Relationship between Opioids and Prostaglandins in Hypoxia-Induced Vasodilation of Pial Arteries in the Newborn Pig (44000)

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Abstract. **Previously, it has been observed that methionine enkephalin and leucine enkephalin contribute to hypoxia-induced pial artery dilation in the newborn pig. It has also been observed that the cyclooxygenase inhibitor indomethacin attenuates hypoxic hyperemia in piglets. The present study was designed to determine the relationship between opioids and prostaglandins in hypoxia-induced pial artery dilation. Newborn pigs equipped with closed cranial windows were used to measure pial artery diameter and collect cortical periarachnoid cerebrospinal fluid (CSF) for assay of opioids and prostaglandins. Hypoxia-induced artery vasodilation was mildly attenuated during moderate hypoxia ($Pa_{O_2} \approx 35$ mm Hg), while this response was blunted during severe hypoxia ($Pa_{O_2} \approx 25$ mm Hg) by indomethacin, 5 mg/kg iv (23% ± 1% vs 18% ± 1% and 33% ± 2% vs 21% ± 2% for moderate and severe hypoxia in the absence and presence of indomethacin, respectively). Hypoxic dilation was accompanied by increased CSF prostaglandin E_2 (PGE_2) concentration (1260 ± 37 vs 1734 ± 67 and 1256 ± 33 vs 2859 ± 189 pg/ml for moderate and severe hypoxia, respectively). Similar changes in CSF 6 keto $PGF_{1\alpha}$ concentration during hypoxia were also observed. Topical PGE_2 (10,100 ng/ml) increased CSF methionine enkephalin (874 ± 35, 1290 ± 44, and 1791 ± 143 pg/ml for control, 10 and 100 ng/ml PGE_2, respectively). Similar increases in CSF methionine enkephalin concentration were observed for topical PGI_2. Additionally, these two prostaglandins also increased CSF leucine enkephalin concentration. Furthermore, while indomethacin had no effect on the release of CSF methionine enkephalin during moderate hypoxia, it attenuated the release of this opioid during severe hypoxia (786 ± 27 and 2633 ± 74 vs 781 ± 51 and 2467 ± 52; 926 ± 15 and 3489 ± 156 vs 898 ± 11 and 2314 ± 124 pg/ml for control and moderate/severe hypoxia before and after indomethacin, respectively). Similar effects of indomethacin on hypoxic release of leucine enkephalin were also observed. These data indicate that prostaglandins contribute to hypoxic pial dilation. Additionally, these data show that prostaglandins release the opioids methionine enkephalin and leucine enkephalin. Finally, these data suggest that elevated prostaglandin concentrations during severe hypoxia release opioids which in turn contribute to hypoxic pial dilation.** [P.S.E.B.M. 1996, Vol 212]

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Although their role in the control of the adult cerebral circulation is somewhat controversial, prostaglandins are prominent in the cerebrovascular physiology of the neonate and contribute to the maintenance of resting cerebral blood flow and cerebrovascular dilation in response to prostaglandin-associated stimuli such as hypercapnia and hemorrhagic hypotension (1, 2). The most abundant prostaglandin present in the piglet cortical periarachnoid ce-

rebrospinal fluid (CSF) is PGE_2, and its concentration is in the vasoactive range (3). In addition to contributing to the vascular effects of physiologic stimuli, prostaglandins may also cause the release of other vasoactive substances (4). These released substances may in turn contribute indirectly to vascular changes produced by prostaglandin-associated stimuli.

Opioids also contribute to the regulation of cerebral hemodynamics. Opioid receptor binding has been demonstrated on cerebral microvessels (5). Enkephalin and dynorphin immunoreactivity, indicative of innervation, has been shown in large cerebral arteries of the pig (6). Moreover, CSF opioid concentrations are in the vasoactive range in the newborn pig during resting conditions (7). For example, opioids such as methionine enkephalin and leucine enkephalin have been observed to produce cerebral vasodilation (7). However, nothing is known about the ability of prostaglandins to influence CSF opioid concentration.

