Role of Carbon Monoxide in Blood Pressure Regulation

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Carbon monoxide (CO) is an odorless, colorless, tasteless gas that is generated in the environment as the result of combustion from stoves and engines among other sources. Approximately 500 people per year in the United States are victims of nonfire-associated CO poisoning according to the Centers for Disease Control and Prevention. CO poisoning is often fatal because of its interaction with hemoglobin, which renders it incapable of carrying oxygen-causing organs to become severely hypoxic. CO inhalation is believed to be fatally toxic at concentrations as little as 800 parts per million (ppm) or 0.08% in the air. Despite the lethal nature of this gas, several recent studies suggest that CO inhalation at low doses (≤250 ppm), as well as increases in CO levels using CO releasing molecules (CORMs), offers protection against ischemic injury in the heart, liver, and kidney.

CO is endogenously produced in the body as a result of the metabolism of heme by heme oxygenase (HO), as well as from lipid peroxidation. The catabolism of heme by HO also produces an equimolar amount of biliverdin, which is rapidly converted in the cell to bilirubin by the enzyme biliverdin reductase. There are 2 major isoforms of HO responsible for CO production. HO-1 is expressed at very low levels under normal conditions but is highly induced by several stimuli, including heavy metals, ultraviolet light, endotoxin, shear stress, hypoxia, and oxidants. HO-2 is the constitutively expressed form of the enzyme with the highest levels observed in the brain and testes. Experimental evidence has demonstrated that systemic induction of HO has several beneficial actions on the cardiovascular system, including lowering of blood pressure, protection against myocardial infarction, and prevention of atherosclerosis.

Although the cardiovascular actions of HO induction have been established, the role of CO in mediating these responses is not clear. The purpose of this review is to outline the potential antihypertensive actions of CO and highlight areas that may pose new opportunities for the development of novel therapeutic targets for the treatment of hypertension.

Altering CO Levels In Vivo: Tools of the Trade

There are 3 main approaches that have been used to alter tissue levels of CO in vivo, which need to be briefly discussed. These include inhibition/induction of HO, CO inhalation, and CORMs. Each of these approaches has its own advantages and limitations depending on the specific experimental settings in which tissue levels of CO are to be altered. HO induction/inhibition has been widely used because of the fact that most of the endogenous CO produced in vivo is derived from HO. Given the routine ability to alter HO in a tissue- and temporal-specific fashion either pharmacologically or genetically, this is an attractive option for examining CO in vivo. However, there are several limitations in altering HO levels to examine the role of CO in vivo. First among these is the specificity of the majority of the metalloporphyrin HO inhibitors, which have nonspecific effects apart from HO inhibition. Secondly, inhibition/induction of HO also alters the levels of bilirubin and free iron, both of which also can have effects on cell and tissue function apart from changes in CO.

CO inhalation therapy is a highly effective means of increasing tissue levels of CO. CO inhalation has been proven to protect against ischemia-reperfusion injury and improve survival of allograft transplants. One limitation with CO inhalation is the high levels of blood carboxyhemoglobin (COHb) that are achieved at CO levels (200 to 500 ppm) required to ensure significant increases in tissue levels of CO. These effects on blood COHb levels may limit the clinical applicability of CO inhalation unless lower levels of CO can achieve similar results. In support of this idea, a recent study by Kobayashi et al demonstrated that low-level CO inhalation (60 ppm), which did not result in a dramatic increase in blood COHb levels, was able to significantly attenuate the development of angiotensin II–dependent hypertension. This study provides the first evidence that chronic low level CO inhalation may be a viable option for the treatment of hypertension.

CORMs are recently described compounds capable of releasing CO at physiological pH. CORMs are available as transition metal carbonyl compounds with a fast rate of CO release (CORM-2, CORM-3, and CORM-F10) or as a sodium boronancarbonate compound (CORM-A1) with a slower rate of CO release. CORMs are advantageous in that they exhibit physiological effects without causing significant increases in blood COHb levels. There are 2 notable
limitations with CORMs. The first being the induction of HO-1 because of the metals in the transition metal carbonyl forms, such as CORM-3.4 This induction of HO-1 can result in a further increase in endogenous CO and bilirubin levels, which may contribute to any observed physiological actions of these compounds. Significant induction of HO-1 can be avoided by use of the nonmetal-containing CORM, CORM-A1; however, this chemical is not currently available from commercial sources and must be synthesized by individual investigators. Another limitation is the inability to infuse these compounds chronically over hours or days because of the relatively short half-life of the compounds in physiological solutions. This is a major obstacle for investigators interested in performing chronic whole-animal integrative studies with these compounds.

