Role of neuronal NO synthase in relationship between NO and opioids in hypoxia-induced pial artery dilation

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Wilderman, M. J., and W. M. Armstead. Role of neuronal NO synthase in relationship between NO and opioids in hypoxia-induced pial artery dilation. Am. J. Physiol. 273 (Heart Circ. Physiol. 42): H1807–H1815, 1997.—Nitric oxide (NO) contributes to hypoxia-induced pial artery dilation, at least in part, via the formation of guanosine 3′,5′-cyclic monophosphate (cGMP) and subsequent release of Met-enkephalin and Leu-enkephalin in the newborn pig. In separate studies, these opioids were also observed to elicit NO-dependent pial dilation. The present study was designed to investigate the role of the neuronal isoform of NO synthase (NOS) in hypoxic pial dilation, associated opioid release, and opioid dilation in piglets equipped with a closed cranial window. Tetrodotoxin (10–6 M) attenuated the dilation resulting from hypoxia (Po2 ~35 torr; 25 ± 1 vs. 14 ± 1%). Similarly, 7-nitroindazole, sodium salt (7-NINA, 10–6 M), a purported neuronal NOS inhibitor, attenuated hypoxic pial dilation (26 ± 1 vs. 14 ± 2%). Hypoxic dilation was accompanied by elevated cerebrospinal (CSF) cGMP, which was blocked by 7-NINA (433 ± 19 and 983 ± 36 vs. 432 ± 19 and 441 ± 19 fmol/ml for control and hypoxia in absence and presence of 7-NINA, respectively). Additionally, hypoxic dilation was also accompanied by elevated CSF Met-enkephalin, which was attenuated by 7-NINA (1,027 ± 47 and 2,871 ± 134 vs. 779 ± 78 and 1,551 ± 42 pg/ml for control and hypoxia in absence and presence of 7-NINA, respectively). In contrast, Met-enkephalin (10–10, 10–9, and 10–8 M) induced dilation that was unchanged by 7-NINA (7 ± 1, 12 ± 1, and 18 ± 1 vs. 6 ± 1, 10 ± 1, and 17 ± 1%, respectively). N-methyl-D-aspartate (NMDA, 10–6 and 10–5 M), an activator of neuronal NOS, induced pial dilation that was blocked by 7-NINA (10 ± 1 and 20 ± 2 vs. 1 ± 1 and 2 ± 1%, respectively). However, sodium nitroprusside-induced dilation was unchanged by 7-NINA. These data indicate that neuronal NOS contributes to hypoxic pial artery dilation but not to opioid-induced dilation. Furthermore, these data suggest that neuronally derived NO contributes to hypoxic dilation, at least in part, via formation of cGMP and the subsequent release of opioids in newborn piglets; cerebral circulation.

Previous studies have examined the role of nitric oxide (NO) in cerebrovasodilation elicited by hypoxia. For example, Iwamoto et al. (18) observed that N- nitro-L-arginine (L-NNA) attenuated the increase in cerebral blood flow during hypoxia in the adult sheep. Conversely, Kozniewska et al. (19) reported that systemic administration of the NO synthase inhibitor, N-nitro-l-arginine (L-NMMA) does not alter the increase in cerebral blood flow elicited by hypoxia in the adult rat. Similarly, Pelligrino et al. (26) reported no attenuation of the elevated cerebral blood flow during hypoxia by N-nitro-L-arginine methyl ester (L-NAME). More recently, however, these authors also observed that L-NAME attenuated severe but not moderate hypoxic pial dilation in the adult rat (27). In the newborn pig, NO has been observed to both contribute to hypoxic pial dilation and not contribute to hypoxic hyperemia (16, 17, 33). These data indicate that there is a case, though controversial, for NO involvement. Discrepancies between these studies could be due to differences in timing or dose of antagonist administered, the species or age of animal investigated, or the effect of antagonist on the oxyhemoglobin dissociation curve (27).

