Published in final edited form as:

Curr Pharm Des. 2008 ; 14(5): 443–453.

Cerebroprotective functions of HO-2

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Abstract

The constitutive isoform of heme oxygenase, HO-2, is highly expressed in the brain and in cerebral vessels. HO-2 functions in the brain have been evaluated using pharmacological inhibitors of the enzyme and HO-2 gene deletion in *in vivo* animal models and in cultured cells (neurons, astrocytes, cerebral vascular endothelial cells). Rapid activation of HO-2 via post-translational modifications without upregulation of HO-2 expression or HO-1 induction coincides with the increase in cerebral blood flow aimed at maintaining brain homeostasis and neuronal survival during seizures, hypoxia, and hypotension. Pharmacological inhibition or gene deletion of brain HO-2 exacerbates oxidative stress induced by seizures, glutamate, and inflammatory cytokines, and causes cerebral vascular injury. Carbon monoxide (CO) and bilirubin, the end products of HO-catalyzed heme degradation, have distinct cytoprotective functions. CO, by binding to a heme prosthetic group, regulates the key components of cell signaling, including BK_{Ca} channels, guanylyl cyclase, NADPH oxidase, and the mitochondria respiratory chain. Cerebral vasodilator effects of CO are mediated via activation of BK_{Ca} channels and guanylyl cyclase. CO, by inhibiting the major components of endogenous oxidant-generating machinery, NADPH oxidase and the cytochrome C oxidase of the mitochondrial respiratory chain, blocks formation of reactive oxygen species. Bilirubin, via redox cycling with biliverdin, is a potent oxidant scavenger that removes preformed oxidants. Overall, HO-2 has dual housekeeping cerebroprotective functions by maintaining autoregulation of cerebral blood flow aimed at improving neuronal survival in a changing environment, and by providing an effective defense mechanism that blocks oxidant formation and prevents cell death caused by oxidative stress.

Keywords

Heme oxygenase; cerebral protection; cerebrovascular disease; oxidative stress; seizures; carbon monoxide; bilirubin

1. Vasodilator role of CO in cerebral circulation

A. Vasoactive effects of exogenous CO in cerebral vessels

Experimental studies in newborn pigs conducted using *in vivo* and *in vitro* approaches demonstrate that exogenous CO is a potent vasodilator in cerebral circulation [1-7]. CO can be delivered to the brain as CO gas dissolved in a physiological buffer or as recently introduced CO-releasing molecules (CO-RMs) that release CO on illumination (dimanganese decacarbonyl, DMDC) or when dissolved in physiologically buffered solutions (sodium boranocarbonate, CO-RM-A1) [8,9]. *In vivo*, CO, DMDC and CORM-A1 applied directly to the brain surface under the cranial window at concentrations 10^{-7} M causes dilation of pial arterioles, major resistance brain vessels that define the cerebral blood

flow [1, 5, 7]. CO can also be delivered to the brain by systemically administered CO-RM-A1 [7]. In newborn pigs, CO-RM-A1 (2 mg/kg iv or ip) increases the CO level in the brain above the threshold level and elicits dilator effects in pial arterioles [7]. *Ex vivo*, pressurized cerebral arterioles from newborn pigs dilate in response to DMDC, a light-activated CO-RM, upon illumination [3,4]. *In vitro*, smooth muscle cells from cerebral arterioles of newborn pigs respond to gaseous CO by activating large conductance Ca^{2+} -activated K^{+} channels (BK_{Ca} channels) [2, 6, 10].

Vasodilator effects of CO in cerebral vessels are developmentally regulated and may vary between different animal species. Moreover, pial arterioles and small cerebral arteries (30-200 μm) may have greater sensitivity to CO than large arteries. The sensitivity of porcine pial arterioles to CO appears to reduce during postnatal development. In juvenile pigs, *in vivo* responses of pial arterioles to gaseous CO were reduced when compared to newborn pigs [11]. In adult rats, topical CO caused dilation of rat pial arterioles, although maximal responses were smaller than in newborn piglets [11]. However, cerebral arteries isolated from mice, rats, and rabbits did not dilate to 10^{-6} - 10^{-4}M CO [12,13]. Conversely, in another study, dog basilar artery segments did dilate to CO [14].

Mechanisms by which CO causes dilation of cerebral arterioles, involves CO interaction with the components of vascular smooth muscle BK_{Ca} channels (see review [2] for details). CO activates BK_{Ca} channels by increasing the Ca^{2+} sensitivity of the α -subunit of the channel, thereby enhancing coupling of Ca sparks to BK_{Ca} channels. CO also increases Ca^{2+} spark amplitude and frequency. Both actions of CO increase opening of BK_{Ca} channels that hyperpolarizes the smooth muscle leading to dilation of cerebral arterioles [6, 10]. The mechanism of BK_{Ca} channel activation by CO is based on the ability of CO to bind to reduced ferrous heme. The pore-forming α -subunits of the BK_{Ca} channel are heme-binding proteins negatively regulated by heme [15]. CO reverses the channel inhibition by binding to the ferrous heme, the inhibitory ligand of the α -subunit, thus leading to the BK_{Ca} channel activation and, finally, to vascular smooth muscle relaxation [16].

Importantly, vasodilator effects of CO in cerebral arterioles are endothelium-dependent and require the presence of intact endothelial lining. Cerebral vessels with damaged endothelium do not dilate to DMDC, a CO donor [3, 4]. Participation of endothelium in CO-mediated dilation involves endothelium-produced vasodilator messenger, NO, that, via activating guanylyl cyclase, provides sufficient background level of cGMP in vascular smooth muscle as required for activation of BK_{Ca} channels by CO [4, 5]. Therefore, NO and cGMP are intrinsic components in the mechanism of endothelium-dependent dilator responses of cerebral arterioles to CO, although the exact mechanism of this interaction has not yet been deciphered [2].

B. Endogenously produced CO and cerebral vascular reactivity

Endogenous CO is produced from heme via the heme oxygenase (HO)-catalyzed reaction. Endogenous CO production by the brain *in vivo* can be estimated by measuring extracellular CO in cortical periarachnoid cerebrospinal fluid (CSF) bathing the brain surface. To date, brain CO measurements *in vivo* have been collected only in newborn pigs. Basal CO concentration in periarachnoid CSF, as detected by gas chromatography/mass spectrometry, is about $5\text{-}8 \times 10^{-8}\text{M}$ [7,17]. Although the measurements of extracellular concentration in CSF reflect the dynamics of overall CO production by the brain, they do not allow direct estimation of the intracellular level of CO. Brain CO production by the brain is rapidly activated (over 3-fold increase in 10-20 min) in response to selected cerebral vasodilatory stimulations, including glutamatergic seizures [17], hypoxia [18], acute hypotension [19], and excitatory neurotransmitter glutamate [20]. The time-dependent increase of CO concentration in periarachnoid CSF during epileptic seizures positively correlates with

cerebral vasodilation consistent with cause-consequence relationship [17]. Furthermore, the brain-permeable HO inhibitor, tin protoporphyrin (SnPP, 3 mg/kg iv), greatly reduced both CO production and cerebral vasodilation initiated by epileptic seizures [17] and acute hypotension [19]. *Ex vivo* isolated cerebral microvessels (60-200 μ m) rapidly respond to vasodilator glutamatergic stimulation by increasing CO production [20-23].

Overall, these experiments identify CO as a potent cerebral vasodilator endogenously produced by the brain and cerebral vasculature. Of particular significance is that immediate activation of brain HO is essential in maintaining blood flow to the brain during selected cerebrovascular insults, including epileptic seizures, hypoxia, and hypotension.

2. Heme oxygenase isoforms in cerebral circulation

Heme oxygenase (HO-1 and HO-2), in concert with NADPH-cytochrome P(450) reductase and biliverdin reductase, catalyzes degradation of heme to final products, CO and bilirubin. Which HO isoform is responsible for high HO activity/CO production in cerebral vessels and brain parenchyma under physiological and pathological conditions?

A. HO-1 in the brain and cerebral vessels

HO-1, the first isoform discovered, that participates in degradation of red blood cells and in elimination of toxic heme from the body, is highly expressed in reticuloendothelial organs, spleen and liver [24, 25]. HO-1 is an inducible protein (an early response gene, also known as heat shock protein Hsp 32) that is induced in response to oxidative stress challenge. Transcriptional induction of HO-1 is regulated via multiple regulatory elements in the promoter region of HO-1 gene, including stress- and antioxidant- response elements in conjunction with the redox-sensitive transcription factor Nrf2 [26-28], nuclear factor kappa B (NF κ B) [30], and cAMP responsive element CRE [31]. HO-1 protein induction may also occur via translation-independent mRNA stabilization [32]. HO-1 can be induced in a variety of organs (kidneys, lungs, heart) and cell types (including vascular cells) by heme, selected metalloporphyrins, transition metals (iron and cobalt), and pro-oxidants, including peroxynitrite, hydrogen peroxide, NO, sodium arsenite, and oxidized lipids [24, 25, 31-36].

In the normal brain and in cerebral vessels, HO-1 is undetectable, with the exception of few neuronal populations, mainly olfactory and hippocampal neurons [37-42]. In sharp contrast to other tissues, brain and cerebrovascular HO-1 is not readily induced by oxidative stress, with the exception of very limited array of strong pro-oxidant signals. Among few described pathophysiological interventions that upregulate HO-1 in the brain *in vivo*, are hemorrhage [41-43] and hyperthermia [38]. However, epileptic seizures that cause a burst of oxidative stress in the brain failed to induce HO-1 in the brain parenchyma or in cerebral vasculature within 4-24 h [39]. HO-1 upregulation in the brain (neurons and glia) has been described in patients with the chronic neurodegenerative disorders, such as Alzheimer's and Parkinson's diseases (reviewed in [42]).

In cerebral vascular endothelial cells, no induction of HO-1 occurred in response to prolonged stimulation (10-24h) by potent pro-oxidants, including arachidonic acid, the inflammatory cytokine, tumor necrosis factor α (TNF α), or the excitatory neuromediator, glutamate [40, 44, 45]. Furthermore, neither the NO donors SIN-1 (3-morpholinosydnonimine) and SNAP (S-nitroso-N-acetylpenicillamine), strong pro-oxidants, nor the endoplasmic reticulum stress signal, thapsigargin, upregulated HO-1 expression [45]. Among potent oxidants, only peroxynitrite in high concentrations (1 mM) but not hydrogen peroxide (up to 10 mM) induced HO-1 in cerebral vascular endothelial cells [40].

Among metalloporphyrins, only cobalt protoporphyrin (CoPP, 10^{-5}M), and, to a much lesser extent, hemin (10^{-4}M) induced HO-1 in cerebral vascular endothelial cells and in astrocytes from newborn pigs within 14-24h [39, 40, 44, 45]. In contrast, other metalloporphyrins, SnPP and ZnPP, and transition metals (CoCl_2 , FeCl_2 , FeCl_3) over a wide concentration range (up to 10^{-4}M) did not upregulate HO-1 in cerebrovascular endothelial cells [45]. Pharmacological intervention with CoPP has been successfully used to induce HO-1 expression in the brain, cerebral vessels, and cerebrovascular endothelial cells and to investigate brain HO-1 functions *in vivo* and *in vitro* [39, 40, 44]. Induction of HO-1 protein and an overall increase in HO activity in the brain and cerebral vessels was produced 24 h after systemic administration of CoPP to newborn pigs (20 mg/kg ip) [39].

