Effect of Carbon Monoxide on Rabbit Cerebral Arteries

Johnny E. Brian, Jr, MD; Donald D. Heistad, MD; Frank M. Faraci, PhD

Background and Purpose Carbon monoxide produces relaxation in some peripheral arteries. Recently it has been suggested that carbon monoxide may be generated in brain tissue. In the present study we examined the hypothesis that carbon monoxide directly relaxes cerebral blood vessels.

Methods The aorta and basilar and middle cerebral arteries were removed from New Zealand White rabbits and mounted for tension recording in vitro. Canine basilar arteries were also studied. After precontraction, cumulative relaxation concentration-response curves to carbon monoxide, nitric oxide, sodium nitroprusside, acetylcholine (rabbit arteries), and ATP (dog basilar artery) were obtained. Maximum relaxation and the concentration of agonists that induced half-maximal relaxation (ED50) were determined.

Results Carbon monoxide (10^-6 to 3x10^-4 mol/L) did not affect tension in rabbit or dog cerebral arteries. In rabbit aorta, carbon monoxide induced 29±4% (mean±SEM) relaxation at the highest concentration used (3x10^-4 mol/L). In contrast, nitric oxide produced 80% to 100% relaxation of all arteries, with ED50 values ranging from 7.1 to 7.4 -log mol/L. Nitroprusside, acetylcholine, and ATP also produced 80% to 100% relaxation of the arteries.

Conclusions Carbon monoxide does not appear to have significant effect on tone in cerebral arteries. In contrast, at high concentrations carbon monoxide produces concentration-dependent relaxation in rabbit aorta. Factors that account for this regional heterogeneity are not clear. Although neurons may produce both nitric oxide and carbon monoxide, our findings suggest that only nitric oxide has direct effects on cerebral vascular tone. (Stroke. 1994;25:639-644.)

Key Words • cerebral arteries • nitric oxide • rabbits

See Editorial Comment, page 643

Experiments, relaxation in response to carbon monoxide correlates with elevation of cGMP levels.6 In cultured aortic smooth muscle, carbon monoxide elevates cGMP levels.10

In vivo, carbon monoxide produces marked increases in cerebral blood flow.11 Although other mechanisms may contribute, a portion of the increase in cerebral blood flow may be due to direct dilator effects on cerebral blood vessels.

Because carbon monoxide induces relaxation in non-cerebral arteries9,9 and because heme oxygenase is abundant in brain,3 we speculated that carbon monoxide may serve as a direct dilator in cerebral arteries. In this study we examined the direct effect of carbon monoxide on vascular tone in rabbit and canine cerebral arteries in vitro. The hypothesis was that carbon monoxide, as well as nitric oxide, is a potent direct dilator of cerebral blood vessels.

Materials and Methods

Arterial Ring Preparation

New Zealand White rabbits (weight, 2.5 to 3.5 kg) were given a lethal dose of sodium pentobarbital (100 mg/kg IV) and the brain and associated vessels removed. In some experiments the thoracic aorta was also removed. In a separate series of experiments, mongrel dogs (weight, 20 to 30 kg) were anesthetized with sodium thiopental (25 mg/kg) and 3-chloralose (100 mg/kg) and exsanguinated and the brain and associated vessels removed. The excised tissues were placed in Krebs buffer (mmol/L): NaCl, 118.3; KCl, 4.7; CaCl2, 2.5; MgSO4, 1.2; KH2PO4, 1.2; NaHCO3, 25; ethylenediaminetetraacetic acid calcium, 0.036; and glucose, 11.1 (pH 7.4) that had been saturated with 95% O2, 5% CO2. With the aid of a microscope, the basilar artery or both middle cerebral arteries...
were dissected from the brain and cleaned of arachnoid and connective tissue. Four ring segments 3 to 4 mm long were cut from each artery and mounted on stainless steel hooks, and the arterial segments were placed in tissue baths containing 25 mL of buffer (37°C) gassed with 95% O2/5% CO2. The upper hook was connected to a force displacement transducer (model BG-25, Kulite Semiconductor Products, Inc), and isometric tension was displayed continuously on a polygraph (model 2200, Gould Inc). Tension was increased to 0.5 g (rabbit cerebral arteries), 1 g (dog basilar artery), or 2 g (rabbit aorta) over 1 hour and maintained for an additional hour for equilibration. All preparations had intact endothelium as demonstrated by relaxation to either acetylcholine (rabbit arteries) or ATP (dog basilar artery).