Several mechanisms have been proposed to account for hypoxia-induced cerebral vasodilation. These possibilities include adenosine, nitric oxide, and vasopressin (8–12). Most studies, however, have investigated mechanisms involved in hypoxic cerebrovasodilation in the adult. Mechanisms involved in hypoxic cerebrovasodilation in the newborn/infant period have received little attention. Recently, it has been observed that the endogenous opioids methionine enkephalin and leucine enkephalin contribute to hypoxic pial vasodilation in the newborn pig (8, 13). Since the cyclooxygenase inhibitor, indomethacin, attenuates hypoxic hyperemia in the piglet (14), prostaglandins may also contribute to hypoxic cerebrovasodilation.

The present study was therefore designed to determine the relationship between opioids and prostaglandins in hypoxia-induced pial artery dilation.

Materials and Methods

Newborn pigs (1–5 days old) of either sex were used in these experiments. They were anesthetized with ketamine hydrochloride (33 mg/kg im) and acepromazine (3.3 mg/kg im). Anesthesia was maintained with α-choloralose (30–50 mg/kg initially, supplemented with 5 mg/kg/hr iv). A catheter was inserted into a 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. 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 consisted of a stainless steel ring containing a glass coverslip to which was attached three metal ports for infusion and sampling of CSF. 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 (37°C) of the following composition (in mg): 220 KCl, 132 $MgCl_2$, 221 $CaCl_2$, 7710 NaCl, 402 urea, 665 dextrose, and 2066 $NaHCO_3$/l, pH 7.33, P_{CO_2} 46 mm Hg, and P_{O_2} 43 mm Hg.

Pial arterioles were observed with a dissecting microscope, a television camera mounted on the microscope, and a video monitor. Vascular diameter was measured with a video microscaler (Model IV550, For-A-Corp, Los Angeles, CA).

Protocol. Two sizes of pial vessels were investigated: small arteries (120–160 μm) and arterioles (50–70 μm). Pial artery diameter was determined every minute for a 10-min exposure period after injection under the window of artificial CSF containing no drug or that containing a drug. Diameters were also measured 10–15 min after the highest concentration of a drug was flushed off the cerebral cortical surface with CSF containing no drug. Typically, 1–2 ml of CSF were flushed through the window over 30 sec. Needles incorporated into the side of the window allowed infusion of CSF under the window and run off of excess CSF. We measured the peak response, and a CSF sample for opioid or prostaglandin analysis was collected at the end of the 10-min exposure period. Cerebral cortical periarachnoid CSF (300 μl) was collected by placing a syringe on an injection port of the cranial window. CSF was collected by slowly infusing artificial CSF into one side of the window and allowing the CSF under the window to drip freely into a collection tube on the opposite side. Hypoxia was produced by decreasing inspired O_2 sufficiently to reduce and maintain Pa_{O_2} at either 35 ± 1 or 25 ± 1 mm Hg while maintaining constant Pa_{CO_2}. Changes in pial artery diameter were noted every minute during a 10 min hypoxic exposure period. A blood sample confirming the hypoxic challenge was taken 3 min after the exposure period was begun. Once blood chemistry data confirmed that the desired level of hypoxia had been achieved, peak dilator responses were recorded. Responses to hypoxia were obtained before and 30 min after indomethacin (5 mg/kg iv; Merck Sharp & Dohme, West Point, PA). Previously, this dose of indomethacin has been shown to reduce cortical periarachnoid CSF prostaglandin concentrations to nondetectable levels and inhibit the conversion of exogenous arachidonic acid to prostaglandins on the cerebral surface by >90% (15). Untreated time control experiments were designed such that responses to hypoxia were obtained initially and again 30 min later.

To investigate the ability of prostaglandins to re-

lease opioids, responses to topical PGI_2, and PGE_2 (1, 10, and 100 ng/ml; Upjohn, Kalamazoo, MI) were obtained in the presence of indomethacin to eliminate endogenous prostaglandins.

Appropriate aliquots of the vehicle for PGE_2 (ethanol) were added to CSF infused under the window. This CSF vehicle had no effect on arteriolar diameter. The vehicle for PGI_2 was a tris (hydroxymethyl) aminomethane (Tris) buffer, pH 8, and addition of this buffer directly under the window had no effect on arteriolar diameter as reported previously (16). In the present study, the effect of Tris on CSF opioid concentration was also studied. All drug solutions were made fresh on the day of use.