Overall, these data demonstrate that, with a few exceptions, HO-1 is not induced in the brain and cerebral vessels by strong oxidant stimulation relevant to various physiological and pathological conditions. Therefore, HO-1 does not contribute to cerebral vascular responses that involve rapid CO-mediated increases in cerebral blood flow essential in brain homeostasis and neuroprotection, including seizures, hypotension, hypoxia, or glutamate.

B. HO-2 expression in the brain and cerebral vessels

The mammalian brain has a uniquely high HO-2 expression. Abundant HO-2 expression in the brain has been detected in a variety of species, including mice, rats, pigs, and humans. In newborn and mature animals, HO-2 is expressed in neurons [37, 38, 43, 46-54], glia [20, 55], and cerebral vessels [1, 3, 20, 40]. In cerebral vessels, HO-2 is detected in both endothelial and in smooth muscle cells [1, 20]. Among other organs, HO-2 is highly expressed also in testis, where it is developmentally regulated and may play a role in male reproduction [56]. Brain HO-2 is the major isoform that mediates multiple cerebrovascular, glial and neuronal functions in healthy and diseased mammalian brain.

3. Regulation of HO-2 activity

HO-2-mediated CO production by the brain is rapidly increased in response to various physiological stimulations, including glutamate, seizures, hypoxia and hypotension, to provide sufficient blood flow to the brain and maintain neuronal functions. In a model of bicuculline-induced neonatal epileptic seizures, brain CO production increased to about 10^{-6}M (about 20-fold) 10-30 min after seizure onset without changes in HO-2 protein expression or HO-1 induction [17]. CO produced via posttranslational activation of HO-2 contributes to a rapid increase in cerebral blood flow in response to epileptic seizures in newborn pigs [2, 17]. Moreover, neuronal HO-2 activation contributes to epileptiform discharges in newborn pigs [57]. SnPP, a brain-permeable HO inhibitor, attenuated neuronal activation and greatly reduced seizure-evoked CO production and pial arteriolar diameter, emphasizing importance of HO-2 contribution to epileptiform activity and cerebral hyperemia response to seizures [17, 57]. Therefore, regulation of HO-2 enzymatic activity is absolutely essential for brain homeostasis.

What are the mechanisms that regulate HO-2 activity?

A. HO-2 Gene Regulation

Analysis of HO-2 gene structure revealed a glucocorticoid response element (GRE) in the promoter region indicating HO-2 expression can be transcriptionally modulated by corticosteroids [56]. Experimental data confirmed this prediction. Postnatal treatment of newborn rats with corticosterone (5 mg/kg over 4 days) increased HO-2 expression in neurons [58-60]. In newborn piglets, prolonged administration of betamethasone (0.2-5.0 mg/kg ip over 2 days) elevated HO-2 protein in cerebral microvessels [20]. In cultured cerebral vascular endothelial cells, betamethasone (10^{-6}M , 48h) upregulated HO-2 but did

not alter HO-1 expression [20]. These findings indicate that glucocorticoid hormones widely used for management of premature babies have a potential to modulate HO-mediated vascular responses in the neonatal cerebral circulation. However, it should be noted that corticosteroids are weak regulators of brain HO-2 gene in both adult [56] and newborn animals (maximal increase, ~1.5 fold in cerebral vessels after a 2 day-treatment, [20]). Therefore, it is unlikely that transcriptional upregulation of HO-2 gene by glucocorticoids contributes to physiologically relevant HO-mediated processes in the brain and in cerebral vasculature.

B. Posttranslational modification of HO-2

HO-2-catalyzed CO production by the brain, isolated cerebral vessels and cultured cerebral vascular endothelial cells is rapidly increased in response to a variety of stimulants, including epileptic seizures, hypoxia, glutamate and ionotropic glutamate receptor agonists without upregulating HO-2/HO-1 proteins [17, 18, 20-23, 61]. These observations suggest that HO-2 can be rapidly activated by posttranslational modifications. HO-2 activity is rapidly stimulated via posttranslational mechanisms that involve protein phosphorylation. In hippocampal and olfactory neurons, HO-2 is activated during neuronal and odorant stimulation by phosphorylation of serine 79 by casein kinase 2 (CK2) via participation of protein kinase C and calmodulin [62, 63]. In cerebral endothelial cells, stimulation of HO-2 activity by glutamate via ionotropic glutamate receptors (iGluRs) involves tyrosine kinase-mediated but no protein kinase C- or CK-2-mediated phosphorylation [21, 22]. We also found that Ca^{2+} /calmodulin-dependent mechanisms are also involved in the regulation of HO-2-mediated CO production in cerebral endothelial responses to iGluRs agonists [21, 22]. Data in neurons and cerebral vessels demonstrate that, indeed, HO-2 activity is rapidly regulated by multiple factors that affect protein phosphorylation, Ca influx, and Ca^{2+} /calmodulin-dependent molecular events. However, details of these cell- and signal-specific mechanisms and events remain to be uncovered.

C. Heme as a regulator of HO-2 activity

HO activity in the brain is limited by the heme substrate availability. HO-2-dependent CO formation and biological effects of CO can be enhanced by delivery of exogenous or endogenous heme. Experimental data in newborn pigs demonstrate that both exogenous and endogenous heme can be used as the HO substrate to produce CO at physiologically active concentrations. In cerebral circulation in newborn pigs *in vivo*, exogenous heme (hemin or heme-L-Lysinate) placed under a closed cranial window increases CO production by the brain concomitantly with cerebral vasodilation; both effects are blocked with SnPP, a HO inhibitor [17]. Exogenous heme also enhances HO-2-mediated CO production by isolated cerebral vessels, glia, cultured cerebral vascular endothelial and smooth muscle cells and astrocytes [20-22, 61]. These data indicate that cerebral vessels and glia can take up exogenous heme and catabolize it to vasoactive CO.

Endogenous heme is formed in the brain and other tissues from glycine and succinyl-CoA [51, 64]. The rate-limiting enzyme of the heme biosynthesis pathway is 5-aminolevulinate synthase (ALAS). ALAS is a mitochondrial enzyme found in hematopoietic and nonhematopoietic tissues as two differently regulated isoforms, ALAS1 and ALAS2 [65-68]. Erythroid-specific ALAS2 (eALAS) contributes to hemoglobin synthesis in developing erythrocytes. ALAS1 (hALAS) is a ubiquitously expressed housekeeping enzyme, with the highest levels found in liver, heart, and brain [69]. In the brain, endogenous heme level is comparable with that of liver (1.3 nmol/mg protein [51]). ALAS1-mediated formation of endogenous heme is required for synthesis and catabolism of hemoproteins, such as nitric oxide synthase, cyclooxygenase, and mitochondrial cytochromes, essential in brain development and functions [51, 64, 70-72]. Moreover, HO-

catalyzed degradation of endogenous heme produces vasodilator, neuromediator, anti-oxidant and cytoprotective compounds critical for brain functioning. There is a striking similarity in distribution of ALAS and HO in the brain and other tissues, indicating co-compartmentalization of heme synthesis and catabolism [51, 73]. In the cerebral circulation, CO formation is limited by endogenous heme availability. 5-aminolevulinate, the product of ALAS-catalyzed reaction of the heme synthesis, stimulated HO-2-mediated CO production by cerebral vessels [22], indicating that functional interplay between ALAS and HO-2 is important in the regulation of CO-mediated cerebral vascular responses.

ALAS1 activity is regulated by heme via a negative feed back mechanism [51, 66, 67, 69, 74, 75]. As the result, reduction of intracellular heme content due to HO-mediated degradation stimulates overall endogenous heme biosynthesis. Most of the studies on ALAS1 regulation have been conducted in hepatocytes. Complex inhibitory effects of heme on hepatic ALAS1 include transcriptional, post-transcriptional and posttranslational events: a) inhibition of ALAS1 gene transcription, b) reducing mRNA stability, c) blocking mitochondrial import of ALAS1 thus preventing processing of the cytosolic precursor to the mature mitochondrial form, and d) inhibition of the ALAS1 enzyme activity by the end product negative feedback mechanism [66, 67, 69, 76, 77]. Heme is also a negative regulator of heme biosynthesis in the brain, but the mechanism of the inhibition is different from that of liver [51, 64]. In olfactory receptor neurons, heme inhibited another enzyme of heme biosynthesis, ALA dehydratase that catalyzes conversion of 5-aminolevulinic acid to porphobilinogen [51]. Complex tissue-specific regulation of ALAS1 gene includes a multitude of other regulatory factors, including insulin and glucose levels, sex hormones, phorbol esters [78-80]. Hepatic ALA synthase is also inhibited by nonheme metalloporphyrins (Zn, Sn and Co porphyrins) [66, 67, 74, 75]. To date, there is no evidence that the heme biosynthesis can be acutely regulated by posttranslational modifications of ALA synthase, such as protein phosphorylation or Ca^{2+} -dependent modifications.

Overall, signaling factors that increase heme availability by increasing exogenous heme delivery, accelerating heme dissociation from hemoproteins, or by *de novo* heme synthesis can increase brain CO production.

4. Glutamate receptors in cerebral vascular endothelium and HO-2 activity

Cerebral vessels express functional glutamate receptors (GluRs), and, therefore, may directly respond to the neurotransmitter stimulation [61]. Glutamate is the principal excitatory neurotransmitter in mammalian brain. Effects of glutamate in the brain are mediated via a diversity of GluRs expressed in neurons [81, 82], glia [83] and in cerebral vascular endothelium [61]. Neurons and glia are commonly recognized as the major targets for glutamate in the brain, but the existence of functional GluRs in cerebral vasculature has been long debated. However, the vast body of evidence accumulated over the recent years clearly demonstrates that GluRs are expressed in cerebral vascular endothelium and contribute to the endothelial functions in rats, humans, and newborn pigs [61, 84-88].

We demonstrated that porcine cerebral vascular endothelium expresses functional GluRs linked to production of CO. The density of functional high-affinity GluRs in cerebral vascular endothelial cells from newborn pigs detected by a radioligand displacement binding of L-[G- ^3H]glutamic acid is higher than in the brain parenchyma [61]. In cerebral vascular endothelial cells, all major types of GluRs, including ionotropic GluRs (iGluRs, NMDA- and AMPA-subtypes) and metabotropic GluRs (mGluRs), are directly identified by the radioligand binding, pharmacological profiling, and immunoblotting. Functionally, cerebral vascular iGluR are linked to HO-2 activation. Glutamate and iGluR (but not mGluR) agonists increase HO-2-mediated CO production by cerebral vessels and cerebral

endothelial cells [21, 22, 61]. Furthermore, isolated pressurized cerebral vessels, in the absence of neuronal input, respond to glutamate and iGluRs stimulation by endothelium-dependent HO-2-mediated dilation [3].

