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Review

Carbon monoxide and the nervous system

J.A. Raub[a,*], V.A. Benignus[b]

[a]*United States Environmental Protection Agency, National Center for Environmental Assessment, Mail Code B-243-01, Research Triangle Park, NC 27711,USA*

[b]*United States Environmental Protection Agency, National Health and Environmental Effects Research Laboratory, Human Studies Division, Mail Code 58B, Research Triangle Park, NC 27711, USA*

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Abstract

Carbon monoxide (CO) is a colorless, tasteless, odorless, and non-irritating gas formed when carbon in fuel is not burned completely. It enters the bloodstream through the lungs and attaches to hemoglobin (Hb), the body's oxygen carrier, forming carboxyhemoglobin (COHb) and thereby reducing oxygen (O_2) delivery to the body's organs and tissues. High COHb concentrations are poisonous. Central nervous system (CNS) effects in individuals suffering acute CO poisoning cover a wide range, depending on severity of exposure: headache, dizziness, weakness, nausea, vomiting, disorientation, confusion, collapse, and coma.

At lower concentrations, CNS effects include reduction in visual perception, manual dexterity, learning, driving performance, and attention level. Earlier work is frequently cited to justify the statement that CO exposure sufficient to produce COHb levels of ca. 5% would be sufficient to produce visual sensitivity reduction and various neurobehavioral performance deficits. In a recent literature re-evaluation, however, the best estimate was that [COHb] would have to rise to 15–20% before a 10% reduction in any behavioral or visual measurement could be observed. This conclusion was based on (1) critical review of the literature on behavioral and sensory effects, (2) review and interpretation of the physiological effects of COHb on the CNS, (3) extrapolation from the effects of hypoxic hypoxia to the effects of CO hypoxia, and (4) extrapolation from rat behavioral effects of CO to humans.

Also covered in this review article are effects of chronic CO exposure, the discovery of neuroglobin, a summary of the relatively new role for endogenous CO in neurotransmission and vascular homeostasis, groups which might be especially sensitive to CO, and recommendations on further research. The interested reader is directed to other published reviews of the literature on CO and historically seminal references that form our understanding of this ubiquitous gas.

Keywords: Central nervous system; Neurobehavior; Carbon monoxide; Carboxyhemoglobin

Contents

* Corresponding author. Tel.: +1-919-541-4157; fax: +1-919-541-1818.
E-mail address: raub.james@epa.gov (J.A. Raub).

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1. Introduction

Carbon monoxide (CO) is a colorless, tasteless, odorless, and non-irritating gas formed when carbon in fuel is not burned completely. Outdoors, the largest source of CO is motor vehicle exhaust. Other sources include industrial processes, non-transportation fuel combustion, and natural sources such as wildfires. Indoors, CO can be found in tobacco smoke and can accumulate from inadequately vented stoves, furnaces, and other combustion sources. Carbon monoxide enters the bloodstream through the lungs and attaches to hemoglobin (Hb), the body's oxygen carrier, forming carboxyhemoglobin (COHb) and thereby reducing oxygen (O_2) delivery to the body's organs and tissues. Equilibrium COHb levels estimated to result from increasing concentrations of CO are given in Table 1. At high concentrations, CO is poisonous. Symptoms in individuals suffering acute CO poisoning cover a wide range, depending on severity of exposure: headache, dizziness, weakness, nausea, vomiting, disorientation, confusion, collapse, and coma. Perhaps the most insidious effect of CO poisoning is the delayed development of central nervous system (CNS) impairment within 1–3 weeks, and the neurobehavioral consequences, especially in children. At lower concentrations, CNS effects include reduction in visual perception, manual dexterity, learning, driving performance, and attention level.

Table 1
Equilibrium COHb levels resulting from steady-state exposure to increasing concentrations of carbon monoxide

CO in atmosphere		Estimated COHb in blood (%)
%	ppm	
0.001	10	2
0.007	70	10
0.012	120	20
0.022	220	30
0.035–0.052	350–520	40–50
0.080–0.122	800–1220	60–70
0.195	1950	80

Source: Raub et al. [48].

2. Acute effects of carbon monoxide on neurobehavior

2.1. Review of the literature

In this section, the arguments from the recent literature about the effect of acute exposure to CO, and its behavioral effects, will be summarized. Not only will conclusions be made and defended, but the uncertainties and their possible explanations will be discussed.

Throughout this review, it will be assumed that the mechanism by which acute CO exposure produces its effects is that of hypoxia [1,2]. This appears to be the case for all of the other effects of acute COHb elevation. For this reason, the term 'CO hypoxia' (COH) will be used frequently, and contrasted with hypoxic hypoxia (HH)-—hypoxia due to reduced inhaled-air O_2 concentration.

Some of the more recent environmental toxicology books [3] continue to state that CO exposure sufficient to produce 5–10% COHb will produce deficits in the ability of humans in visual function, task performance, and maintaining alertness. Others [4] note the historical problems with the human CO-behavioral literature, but do not review other available information. More critical reviews [1,2,5,6] have remarked in detail upon the disagreement among reported findings in the literature. The following is a condensed version of these works. It is easy to dispense with some of the problems in the literature because they were simply unreplicable. One early work (and still probably the most often cited) by McFarland et al. [7] reported a dose-related decline in

visual sensitivity in three subjects with actual data given for only one subject. The (apparently) same data were reported twice again in separate venues [8,9]. Four other studies were unable to find any effects of elevated COHb on visual thresholds, despite more extensive and careful work [10–13]. Similarly, the decline in time estimation ability reported by Beard and Wertheim [14] was not replicable by others [15–20].

Other reported effects of CO exposure also proved to be difficult to replicate, but not as clearly as these examples. In an effort to find an explanation for the variability among the reports, Benignus [21] found that experiments conducted in a single-blind manner were significantly more likely to find effects of CO than those conducted double-blind (i.e. both the researcher and the subject were unaware of the experimental protocol). For the majority of dependent variables, a number of studies that were not double-blind reported effects while none of the double-blind studies did. When non-double-blind studies were removed from consideration, only three dependent variables remained with some statistically significant findings. These were tracking (a visuo-motor coordination task), vigilance (a low-demand, long-duration task), and continuous performance (any task in which the subject is continuously engaged for a period of time). Among double-blind reports on these three, however, there was still a disagreement. Table 2 is a summary in which it is seen that less than 50% of studies reported significant effects. These conclusions are discussed in detail elsewhere [2,5].

The double-blind studies that reported significant results had about the same variance as did studies that found no significant effects. There were no important differences in methodology or experimental design. In fact, non-significant findings in studies were frequently failures to replicate the original studies, sometimes by the original workers. Those studies reporting significant results found them because of larger effects.

It is difficult to conclude very much about the behavioral literature on acute CO effects in humans. Many, but by no means all, of the studies were methodologically questionable. Among the double-blind studies, most were performed in a sophisticated and careful manner. Yet, some reported significant effects, although the majority did not. Among the double-blind studies, the one with the widest range of COHb (up to 16.6%) found no significant effects on tracking [22] even at the highest level of COHb and despite the fact that the variability in the results was not greater than other studies. At the very least, it is reasonable to claim that the oft-reported visual sensitivity effects are not correct because of the inability to replicate them by others. It is also reasonable that none of reported CO behavioral effects in humans are, without further work, entirely credible. The problem can be approached from several other viewpoints, as follows.

