Carbon monoxide and Ca\(^{2+}\)-activated K\(^{+}\) channels in cerebral arteriolar responses to glutamate and hypoxia in newborn pigs

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Kanu A, Leffler CW. Carbon monoxide and Ca\(^{2+}\)-activated K\(^{+}\) channels in cerebral arteriolar responses to glutamate and hypoxia in newborn pigs. Am J Physiol Heart Circ Physiol 293: H3193–H3200, 2007. First published August 31, 2007; doi:10.1152/ajpheart.00274.2007.—Large-conductance calcium-activated potassium (K\(_{Ca}\)) channels regulate the physiological functions of many tissues, including cerebrovascular smooth muscle. L-Glutamic acid (glutamate) is the principal excitatory neurotransmitter in the central nervous system, and oxygen tension is a dominant local regulator of vascular tone. In vivo, glutamate and hypoxia dilate newborn pig cerebral arterioles, and both dilatations are blocked by inhibition of carbon monoxide (CO) production. CO dilates cerebral arterioles by activating K\(_{Ca}\) channels. Therefore, the present study was designed to investigate the effects of glutamate and hypoxia on cerebral CO production and the role of K\(_{Ca}\) channels in cerebral arteriolar dilations to glutamate and hypoxia. In the presence of ibiotoxin or paxilline that block dilation to the K\(_{Ca}\) channel opener, NS-1619, neither CO nor glutamate dilated pial arterioles. Conversely, neither paxilline nor ibiotoxin inhibited dilation to acute severe or moderate prolonged hypoxia. Both glutamate and hypoxia increased cerebrospinal fluid (CSF) CO concentration. Ibiotoxin that blocked dilation to glutamate did not attenuate the increase in CSF CO. The guanylyl cyclase inhibitor, 1H-(1,2,4)oxadiazolo(4,3-a)quinoxalin-1-one (ODQ), which blocked dilation to sodium nitroprusside, did not inhibit dilation to hypoxia. These data suggest that dilation of newborn pig pial arterioles to glutamate is mediated by activation of K\(_{Ca}\) channels, consistent with the intermediary signal being CO. Surprisingly, although 1) heme oxygenase (HO) inhibition attenuates dilation to hypoxia, 2) hypoxia increases CSF CO concentration, and 3) K\(_{Ca}\) channel antagonists block dilation to CO, neither K\(_{Ca}\) channel blockers nor ODQ altered dilation to hypoxia, suggesting the contribution of the HO/CO system to hypoxia-induced dilation is not by stimulating vascular smooth muscle K\(_{Ca}\) channels or guanylyl cyclase.

VASCULAR TONE in the cerebral circulation is regulated by numerous cellular mechanisms including modification of activity of potassium channels (6, 7). Activation of potassium channels mediates relaxation of cerebral blood vessels in response to a diverse group of important stimuli. One such stimulus is carbon monoxide (CO), which is produced physiologically from the degradation of heme to biliverdin, iron, and CO by heme oxygenase (HO) (32), and can be produced in high enough concentration to cause vasodilation (10, 21, 25, 26). CO is a dilator of newborn cerebral arterioles in vivo and contributes to cerebral arteriolar dilation induced by excitatory amino acids in newborn pigs (13, 26). In adult rabbits and newborn pigs, HO inhibition reduces the elevation of cerebral blood flow during epileptic seizures but has no effect on cerebral hyperemia, caused by hypercapnia, or in basal conditions (9, 36). CO activates large-conductance calcium-activated potassium (K\(_{Ca}\)) channels in intact newborn cerebral arteriole smooth muscle cells (18), and dilations to CO are blocked by inhibitors of K\(_{Ca}\) channels (26).

L-Glutamic acid (glutamate) is a dilator of newborn cerebral arterioles (8) and the major excitatory neurotransmitter in the central nervous system (34). In the cerebral microcirculation of newborn pigs, glutamate stimulates CO production and causes HO-dependent vasodilation (43). Dilator responses to glutamate in vivo are blocked by the HO inhibitor, chromium mesoporphyrin (26, 43).

Mechanisms involved in hypoxia-induced vasodilation remain poorly understood. Even less is known about the mechanisms that mediate vasodilation to hypoxia in the newborn cerebral circulation. Most data are consistent with a local response (7), but brain-stem neuronal involvement in hypoxia-induced cerebral hyperemia has also been reported (48). The HO/CO system seems to be involved because, similar to glutamate, chromium mesoporphyrin inhibits pial arteriolar dilations to hypoxia in piglets (26).