Opioid Analysis. CSF samples collected were immediately acidified with 1 *N* acetic acid to prevent peptide degradation, rapidly frozen, and stored at −20°C. RIA kits for methionine enkephalin and leucine enkephalin are commercially available (Inc Star, Stillwater, MN; Peninsula Lab, Belmont, CA). The RIA uses simultaneous addition of sample, rabbit antiopioid antibody, and the I^{125} derivative of the opioid. After an overnight incubation at 4°C, free opioid was separated from opioid bound to antibody by the addition of saturated ammonium sulfate in the presence of rabbit carrier gamma globulin. Following centrifugation at 760*g* for 10 min, the supernatant was decanted and the pellet counted using a gamma scintillation counter. All samples and standards were assayed in duplicate. Data are calculated as %B/T versus concentration, where:

$$\%B/T = \frac{\text{CPM of standard or unknown tube}}{\text{CPM of total count tube}}$$

We have used these methods previously to quantify opioid concentration in periarachnoid cortical CSF (7).

Prostaglandin Analysis. CSF samples collected were analyzed for PGE_2 and 6 keto $PGF_{1\alpha}$ using scintillation proximity assay methods. Commercially available kits for PGE_2 and 6 keto $PGF_{1\alpha}$ (Amersham, Arlington Heights, IL) were used. Briefly, this assay determines prostaglandin concentration for binding to an antiserum, which has a high specificity for either PGE_2 or 6 keto $PGF_{1\alpha}$. The antibody-bound prostaglandin is then reacted with an anti-rabbit second antibody bound to fluoromicrospheres. Labeled prostaglandin bound to the primary rabbit antibody can then be measured by determining the amount of light emitted by the fluoromicrospheres using a beta scintillation counter. All unknowns were assayed at two dilations. The concentration of unlabeled prostaglandin is calculated from the standard curve *via* linear regression analysis. The intraassay variability for the opioid and prostaglandin assays was less than 5% and the interassay variability less than 8%.

Statistical Analysis. Pial arteriolar diameter, systemic arterial pressure, prostaglandin, and opioid levels were analyzed using analysis of variance for repeated measures. If the value was significant, the Fisher test was performed. An a level of $P < 0.05$ was considered significant in all statistical tests. Values are represented as means ± standard error of absolute values or as fold change from control values.

Results

Influence of Hypoxia on Cortical Periarachnoid CSF Prostaglandin Concentration. Hypoxia increased CSF PGE_2 and 6 keto $PGF_{1\alpha}$ by 1.4 ± 0.1– and 1.8 ± 0.1–fold during moderate hypoxia ($Pa_{O_2} \approx 35$ mm Hg) (Fig. 1, A and B). During severe hypoxia ($Pa_{O_2} \approx 25$ mm Hg), these same prostaglandins were increased by 2.3 ± 0.2– and 3.0 ± 0.1–fold, respectively (Fig. 1, A and B).

Influence of Indomethacin on Pial Artery Diameter and CSF Prostaglandin Concentration during