Similarly, other studies have also examined the role of opioids in hypoxic cerebrovasodilation. For example, it was observed that hypoxia increases plasma Met-enkephalin in fetal sheep (22) and plasma β-endorphin in human newborns at delivery (35) and in those infants with hypoxic-ischemic encephalopathy with ongoing hypoxia (21). In the piglet, hypoxia increases cortical periarachnoid cerebrospinal fluid (CSF) Met-enkephalin and Leu-enkephalin concentrations, whereas antagonists of these opioids diminish hypoxic pial artery dilation, indicating their involvement in the vascular response to this stimulus (6, 7). In addition to acting separately, NO and opioids also interact in the modulation of hypoxic pial dilation in the piglet. For example, sodium nitroprusside (SNP) increases CSF Met-enkephalin and Leu-enkephalin concentrations via the formation of guanosine 3′,5′-cyclic monophosphate (cGMP) (33). Additionally, hypoxic elevation of the CSF concentration for both opioids was attenuated by L-NAME, suggesting that NO contributes to hypoxic dilation, at least in part, via formation of cGMP and the subsequent release of opioids. In separate studies, Met-enkephalin and Leu-enkephalin were also observed to elicit dilation in an NO-dependent manner (10). Because NO therefore contributes to both the release of these opioids as well as their vascular response, these data create a feed-forward cycle. If the pools of NO used for opioid release and dilation were compartmentalized, however, such a situation may not occur.

In the cerebral circulation, morphological evidence supports the idea that neuronally derived NO participates in the control of cerebral blood flow. For example, the close association of NO synthase-containing neurons with the basilar lamina of intraparenchymal microvessels (15) and the location of NO synthase immunoreactivity in adventitial nerve fibers of cerebral arteries (25) support the possibility that NO mediates neurogenic vasodilation. Recently, 7-nitroindazole has been reported to inhibit brain NO synthase (13) and may therefore be a useful probe for characterizing the contribution of neuronal NO synthase to the control of the cerebral circulation.
The present study was therefore designed to investigate the role of the neuronal isoform of NO synthase in hypoxic pial artery dilation, associated opioid release, and opioid dilation.

METHODS

All experiments have been approved by the Institutional Animal Care and Use Committee. Pigs (1–5 days old) of either sex were used in these experiments. They were first anesthetized with ketamine hydrochloride-deacetrormazine (3.3 and 33 mg/kg im). Anesthesia was maintained with α-chloralose (30–50 mg/kg initially, supplemented with 5 mg/kg iv). A catheter was inserted into the femoral artery to record blood pressure and to sample for blood gases and pH. Another catheter was placed in a femoral vein for injection of drugs. The trachea was cannulated, and the animals were ventilated with room air. The body temperature was maintained at 37–38°C with a heating pad.

For insertion of the cranial window, the scalp was removed, and an opening was made in the skull over the parietal cortex. The dura was cut and retracted over the cut bone edge. The cranial window was placed in the hole and cemented in place with dental acrylic. The space under the window was filled with artificial CSF of the following composition (in mM): 3.0 KCl, 1.5 MgCl₂, 1.5 CaCl₂, 132 NaCl, 6.6 urea, 3.7 dextrose, and 24.6 NaHCO₃ (with pH 7.30–7.36, P CO₂ 42–49 mmHg, and P O₂ 40–50 mmHg).

Pial arteries were observed with a dissecting microscope, a television camera mounted on the microscope, and a video monitor. Vascular diameter was measured with a video microsolar.

Protocol. Pial artery diameter (small artery 120–160 µm and arteriole 50–70 µm) was determined every minute for a 10-min exposure period after injection under the window of artificial CSF containing no drug and CSF containing a drug. Diameters were also measured 10–15 min after the highest arteriole dilation (small artery 50–70 µm) was determined every minute for a 10-min exposure period after injection under the window of artificial CSF containing no drug and CSF containing a drug.