The mechanisms by which cerebral endothelial iGluR are coupled to HO-2 enzymatic activity are complex and not yet completely understood. iGluR-mediated increases in CO production by cerebral microvessels involve protein tyrosine kinase- and Ca^{2+} -mediated pathways leading to posttranslational activation of HO-2 and, possibly, enhanced endogenous heme delivery [21, 22]. It has been reported that neuronal HO-2 is activated by posttranslational modifications via calcium/calmodulin, casein kinase CK2 and PI3 kinase signaling [62, 63]. In cerebral vessels, we found no evidence that casein kinase CK2 or PI3 Kinase are involved in the mechanism of CO increase by glutamate [21, 22] indicating cell-specific regulatory mechanisms of HO-2 activation. Recent evidence suggests that the endothelial NO synthase (eNOS)/guanylyl cyclase ensemble is required for glutamate-increased CO production in cerebral vessels [90]. Inhibition of NOS or soluble guanylyl cyclase blocked, while 8-bromo cGMP mimicked, the ability of glutamate to stimulate cerebrovascular CO production. Therefore, eNOS, by elevating cGMP level, is a likely key upstream player in the signaling mechanism of HO-2 activation. eNOS, a Ca^{2+} /calmodulin-dependent enzyme, is highly regulated by intracellular Ca^{2+} [91, 92] and, therefore, it is possible that glutamate, via iGluR-mediated Ca^{2+} entry and/or protein phosphorylation, initiates eNOS-mediated cGMP-dependent signaling cascade leading to HO-2 activation.

Neurons and glia co-express GluRs and HO-2 and, therefore, may potentially respond to glutamate by increasing CO production. Indeed, astrocytes from the newborn piglet brain (freshly isolated or in primary cultures) respond to glutamate by rapidly increasing CO production via post-translational activation of HO-2 [93]. Interestingly, HO-1 pharmacologically induced in astrocytes by CoPP, was not further activated by glutamate, indicating that HO-1, in contrast to HO-2, is not regulated post-translationally [93]. It is not known whether glutamate stimulation leads to activation of neuronal HO-2. Although neurally-released CO can influence cerebral blood flow, no experimental data supporting activation of neuronal HO-2 by glutamate have been collected to date. *In vivo*, brain glia appears to be a major cellular source of glutamate-increased CO production by the brain, because a selective glia toxin, L-aminoadipic acid, blocks the CO responses to topical glutamate [20].

Overall, cerebral vessels and cerebral vascular endothelial cells, as well as neurons and glia, via GluRs, directly respond to glutamatergic stimulation. In cerebral vascular endothelium and in astrocytes, GluRs are functionally linked to HO-2 activation. Glutamatergic stimulation, via a GluR-mediated posttranslational HO-2 activation in the brain, contributes to immediate cerebral vascular responses and modulation of cerebral blood flow.

5. Seizures, neuronal injury, and cerebral vascular function

Seizures are the manifestation of abnormal synchronous neuronal discharges as a result of imbalance between the excitatory and inhibitory transmissions in favor of net glutamatergic excitation of cortical neurons. Seizures are accompanied by a marked increase in cerebral metabolic rate as indicated by increased glucose and oxygen utilization [81, 94-98]. The cerebral cortex is among the brain areas with the highest increase in metabolic rate. The brain glucose and high-energy phosphates (ATP and phosphocreatine) levels decrease significantly when seizures are extended for over 30 min (epileptic seizures), indicating that energy demands may exceed glucose supply to the brain [98].

Increased cerebral blood flow acts to compensate for the extreme metabolic demands of hyperactive neurons of the brain. During seizures, dramatic increases in cerebral blood flow

(up to 7-fold) have been documented in adult and newborn animals [81, 94, 95, 97-99] and in human neonates [100, 101]. Cerebral blood flow is tightly coupled to seizure activity and increases immediately with the onset of electrical discharge [57, 102]. In human patients, a substantial increase in cerebral blood flow within 6-10 sec after the seizure onset was terminated within 1 min after the end of seizures [102].

Loss of coupling between cerebral blood flow and metabolic rate that may occur during epileptic seizures is likely to result in brain damage and adverse clinical outcome [81, 96, 97, 100]. Seizures can damage the brain cells, modify brain functions, and increase the risk for persistent epilepsy [81, 100, 103-107]. In young experimental animals, repeated seizures have long-lasting debilitating effects on brain development, learning abilities, and behavioral milestones [106-108]. Therefore, an increase in cerebral blood flow is required to match brain metabolism to protect the brain from the damaging effects of extended seizures, while insufficient elevation of cerebral blood flow would exacerbate the neuronal damage.

Clinical and experimental evidence strongly suggests that seizures are associated with cerebral vascular injury manifested morphologically and functionally. Ultrastructural changes in brain capillaries, vascular lesions and malformations have been reported in relation to epileptic seizures [81, 103, 105, 109]. Endothelial cells of the brain microvessels comprise the blood brain barrier that limits the transport of nutrients and metabolites to and from the brain. Blood brain barrier malfunctioning or severe disruption has been observed in animal seizure models [109, 110] and in epileptic patients [103, 111]. Functionally, epileptic seizures cause a sustained loss of cerebral vascular function that is extended for at least two days after seizures (delayed postictal period) [17]. Sustained postictal cerebral vascular dysfunction is characterized by reduced vasoreactivity to physiologically relevant dilators, including hyperapnia and bradykinin [17, 39]. Postictal cerebral vascular dysfunction and subsequent loss of blood flow autoregulation may substantially contribute to neurological problems that are frequently observed in epilepsy patients during postictal period.

Seizures cause extensive formation of reactive oxygen species (ROS) and oxidative stress [112, 113]. Oxidative damage to cell proteins, lipids, and DNA may explain long-term debilitating consequences of seizures to the brain [81, 96, 107, 114]. Glutamate excitotoxicity is a primary mechanism in seizure- and oxidative stress-induced brain injury [81, 82, 115-117]. In the brain, the intracellular concentration of glutamate is in the 10^{-3} M range, and extracellular concentration is maintained at 10^{-6} - 10^{-5} M [81, 83, 115]. Massive release of glutamate is a central event in recruiting neurons into epileptic discharges, and glutamate receptors and transporters play crucial roles in seizures and epilepsy [95, 116-118]. During seizures, the glutamate concentration in the brain can be elevated to a cytotoxic level [83, 117]. Excitatory neurotransmitters at excitotoxic concentrations (10^{-4} - 10^{-3} M) act as endogenous neurotoxins [47, 48, 83, 95, 113, 117, 119]. Disruption of Ca^{2+} homeostasis and oxidative stress appear to be key mechanisms in glutamate cytotoxicity [113, 119, 120]. Neurotoxic effects of glutamate are mediated via glutamate receptors, and the most rapid neuronal injury results from activation of NMDA receptors and AMPA/Kainate receptors and massive Ca^{2+} entry [81, 95, 113]. Ca^{2+} overload of mitochondria leads to ROS generation, cytochrome c release and activation of Ca^{2+} -dependent proteases that promote apoptosis [113, 121]. Cerebrovascular endothelial cells are also major targets for glutamate toxicity [40, 88]. In cerebral vascular endothelial cells from newborn pigs, glutamate (10^{-4} - 10^{-3} M) increases superoxide production and causes oxidative stress-related cell death by apoptosis [40].

Another seizure-related stress factor, $\text{TNF}\alpha$, is a proinflammatory cytokine that causes oxidative stress and apoptosis in the brain [122-124]. Brain $\text{TNF}\alpha$ production is increased during seizures and contributes to neuronal death [122, 125, 126]. $\text{TNF}\alpha$ also directly

targets the cerebral vasculature [44]. In cerebral vascular endothelial cells from newborn pigs and adult rats, TNF α increases ROS formation and causes oxidative-stress-mediated apoptosis [44].

Recently accumulated evidence reviewed below indicates that HO provide an effective cerebroprotective mechanism against damaging effects of seizures by a) increased cerebral blood flow to the brain during seizures, and b) protecting against cerebral vascular endothelial damage caused by major seizure-related stress factors, glutamate and TNF α .

6. Cerebroprotective role of HO-1

It has been widely accepted that HO-1 upregulated in response to a multitude of oxidative stress factors provides a potent endogenous antioxidant protection in a variety of cells and tissues [26, 27, 127]. However, little is known about the physiological importance of HO-1 in the brain. A patient with HO-1 deficiency manifested hemolytic anemia, iron deposition and kidney damage, but no brain abnormalities have been reported [128]. Brain HO-1 can be induced by hemorrhage related to brain trauma or cerebral infarctions [129], but physiological implications are vague.

Pharmacological induction of HO-1 in the brain prevents long-term cerebrovascular injury caused by epileptic seizures [39]. In the newborn pig model, bicuculline-induced epileptic seizures cause a loss of cerebral vascular responsiveness to physiologically relevant dilator stimuli sustained for at least 2 days after the ictal episode [17]. Inadequate vasoreactivity, a functional manifestation of cerebral vascular injury, can lead to disturbances in cerebral blood flow regulation and is likely to contribute to neuronal injury. Neuronal sequelae, including learning and memory deficits, occur as a consequence of epileptic seizures in adult and newborn animals and in human patients [81, 100-108]. Remarkably, in piglets with pharmacologically induced brain HO-1 (by systemic CoPP), seizures did not cause cerebral vascular injury [39]. However, taking into account the fact that HO-1 is not induced in the brain and cerebral vessels by epileptic seizures [39], physiological significance of HO-1 in preventing postictal cerebral vascular injury is obscure.

7. Cerebroprotective role of HO-2

Because HO-2 is the only isoform expressed in the brain and in cerebral vasculature (as discussed above), it was reasonable to ask the question whether HO-2 is essential in brain cell survival under conditions of excitotoxic and oxidative stress. Using *in vivo* and *in vitro* approaches, we investigated the potential cerebroprotective role of HO-2 against cerebral vascular injury caused by seizures, as well as glutamate and TNF α , major seizure-related excitotoxic and inflammatory stress factors.

A. HO-2 protection against seizure-induced cerebral vascular injury in vivo

In vivo, inhibition of brain HO-2 enzymatic activity by systemic administration of brain-permeable HO inhibitors, SnPP or chromium mesoporphyrin (CrMP), greatly attenuates cerebral vasodilation in response to bicuculline-induced seizures in newborn pigs, thus creating a harmful mismatch between neuronal activation and cerebral blood flow responses [7, 17, 130]. Moreover, inhibition of HO-2 activity immediately before seizures has long-term consequences for the brain because it greatly exacerbates postictal cerebrovascular injury. A substantial loss of cerebral vascular responsiveness to major endothelium-dependent dilator stimuli, hypercapnia, bradykinin, and heme, indicative of severe sustained endothelial dysfunction, is observed two days after the seizure episode in SnPP-pretreated animals [7, 17, 39]. Postictal endothelium-independent responses of cerebral arterioles to isoproterenol and sodium nitroprusside are minimally affected by HO-2 inhibition during

pre-ictal state, suggesting a specific importance of HO-2 in protecting endothelial function in cerebral circulation.