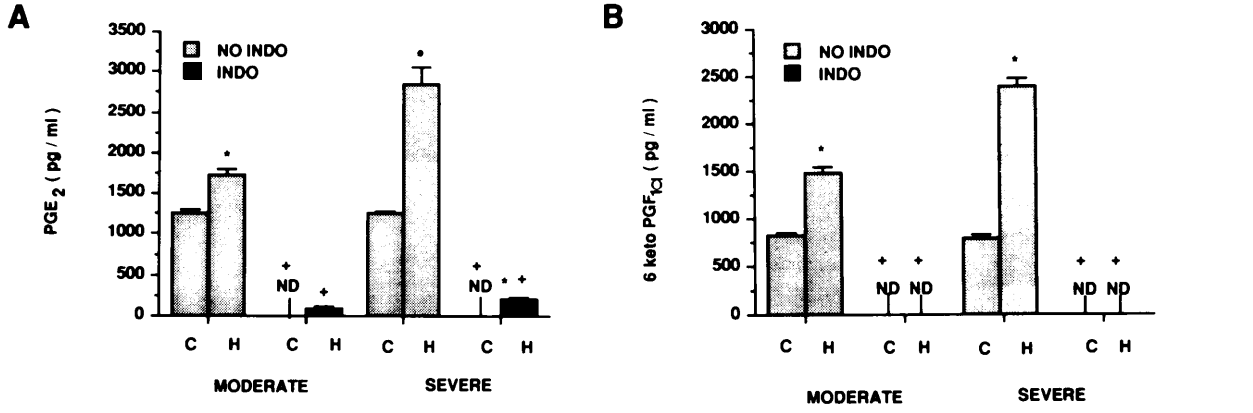

Figure 1. (A) Influence of moderate hypoxia ($Pa_{O_2} \approx 35$ mm Hg) and severe hypoxia ($Pa_{O_2} \approx 25$ mm Hg) on cortical periarachnoid CSF PGE_2 (pg/ml) in the absence and presence of indomethacin (5 mg/kg iv). (B) Influence of moderate and severe hypoxia on CSF 6 keto $PGF_{1\alpha}$ (pg/ml) in the absence and presence of indomethacin. Values are mean ± SEM; $n = 6$. ND, less than 100 pg/ml. *$P < 0.05$ compared with corresponding control value (C); †$P < 0.05$ compared with absence of indo (NO INDO).

Control and Hypoxic Conditions. Indomethacin decreased pial artery diameter from 152 ± 6 to 134 ± 9 μm. Also indomethacin decreased control cortical periarachnoid CSF prostaglandin concentration to nondetectable levels (Fig. 1, A and B) as previously observed (3, 15). Furthermore, elevated CSF PGE_2 and 6 keto $PGE_{1\alpha}$ levels observed during hypoxia were reduced by indomethacin administered prior to the hypoxic stimulus (Fig. 1, A and B).

Influence of Cyclooxygenase Inhibition on Hypoxia-Induced Pial Artery Vasodilation. Moderate and severe levels of hypoxia elicited reproducible pial small artery (120–160 μm) and arteriole (50–70 μm) vasodilation (Fig. 2A).

Hypoxia-induced dilation of small pial arteries and arterioles was modestly attenuated by indomethacin (5 mg/kg iv) during moderate hypoxia and blunted by indomethacin during severe hypoxia (Fig. 2B). On a percentage basis, indomethacin attenuated moderate hypoxia-induced small pial artery and arteriole dilation by 22% ± 4% and 23% ± 3%, whereas severe hypoxia-induced dilation was decreased by 36% ± 6% and 39% ± 4% for small arteries and arterioles, respectively.

Influence of Prostaglandins on Pial Artery Diameter and CSF Opioid Concentration. Topical PGE_2 and PGI_2 (1–100 ng/ml) elicited pial artery dilation (Table I). PGE_2 and PGI_2 also increased CSF methionine enkephalin and leucine enkephalin concentration (Fig. 3, A and B). In each case, only two of the three prostaglandin concentrations (10 and 100 ng/ml) had a significant influence on CSF opioid concentration, the 1 ng/ml concentration having no effect. PGE_2 (10 and 100 ng/ml) increased methionine enkephalin by 1.6 ± 0.1– and 2.1 ± 0.1–fold while increasing leucine enkephalin by 1.5 ± 0.1– and 1.9 ± 0.1–fold. Similarly, PGI_2 (10 and 100 ng/ml) increased methionine enkephalin by 1.7 ± 0.1– and 2.2 ± 0.1–fold while increasing leucine enkephalin by 1.4 ± 0.1– and 1.7 ± 0.1–fold.