Therefore, experiments were designed and conducted to test the hypothesis that both hypoxia and glutamate increase cerebral CO production, leading to K\(_{Ca}\) channel-induced cerebrovascular dilation. The following results are consistent with the hypothesis when the stimulus is glutamate but do not support this direct action hypothesis when the dilator stimulus is hypoxia, suggesting that the role of the HO/CO system in hypoxia-induced vasodilation is upstream from the mediator in the dilator pathway.

MATERIALS AND METHODS

All procedures that involved animals were reviewed and approved by the Animal Care and Use Committee of the University of Tennessee Health Science Center. Newborn pigs (1 to 3 days old) 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). Because ketamine appears to cause anesthesia by noncompetitive inhibition of N-methyl-D-aspartate receptors, its use should be carefully considered in experiments that involve glutamatergic transmission. However, we have compared newborn piglets anesthetized with either ketamine or thiopenthal and could not detect any differences in glutamatergic seizure-induced cerebral hemodynamics, responses to topical glutamate, or even responses to topical N-methyl-
Pial arteriolar response to NS-1619. Responses of pial arterioles to topical application of the benzimidazolone compound NS-1619 (at \(10^{-6}\) M), an activator of \(K_{\text{Ca}}\) channels, were measured before and after topical application of \(4 \times 10^{-5}\) M paclinil, a selective \(K_{\text{Ca}}\) channel blocker. This test was done to confirm the efficacy of paxilline.

**Pial arteriolar response to CO.** Treatments with topical CO at concentrations of \(10^{-7}\) and \(10^{-6}\) M were given before and during treatment with paclinil (\(4 \times 10^{-5}\) M topically). CO was purchased in a compressed gas cylinder of 100% CO. The initial stock solution (\(10^{-3}\) M) was produced by saturation of water with CO using solubilities from the Handbook of Chemistry and Physics. Dilutions were produced in gas-tight containers without a gaseous interface.

**Pial arteriolar responses to glutamate and SNP.** Responses to topical application of \(2 \times 10^{-7}\) M SNP and glutamate (\(10^{-6}\) and \(10^{-5}\) M) were determined before and during treatment with paclinil (\(4 \times 10^{-5}\) M or iiberiotoxin (\(10^{-6}\) M). CSF collections for CO determinations were made at the end of 7 min of treatment or control.

**Pial arteriolar responses to hypoxia.** Acute hypoxia was produced by ventilation with 10% O\(_2\) in N\(_2\). Hypoxia was maintained for 5 min, and the maximal dilation was recorded as the response. This treatment caused a decrease in arterial partial pressure of O\(_2\) (PaO\(_2\)) to \(\approx 30\) mmHg within 5 min. Pial arteriolar diameters and arterial pressures were measured. For CSF CO determination, hypoxia was maintained for 7 min, at which time CSF from under the window was collected.

In another group of piglets, hypoxia was given and measurements were taken over 30 min of hypoxia (13% O\(_2\)). Hypoxia challenges were given to each piglet. One group then received paclinil and the other group vehicle during the second hypoxic challenge. We used 13% O\(_2\) instead of 10% O\(_2\) in these experiments because piglets deteriorate rapidly as ventilation with 10% O\(_2\) is prolonged.

For CO measurement, CO in a CSF was measured using gas chromatography-mass spectrometry and \(^{31}\)CO as the internal standard as described previously (22, 23, 26, 43). Confirmation of the accuracy of CO measurements in CSF under the cranial window was obtained by filling the windows with known concentrations and then collecting and measuring the CO in the CSF that was collected (Table 1).

**Statistical analysis.** Values are presented as means ± SE. For comparisons among groups, results were subjected to a one-way ANOVA for repeated measures with Tukey’s post hoc test to isolate differences between groups. Paired r-tests were used to compare two groups. A level of \(P < 0.05\) was considered significant.

**RESULTS**

There were no significant changes in arterial blood gas levels, pH, pressure, or body temperature when the values were compared at the beginning and end of the experiments.

**Effects of NS-1619 on newborn pig pial arterioles.** NS-1619, which opens \(K_{\text{Ca}}\) channels, caused dilation of pial arterioles that was abolished by paclinil, a \(K_{\text{Ca}}\) channel blocker (Fig. 1).

