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Forum Review

Cerebrovascular Effects of Carbon Monoxide

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ABSTRACT

This review examines the influence of endogenous and exogenous carbon monoxide (CO) on the cerebral circulation. Although CO generated from neuronal heme oxygenase can modulate neurotransmission, evidence supporting its role in cerebral vasodilation is limited. In newborn piglets, heme oxygenase is enriched in microvessels and contributes to hypoxic vasodilation. Low CO concentrations dilate piglet arterioles by opening calcium-activated potassium channels. With inhalation of CO and formation of carboxyhemoglobin, cerebral vasodilation can be greater than that occurring with hypoxic hypoxia at equivalent reductions of arterial oxygen content. This additional vasodilation is probably attributable to additional release of hypoxic vasodilators secondary to increased oxyhemoglobin affinity, although direct effects of CO on cerebral arterioles may also occur. When CO exposure is prolonged, cerebral endothelium undergoes oxidant stress as evident by nitrotyrosine formation. As CO levels increase, modest decreases in oxygen consumption are detectable, which may reflect CO or nitric oxide interactions with cytochrome oxidase in regions with very low oxygen availability. If subsequent CO concentration increases sufficiently to depress cardiac function and limit cerebral perfusion, cerebral oxygen consumption becomes further reduced, and oxidant stress becomes amplified by leukocyte sequestration and xanthine oxidase activity with consequent lipid peroxidation. Specific regions of the brain, such as central white matter, globus pallidus, and hippocampus, are selectively vulnerable to CO toxicity, but whether the mechanisms involved in selective injury differ from other forms of hypoxia–ischemia needs to be clarified. Antioxid. Redox Signal. 4, 279–290.

INTRODUCTION

EARLY WORK ON THE EFFECT OF CARBON MONOXIDE (CO) on the cerebral circulation stemmed from an interest in understanding the mechanisms of CO toxicity and its neurological consequences. More recent interest has been spurred by the findings that CO derived from heme oxygenase in neurons and endothelium can act as a neurotransmitter and vasodilator. This review examines the response of the cerebral circulation to inhaled CO and considers the potential role of heme oxygenase in cerebrovascular regulation.

HEME OXYGENASE IN BRAIN

Heme oxygenase cleaves the heme ring to produce ferrous iron, CO, and biliverdin, which is rapidly reduced to bilirubin. Three isoforms have been described. Heme oxygenase-1 is the primary isoform in liver and spleen, but it is normally not significantly expressed in brain. Heme oxygenase-1 is a stress protein that can be induced in brain in specific cell types by hyperthermia (46), glutathione depletion (18), Alzheimer's disease (66), Parkinson's disease (67), focal cerebral ischemia (36, 54), transient global cerebral ischemia (19, 75), subarachnoid hemorrhage (47, 55, 87), and intracerebral hemorrhage (48). After ischemia or introduction of blood, expression is localized primarily in microglia. CO generated by delayed induction of heme oxygenase-1 in microglia has not been considered important for cerebrovascular regulation in these pathophysiological conditions, although bilirubin and its breakdown products may be important in vasospasm after subarachnoid hemorrhage.

Heme oxygenase-2 is expressed constitutively in neurons in a topographical pattern that parallels soluble guanylyl cyclase

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expression (90, 91). Expression is particularly high in olfactory bulb, pyramidal cell layer and dentate gyrus of hippocampus, cerebellar granule and Purkinje cell layers, and brainstem regions, some of which are involved in cardiorespiratory control. Some evidence supports a role for CO in long-term potentiation in hippocampal neurons and in modulating effects of metabotropic glutamate receptor activation (1, 21, 72, 96). These actions are thought to be mediated by modulating guanylyl cyclase activity (28). However, CO is a widely diffusible molecule, and its precise function in neurotransmission is not completely understood. The role of CO released from neurons in regulating the cerebral vasculature has not been as well studied as that of nitric oxide (NO). In addition to neurons, the endothelium is a potential source of CO. Heme oxygenase-2 expression has been reported in dog basilar artery (95) and piglet cerebral endothelial cell culture (56). Whether expression is present throughout the cerebral endothelium in mature brain of most species is unknown. The function of a third isoform, heme oxygenase-3, has not been clarified.

DIRECT EFFECTS OF CO ON CEREBRAL VESSELS

In isolated rings of basilar artery from dog and rabbit and of middle cerebral artery from rabbit, Brian *et al.* (9) could not detect a decrease in tension with CO concentrations in the micromolar range, whereas relaxation of aortic rings could be demonstrated. Because of this negative finding, there was not much interest in investigating direct vascular effects of CO in the cerebral circulation. However, in 1999 Leffler *et al.* (41) reported that nanomolar concentrations of CO produced dose-dependent dilation of pial arterioles *in vivo* when solutions of artificial cerebrospinal fluid containing CO were superfused over the cortical surface of newborn piglets. The reason for the differences in the response to CO between the studies of Brain *et al.* and Leffler *et al.* is unclear, but could be related to effects of development, vessel size, or methodology (*in vitro* versus *in vivo*). In piglets no increases in perivascular cyclic GMP (cGMP) could be detected in the cerebrospinal fluid in the cranial window during CO superfusion. Increases in cyclic AMP were modest. However, the dilation to CO could be blocked by tetraethylammonium chloride or iberiotoxin, thereby suggesting that dilation was mediated by calcium-activated potassium (K_{Ca}) channels. This finding is supported by patch-clamp studies on rat tail artery smooth muscle where CO increases the open state probability of large conductance K_{Ca} channels, possibly through a direct interaction with histidine residues (92, 93).

Cerebral micovessels in the piglet are enriched with heme oxygenase-2 (41). Superfusing the cortical surface with heme-L-lysinate, a substrate for heme oxygenase, produces dose-dependent dilation of piglet pial arterioles, which is blocked by K_{Ca} antagonists. The dilation is also blocked by the heme oxygenase inhibitor, chromium mesoporphyrin. Thus, K_{Ca} channel activation appears to be the primary mechanism of cerebral vasodilation to both endogenous and exogenous CO in the piglet.

