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The permissive role of endothelial NO in CO-induced cerebrovascular dilation

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Barkoudah, Ebrahim, Jonathan H. Jaggar, and Charles W. Leffler. The permissive role of endothelial NO in CO-induced cerebrovascular dilation. *Am J Physiol Heart Circ Physiol* 287: H1459–H1465, 2004. First published June 10, 2004; 10.1152/ajpheart.00369.2004.—Carbon monoxide (CO) and nitric oxide (NO) are important paracrine messengers in the newborn cerebrovasculature that may act as comessengers. Here, we investigated the role of NO in CO-mediated dilations in the newborn cerebrovasculature. Arteriolar branches of the middle cerebral artery (100–200 μm) were isolated from 3- to 7-day-old piglets and cannulated at each end in a superfusion chamber, and intravascular pressure was elevated to 30 mmHg, which resulted in the development of myogenic tone. Endothelium removal abolished dilations of pressurized pial arterioles to bradykinin and to the CO-releasing molecule $\text{Mn}_2(\text{CO})_{10}$ [dimanganese decacarbonyl (DMDC)] but not dilations to isoproterenol. With endothelium intact, N^{ω} -nitro-L-arginine (L-NNA), 1*H*-[1,2,4]oxadiazolo[4,3-*a*]quinoxalin-1-one (ODQ), or tetraethylammonium chloride (TEA^+), inhibitors of NO synthase (NOS), guanylyl cyclase, and large-conductance Ca^{2+} -activated K^+ (K_{Ca}) channels, respectively, also blocked dilation induced by DMDC. After inhibition of NOS, a constant concentration of sodium nitroprusside (SNP), a NO donor that only dilated the vessel 6%, returned dilation to DMDC. The stable cGMP analog 8-bromo-cGMP also restored dilation to DMDC in endothelium-intact, L-NNA-treated, or endothelium-denuded arterioles, and this effect was blocked by TEA^+ . Similarly, in the continued presence of ODQ, 8-bromo-cGMP restored DMDC-induced dilations. These findings suggest that endothelium-derived NO stimulates guanylyl cyclase in vascular smooth muscle cells and, thereby, permits CO to cause dilation by activating K_{Ca} channels. Such a requirement for NO could explain the endothelium dependency of CO-induced dilation in piglet pial arterioles.

nitric oxide; carbon monoxide

CARBON MONOXIDE (CO) is produced endogenously by catabolism of heme to CO, free iron, and biliverdin during enzymatic degradation of heme by heme oxygenase (HO) (24). The constitutive isoform, HO-2, is highly expressed in the newborn piglet brain (29). Furthermore, in newborns, CO appears to be an important paracrine messenger in controlling cerebrovascular circulation. Both the cGMP/PKG signaling pathway (27) and cGMP/PKG-independent mechanisms via activation of Ca^{2+} -activated K^+ (K_{Ca}) channels (15, 41) have been proposed to explain the vascular dilatory action of CO. In the newborn piglet cerebral circulation, CO causes vascular smooth muscle hyperpolarization via a mechanism requiring functional large-conductance K_{Ca} channels (43). CO is a potent dilator of cerebral arterioles in vivo and contributes to cerebrovascular dilation induced by hypoxia and excitatory amino acids (10, 22).

Another potential paracrine dilator in the newborn cerebral circulation is nitric oxide (NO) (20). NO released from the endothelium or from NO donors is an effective vasodilator. In piglets, neurally mediated dilation (26) and possibly dilation to hypoxia (1) appear to involve NO. Although guanylyl cyclase is markedly more sensitive to NO than to CO (27), both gases can activate soluble guanylyl cyclase (9), and both molecules appear to be modulators of neuronal activity, particularly in the brain (45). NO appears to be involved in maintenance of low basal cerebrovascular tone (7, 25). NO can interact with CO to control the cerebral circulation. Inhibition of NO synthase (NOS) with N^{ω} -nitro-L-arginine (L-NNA) blocks CO-induced cerebral vasodilation in piglets, and the inhibition of dilation is reversed by sodium nitroprusside (SNP) (21). In newborn pig cerebral arterioles, glutamate induces dilation by stimulating CO production via the HO pathway (10). Most available data suggest that CO can induce vasodilation independently of endothelium. The vasodilatory effect of CO has been attributed to endothelium-independent mechanisms in the rat tail artery (39), porcine coronary artery and vein (11), dog carotid and coronary arteries (37), and rat thoracic aorta (23). However, recent data indicate that glutamate-induced dilations mediated by CO in piglet cerebral arterioles are endothelium dependent (10). Furthermore, L-NNA, a NOS blocker, inhibits glutamate-induced pial arteriolar dilation (18, 26). The dilation to glutamate can be partially restored with 8-bromo-cGMP and completely restored with SNP (18). These data suggest the permissive role of NO in CO and glutamate-induced vasodilations (18) may involve a minimum necessary background cellular level of cGMP to allow CO to cause dilation. Similarly, in the adult rat cerebral circulation, hypercapnia-induced dilation requires NO to maintain a minimal background cGMP level (12, 13, 38, 38a). The dilator actions of CO involve both prostacyclin and NO as permissive enablers (21). Interestingly, both NO and prostacyclin are produced in the endothelium and can increase cGMP in vascular smooth muscle (2, 3).