RESULTS

Influence of tetrodotoxin on hypoxia-induced pial artery dilation and opioid release. Two levels of hypoxia, moderate (P O₂ of 35 ± 3 mmHg) and severe (P O₂ of 25 ± 3 mmHg), elicited reproducible pial artery
and arteriole dilation (Table 1). Dilation in response to moderate and severe hypoxia was attenuated by tetrodotoxin (10⁻⁶ M; Fig. 1). In contrast, hypoxia-associated elevation of CSF cGMP was blocked by tetrodotoxin (Fig. 1). Additionally, increases in CSF Met-enkephalin associated with both moderate and severe hypoxia were attenuated by tetrodotoxin (Fig. 2A). For moderate hypoxia, these values on the basis of magnitude were 2.7 ± 0.1 vs. 2.2 ± 0.1, whereas, for severe hypoxia, they were 4.2 ± 0.1 vs. 3.0 ± 0.1 in the absence and presence of tetrodotoxin, respectively. Tetrodotoxin also attenuated both the moderate and severe hypoxic release of Leu-enkephalin (Fig. 2B).

Influence of tetrodotoxin on opioid-induced pial artery dilation. Met-enkephalin or Leu-enkephalin (10⁻¹⁰, 10⁻⁸, and 10⁻⁶ M) elicited reproducible pial small artery (120–160 µm) and arteriole (50–70 µm) vasodilation (Table 2). Dilation produced by these opioids was unchanged by tetrodotoxin (Fig. 3).

Role of neuronal NO synthase in hypoxia-induced pial artery dilation and opioid release. Dilation in response to moderate and severe hypoxia was attenuated by 7-NINA (10⁻⁶ M; Fig. 4). Increases in CSF cGMP associated with hypoxia, however, were blocked by 7-NINA (Fig. 4). The coadministration of 7-NINA with tetrodotoxin, however, did not further decrease hypoxic pial dilation compared with the response seen in the presence of tetrodotoxin alone. In these experiments, the percent dilation for pial small arteries and arterioles during moderate hypoxia was 25 ± 1 and 32 ± 1% vs. 14 ± 1 and 19 ± 1% vs. 15 ± 1 and 19 ± 1% for control conditions, tetrodotoxin, and tetrodotoxin plus 7-NINA, respectively (n = 7). Similarly, values during severe hypoxia for the above respective categories were 34 ± 1 and 44 ± 2% vs. 21 ± 1 and 23 ± 1% vs. 21 ± 2 and 23 ± 2% (n = 7). Increases in CSF Met-enkephalin associated with both moderate and severe hypoxia were attenuated by 7-NINA (Fig. 5A). On the basis of magnitude, these values were 2.9 ± 0.1 vs. 2.1 ± 0.1 and 3.9 ± 0.1 vs. 2.7 ± 0.1 for moderate and severe hypoxia in the absence and presence of 7-NINA, respectively. Hypoxic release of Leu-enkephalin was similarly attenuated by 7-NINA (Fig. 5B).

Table 1. Influence of moderate and severe hypoxia on pial artery diameter

<table>
<thead>
<tr>
<th>Time</th>
<th>Control</th>
<th>Hypoxia</th>
<th>Control</th>
<th>Hypoxia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
<td>First</td>
<td>Second</td>
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<td></td>
<td>Control</td>
<td>Hypoxia</td>
<td>Control</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Artery</td>
<td>Moderate (PO₂ = 35 mmHg)</td>
<td>139 ± 3</td>
<td>174 ± 5*</td>
<td>140 ± 4</td>
</tr>
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<td>Arteriole</td>
<td>67 ± 2</td>
<td>92 ± 3*</td>
<td>63 ± 2</td>
<td>85 ± 3*</td>
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<tr>
<td>Severe (PO₂ = 25 mmHg)</td>
<td>136 ± 3</td>
<td>178 ± 4*</td>
<td>140 ± 5</td>
<td>187 ± 8*</td>
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<tr>
<td>Artery</td>
<td>54 ± 4</td>
<td>89 ± 7*</td>
<td>62 ± 3</td>
<td>94 ± 5*</td>
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</table>

Values are mean diameters ± SE in µm; n = 5. *P < 0.05 compared with corresponding control value.