Table 2
Significant effects of elevated COHb in double blind studies

Dependent variable	Number of reports	Probability of reported significant effects
Tracking	7	0.43
Vigilance	4	0.25
Continuous performance	5	0.40

2.2. Re-analysis of the literature

It is well known that as the capacity for arterial blood to carry O_2 is reduced by either HH or COH. To compensate, a cerebrovascular vasodilation occurs which is sufficient to maintain O_2 delivery to the CNS. This phenomenon has been demonstrated in dogs, sheep, goats, and humans [23]. Despite constant supply of O_2, however, metabolism of O_2 ($CMRO_2$) begins to decline in a statistically significant manner as COHb approaches 30–50% [23–27].

2.2.1. Mechanism of CO and behavioral effects

The available physiological data have been re-analyzed and synthesized to provide insight to behavioral effects of CO exposure [2]. The data for the effect of COHb on $CMRO_2$ were reported as means for each COHb level. To achieve more generalized information, $CMRO_2$ means from two studies [24,27] were converted to percentages of baseline and pooled into one data set and plotted against COHb. Other studies were not included in the analysis because important data needed to transform the means to percent of baseline were not reported. A logit function was fitted to the percent of baseline data and the effective dose causing a 10% change from baseline (ED-10) could be determined. The data and the curve fit, with associated 95% confidence limits are plotted in Fig. 1. It appears that a 10% decrement in $CMRO_2$ should not be expected until COHb reaches ca. 27%. The confidence limits for the ED-10 were about 22–32%.

Assuming that hypoxia is the mechanism by which CO impacts CNS function, it appears that effects of COHb so small as to not detectably impair O_2 metabolism would similarly not detectably affect behavior. Perhaps a reduction in $CMRO_2$ of a greater size is required to produce an ED-10 on behavior. In any event, the size of reduction in $CMRO_2$ at 5–10% COHb is quite small, and the probable behavioral effect is correspondingly small. The physiological data on $CMRO_2$ effects of COHb, therefore, appear to lend support to the argument that the significant effects on behavior reported in some studies were Type II errors (i.e. reporting significant effects when there were no effects of that size in the population).

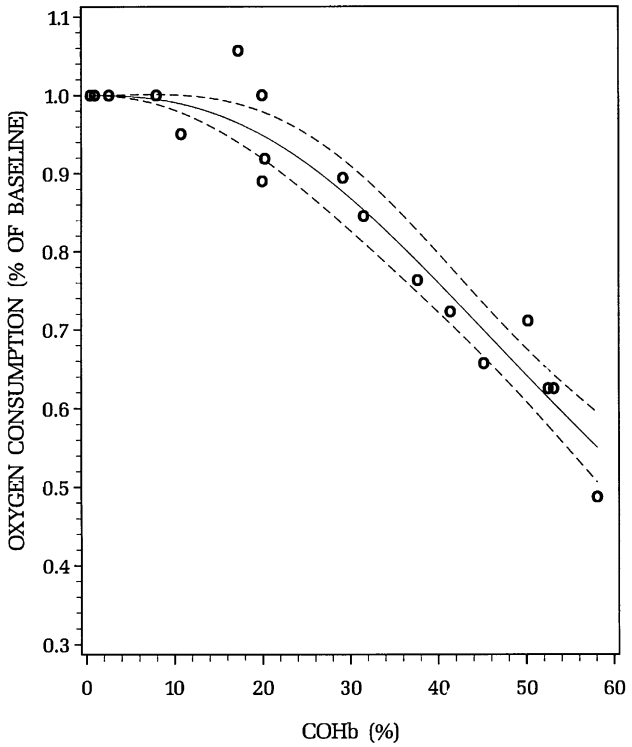

Fig. 1. Brain oxygen consumption as a function of COHb. Data from the literature with fitted line and upper and lower 95% confidence bounds.

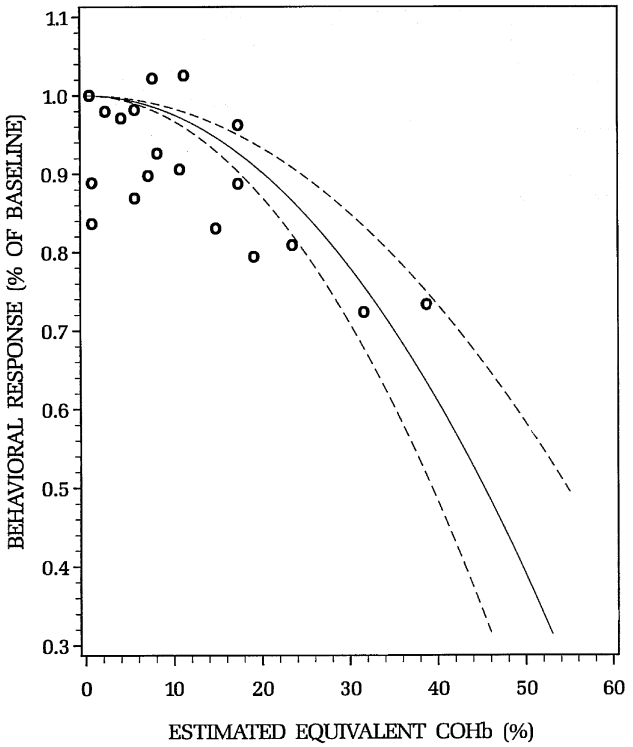

Fig. 2. Behavioral decrement for humans in HH, which has been converted to equivalent COHb, and behavioral effects have been corrected for the effects of hyperventilation which occurs in hypoxia but not with elevated COHb.

2.2.2. Comparison of COH and HH effects on behavior

COH and HH both produce behavioral effects that were compared [6] as follows. The two forms of hypoxia were expressed in a scale of measurement that is physiologically comparable by making all measures equivalent in terms of arterial O_2 content (C_aO_2) which can be found for either one. A problem for such comparison was that HH produces hyperventilation, which reduces the arterial CO_2 (hypocapnia), but COH has no such effect. Hypocapnia also has a behavioral effect. To make a properly adjusted comparison, (1) the HH behavioral literature was reviewed, and results were pooled and recast as continuous functions of C_aO_2, (2) the hypocapnia behavioral literature was reviewed, pooled, and cast into a continuous function, (3) the amount of hypocapnia was estimated as a continuous function of C_aO_2, and then (4) the magnitude of the behavioral effects of the estimated hypocapnia (due to HH) were subtracted from the magnitude of the behavioral effects due to the HH. Thus, the HH behavioral decrements were corrected for hypocapnia and thereby made comparable to COH. Finally, the C_aO_2 for the HH was converted to equivalent COH for ease of comparison.

Fig. 2 is a plot of the estimated corrected HH behavioral effect. The ED-10 for hypocapnia-corrected HH is seen to be equivalent to ca. 20% COHb. From the earlier comparison of HH to COH, additional weight is provided to the argument that the ED-10 for acute CO effects is much higher than reported by some studies and that the studies reporting such effects were probably Type II errors.

2.2.3. Comparison of rat CO behavioral results to those of humans

There is an extensive database of literature on the effects of acute CO exposure on behavior in rats that was reviewed and synthesized by Benignus [6]. Direct comparison of rat results to human results is complicated by the fact that rats become poikilothermic when subjected to almost any toxicant. Because of typical laboratory temperatures, this means that they become hypothermic as a function of the dose of the toxicant. Hypothermia has an effect on behavior. Humans, however, do not become hypothermic under similar COHb elevations (at least not for the time durations of experiments) because of their higher mass. Thus to compare rat data to humans, a correction should be made for hypothermia effects.