The present study was designed to address the hypothesis that CO-induced dilation of piglet pial arterioles depends on endothelial factors. CO may directly increase smooth muscle cell K_{Ca} channel Ca^{2+} sensitivity, but a minimum necessary background level of cGMP, which can be produced by endothelial NO, is necessary for CO to cause vasodilation.

MATERIALS AND METHODS

Pressurized Arteriole Preparation

Protocols using piglets were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Arterioles were collected from 3- to 7-day-old piglets

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of either gender. Ketamine (33 mg/kg im) and acepromazine (3.3 mg/kg im) were used to anesthetize the animals. The brain was removed and placed in 4°C physiological saline solution (PSS) of the following composition (in mM): 4.8 KCl, 112 NaCl, 26 NaHCO₃, 1.2 MgSO₄, 1.2 KH₂PO₄, 10 glucose, and 1.8 CaCl₂. The PSS was equilibrated with 6% O₂-6% CO₂-88% nitrogen, with pH maintained at 7.4. The PSS was equilibrated with 6% O₂ because we measured the PO₂ in cerebrospinal fluid (CSF) that had been placed under the cranial window of piglets for 30 min and found the PO₂ in the CSF mirrors the arterial PO₂. The arterial PO₂ of newborn piglets before mechanical ventilation is usually 80–90 mmHg; thus we presumed that the normal pressure to which the arteriole is exposed in vivo from both the luminal and abluminal side would be in this range. With the use of 6% O₂, the PO₂ when the liquid is equilibrated with the gas is ~43 mmHg (compared with 150 mmHg if 21% O₂ is used). Because the superfusion chamber is not covered, O₂ increases in the bath during superfusion to around the normal for the vessel in vivo. It is reasonable to presume and we have found anecdotally that lower PO₂ results in more stable and apparently endothelium-intact preparations, probably as a result of the proportional reductions in ROS. Bath temperature was maintained at 35–36°C.

Branches of the middle cerebral artery (100–200 μm) were isolated and cleaned of basolateral connective tissue. An arteriolar segment was cannulated at each end and placed in a superfusion chamber (Living Systems), where it was maintained at 35–36°C and pressurized to 10 mmHg for ~10 min. After the diameter stabilized, intravascular pressure was increased to 30 mmHg, and the arteriole was allowed to develop a stable diameter (~30–50 min). The intravascular pressure used was lower than what is customary because the newborn has a lower arterial pressure. Sixty millimeters of mercury is a typical mean arterial pressure for a newborn pig resulting in an approximate pressure in a first-order pial arteriole of 30 mmHg. The arteriole wall was observed using a charge-coupled device camera (Sanyo), and diameter was monitored continuously using the automatic edge detection of Ion Wizard software (Ionoptix; Milton, MA).

To denude endothelium, air was perfused through the arteriole lumen for 5–10 min, followed by PSS. Denudation was confirmed by the absence of a dilatory response to bradykinin, an endothelium-dependent vasodilator, and a normal dilation to isoproterenol, an endothelium-independent vasodilator.

Chemicals

Isoproterenol, bradykinin, 8-bromo-cGMP, SNP, Mn₂(CO)₁₀ [dimanganese decacarbonyl (DMDC)], tetraethylammonium chloride (TEA⁺), L-NNA, and 1*H*-[1,2,4]oxadiazolo-[4,3-*a*]quinoxalin-1-one (ODQ) were purchased from Sigma-Aldrich (St. Louis, MO).

DMDC was prepared in DMSO (10 mM stock), stored in a closed vial wrapped with foil, and kept at room temperature. After DMDC was mixed with PSS, the release of CO progressively increases upon light exposure and peaks at ~45 min. We previously reported values for CO released from DMDC in sealed vials measured using gas chromatography/mass spectroscopy analysis of the head space gas (43). These measurements showed that 1 μM DMDC produced a CO concentration of 3 μM in a sealed container after 10 min of light activation.

ODQ was used to inhibit guanylyl cyclase. ODQ was dissolved in DMSO (25 mM). ODQ (25 μM) was superfused over the arteriole for ~10 min before and during the experiments to achieve guanylyl cyclase inhibition.