### Table 1. CO concentrations calculated to be in solution and concentrations measured in aCSF collected following placement of each concentration under cranial windows implanted in newborn pigs

<table>
<thead>
<tr>
<th>Concentration Calculated, nM</th>
<th>Concentration Measured, nM</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>49±6</td>
</tr>
<tr>
<td>100</td>
<td>136±12</td>
</tr>
<tr>
<td>1,000</td>
<td>1,004±89</td>
</tr>
<tr>
<td>10,000</td>
<td>13,936±1,052</td>
</tr>
</tbody>
</table>

Values of concentrations measured are means ± SE; \(n = \) collections at each concentration from 9 piglets. CO, carbon monoxide; aCSF, artificial cerebrospinal fluid.
Effects of KCa channel blockers in pial arteriolar diameter. Pial arterioles previously treated with glutamate, following washout and return to resting diameters, constricted in response to paxilline and iberiotoxin (Figs. 2 and 3). Conversely, in groups of piglets in which pial arterioles had not had previous exogenous glutamate exposure, neither paxilline nor iberiotoxin caused constriction (Figs. 4 and 5).

Effects of paxilline on vasodilation in response to CO and isoproterenol. As shown in Fig. 2, CO-induced dilation was abolished by paxilline (Fig. 2), as shown previously for another KCa channel blocker, iberiotoxin (25), but paxilline did not affect dilation to isoproterenol (10⁻⁷ M) (increase in pial arteriolar diameter, 22 ± 3% and 22 ± 6%, without and with paxilline, respectively).

Effects of paxilline and iberiotoxin on dilations to glutamate and SNP. Both glutamate and SNP dilated newborn pig cerebral arterioles in vivo (Fig. 3). Paxilline and iberiotoxin blocked the dilation to glutamate but did not affect the dilation to SNP.

Effects of paxilline and iberiotoxin on pial arteriolar dilations to hypoxia. Hypoxia accomplished by ventilation with 10% O₂ for 5 min caused dilation of pial arterioles (Fig. 4). PaO₂, arterial partial pressure of CO₂ (PaCO₂), and arterial pH before hypoxia were 103 ± 4, 32 ± 1, 7.40 ± 0.01 mmHg and, after 5 min of 10% O₂, were 33 ± 2, 36 ± 2, 7.35 ± 0.02 mmHg. The hypoxia-induced increase of pial arteriolar diameter was unaltered by paxilline or iberiotoxin.
The effects of prolonged hypoxia (13% oxygen) on diameters of pial arterioles of newborn pigs are shown in Fig. 5. PaO₂, PaCO₂, and pH were 91 ± 11003 4, 38 ± 11003 2, 7.40 ± 0.02 before and 51 ± 2, 30 ± 2, and 7.40 ± 0.08 mmHg after 30 min ventilation with 13% O₂. Dilation to hypoxia progressed over about the first 15 min, and dilations were then sustained for the next 15 min. Following the return to normoxia, repeated ventilation with 13% O₂ for 30 min produced dilation identical to the first hypoxic challenge (Fig. 5A). Similarly, when paxilline was included during the second hypoxic challenge, dilation to hypoxia was unaltered (Fig. 5B).

Effects of ODQ on dilation to hypoxia. Because HO inhibition inhibits dilation to hypoxia, KCa channel inhibitors had no effect on the dilation, and because CO may stimulate guanylyl cyclase and increase cGMP, we examined the effect of ODQ on dilation of pial arterioles to hypoxia. Efficacy of ODQ was demonstrated by blocking dilation to SNP (Fig. 6). ODQ did not alter pial arteriolar dilation to hypoxia (Fig. 6).

Effects of glutamate and hypoxia on CSF CO concentration. As shown previously (43), glutamate increases CO concentration in CSF collected from under the cranial window (Fig. 7). Iberiotoxin, which blocked dilation to glutamate (Fig. 3), did not change the increase in CSF CO concentration to glutamate (Fig. 7). Hypoxia also increased CSF CO concentration before and following iberiotoxin (Fig. 7), although dilation to hypoxia was not blocked by iberiotoxin or paxilline (Fig. 4).