The dilation to exogenous CO and to heme-L-lysinate is blocked by the cyclooxygenase inhibitor indomethacin and by the NO synthase (NOS) inhibitor nitroarginine (42). However, in the presence of indomethacin, coapplication of 1 pmol/L iloprost restored dilation to CO and heme-L-lysinate. In the presence of nitroarginine, coapplication of 100 nmol/L sodium nitroprusside restored dilation to CO and heme-L-lysinate. These small doses of iloprost and nitroprusside by themselves had no effect on baseline diameter. Thus, it appears that prostacyclin and NO play obligatory permissive roles in enabling dilation to CO rather than mediating the dilation to CO.

The role of CO generated by heme oxygenase in physiological responses of the cerebral circulation has not been well investigated. Only a few studies report positive effects of heme oxygenase inhibitors. In the piglet, pial arteriolar dilation to hypoxic hypoxia was attenuated by chromium mesoporphyrin (41), thereby suggesting a role for CO in hypoxic dilation. However, regulation of the cerebrovascular response to hypoxia in the piglet is complex because the dilator response can also be attenuated by the P450 inhibitor, miconazole (40), NOS inhibitors (3, 4), inhibitors of ATP-sensitive K^+ channels (5), and opioid antagonists (3). It is known that mechanisms that modulate cerebrovascular reactivity to other stimuli, such as CO_2 and acetylcholine, change during development in the piglet (97). Thus, modulators of hypoxic reactivity may also change during development. In guinea pig brain, heme oxygenase activity is greater in mature fetuses than in adults (11). Whether there is a postnatal developmental decrease in cerebrovascular heme oxygenase activity in cerebral vessels is unknown.

In the rat, increases in cerebral blood flow (CBF) evoked by kainate-induced seizures are attenuated by the heme oxygenase inhibitor, tin protoporphyrin (53). A preliminary report in piglets indicates that pial arteriolar dilation to bicuculline-induced seizures is attenuated by chromium mesoporphyrin and tin protoporphyrin (64). Thus, CO may contribute to cerebral vasodilation during seizures together with other mediators.

In piglets, application of chromium mesoporphyrin caused a modest increase in baseline diameter of pial arterioles (41). This increase was blocked by nitroarginine. The authors suggest that tonic production of CO acts to inhibit NO synthesis, and that inhibiting CO production will increase NO availability and cause the observed vasodilation. However, inhibition of NOS directly by CO may require nonphysiological concentrations of CO (23, 24). An alternative explanation is an interaction of CO with NO on activation of soluble guanylyl cyclase. In cerebellar granule cell cultures, adding 1.5–5 μmol/L CO suppressed NO-induced increases in cGMP, whereas 150–500 μmol/L CO potentiated the increase in cGMP (27). Inhibition of heme oxygenase resulted in an augmentation of the NO-mediated increase in cGMP. If similar interactions occur in vascular smooth muscle, interpretations of data using heme oxygenase inhibitors will have to take into account the local concentrations of CO and NO that are normally present in the physiological response.

Some information is available on the cerebral effects of heme oxygenase-2 gene deletion under conditions of cerebral ischemia. With deletion of the heme oxygenase-2 gene but not the heme oxygenase-1 gene, mice have a larger infarct in the brain after transient occlusion of the middle cerebral artery (15). Inhibition of heme oxygenase with tin protoporphyrin IX also enlarges infarct size in wild-type mice, but not in heme oxygenase-2 knockout mice (17). However, this effect on infarct size was not attributable to differences in in-

traischemic blood flow. Blood flow was not different between wild-type and heme oxygenase-2 knockout mice in either the ischemic or nonischemic hemisphere (15). Thus, one interpretation is that CO derived from heme oxygenase-2 does not make a major contribution to basal cerebrovascular tone or to vasodilation during ischemia. Alternatively, other pathways may compensate for the loss of heme oxygenase-2. The protective effect of heme oxygenase-2 in focal cerebral ischemia appears to be related to bilirubin availability. Activation of heme oxygenase or addition of low concentrations of bilirubin to neuronal cell culture protects neurons from hydrogen peroxide-induced injury (16). Heme oxygenase activity can limit neuronal apoptosis in cell culture and may also limit cell death in ischemic border regions where some neurons undergo an apoptotic form of cell death (17).

CO INHALATION

As the partial pressure of CO in the blood increases during CO inhalation, the amount of CO bound to the four heme groups in hemoglobin increases. The formation of carboxyhemoglobin (COHb) results in a decreased O_2 carrying capacity and an increased O_2 affinity of the remaining heme groups that are not bound by CO. Consequently, the oxyhemoglobin P_{50} [partial pressure of O_2 (PO_2) at 50% oxyhemoglobin saturation] decreases. The combination of a decrease in O_2 carrying capacity and a decrease in P_{50} promotes tissue hypoxia and is thus referred to as CO hypoxia.

The brain has a relatively high metabolic rate of O_2 consumption ($CMRO_2$). Complete cessation of blood flow causes a rapid loss of consciousness and electroencephalographic activity within ~1 min, followed by depletion of high-energy phosphates and cellular depolarization within 2–3 min. To defend against even small changes in tissue oxygenation, cerebral arterioles are equipped with an array of complex mechanisms to respond within seconds to various forms of hypoxia. Because nearly all capillaries in the brain are perfused continuously, capillary recruitment is not available as a mechanism to maintain O_2 flux to cells during hypoxic states. Rather, arteriolar dilation is required to maintain O_2 transport to the capillaries such that the capillary PO_2 is sufficient to maintain the O_2 gradient required for O_2 flux to the most distant mitochondria. The various forms of tissue hypoxia, including hypoxic hypoxia, anemic hypoxia, and histotoxic hypoxia (*e.g.,* cyanide poisoning), all evoke an increase in CBF (57, 63, 68). In the case of CO hypoxia, dose-dependent increases in CBF related to the amount of COHb have been reported in a variety of species including humans (8, 57), dogs (86), sheep (34), goats (13), rabbits (52), and rats (44). To understand the mechanisms of the increase in CBF during CO hypoxia, it is instructive to compare the response with that of other forms of hypoxia.