Tissue levels of CO are consistent between the rat and the mouse averaging 1 to 5 pmol/mg of tissue under control conditions.19 Treatment with the HO inducer heme arginate, which doubles blood COHb levels, modestly increases tissue CO levels primarily in the muscle, heart, and lung by 2-fold.19 However, inhalation of 500 ppm CO, which increases COHb levels >50-fold, increases CO levels in the brain, heart, kidney, liver, and lung by >15-fold.19 Specific studies examining tissue CO levels after administration of CORMs have not yet been performed. CO inhalation at high levels results in a larger increase in CO levels in more tissues than induction of HO-1 but at the expense of greatly increase blood COHb levels, which could be a significant concern clinically.

**CO and the Brain: CO as a Modulator of Baroreceptor Function**

Over the last 20 years, several studies have demonstrated that CO is an important signaling molecule in the brain.26,27 CO generated in the brain is generated from the metabolism of heme by HO-2, which is the major isoform of HO expressed in the brain.28,29 Both HO-2 and HO-1 are expressed in neurons and glia, with HO-1 being induced in these cells types in response to several types of brain injuries, including stroke.30–32 CO modulates the release of neurotransmitters such as oxytocin and corticotropin (adrenocorticotropic hormone)-releasing hormone.33,34 Neural generation of CO can decrease the firing rates of neurons in several nuclei of the brain, including the locus coeruleus and the nucleus tractus solitarii (NTS).35,36

The NTS is an important area involved in the integration of autonomic control of the cardiovascular system, where afferent fibers from peripheral cardiovascular baroreceptors and chemoreceptors make their first central synapses.37 It is in this region of the brain where alterations in HO and CO decrease baroreceptor sensitivity. Johnson et al38 were the first to demonstrate that systemic inhibition of HO with zinc deuteroporphyrin 2,4-bis glycol (ZnDPBG) was associated with an increase in mean arterial pressure and a decrease in the gain of the baroreflex. More importantly, they were also able to increase blood pressure by unilateral or bilateral administration of ZnDPBG directly into the NTS.39 The increase in blood pressure elicited by direct NTS administration of ZnDPBG was reversed by direct injection of saline saturated with CO, indicating that decreases in the levels of CO are responsible for the pressor actions of ZnDPBG administration into the NTS. These studies were significant in that they provided the first experimental evidence that CO produced by HO may play a role in central regulation of blood pressure. Additional studies by Lo et al38 also demonstrated that direct injection of ZnDBPG into the NTS decreased baroreflex sensitivity. It should again be noted that the use of metalloporphyrins as HO inhibitors is not without controversy. Several nonspecific effects, such as direct inhibition of guanylate cyclase, inhibition of NO synthase, and induction of HO-1 have been attributed to metalloporphyrins.13,34 However, of the wide variety of metalloporphyrins, ZnDPBG has been found to be among the most specific for inhibition of HO.14,39

Further evidence for a role of CO in the central regulation of blood pressure comes from studies in which hematin, an alternative form of the HO substrate hemin, was directly injected into the NTS. Direct injection of hematin into the NTS consistently lowers blood pressure and heart rate.40,41 The depressor effects of hematin injection into the NTS are similar to those observed with injection of the excitatory amino acid glutamate. Glutamate is the principle neurotransmitter of baroreceptor afferent fibers, which terminate in the NTS.42 Glaun and Miller43 were the first to provide experimental evidence linking CO and glutamate receptors in the NTS. This report was confirmed by several studies, which have reported that blockade of HO with zinc protoporphyrin IX or ZNDPBG results in a significant decrease in the depressor and bradycardic response to 1-glutamate and group II and III metabolotropic glutamate receptor agonists.44–46