Influence of Indomethacin on Hypoxia-Induced Release of CSF Opioids. Indomethacin had no effect on the release of CSF methionine enkephalin or leucine enkephalin during moderate hypoxia ($Pa_{O_2} \approx 35$ mm Hg) (Fig. 4, A and B). However, indomethacin attenuated the release of these opioids during severe hypoxia ($Pa_{O_2} \approx 25$ mm Hg) (Fig. 4, A and B). On a fold basis, these increases in CSF methionine enkephalin reflect 3.4 ± 0.1– and 3.2 ± 0.2– vs 3.8 ± 0.2– and 2.6 ± 0.1–fold increases for moderate and severe hypoxia before and after indomethacin, respectively. Similarly, leucine enkephalin was increased by 2.9 ± 0.2– and 2.7 ± 0.1–fold during moderate hypoxia and by 3.6 ± 0.2– and 2.5 ± 0.2–fold during severe hypoxia before and after indomethacin, respectively. The basal CSF concentrations of methionine enkephalin and leucine enkephalin were unchanged by indomethacin (Fig. 4, A and B).

Influence of Indomethacin on Prostaglandin-Induced Vasodilation. Responses to topical PGI_2 were unchanged in the presence of indomethacin (5 mg/kg iv) (139 ± 6 and 162 ± 7 vs 127 ± 6 and 152 ± 7 μm, for the response to PGI_2 [10 ng/ml] in the absence and presence of indomethacin, respectively; $n = 6$). Similarly, responses to PGE_2 were unchanged by indomethacin (133 ± 6 and 164 ± 4 vs 122 ± 4 and 150 ± 3 μm for PGE_2 [10 ng/ml] in the absence and presence of indomethacin, respectively; $n = 6$).

Influence of the PGI_2 Vehicle on Pial Artery Diameter and CSF Opioid Concentration. The Tris-CSF vehicle for PGI_2 had no effect on pial artery diameter (141 ± 6 vs 141 ± 4 μm) or CSF opioid concentration (917 ± 37 vs 886 ± 25 pg/ml and 33 ± 2 vs 32 ± 1 pg/ml for control and vehicle concentrations of methionine enkephalin and leucine enkephalin, respectively; $n = 5$).

Blood Gases. Blood gas values were obtained at the beginning and at the end of all normoxia experi-

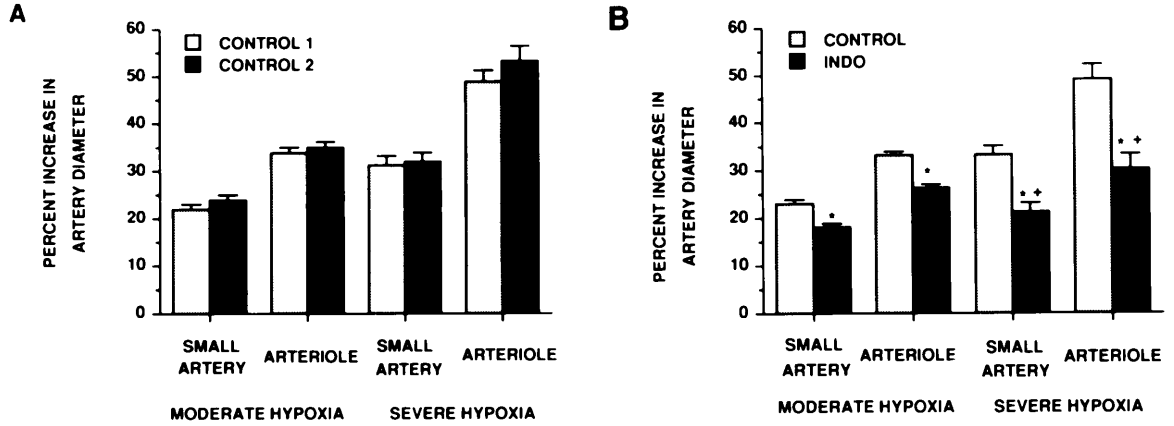

Figure 2. (A) Influence of moderate hypoxia ($Pa_{O_2} \approx 35$ mm Hg) and severe hypoxia ($Pa_{O_2} \approx 25$ mm Hg) on small pial arteries and arterioles. (B) Influence of moderate and severe hypoxia on small pial arteries and arterioles in the absence (CONTROL) and presence of indomethacin (5 mg/kg iv). Values are mean ± SEM, $n = 6$. *$P < 0.05$ compared with corresponding control; †$P < 0.05$ compared with the difference between corresponding control during moderate hypoxia.