COMPARISON WITH HYPOXIC AND ANEMIC HYPOXIA

For equivalent decreases in arterial O_2 content (CaO_2) produced by low arterial PO_2, low hematocrit, or both low PO_2 and low hematocrit, CBF is generally found to increase by an equivalent amount (30, 89), although some reports indicate a smaller increase with anemia (84). The relationship of CBF to CaO_2 can be fitted with a rectangular hyperbola, such that CBF $\times$ CaO_2 = constant. Because CBF $\times$ CaO_2 equals bulk O_2 transport to the cerebral microcirculation, this relationship implies that cerebral O_2 transport remains unchanged during both hypoxic and anemic hypoxia over a wide range of CaO_2. Statistically, the calculated O_2 transport does indeed remain unchanged in most studies of hypoxic and anemic hypoxia (26, 30, 32, 35, 89).

With anemia the amount of vasodilation required to increase CBF is less than that with hypoxia because of the decrease in blood viscosity that accompanies hemodilution. Depending upon the baseline conditions, the degree of hemodilution, and possibly other factors, hemodilution can cause constriction, no change in diameter, or dilation of pial arterioles on the surface of the cortex (6, 25, 26). The response of the pial arterioles to the change in viscous load appears to be appropriate for maintaining cerebral O_2 transport at a constant level. When red cell-based hemoglobin is replaced by plasma-based hemoglobin, pial arterioles constrict to counteract the decrease in blood viscosity at low hematocrit with near-normal CaO_2 (6). Consequently, increases in CBF are small such that O_2 transport is unchanged (88). Furthermore, producing hypoxic hypoxia after cell-free hemoglobin exchange transfusion results in an increase in CBF that keeps O_2 transport constant (89). Thus, with different combinations of arterial PO_2, hematocrit, and hemoglobin concentration, CBF can be described by a single relationship of CaO_2 in which cerebral O_2 transport is well regulated by appropriate changes in arteriolar diameter.

In the case of CO hypoxia, the increase in CBF is as great or greater than the increase seen during hypoxic or anemic hypoxia at comparable levels of CaO_2 (57, 86). This increase in CBF is not influenced by interruption of the peripheral chemoreflexes or baroreflexes (85, 86), thereby supporting a local vascular mechanism of vasodilation. In the anesthetized dog with mechanical ventilation, hypoxic hypoxia increased arterial pressure whereas CO hypoxia decreased arterial pressure (85, 86). Thus, comparable increases in CBF during hypoxic and CO hypoxia actually required greater vasodilation during CO hypoxia. In unanesthetized sheep in which arterial pressure did not decrease during CO hypoxia (34), the increase in CBF was greater than that during isocapnic hypoxic hypoxia (Fig. 1). As a result, O_2 transport to the cerebral microcirculation actually increases during CO hypoxia because of greater cerebral vasodilation. This enhanced vasodilation to CO hypoxia compared with hypoxic hypoxia at equivalent CaO_2 could be attributed to enhanced tissue hypoxia secondary to the decrease in P_{50} or to direct effects of CO on the cerebral arterioles.

EFFECT OF OXYHEMOGLOBIN AFFINITY

Manipulating P_{50} without CO inhalation produces changes in cerebrovascular resistance and CBF. For example, decreasing P_{50} in rats results in an increase in CBF at both normal and reduced hematocrit (94). Increasing P_{50} in either fetal sheep or newborn lambs by replacing fetal hemoglobin with adult hemoglobin decreases CBF both when CaO_2 is normal

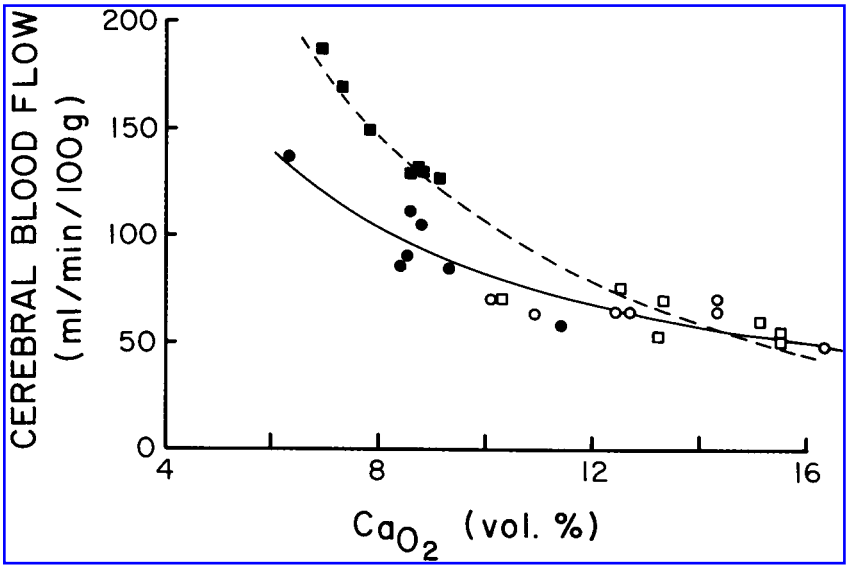

FIG. 1. Cerebral blood flow versus arterial O_2 content (CaO_2) in unanesthetized sheep during hypoxic hypoxia (●) and CO hypoxia (■). Baseline control values on room air ventilation are shown for hypoxic hypoxia experiments (○) and CO hypoxia experiments (□). The slope of the regression line fitted to the reciprocal of O_2 content for CO hypoxia (----) was significantly greater than the regression line for hypoxic hypoxia (——). Reprinted with permission from reference (34).

and when CaO_2 is decreased during hypoxic hypoxia (35, 65). These results are consistent with the concept that P_{50} influences the PO_2 gradient from the blood vessel to the tissue and thereby resets tissue PO_2. Tissue PO_2 feeds back on regulating arteriolar tone and adjusts CBF and bulk O_2 transport in a compensatory manner to limit variations in tissue PO_2. Thus, under conditions of constant arterial partial pressure of CO_2, $CMRO_2$, and cerebral perfusion pressure, CBF can be described as a function of CaO_2 and P_{50}.

