Contributions of prostacyclin and nitric oxide to carbon monoxide-induced cerebrovascular dilation in piglets

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Leffler, Charles W., Alberto Nasjletti, Robert A. Johnson, and Alexander L. Fedinec. Contributions of prostacyclin and nitric oxide to carbon monoxide-induced cerebrovascular dilation in piglets. Am J Physiol Heart Circ Physiol 280: H1490–H1495, 2001.—Carbon monoxide (CO) is an endogenous dilator in the newborn cerebral microcirculation. Other dilators include prostanooids and nitric oxide (NO), and interactions among the systems are likely. Experiments on anesthetized piglets with cranial windows address the hypothesis that CO-induced dilation of pial arterioles involves interaction with the prostanooid and NO systems. Topical application of CO or the heme oxygenase substrate heme-1-lysinate (HLL) produced dilation. Indomethacin, N^N-nitro-L-arginine (1-NNA), and either iberiotoxin or tetraethylammonium chloride (TEA) were used to inhibit prostanooids, NO, and Ca^{2+}-activated K^+ (K_{Ca}) channels, respectively. Indomethacin, 1-NNA, iberiotoxin, or TEA blocked cerebral vasodilation to CO and HLL. Vasodilations to both CO and HLL were returned to indomethacin-treated piglets by sodium nitroprusside but not iloprost. In iberiotoxin- or TEA-treated piglets, dilations to CO and HLL could not be restored by either iloprost or sodium nitroprusside. The dilator actions of CO involve prostacyclin and NO as permissive enablers. The permissive actions of prostacyclin and NO may alter the KCa channel response to CO because neither iloprost nor sodium nitroprusside could restore dilation to CO when these channels were blocked.

CONSIDERABLE EVIDENCE is accumulating that carbon monoxide (CO) can be an important vascular paracrine factor. CO is produced physiologically via metabolism of heme to CO, biliverdin, and free iron by heme oxygenase (HO) (18). Both HO-1 and HO-2 have been identified in vascular endothelial and smooth muscle cells (4, 31). Endogenously produced CO and exogenous CO can cause endothelium-independent dilation of arteries and arterioles (4, 5, 9, 10), and endogenous CO appears to provide a tonic vasodepressor effect via inhibition of an autonomic pressor mechanism (8). CO has been suggested as an additional endotheliadervived relaxing factor important in vascular regulation (31). HO-2 is strongly expressed in the piglet cerebral microvasculature (16). Exogenous CO and topical application of HO substrate dilate piglet cerebral arterioles. The dilation to heme, but not to CO, is blocked by the HO inhibitor chromium mesoporphyrin, suggesting endogeneous HO can produce sufficient CO to dilate the cerebral microvasculature. It has been suggested that the similarity of cellular locations of HO and nitric oxide (NO) synthase (NOS), to which we add prostaglandin cyclooxygenase (COX), may imply coordinated and potentially complementary roles of the paracrine mediators that are the currently identified endothelium-derived relaxing factors (31).

Prostacyclin plays an important role in control of the newborn cerebral circulation (14). Prostacyclin can cause dilation directly via increases in vascular smooth muscle cAMP (21). Endothelial prostacyclin can also provide a permissive signal to cerebral microvascular smooth muscle allowing dilation in response to another stimulus (15), hypothetically communicating confirmation of the functional integrity of the vessel wall (12). Extensive cross-talk occurs between this prostanoid and NO systems in control of vascular tone (25). Recently, it has been shown that CO may join the group because CO produced from endogenous HO can stimulate prostaglandin synthesis in the rat hypothalmus (19). Furthermore, NO can cause upregulation of HO-1 in vascular smooth muscle independantly of cGMP (6). Finally, it has been suggested that the effects of CO on cerebral blood flow may be mediated by NO (20).

Therefore, the present experiments were designed to address the hypothesis that CO-induced dilation of newborn cerebral arterioles in vivo involves interaction with the cerebral prostanoand and NO systems.
METHODS