Experimental Design

Dilatory responses to bradykinin, isoproterenol, and DMDC. First, the arteriole was superfused with bradykinin (10 μM) for 3 min. Arteriolar diameter was continually recorded, and the maximal diameter was taken as the response. The superfusion solution was then returned to PSS, and the diameter of the arteriole was monitored until

the baseline diameter was again achieved. Isoproterenol (1 μM) was then superfused over the arteriole for 3 min, maximum dilation was recorded as the response, and the arteriole was superfused with PSS until a return to baseline diameter was observed.

Next, the arteriole was superfused with DMDC (1 μM) for 5 min under subdued light. An incandescent light (General Electric 60-W soft white light at 40 cm and at a 45° angle along the longitudinal vector of the arteriole) was then shown on the vessel chamber for 5 min. The light was then turned off, and the arteriole was allowed to return to its basal myogenic tone while being superfused with PSS (~35–40 min). We found that this procedure was most effective at producing reversible and repeatable dilation.

In other arterioles, the response to DMSO (100 μM) was determined by adding DMSO to the PSS without DMDC and superfusing the arteriole. DMSO did not affect arteriolar diameter. Furthermore, DMSO alone had no effect on responses to either isoproterenol or bradykinin.

Inhibition of NOS and NO clamp with SNP. First, the dilatory response to DMDC was determined as above. The arteriole was then superfused with L-NNA (1 mM) for 15 min to inhibit NOS, followed by L-NNA and DMDC in combination for 5 min. DMDC was activated as above in the continued presence of L-NNA. Superfusion was returned to L-NNA, and, when a stable baseline was evident, SNP (50–100 nM to produce detectable dilation) was added. When a stable baseline was observed, the arteriole was superfused with a combination of DMDC, L-NNA, and SNP for 5 min, followed by light activation for 5 min.

Inhibition of guanylyl cyclase and cGMP clamp. Dilation to DMDC was determined. To inhibit guanylyl cyclase, the arteriole was then superfused with ODQ (25 μM) for 9–14 min until diameter stabilized. Next, the arteriole was superfused with a combination of DMDC and ODQ, the bright light was turned on for 5 min, and the response was recorded. DMDC was washed away with PSS-containing ODQ, and the arteriole was then superfused with ODQ (25 μM) and 8-bromo-cGMP (10 μM). After 10–15 min of stabilization, DMDC (1 μM), along with 8-bromo-cGMP and ODQ, was superfused over the arteriole for 5 min under subdued light. The bright light was then turned on for 5 min to activate the accumulated DMDC.

Additional experiments used different combinations of the above treatments (L-NNA plus 8-bromo-cGMP, endothelium denudation plus SNP, etc.). To determine whether cGMP supplementation could bypass K_{Ca} channels and allow dilation to DMDC to occur, TEA⁺ (1 mM) was used in the superfusion to inhibit K_{Ca} channels, and responses to DMDC were measured in the absence and presence of 8-bromo-cGMP.

Statistical Analysis

Data are expressed as means ± SE. Statistical comparisons of independent populations among all groups were made with one-way ANOVA. Comparisons between two groups were made with the Tukey-Kramer multiple-comparison test. *P* < 0.05 was considered statistically significant.

RESULTS

CO-Induced Dilation is Endothelium Dependent in Piglet Cerebral Arterioles

An elevation in intravascular pressure from 10 to 30 mmHg resulted in the development of myogenic tone that was 79 ± 12% of passive diameter. Although endothelium removal appeared to increase the level of myogenic tone induced by a pressure of 30 mmHg (70 ± 15% of passive diameter), the differences were not significant at *P* < 0.05. In endothelium-intact arterioles, bradykinin, DMDC, and isoproterenol each dilated the arterioles (Figs. 1 and 2). Endothelium removal