Fractional O_2 extraction is a useful parameter that normalizes for differences in $CMRO_2$. Fractional O_2 extraction equals the arteriovenous O_2 content difference divided by CaO_2. This is equivalent to the ratio of $CMRO_2$ to cerebral O_2 transport. When $CMRO_2$ and cerebral O_2 transport are maintained at constant levels during hypoxic hypoxia and anemia, fractional O_2 extraction is unchanged (30, 32, 35). Even when $CMRO_2$ is reduced by pentobarbital coma, fractional O_2 extraction is unchanged during normoxia and hypoxic hypoxia (14). In contrast, CO hypoxia results in a decrease in fractional O_2 extraction as cerebral O_2 transport increases (32, 34). Decreases in P_{50} largely account for the decrease in fractional O_2 extraction (Fig. 2). When P_{50} is increased by replacing fetal hemoglobin with adult hemoglobin in fetal or newborn sheep, under either normoxic or hypoxic conditions, fractional O_2 extraction increases to levels normally seen in adult sheep. The relationship of fractional O_2 extraction to P_{50} during CO hypoxia in newborn or adult sheep is similar to the relationship obtained with fetal hemoglobin replacement (Fig. 2). Therefore, changes in P_{50} appear to be an important determinant of the CBF response to inhaled CO.

To see if CaO_2 and P_{50} predict the CBF response to CO hypoxia, P_{50} was varied independently of COHb. Newborn lambs that had their P_{50} increased by replacing fetal hemoglobin with adult hemoglobin were ventilated with CO (35). Increasing P_{50} with adult hemoglobin transfusion decreased CBF and cerebral O_2 transport. Increasing COHb to a level sufficient to restore P_{50} to the original baseline value resulted in an increase in CBF that restored cerebral O_2 transport back to the original baseline level without a change in $CMRO_2$ or arterial pressure. By not letting P_{50} fall below the original baseline P_{50}, O_2 transport did not increase above the original baseline O_2 transport as it normally would have during CO hypoxia. Thus, the increase in CBF that occurs during CO hypoxia does not disturb the relationship between O_2 transport and P_{50}. In other words, CBF during CO hypoxia can be predicted by CaO_2 and P_{50} when perfusion pressure and $CMRO_2$ are maintained. Therefore, the augmented CBF response to CO hypoxia compared with hypoxic hypoxia and the consequent increase in cerebral O_2 transport are consistent with effects of CO on P_{50}. However, these results do not disprove that CO may have a direct dilating effect on cerebral arterioles in addition to the predicted effects on a tissue PO_2 feedback mechanism.

SIMULATION OF TISSUE PO_2

To evaluate the effects of CaO_2 and P_{50} on tissue PO_2, a compartmental model of O_2 transport was developed that allowed for precapillary O_2 loss in five orders of branching arterioles (69). The relationship of the experimentally determined CBF values to the simulated tissue PO_2 values was determined during hypoxic hypoxia for two different levels of P_{50} in lambs with fetal and adult hemoglobin (70). The relationship of CBF to simulated tissue PO_2 at low P_{50} (26 mm Hg) was within 1–3 mm Hg of the relationship at high P_{50} (37 mm Hg). Because 1–3 mm Hg difference was less than the 11 mm Hg difference in P_{50}, the model gave a reasonably close

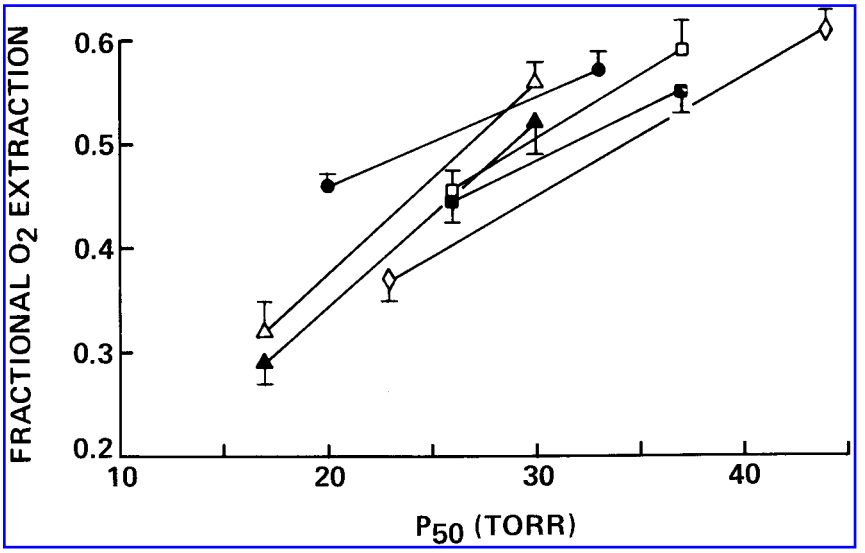

FIG. 2. Cerebral fractional O_2 extraction versus P_{50} with increasing P_{50} when fetal hemoglobin is replaced by adult hemoglobin in normoxic newborn lambs (■), in hypoxic newborn lambs (□), in normoxic fetal sheep (▲), and in hypoxic fetal sheep (Δ). Decreasing P_{50} with CO hypoxia in newborn lambs (●) and adult sheep (◇) resulted in decreases in fractional O_2 extraction. The relationship of fractional O_2 extraction versus P_{50} with CO hypoxia paralleled that of exchange of adult for fetal hemoglobin. Reprinted with permission from reference (35).

prediction of CBF on the basis of tissue PO_2. Further work is needed to consider the complex three-dimensional geometry of the cerebral microcirculation and spatial variations in tissue PO_2.