Influence of 7-NINA on opioid-induced pial artery dilation and increased CSF cGMP concentration. Dilation produced by Met-enkephalin and Leu-enkephalin was unchanged after 7-NINA administration (Fig. 6). Similarly, opioid dilation was associated with the release of cGMP that was also unchanged by 7-NINA (Fig. 6).
concentration. SNP and NMDA (10^{-8} and 10^{-6} M) elicited reproducible pial small artery and arteriole vasodilation (Table 2). SNP-induced pial dilation and associated CSF cGMP release were unchanged by 7-NINA (Fig. 7). In contrast, NMDA pial dilation and associated CSF cGMP release were blocked by 7-NINA (Fig. 7). However, substance P (10^{-8} and 10^{-6} M) pial artery dilation and associated cGMP release were unchanged by 7-NINA. For example, pial small arteries dilated by 10 ± 1 and 19 ± 1% and arterioles by 14 ± 1 and 27 ± 1% for substance P (10^{-6} and 10^{-6} M) before 7-NINA.

In the presence of 7-NINA, the small arteries dilated by 10 ± 1 and 17 ± 1% and the arterioles by 14 ± 1 and 25 ± 2% (n = 8). Similarly, substance P (10^{-6} M) increased CSF cGMP from 444 ± 9 to 750 ± 51 fmol/ml.

### Table 2. Influence of methionine enkephalin, leucine enkephalin, SNP, and NMDA on pial artery diameter

<table>
<thead>
<tr>
<th>Conc, -log M</th>
<th>Time Control</th>
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<th>Arteriole</th>
<th>Small artery</th>
<th>Arteriole</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
<td>First</td>
<td>Second</td>
<td>First</td>
</tr>
<tr>
<td>C</td>
<td>137 ± 3</td>
<td>64 ± 3</td>
<td>141 ± 4</td>
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<tr>
<td>10</td>
<td>145 ± 4*</td>
<td>72 ± 4*</td>
<td>152 ± 4*</td>
<td>78 ± 4*</td>
<td>152 ± 4*</td>
</tr>
<tr>
<td>8</td>
<td>152 ± 4*</td>
<td>76 ± 4*</td>
<td>158 ± 4*</td>
<td>80 ± 4*</td>
<td>158 ± 4*</td>
</tr>
<tr>
<td>6</td>
<td>159 ± 5*</td>
<td>80 ± 4*</td>
<td>167 ± 5*</td>
<td>84 ± 4*</td>
<td>167 ± 5*</td>
</tr>
<tr>
<td>C</td>
<td>148 ± 3</td>
<td>66 ± 3</td>
<td>146 ± 3</td>
<td>68 ± 3</td>
<td>146 ± 3</td>
</tr>
<tr>
<td>10</td>
<td>157 ± 3*</td>
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<tr>
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<td>75 ± 4*</td>
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</tr>
<tr>
<td>C</td>
<td>138 ± 5</td>
<td>64 ± 3</td>
<td>140 ± 4</td>
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<tr>
<td>8</td>
<td>153 ± 6*</td>
<td>72 ± 3*</td>
<td>153 ± 8*</td>
<td>72 ± 4*</td>
<td>153 ± 8*</td>
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<tr>
<td>6</td>
<td>170 ± 7*</td>
<td>66 ± 3*</td>
<td>168 ± 8*</td>
<td>72 ± 5*</td>
<td>168 ± 8*</td>
</tr>
</tbody>
</table>

Values are mean diameters ± SE in µm; n = 5. SNP, sodium nitroprusside; NMDA, N-methyl-D-aspartate. *P < 0.05 compared with corresponding control value (C).