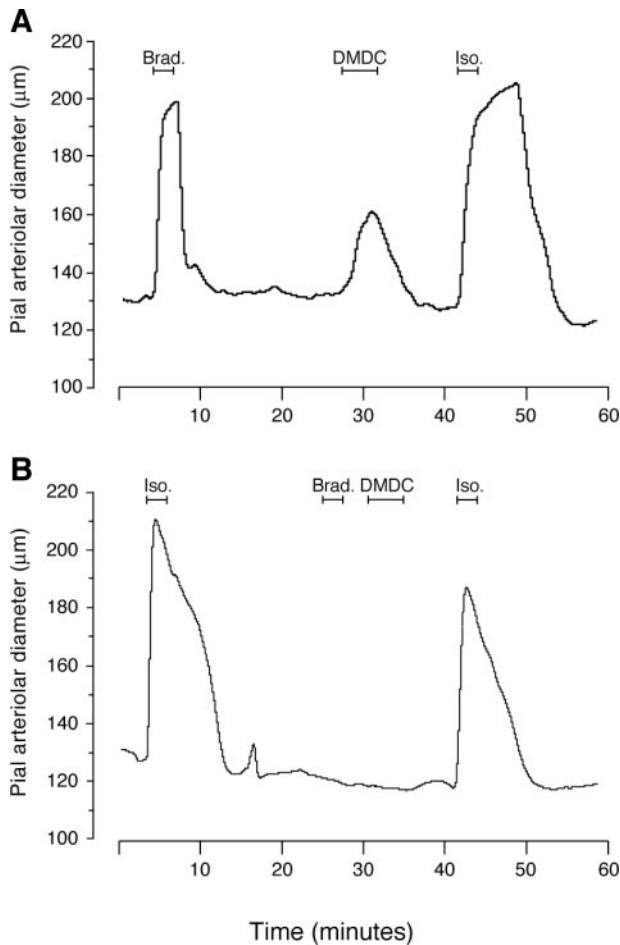


Fig. 1. Diameter tracings of newborn piglet pressurized cerebral arterioles. *A*: arteriole with intact endothelium superfused with bradykinin (Brad; 10 μ M), dimanganese decacarbonyl (DMDC; 1 μ M), and isoproterenol (Iso; 1 μ M), respectively. *B*: arteriole denuded of endothelium superfused with Iso (1 μ M), Brad (10 μ M), and DMDC (1 μ M).

abolished dilations of isolated pial arterioles to bradykinin and DMDC but not to isoproterenol (Figs. 1 and 2). Figure 1 shows representative recordings of two pial arterioles, one with intact endothelium (*A*) and another denuded of endothelium (*B*). In the endothelium-intact arteriole, isoproterenol, bradykinin, and DMDC caused reversible dilations. Dilations to isoproterenol were reproducible, even when applied 2 h apart. In contrast, neither bradykinin nor DMDC dilated endothelium-denuded arterioles (Fig. 1*B*). Figure 2 shows the mean percent dilations caused by bradykinin, DMDC, and isoproterenol in intact arterioles and those denuded of endothelium. These data show that CO-induced dilations require the presence of an intact endothelium. Bradykinin and isoproterenol were used to verify the intact endothelium and smooth muscle function, respectively.

L-NNA Blocks CO-Induced Dilatation and a Constant Dilator Threshold Concentration of SNP Restores the CO-Induced Dilatation

In endothelium-intact arterioles, application of L-NNA, a NOS inhibitor, produced a small constriction (from 178 \pm 12 to 172 \pm 17 μ m). Similarly to endothelium removal, L-NNA

blocked DMDC-induced dilations (Fig. 3). However, in the continued presence of L-NNA, a low concentration of SNP (50–100 nM) that dilated the arteriole from 184 \pm 13 to 188 \pm 13 μ m returned a strong dilation to DMDC (Fig. 3). Neither L-NNA nor application of L-NNA and SNP blocked isoproterenol-induced dilations (36 \pm 1%, 26 \pm 1%, and 36 \pm 2% for control, L-NNA, and L-NNA+SNP, respectively). These data show that dilation to CO does not result from stimulation of NO production, but, nevertheless, complete removal of NO production prevents CO from causing dilation.

SNP Restores CO Vasodilatory Response in Endothelium-Denuded Piglet Pial Arterioles

Similarly to after L-NNA, a low concentration of SNP (50–100 nM) that caused only a small dilation (~6%) by itself restored vasodilation to DMDC in endothelium-denuded arterioles (Fig. 4). These data suggest the role of endothelium in CO-induced dilation involves providing a necessary basal level of NO.

cGMP Analog Restores Vasodilation to CO to Vessels Treated With L-NNA

After L-NNA (1 mM) blocked the dilatory effect of DMDC in arterioles with intact endothelium, the dilatory response to DMDC was returned when 8-bromo-cGMP (10 μ M) was added to the superfusion solution (Fig. 5). 8-Bromo-cGMP itself caused no sustained change in arteriole diameter (Fig. 5). These data suggest the permissive contribution of NO involves stimulation of cGMP production.