DISCUSSION

The new findings of the present study of newborn pigs are as follows. First, the selective KCa channel blockers, paxilline and iberiotoxin, block the dilation of pial arterioles to glutamate but not the nitric oxide (NO)-releasing molecule, SNP, as shown previously (1, 2), nor to the β-adrenergic agonist, isoproterenol. Second, neither paxilline nor iberiotoxin alters dilation to
either short, severe hypoxia or longer-term moderate hypoxia (paxilline only). Third, ODQ does not inhibit dilation to hypoxia. Finally, hypoxia increases cerebral production of CO, similarly to glutamate, and these increases in CO are not affected by iberiotoxin that blocks the dilation of glutamate.

The efficacy of paxilline was confirmed with NS-1619, a vasodilator that opens KCa channels at micromolar concentrations, which neither activate voltage-gated or ATP-dependent potassium channels nor block L-type Ca2+ channels (14, 44). The concentration of paxilline was chosen as the minimal concentration used previously by others. Paxilline is as selective and can be more effective than the more commonly employed KCa channel blocker, iberiotoxin (15). The increased efficacy of paxilline can be related to reduced affinity of specific KCa channels for iberiotoxin, particularly when complexed with β-subunits, diffusion limitations, and peptide adhesion sites of equipment. Nevertheless, in the present experiments, using paxilline and iberiotoxin in identical protocols produced the same results in each case. Furthermore, since paxilline and iberiotoxin did not alter the dilation to SNP or isoproterenol, it appears that the loss of responses to NS-1619 and CO (Ref. 26, and in present study) are selective rather than a paxilline- or iberiotoxin-induced generalized depression in pial arteriolar responsiveness.

Inhibition of KCa channels with paxilline or iberiotoxin did not alter basal pial arteriolar diameters in any group in the present study, except for piglets that had previously received topical application of glutamate. Since dilation to glutamate was blocked by both iberiotoxin and paxilline, this dilation clearly involves KCa channels. However, removal of glutamate indicated that the dilation of pial arterioles was completely reversible, suggesting the termination of the KCa channel activity elevation. The consistent constriction produced by KCa channel inhibition even 30 min or more following removal of the glutamate suggests that topical exogenous glutamate stimulation changes the contribution of KCa channels to the regulation of vascular tone. The increased contribution could be explained by either an increase of constrictor that is counterbalanced by increased KCa activity or a prolonged activation of KCa channels from the initial signaling pathway with compensatory elevation of a constrictor influence. Neuronal activation, which could be caused by glutamate, was not measured in the present experiments. It is conceivable that initial topical glutamate altered baseline neuronal activity. These data emphasize the potential for complex compensatory mechanisms to contribute to the establishment of final vascular tone and that a simple reversal of a single response upon removal of a stimulus does not guarantee that original conditions are achieved.

Evidence for an involvement of K+ channel activation in CO-induced vasodilation has arisen from various studies employing intact circulation in vivo, isolated vessels, and patch-clamp techniques. For example, CO administration produced dose-dependent increases of piglet pial arteriolar diameters in vivo that were abolished in the presence of tetraethylammonium and iberiotoxin (26). Furthermore, CO has a direct effect
on the vascular smooth muscle KCa channels (49, 50, 52) increasing the open probability of the KCa channels (17).

Topical application of agonists and antagonists to the brain surface impacts brain and vascular cells. Of particular note, in addition to vascular smooth muscle, astrocytes and endothelial cells have KCa channels. CO can dilate cerebral arterioles by activating KCa channels on the smooth muscle, specifically by binding to heme attached to the α-subunit of the KCa channel, increasing its calcium sensitivity, and thereby increasing spark to STOC coupling (17, 18). This mechanism can be observed in isolated cerebrovascular smooth muscles and intact pressurized arterioles (17) and is the most likely explanation for the ability of KCa channel inhibitors to abolish cerebral arteriolar dilation to CO in situ (present study, and Ref. 26). Nevertheless, activation of KCa channels on astrocytes, neurons, and/or endothelium by CO may contribute to CO-induced cerebrovascular dilation by enhancing the production of dilatory signals to the vascular smooth muscle. Therefore, although CO can cause changes in isolated myocytes consistent with dilation, multiple additional signaling mechanisms could contribute to the dilation in the intact brain and the relative contributions of these mechanisms could be stimulus specific. However, the observation that pial arteriolar dilation to topical CO is abolished by KCa channel inhibition results in the extrapolation that a stimulus producing cerebrovascular dilation using CO as the transmitter would also be blocked by KCa channel inhibition.