After equilibration, vessels were contracted with histamine (rabbit cerebral arteries), prostaglandin F2α (dog basilar artery), or phenylephrine (rabbit aorta). When stable tone had developed, a relaxation concentration-response curve was obtained by cumulative addition of carbon monoxide, nitric oxide, sodium nitroprusside (rabbit arteries), and ATP (dog basilar artery) in random order. After the concentration-response curve was obtained, vessels were washed with buffer and allowed to return to resting tension for at least 30 minutes. This procedure was repeated for each vasodilator. Data are expressed as percentage of tension present before initiation of the vasodilator dose-response curve. At the end of the experimental protocol, maximum tension was determined in rabbit cerebral arteries with histamine (300 μmol/L) and in dog basilar artery and rabbit aortic segments with KCl (40 mmol/L).

Preparation of Carbon Monoxide and Nitric Oxide Solutions

A stock solution of deoxygenated water saturated with nitric oxide gas was prepared anaerobically as described previously.12 The concentration of nitric oxide in the saturated solution was calculated based on known physical constants for nitric oxide. Aliquots of the saturated nitric oxide solution were withdrawn anaerobically from the stock and added to the tissue baths. Because the response to nitric oxide is transient, the nitric oxide concentration-response curve was obtained in whole-log steps rather than half-log steps as used with the other vasodilators.

A similar method was used for preparation of a saturated solution of carbon monoxide. Because relatively high concentrations of carbon monoxide were needed to produce relaxation, large amounts of saturated carbon monoxide solution were required to achieve the final concentration in the baths. To prevent dilutional, pH, or temperature effects when the carbon monoxide solution was added to the tissue baths, the saturated solution of carbon monoxide was prepared in buffer saturated with 95% O2/5% CO2 to which had been added the concentration of the vasoconstrictor in use for the particular vessel. This solution was warmed to 37°C before addition to the tissue baths. The concentration of carbon monoxide in the saturated solution was calculated from known physical constants for carbon monoxide, and the solution was prepared daily.

Statistical Methods

Relaxation responses for vessel segments from each animal were averaged to yield a mean response for the animal, which was then used for further analysis (n indicates the number of animals in each group). Relaxation concentration-response curves were analyzed for maximal relaxation and the concentration of vasodilator that induced half-maximal relaxation (ED50). Values are presented as mean±1 SEM. ANOVA was used to compare values between vasodilators for the same artery and between arteries for the same vasodilator, as well as the percentage of maximum tension that the arteries developed. ED50 values were converted to −log values for analysis and are presented as mean with 95% confidence intervals. A value of P<.05 was considered significant.

Results

In rabbit basilar and middle cerebral arteries and in dog basilar artery, carbon monoxide did not affect vascular tension even at the highest concentration used (3×10−4 mol/L; Figs 1 and 2). Acetylcholine produced concentration-dependent relaxation with a maximal effect of 97±2% and 97±3%, respectively, in rabbit basilar and middle cerebral arteries. In dog basilar
TABLE 1. Maximum Relaxation Induced by Carbon Monoxide, Nitric Oxide, and Other Relaxants

<table>
<thead>
<tr>
<th>Artery</th>
<th>CO</th>
<th>NO</th>
<th>NP</th>
<th>ACh</th>
<th>ATP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rabbit basilar</td>
<td>0±6</td>
<td>99±1†</td>
<td>100±0.2</td>
<td>97±2</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>5</td>
<td>6</td>
<td>6</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>Rabbit middle cerebral</td>
<td>1±3</td>
<td>80±3</td>
<td>92±5</td>
<td>97±3</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Dog basilar</td>
<td>-1±5</td>
<td>98±2†</td>
<td>97±2</td>
<td>87±2</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>4</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Rabbit aorta</td>
<td>29±4*</td>
<td>81±3</td>
<td>97±1</td>
<td>82±3*</td>
<td></td>
</tr>
<tr>
<td>n</td>
<td>6</td>
<td>6</td>
<td>5</td>
<td>7</td>
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</table>

Data are maximum relaxation (expressed as percentage of precontracted value). Values are presented as mean±SEM; n represents number of animals. Rabbit cerebral arteries were precontracted with histamine, dog basilar artery with prostaglandin F₂₀, and rabbit aorta with phenylephrine. Average tension (as percentage of maximal tension) before relaxation concentration-response curves was as follows: rabbit basilar, 65±3%; rabbit middle cerebral, 83±3%; dog basilar, 25±3%; and rabbit aorta, 70±3%. CO indicates carbon monoxide; NO, nitric oxide; NP, nitroprusside; and ACh, acetylcholine.