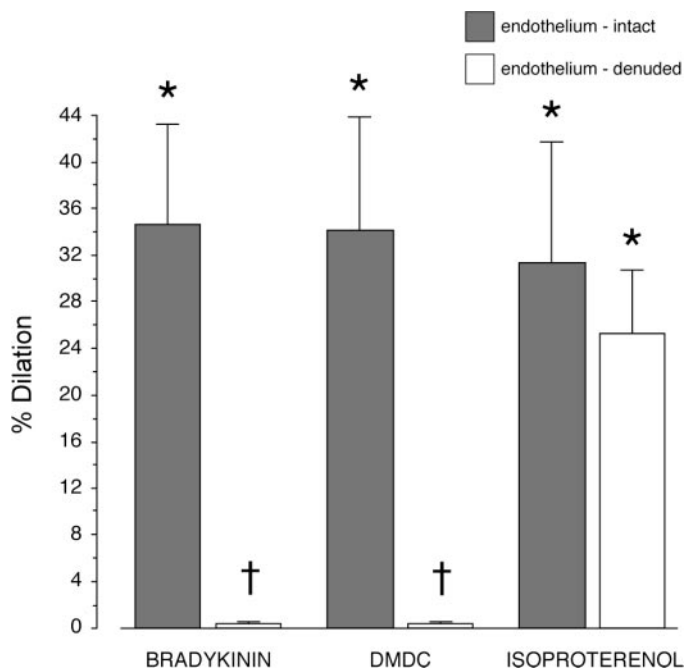


Fig. 2. Responses of pressurized piglet cerebral arterioles with and without endothelium to Brad (10 μ M), DMDC (1 μ M), and Iso (1 μ M) (*n* = 5 in each group). Shown are the percent diameter changes before and after the treatment. %Dilatation is the maximum diameter achieved during treatment minus the diameter immediately before the treatment was applied (control diameter) divided by control diameter times 100. Values are means \pm SE. **P* < 0.05 compared with zero change in diameter; †*P* < 0.05 compared with endothelium intact.

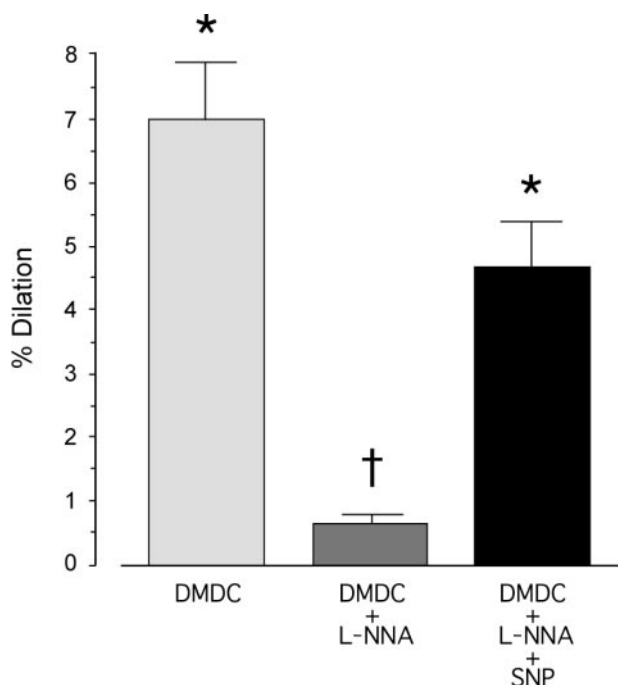


Fig. 3. Effect of DMDC (1 μ M) on endothelium-intact arterioles before and in the presence of *N*^o-nitro-L-arginine (L-NNA; 1 mM) and L-NNA with sodium nitroprusside (SNP) (100 nM). Shown are percent diameter changes comparing the diameters before and after the treatment ($n = 5$). Values are means \pm SE. * $P < 0.05$ compared with zero change in diameter; † $P < 0.05$ compared with before L-NNA.

cGMP Returns Dilation to CO in Endothelium-Denuded Pial Arterioles

DMDC did not dilate endothelium-denuded arterioles. However, in the presence of 8-bromo-cGMP (10 μ M), which only

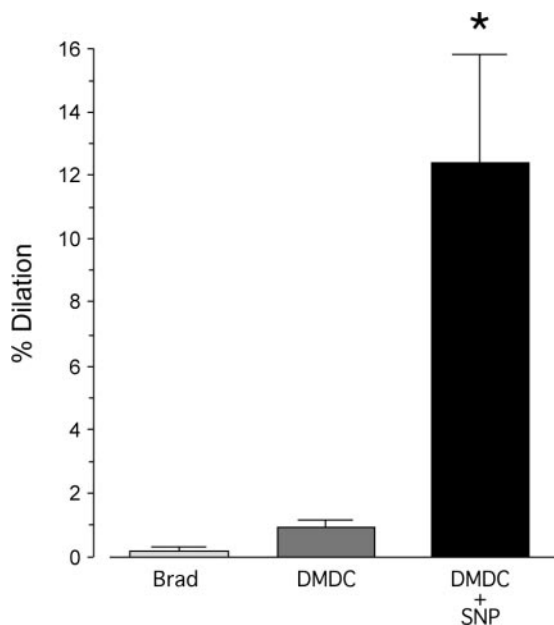


Fig. 4. Effect of SNP (100 nM) on dilation of endothelium-denuded pial arterioles to DMDC (1 μ M). Dilation to Brad (10 μ M) is shown to confirm removal of endothelium. Shown are the percent diameter changes comparing the diameter before and after every treatment ($n = 5$). Values are means \pm SE. * $P < 0.05$ compared with no change in diameter and before SNP treatment.