The rat data were reviewed, pooled, and adjusted for the effect of hypothermia by (1) evaluating the literature on hypothermia effects on behavior, pooling the results, and casting them as a continuous function of body temperature, (2) estimating the body temperature as a function of COHb, and finally (3) subtracting the behavioral effect of hypothermia (due to COHb elevation) from the behavioral results in the literature. To cast rat behavior as a function of COHb and to make corrections, COHb had to be estimated

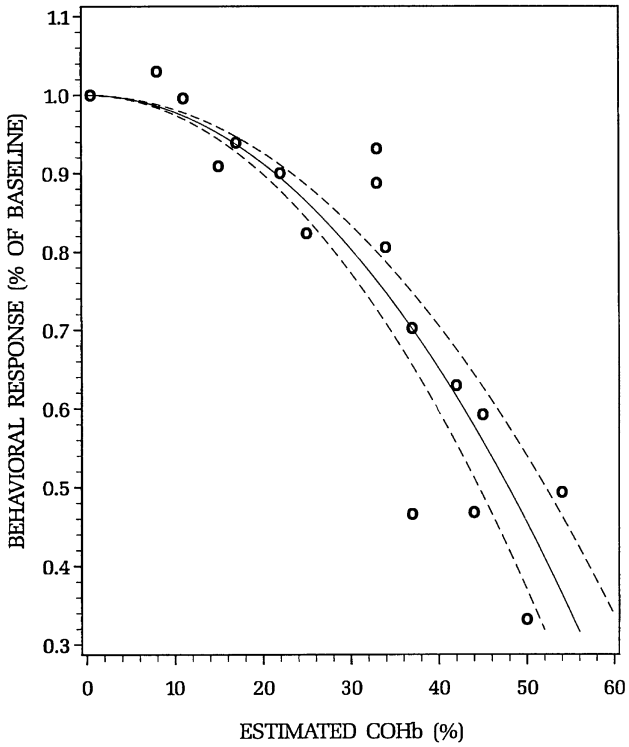

Fig. 3. Behavioral decrement in rats as a function of estimated COHb.

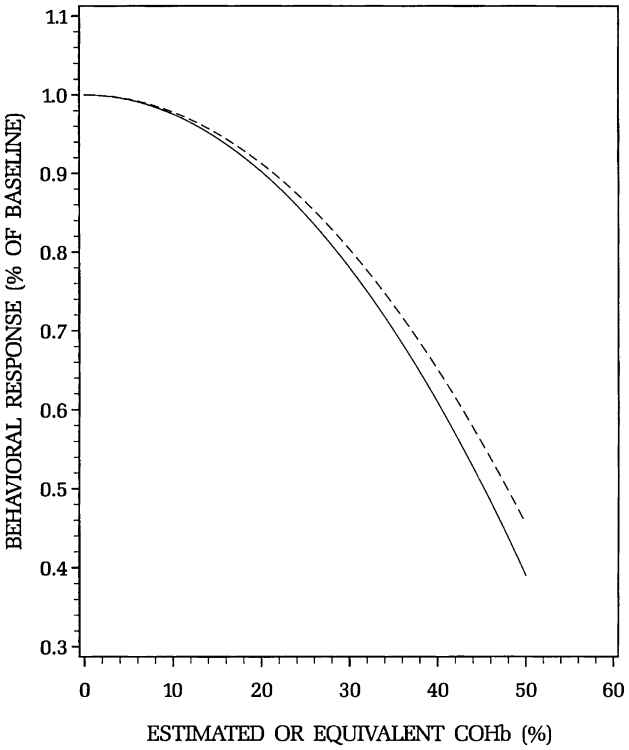

Fig. 4. Behavioral decrements in rats with elevated COHb (dashed line) compared to behavioral effects in hypoxic humans (solid line) in which hypoxia has been expressed in equivalence to COHb.

from a rat version of the physiologically based pharmacokinetic model for CO uptake and elimination [28].

Fig. 3 is a plot of the estimated behavioral effects of COHb elevation in rats as adjusted for hypothermia. The ED-10 for these data is ca. 21% COHb. It is possible that rats are less sensitive to behavioral disruption by elevated COHb or that tests used in rats are not as sensitive as those used in humans. The rat ED-10 data for COHb, however, agree with the HH data from humans. The extent of this agreement is depicted in Fig. 4 where the solid line is the curve fitted to hypocapnia-corrected HH data in humans and the broken line is the curve fitted to the hypothermia-corrected rat COHb data.

2.2.4. Plot of human COHb behavior data

Human data on behavioral effects of COHb elevation were compared with the curve estimated from HH effects in humans [6]. Because there were no high level data, fitting a curve to the human COHb data only was considered to be prone to error. The comparison was made qualitatively by plotting the means from all of the available double-blind COHb studies on the same graph as the curve fitted to human HH studies. These means were plotted as closed points for studies which reported significant results and as open points for those which were not significant. Because the rat COHb data and human HH data were so similar, results were compared only to the human HH data (Fig. 5).

Most of the statistically significant points in Fig. 5 are clustered about the lower COHb end of the plot. However,

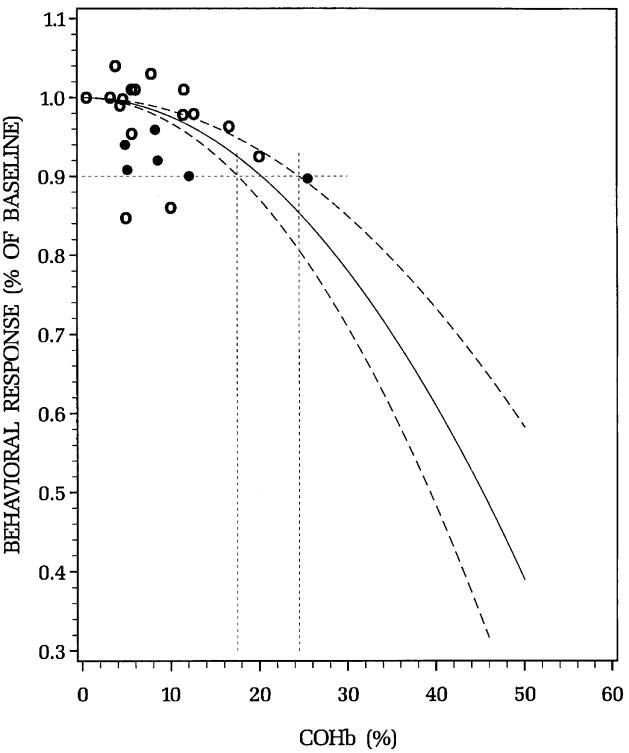

Fig. 5. Behavioral decrements in humans (circles) with elevated COHb compared to behavioral effects in hypoxic humans (solid and dotted lines) in which hypoxia has been expressed in equivalence to COHb. Closed and open circles were the mean values for studies reporting statistically and non-significant results, respectively.

more of the means are non-significant in the same area and at even higher COHb levels. The highest level of COHb (ca. 25%) was statistically significant. It appears that most of the means, except for the very low COHb points, did fall below baseline even though they were not found to be significant. Taken as a group, the points tend to fall around the line fitted to human HH data and (because of their similarity) the rat COHb data.