The increase in bulk O_2 transport to the cerebral microcirculation with CO hypoxia will act to mitigate the fall in tissue PO_2, but tissue PO_2 is still predicted to be lower than that with hypoxic hypoxia at an equivalent CaO_2. For example, Fig. 3 shows the model prediction of PO_2 in the five successive branches of arterioles, capillaries, five branching orders of venules, and a single tissue compartment for adult sheep, which have a relatively high baseline P_{50} of 41 mm Hg and a low hematocrit of 30% (70). With hypoxic hypoxia at 50% arterial O_2 saturation, tissue PO_2 is predicted to fall from 42 to 21 mm Hg. The decrease in PO_2 in arterioles is small because arterial PO_2 is on the steep portion of the oxygen dissociation curve where decreases in PO_2 are buffered. In contrast, with 50% COHb and a decrease in P_{50} to 19 mm Hg, PO_2 is predicted to decrease rapidly along the arteriolar network because of low O_2 carrying capacity and low P_{50}. Capillary and venular PO_2 decrease to values lower than the corresponding values for hypoxic hypoxia. Tissue PO_2 is predicted to fall to 19 mm Hg, which is slightly less than the 21 mm Hg value predicted for hypoxic hypoxia. If CBF during CO hypoxia is set at the level measured during hypoxic hypoxia with constant O_2 transport, tissue PO_2 would be predicted to fall to 15 mm Hg. Thus, the enhanced CBF response and increased O_2 transport with CO hypoxia attenuate the additional drop in tissue PO_2 associated with the decrease in P_{50} by about two-thirds compared with the expected tissue PO_2 during hypoxic hypoxia with constant O_2 transport [(19 − 15)/(21 − 15) = 2/3]. In contrast, the relationship of CBF to PO_2 in the terminal arteriole did not provide a unifying relationship (70). Although isolated arteries can dilate during

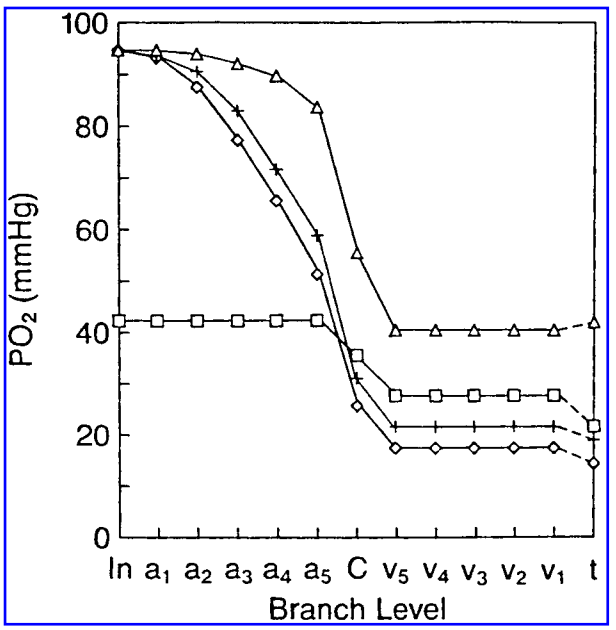

FIG. 3. Computer simulation of PO_2 in five successive branches of arterioles (a_1–a_5), capillary (C), five converging branches of venules (v_5–v_1) and a single tissue (t) compartment with different arterial input (In) PO_2. Model parameters were obtained from the literature. Experimental blood flow and O_2 consumption data were obtained from sheep. Precapillary decrease in PO_2 with CO hypoxia (+) was greater than during room air ventilation (Δ), or hypoxic hypoxia (□). Arterial O_2 content in both hypoxic and CO hypoxia was 50% of normal. Although blood flow during CO hypoxia was greater than during hypoxic hypoxia, tissue PO_2 was predicted to be lower. If blood flow during CO hypoxia was set to the level observed during hypoxic hypoxia (◇), tissue PO_2 would be even lower. Reprinted with permission from reference 70.

hypoxia, oxygen sensing within parenchymal cells appears to play a greater role in regulating vasodilation than vascular oxygen sensors. Therefore, cerebral vasodilation to CO hypoxia can be largely predicted by tissue PO_2 in a simplified compartmental model in which CaO_2 and P_{50} are varied independently at a particular level of $CMRO_2$ and perfusion pressure. However, the critical level of PO_2 below which oxidative phosphorylation becomes limited with CO hypoxia is predicted to require less of a decrease in CaO_2 than with hypoxic hypoxia.

EFFECTS ON BRAIN FUNCTION, METABOLISM, AND OXIDATIVE STRESS

It is important to consider the level of COHb when considering toxic effects of CO on brain function and metabolism. As reviewed by Benignus (7), several studies in humans reported changes in visual detection thresholds, fine motor skills, and reaction times at COHb less than 20%. However, these changes could not be confirmed by others. Differences in findings among studies could be due to differences in experimental design, intersubject variability, and the range and duration of COHb. The threshold for obtaining consistent changes in cognitive function among subjects may be greater than 20% COHb. Tissue hypoxia is probably too mild to explain changes in cognitive function at COHb less than 20%. On the other hand, it is now appreciated that CO can act as an endogenous neurotransmitter (71). Acute inspiration of CO may interfere with normal signaling processes evoked by endogenous CO. Chronic exposure to low levels of CO from cigarette smoke or other sources in the environment may upregulate pathways that modulate neurotransmitter processing of CO. In this way, environmental factors could contribute to intersubject variability in cognitive deficits during CO inhalation. However, this hypothesis has not been investigated.