B. HO-2 protection against glutamate-induced cerebral endothelial injury *in vitro*

Glutamate, the major excitatory neurotransmitter in the central nervous system, is also a potent excitotoxic factor that triggers neuronal damage and death (as discussed above). Massive release of glutamate to excitotoxic level occurs during seizures, ischemia, hypoxia, and brain injury [115]. Glutamate-induced brain cell injury is involved in the pathogenesis of neurodegenerative disorders, stroke, seizures and epilepsy [95, 115, 119, 131].

Cerebral vascular endothelial cells express functional glutamate receptors (as discussed above) and, therefore, can be directly targeted by glutamate. In primary cultures of cerebral vascular endothelial cells from newborn pigs and adult mice, glutamate (10^{-4} - 10^{-3} M) increases formation of various ROS, including superoxide anion, and causes cell death by apoptosis [40]. Apoptosis is characterized by a series of highly regulated events that result in DNA laddering, chromatin condensation, cell shrinkage, membrane blebbing, and, finally, cell death by fragmentation into apoptotic bodies, and phagocytosis of the cell remnants [132, 133]. Glutamate-induced hallmark apoptotic events in cerebrovascular endothelial cells include nuclear translocation of the transcription factor NF κ B, caspase-mediated proteolysis and executioner caspase-3 activation, DNA fragmentation, loosening of cell-cell and cell-matrix contacts and cell detachment [40]. Glutamate-induced apoptosis in cerebrovascular endothelial cells is prevented by treatment with superoxide dismutase (SOD) and, therefore, is mediated via oxidative stress, mainly, by superoxide anion.

HO-2 gene deletion (HO-2 knock-out mice) or pharmacological inhibition of HO-2 activity by SnPP (newborn pigs) greatly exacerbated glutamate-induced apoptosis [40]. Pharmacological induction of HO-1 induced by CoPP treatment 24 h before the insult provided resistance to cerebral vascular endothelial cells against glutamate-induced death [40]. Taking into account that HO-1 is not induced in cerebral vasculature by glutamate *in vitro* or glutamatergic seizures *in vivo* [17, 39, 40], HO-1 would not appear to provide endogenous protection against glutamate toxicity in the cerebral endothelium. In contrast, HO-2 emerges as a critical essential physiologically relevant endogenous cytoprotective system against glutamate-induced apoptosis in cerebral vascular endothelium.

C. HO-2 protection against oxidative stress-related neuronal injury

Oxidative stress is a common cause of neuronal injury and death, and potent anti-oxidant defense in the brain is absolutely essential for normal brain functioning. Evidence accumulated in *in vivo* and *in vitro* experimental models indicates that HO-2 is an essential and powerful component of anti-oxidant defense in the brain. In HO-2 knock-out mice (HO2-KO), oxidative stress-related neuronal damage caused by intracranial injection of NMDA or by focal ischemia/reperfusion was substantially worsened [47, 48]. In a model of focal ischemia/vascular stroke by middle cerebral artery occlusion and reperfusion, HO2-KO mice had significantly increased incidence of neuronal death via apoptosis, but not necrosis, indicating that HO-2 protection is predominantly linked to apoptotic neuronal death [47]. The cerebral infarction volume was greatly increased in HO2-KO mice as well as in WT mice with SnPP-inhibited HO-2 enzymatic activity [47]. In a model of glutathione depletion-induced oxidative injury, brain neuronal damage and olfactory neuronal death were also greatly aggravated in HO2-KO mice [37].

D. HO-2 protection against inflammation-induced endothelial injury

Pro-inflammatory cytokines mediate brain inflammatory disease related to seizures, sepsis, stroke, and brain trauma [123, 124]. Brain endothelium is highly susceptible to injury caused

by the pro-inflammatory mediator, TNF α , abundantly produced by injured brain. In porcine and murine cerebral vascular endothelial cells, TNF α is a potent pro-oxidant and pro-apoptotic signal that induces key events of apoptosis within 1-3h [44]. NADPH oxidase is identified as the major superoxide-producing enzyme in cerebral vascular endothelial cells that is immediately activated by the cytokine and initiates a signaling cascade leading to apoptosis [134]. We investigated whether HO-2 is cytoprotective against TNF α -induced oxidative stress-mediated endothelial injury in cerebral circulation. HO-2 gene deletion (HO2-KO mice) greatly sensitized cerebral endothelial cells to TNF α -induced apoptosis [44]. In cerebral vascular endothelial cells from HO-2 KO mice, all TNF α -induced events of apoptosis, including DNA fragmentation, caspase-3 activation, nuclear translocation of pro-apoptotic nuclear transcription factor NF κ B, and disruption of cell-cell and cell-matrix contacts were greatly exacerbated compared to WT mice. Pharmacological inhibition of HO activity in cerebral vascular endothelial cells mimicked the effects HO-2 gene deletion. In porcine cerebral vascular endothelial cells, SnPP greatly potentiated apoptotic effects of TNF α , suggesting that the constitutive HO-2 enzymatic activity provides an immediate cerebroprotection against inflammation-induced injury [44].

Cerebral vascular endothelial cells with pharmacologically induced HO-1 were completely resistant against TNF α -induced apoptosis [44]. However, because TNF α itself does not induce HO-1 in cerebral vascular endothelial cells even with 24 h of treatment [44], while irreversible events of apoptotic cell death occur within 3h, a physiological contribution of HO-1 to anti-oxidant defense against inflammation-induced endothelial injury is unlikely.

Overall, HO-2 is an essential component of the cerebral vascular defense system that provides resistance of cerebral vascular function to stress, excitotoxic amino acids and inflammatory mediators by enhancing anti-oxidant defense and promoting cell survival mechanisms.

E. HO-2 protection against hemorrhage and traumatic brain injury

Extracellular heme is a potent pro-oxidant that may cause long-term neuronal damage during hemorrhage, stroke, and traumatic brain injury [135, 136]. Heme is a potent HO-1 inducer, and HO-1 is induced in the brain following traumatic brain injury and hemorrhage [43]. However, recent studies pinpoint to importance of HO-2 in protecting the brain from consequences of hemorrhage and traumatic brain injury [43, 135-139]. In HO2-KO mice, oxidative stress estimated by lipid peroxidation in the hemorrhage-injured brain was greatly increased [43, 137, 138]. HO-2 gene deletion also increased brain injury volume and potentiated extended neuronal damage caused by brain hemorrhage [43, 137-139]. These studies emphasize that HO-2 is a crucial neuroprotective enzyme in detoxifying high levels of heme in the brain [139].

8. Cerebroprotective properties of the end products of HO-catalyzed heme degradation

Cytoprotective effects of HO-2 require the enzyme activity because HO inhibitors mimic HO-2 gene deletion in neurons and cerebral endothelial cells. The products of HO-catalyzed heme degradation, bilirubin and CO, have multiple anti-oxidant properties that include inhibiting formation of ROS, quenching free radicals and, therefore, blocking key signaling pathways leading to oxidative stress-induced cell death by apoptosis.

A. Anti-oxidant and anti-apoptotic properties of bilirubin

Bilirubin is formed by enzymatic reduction of biliverdin by biliverdin reductase abundantly expressed in tissues with high content of HO [24, 25, 141]. Bilirubin, largely formed from

hemoglobin degradation in the spleen, in a complex with albumin is transported by blood to the liver for conversion to bilirubin diglucuronide (conjugated bilirubin) and further excretion from the body. Under physiological conditions, bilirubin concentrations in plasma range from 5 to 17 μM (<1 mg/100 ml [141]). Bilirubin from the blood stream can penetrate the brain with normal or compromised blood brain barrier and accumulate in all cell compartments, including cytosol, nuclei, and membranes [142]. Bilirubin at high concentrations (>300 μM , or 25 mg/100 ml plasma level) has been long considered as a cytotoxic factor that deposits in the brain and causes encephalopathy in severely jaundiced newborn babies [143]. High concentrations of bilirubin (>50 μM) are cytotoxic and cause neuronal and glial cell death by apoptosis and necrosis [144, 145]. However, cerebral vascular endothelial cells are resistant to bilirubin cytotoxicity: 3h-exposure to 50 μM unconjugated bilirubin does not cause apoptosis (Parfenova, unpublished observations).

Conversely, at low concentrations ($<1\mu\text{M}$), bilirubin has potent anti-oxidant properties, as has been demonstrated in cells, animal models, and in clinical studies [25, 47, 140, 144, 146-148]. The mechanism of its antioxidant effects involves effective scavenging of oxidant free radicals by bilirubin/biliverdin redox cycling. Bilirubin is oxidized by ROS to biliverdin, and then recycled by abundantly expressed biliverdin reductase back to bilirubin [140, 149]. Bilirubin may also quench radicals by donating a reactive hydrogen atom thus forming a bilirubin radical rather than biliverdin [141]. Bilirubin exerts its anti-oxidant effects most effectively in albumin-bound form, which provides most favorable configuration of the reactive hydrogen atoms of bilirubin for participation in redox reactions [141]. Submaximal concentrations of bilirubin in newborn babies are associated with the overall high anti-oxidant capacity of plasma, a lower incidence of oxidative stress injury and protection against retinopathy [146, 147]. We demonstrated potent oxidant-quenching properties of bilirubin by measurements of oxidative stress in cerebral vascular endothelial cells caused by glutamate and $\text{TNF}\alpha$ [40, 44]. We found that albumin-bound bilirubin (0.1-10 μM) eradicated superoxide and other ROS produced by cerebral vascular endothelial cells stimulated by these potent pro-oxidants.

Oxidative stress may cause cell death by apoptosis and necrosis. Therefore, as an effective oxidant scavenger, bilirubin may be beneficial for cell survival. This notion is supported by evidence in neurons and cerebral endothelial cells. Bilirubin protected cultured neurons against oxidative stress-related death caused by glutamate and hydrogen peroxide [140, 144]. In cerebral vascular endothelial cells, bilirubin (0.1-10 μM) blocked early and late apoptotic events initiated by glutamate and $\text{TNF}\alpha$ [40, 44]. Bilirubin provided even better cytoprotection from oxidative stress-induced cerebral endothelial death than SOD and Tiron, potent anti-oxidants [40, 44].

Overall, bilirubin has dual actions in the brain. At high concentrations (10^{-4}M) and, especially, following the extended exposure (up to several days), bilirubin deposits in brain cells and causes apoptosis and necrosis. Bilirubin-related encephalopathy has been described in severely jaundiced newborn babies when in blood the bilirubin level exceeds 25 mg/dL ($4\times 10^{-4}\text{M}$). Conversely, at low concentrations (10^{-8} - 10^{-6}M), bilirubin is a potent oxidant scavenger that may rescue cells from oxidative stress-induced apoptosis.

B. Anti-oxidant and anti-apoptotic properties of CO

Data accumulated over last several years indicate that exogenous CO, at low concentrations, has potent anti-oxidant and anti-apoptotic effects in vivo and in vitro [7, 40, 44, 150]. Gaseous CO (200 ppm for 48h) blocked vascular smooth muscle cell apoptosis caused by inflammatory cytokines [150]. Introduction of CO-RMs that provide a sustained CO delivery upon systemic administration and in cultured cells has greatly advanced our knowledge of CO-mediated cytoprotection [7, 40, 44, 151-153]. Exogenous CO, delivered

either as CO gas or by CO-RMs, exhibited cardioprotective effects in hypoxia-reoxygenation model in isolated hearts [151, 152] and during heart transplantation [151, 152, 154].