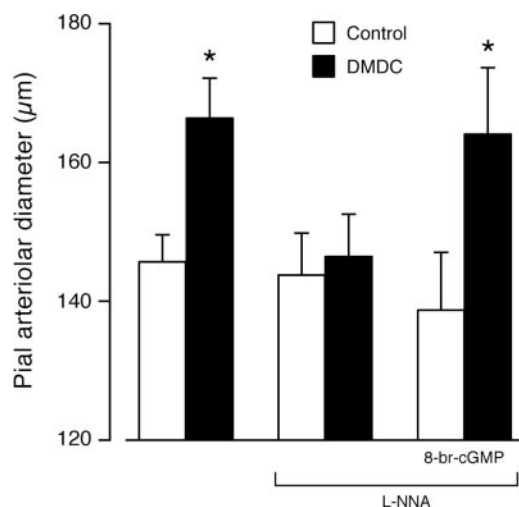


Fig. 5. Pial arteriolar diameter responses to DMDC (1 μ M) before and after treatment with L-NNA (1 mM) and L-NNA plus 8-bromo-cGMP (8-br-cGMP; 10 μ M). Values are means \pm SE; $n = 5$. * $P < 0.05$ compared with the preceding control.

dilated endothelium-denuded pial arterioles 2 \pm 1%, DMDC caused strong dilation (Fig. 6). These data suggest that an endothelium-derived factor(s), presumably NO, activates smooth muscle guanylyl cyclase to produce a necessary basal cGMP level and permits CO-induced dilation.

8-Bromo-cGMP Restores Dilation to CO that is Blocked by ODQ

In endothelium-intact arterioles, ODQ (25 μ M), a guanylyl cyclase inhibitor, blocked the dilatory effect of DMDC. However, in the continued presence of ODQ, 8-bromo-cGMP (10 μ M), but not SNP (data not shown), restored DMDC-induced

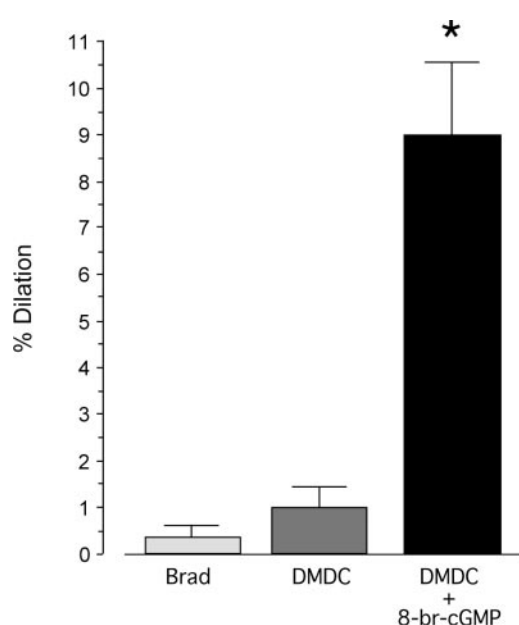


Fig. 6. Dilation to DMDC (1 μ M) in endothelium-denuded pial arterioles before and in the presence of 8-br-cGMP (10 μ M). Dilation to Brad (10 μ M) is shown to confirm absence of endothelium ($n = 5$). Values are means \pm SE of percent dilations. * $P < 0.05$ compared with zero change and to without 8-br-cGMP.

dilations (Fig. 7). These data further support the hypothesis that a minimum necessary background level of cGMP is necessary to permit CO to cause dilation.

8-Bromo-cGMP Does Not Restore Dilation to CO After TEA⁺

TEA⁺ (1 mM), a K_{Ca} channel blocker, prevented DMDC-induced arteriolar dilation (Fig. 8). Moreover, the concentration of 8-bromo-cGMP that restored the dilatory effect of CO in endothelium-denuded, L-NNA-treated, and ODQ-treated arterioles did not return the arteriolar response to CO in the presence of TEA⁺. These data suggest the final effector of CO-induced dilation is activation of K_{Ca} channels and the contributions of cGMP/PKG are upstream of this activation.

DISCUSSION

The major novel findings of the present study using isolated pressurized piglet pial arterioles are as follows: 1) the vasodilatory action of CO is endothelium dependent and 2) the endothelial dependence of CO-induced dilation includes a permissive role of NO, which involves maintaining a necessary cGMP concentration in smooth muscle cells.