All procedures that involve animals were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Newborn pigs (1–3 days old; 1–2.5 kg) were anesthetized with ketamine hydrochloride (33 mg/kg im) and acepromazine (3.3 mg/kg im) and maintained on α-chloralose (50 mg/kg iv). The animals were intubated and ventilated with air. Catheters were inserted into the femoral vein for maintenance of anesthesia and drug injections and into the femoral artery to record blood pressure and draw samples for blood gases and pH analysis. Blood gases and pH were maintained within normal ranges. Body temperature was maintained at 37–38°C. The scalp was retracted, and a hole 2 cm in diameter was made in the skull over the parietal cortex. The dura was cut without touching the brain, and all cut edges were retracted over the bone so that the periarachnoid space was not exposed to bone or damaged membranes. A stainless steel and glass cranial window was placed in the hole and cemented into place with dental acrylic. The space under the window was filled with artificial cerebrospinal fluid (aCSF), which was equilibrated with 6% CO_2-6% O_2-88% N_2 and produced gases and pH within the normal range for CSF (pH = 7.33–7.40, P CO_2 = 42–46 mmHg, and P O_2 = 43–50 mmHg). aCSF could be injected and samples could be collected from needle ports on the sides of the window. The volume of the fluid directly beneath the window was 500 μl and was contiguous with the periarachnoid space. Pial vessels were observed with a dissecting microscope. Diameters were measured with a video micrometer coupled to a television camera mounted on the microscope and to a video monitor. Two pial arterioles of different sizes were measured in each piglet.

Materials. CO was purchased as compressed gas (99.5%). A saturated solution was produced in ethanol at 37°C (7 × 10^{-3} M). The stock was diluted in aCSF for injection under the cranial window at concentrations from 10^{-12} to 10^{-5} M. The HO substrate heme-L-lysinate (38 mM) was prepared using methods described by Tenhunen et al. (27). It was protected from light at all times until placement beneath the cranial window, and the cranial window was only illuminated during vessel diameter measurements. Heme-L-lysinate was stored at -30°C. Lysinate vehicle (L-lysine, H_2O, propylene glycol, and ethanol) was used as 0 M heme-L-lysinate at a dilution equal to that with heme-L-lysinate at 2 × 10^{-6} M. Heme-L-lysinate was diluted in aCSF (10^{-9}-2 × 10^{-6} M) for placement under the cranial window. The HO inhibitor chromium mesoporphyrin was purchased from Porphyrin Products (Logan, UT). Water-soluble indomethacin (indomethacin trihydrate) was a gift from Merck (Rahway, NJ). Iloprost was a gift from Schering AG (Berlin, Germany). Other reagents were purchased from Sigma Chemical (St. Louis, MO).

Experiments. CO and heme-L-lysinate were applied directly to pial arterioles, and the maximal diameter attained over a 10-min period was recorded as the response to each dose. Repeat ascending dose-response curves to CO or heme-L-lysinate were produced before and after no treatment or treatment with indomethacin (5 mg/kg iv) (14) or N^ω-nitro-L-arginine (L-NNA; 10^{-3} M topically) (2). Collections of CSF (300 of 500 μl total) were made from beneath the cranial window at the end of each 10-min period for later measurements of 6-keto-PGF_1α.

Responses to either isoproterenol (10^{-6} M topically for 5 min), hypercapnia (10% CO_2 ventilation for 5 min), or sodium nitroprusside (10^{-7} M topically for 5 min) were measured before and after treatments.

Experiments to investigate potential permissive contributions of prostacyclin and NO (see RESULTS) were conducted by applying either iloprost (10^{-12} M) or sodium nitroprusside topically during treatment with either CO or heme-L-lysinate after inhibition of COX or NOS with indomethacin or L-NNA, respectively. In experiments in which responses to CO were determined in the presence of sodium nitroprusside and L-NNA, the concentration of sodium nitroprusside used was 10^{-7} M. When responses to heme-L-lysinate were determined with sodium nitroprusside and L-NNA, the concentration of sodium nitroprusside was 2 × 10^{-7} M, because in four preliminary experiments the results with 10^{-7} M were not consistent.

Also, either iloprost or sodium nitroprusside was topically given during treatment with CO after inhibition of K_Ca channels with iberiotoxin (10^{-6} M) or tetraethlylammonium chloride (TEA) (10^{-3} M) (16).

6-keto-PGF_1α, was measured in the CSF by radioimmunoassay as described previously (21).

Statistical analysis. Values for each variable are presented as means ± SE. Comparisons among populations within each experimental group used ANOVA with repeated measures. Fisher’s protected least-significant difference test was used to determine differences between populations within each group. P < 0.05 was considered significant.

RESULTS

CO produced dose-dependent dilation of larger and smaller piglet pial arterioles (Fig. 1). Repeat dose-
response curves were virtually superimposable (data not shown) \((n = 5)\). Heme-L-lysinate also caused dose-dependent dilation of pial arterioles (Fig. 2). Repeat dose-response curves were superimposable and larger and smaller arterioles responses were similar, so only data from \( \sim 60 \mu \text{m} \) arterioles are shown.