Fig. 2. Influence of moderate hypoxia (P_{O2} = 35 mmHg) and severe hypoxia (P_{O2} = 25 mmHg) on CSF opioid concentration in absence (vehicle) and presence of tetrodotoxin (10^{-6} M). A: Met-enkephalin; B: Leu-enkephalin. Values are means ± SE; n = 6. *P < 0.05 compared with corresponding control value; +P < 0.05 compared with absence of tetrodotoxin.

Fig. 3. Influence of Met-enkephalin and Leu-enkephalin (10^{-10}, 10^{-8}, and 10^{-6} M) on pial small artery and arteriole diameter in absence (vehicle) and presence of tetrodotoxin (10^{-6} M). Values are means ± SE; n = 7.
in the absence of 7-NINA and from 458 ± 7 to 744 ± 24 fmol/ml in the presence of 7-NINA (n = 6).

Influence of 7-NINA on pial artery diameter and CSF cGMP concentration. The administration of 7-NINA had no effect on pial small artery or arteriole diameter (130 ± 5 vs. 130 ± 5 and 54 ± 2 vs. 54 ± 2 µm before and after 7-NINA, respectively, n = 5). Control CSF values for cGMP were also unchanged by 7-NINA (Figs. 4, 6, and 7).

Blood chemistry. Blood chemistry values were obtained at the beginning and end of all normoxia experiments. These values were unchanged at the end compared with the values obtained at the beginning (7.42 ± 0.01 and 32 ± 1, 95 ± 2, and 68 ± 2 mmHg vs. 7.41 ± 0.01 and 33 ± 1, 97 ± 2, and 69 ± 2 mmHg for pH, PCO2, PO2, and mean arterial blood pressure, respectively, n = 38). The blood chemistry was also obtained during normoxia and hypoxia in experiments designed to investigate the cerebrovascular effects of hypoxia. Hypoxia decreased PO2 from 95 ± 2 to 35 ± 3 (n = 45) for moderate hypoxia and from 95 ± 2 to 25 ± 3 mmHg (n = 45) for severe hypoxia, whereas pH, PCO2, and mean arterial blood pressure were unchanged.

**DISCUSSION**

Results of the present study show that neurogenic mechanisms contribute to hypoxic pial artery dilation. First, tetrodotoxin, which selectively blocks Na+ channels and can thereby abolish neurally mediated responses, attenuated hypoxic pial dilation, and blocked the hypoxia-associated rise in cortical periarachnoid CSF cGMP concentration. Second, 7-NINA, the watersoluble Na+ salt of the purported neuronal NO synthase inhibitor 7-nitroindazole (8, 24), similarly attenuated hypoxic pial dilation and blocked the hypoxia-
Additional results of this study show that neurogenic mechanisms contribute to hypoxia-associated elevation in CSF Met-enkephalin and Leu-enkephalin concentrations. Similar to the paradigm above, tetrodotoxin and 7-NINA attenuated hypoxic release of these two opioids. It had previously been observed that SNP, a releaser of NO, increased CSF Met-enkephalin and Leu-enkephalin concentrations and that coadministration associated rise in cortical periarachnoid CSF cGMP concentration. Coadministration of 7-NINA with tetrodotoxin did not further decrease the already attenuated response observed in the presence of tetrodotoxin alone. These data suggest that tetrodotoxin and 7-NINA effects on hypoxic dilation are at the same location. As such, therefore, these results are the first to report that the neuronal isoform of NO synthase is an important contributor for hypoxic pial artery dilation in the newborn, consistent with previous data from the adult rat (27).

![Fig. 6. A: influence of Met-enkephalin and Leu-enkephalin (10^{-10}, 10^{-8}, and 10^{-6} M) on cortical periarachnoid CSF cGMP concentration in absence (vehicle) and presence of 7-NINA (10^{-6} M). Values are means ± SE; n = 7. B: influence of Met-enkephalin and Leu-enkephalin on pial small artery and arteriole diameter in absence (vehicle) and presence of 7-NINA (10^{-6} M). Values are means ± SE; n = 7.

