-synuclein gene identified in families with Parkinson's disease. *Science* **276**, 2045–2047.

Premkumar D. R., Smith M. A., Richey P. L., Petersen R. B., Castellani R., Kutty R. K., Wiggert B., Perry G. and Kalaria R. N. (1995) Induction of heme oxygenase-1 mRNA and protein in neocortex and cerebral vessels in Alzheimer's disease. *J. Neurochem.* **65**, 1399–1402.

Qato M. K. and Maines M. D. (1985) Prevention of neonatal hyperbilirubinaemia in non-human primates by Zn-protoporphyrin. *Biochem. J.* **226**, 51–57.

Refolo L. M., Pappolla M. A., LaFrancois J. *et al.* (2001) A cholesterol-lowering drug reduces beta-amyloid pathology in a transgenic mouse model of Alzheimer's disease. *Neurobiol. Dis.* **8**, 890–899.

Reichmann H. and Riederer P. (1994) Mitochondrial Disturbances in Neurodegeneration: Neurodegenerative Diseases. Saunders, Philadelphia, PA.

Rieder C. R., Williams A. C. and Ramsden D. B. (2004) Selegiline increases heme oxygenase-1 expression and the cytotoxicity produced by dopamine treatment of neuroblastoma SK-N-SH cells. *Braz. J. Med. Biol. Res.* **37**, 1055–1062.

Rizzu P., Joosse M., Ravid R., Hoogeveen A., Kamphorst W., van Swieten J. C., Willemsen R. and Heutink P. (2000) Mutation-dependent aggregation of tau protein and its selective depletion from the soluble fraction in brain of P301L FTDP-17 patients. *Hum. Mol. Genet.* **9**, 3075–3082.

Rozema D. B., Lewis D. L., Wakefield D. H. *et al.* (2007) Dynamic PolyConjugates for targeted in vivo delivery of siRNA to hepatocytes. *Proc. Natl Acad. Sci. USA* **104**, 12982–12987.

Rubinsztein D. C. (2006) The roles of intracellular protein-degradation pathways in neurodegeneration. *Nature* **443**, 780–786.

Ryter S. W. and Tyrrell R. M. (2000) The heme synthesis and degradation pathways: role in oxidant sensitivity. Heme oxygenase has both pro- and antioxidant properties. *Free Radic. Biol. Med.* **28**, 289–309.

Sahlas D. J., Liberman A. and Schipper H. M. (2002) Role of heme oxygenase-1 in the biogenesis of corpora amylacea. *Biogerontology* **3**, 223–231.

Saunders A. M., Strittmatter W. J., Schmechel D. *et al.* (1993) Association of apolipoprotein E allele epsilon 4 with late-onset familial and sporadic Alzheimer's disease. *Neurology* **43**, 1467–1472.

Sayre L. M., Smith M. A. and Perry G. (2001) Chemistry and biochemistry of oxidative stress in neurodegenerative disease. *Curr Med Chem*, **8**, 721–738.

Scapagnini G., D'Agata V., Calabrese V., Pascale A., Colombrita C., Alkon D. and Cavallaro S. (2002) Gene expression profiles of heme oxygenase isoforms in the rat brain. *Brain Res.* **954**, 51–59.

Schipper H. M. (1996) Astrocytes, brain aging, and neurodegeneration. *Neurobiol. Aging* **17**, 467–480.

Schipper H. M. (1998a) Experimental induction of corpora amylacea in adult rat brain. *Microsc. Res. Tech.* **43**, 43–48.

Schipper H. M. (1998b) Glial Iron Sequestration and Neurodegeneration, in Astrocytes in Brain Aging and Neurodegeneration (Schipper H. M., ed.), pp. 235–251. R.G. Landes Co., Austin, TX.

Schipper H. M. (1999) Glial HO-1 expression, iron deposition and oxidative stress in neurodegenerative diseases. *Neurotox. Res.* **1**, 57–70.

Schipper H. M. (2000) Heme oxygenase-1: role in brain aging and neurodegeneration. *Exp. Gerontol.* **35**, 821–830.

Schipper H. M. (2001) Mitochondrial iron deposition in aging astroglia: mechanisms and disease implications, in Mitochondrial Ubiquinone (Coenzyme Q): Biochemical, Functional, Medical, and Therapeutic Aspects in Human Health and Disease (Ebadi M. and Chopra R. K., eds), pp. 267–280. Prominent Press, Scottsdale, AZ.