*P<.05 vs other arteries for the same relaxant.
†P<.05 vs rabbit middle cerebral and aorta.

artery, ATP produced 87±2% relaxation, indicating intact endothelium in all groups (Table 1).

In contrast to the basilar and middle cerebral arteries, carbon monoxide induced concentration-dependent relaxation of the aorta, with 29±4% reduction of tension at 3 x 10⁻⁴ mol/L (Table 1). Carbon monoxide–induced relaxation of the aorta was readily reversed by increasing the rate of O₂/CO₂ flow into the tissue baths with washout of carbon monoxide (Fig 3).

Concentrations of vasodilators that induced half-maximal relaxation are shown in Table 2. Values for ED₅₀ could not be calculated for effects of carbon monoxide on cerebral arteries. Sensitivity of the rabbit aorta to nitric oxide was more than 3 logs greater than that to carbon monoxide.

Discussion

The major new finding of this study is that carbon monoxide does not produce direct relaxation of large cerebral arteries. Nitric oxide produced marked relaxation of cerebral arteries. In contrast to cerebral arteries, carbon monoxide produced relaxation in the rabbit aorta, although carbon monoxide was much less potent than nitric oxide. These findings suggest that, although carbon monoxide can function as a vasodilator in at least one noncerebral blood vessel, carbon monoxide does not appear to have an important direct dilator effect in large cerebral arteries.

Factors responsible for this regional heterogeneity of response to carbon monoxide are not clear, particularly since nitric oxide was an effective vasodilator in all vessels. Heterogeneous responses of different vascular preparations to carbon monoxide have been reported by Furchgott and Jothianandan, who observed that the relative potency of carbon monoxide to nitric oxide varied from approximately 1:1000 for rabbit aorta to 1:1 for dog circumflex coronary artery. Heterogeneity of soluble guanylate cyclase has been suggested and could contribute to regional differences in response to carbon monoxide. An alternative hypothesis is that carbon monoxide–induced relaxation is not mediated by activation of guanylate cyclase, as has been proposed in some studies, and mechanisms responsible for dilator responses to carbon monoxide in peripheral arteries are not present in cerebral arteries.

We considered the possibility that failure of carbon monoxide to induce relaxation in cerebral arteries could be related to lack of penetration of carbon monoxide into smooth muscle cells. This possibility seems unlikely, however, because the water solubility and diffusion coefficients for carbon monoxide and oxygen are approximately equal. Carbon monoxide is more soluble in organic solvents than in water, and the half-time for equilibration with hemoglobin in red blood cells is approximately 80 milliseconds. Thus, it seems likely that carbon monoxide diffuses rapidly into smooth muscle cells.

Carbon monoxide could fail to remain in solution long enough to affect cerebral arteries. The solutions of carbon monoxide, however, produced relaxation in aortic preparations that have a greater diffusion distance to smooth muscle. In addition, relaxation of the aorta was readily reversed by washout of carbon monoxide in the tissue baths.

Dilator capacity of cerebral vessels was not impaired; both endothelium-dependent and -independent relaxants produced 80% to 100% relaxation. Finally, we
considered the possibility that species differences could influence our results because Furchgott and Jothianandan\(^b\) had observed differences in the relative potency of nitric oxide to carbon monoxide in different species. However, we found no response to carbon monoxide in rabbit or dog cerebral arteries. In addition, there was no regional heterogeneity of responses of large brain arteries in the rabbit, because neither the basilar nor middle cerebral artery relaxed in response to carbon monoxide.

In contrast to carbon monoxide, nitric oxide produced rapid relaxation in all vessels, and ED\(_{50}\) values for nitric oxide were not different between vessels (Table 2). Others have demonstrated that nitric oxide induces relaxation in rabbit middle cerebral artery in vitro\(^4,18\) and in rat and cat pial arterioles in vivo\(^20,21\). Previously reported ED\(_{50}\) values for nitric oxide in rabbit middle cerebral artery\(^18\) are similar to values determined in the current study.