As outlined in Figure 1, increases in CO in the NTS occur after stimulation of glutamate receptors after release of glutamate from cardiovascular afferents; however, the mechanism by which increased CO affects NTS neuronal activity (ie, increases in cGMP or direct action on K+ channels) is not known. Blockade of HO reduces CO release and decreases postsynaptic neuron activity in the NTS, which leads to increases in blood pressure. Despite our increased understanding of the acute role of CO in the central regulation of blood pressure, little information is available regarding whether alterations of central HO/CO exist in experimental and genetic models of hypertension, especially those with increase sympathetic activity, such as the spontaneously hypertensive rat. Also, the potential antihypertensive actions of chronic increases in HO/CO in the NTS have not been examined in hypertensive models, so it is difficult to determine the importance of CO in this region in the long-term control of blood pressure. Advances in gene delivery techniques in the brain, as well as brain-specific transgenic overexpression approaches, which chronically increase HO/CO levels in neurons or glia, should allow for the importance of altered central CO in the regulation of blood pressure to be determined in the future.

**CO and the Vasculature: Does the Good Outweigh the Bad?**

One of the first reported biological functions of CO is its ability to dilate blood vessels in several organs.47,48 The major
source of CO in the vasculature is HO-2, which is expressed in both endothelial and vascular smooth muscle cells. Like NO, the ability of CO to relax blood vessels occurs through activation of soluble guanylyl cyclase (sGC), increases in cGMP, and activation of high-conductance Ca\(^{2+}\)-activated K\(^+\) channels.\(^{52-54}\) The activation of K\(^+\) channels leads to membrane hyperpolarization, which inhibits calcium entry from voltage-activated Ca\(^{2+}\) channels.\(^{50,52}\) However, there is evidence that CO may act directly or via pathways other than cGMP to cause vasodilatation in certain vascular beds.\(^{52-54}\) In the kidney, studies using specific CORMs have found that increases in CO produce increases in renal blood flow that are significantly but not totally reduced by blockade of sGC and completely blocked by inhibition of K\(^+\) channels.\(^{55,56}\)

CO provides an important counterbalance against vasoconstriction mediated by such agents as Ang II and 20-hydroxyecosatetraenoic acid, especially in the renal vasculature.\(^{54,57}\) CO, derived from either endothelial or smooth-muscle HO, can directly inactivate P450 enzymes in the vasculature by binding to the heme moiety in the enzyme. Induction of HO-1 in the vasculature can also directly decrease the activity of P450 enzymes by reducing the availability of heme, which is required for the function of P450 enzymes. Thus, increases in vascular CO/HO can result in decreased production of vasoconstrictors such as 20-hydroxyecosatetraenoic acid, but it can also inhibit production of P450 enzymes such as prostaglandin \(I_2\) and prostaglandin \(E_2\).\(^{58,59}\) Interestingly, CO produced from HO can also act as a second messenger for P450 enzymes. For example, incubation of rat mesenteric arterioles with 11,12-epoxyeicosatrienoic acid stimulates CO release, and vasodilatation to 11,12-epoxyeicosatrienoic acid is abolished by inhibition of HO activity.\(^{60}\) Because vasodilatation to 11,12-epoxyeicosatrienoic acid and CO are both inhibited by iiberotoxin,\(^{56,60}\) it is likely that CO mediates dilatation to 11,12-epoxyeicosatrienoic acid through activation of K\(^+\) channels.