The mechanism(s) by which CO causes vasodilation remains under investigation. CO can hyperpolarize vascular smooth muscle via the activation of K_{Ca} channels (15, 39–41, 43). In piglet cerebral arterioles, CO increases the Ca²⁺ sensitivity of K_{Ca} channel α -subunits (43). In smooth muscle cells, K_{Ca} channels are sensitive to Ca²⁺ in the micromolar range, such as that produced by a Ca²⁺ spark (30). Ca²⁺ sparks induce arterial hyperpolarization by activating K_{Ca} channels (16). Membrane hyperpolarization reduces L-type voltage-dependent Ca²⁺ channel activity, leading to a decrease in global intracellular Ca²⁺ concentration and dilation (16). With the elevation

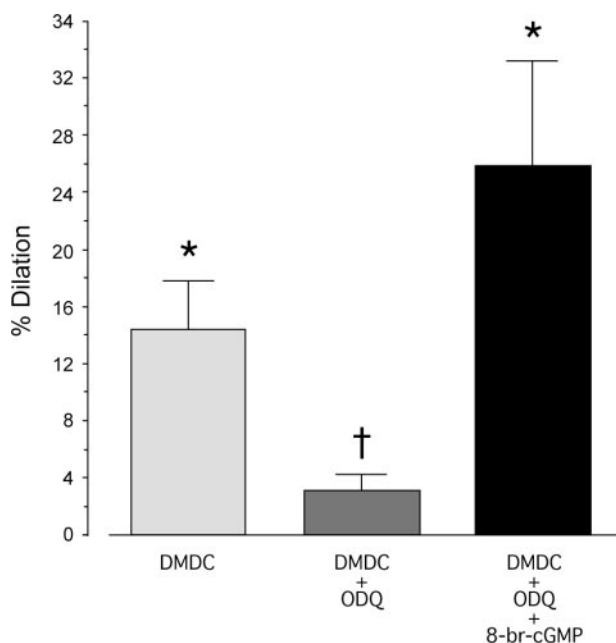


Fig. 7. Dilation to DMDC (1 μ M) in pressurized pial arterioles with intact endothelium before and in the presence of 1*H*-[1,2,4]oxadiazolo-[4,3-*a*]quinoxalin-1-one (ODQ; 10 μ M) and ODQ plus 8-br-cGMP (10 μ M). Shown are percent diameter changes from the same conditions without DMDC ($n = 5$). Values are means \pm SE. * $P < 0.05$ compared with zero change; † $P < 0.05$ compared with DMDC and DMDC + ODQ + 8-br-cGMP.

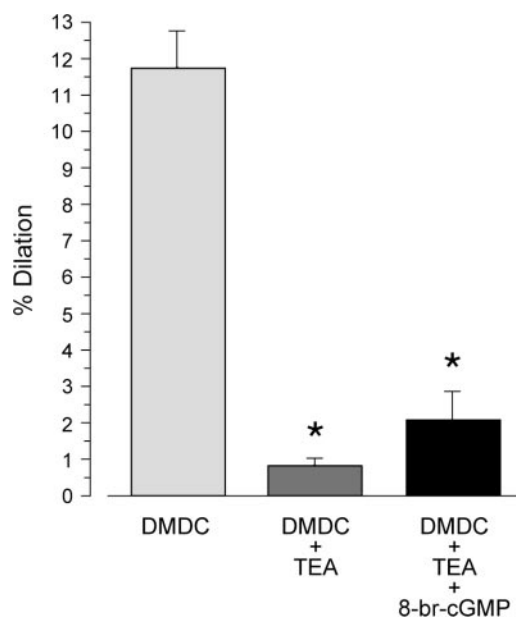


Fig. 8. Effect of TEA⁺ on dilations of piglet pial arterioles to DMDC. The percent changes of endothelium-intact arteriolar diameters to DMDC (1 μ M) are presented before and in the presence of TEA⁺ (1 mM) and TEA⁺ plus 8-br-cGMP (10 μ M). DMDC values shown are from the new baselines ($n = 5$). Values are means \pm SE. * $P < 0.05$ compared with before TEA⁺.

of K_{Ca} channel Ca²⁺ sensitivity, CO enhances effective coupling to Ca²⁺ sparks (15). CO also increases Ca²⁺ spark frequency, which would contribute to the increase in K_{Ca} channel activity (15). In the rat aorta, CO elevates cGMP in both autocrine and paracrine fashions (6), but CO-induced dilation can be produced without elevating cGMP (18, 22). In fact, exogenous administration of NO and CO to cause equivalent dilations is accompanied by elevations of cGMP when NO is applied but not when CO is applied (4, 22). Nevertheless, CO-induced vasorelaxation is inhibited by blocking guanylyl cyclase (18). Thus, even though dilator concentrations of CO do not activate guanylyl cyclase, paradoxically, cGMP appears necessary for CO to cause dilation.