![Fig. 7. A: influence of N-methyl-D-aspartate (NMDA, 10^{-8} and 10^{-6} M) and sodium nitroprusside (SNP, 10^{-8} and 10^{-6} M) on cortical periarachnoid CSF cGMP concentration in absence (vehicle) and presence of 7-NINA (10^{-6} M). Values are means ± SE; n = 7. *P < 0.05 compared with corresponding vehicle value. B: influence of NMDA and SNP on pial small artery and arteriole diameter in absence (vehicle) and presence of 7-NINA (10^{-6} M). Values are means ± SE; n = 7. *P < 0.05 compared with corresponding vehicle value.](http://ajpheart.physiology.org/)
tion of LY-83583 or Rp-8-bromoguanosine 3',5'-cyclic monophosphothioate (Rp-8-bromo-cGMPS), soluble guanylate cyclase and cGMP antagonists, respectively, blocked these increases in CSF opioid concentration (33). These opioids are vasodilators and have themselves been observed to contribute to hypoxic pial dilation (6, 7). Because hypoxic release of these opioids was attenuated by L-NNA, it had been concluded that NO-induced release of cGMP contributed to hypoxic opioid release (33). Data from the present study therefore extend these previous observations and indicate that activation of neuronal NO synthase contributes to hypoxia-associated opioid release and thereby to the resulting pial artery dilation.

In contrast to its role in hypoxic pial dilation and opioid release, results of the present study show that activation of neuronal NO synthase does not contribute to Met-enkephalin and Leu-enkephalin pial artery dilation. Additionally, tetrodotoxin had no effect on opioid-induced dilation. Because L-NNA has previously been observed to blunt dilation and block associated rises in CSF cGMP concentration by these opioids (10), the present data suggest that activation of endothelial NO synthase contributes to these vascular and biochemical events. More importantly, because the pools of NO synthase utilized for opioid release and dilation therefore appear to be different, a feed-forward cycle linking these biochemical and vascular events does not exist, a situation that makes somewhat more physiological sense.

Finally, this study was designed to make certain that the probe used to discern the role of neuronal NO synthase in hypoxic pial dilation, associated opioid release, and opioid dilation was selective in its action. NMDA causes neuronal production and release of NO in brain tissue (13), whereas it produces dilation of cerebral arterioles in vivo that is attenuated by inhibitors of NO synthase (12, 22). More recently, 7-nitroindazole has been shown to inhibit brain NO synthase and cerebral vasodilation in response to NMDA (13). Alternatively, SNP has been well known to elicit dilation via the release of NO and not the activation of NO synthase. Substance P, on the other hand, is thought to elicit dilation via activation of the endothelial isoform of NO synthase. Results of these selectivity experiments in the present study show that pial artery dilation elicited by NMDA was blocked by 7-NINA, whereas responses to SNP and substance P were unchanged, supportive of its action as a neuronal NO synthase inhibitor.