With COHb in the range of 35–55% in conscious adult sheep, a 16% decrease in $CMRO_2$ was observed despite the increase in cerebral O_2 transport and maintenance of arterial blood pressure (34). At comparable levels of CaO_2 with hypoxic hypoxia, $CMRO_2$ was unchanged. Using a different blood-flow methodology in sheep, progressive decreases in $CMRO_2$ were found over time as COHb increased from 40 to 65% prior to a drop in arterial pressure (38). Behavioral changes were observed as $CMRO_2$ declined with eventual loss of consciousness. Likewise, CO hypoxia in the rabbit produces decreases in somatosensory evoked potential amplitude despite maintained cerebral O_2 transport at levels of CaO_2 that produce no change in evoked potentials with hypoxic hypoxia (43). Thus, metabolic and functional disturbances do not require a drop in arterial pressure and cerebral O_2 transport.

Because of spatial heterogeneity of PO_2 at the cellular level related to distance from the capillary and to red cell flux heterogeneity among capillaries, the simplest explanation for the decrease in $CMRO_2$ is that tissue PO_2 declines below critical levels in a subpopulation of cells. Based on the aforementioned simulation model with a single tissue compartment, mean tissue PO_2 remains lower with CO hypoxia than with hypoxic hypoxia even when the increase in CBF is greater with CO hypoxia. Thus, a subpopulation of neurons and glia is more likely to reach critically low PO_2 values with CO hypoxia than with hypoxic hypoxia. It is interesting that newborn lambs, which have a lower P_{50} and slightly higher $CMRO_2$ than adult sheep, do not exhibit a decrease in $CMRO_2$ over levels of COHb comparable to adult sheep (34). It is conceivable that the increase in P_{50} and the decrease in $CMRO_2$ during maturation in sheep result in a decrease in capillary density, in contrast to the postnatal increase in cerebral capillary density seen in rodents (12). A 40% lower cerebral plasma volume in adult sheep compared with newborn lambs (73) supports the possibility of a postnatal decrease in vascular density in sheep. Thus, there could be developmental and species differences in the threshold for metabolic disturbances during CO hypoxia.

In addition to tissue hypoxia, decreases in $CMRO_2$ could be the result of direct effects of CO on the mitochondrial respiratory chain. At high concentrations, CO can exert direct effects on cytochrome oxidase. Using reflectance spectrophotometry on the brain surface in which the hemoglobin signal was suppressed by exchange transfusion with a fluorocarbon solution, Piantadosi *et al.* (58, 59) demonstrated cytochrome interactions with CO when rats were ventilated with at least 3% CO. Increases in phosphocreatine and an alkaline shift in extramitochondrial pH occurred without a change in $CMRO_2$ or ATP and were thought to reflect an increased efficiency of oxidative phosphorylation by unblocked respiratory chains (60). The importance of this cytochrome interaction with CO in the normal, hemoglobin-perfused brain is unclear. Inspiring 3% CO results in COHb greater than 70% in the rat (44). At these high levels of COHb, arterial pressure usually falls, the increase in CBF is attenuated, and high-energy phosphates in brain decrease (44, 45). Thus, effects of tissue hypoxia on mitochondrial respiration may precede interactions of CO with respiratory cytochromes. On the other hand, the interaction of CO with cytochromes seen in fluorocarbon-perfused rats occurs at a relatively high tissue PO_2. If the interaction depends on the ratio of CO to O_2, the interaction may become significant at a lower partial pressure of CO in the hemoglobin-perfused brain because of the presence of low tissue PO_2. In addition, low PO_2 may permit NO to interact with cytochrome oxidase (10).

Interactions of CO with respiratory cytochromes at moderate levels of CO may not substantially decrease oxidative phosphorylation because of the "cushioning" effect of unblocked electron transport chains. Cyanide would be anticipated to decrease this cushioning effect. Cyanide infusion by itself causes an increase in CBF (63) and a reduction of cytochrome aa_3 that precedes the reduction in $CMRO_2$ (39). Combining cyanide infusion with CO ventilation produced additive effects on CBF, but synergistic decreases in $CMRO_2$ (63). Because cyanide augmented CBF and cerebral O_2 transport during CO exposure, the decrease in $CMRO_2$ is more readily explained by a loss of this cushioning effect than by a decrease in O_2 availability. Because firefighters and other victims of CO inhalation can be exposed to a variety of toxins such as cyanide, it is important to consider that toxicity to

CO may be potentiated by the presence of other toxins in the environment.

Exposure to CO may also act to increase NO availability and lead to endothelial oxidant stress, as described in a series of studies by Thom, Ischiropoulos, and colleagues. Ventilating rats with as little as 50 ppm of CO for 1 h has been reported to increase nitrotyrosine immunoreactivity in aortic endothelium and result in transcapillary efflux of albumin (82). In endothelial cell culture, exposure to 100 ppm of CO (110 nmol/L) produces peroxynitrite (81) and eventual apoptosis (83). Exposure to 10 ppm of CO for 1 h increases manganese superoxide dismutase and provides tolerance to subsequent exposure to 100 ppm of CO. Thus, relatively low concentrations of CO can produce oxidant stress and cell death in aortic endothelial cells, but prior exposure to small, sublethal concentrations of CO provides protection. Whether CO preconditioning provides protection in brain from lethal levels of CO has not been investigated.

In brain, oxidant stress has been examined at higher levels of CO than in aortic endothelial cells. When rats are exposed to 1,000 ppm of CO for 40 min, nitrotyrosine concentration in brain increases and the increase is attenuated by removal of platelets (29). However, platelet removal had no effect on the increase in nitrotyrosine when the CO exposure was extended to 60 min. Thus, platelets may act as a source of NO during early exposure to CO (78), whereas other cells may be the primary source when exposure is prolonged. As CO did not increase NOS catalytic activity in platelets, a decrease in NO binding to heme proteins in platelets was postulated to cause the increase in NO availability (77, 78).