In cerebral vascular endothelial cells, CO released from a water-soluble CO-RM-A1 (1 μ M) completely blocked ROS formation evoked by glutamate and TNF α [40, 44]. Furthermore, CO-RM-A1 prevented oxidative stress-induced apoptosis induced by glutamate and TNF α in cerebral endothelial cells [40, 44]. CO also has potent cerebroprotective properties. *In vivo*, systemically administered CO-RM-A1 effectively delivers CO to the brain as demonstrated by direct measurements of CO in newborn pigs [7]. CO-RM-A1 (2 mg/kg iv or ip) administered shortly before epileptic seizures prevented sustained postictal cerebral vascular injury in newborn pigs [7]. Overall, these results provide evidence that CO is an effective anti-oxidant and cytoprotective factor in the brain, heart and kidney.

What are the mechanisms by which CO blocks oxidative stress and exhibits cytoprotective effects? Cytoprotective effects of CO are based on its ability to interact with heme that constitutes a prosthetic group of major ROS-generating systems, including NADPH oxidase and the mitochondrial respiratory chain. Inhibition of NADPH oxidase activity by CO has been demonstrated in vascular smooth muscle [155] and cerebral vascular endothelial cells (Basuroy, unpublished observations). NADPH oxidase activation is a major early event in a signaling cascade leading to apoptosis of cerebral vascular endothelial cells caused by TNF α [134] and therefore, CO, by inhibiting NADPH oxidase, prevents apoptosis initiated by TNF α (Basuroy, unpublished observations). Conversely, activation of mitochondrial pathway is a major cause of cerebral endothelial death induced by excitotoxic glutamate [134]. CO inhibits activation of mitochondrial respiratory chain-mediated ROS production in response to glutamate thus leading to prevention of glutamate-induced apoptosis in cerebral vascular endothelium [134]. CO inhibition of the mitochondrial pathway of apoptosis (cytochrome C release and activation of p52 transcription factor) has been demonstrated in vascular smooth muscle [150] and in isolated mitochondria [156]. CO, by inhibiting mitochondrial oxidative phosphorylation, promoted survival of pre-transplanted kidneys [153]. The ability of CO to stimulate the activity of heme-containing soluble guanylyl cyclase in vascular smooth muscle has been linked to anti-apoptotic cell defense against inflammatory cytokines [150].

Overall, the products of HO activity, CO and bilirubin, via different mechanisms, have potent antioxidant and anti-apoptotic capacities. CO blocks the formation of free oxygen radicals by inhibiting the activity of major ROS-producing systems, NADPH oxidase and the mitochondria respiratory chain. Bilirubin, via the redox cycling reaction, effectively removes preformed ROS directly by scavenging. In ensemble, CO and bilirubin, by inhibiting formation and quenching oxidant radicals, prevent oxidative stress-induced signaling cascade leading to apoptosis.

9. Conclusion

The heme oxygenase (HO)-catalyzed reaction of heme degradation produces compounds that uniquely combine vasodilatory (CO), anti-oxidant (CO and bilirubin) and anti-apoptotic (CO, bilirubin) properties. Overall, HO has a well-deserved reputation of a potent endogenous anti-oxidant cell defense system. Until recently, HO-1, transcriptionally upregulated in response to oxidative stress, was considered the only HO isoform that provided cytoprotection. However, it is becoming evidently clear that, in the brain, HO-2, rather than HO-1, participates in a multitude of housekeeping functions directed at maintaining brain homeostasis and protecting against oxidative stress under constantly changing conditions. The ability of HO-2 to be posttranscriptionally activated provides

immediate actions including cerebral vasodilation, blocking oxidant-producing systems, scavenging ROS, and, therefore, preventing and eliminating further damage caused by ROS. Cerebroprotective functions of HO-2 include CO-mediated increases in cerebral blood flow in response to seizures, hypoxia, hypotension, and glutamate aimed at providing neurons with nutrients and oxygen. In addition, CO, via its ability to strongly bind to heme, inhibits heme-containing ROS-generating systems, NADPH oxidase and the mitochondrial respiratory chain, thus reducing oxidative stress. Furthermore, bilirubin, a potent ROS scavenger, eliminates preformed oxidant radicals thus strengthening the anti-oxidant effects of CO. Therefore, HO-2 builds a strong cerebroprotective system in the brain and cerebral circulation that, in contrast to HO-1, provides immediate responses to cerebrovascular stress and prevents potential damage to neurons, astrocytes, and cerebral vascular endothelium.

References

1. Leffler CW, Nasjletti A, Yu C, Johnson RA, Fedinec AL, Walker N. Carbon monoxide and cerebral microvascular tone in newborn pigs. *Am J Physiol*. 1999; 276:H1641–6. [PubMed: 10330249]
2. Leffler CW, Parfenova H, Jaggar JH, Wang R. Carbon monoxide and hydrogen sulfide: gaseous messengers in cerebrovascular circulation. *J Appl Physiol*. 2006; 100:1065–76. [PubMed: 16467393]
3. Fiumana E, Parfenova H, Jaggar JH, Leffler CW. Carbon monoxide mediates vasodilator effects of glutamate in isolated pressurized cerebral arterioles of newborn pigs. *Am J Physiol Heart Circ Physiol*. 2003; 284:H1073–9. [PubMed: 12666665]
4. Barkoudah E, Jaggar JH, Leffler CW. The permissive role of endothelial NO in CO-induced cerebrovascular dilatation. *Am J Physiol*. 2004; 287:H1459–65.
5. Koneru P, Leffler CW. Role of cGMP in carbon monoxide-induced cerebral vasodilation in piglets. *Am J Physiol Heart Circ Physiol*. 2004; 286:H304–9. [PubMed: 14684363]
6. Jaggar JH, Leffler CW, Cheranov SY, Tcheranova DES, Cheng X. Carbon monoxide dilates cerebral arterioles by enhancing the coupling of Ca^{2+} sparks to Ca^{2+} -activated K^{+} channels. *Circ Res*. 2002; 91:610–7. [PubMed: 12364389]
7. Zimmermann A, Leffler CW, Tcheranova D, Fedinec A, Parfenova H. Cerebroprotective effects of the CO-releasing molecule, CORM-A1, against seizure-induced neonatal vascular injury. *Am J Physiol Heart Circ Physiol*. 2007 Epub July 2007.
8. Motterlini R, Clark JE, Foresti R, Sarathchandra P, Mann BE, Green CJ. Carbon monoxide-releasing molecules: characterization of biochemical and vascular activities. *Circ Res*. 2002; 90:E17–24. [PubMed: 11834719]
9. Motterlini R, Sawle P, Hammad J, Bains S, Alberto R, Foresti R, Green CJ. CORM-A1: a new pharmacologically active carbon monoxide-releasing molecule. *FASEB J*. 2005; 19:284–6. [PubMed: 15556971]
10. Xi Q, Tcheranova D, Parfenova H, Horowitz B, Leffler CW, Jaggar JH. Carbon monoxide activates KCa channels in newborn arteriole smooth muscle cells by increasing apparent Ca^{2+} sensitivity of α -subunits. *Am J Physiol Heart Circ Physiol*. 2004; 286:H610–8. [PubMed: 14563665]
11. Holt DC, Fedinec AL, Vaughn AN, Leffler CW. Age and species dependence of pial arteriolar responses to topical carbon monoxide in vivo. *Exp Biol Med*. 2007 in press.
12. Andresen JJ, Shafi NI, Durante W, Bryan RM Jr. Effects of carbon monoxide and heme oxygenase inhibitors in cerebral vessels of rats and mice. *Am J Physiol*. 2006; 291:H223–30.
13. Brian JE Jr, Heistad DD, Faraci FM. Effect of carbon monoxide on rabbit cerebral arteries. *Stroke*. 1994; 25:639–43. [PubMed: 8128519]
14. Komuro T, Borsody MK, Onon S, Marton LS, Weir BK, Zhang ZD, Paik E, MacDonald RL. The vasorelaxation of cerebral arteries by carbon monoxide. *Exp Biol Med*. 2001; 226:860–5.
15. Tang XD, Xu R, Reynolds MF, Garcia ML, Heinemann SH, Hoshi T. Haem can bind to and inhibit mammalian calcium-dependent Slo1 BK channels. *Nature*. 2003; 425:531–5. [PubMed: 14523450]

16. Jaggar JH, Li A, Parfenova H, Liu J, Umstot ES, Dopico AM, Leffler CW. Heme is a carbon monoxide receptor for large-conductance Ca^{2+} -activated K^{+} channels. *Circ Res*. 2005; 97:805–12. [PubMed: 16166559]
17. Carratu P, Pourcyrus M, Fedinec A, Leffler CW, Parfenova H. Endogenous heme oxygenase prevents impairment of cerebral vascular functions caused by seizures. *Am J Physiol Heart Circ Physiol*. 2003; 285:H1148–57. [PubMed: 12915392]
18. Kanu A, Leffler CW. Carbon monoxide and Ca^{2+} -activated K^{+} channels in cerebral arteriolar responses to glutamate and hypoxia in newborn pigs. *Am J Physiol Heart Circ Physiol*. 2007 (in press).
19. Kanu A, Whitfield J, Leffler CW. Carbon monoxide contributes to hypotension-induced cerebrovascular vasodilation in piglets. *Am J Physiol Heart Circ Physiol*. 2006; 291:H2409–14. [PubMed: 16751286]
20. Parfenova H, Neff RA III, Alonso JS, Shlopov BV, Jamal CN, Sarkisova SA, Leffler CW. Cerebral vascular endothelial heme oxygenase: expression, localization, and activation by glutamate. *Am J Physiol Cell Physiol*. 2002; 281:C1954–63. [PubMed: 11698254]
21. Leffler CW, Balabanova L, Fedinec AL, Waters CM, Parfenova H. Mechanism of glutamate stimulation of CO production in cerebral microvessels. *Am J Physiol Heart Circ Physiol*. 2003; 285:H74–80. [PubMed: 12623781]
22. Leffler CW, Balabanova L, Sullivan D, Wang X, Fedinec AL, Parfenova H. Regulation of CO production in cerebral microvessels of newborn pigs. *Am J Physiol Heart Circ Physiol*. 2003; 285:H292–7. [PubMed: 12623784]
23. Robinson JS, Fedinec AL, Leffler CW. Role of carbon monoxide in glutamate receptor-induced dilation of newborn pig pial arterioles. *Am J Physiol Heart Circ Physiol*. 2002; 282:H2371–6. [PubMed: 12003848]
24. Maines MD. Heme oxygenase: function, multiplicity, regulatory mechanisms, and clinical applications. *FASEB J*. 1988; 2:2557–68. [PubMed: 3290025]
25. Maines MD. The heme oxygenase system and its functions in the brain. *Cell Molec Biol*. 2000; 46:573–85. [PubMed: 10872744]
26. Alam J, Cook JL. Transcriptional regulation of the heme oxygenase-1 gene via the stress response element pathway. *Curr Pharm Des*. 2003; 9:2499–511. [PubMed: 14529549]
27. Alam J, Cook JL. How many transcription factors does it take to turn on the heme oxygenase-1 gene? *Am J Respir Cell Mol Biol*. 2007; 36:166–74. [PubMed: 16990612]
28. Martin D, Rojo AI, Salinas M, Diaz R, Gallardo G, Alam J, De Galarreta CM, Cuadrado A. Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. *J Biol Chem*. 2004; 279:8919–29. [PubMed: 14688281]
29. Nguyen T, Sherratt PJ, Pickett CB. Regulatory mechanisms controlling gene expression mediated by the antioxidant response element. *Annu Rev Pharmacol Toxicol*. 2003; 43:233–60. [PubMed: 12359864]
30. Lavrovsky Y, Schwartzman ML, Abraham NG. Novel regulatory sites of the human heme oxygenase-1 promoter region. *Biochem Biophys Res Commun*. 1993; 196:336–41. [PubMed: 8216309]
31. Kronke G, Bochkov VN, Huber J, Gruber F, Bluml S, Furnkranz A, Kadl A, Binder BR, Leitinger N. Oxidized phospholipids induce expression of human heme oxygenase-1 involving activation of cAMP-responsive element-binding protein. *J Biol Chem*. 2003; 278:51006–14. [PubMed: 14523007]
32. Bouton C, Demple B. Nitric oxide-inducible expression of heme oxygenase-1 in human cells. Translation-independent stabilization of the mRNA and evidence for direct action of nitric oxide. *J Biol Chem*. 2000; 275:32688–93. [PubMed: 11032845]
33. Abraham NG, Botros FT, Rezzani R, Rodella L, Bianchi R, Goodman AI. Differential effect of cobalt protoporphyrin on distributions of heme oxygenase in renal structure and on blood pressure in SHR. *Cell Mol Biol (Noisy-le-grand)*. 2002; 48:895–902. [PubMed: 12699248]