Other studies have also indicated that 7-nitroindazole is a selective inhibitor of neuronal NO synthase. For example, this agent has been reported to inhibit brain NO synthase (13, 23, 24) and has no effect on arterial blood pressure when systemically administered (13, 23), unlike L-NAME and other nonspecific NO synthase inhibitors. Additionally, 7-nitroindazole has no effect on the vascular response to the gold standard endothelium-dependent dilator acetylcholine in isolated rabbit aorta rings (24) or in situ rat pial vessels (13, 31, 34) or on rat cerebellar blood flow (16). Although the above observations are supportive of 7-nitroindazole’s role as a selective neuronal NO synthase inhibitor, other published work opposes that view. For example, it has been noted that 7-nitroindazole attenuated endothelial and neuronal NO synthase activity equally well and also inhibited the cerebral blood flow response to acetylcholine in the adult rat (11). Similarly, this agent was observed to inhibit endothelial NO synthase in homogenates of bovine aortic endothelial cells (8). On the other hand, however, it has also been observed that 7-nitroindazole, when given intraperitoneally, had little effect on mean arterial blood pressure and did not affect NO synthase activity on subsequent ex vivo analysis of aortic endothelial cells (11). Because all the studies to date demonstrating selectivity of 7-nitroindazole for inhibition of neuronal NO synthase in vivo have occurred with systemic administration of this antagonist, it has been suggested that systemic and not local NO synthase activity may be more important for modulation of cerebrovascular reactivity (11). However, data in the present study showing that substance P pial dilation was unchanged while NMDA dilation was blunted by topical 7-NINA suggest that local administration of this compound results in a selective activity profile in the newborn pig. Additionally, the similarity and nonadditive nature of tetrodotoxin and 7-NINA actions were also consistent with the neuronal NO synthase selectivity of this compound. Therefore, the requirement for systemic administration remains uncertain. Of note is the additional observation that such selectivity problems have only been described for 7-nitroindazole and not for 7-NINA.

Previous studies in the newborn pig have attempted to characterize the signal transduction pathways utilized by NO and cGMP in their contributions to hypoxic pial artery dilation. For example, pial dilation by the NO releaser SNP and the cGMP analog, 8-bromocGMP, was attenuated by the ATP-sensitive K+ channel (KATP) antagonist glibenclamide (3), whereas hypoxic pial dilation was similarly attenuated by this antagonist (30). Because it was also observed that LY-83583 and Rp-8-bromo-cGMPS, soluble guanylate cyclase and protein kinase G inhibitors, respectively, blunted SNP-induced pial dilation, it has been suggested that NO primarily elicits its vascular effects via cGMP production in the piglet cerebral circulation (3, 32, 33). In contrast, responses to SNP and 8-bromo-cGMP were unchanged in the presence of the Ca2+-sensitive K+ channel antagonist iberiotoxin (1). Taken together, these data therefore indicate that neuronal NO-induced production of cGMP results in activation of the KATP channel and pial artery dilation during hypoxia. It is presently uncertain why it has also been observed that SNP-induced pial dilation was unchanged by glibenclamide in the piglet (9).

Because 7-NINA attenuated but did not eliminate hypoxic pial artery dilation and associated opioid release, these data suggest that other mechanisms are involved as well. For example, it has recently been observed that adenosine 3',5'-cyclic monophosphate
(cAMP) contributes to both hypoxic pial dilation and associated opioid release in the piglet (32). Similarly, prostaglandins and vasopressin also contribute to hypoxic dilation and associated opioid release via both cGMP- and cAMP-dependent mechanisms (4, 5, 28). In contrast, adenosine contributed to hypoxic pial dilation via NO, cyclic nucleotide, and KATP channel-dependent mechanisms independent of the release of opioids (2). Because in all of the above studies pharmacological probes for each vasoactive system could attenuate but not completely eliminate hypoxic pial dilation, these data taken together indicate that dilation to this stimulus is multifactorial. It is speculated that as one vasoactive system is eliminated, others are upregulated to buffer the removal of the contributing mechanism.

Potential sources of opioids and cyclic nucleotides in cortical periarachnoid CSF are neurons, glia, vascular smooth muscle, and endothelial cells. However, the source of these substances cannot be determined from the present experimental design. Similarly, it is uncertain what stimulates the neuronal NO synthase to release NO, which in turn activates opioid release via cGMP.

In conclusion, results of the present study show that neuronal NO synthase contributes to hypoxic pial artery dilation but not to opioid-induced dilation. Furthermore, these data suggest that neurally derived NO contributes to hypoxic dilation, at least in part, via formation of cGMP and the subsequent release of opioids.

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REFERENCES