Interestingly, NOS inhibition blocks not only nitrotyrosine formation (29), but also the increase in red-cell perfusion (50). These results suggest that NO either mediates or permits cerebral vasodilation at 1,000 ppm exposure in the rat, although the lack of arterial pressure data does not permit definitive conclusions. Dilation of cerebral vessels by NO not only involves a cGMP-dependent pathway, but may also involve NO inhibition of cytochrome P450 ω-hydroxylase activity, which produces the cerebral vasoconstrictor 20-hydroxyeicosatetraenoic acid (20-HETE) (2, 74). 20-HETE prevents hyperpolarization of arteriolar smooth muscle by closing K_{Ca} channels (37). Therefore, it is possible that increased NO during CO exposure inhibits 20-HETE formation and permits CO to directly open K_{Ca} channels (92).

When CO exposure is increased to 2000 ppm for 60 min, red-cell perfusion has been observed to increase over a 30-min period and then to decline toward baseline, possibly reflecting the onset of arterial hypotension (51). Small increases in extracellular K^+ activity occurred as perfusion declined from the peak value. In aged rats, the increase in red cell flux was blunted, but it is unclear if this blunted flow response is attributable to lower NO or greater hypotension, or is specific to this form of hypoxia.

When rats are exposed to 1,000 ppm of CO for 40 minutes followed by exposure to 3,000 ppm of CO for 20 min, COHb reaches 66% and the rats often become hypotensive and lose consciousness (29). As the electroencephalographic activity becomes depressed, increases in NADH fluorescence, decreases in extracellular pH, and increases in extracellular K^+ activity become evident, thereby indicating significant tissue hypoxia (49). Electron paramagnetic resonance spectroscopy indicates a large increase in NO in brain (29). Immunocytochemistry indicates enriched localization of nitrotyrosine in cerebral vessels. Plasma markers of oxidative stress; including oxidized glutathione, are elevated (80). Inhibition of NOS or leukocyte depletion reduces the observed increases in xanthine oxidase, which contributes to lipid peroxidation in this model (29, 76). Lipid peroxidation is also reduced by NOS inhibition. In a slightly different model of CO toxicity, hydroxyl radicals can be detected in cerebral microdialysates using the salicylate trapping technique (61).

Therefore, this series of studies by Thom *et al.* indicates that exposure to moderate levels of CO for at least 40 minutes without hypoperfusion appears to induce oxidant stress in the cerebral endothelium, possibly mediated in part by NO release from platelets. Subsequent exposure to higher levels of CO is postulated to cause myocardial dysfunction, cerebral hypoperfusion, leukocyte sequestration, and conversion of xanthine dehydrogenase to xanthine oxidase. Concurrent energy loss leads to release of adenine nucleotides, increased hypoxanthine availability, generation of superoxide anion, oxidant stress, and brain lipid peroxidation. Hence, moderate levels of CO exposure of sufficient duration appear to prime the endothelium for a cascade of oxidative damage. If COHb continues to increase to levels sufficient to cause energy imbalance, a cascade of prooxidative processes is triggered and amplified, leading to brain injury.

NEUROPATHOLOGY

Specific areas of the brain are more vulnerable than others to damage by CO exposure, as reviewed by Kindwall (31). Delayed white matter injury, particularly central white-matter (20), is most prevalent and probably contributes to neurological deficits with moderate CO poisoning. As CO levels and duration increase, selective injury in globus pallidus, hippocampus, and cerebellar Purkinje neurons becomes prominent. Heterogeneous cell loss can extend into cortex. Morphological features of both necrosis and apoptosis can be detected during delayed injury (62), analogous to that seen after other forms of hypoxia–ischemia injury. Selective injury in hippocampus and Purkinje neurons is consistent with their selective vulnerability to other forms of hypoxia–ischemia. Basal ganglia are also selectively vulnerable to ischemia, but generally require somewhat longer ischemic durations than that required for hippocampal injury. Damage in basal ganglia can be quite prominent in models of partial ischemia where oxidative stress is enhanced. Interaction of the dopaminergic and glutamatergic systems in enhancing excitotoxicity is thought to contribute to selective vulnerability of basal ganglia (22). However, other forms of hypoxia–ischemia typically involve injury to the caudate and putamen. The reasons for the apparent selectivity for globus pallidus injury with CO toxicity are unclear. Therefore, more work is needed to understand whether the mechanisms involved in other forms of hypoxic–ischemic brain injury explain the pattern of

selective vulnerability in CO toxicity, or if additional mechanisms such as endothelial oxidant stress contribute to selective neuronal and white matter degeneration.

SUMMARY

The interaction of pathways involved in cerebrovascular regulation by CO and in CO toxicity are summarized schematically in Fig. 4. Some of these interactions remain speculative at this time. CO derived from heme oxygenase in neurons and endothelium potentially can regulate cerebral vasodilation. However, the evidence available to date supporting a role for endogenous CO is derived primarily from newborn piglets, where activation of K_{Ca} channels appears to be more important than increasing cGMP. Evidence for a regulatory role for CO in mature brain is limited. More work is needed to understand how CO functions in the physiological regulation of the cerebral circulation in mature brain, and how it might interact with the NOS–guanylyl cyclase pathway and with pathways that control K_{Ca} channels, such as the P450 ω-hydroxylase and epoxygenase pathways.