Table I. Influence of PGE_2 and PGI_2 on Pial Artery Diameter

	PGE_2 (ng/ml)				PGI_2 (ng/ml)			
	Control	1	10	100	Control	1	10	100
Small artery	135 ± 2	144 ± 3[a]	152 ± 4[a,b]	167 ± 2[a,b]	151 ± 4	166 ± 6[a]	76 ± 3[a,b]	187 ± 3[a,b]
Arterioles	62 ± 2	72 ± 4[a]	77 ± 4[a,b]	86 ± 4[a,b]	66 ± 2	76 ± 2[a]	82 ± 3[a,b]	94 ± 4[a,b]

Note. Values are expressed as μm ± SEM; $n = 6$.
[a] $P < 0.05$ compared with corresponding control value.
[b] $P < 0.05$ compared with value for next lowest concentration.

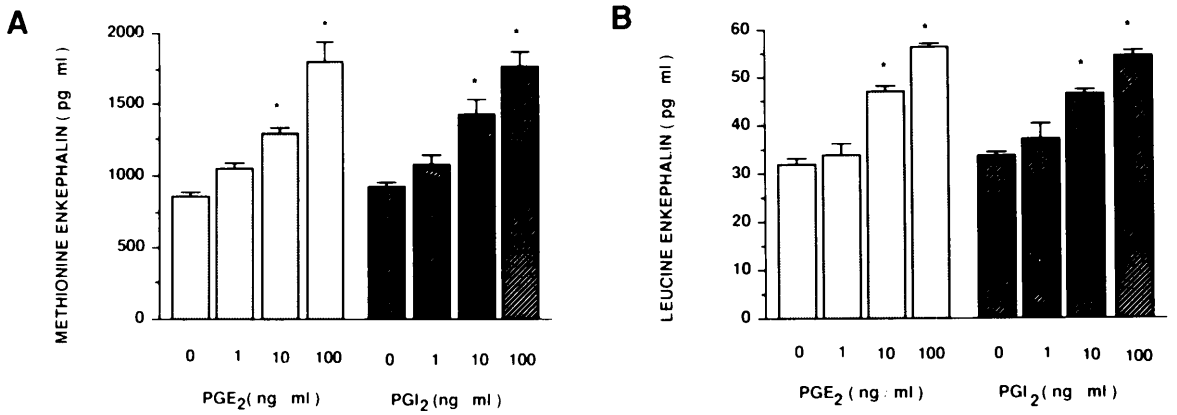

Figure 3. (A) Influence of PGE_2 and PGI_2 (1–100 ng/ml) on periarachnoid cortical CSF methionine enkephalin concentration (pg/ml). (B) Influence of PGE_2 and PGI_2 (1–100 ng/ml) on periarachnoid cortical CSF leucine enkephalin concentration (pg/ml). Values are mean ± SEM; $n = 6$. *$P < 0.05$ compared with corresponding control (0).

ments as well as during normoxia and hypoxia in experiments designed to investigate the cerebrovascular effects of hypoxia (Table II). Hypoxia decreased P_{O_2}, while P_{CO_2}, pH, and mean arterial blood pressure were unchanged.

Discussion

Results of the present study show that the prostaglandins PGE_2 and PGI_2 contribute to hypoxia-induced pial artery dilation. On the basis of Poiseuille's law, pial dilation of the magnitude induced by hypoxia would have substantial effects on cerebrovascular resistance. Hypoxic pial artery dilation was mildly attenuated during moderate hypoxia, while this response was blunted during severe hypoxia by the cyclooxygenase inhibitor, indomethacin. These pharmacologic data support and corroborate the biochemical data showing that hypoxic pial dilation was associated with increased cortical periarachnoid CSF PGE_2 and 6 keto $PGF_{1\alpha}$ concentration. The present data suggest that prostaglandins contribute to pial vasodilation during severe hypoxia, but are less important during moderate hypoxia. Because hypoxic pial dilation was not completely blocked by indomethacin, even during severe hypoxia, these data further suggest that other mechanisms (e.g., adenosine, nitric oxide, vasopressin) (8–12) also contribute to that dilation.