2.3. Conclusions about acute effects of carbon monoxide on neurobehavior

The literature on the effects of acute COHb elevation on behavior in humans is inconsistent. Some studies report relatively large effects at low COHb (5–10%), although the majority of reports show much smaller, and (usually) statistically non-significant, effects until much larger COHb. Furthermore, both physiological data on $CMRO_2$ reduction as a function of COHb and behavioral data from humans in HH support the idea that COHb should not be expected to produce ED-10 sized effects until it approaches 20%. Behavioral data from rats exposed to CO agree with the latter concept. It does not seem reasonable to accept the effects reported as statistically significant at low COHb at face value. Yet, it is difficult to explain such effects from well planned and executed studies.

It is possible that some of the reported statistically significant, low-level COHb results were simple Type II errors; other results may be due to inadvertent leaks in blinding or because of under-reported, non-significant findings [85]. Regardless of the reason for these reports, it appears unlikely that the findings should be taken at face value. The preponderance of the evidence is against it.

Having concluded that there are good reasons to believe that acutely elevated COHb must approach 20% before the behavioral ED-10 is reached, it must be emphasized that effects of smaller size are predicted as a continuous function of COHb. What is implied, is that effects are: (1) much smaller than previously thought, (2) much less likely to be found significant in studies of typical size, and (3) less important to successful task performance in non-laboratory (i.e. real-world) work. It is always possible to imagine some scenario in which such very small effects could be important, but such cases would be exceedingly rare.

These studies were all conducted on normal, young subjects except Harbin et al. [29] who studied elderly and young subjects in signal detection tasks and found no effects in either group. It is possible that some form of compromised health would make persons more sensitive to acute COHB elevation. If, e.g. a person's cerebrovasculature was unable to dilate as a function of increasing COHb, there would be no compensatory increase in blood supply and a much greater decline in $CMRO_2$. There has been no attempt to identify such specially sensitive subjects. Similarly, if cardiac function was impaired, then it might become impossible to deliver more blood to the brain without impairing blood supply to other tissue. If impairment were sufficiently severe, blood supply to the brain might become inadequate for compensatory purposes (See Section 5 for additional information about sensitive population groups).

There has been no definitive research in the area of acutely high COHb elevation and human behavioral effects. Behavioral studies, with few exceptions, have relied on designs in which only one or two low-level exposures have been employed. A wide-range, dose-effect study in which COHb were elevated sufficiently to produce easily and reliably detected effects would be more convincing if effects were dose-related and did not reach large size until large COHb. If only low levels of exposure are to be used, and if the results were to be convincing, it would be necessary to do a number of studies using the same design and to assure that the rate of non-significant results were known.

3. Chronic effects of carbon monoxide

There are many published studies on acute experimental and accidental exposures to CO (Section 6); however, there is not enough reliable information on effects of chronic exposures to low concentrations from either controlled-human studies, ambient population-exposure studies, or from occupational studies. Further work is needed, therefore, to determine the potential for long-term exposures in the population and to develop reliable dose-response relationships for at-risk groups. This information currently is missing from the published literature. Some of the issues associated with chronic CO exposures are discussed later.

None of the available time series studies of ambient CO exposures in the population is capable of assessing the incremental effect of pollutants over extended time periods. For example, current models cannot confidently predict whether reduction in pollution will decrease monthly rates of hospital admissions or mortality, even if they imply a reduction of admissions on days with low pollution. This public-health-related issue cannot be addressed by daily time series analysis, using only admission or mortality counts. In future studies, investigators also could consider time-averaged health effects over, say, 1 or 3 months, in relation to pollution exposure metrics for the corresponding periods. Consideration of extended time-averaged health effects would tend to allow for detection of more chronic impacts beyond any short-term 'harvesting'[1] that might be observed in daily analyses.

[1] In time series studies, harvesting is a short-term elevation in the frequency of a health outcome during or just after a short period of elevated exposure, followed quickly by a short-term reduction in frequency of the same outcome below baseline frequency, then by a return to baseline. It has been argued that presence of harvesting would suggest that elevated exposures hasten occurrence of the health outcome by only a short time, but bring about little or no net increase in occurrence of the outcome. It also has been argued that absence of harvesting would suggest that, without the elevated exposures, the outcome might have been delayed for a long time or might not have occurred at all.

The potential effect of CO on brain growth and function of the developing fetus, infant, and child have been primarily determined through controlled studies on laboratory animals. From all these studies, it is clear that severe, acute CO poisoning can be fetotoxic, although specification of maternal and fetal COHb levels is difficult because, as in almost all CO poisoning reports, such exposures rarely involve the achievement of steady-state COHb levels or permit careful and rapid determination of COHb levels. Available data [2] provide strong evidence that maternal CO exposures of 150–200 ppm, leading to approximately 15–25% COHb, produce reductions in birth weight, delays in behavioral development, and disruption of cognitive function in laboratory animals of several species. Cardiovascular effects, including cardiomegaly, also are found. Isolated experiments suggest that some of these effects may be present at concentrations as low as 60–65 ppm (ca. 6–11% COHb) maintained throughout gestation.

Studies relating human subchronic CO exposure from ambient sources or cigarette smoking to reduced birth weight are of concern because of the risk for developmental disorders; however, many of these studies have not considered all sources of CO exposure, other pollutants, or other risk factors during gestation. A few available studies suggest that subchronic ambient CO exposure averaged over about 3 month may be associated with increased incidence of low birth weight (typically defined as birth weight $\leq$2500 g). At the same time, these studies are inconclusive, and they are subject to potential confounding by unmeasured factors, such as maternal smoking and nutrition, that are known to influence birth weight. Also, outdoor CO levels may be correlated with indoor levels of CO and other pollutants, which could be higher than outdoor levels, and which were not measured in these studies. Common socioeconomic factors could be associated with both ambient CO levels and such potential confounding variables. These studies are potentially important, however, because low birth weight is associated with infant mortality and childhood morbidity and may predict increased risk of morbidity into adulthood. Although low birth weight is probably not a direct cause of these harmful outcomes, it is a useful marker for developmental disturbances that are more directly responsible.

The developing fetus is at risk from hypoxemia because fetal Hb F binds CO somewhat more strongly than does adult Hb A and because increased COHb saturation would be expected to impair tissue molecular O_2 delivery more in the fetus than in the child or the adult. Results from laboratory animal studies suggest that acute exposure to lower levels of CO, leading to $\leq$10% COHb should not have much of an effect on the developing fetus until possibly later in gestation when the embryo is much larger and more dependent on transport of oxygen by red blood cells. In addition, results from studies of fetal outcome following mild to moderate accidental CO poisoning in pregnancy suggest that hypoxemia associated with measured COHb saturations of up to 18% (or even higher estimated levels) does not impair the growth potential of the fetus when pregnancy continues normally. Therefore, it is unlikely that low levels of CO typically encountered by pregnant women would cause increased fetal risk. It is necessary, however, to consider the combined effects of CO with the other common risk factors that may cause adverse fetal outcome (e.g. tobacco use, lead exposure, alcohol consumption, genetic background, maternal general health, obstetric history).

4. Physiologic responses to carbon monoxide exposure

One of the possible reasons why chronic exposures to low CO concentrations may not pose as much a problem as high, acute exposure is due to physiological compensation, tolerance, or adaptation. Smokers show an adaptive response to elevated COHb levels, as evidenced by increased red blood cell volumes (through increased hemopoiesis) or reduced plasma volumes. The major source of total exposure to CO for smokers comes from active tobacco smoking. Baseline COHb concentrations in smokers average 4% (compared to $<$2% for non-smokers), with a usual range of 3–8% for one to two pack-per-day smokers, reflecting absorption of CO from inhaled smoke. COHb levels as high as 15% have been reported for chain smokers.