Schipper H. M. (2004a) Brain iron deposition and the free radical-mitochondrial theory of ageing. *Ageing Res. Rev.* **3**, 265–301.

Schipper H. M. (2004b) Heme oxygenase-1: transducer of pathological brain iron sequestration under oxidative stress, in Redox-Active Metals in Neurological Disorders (LeVine S., Connor J. R. and Schipper H. M., eds), 1012, pp. 84–93, Ann NY Acad Sci 1012, New York.

Schipper H. M. (2004c) Heme oxygenase expression in human central nervous system disorders. *Free Radic. Biol. Med.* **37**, 1995–2011.

Schipper H. M. (2007) Biomarker potential of heme oxygenase-1 in Alzheimer disease and mild cognitive impairment. *Biomarkers Med.* **1**, 375–385.

Schipper H. M. and Cissé S. (1995) Mitochondrial constituents of corpora amylacea and autofluorescent astrocytic inclusions in senescent human brain. *Glia* **14**, 55–64.

Schipper H., Brawer J. R., Nelson J. F., Felicio L. S. and Finch C. E. (1981) Role of the gonads in the histologic aging of the hypothalamic arcuate nucleus. *Biol. Reprod.* **25**, 413–419.

Schipper H. M., Scarborough D. E., Lechan R. M. and Reichlin S. (1990) Gomori-positive astrocytes in primary culture: effects of in vitro age and cysteamine exposure. *Brain Res. Dev. Brain Res.* **54**, 71–79.

Schipper H. M., Kotake Y. and Janzen E. G. (1991) Catechol oxidation by peroxidase-positive astrocytes in primary culture: an electron spin resonance study. *J. Neurosci.* **11**, 2170–2176.

Schipper H. M., Mydlarski M. B. and Wang X. (1993) Cysteamine gliopathy in situ: a cellular stress model for the biogenesis of astrocytic inclusions. *J. Neuropathol. Exp. Neurol.* **52**, 399–410.

Schipper H. M., Cisse S. and Stopa E. G. (1995) Expression of heme oxygenase-1 in the senescent and Alzheimer-diseased brain. *Ann. Neurol.* **37**, 758–768.

Schipper H. M., Liberman A. and Stopa E. G. (1998a) Neural heme oxygenase-1 expression in idiopathic Parkinson's disease. *Exp. Neurol.* **150**, 60–68.

Schipper H. M., Vininsky R., Brull R., Small L. and Brawer J. R. (1998b) Astrocyte mitochondria: a substrate for iron deposition in the aging rat substantia nigra. *Exp. Neurol.* **152**, 188–196.

Schipper H. M., Bernier L., Mehindate K. and Frankel D. (1999) Mitochondrial iron sequestration in dopamine-challenged astroglia: role of heme oxygenase-1 and the permeability transition pore. *J. Neurochem.* **72**, 1802–1811.

Schipper H. M., Chertkow H., Mehindate K., Frankel D., Melmed C. and Bergman H. (2000) Evaluation of heme oxygenase-1 as a systemic biological marker of sporadic AD. *Neurology* **54**, 1297–1304.

Schipper H. M., Mawal Y., Chertkow H. and Bergman H. (2001) Blood HO-1 mRNA in AD and MCI. *Neurology* **57**, 2142–2143.

Schipper H. M., Bennett D. A., Liberman A., Bienias J. L., Schneider J. A., Kelly J. and Arvanitakis Z. (2006) Glial heme oxygenase-1 expression in Alzheimer disease and mild cognitive impairment. *Neurobiol. Aging* **27**, 252–261.

Schipper H. M., Gupta A. and Szarek W. A. (2009) Suppression of glial HO-1 activitiy as a potential neurotherapeutic intervention in AD. *Curr. Alzheimer Res.*, in press.

Selkoe D. J. (1991) The molecular pathology of Alzheimer's disease. *Neuron* **6**, 487–498.

Shastry B. S. (2003) Neurodegenerative disorders of protein aggregation. *Neurochem. Int.* **43**, 1–7.

Shibata N., Ohnuma T., Baba H. and Arai H. (2009) No genetic association between polymorphisms of heme oxygenase 1 and 2 and

Alzheimer's disease in a Japanese population. *Dement. Geriatr. Cogn. Disord.* **27**, 273–277.