With inhalation of CO and formation of COHb, increases in CBF can be predicted on the basis of the decrease in CaO_2 and P_{50} as long as arterial blood pressure does not fall. Computer simulations indicate a lower tissue PO_2 with COHb than with hypoxic hypoxia at equivalent reductions in CaO_2 because of accelerated precapillary O_2 loss and the leftward shift of the oxygen dissociation curve with COHb. The lower tissue PO_2 is thought to result in a greater release of media-

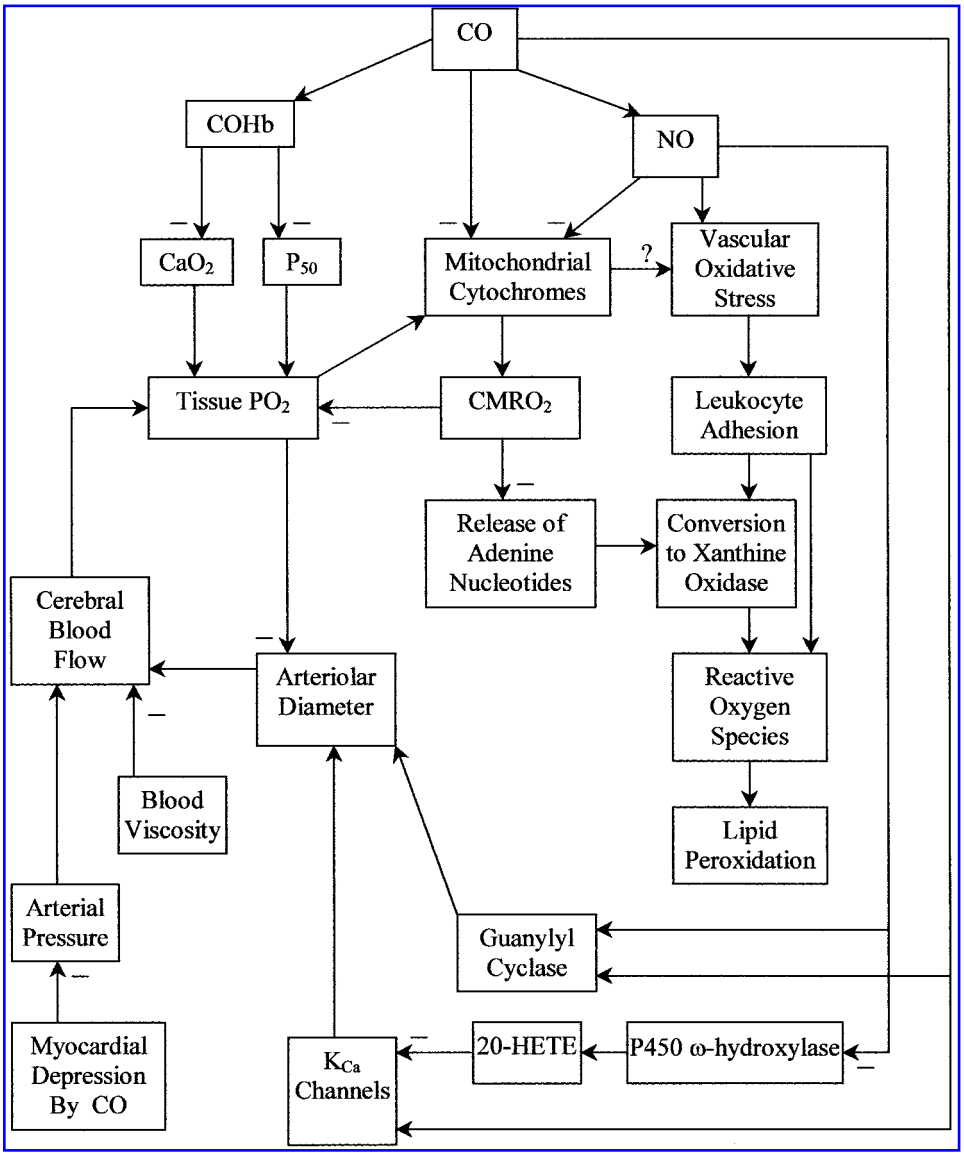

FIG. 4. Schematic summary of effects of CO on the cerebral circulation and potential mechanisms of CO toxicity. A negative sign implies an inverse relationship between variables. The contribution of superoxide or other reactive oxygen species from mitochondrial sources on vascular oxidative stress is speculative.

tors of hypoxic vasodilation, a greater increase in CBF, and an increase in bulk O_2 transport into the cerebral microcirculation, which, in turn, acts to attenuate the decrease in tissue PO_2.

When the duration of CO inhalation extends beyond an hour, the cerebral endothelium displays evidence of oxidant stress as indicated by nitrotyrosine immunoreactivity. If the level of COHb reaches a point where cardiac function is compromised and arterial blood pressure falls, the increase in CBF becomes attenuated and tissue PO_2 will fall further. The combination of low O_2, high CO, and apparently high NO will eventually inhibit cytochrome oxidase and $CMRO_2$, leading to release of adenine nucleotides and hypoxanthine. Impaired mitochondrial respiration might also lead to increased free radical generation, although the contribution of mitochondria to oxidant stress under conditions of CO hypoxia has not been delineated. As cerebral perfusion declines, leukocytes are sequestered, xanthine dehydrogenase is converted to xanthine oxidase, and a cascade of reactive oxygen species and lipid peroxidation are generated. This scenario may represent one mechanism by which CO toxicity causes neuronal injury. Interestingly, hyperbaric oxygenation, which might be expected to promote the formation of reactive oxygen species, reduced delayed neurologic deficits compared with 100% O_2 therapy at ambient pressure in patients who presented within 6 h of CO poisoning (79). However, patients in this study had not lost consciousness and presumably had an adequate increase in CBF for maintaining $CMRO_2$. Thus, sustaining a high tissue PO_2, together with rapid clearance of CO, might be important for preventing progression of the CO-induced oxidant injury cascade in patients with mild-to-moderate CO poisoning.

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ABBREVIATIONS

CaO_2, arterial oxygen content; CBF, cerebral blood flow; cGMP, cyclic GMP; $CMRO_2$, cerebral oxygen consumption; CO, carbon monoxide; COHb, carboxyhemoglobin; 20-HETE, 20-hydroxyeicosatetraenoic acid; K_{Ca} channels, calcium-activated potassium channels; NO, nitric oxide; NOS, nitric oxide synthase; P_{50}, partial pressure of oxygen at 50% oxyhemoglobin saturation; PO_2, partial pressure of oxygen.

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