The only experimental evidence for short- or long-term compensation to increased COHb levels in the blood from exogenous sources other than smoking is indirect. Experimental animal data indicate that incremental increases in COHb produce physiological responses that tend to offset the deleterious effects of CO exposure on oxygen delivery to the tissues. Experimental human data indicate that compensatory cardiovascular responses to submaximal upper and lowerbody exercise (e.g. increased heart rate, cardiac contractility, cardiac output) occur after CO exposures. These changes were highly significant for exposures attaining 20% COHb. Other compensatory responses are increased coronary blood flow, cerebral blood flow, Hb, and oxygen consumption in muscle.

Short-term compensatory responses in blood flow or oxygen consumption may not be complete or may even be absent in certain persons. For example, from the laboratory animal studies, it is known that coronary blood flow is increased with COHb; and, from human clinical studies, it is known that subjects with ischemic heart disease respond to the lowest levels of COHb (6% or less). The implication is that, in some cases of cardiac impairment, the short-term compensatory mechanism is impaired.

From neurobehavorial studies (see earlier sections), it is apparent that decrements resulting from CO exposure have not been consistent in all subjects, even in the same studies, and have not demonstrated a dose-response relationship with increasing COHb levels. The implication from these data suggests there may be some threshold or time lag in

a compensatory mechanism such as increased blood flow. Without direct physiological evidence in either laboratory animals or humans, this concept can only be hypothesized.

The mechanism by which long-term adaptation may occur, if it can be demonstrated in humans, is assumed to be increased Hb concentration via an increase in hemopoiesis. This alteration in Hb production has been demonstrated repeatedly in laboratory animal studies, but no recent studies have been conducted that indicate the occurrence of some adaptational benefit. Even if the Hb increase is a signature of adaptation, it has not been demonstrated at low ambient concentrations of CO.

5. Sensitive population groups

Health effects caused by CO are most likely to occur in individuals who are physiologically stressed, either by exercise or by medical conditions that can make them more susceptible to low levels of CO. Most of the known quantitative concentration–response relationships regarding the human health effects of CO come from carefully controlled studies in healthy, predominantly male, young adults and in patients with diagnosed cardiovascular diseases (e.g. coronary artery disease). It can be hypothesized, however, from both clinical and theoretical work, anecdotal reports, and from experimental research in laboratory animals, that certain other groups in the population are at potential risk to exposure from CO. Probable risk groups that have not been studied adequately, but that could be expected to be susceptible to CO because of gender differences, aging, or preexisting disease, or because of the use of medications or alterations in their environment, include fetuses and young infants; children and adolescents; pregnant women; the elderly, especially those with compromised cardiovascular function; individuals with peripheral vascular or cerebrovascular disease; individuals with hematologic diseases (e.g. anemia) that affect oxygen-carrying capacity or transport in the blood; individuals with genetically unusual forms of Hb associated with reduced oxygen-carrying capacity; those with chronic obstructive pulmonary disease; people using medicinal or recreational drugs with CNS depressant properties; individuals exposed to other chemical substances (e.g. methylene chloride) that increase endogenous formation of CO; and individuals who have not adapted to high altitude and are exposed to a combination of high altitude and CO. Little empirical evidence is available by which to specify health effects associated with CO exposures in these probable risk groups (Section 8).

6. Intracellular effects of carbon monoxide

Traditional concepts for CO pathophysiology have been based on the high affinity of CO for Hb and consequent reduction of O_2 delivery. Recently published information suggests the possibility for biochemical mechanisms that are not necessarily related to an impairment of oxygen delivery from elevations in COHb. Most of this research was done with cells in culture and with laboratory animals. To be relevant to human exposures from environmental contamination, it is important to note what concentrations of CO are likely to occur in vivo. Lung parenchyma represents a special situation where cells may be exposed to ambient CO without the reduction in concentration associated with Hbbound CO. Elsewhere in the body, only a fraction of COHb will dissociate to elevate extravascular CO concentrations. This elevation is in the range of approximately 2–10 nmol when the COHb concentration is from 0.8 to 3.8% [30,31]. The COHb values near steady-state conditions in laboratory rats are close to values for humans [32]. This strengthens the potential for human relevance in recent animal studies that show that newly identified biochemical mechanisms do have adverse physiological effects. However, caution still is warranted because direct evidence for the occurrence of these mechanisms in humans has not been shown.

It is unclear whether disturbances in vascular tone by CO are a generalized, systemic response. The impact of variables such as the duration of exposure have not been adequately investigated. Cerebral vasodilation is a well-established effect caused by exposure to CO. At high CO concentrations, on the order of 500–2000 ppm, the mechanism is related to impairment of O_2 delivery. However, a portion of the observed increases in cerebral blood flow are independent of perturbations in O_2 supply. In a setting where cellular oxidative metabolism was not impaired by CO, elevations in cerebral blood flow appeared to be mediated by other, perhaps cellular, mechanisms.

Animals exposed to very high CO concentrations (e.g. 3000–10,000 ppm) have diminished brain blood flow, which contributes to CO-mediated tissue injury [33,34]. The mechanism is based on CO-mediated hypoxic stress. Intracellular mechanisms are discussed later.

6.1. Inhibition of hemoprotein function

Carbon monoxide can inhibit a number of hemoproteins found in cells, such as myoglobin found in heart and skeletal muscle, neuroglobin found in the brain, as well as cytochrome c oxidase, cytochrome P450, dopamine β hydroxylase, and tryptophan oxygenase. Inhibition of these enzymes could have adverse effects on cell function.

6.1.1. Myoglobin

Carbon monoxide acts as a competitive inhibitor, hence biological effects depend on the partial pressures of both CO and O_2. The cellular hemoprotein with the highest relative affinity for CO over that for O_2 is myoglobin (Mb). Carbon monoxide will inhibit Mb-facilitated oxygen diffusion, but physiological compromise is seen only with

high concentrations of COMb. High-energy phosphate production was shown to be inhibited in isolated cardiac myocytes, maintained at a physiologically relevant oxygen concentration, when COMb exceeded 40%. Formation of sufficient COMb to impair oxidative phosphorylation in vivo has been estimated to require at least a COHb level of 20–40%.

6.1.2. Neuroglobin

Like heart and skeletal muscle, brain tissue also has high oxygen demand for energy production; however, a specialized family of brain globins was not previously identified in vertebrates[2] until a recent series of publications [35–37] demonstrated the presence of a neuroglobin (Nb) in man and mouse. A predominant RNA expression pattern was reported in the human brain, with the strongest signals in the frontal lobe, subthalamic nucleus, and thalamus. Recombinant human Nb has an oxygen affinity of approximately 1 torr, which is higher than mammalian Hb (≈26 torr), but the same as Mb (≈1 torr).

Neuroglobin is a structurally different protein[3] from the other human globins and from the neuroglobins of some invertebrates. This aberration gives human Nb a higher oxygen affinity, making it difficult to determine if Nb can meet the kinetic and equilibrium requirements to function in facilitated oxygen transport or storage under known physiological conditions, as demonstrated for Mb. Rather, Nb may be a scavenger or sensor for CO, NO, or O_2 as are other globins. Because Nb is expressed in lower concentrations in areas of the brain that are sensitive to hypoxia, some researchers believe that the specialized configuration of Nb favors facilitated O_2 delivery under conditions of high oxygen demand, and prevents rapid re-binding of the O_2, so that it is favorably utilized by the mitochondria.