In the cerebral microcirculation, glutamate is a vasodilator (35, 43). The increased neuronal activity caused by glutamate requires increased cerebral blood flow that involves glutamate stimulation of CO production from heme by HO-2 in newborn pigs (23, 26). In the present study, the blockade of dilation to glutamate by paxilline and iberiotoxin is consistent with a mechanism involving CO as a messenger, since CO-induced dilation is mediated by KCa channels. Furthermore, as reported previously (43), topical application of glutamate to the brain surface increased CO accumulation in the CSF. Inhibition of the glutamate-induced dilation with iberiotoxin did not affect the increase in CSF CO. These data further suggest that glutamate increases CO production and that CO then causes the dilation by activating KCa channels, rather than KCa channel activation causing the increase in CO.

Although both glutamate and hypoxia increase cerebral production of CO, the inhibition of the KCa channel that serves as the receptor for the direct effect of CO on cerebral arteriolar smooth muscle cells (18) blocks only the dilation to glutamate, not to hypoxia. This apparent inconsistency has important conceptual connotation. If CO produced by brain cells was delivered to target cells by release and transport in the CSF, CSF CO concentrations during stimulation would be sufficient to produce equivalent dilation when applied topically to the brain surface. However, this is not the case. For example, although glutamate (10−5 M) nearly doubles CSF CO concentration, the resultant CSF concentration is <10−7 M (Fig. 7). The dilation to 10−5 M glutamate (Fig. 3) is similar to that produced by a CO concentration of 10−6 M (Fig. 2). Because the CSF CO concentration is insufficient to account for the dilation, it appears reasonable to propose that, although CSF CO concentration under the window reflects changes in production by cells at the brain surface, concentrations at the sites of production are higher where CO can function as a precision gasotransmitter. Although both glutamate and hypoxia increase CSF CO concentration and produce dilation that is blocked by HO inhibition, available data implicate astrocytes as the signaling cells in the case of glutamate (28) and endothelium in the case of hypoxia (27). Because KCa channel inhibition blocks dilation to glutamate, but not to hypoxia, CO produced by astrocytes in response to glutamate may dilate adjacent arterioles by activating the smooth muscle KCa channels, whereas CO produced in endothelium may be involved in an endothelium-derived signal for hypoxic vasodilation.

Hypoxia stimulates the production by endothelium, astrocytes, and neurons of a wide variety of vasodilator metabolites, including potassium and hydrogen ions, prostaglandins, excitatory amino acids, NO, endothelial-derived hyperpolarizing factor, and adenosine that can work in concert to produce the final dilatory response (19, 37, 39, 40). The present results indicate that neither the initial onset of dilation to hypoxia nor the maintenance of the dilation requires functional KCa channels in newborn pigs. Furthermore, the lack of effect of paxilline or iberiotoxin on responses to hypoxia coupled with blockade of glutamate-induced dilation suggests that hypoxic stimulation of glutamatergic signaling plays a minimal role in dilation to hypoxia in the newborn cerebral vasculature.

In response to 10% O2 ventilation, pial arterioles dilate rapidly over the first 2 min, with only a slight further dilation between 2 and 5 min (data not shown). Five minutes of hypoxia produces minimal changes in arterial pressure. With longer hypoxia periods using 10% O2, arterial pressure will drop without support. Although 5 min are clearly sufficient for activation of peripheral reflex pathways, it is probably insufficient to involve numerous humoral regulators such as vasopressin and opioids (27). Therefore, to sustain a longer period of hypoxia, 13% O2 in the inspired air was used. Similar to 5 min of severe hypoxia, cerebrovascular dilation to 30 min of moderate hypoxemia was totally unaffected by inhibition of KCa channels. Although, when KCa channel activity is blocked, the influence of other dilator pathways could be accentuated masking a normal contribution of KCa channels, this possibility appears unlikely because HO inhibition blocks dilation to hypoxia (26).

In newborn pigs, in contrast to the KCa channel blocker, a HO inhibitor did reduce the increase of pial arteriolar diameter in response to hypoxia (26). Furthermore, although blockade of neither of the most appreciated mediators of CO-induced vascular responses, KCa channels and cGMP, altered hypoxia-induced cerebral vasodilation, hypoxia did increase CSF CO concentration. The correlation of the CSF elevation with response blockade by KCa channel inhibition in the case of glutamate but not hypoxia emphasizes the point that CSF CO concentration indicates that brain cells on the surface produced CO in response to a stimulus but does not indicate which cells or whether the producing cells are in sufficient proximity to the vascular smooth muscle to signal dilation.