Indomethacin treatment blocked vasodilations to both CO (Fig. 1) and heme-L-lysinate (Fig. 2). \( \text{L-NNA} \) also blocked vasodilation to both CO (Fig. 3) and heme-L-lysinate (Fig. 4). Neither indomethacin nor \( \text{L-NNA} \) altered dilatory responses to sodium nitroprusside \((10^{-7} \text{ M})\) or isoproterenol \((10^{-6} \text{ M})\) (data not shown).

Dilation to CO that had been blocked by indomethacin treatment was partially restored in the presence of \(10^{-12} \text{ M} \) iloprost (which did not cause significant dilation on its own) and was again absent after removal of the iloprost (Fig. 5). When sodium nitroprusside \((10^{-7} \text{ M})\) was given with CO, no dose-dependent restoration of dilation to CO could be documented (Fig. 6). In contrast, sodium nitroprusside \((10^{-7} \text{ M})\) totally restored vasodilation to CO after inhibition of NOS by \( \text{L-NNA} \) (Fig. 7). Removal of topical sodium nitroprusside eliminated the dilation to CO, and the dilation was again restored when sodium nitroprusside was reapplied.

As with CO responsiveness, cerebral vasodilation to topical heme-L-lysinate that had been blocked by indomethacin returned when a prostacyclin (IP) receptor agonist background was provided in the form of iloprost \((10^{-12} \text{ M})\) (Fig. 8). In the case of heme-L-lysinate-induced dilation, iloprost cotreatment completely restored dilation to indomethacin-treated piglets. On removal of iloprost, as with CO, vasodilation to heme-L-lysinate was absent. Application of sodium nitroprusside to indomethacin-treated piglets appeared to partially allow dilation to heme-L-lysinate to occur (Fig. 9).

As with CO, \( \text{L-NNA} \) blocked responses to heme-L-lysinate. Sodium nitroprusside \((2 \times 10^{-7} \text{ M})\) cotreatment restored dilation in response to heme-L-lysinate (Fig. 10).

Results for iberiotoxin and TEA were identical and thus were combined. Inhibition of \( K_{\text{Ca}} \) channels blocked dilation to CO. Neither iloprost nor SNP restored any dilation to CO in piglets treated with iberiotoxin or TEA (Fig. 11).
Neither CO nor heme-L-lysinate had effects on cerebral prostacyclin production that could be detected by measuring 6-keto-PGF$_{1\alpha}$ in cortical periarachnoid fluid. CO caused no significant change at any concentration from $10^{-12}$ to $10^{-7}$ M ($n = 13$) (data not shown). Although heme-L-lysinate appeared to increase cortical periarachnoid 6-keto-PGF$_{1\alpha}$ concentration slightly, significance was reached only at the highest concentration [6-keto-PGF$_{1\alpha}$ concentration: vehicle, $1,050 \pm 148$ pg/ml; and heme-L-lysinate ($2 \times 10^{-6}$ M), $1,699 \pm 539$ pg/ml].

**DISCUSSION**

The new findings of the current study are that 1) both prostanoids and NO appear to contribute to CO-induced cerebral vasodilator responses via permissive signals, and 2) $K_{Ca}$ channels mediate vasodilations to CO downstream of the actions of prostanoids and NO.

CO is a potential paracrine mediator with multiple similarities to NO. Constitutively expressed enzymes responsible for the generation of both gases are found in the endothelium, vascular smooth muscle, and perivascular neurons (3, 18, 31). Both can produce vasodilation and inhibit platelet activation via activation of soluble guanylyl cyclase (4, 11, 18, 28), although considerable evidence suggests CO can produce dilation independently of cGMP (7, 26). Dilation to CO and NO can involve vascular smooth muscle hyperpolarization via $K_{Ca}$ channel activity (23, 24, 28, 29, 30). CO and NO appear to have special roles in the brain where they can function as cotransmitters or as modulators of neuropeptides. The localizations of HO-2 and brain NOS (bNOS) in the brain suggest special roles for CO and NO in cerebral function, including regulation of cerebrovascular circulation.