CO and NO have been implicated as mediators of the increased cerebral blood flow in response to elevated brain activity (5, 8). NOS and HO colocalize in endothelial cells and neurons, which may provide the potential for complimentary, coordinated, and modulatory interactions between the two gaseous mediators (14, 36, 44, 45). CO can inhibit endothelial NOS, thus causing a reduction in NO production (35). Conversely, inhibiting NOS with L-NNA blocks cerebral vasodilation to CO in piglets (21). This blockade can be reversed by a constant application of SNP to provide a constant background NO concentration. Previous data clearly demonstrated that the effect of NO in the cerebral microcirculation is connected to guanylyl cyclase (28). Thus we hypothesize that NOS is necessary for CO dilation because it provides CO with the permissive molecule cGMP.

CO can directly activate TEA-sensitive K⁺ channels even in the presence of ODQ (17) and even in pulled patches where neither NO nor cGMP would be available (43). These data, on the surface, appear at odds with the present report. However, increasing the open probability (P_o) of K_{Ca} channels in a whole cell patch of an isolated myocyte and producing dilation of an

intact pressurized arteriole are very different. Kaide et al. (17) demonstrated that CO could increase P_o without activating guanylyl cyclase. They also showed that channel activity was very low when neither cGMP nor CO was present. When 10 μM CO was added, P_o of K_{Ca} channels was increased, but it is far from certain whether the elevation of P_o would be sufficient to cause enough hyperpolarization to produce the marked dilation obtained in pressurized arterioles with 1 μM DMDC (equivalent to maximally 3 μM CO). As we have shown before, CO can increase the P_o even of channels composed of only the α -subunit expressed in HEK cells (43). However, in the studies on isolated myocytes, transient K_{Ca} channel current frequency is relatively low but increases with exposure to CO. In the presence of cGMP, and probably multiple additional inputs that would be present in intact arterioles, the activation of transient K_{Ca} channel currents by CO may be much greater. Therefore, although CO can increase the Ca^{2+} sensitivity of K_{Ca} channels by affecting the α -subunit and this action is the final effector mechanism for CO dilation, it is likely additional conditions are required for CO to dilate an intact arteriole. As discussed in more detail below, potential targets for PKG-catalyzed phosphorylation that would modify transient K_{Ca} channel currents include K_{Ca} channels themselves, an associated protein, and sarcoplasmic reticulum ryanodine receptors.

CO has been reported to cause endothelium-independent dilation (39), but we report here that in isolated piglet cerebral arterioles, CO requires an intact endothelium to cause dilation. As noted above, the vasodilatory effect of CO can be blocked by L-NNA. In piglet pressurized pial arterioles treated with L-NNA, dilation to CO was restored either by providing constant NO in the form of SNP or constant cGMP in the form of 8-bromo-cGMP. Consistent with a critical role for cGMP, ODC, a soluble guanylyl cyclase inhibitor, inhibited dilation to CO (18). The CO-induced dilation was restored to ODC-treated arterioles when 8-bromo-cGMP was provided. Furthermore, both NO and 8-bromo-cGMP restored CO-induced dilation in endothelium-denuded pial arterioles. These data suggest that the endothelium generates NO that activates vascular smooth muscle guanylyl cyclase, thereby providing the background cGMP/PKG activity needed to permit CO to dilate pial arterioles.

Neither CO (22) nor DMDC (18) elevate cGMP production in the newborn brain and cerebral arterioles at concentrations that cause maximal dilation. Nevertheless cGMP is clearly involved in the piglet cerebrovascular dilation to CO. As noted above, the vascular smooth muscle response to CO is due primarily to an effect on K_{Ca} channels (16, 18, 43). cGMP, via activation of PKG and phosphorylation of the K_{Ca} channel itself or an associated protein, may increase K_{Ca} channel Ca^{2+} or CO sensitivity (32). If the action of cGMP is on K_{Ca} channel Ca^{2+} sensitivity, it may be indirect because it has been reported that cGMP does not directly activate K_{Ca} channels in arterial smooth muscle cells (32). Also, phosphorylation of sarcoplasmic reticulum ryanodine receptors would activate Ca^{2+} sparks (31), which would facilitate CO-induced dilation. The effects of NO and 8-bromo-cGMP appear to be all or none (18), suggesting the minimum necessary level of NO/cGMP must be a maximal response.

In summary, the vasodilatory effect of CO on newborn cerebral arterioles involves a permissive role of NO. Endothelial cells appear to be necessary to produce NO to stimulate the

production of cGMP in vascular smooth muscle cells. Such a requirement for NO would explain the endothelium dependency of CO-induced dilation and the endothelium and CO dependence of glutamatergic dilation of piglet pial arterioles (10, 33).

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