The ability of KCa channel inhibitors to block CO-induced dilation, but not hypoxia-induced dilation, might suggest that the contribution of HO to hypoxia-induced dilation is indirect. HO-2 has been proposed to be a cellular O2 sensor (27), suggesting that the sensing of hypoxia may involve HO/CO, but the dilation in response may not. Recently, O2 has been reported to stimulate KCa channels by activating HO-2, leading to the generation of CO, the downstream channel activator (51). The presence of HO-2 in the KCa channel complex
provides a molecular explanation for the observation that HO inhibition results in carotid body excitation (42). Hypoxic depression of \( \text{K}_\text{Ca} \) channel activity in neurons of the central nervous system may also contribute to the excitotoxicity that results from increased neuronal excitability (31). Nevertheless, the present data suggest a lack of \( \text{K}_\text{Ca} \) channel involvement in pial arteriolar dilation to hypoxia in newborn pigs.

The results of the present report are consistent in many respects with those of another group using the same model (1, 2) but also different with regard to the effects of iberiotoxin on the responses to both hypoxia and glutamate. Both these previous studies and the present one find no effect of \( \text{K}_\text{Ca} \) channel inhibition on dilation to SNP. However, when hypoxia equivalent to the 5-min hypoxia level in the present study was administered for 10 min, Armstead et al. (1, 2) detected about a 20% reduction in hypoxia-induced vasodilation following iberiotoxin. Although the precise timing of the measurements differ between the studies, it is difficult to detect any differences between this study and the present one to explain our total lack of effect of iberiotoxin at a higher concentration on responses to hypoxia given for 5 min at the same \( P_O_2 \). Our data with iberiotoxin are identical to those using another highly selective and potent \( \text{K}_\text{Ca} \) channel inhibitor, which also had no effect on hypoxia-induced dilation. Also, Phillip and Armstead (41) found only about 50% inhibition of the dilator response to glutamate with iberiotoxin that abolished dilation to NS-1619. Experimental design and methods appear identical, leaving no obvious explanation for the greater efficacy of \( \text{K}_\text{Ca} \) channel blockers, iberiotoxin and paxilline, on glutamate-induced dilation in the present study. Consistent between both groups is the conclusion that the contribution of \( \text{K}_\text{Ca} \) channels to glutamate-induced dilation is greater than it is to dilation to moderately severe acute hypoxia.

As reported previously (1, 2), the present results indicate that the mechanism by which NO dilates piglet pial arterioles does not involve \( \text{K}_\text{Ca} \) channels because dilation to SNP was unaltered by paxilline or iberiotoxin. NO-induced dilation is associated with increased levels of cGMP in vascular smooth muscles cells (16, 37, 39), and ODQ blocked dilation to SNP. It has also been suggested that the effects of CO on cerebral blood flow may be mediated by NO (33). However, the ability of \( \text{K}_\text{Ca} \) channel inhibition to block dilation to CO but not to NO indicates distinct mechanisms and argues strongly against the dilation to either of these gaseous messengers being mediated by the other. Similarly, dilation to the \( \beta \)-adrenergic agonist, isoproterenol, was not affected by paxilline, indicating that cAMP-induced cerebrovascular dilation does not involve \( \text{K}_\text{Ca} \) channels.

In conclusion, the present study shows that \( \text{K}_\text{Ca} \) channels account for the relaxation induced in pial arterioles by CO and glutamate in newborn pigs. Because glutamate increases CO production (Ref. 43, and the present study) and CO activates \( \text{K}_\text{Ca} \) channels (17), it is reasonable to propose that CO is the messenger by which glutamate dilates piglet cerebral arterioles. Conversely, the inhibition of \( \text{K}_\text{Ca} \) channels did not alter cerebrovasodilation to hypoxia, SNP, or isoproterenol. These data suggest that dilation to hypoxia is not caused by hypoxia increasing CO that activates \( \text{K}_\text{Ca} \) channels. The present results also suggest a lack of involvement of guanylyl cyclase. Previous data show that an inhibition of CO production attenuates dilation to hypoxia, and the present data show that hypoxia increases cerebral CO production. We speculate that HO/CO is acting as an \( O_2 \) sensor rather than a dilator mechanism in this instance.

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GRANTS

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