Prostacyclin provides a predominant dilator influence in the neonatal cerebral vasculature (14). This influence was originally conceived to involve cAMP-dependent modulation of cerebrovascular tone. However, it is becoming increasingly clear that, although prostacyclin may allow specific cAMP-dependent responses to occur, the physiological role of prostacyclin in the newborn circulation can be a permissive one that is independent of direct coupling between the IP recep-
tor and adenylyl cyclase (12). It is proposed that endothelially derived prostacyclin communicates the functional integrity of the arteriolar wall to the vascular smooth muscle, greatly modifying vascular smooth muscle responses to additional inputs. NO signals from bNOS may provide additional confirmation of perivascular neuronal integrity in the older individual (13). Concerning the question of “permissive” versus “conventional” actions of prostacyclin and NO, one must also consider the possibility that elevations of production of mediators in very localized areas could be occurring, allowing conventional function in either an autocrine or a paracrine manner. Such small increases might not be detectable by collections of periarachnoid fluid. Nevertheless, it is clear that both prostacyclin and NO can act in a permissive enabling manner, because with production systems blocked and only a constant exogeneously supplied addition of either iloprost or sodium nitroprusside the full dose-response relationship to CO or heme-L-lysinate can be restored.

We now report that CO appears to interact with both of these other classical autocrine/paracrine mediators in producing cerebral vasodilator responses. Interactions between the CO and NO systems have been reported before (18). In fact, it has been reported previously that CO-induced cerebral vasodilation in the adult rat appears to be mediated by NO (20), although the mechanism is not known. Over a longer period NO can cause upregulation of HO-1 (6), but the time course of the present experiments obviates any such mechanism being involved in our data. Whereas NO and CO are both capable of activating soluble guanylyl cyclase and thus potentially summatting in producing cGMP-dependent dilation, we could detect no increase in cerebral cGMP production coincident with CO- or heme-L-lysinate-induced dilation (16). CO has been shown to stimulate prostaglandin synthesis (19). However, increased prostanoid production does not appear to be necessary for CO-induced cerebral vasodilation, because a background of only $10^{-12}$ M iloprost is sufficient to promote CO and heme-L-lysinate dilations in piglets treated with indomethacin and neither heme-L-lysinate nor CO increased cerebral prostacyclin production markedly. cAMP does not appear to play a major role in the dilator responses to CO because increases in cerebral cAMP production in response to CO or heme-L-lysinate were small and the dose dependency was poor (16) in contrast to dose-dependent dilations produced by iloprost or isoproterenol (22). Therefore, it appears that, while CO-induced cerebral vasodilation involves interactions with both the prostanoid and nitric oxide systems, cyclic nucleotides are not the primary second messengers in CO-induced dilation in this system. Particularly likely is involvement of hyperpolarization via a KCa channel (17, 29, 30).

Many unanswered questions remain. Surprisingly, both indomethacin and l-NNA totally abolished dilation to either CO itself or heme-L-lysinate. Iloprost alone restored much of the dilation after indomethacin treatment but not after l-NNA. Similarly, sodium nitroprusside alone restored dilation to CO and heme-L-lysinate after l-NNA, but iloprost was without effect. It appears that obligatory permissive enabling roles are played by both prostacyclin and NO. It has been reported that dilation to prostacyclin can be mediated by NO but that such dilation involved vasodilator levels of prostacyclin and increased cGMP (1). Therefore, although the present experiments strongly suggest a contribution of endogenous NO to CO-induced dilation, the mechanisms remain illusive.

Previous permissive actions of IP receptor activation have involved cAMP-dependent dilators. Iloprost appears to increase the cAMP production in response to, for example, hypercapnia, histamine, and epoxyeicosatrienoic acids by a mechanism involving protein kinase C (12). However, vasodilation to CO is not coincident with a detectable elevation of cAMP. Instead, the dila-

![Fig. 10. Pial arteriolar dilation to HLL. The control curve was generated, l-NNA was topically applied, and further HLL dose-response curves were then generated in the presence of l-NNA and L-NNA with SNP ($n = 5$ arterioles). *$P < 0.05$ compared with no HLL.](image1)

![Fig. 11. Pial arteriolar dilation to CO. The control curve was generated, and tetraethylammonium chloride (TEA) or iberiotoxin was then applied. CO dose-response curves to SNP and iloprost were performed in the presence of TEA or iberiotoxin ($n = 7$ arterioles with SNP and $n = 6$ arterioles with iloprost). *$P < 0.05$ compared with no CO.](image2)
tion to CO appears to be mediated by KCa channels, because the dilation was totally abolished by either TEA or iberiotoxin. The permissive actions of prostacyclin and NO may alter the KCa channel response to CO because neither iloprost nor sodium nitroprusside could restore dilation to CO when KCa channels were blocked.

The dilator actions of CO appear to be intimately involved with the prostanoitid and NO systems and subsequent activation of KCa channels by CO.

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REFERENCES