6.1.3. Other hemoproteins

Coefficients for binding CO versus O_2 among cytochrome P-450-like proteins vary between 0.1 and approximately 12, and there have been recent discussions suggesting that CO-mediated inhibition of these proteins could cause smooth muscle relaxation in vivo [38]. The issue relates to inhibition of cytochrome P-450-dependent synthesis of several potent vasoconstricting agents [39]. Vasodilation has been shown via this mechanism with high concentrations of CO (ca. 90,000 ppm). It is unclear, however, whether this could arise under physiological conditions and CO concentrations produced endogenously. The competition between CO and O_2 for cytochrome c oxidase has been known for a long time, but some new information has been published over the intervening years. Based on its Warburg partition coefficient of between 5 and 15, CO binding is favored only in situations where oxygen tension is extremely low [38]. Carbon monoxide binding to cytochrome c oxidase in vivo will occur when COHb is high (ca. 50%), a level that causes both systemic hypotension as well as impaired oxygen delivery [33]. Mitochondrial dysfunction, possibly linked to cytochrome inhibition, has been shown to inhibit energy production, and it also may be related to enhanced free radical production [40–42].

6.2. Free radical production

Laboratory animal studies indicate that nitrogen- and oxygen-based free radicals are generated in vivo during CO exposures [2]. Exposure to CO at concentrations of 20 ppm or more for 1 h will cause platelets to become a source of the nitric oxide free radical ($^{\cdot}NO$) in the systemic circulation of rats [43,44]. Studies with cultured bovine pulmonary endothelial cells have demonstrated that exposures to CO at concentrations as low as 20 ppm cause cells to release $^{\cdot}NO$ and the exposure will cause death by a $^{\cdot}NO$ process that is manifested 18–24 h after the CO exposure. The mechanism is based on elevations in steady-state $^{\cdot}NO$ concentration and production of peroxynitrite [43,45]. Peroxynitrite is a relatively long-lived, strong oxidant that is produced by the near diffusion-limited reaction between $^{\cdot}NO$ and superoxide radical.

The mechanism for enhanced H_2O_2 production in CO-exposed rats is not clear. It is possible that $^{\cdot}NO$ or peroxynitrite may perturb mitochondrial function. Peroxynitrite inhibits electron transport at complexes I through III, and $^{\cdot}NO$ targets cytochrome oxidase. It is important to note, however, that alterations in mitochondrial function and an increase of cellular H_2O_2 were not found in studies where cultured bovine endothelial cells were exposed to similar CO concentrations [45]. An alternative possible mechanism to mitochondrial dysfunction is that exposure to CO may inhibit antioxidant defenses. Mechanisms linked to elevations in $^{\cdot}NO$ could be responsible for inhibiting one or more enzymes.

Exposure to high CO concentrations (2500–10,000 ppm) cause mitochondria in brain cells to generate hydroxyl-like radicals [40–42]. An additional source of partially reduced O_2 species found in animals exposed to CO is xanthine oxidase. Conversion of xanthine dehydrogenase, the enzyme normally involved with uric acid metabolism, to xanthine oxidase, the radical-producing form of the enzyme, occurred in the brains of rats exposed to approximately 3000 ppm CO [46]. Lower CO concentrations did not trigger this change. Therefore, xanthine oxidase is unlikely to be a free radical source following exposures to CO at concentrations found in ambient air. Moreover, enzyme

[2] Globin-like proteins have previously been identified in invertebrates (e.g. annelids, molluscs, nemotodes, and nemerteans) [86,87].

[3] Globin binding schemes are commonly described in terms of the number of occupied binding sites on the heme iron when in the deoxy ferrous (Fe^{2+}) form; Hb and Nb have five (pentacoordinate) and six (hexacoordinate) sites occupied, respectively. The identification and importance of the heme pocket conformation for different globins is controversial and not well understood.

conversion was not a primary effect of CO; rather, it occurred only following sequestration and activation of circulating leukocytes [47].

6.3. Other CNS effects of carbon monoxide

Among the most concerning pathophysiological effects of CO is its propensity for causing severe neurological symptoms, brain damage, or even death after exposure to high CO concentrations [48]. There has been considerable effort focused on potential mechanisms for this process. Carbon monoxide poisoning is not a 'pure' pathological process because injuries may be precipitated by a combination of cardiovascular effects linked to hypoperfusion or frank ischemia, COHb-mediated hypoxic stress, and intracellular effects, including free radical production and oxidative stress. For example, CO poisoning causes elevations of glutamate and dopamine in experimental models and human fatalities [41,42]. These elevations occur because of the CO-associated cardiovascular compromise and, possibly, other direct CO-mediated effects. Based on the effects of agents that block the NMDA receptor, elevations of glutamate in experimental CO poisoning have been linked to a delayed type (but not an acute type) of amnesia, to loss of CA1 neurons in the hippocampus of mice, and to loss of glutamate-dependent cells in the inner ear of rats [49]. Antioxidants and free radical scavengers can protect against CO-mediated cytotoxicity of glutamate-dependent nerve cells [50,51]. Mechanisms of glutamate neurotoxicity include excessive calcium influx, free-radical-mediated injury that may include calcium–calmodulin-dependent activation of cytosolic NO synthase, and lipid peroxidation. Moderate stimulation by excitatory amino acids may cause mitochondrial dysfunction with impaired synthesis of adenosine triphosphate and production of reactive O_2 species. Cell death can be through necrosis or programmed cell death, depending on the intensity of the stimulus. There also may be a synergistic injury with other forms of oxidative stress because reactive O_2 species can intensify excitotoxicity. Glutamate also can injure cells in the CNS that do not have NMDA receptors by competing for cysteine uptake, which inhibits synthesis of glutathione.

7. Physiologic role of endogenous carbon monoxide

A number of endogenously produced gases have modulation functions in biological systems (Table 3). Of these, the two diatomic gases, CO and NO, have important physiological roles in neuronal signal transduction and in the maintenance of vascular tone. The intercellular roles of these two gases are often difficult to separate.

Carbon monoxide is produced endogenously by oxidation of organic molecules, but the predominant source is from the degradation of heme. The rate-limiting enzyme for heme metabolism is heme oxygenase (HO), which converts heme to biliverdin, free iron, and CO. Three distinct isoforms of HO have been characterized. The inducible enzyme (HO-1) is found in vascular endothelial cells, smooth muscle cells, bronchoalveolar epithelium, and pulmonary macrophages. This isoform can be induced by its substrate, heme, as well as NO, H_2O_2, several cytokines, and lipopolysaccharide. The constitutive enzyme (HO-2) is found in certain neurons within the nervous system, testicular cells, and vascular smooth muscle cells [52]. Little is known about a third isoform, HO-3, which has been identified in homogenates from a number of organs. The rate of CO synthesis varies for nerve cells; from 3 fmol/mg protein/min in cerebellar granule cells to 4.7 pmol/mg protein/min in olfactory nerve cells, whereas rat cerebellar homogenates can generate as much as 56.6 pmol/mg protein/min [53,54].

Nitric oxide is produced endogenously by nitric oxide synthase (NOS) oxidizing arginine to NO with the stoichiometric formation of citrulline. Three distinct isoforms of NOS have been characterized. Inducible NOS, when activated, enables pulmonary macrophages to form NO that kills tumor cells and bacteria. Endothelial NOS (eNOS) produces the NO that has been shown to relax blood vessels (see discussion later). Of particular significance to this discussion is neuronal NOS (nNOS) that, when activated, produces the neural transmitter, NO.