There is a very complicated relationship between vascular NO and CO (Figure 2). Both gases can activate sGC to increase cGMP levels and cause vasodilation.\(^{61,62}\) Previous studies have shown that low levels of CO (0.001 to 0.1 \(\mu\)mol/L) can stimulate NO release, whereas higher levels of CO (\(\geq 1 \mu\)mol/L) inhibit NO synthase.\(^{63}\) In the renal circulation, CO buffers against excessive vasoconstriction observed after blockade of NO.\(^{64,65}\) This suggests that a major role for CO in the renal vasculature is to provide protection against excessive vasoconstriction when the renal NO system is deficient. In contrast, vascular smooth muscle–specific overexpression of HO-1, resulting in a 3-fold increase in vascular HO activity, attenuates cGMP production in response to NO, impairs NO-mediated vasodilatation, and causes hypertension.\(^{66}\) Additional evidence for deleterious effects of excessive CO production in the vasculature is supported by studies on Dahl salt-sensitive rats in which responses to acetylcholine are restored upon inhibition of HO.\(^{67}\) In the obese Zucker rat model of the metabolic syndrome, inhibition of HO activity results in an enhancement of acetylcholine-induced vasodilatation and lowering of blood pressure.\(^{68}\) All of these studies clearly indicate that excessive production of CO in the vasculature leads to alterations in NO production, endothelial dysfunction, and hypertension. However, there are also beneficial effects of increased HO/CO in the vasculature. Increases in vascular HO/CO improve acetylcholine-mediated vasodilatation in diabetes.\(^{25}\) One potential mechanism for the improvement in vasodilatation by CO in diabetes is a decrease in the levels of oxidants with HO-1 induction or increases in CO. This hypothesis is supported by data from endothelial cells in which HO-1 or CO reduces oxidant damage and endothelial cell sloughing in streptozotocin-induced diabetes.\(^{69-71}\)

There are several potential therapeutic applications for increases in vascular CO that are somewhat limited by the multifaceted nature of the actions of CO with NO. Increases in vascular CO would most likely be beneficial in states where endogenous NO production is reduced. The kidney would likely be a good target for increases in vascular CO, especially in conditions of excessive renal vasoconstriction. However, increases in vascular CO need to be tempered as to not interfere with endogenous NO production, which can lead to impaired vascular relaxation and increases in blood pressure. Increases in vascular CO may also be beneficial to protect the vasculature against oxidant damage and to repair
CO and the Kidney: Beyond the Vasculature

As outlined above, CO has an important role in the regulation of vascular tone, and this is especially true in the renal vasculature. HO–derived CO is critical for the maintenance of renal medullary blood flow. Because decreases in renal medullary blood flow are observed in several forms of hypertension, preservation of renal medullary blood flow by CO is an important adaptive mechanism to promote sodium excretion by the kidney. Another mechanism by which CO regulates renal vascular resistance is by modulation of afferent arteriolar tone, as described in the previous section. Another important mechanism that regulates the afferent arteriolar diameter is tubuloglomerular feedback (TGF). One hypothesis for the mechanism of TGF is that a rise in sodium delivery to the early distal tubule results in an increase in ADP release by the macula densa cells. ADP then acts on the A1 receptor causing contraction of the afferent arteriole, a drop in glomerular pressure, and a reduction in the single-nephron GFR (Figure 3). The increase in sodium delivery into this segment also activates neuronal NO synthase activity in macula densa cells. The NO produced is thought to regulate the TGF sensitivity by dilating the afferent arteriole to prevent excessive vasoconstriction. As mentioned earlier, there are considerable similarities in the actions of CO and NO on the vasculature; however, the role of CO on TGF regulation is not clearly understood. CO at low concentrations releases NO from intracellular stores, whereas at high concentration it inhibits NO synthase activity. HO inhibition alone has no effect on TGF sensitivity, but inhibition of HO activity after NO synthase inhibition further increases TGF sensitivity. Therefore, in models of hypertension where NO bioavailability is reduced, CO production may be critical to maintain an afferent arteriolar diameter against excessive TGF-mediated constriction. Most of the CO generated in the renal vasculature is derived from HO–2; however, it is possible that HO–1 may be an important source of vascular CO, especially in hypertensive models, such as Ang II hypertension, where HO–1 is induced. CO may further regulate TGF sensitivity by inhibiting superoxide production from reduced nicotinamide-adenine dinucleotide phosphate oxidase, consequently increasing NO availability by reducing its conversion to peroxynitrite. Although the vascular functions of CO have been established in the kidney, the role of CO in tubule cells has not been thoroughly examined.