Shobab L. A., Hsiung G. Y. and Feldman H. H. (2005) Cholesterol in Alzheimer's disease. *Lancet Neurol.* **4**, 841–852.

Smith M. A., Kutty R. K., Richey P. L., Yan S. D., Stern D., Chader G. J., Wiggert B., Petersen R. B. and Perry G. (1994) Heme oxygenase-1 is associated with the neurofibrillary pathology of Alzheimer's disease. *Am. J. Pathol.* **145**, 42–47.

Snyder S. H., Jaffrey S. R. and Zakhary R. (1998) Nitric oxide and carbon monoxide: parallel roles as neural messengers. *Brain Res. Brain Res. Rev.* **26**, 167–175.

Sohal R. S., Mockett R. J. and Orr W. C. (2002) Mechanisms of aging: an appraisal of the oxidative stress hypothesis. *Free Radic. Biol. Med.* **33**, 575–586.

Song W., Su H., Song S., Paudel H. K. and Schipper H. M. (2006) Over-expression of heme oxygenase-1 promotes oxidative mitochondrial damage in rat astroglia. *J. Cell. Physiol.* **206**, 655–663.

Song L., Song W. and Schipper H. M. (2007) Astroglia overexpressing heme oxygenase-1 predispose co-cultured PC12 cells to oxidative injury. *J. Neurosci. Res.* **85**, 2186–2195.

Song W., Patel A., Qureshi H. Y., Han D., Schipper H. M. and Paudel H. K. (2009) The Parkinson disease-specific A30P mutation stabilizes alpha-synuclein against proteosomal degradation triggered by heme oxygenase 1 overexpression in human neuroblastoma cells. *J. Neurochem.*, in press.

Srebro Z. (1971) Periventricular Gomori-positive glia in brains of X-irradiated rats. *Brain Res.* **35**, 463–468.

Stahnke T., Stadelmann C., Netzler A., Bruck W. and Richter-Landsberg C. (2007) Differential upregulation of heme oxygenase-1 (HSP32) in glial cells after oxidative stress and in demyelinating disorders. *J. Mol. Neurosci.* **32**, 25–37.

Stocker R., Yamamoto Y., McDonagh A. F., Glazer A. N. and Ames B. N. (1987) Bilirubin is an antioxidant of possible physiological importance. *Science* **235**, 1043–1046.

Stokes A. H., Hastings T. G. and Vrana K. E. (1999) Cytotoxic and genotoxic potential of dopamine. *J. Neurosci. Res.* **55**, 659–665.

Strittmatter W. J., Saunders A. M., Schmechel D., Pericak-Vance M., Enghild J., Salvesen G. S. and Roses A. D. (1993) Apolipoprotein E: high-avidity binding to beta-amyloid and increased frequency of type 4 allele in late-onset familial Alzheimer disease. *Proc. Natl Acad. Sci. USA* **90**, 1977–1981.

Sun J., Hoshino H., Takaku K. *et al.* (2002) Hemoprotein Bach1 regulates enhancer availability of heme oxygenase-1 gene. *EMBO J.* **21**, 5216–5224.

Suttner D. M. and Dennery P. A. (1999) Reversal of HO-1 related cytoprotection with increased expression is due to reactive iron. *FASEB J.* **13**, 1800–1809.

Suzuki H., Kanamaru K., Tsunoda H., Inada H., Kuroki M., Sun H., Waga S. and Tanaka T. (1999) Heme oxygenase-1 gene induction as an intrinsic regulation against delayed cerebral vasospasm in rats. *J. Clin. Invest.* **104**, 59–66.

Suzuki H., Tashiro S., Sun J., Doi H., Satomi S. and Igarashi K. (2003) Cadmium induces nuclear export of Bach1, a transcriptional repressor of heme oxygenase-1 gene. *J. Biol. Chem.* **278**, 49246–49253.

Syapin P. J. (2008) Regulation of haeme oxygenase-1 for treatment of neuroinflammation and brain disorders. *Br. J. Pharmacol.* **155**, 623–640.

Takahashi M., Dore S., Ferris C. D. *et al.* (2000) Amyloid precursor proteins inhibit heme oxygenase activity and augment neurotoxicity in Alzheimer's disease. *Neuron* **28**, 461–473.