We did not examine the effects of carbon monoxide on cerebral arterioles and cannot exclude the possibility that carbon monoxide produces direct relaxation of cerebral arterioles. However, large cerebral arteries contribute a significant proportion of overall cerebral vascular resistance,\(^22\) and lack of effect on large arteries by carbon monoxide would lessen any dilator influence on cerebral arterioles. A prior investigation in vivo demonstrated marked increases in cerebral blood flow in animals exposed to carbon monoxide.\(^11\) The present study suggests that a direct effect of carbon monoxide on cerebral vessels is not likely and that a reduction in oxygen delivery due to changes in the oxyhemoglobin dissociation curve may be responsible for increases in cerebral blood flow.

The current data and data from others\(^18-21\) indicate that nitric oxide is a potent dilator of cerebral vessels. We have suggested that neuronally derived nitric oxide may influence cerebral vascular tone.\(^23\) Although both nitric oxide and carbon monoxide may be produced in brain and act as intracellular messengers,\(^7\) the present study suggests that, in contrast to nitric oxide, neuronally derived carbon monoxide may not play an important role in producing vasodilation in brain. Although this study provides strong evidence that carbon monoxide does not have a direct effect on large brain arteries, we cannot exclude indirect effects of carbon monoxide or effects on cerebral arterioles.

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### References

8. Gräser T, Vedernikov YP, Li DS. Study on the mechanism of carbon monoxide induced endothelium-independent relaxation in
Carbon monoxide (CO) is a highly unlikely candidate for a putative messenger molecule. However, just a few years ago nitric oxide (NO) was an unfavorable candidate as well. The criteria for a chemical's candidacy as a messenger molecule have been revolutionized by the discovery of NO's involvement in a variety of physiological processes. Since neurotransmitters run in families, it is not surprising that there may be other potential gaseous, labile molecules that function as neurotransmitters. CO is produced by the enzyme heme oxygenase (HO), which cleaves heme into biliverdin and CO. Two isoforms of HO have been identified. The heme of HO-1 (HO1) is an inducible enzyme, with HO1 levels regulated by heme and oxidative stressors. HO1 is concentrated in peripheral tissues such as the liver and spleen. HO type 2 (HO2) is constitutively expressed and particularly abundant in brain. HO resembles NO synthase (NOS) in that the electrons for CO synthesis are donated by cytochrome P450 reductase (CPR), resembling the CPR activity of NOS. CO, like NO, binds to the heme moiety of guanylyl cyclase (GC) to increase cGMP levels. CO's propensity for heme accounts for its toxic effect by reducing oxygen delivery through changes in the oxyhemoglobin dissociation curve.

Despite CO's potential toxicity, it is also emerging as another gaseous messenger molecule in the brain. For instance, endogenous levels of cGMP as well as odorant-induced increases in cGMP in olfactory neurons are maintained and regulated by CO, since potent and selective inhibitors of HO deplete basal cGMP levels and prevent odorant-induced increases in cGMP, whereas inhibitors of NOS are ineffective. HO2 also has discrete neuronal localizations that suggest specialized neuronal functions. HO2 is concentrated in hippocampal pyramidal cells, where CO may function as a retrograde messenger in long-term potentiation. In the nucleus tractus solitarius where HO2 is concentrated, metabotropic glutamate receptor activation regulates a specific cation channel conduction through an HO- and cGMP-dependent mechanism. HO2 is also enriched in the carotid body (Dinerman JL, Prabhakar NR, Snyder SH. Unpublished data), where CO inhibits carotid body activity.

In the accompanying article, Brian et al attempted to expand the postulated functions of CO to include relaxation of cerebral arteries by examining the effect of CO and comparing it with that of NO on rabbit and dog arteries. They showed that both NO and CO dilate aortic preparations, but only NO relaxes cerebral arteries. In vitro experiments show that CO relaxes a variety of smooth muscle preparations through increases in cGMP, including guinea pig ileal strips; guinea pig pulmonary artery; canine femoral, carotid, and coronary arteries; and lamb ductus arteriosus. CO, like NO, also inhibits platelet aggregation due to the activation of GC. The reason that cerebral arteries are unresponsive to CO is not known. Further studies are required to confirm these initial observations and determine the potential mechanisms underlying cerebral arterial smooth muscle resistance to CO.

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References
2. Cruse I, Maines MD. Evidence suggesting that the two forms of heme oxygenase are products of different genes. J Biol Chem. 1988;263:3349-3353.
Effect of carbon monoxide on rabbit cerebral arteries.
J E Brian, Jr, D D Heistad and F M Faraci

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