There have been conflicting reports on the role of CO as compared with HO induction in the regulation of tubular function. In isolated thick-ascending loop of Henle (TALH) tubule segments, CO stimulates the apical 70-pS K+ channel. Apical K+ channels are essential for the recycling of K+ to maintain the activity of the Na+–K+–2Cl– cotransporter, which is the major transporter involved in Na+ reabsorption in the TALH. CO-mediated stimulation of apical K+ channel activity increases K+ availability for the Na+–K+–2Cl– cotransporter, resulting in increased Na+ reabsorption in the TALH. This observation is supported by additional data from in vivo microperfusion experiments, which demonstrate that blockade of HO activity results in a decrease in sodium and fluid reabsorption in the TALH. However, results from whole-animal studies in which the levels of HO are altered have not supported an antinaturetic function of CO in the kidney. Recent studies by Li et al have documented an increase in CO production in the renal medulla in response to increases in renal perfusion pressure. Because increases in
renal perfusion pressure lead to increases in sodium and water excretion, the reported antinatriuretic action of CO in the TALH does not correspond with what is occurring in this nephron segment physiologically. HO induction with hemin increases sodium and water excretion in the absence of any effects on glomerular filtration rate, and this effect is blocked by previous HO inhibition. Lastly, chronic inhibition of HO in the renal medulla attenuates pressure-natriuresis and hypotension in response to increases in sodium intake. Whether salt-sensitive hypertension resulting from inhibition of HO in the medulla is the result of changes in medullary blood flow or alterations in tubular function is not known and warrants further investigation.

CO signaling in renal tubular epithelial cells has not been extensively studied; however, because it uses similar signaling pathways as NO in tubular epithelial cells. For example, both CO and NO increase cGMP levels in renal tubules, which activates cGMP-stimulated phosphodiesterase (phosphodiesterase II), enhancing the degradation of cAMP. Because cAMP levels have been linked to apical positioning of the Na⁺-K⁺-2Cl⁻ cotransporter, it is possible that chronic increases in CO levels may decrease Na⁺ reabsorption via alterations in apical levels of the Na⁺-K⁺-2Cl⁻ cotransporter. This possibility remains to be tested in vivo.

Another possible pathway by which increases in CO may affect renal tubular epithelial cell function is through interactions with superoxide anion generation. Superoxide generation has been reported to stimulate sodium reabsorption through direct actions, as well as by decreasing the availability of NO in the tubule. CO has not been traditionally looked on as an antioxidant; however, recent studies have reported that CO can decrease superoxide generation via inhibition of reduced nicotinamide-adenine dinucleotide phosphate oxidase activity. Recently, we demonstrated that previous induction of HO in the kidney was associated with a decrease in Ang II–stimulated superoxide production in a model of Ang II hypertension. We also have additional experimental evidence that increases in CO alone can attenuate Ang II–stimulated superoxide production in both cultured TALH and mouse inner medullary collecting duct cells (unpublished observation). Collectively, these results suggest that decreases in superoxide anion production may be a potential mechanism by which chronic increases in CO can affect sodium transport, especially in cases in which renal Ang II levels are increased. However, the role of CO as a potential antioxidant in the kidney is an emerging concept, and its importance remains to be tested in vivo.

Perspectives
Paracelsus, often referred to as the father of toxicology, wrote, “All things are poison and nothing is without poison, only the dose permits something not to be poisonous.” This statement is especially true for CO, because levels as low as 800 ppm in the air can be fatal. However, a growing body of literature indicates that low-level CO inhalation (<100 ppm) or treatment with CO donors has several beneficial cardiovascular actions, including lowering of blood pressure, protection against cardiac ischemia, and protection against diabetes induced vascular injury. There are also substantial reports that induction of HO-1, which results in increased CO production, also lowers blood pressure in several forms of hypertension. To what extent do increases in CO
specifically contribute to the blood pressure lowering observed with induction of HO? It has been difficult to specifically address this question in the past given the limited ability to specifically increase CO levels in various tissues either chemically or genetically. However, with the emerging use of genetically engineered animal models and further development of CORMs, it will soon be possible to explore the potential antihypertensive properties of specific increases in CO production. Translational studies examining the efficacy of CO inhalation therapy are warranted to further develop CO as a novel antihypertensive agent.

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References


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