The physiological role of NO has been emerging for some time, but the endogenous role of CO was more surprising [55,56]. The main physiological roles for both gases is thought to be through the regulation of soluble

Table 3
Role of endogenous gases in biological systems

Gas	Base source	Enzyme generator	Enzyme receptor	Effector pathway	Effector organ	Physiological function
NO	Arginine	NO synthase (NOS)	Guanylyl cyclase (sGC)	Increased cGMP formation	Brain Vascular system	Neurotransmitter Vasodilator
CO	Heme	Heme oxygenase (HO)	Guanylyl cyclase (sGC)	Increased cGMP formation	Brain Vascular system	Neurotransmitter Vasodilator
H_2S	Cysteine	Cystathionine B-synthase (CBS)	NMDA receptors	LTP induction	Brain	Neuromodulator

NMDA, *N*-methyl-D-aspartate ;c-GMP, cyclic guanosine monophosphate, LTP, longterm potentiation.

guanylate cyclase (sGC) activity. Both CO and NO can activate sGC, although activation by CO is approximately 30-fold lower. In neuronal cells possessing both HO and NOS, regulation of cyclic guanosine monophosphate (cGMP) synthesis is mediated in a reciprocal fashion by producing either CO or NO [53]. Both HO and NOS are oxidative enzymes using NADPH as an electron donor and are rapidly synthesized by activation of constitutive and inducible isoenzymes. A compensatory interrelationship between HO and NOS also has been found in other cells (e.g. endothelial cells and activated macrophages), although the functional significance is unknown. Therefore, the following discussion will include both of these endogenously produced gases.

7.1. Role of carbon monoxide as a neurotransmitter

Both HO-2 and NOS occur in multiple neuronal pathways throughout the nervous system with noted overlap between them; however, they differ when compared to localization with cGMP. The areas enriched in HO-2 neurons also are enriched with cGMP, especially in olfactory neuronal tissue. The same is not always true for NOS, implying that NO may be acting through other regulatory pathways. Determining neurotransmitter and behavioral functions for CO and NO, however, is very difficult and is specific to the area of the nervous system being evaluated [57]. Advances in studies utilizing genomic deletion of HO and NOS (e.g. knock-out mice) and specific inhibition of endogenous CO and NO formation have provided valuable new information about the many possible neural functions for CO.

7.1.1. Central and peripheral nervous system

Carbon monoxide has been implicated in the stimulated release of stress neuropeptides from the hypothalamo-pituitary-adrenal axis, as well in cholinergic stimulation of the autonomic nervous system. Carbon monoxide also has been proposed to mediate cGMP formation and sensory adaptation in vertebrate olfactory receptor neurons. The isoenzyme, HO-2, may be neuroprotective in cerebral ischemia.

The inhibition of HO-2 in rat hypothalamus in vivo studies [58] has shown that endogenous CO is involved in regulation of stimulated vasopressin secretion under basal conditions. Another gaseous transmitter, H_2S (Table 2), also plays a role, especially in the release of corticotropin-releasing hormone.

Both HO-2 and NOS enzymes that synthesize CO and NO, respectively, have been localized and are expressed in the suprachiasmatic nucleus (SCN), the master circadian clock in the CNS of mammals. Cholinergic stimulation adjusts the clock timing through activation of sGC and cGMP synthesis. Hb, which avidly binds CO and NO, blocked the stimulation of cGMP synthesis.

Odor response adaptation (LLA) in olfactory receptor neurons of rats and salamanders, characterized by reduced amplitude and prolonged kinetics of the cAMP-mediated excitatory odor response and the generation of a persistent cGMP-activated current component, may be mediated by endogenous CO [53]. Similar effects can be produced by micromolar amounts of exogenous CO or cGMP. Inhibitors of cGMP abolish LLA, consistent with prevention of CO release.

A retrograde synaptic messenger role for CO has been suggested for the induction of long-term potentiation in the mammalian superior cervical ganglion [59] and the hippocampus [60]. The maintenance phase of ganglionic LPT requires the presence of NO. The introduction of Hb blocked the induction of LPT.

In the process of heme degradation by HO-2, the byproduct biliverdin is rapidly oxidized to bilirubin. Studies by Dore et al. [61] demonstrated that bilirubin is a neuroprotectant. In a model of focal ischemia of vascular stroke, neural damage is increased in knock-out mice deficient in HO-2 after cerebral artery occlusion and after intracranial injection of *N*-methyl-D-aspartate (NMDA).

7.1.2. Enteric nervous system

The first direct evidence for CO as a neurotransmitter came from innervation studies of the smooth muscle relaxation stages of intestinal peristalsis in knock-out mice [62]. Immuno-histochemical staining demonstrated colocalization of HO-2 and nNOS to the same myenteric plexus neurons where CO and NO act as coneurotransmitters to activate sGC, and thereby mediate intestinal inhibitory neurotransmission. Modulation of intestinal smooth muscle activity by endogenously produced CO and increased activation of HO-2 also has been demonstrated in canine jejunum.

7.2. Role of carbon monoxide in vascular homeostasis

Both NO and CO have important roles in the maintenance of vascular tone (Figs. 6 and 7). In fact, as new evidence emerges, both have been found to serve as ideal paracrine endothelial modulators, one possibly as a 'backup' to the other [63]. They are endogenously synthesized in low concentrations and both share similar properties due to activation of sGC, such as platelet disaggregation and relaxation of vascular smooth muscle. They differ in kinetics and in the substrates required for endogenous generation.

More information is available on NO, especially since it was discovered to be identical to the endothelium-derived relaxation factor (EDRF) that was responsible for relaxation of vascular smooth muscle [64–66]. The NO syntheses are responsible for the synthesis of NO from L-arginine in endothelial cells. Nitric oxide diffuses into adjacent smooth muscle cells where it activates sGC, resulting in increased

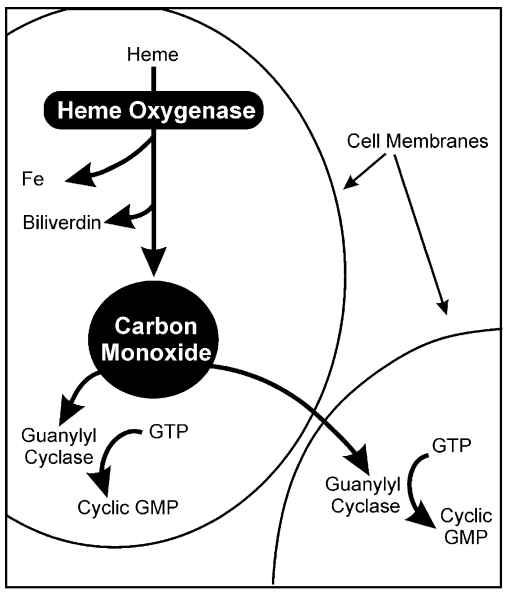

Fig. 6. Diffuse gas messenger. By diffusing through the vascular endothelial and smooth muscle cell membranes and interacting with the enzyme guanylyl cyclase, endogenously produced carbon monoxide may act as a biological signal for vascular homeostasis. Source: Barinaga (1993) [55].