34. Dwyer BE, Nishimura RN, Lu SY. Differential expression of heme oxygenase-1 in cultured cortical neurons and astrocytes determined by the aid of a new heme oxygenase antibody. Response to oxidative stress. *Brain Res Mol Brain Res.* 1995; 30:37–47. [PubMed: 7609642]
35. Foresti R, Sarathchandra P, Clark JE, Green CJ, Motterlini R. Peroxynitrite induces haem oxygenase-1 in vascular endothelial cells: a link to apoptosis. *Biochem J.* 1999; 339:729–36. [PubMed: 10215613]
36. Kietzmann T, Samoylenko A, Immenschuh S. Transcriptional regulation of heme oxygenase-1 gene expression by MAP kinases of the JNK and p38 pathways in primary cultures of rat hepatocytes. *J Biol Chem.* 2003; 278:17927–36. [PubMed: 12637567]
37. Chen J, Tu Y, Moon C, Nagata E, Ronnett GV. Heme oxygenase-1 and heme oxygenase-2 have distinct roles in the proliferation and survival of olfactory receptor neurons mediated by cGMP and bilirubin, respectively. *J Neurochem.* 2003; 85:1247–61. [PubMed: 12753084]
38. Ewing JF, Maines MD. Rapid induction of heme oxygenase 1 mRNA and protein by hyperthermia in rat brain: heme oxygenase 2 is not a heat shock protein. *Proc Natl Acad Sci U S A.* 1991; 88:5364–8. [PubMed: 2052613]
39. Parfenova H, Carratu P, Tcheranova D, Fedinec A, Pourcyrus M, Leffler CW. Epileptic seizures cause extended postictal cerebral vascular dysfunction that is prevented by HO-1 overexpression. *Am J Physiol Heart Physiol.* 2005; 288:2843–50.
40. Parfenova H, Basuroy S, Bhattacharya S, Tcheranova D, Qu Y, Regan RF, Leffler CW. Glutamate induces oxidative stress and apoptosis in cerebral vascular endothelial cells: contributions of HO-1 and HO-2 to cytoprotection. *Am J Physiol Cell Physiol.* 2006; 290:C1399–410. [PubMed: 16371440]
41. Schipper HM. Heme oxygenase-1: transducer of pathological brain iron sequestration under oxidative stress. *Ann N Y Acad Sci.* 2004; 1012:84–93. [PubMed: 15105257]
42. Schipper HM. Heme oxygenase expression in human central nervous system disorders. *Free Radic Biol Med.* 2004; 37:1995–2011. [PubMed: 15544918]
43. Chang EF, Wong RJ, Vreman HJ, Igarashi T, Galo E, Sharp FR, Stevenson DK, Noble-Haesslein LJ. Heme oxygenase-2 protects against lipid peroxidation-mediated cell loss and impaired motor recovery after traumatic brain injury. *J Neurosci.* 2003; 23:3689–96. [PubMed: 12736340]
44. Basuroy S, Bhattacharya S, Tcheranova D, Qu Y, Regan RF, Leffler CW, Parfenova H. Heme oxygenase-2 provides endogenous protection against oxidative stress and apoptosis caused by TNF α in cerebral vascular endothelial cells. *Am J Physiol Cell Physiol.* 2006; 291:C897–908. [PubMed: 16822952]
45. Tcheranova D, Leffler CW, Parfenova H. Distinct protein kinase pathways are involved in HO-1 induction by severe oxidative stress in cerebral microvascular endothelium of newborn pigs. *FASEB J.* 2005; 19(5):A1252.
46. Chen J, Tu Y, Connolly EC, Ronnett GV. Heme oxygenase-2 protects against glutathione depletion-induced neuronal apoptosis mediated by bilirubin and cyclic GMP. *Curr Neurovasc Res.* 2005; 2:121–31. [PubMed: 16181104]
47. Doré S, Goto S, Sampei K, Blackshaw S, Hester LD, Ingi T, Sawa A, Traystman RJ, Koehler RC, Snyder SH. Heme oxygenase-2 acts to prevent neuronal death in brain cultures and following transient cerebral ischemia. *Neuroscience.* 2000; 99:587–92. [PubMed: 10974422]
48. Doré S, Sampei K, Goto S, Alkayed NJ, Guastella D, Blackshaw S, Gallagher M, Traystman RJ, Hurn PD, Koehler RC, Snyder SH. Heme oxygenase-2 is neuroprotective in cerebral ischemia. *Mol Med.* 1999; 5:656–63. [PubMed: 10602774]
49. Sun Y, Rotenberg MO, Maines MD. Developmental expression of heme oxygenase isozymes in rat brain. Two HO-2 mRNAs are detected. *J Biol Chem.* 1990; 265:8212–7. [PubMed: 2186037]
50. Snyder SH, Jaffrey SR, Zakhary R. Nitric oxide and carbon monoxide: parallel roles as neural messengers. *Brain Res Brain Res Rev.* 1998; 26:167–75. [PubMed: 9651518]
51. Ingi T, Chiang G, Ronnett GV. The regulation of heme turnover and carbon monoxide biosynthesis in cultured primary rat olfactory receptor neurons. *J Neurosci.* 1996; 16:5621–5628. [PubMed: 8795618]

52. Zakhary R, Gaine SP, Dinerman JL, Ruat M, Flavahan NA, Snyder SH. Heme oxygenase 2: endothelial and neuronal localization and role in endothelium-dependent relaxation. *Proc Natl Acad Sci U S A*. 1996; 93:795–8. [PubMed: 8570637]
53. Zakhary R, Poss KD, Jaffrey SR, Ferris CD, Tonegawa S, Snyder SH. Targeted gene deletion of heme oxygenase 2 reveals neural role for carbon monoxide. *Proc Natl Acad Sci U S A*. 1997; 94:14848–53. [PubMed: 9405702]
54. Zhao H, Wong RJ, Nguyen X, Kalish F, Mizobuchi M, Vreman HJ, Stevenson DK, Contag CH. Expression and regulation of heme oxygenase isozymes in the developing mouse cortex. *Pediatr Res*. 2006; 60:518–23. [PubMed: 16966352]
55. Scapagnini G, D'Agata V, Calabrese V, Pascale A, Colombrita C, Alkon D, Cavallaro S. Gene expression profiles of heme oxygenase isoforms in the rat brain. *Brain Res*. 2002; 954:51–9. [PubMed: 12393232]
56. Liu N, Wang X, McCoubrey WK, Maines MD. Developmentally regulated expression of two transcripts for heme oxygenase-2 with a first exon specific to rat testis; control by corticosterone of the oxygenase protein expression. *Gene*. 2000; 241:175–83. [PubMed: 10607912]
57. Parfenova H, Daley ML, Carratu P, Leffler CW. Heme Oxygenase Inhibition Reduces Neuronal Activation Evoked by Bicuculline in Newborn Pigs. *Brain Res*. 2004; 1014:87–96. [PubMed: 15212995]
58. Maines MD, Eke BC, Zhao X. Corticosterone promotes increase heme oxygenase-2 protein and transcript expression in newborn rat. *Brain Res*. 1996; 722:83–94. [PubMed: 8813353]
59. Raju VS, McCoubrey WK Jr, Maines MD. Regulation of heme oxygenase-2 by glucocorticoids in neonatal rat brain: characterization of a functional glucocorticoid response element. *Biochim Biophys Acta*. 1997; 1351:89–104. [PubMed: 9116047]
60. Weber CM, Eke BC, Maines MD. Corticosterone regulates heme oxygenase-2 and NO synthase transcription and protein expression in rat brain. *J Neurochem*. 1994; 63:953–62. [PubMed: 7519667]
61. Parfenova H, Fedinec A, Leffler CW. Ionotropic glutamate receptors in cerebral microvascular endothelium are functionally linked to heme oxygenase. *J Cereb Blood Flow Metab*. 2003; 23:190–7. [PubMed: 12571450]
62. Boehning D, Moon C, Sharma S, Hurt KJ, Hester LD, Ronnett GV, Shugar D, Snyder SH. Carbon monoxide neurotransmission activated by CK2 phosphorylation of heme oxygenase-2. *Neuron*. 2003; 40:129–37. [PubMed: 14527438]
63. Boehning D, Sedaghat L, Sedlak TW, Snyder SH. Heme oxygenase-2 is activated by calcium-calmodulin. *J Biol Chem*. 2004; 279:30927–30. [PubMed: 15175337]
64. De Matteis F, Zetterlund P, Wetterberg L. Brain 5-aminolaevulinate synthase. Developmental aspects and evidence for regulatory role. *Biochem J*. 1981; 196:811–7. [PubMed: 7317018]
65. Astner I, Schulze JO, van den Heuvel J, Jahn D, Schubert WD, Heinz DW. Crystal structure of 5-aminolevulinate synthase, the first enzyme of heme biosynthesis, and its link to XLSA in humans. *EMBO J*. 2005; 24:3166–77. [PubMed: 16121195]
66. Cable EE, Cable JW, Bonkovsky HL. Repression of hepatic delta-aminolevulinate synthase by heme and metalloporphyrins: relationship to inhibition of heme oxygenase. *Hepatology*. 1993; 18:119–27. [PubMed: 8325603]
67. Cable EE, Gildemeister OS, Pepe JA, Donohue SE, Lambrecht RW, Bonkovsky HL. Hepatic 5-aminolevulinic acid synthase mRNA stability is modulated by inhibitors of heme biosynthesis and by metalloporphyrins. *Eur J Biochem*. 1996; 240:112–7. [PubMed: 8797843]
68. May BK, Dogra SC, Sadlon TJ, Bhasker CR, Cox TC, Bottomley SS. Molecular regulation of heme biosynthesis in higher vertebrates. *Prog Nucleic Acid Res Mol Biol*. 1995; 51:1–51. [PubMed: 7659773]
69. Srivastava G, Borthwick IA, Maguire DJ, Elferink CJ, Bawden MJ, Mercer JF, May BK. Regulation of 5-aminolevulinate synthase mRNA in different rat tissues. *J Biol Chem*. 1988; 263:5202–9. [PubMed: 3356687]
70. Chernova T, Nicotera P, Smith AG. Heme deficiency is associated with senescence and causes suppression of N-methyl-D-aspartate receptor subunits expression in primary cortical neurons. *Mol Pharmacol*. 2006; 69:697–705. [PubMed: 16306232]