Takeda A., Perry G., Abraham N. G., Dwyer B. E., Kutty R. K., Laitinen J. T., Petersen R. B. and Smith M. A. (2000) Overexpression of heme oxygenase in neuronal cells, the possible interaction with Tau. *J. Biol. Chem.* **275**, 5395–5399.

Tanaka T., Nishimura Y., Tsunoda H. and Naka M. (2000) Pharmacogenomics and therapeutic target validation in cerebral vasospasm. *J. Cardiovasc. Pharmacol.* **36** (Suppl. 2), S1–S4.

Trakshel G. M., Kutty R. K. and Maines M. D. (1988) Resolution of the rat brain heme oxygenase activity: absence of a detectable amount of the inducible form (HO-1). *Arch. Biochem. Biophys.* **260**, 732–739.

Trapp B. D. and Nave K. A. (2008) Multiple sclerosis: an immune or neurodegenerative disorder? *Annu. Rev. Neurosci.* **31**, 247–269.

Vasilev V., Veskov R., Janac B., Rakic L. and Stojiljkovic M. (2003) Age-related differences in MK-801- and amphetamine-induced locomotor and stereotypic activities of rats. *Neurobiol. Aging* **24**, 715–723.

Vaya J. and Schipper H. M. (2007) Oxysterols, cholesterol homeostasis, and Alzheimer disease. *J. Neurochem.* **102**, 1727–1737.

Vaya J., Song W., Khatib S., Geng G. and Schipper H. M. (2007) Effects of heme oxygenase-1 expression on sterol homeostasis in rat astroglia. *Free Radic. Biol. Med.* **42**, 864–871.

Verma A., Hirsch D. J., Glatt C. E., Ronnett G. V. and Snyder S. H. (1993) Carbon monoxide: a putative neural messenger. *Science* **259**, 381–384.

Vincent S. R., Das S. and Maines M. D. (1994) Brain heme oxygenase isoenzymes and nitric oxide synthase are co-localized in select neurons. *Neuroscience* **63**, 223–231.

Vogelsberg-Ragaglia V., Bruce J., Richter-Landsberg C., Zhang B., Hong M., Trojanowski J. Q. and Lee V. M. (2000) Distinct FTDP-17 missense mutations in tau produce tau aggregates and other pathological phenotypes in transfected CHO cells. *Mol. Biol. Cell* **11**, 4093–4104.

Volles M. J. and Lansbury P. T. Jr (2002) Vesicle permeabilization by protofibrillar alpha-synuclein is sensitive to Parkinson's disease-linked mutations and occurs by a pore-like mechanism. *Biochemistry* **41**, 4595–4602.

Wang J. and Dore S. (2007) Heme oxygenase-1 exacerbates early brain injury after intracerebral haemorrhage. *Brain* **130**, 1643–1652.

Wang X., Manganaro F. and Schipper H. M. (1995) A cellular stress model for the sequestration of redox-active glial iron in the aging and degenerating nervous system. *J. Neurochem.* **64**, 1868–1877.

Williams G. C. (1957) Pleiotropy, natural selection, and the evolution of senescence. *Evolution* **11**, 398–411.

Yan S. D., Chen X., Schmidt A. M. *et al.* (1994) Glycated tau protein in Alzheimer disease: a mechanism for induction of oxidant stress. *Proc. Natl Acad. Sci. USA* **91**, 7787–7791.

Yu H.-L., Chertkow H. M., Bergman H. and Schipper H. M. (2003) Aberrant profiles of native and oxidized glycoproteins in Alzheimer plasma. *Proteomics* **3**, 2240–2248.

Yuan Y., Guo J. Z. and Zhou Q. X. (2008) The homeostasis of iron and suppression of HO-1 involved in the protective effects of nimodipine on neurodegeneration induced by aluminum overloading in mice. *Eur. J. Pharmacol.* **586**, 100–105.

Zhang J. and Piantadosi C. A. (1992) Mitochondrial oxidative stress after carbon monoxide hypoxia in the rat brain. *J. Clin. Invest.* **90**, 1193–1199.

Zukor H., Song W., Liberman A. *et al.* (2009) HO-1-mediated macroautophagy: a mechanism for unregulated iron deposition in aging and degenerating neural tissues. *J. Neurochem.* **109**, 776–791.