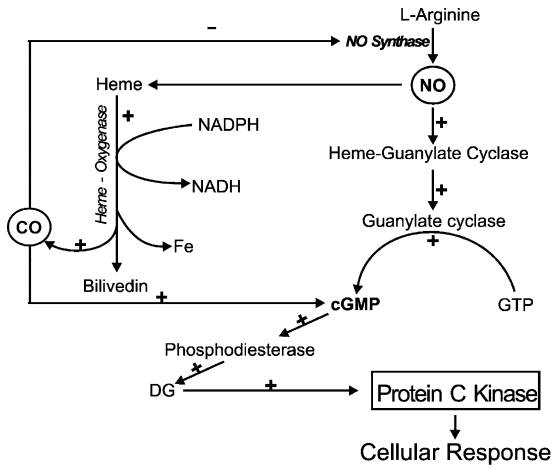

Fig. 7. Proposed mechanism for the regulation of NO signaling by CO. Activation of heme oxygenase by NO produces CO which has been shown to inhibit NO synthase. It is possible that a NO–CO feed-back loop exists in the vascular system for the regulation of NO production under normal and hypoxic conditions. Source: Maulik et al. (1996) [84].

levels of cellular cGMP and vasorelaxation of smooth muscle.[4] Nitric oxide also influences platelet and leukocyte adhesion, as well as other vascular properties, to facilitate vasodilation. During hypoxia, the basal levels of NO decrease significantly because of a decrease in the expression of the isoenzyme NOS III.

In the endothelium, numerous localizations of HO to autonomic nerves parallel those of NOS, suggesting that NO and CO may act in concert throughout the body [67,68]. During hypoxia, the expression of the isoform HO-1 increases, leading to increased basal levels of CO. Like NO, CO activates sGC in smooth muscle cells (Fig. 6), leading to increased levels of cGMP, vasorelaxation of smooth muscle, and other vascular properties including the prevention of platelet aggregation and adhesion [69,70]. There are some data indicating that CO influences vascular tone by modulation of ion channels[5] in vascular smooth muscle cells [71,72] and by the same mechanisms as proposed for NO [73]. Induction of HO-1 also may protect tissues from subsequent inflammatory stress [74] after ischemic injury when NO levels are quenched by increased superoxide during vascular re-perfusion [75].

Hypoxia can affect vascular smooth muscle and endothelial cells leading to a number of vascular disorders, including vasoconstriction, edema, and thrombus formation. Under normal physiological conditions, endogenously produced NO provides for the maintenance of vascular tone because NO is a more potent vasodilator than CO. Under hypoxic stress, however, or in the vascular re-perfusion after tissue ischemia, when there is a decline in NO bioavailability, the regulation of cGMP is dominated by CO [76,77]. In fact, hypoxia-induced CO may shut down NO production by binding to the heme group of NOS [78,79], thereby perpetuating the effect on vessel integrity (Fig. 7). Thus, endogenously produced CO provides a backup mechanism for vascular homeostasis under less than ideal physiologic conditions.

8. Speculation and recommendations

8.1. Specially sensitive persons

Anyone with the inability to adequately regulate O_2 supply or metabolism would seem to be specially sensitive to elevation in COHb. This has been empirically demonstrated for the onset of chest pain during exercise in angina patients. There are a number of physical conditions which impair the ability of O_2 to reach the brain and thus could make persons with such an affliction more sensitive to elevated COHb. Among these are atherosclerotic lesions [80], non-insulin dependent diabetes [81], and cerebral microvascular pathology of less well-known etiology [82]. There also have been reports of gender differences in cerebrovascular reactivity. Females are more reactive during ovulation, a response that disappeared after menopause and during menstruation [83].

[4] Several pharmacological vasodilators (e.g. nitroglycerine) produce their effect by activating guanylyl cyclase.

[5] Depleted ATP concentrations inside arterial smooth muscle cells may signal ATP-sensitive potassium channels to open; the reduction of potassium ions would increase the negative polarity of cell membranes and close voltage-dependent calcium ion channels. Subsequent reduction in intracellular calcium ions would cause blood vessels to relax and dilate.

Also, some complications of pregnancy (e.g. preeclampsia) cause cerebral vasoconstriction that is unresponsive to stimuli that under normal circumstances result in vasodilation. New studies suggest that some of progressive morphological changes within the brain and consecutive neuropsychological symptoms found in immune deficiency diseases, and in diseases of aging (e.g. Alzheimer's), are of vascular origin. Therefore, older people may be, as a group, more sensitive to COHb elevation. This hypothesis has not been adequately tested in either humans or laboratory animals. One previous study of effects of COHb elevation on elderly persons did not show any differences with respect to a control group of young subjects [29], but the elderly subjects had been selected for excellent health.

8.2. Interaction with drugs

There remains little direct information on the possible enhancement of CO toxicity by concomitant drug use or abuse; however, there are some data suggesting cause for concern. There is some evidence that interactions of drug effects with CO exposure can occur in both directions; that is, CO toxicity may be enhanced by drug use, and the toxic or other effects of drugs may be altered by CO exposure. Nearly all published data available on CO combinations with drugs concern psychoactive drugs. Descriptions of these studies were provided in available reviews [5].

The use and abuse of psychoactive drugs and alcohol are widespread. Because of the effect of CO on brain function, interactions between CO and psychoactive drugs could be anticipated. However, very little systematic research has addressed this question. In addition, very little of the research that has been done has utilized models for expected effects from treatment combinations. Thus, often it is not possible to assess whether the combined effects of drugs and CO exposure are additive or synergistic. It is important to recognize that even additive effects of combinations can be of clinical significance, especially when the individual is unaware of the combined hazard. The greatest evidence for a potentially important interaction of CO comes from studies with alcohol in both laboratory animals and humans, where at least additive effects have been obtained. The significance of these effects is augmented by the probable high incidence of combined alcohol use and CO exposure in the population.

Besides interaction with psychoactive drugs, there is growing concern that prescribed medications, especially those used by the elderly population (e.g. nitric oxide blockers and calcium channel blockers), could interact with CO. There are no known published data available, however, on CO combinations with these drugs.

8.3. What topics need to be pursued in research

Newly emerging work on the intracellular effects of CO, especially the binding of CO by myoglobin and neuroglobin and the role of CO as a neurotransmitter, should (and probably will) continue. This new research will eventually provide sufficient mechanistic understanding to solve standing problems in the literature about the effects of CO on behavior. Further work to elucidate the mechanisms of cerebrovascular responsiveness to COHb elevation needs to continue so as to be able to predict specially sensitive groups. It also seems important to test persons or laboratory animals that have impaired cerebrovascular reactivity in behavioral performance situations to determine if these impairments increase sensitivity to COHb elevation.

Clearly, new research is needed on the potential effects of chronic exposure to low levels of CO, especially levels typically released from malfunctioning or improperly vented combustion appliances used in homes. Anecdotal reports of CO poisoning due to these sources are numerous, but exposure conditions are unknown. Better controlled studies, either through the use of laboratory animals, or through controlled simulation–exposures in humans, are needed to understand the potential effects of COHb accumulation over time, the physiological responses to chronically elevated COHb in healthy children and adults and in people with impaired cerebrovascular responsiveness to COHb elevation.

It may be equally important to consider what kinds of research have low probability of producing decisive outcomes. With respect to the disparate results in the acute effects of COHb elevation on behavior, it would seem that new experiments which demonstrated effects around 5–10% COHb would not be decisive because of the large number of extant articles on each side of this issue. To affect the scientific judgement about these results would require a large number of experiments showing effects, or a large number of independent replications before opinions could be changed. This would be a questionable expenditure of human experimental resources. To be sure, such results would be important and would have great value. It is probable, however, that the shorter route to a consensus would be through a mechanistic understanding of CO effects.

9. Disclaimer

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