71. Jaronczyk K, Bui L, Soong JM, McLaughlin BE, Marks GS, Brien JF, Nakatsu K. The source of heme for vascular heme oxygenase II: de novo heme biosynthesis in rat aorta. *Can J Physiol Pharmacol*. 2004; 82:218–24. [PubMed: 15181459]
72. Shinjyo N, Kita K. Up-regulation of heme biosynthesis during differentiation of Neuro2a cells. *J Biochem (Tokyo)*. 2006; 139:373–81. [PubMed: 16567402]
73. Verma A, Hirsch DJ, Glatt CE, Ronnett GV, Snyder SH. Carbon monoxide: a putative neural messenger. *Science*. 1993; 259:38–4.
74. Cannon JB, Kuo FS, Vatandoust F, Liem HH, Muller-Eberhard U. The effect of metalloporphyrins and heme liposomes on delta-aminolevulinate synthase activity in rat liver. *Biochem Biophys Res Commun*. 1985; 130:306–12. [PubMed: 4026834]
75. Maines MD, Janousek V, Tomio JM, Kappas A. Cobalt inhibition of synthesis and induction of delta-aminolevulinate synthase in liver. *Proc Natl Acad Sci U S A*. 1976; 73:1499–503. [PubMed: 818637]
76. Hamilton JW, Bement WJ, Sinclair PR, Sinclair JF, Alcedo JA, Wetterhahn KE. Heme regulates hepatic 5-aminolevulinate synthase mRNA expression by decreasing mRNA half-life and not by altering its rate of transcription. *Arch Biochem Biophys*. 1991; 289:387–92. [PubMed: 1898078]
77. Cable EE, Miller TG, Isom HC. Regulation of heme metabolism in rat hepatocytes and hepatocyte cell lines: delta-aminolevulinic acid synthase and heme oxygenase are regulated by different heme-dependent mechanisms. *Arch Biochem Biophys*. 2000; 384:280–95. [PubMed: 11368315]
78. Guberman AS, Scassa ME, Cánepa ET. Repression of 5-aminolevulinate synthase gene by the potent tumor promoter, TPA, involves multiple signal transduction pathways. *Arch Biochem Biophys*. 2005; 436:285–96. [PubMed: 15797241]
79. Roberts AG, Elder GH. Alternative splicing and tissue-specific transcription of human and rodent ubiquitous 5-aminolevulinate synthase (ALAS1) genes. *Biochim Biophys Acta*. 2001; 1518:95–105. [PubMed: 11267664]
80. Scassa ME, Guberman AS, Varone CL, Canepa ET. Phosphatidylinositol 3-kinase and Ras/mitogen-activated protein kinase signaling pathways are required for the regulation of 5-aminolevulinate synthase gene expression by insulin. *Exp Cell Res*. 2001; 271:201–13. [PubMed: 11716532]
81. Meldrum BS. Concept of activity-induced cell death in epilepsy: historical and contemporary perspectives. *Prog Brain Res*. 2002; 135:3–11. [PubMed: 12143350]
82. Nakanishi S. Molecular diversity of glutamate receptors and implications for brain function. *Science*. 1992; 258:597–603. [PubMed: 1329206]
83. Nedergaard M, Takano T, Hansen AJ. Beyond the role of glutamate as a neurotransmitter. *Nat Rev Neurosci*. 2002; 3:748–55. [PubMed: 12209123]
84. Collard CD, Park KA, Montalto MC, Alapati S, Buras JA, Stahl GL, Colgan SP. Neutrophil-derived glutamate regulates vascular endothelial barrier function. *J Biol Chem*. 2002; 277:14801–11. [PubMed: 11847215]
85. Krizbai IA, Deli MA, Pestenacz A, Siklos L, Szabo CA, Andras I, Joo F. Expression of glutamate receptors on cultured cerebral endothelial cells. *J Neurosci Res*. 1998; 54:814–9. [PubMed: 9856865]
86. Koenig H, Trout JJ, Goldstone AD, Lu CY. Capillary NMDA receptors regulate blood-brain barrier function and breakdown. *Brain Res*. 1992; 588:297–303. [PubMed: 1393582]
87. Lin SH, Maiese K. The metabotropic glutamate receptor system protects against ischemic free radical programmed cell death in rat brain endothelial cells. *J Cereb Blood Flow Metab*. 2001; 21:262–75. [PubMed: 11295881]
88. Sharp CD, Houghton J, Elrod JW, Warren A, Jackson TH 4th, Jawahar A, Nanda A, Minagar A, Alexander JS. N-methyl-D-aspartate receptor activation in human cerebral endothelium promotes intracellular oxidant stress. *Am J Physiol Heart Circ Physiol*. 2005; 288:H1893–9. [PubMed: 15576430]
89. Pliss L, Jezova D, Mares V, Balcar VJ, St'astny F. N-Acetyl-L-aspartyl-L-glutamate changes functional and structural properties of rat blood-brain barrier. *Neurosci Lett*. 2002; 317:85–8. [PubMed: 11755246]

90. Leffler CW, Balabanova L, Fedinec AL, Parfenova H. Nitric Oxide increases carbon monoxide production by piglet cerebral microvessels. *Am J Physiol Heart Circ Physiol.* 2005; 289:H1442–7. [PubMed: 15964921]
91. Fleming I, Busse R. Molecular mechanisms involved in the regulation of the endothelial nitric oxide synthase. *Physiol Regul Integr Comp Physiol.* 2003; 284:R1–12.
92. Lin S, Fagan KA, Li KX, Shaul PW, Cooper DM, Rodman DM. Sustained endothelial nitric oxide synthase activation requires capacitative Ca^{2+} entry. *J Biol Chem.* 2000; 275:17979–85. [PubMed: 10849433]
93. Tcheranova D, Basuroy S, Parfenova H, Leffler CW. Regulation of CO production by glutamate in cultured astrocytes from the cerebral cortex. *FASEB J.* 2006; 20(4):A292.
94. Borgström L, Chapman AG, Siesjö BK. Glucose consumption in the cerebral cortex of rat during bicuculline-induced status epilepticus. *J Neurochem.* 1976; 27:971–3. [PubMed: 966032]
95. Chapman AG. Glutamate receptors in epilepsy. *Prog Brain Res.* 1998; 116:371–83. [PubMed: 9932389]
96. Ingvar M. Cerebral blood flow and metabolic rate during seizures. Relationship to epileptic brain damage. *Ann N Y Acad Sci.* 1986; 462:194–206. [PubMed: 3518570]
97. Pereira de Vasconcelos A, Ferrandon A, Nehlig A. Local cerebral blood flow during lithium-pilocarpine seizures in the developing and adult rat: role of coupling between blood flow and metabolism in the genesis of neuronal damage. *J Cereb Blood Flow Metab.* 2002; 22:196–205. [PubMed: 11823717]
98. Fujikawa DG, Dwyer BE, Lake RR, Wasterlain CG. Local cerebral glucose utilization during status epilepticus in newborn primates. *Am J Physiol.* 1989; 256:C1160–7. [PubMed: 2735393]
99. Montecot C, Seylaz J, Pinard E. Carbon monoxide regulates cerebral blood flow in epileptic seizures but not in hypercapnia. *Neuroreport.* 1998; 9:2341–6. [PubMed: 9694225]
100. Borch K, Pryds O, Holm S, Lou H, Greisen G. Regional cerebral blood flow during seizures in neonates. *J Pediatr.* 1998; 132:431–435. [PubMed: 9544896]
101. Perlman JM, Volpe JJ. Seizures in the preterm infant: effects on cerebral blood flow velocity, intracranial pressure, and arterial blood pressure. *J Pediatr.* 1983; 102:288–93. [PubMed: 6822940]
102. Niehaus L, Wiesmann UC, Meyer B. Changes in cerebral hemodynamics during simple partial motor seizures. *Eur Neurol.* 2000; 44:8–11. [PubMed: 10894989]
103. Cornford EM. Epilepsy and the blood brain barrier: endothelial cell responses to seizures. *Adv Neurol.* 1999; 79:845–62. [PubMed: 10514868]
104. Lombroso CT. Neonatal seizures: a clinician overview. *Brain Dev.* 1996; 18:1–28. [PubMed: 8907338]
105. Turjman F, Massoud TF, Sayre JW, Vinuela F, Guglielmi G, Duckwiler G. Epilepsy associated with cerebral arteriovenous malformations: a multivariate analysis of angioarchitectural characteristics. *Am J Neuroradiol.* 1995; 16:345–50. [PubMed: 7726084]
106. Wasterlain CG, Niquet J, Thompson KW, Baldwin R, Liu H, Sankar R, et al. Seizure-induced neuronal death in the immature brain. *Prog Brain Res.* 2002; 135:335–53. [PubMed: 12143353]
107. Söderfeldt B, Kalimo H, Olsson Y, Siesjö BK. Bicuculline-induced epileptic brain injury. Transient and persistent cell changes in rat cerebral cortex in the early recovery period. *Acta Neuropathol.* 1983; 62:87–95. [PubMed: 6659880]
108. Sankar R, Shin DH, Liu H, Mazarati A, de Vasconcelos AP, Wasterlain CG. Pattern of status epilepticus-induced neuronal injury during development and long-term consequences. *J Neurosci.* 1998; 18:8382–93. [PubMed: 9763481]
109. Bolwig TG. Blood-brain barrier studies with special reference to epileptic seizures. *Acta Psychiatr Scand Suppl.* 1988; 345:15–20. [PubMed: 3067537]
110. Oztas B, Kilic S, Dural E, Ispir T. Influence of antioxidants on the blood-brain barrier permeability during epileptic seizures. *Int J Neurosci.* 2000; 105:27–35. [PubMed: 11069044]
111. Janigro D. Blood-brain barrier, ion homeostasis and epilepsy: possible implications towards the understanding of ketogenic diet mechanisms. *Epilepsy Res.* 1999; 37:223–32. [PubMed: 10584972]

112. Armstead WM, Mirro R, Leffler CW, Busija DW. Cerebral superoxide anion generation during seizures in newborn pigs. *J Cereb Blood Flow Metab.* 1989; 9:175–9. [PubMed: 2921292]
113. Patel M. Mitochondrial dysfunction and oxidative stress: cause and consequence of epileptic seizures. *Free Radic Biol Med.* 2004; 37:1951–62. [PubMed: 15544915]
114. Fujikawa DG. Prolonged seizures and cellular injury: understanding the connection. *Epilepsy Behav.* 2005; 7:S3–11. [PubMed: 16278099]
115. Coyle JT, Puttfarcken P. Oxidative stress, glutamate, and neurodegenerative disorders. *Science.* 1993; 262:689–95. [PubMed: 7901908]
116. Scheyer RD. Involvement of glutamate in human epileptic activities. *Prog Brain Res.* 1998; 116:359–69. [PubMed: 9932388]
117. Meldrum BS. Glutamate as a neurotransmitter in the brain: review of physiology and pathology. *J Nutr.* 2000; 130:1007S–15S. [PubMed: 10736372]
118. Tanaka K, Watase K, Manabe T, Yamada K, Watanabe M, Takahashi K, et al. Epilepsy and exacerbation of brain injury in mice lacking the glutamate transporter GLT-1. *Science.* 1997; 276:1699–702. [PubMed: 9180080]
119. Nakao N, Brundin P. Neurodegeneration and glutamate induced oxidative stress. *Prog Brain Res.* 1998; 116:246–63.
120. Berg-Johnsen J, Haugstad TS, Langmoen IA. Glutamate in the human brain: possible role in synaptic transmission and ischemia. *Prog Brain Res.* 1998; 116:287–302. [PubMed: 9932384]
121. Reynolds IJ. Intracellular calcium and magnesium: critical determinants of excitotoxicity? *Prog Brain Res.* 1998; 116:226–43.
122. Bengzon J, Mohapel P, Ekdahl CT, Lindvall O. Neuronal apoptosis after brief and prolonged seizures. *Prog Brain Res.* 2002; 135:111–9. [PubMed: 12143333]
123. Benveniste EN. Inflammatory cytokines within the central nervous system: sources, function, and mechanism of action. *Am J Physiol.* 1992; 263:C1–16. [PubMed: 1636671]
124. Chen G, Goeddel DV. TNF-R1 signaling: a beautiful pathway. *Science.* 2002; 296:1634–5. [PubMed: 12040173]
125. Shinoda S, Skradski SL, Araki T, Schindler CK, Meller R, Lan JQ, et al. Formation of a tumor necrosis factor receptor 1 molecular scaffolding complex and activation of apoptosis signal-regulating kinase 1 during seizure-induced neuronal death. *Eur J Neurosci.* 2003; 17:2065–76. [PubMed: 12786973]
126. Vezzani A, Moneta D, Richichi C, Aliprandi M, Burrows SJ, Ravizza T, et al. Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis. *Epilepsia.* 2002; 43(5):30–5. [PubMed: 12121291]
127. Alcaraz MJ, Fernandez P, Guillen MI. Anti-inflammatory actions of the heme oxygenase-1 pathway. *Curr Pharm Des.* 2003; 9:2541–51. [PubMed: 14529552]
128. Yachie A, Niida Y, Wada T, Igarashi N, Kaneda H, Toma T, et al. Oxidative stress causes enhanced endothelial cell injury in human heme oxygenase-1 deficiency. *J Clin Invest.* 1999; 103:129–35. [PubMed: 9884342]
129. Beschoner R, Adjodah D, Schwab JM, Mittelbronn M, Pedal I, Mattern R, et al. Long-term expression of heme oxygenase-1 (HO-1, HSP-32) following focal cerebral infarctions and traumatic brain injury in humans. *Acta Neuropathol (Berl).* 2000; 100:377–84. [PubMed: 10985695]
130. Poucyrus M, Bada H, Parfenova H, Daley M, Korones S, Leffler CW. Cerebrovasodilatory contribution of endogenous carbon monoxide during seizures in newborn pigs. *Pediatr Res.* 2002; 51:579–85. [PubMed: 11978880]
131. Henshall DC, Simon RP. Epilepsy and apoptosis pathways. *J Cereb Blood Flow Metab.* 2005; 25:1557–72. [PubMed: 15889042]
132. Meier P, Finch A, Evan G. Apoptosis in development. *Nature.* 2000; 407:796–801. [PubMed: 11048731]
133. Hengartner MO. The biochemistry of apoptosis. *Nature.* 2000; 407:770–6. [PubMed: 11048727]

134. Basuroy S, Leffler CW, Parfenova H. Signal-specific contributions of NADPH oxidase and mitochondria to oxidative-stress induced cerebral vascular endothelial injury. *FASEB J.* 2007; 21(5):A1383.
135. Wagner KR, Dwyer BE. Hematoma removal, heme, and heme oxygenase following hemorrhagic stroke. *Ann N Y Acad Sci.* 2004; 1012:237–51. [PubMed: 15105270]
136. Wagner KR, Sharp FR, Ardizzone TD, Lu A, Clark JF. Heme and iron metabolism: role in cerebral hemorrhage. *J Cereb Blood Flow Metab.* 2003; 23:629–52. [PubMed: 12796711]
137. Qu Y, Chen J, Benvenisti-Zarom L, Ma X, Regan RF. Effect of targeted deletion of the heme oxygenase-2 gene on hemoglobin toxicity in the striatum. *J Cereb Blood Flow Metab.* 2005; 25:1466–75. [PubMed: 15902196]
138. Qu Y, Chen-Roetling J, Benvenisti-Zarom L, Regan RF. Attenuation of oxidative injury after induction of experimental intracerebral hemorrhage in heme oxygenase-2 knockout mice. *J Neurosurg.* 2007; 106:428–35. [PubMed: 17367065]
139. Wang J, Zhuang H, Doré S. Heme oxygenase 2 is neuroprotective against intracerebral hemorrhage. *Neurobiol Dis.* 2006; 22:473–6. [PubMed: 16459095]
140. Baranano DE, Rao M, Ferris CD, Snyder SH. Biliverdin reductase: a major physiologic cytoprotectant. *Proc Natl Acad Sci U S A.* 2002; 99:16093–8. [PubMed: 12456881]
141. Stocker R. Antioxidant activities of bile pigments. *Antioxid Redox Signal.* 2004; 6:841–9. [PubMed: 15345144]
142. Hansen T, Tommarello S, Allen J. Subcellular localization of bilirubin in rat brain after in vivo i.v. administration of [³H]bilirubin. *Pediatr Res.* 2001; 49:203–7. [PubMed: 11158514]
143. Hansen TW. Mechanisms of bilirubin toxicity: clinical implications. *Clin Perinatol.* 2002; 29:765–78. [PubMed: 12516745]
144. Doré S, Takahashi M, Ferris CD, Hester LD, Guastella D, Snyder SH. Bilirubin, formed by activation of heme oxygenase-2, protects neurons against oxidative stress injury. *Proc Natl Acad Sci USA.* 1999; 96:2445–50. [PubMed: 10051662]
145. Falcão AS, Silva RF, Fernandes A, Brito MA, Brites D. Influence of hypoxia and ischemia preconditioning on bilirubin damage to astrocytes. *Brain Res.* 2007; 1149:191–9. [PubMed: 17376407]
146. Belanger S, Lavoie JC, Chessex P. Influence of bilirubin on the antioxidant capacity of plasma in newborn infants. *Biol Neonate.* 1997; 71:233–8. [PubMed: 9129792]
147. Hegyi T, Goldie E, Hiatt M. The protective role of bilirubin in oxygen-radical diseases of the preterm infant. *J Perinatol.* 1994; 14:296–300. [PubMed: 7965225]
148. Minetti M, Mallozzi C, Di Stasi AM, Pietraforte D. Bilirubin is an effective antioxidant of peroxynitrite-mediated protein oxidation in human blood plasma. *Arch Biochem Biophys.* 1998; 352:165–74. [PubMed: 9587403]
149. Sedlak TW, Snyder SH. Bilirubin benefits: cellular protection by a biliverdin reductase antioxidant cycle. *Pediatrics.* 2004; 113:1776–82. [PubMed: 15173506]
150. Liu XM, Chapman GB, Peyton KJ, Schafer AI, Durante W. Carbon monoxide inhibits apoptosis in vascular smooth muscle cells. *Cardiovasc Res.* 2002; 55:396–405. [PubMed: 12123779]
151. Clark JE, Naughton P, Shurey S, Green CJ, Johnson TR, Mann BE, et al. Cardioprotective actions by a water-soluble carbon monoxide-releasing molecule. *Circ Res.* 2003; 93:e2–8. [PubMed: 12842916]
152. Stein AB, Guo Y, Tan W, Wu WJ, Zhu X, Li Q, et al. Administration of a CO-releasing molecule induces late preconditioning against myocardial infarction. *J Mol Cell Cardiol.* 2005; 38:127–34. [PubMed: 15623429]
153. Sandouka A, Balogun E, Foresti R, Mann BE, Johnson TR, Tayem Y, et al. Carbon monoxide-releasing molecules (CO-RMs) modulate respiration in isolated mitochondria. *Cell Mol Biol (Noisy-le-grand).* 2005; 51:425–32. [PubMed: 16309593]
154. Nakao A, Choi AM, Murase N. Protective effect of carbon monoxide in transplantation. *J Cell Mol Med.* 2006; 10:650–71. [PubMed: 16989726]
155. Taille C, El-Benna J, Lanone S, Boczkowski J, Motterlini R. Mitochondrial respiratory chain and NAD(P)H oxidase are targets for the antiproliferative effect of carbon monoxide in human airway smooth muscle. *J Biol Chem.* 2005; 280:25350–60. [PubMed: 15863496]

156. Alonso JR, Cardellach F, López S, Casademont J, Miró O. Carbon monoxide specifically inhibits cytochrome c oxidase of human mitochondrial respiratory chain. *Pharmacol Toxicol*. 2003; 93:142–6. [PubMed: 12969439]

List of Abbreviations

ALAS	Aminolevulinate synthase
BK_{Ca} channels	Large conductance Ca ²⁺ -activated potassium channels
CO	Carbon monoxide
CoPP	Cobalt protoporphyrin
CO-RM	CO-releasing molecule
CSF	Cerebrospinal fluid
DMDC	Dimanganese decacarbonyl
eNOS	Endothelial NO synthase
GluRs	Glutamate receptors
iGluRs	Ionotropic glutamate receptors
mGluRs	Metabotropic glutamate receptors
HO	Heme oxygenase
ROS	Reactive oxygen species
SnPP	Tin protoporphyrin
SOD	Superoxide dismutase
TNFα	Tumor necrosis factor alpha
ZnPP	Zink protoporphyrin

Fig. (1).

Cerebroprotective functions of HO-2 against immediate and delayed effects of epileptic seizures. Brain HO-2 activation during seizures: A) mediates the cerebral flow increase to match metabolic demands of hyperactive neurons and prevent neuronal damage during the ictal period, and B) inhibits the formation and effects of reactive oxygen species caused by seizure-related excitotoxic and inflammatory mediators, glutamate and TNF- α , thus reducing oxidative stress-mediated sustained cerebral endothelial injury and preserving the cerebral blood flow autoregulation thereby preventing subsequent neuronal damage during the delayed postictal period.