Since it has been observed previously that methionine enkephalin and leucine enkephalin contribute to hypoxia-induced pial artery dilation (8, 13), the relationship between opioids and prostaglandins in that dilation was also investigated. Results of this study show that topical PGE_2 and PGI_2 increased the CSF concentrations of methionine enkephalin and leucine enkephalin. While indomethacin had no effect on the release of CSF methionine enkephalin and leucine enkephalin during moderate hypoxia, it attenuated the release of these opioids during severe hypoxia. However, basal CSF concentrations of opioids were unchanged by indomethacin and the lowest concentration of the prostaglandins topically applied to the cerebral cortical surface (1 ng/ml) did not significantly alter CSF opioid concentration. The concentration of prostaglandins used in this study were chosen on the basis that these concentrations produced comparable changes in pial artery diameter and encompass concentrations for resting, physiologic, and pharmacologic stimulation conditions. Although it is uncertain what concentration of active prostaglandin exists at the receptor level, PGE_2 and the inactive metabolite for PGI_2, 6 keto $PGF_{1\alpha}$, have been observed in cortical periarachnoid CSF at a concentration of approximately 1 ng/ml under resting conditions and at approximately 10 ng/ml in response to a variety of strong physiological stimulatory treatments (1, 2, 15). Since results of the present study show a somewhat greater

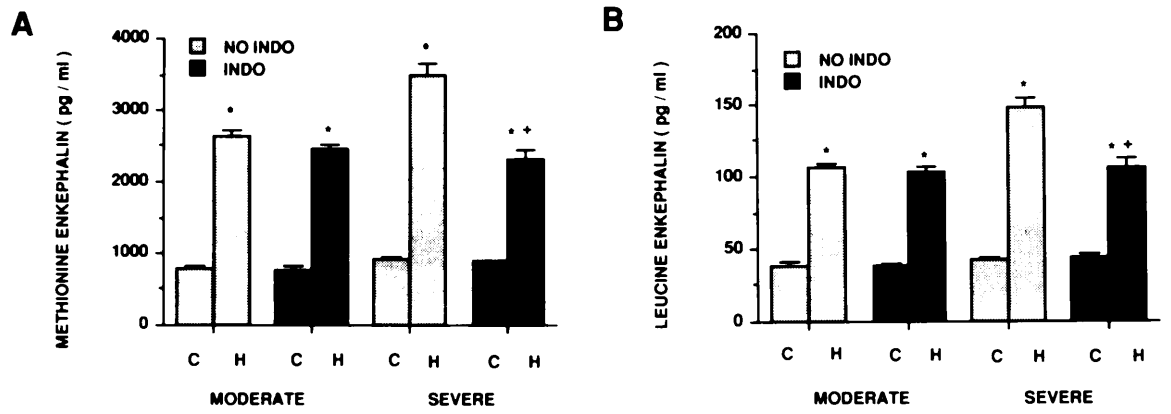

Figure 4. (A) Influence of moderate hypoxia ($Pa_{O_2} \approx 35$ mm Hg) and severe hypoxia ($Pa_{O_2} \approx 25$ mm Hg) on cortical periarachnoid CSF methionine enkephalin (pg/ml) in absence (NO INDO) and presence of indomethacin (5 mg/kg iv). (B) Influence of moderate hypoxia and severe hypoxia on cortical periarachnoid CSF leucine enkephalin (pg/ml) in absence (NO INDO) and presence of indomethacin (5 mg/kg iv). Values are mean ± SEM; $n = 6$. $^*P < 0.05$ compared with corresponding control; $\dagger P < 0.05$ compared with absence of indo (NO INDO).

effect of indomethacin on hypoxia-induced release of CSF opioids (Fig. 4, A and B) than what would have been anticipated by consideration of data concerning the effects of prostaglandins on opioid release (Fig. 3, A and B), it may not be possible to correlate absolute prostaglandin levels in CSF with the vascular/biochemical response. Assuming that PGE_2 and PGI_2 act in a paracrine manner, during active synthesis the local concentration of these prostaglandins would be much higher and could account for the above underestimation. Therefore, the direction of the change may be more important in suggesting whether local concentrations are increasing or decreasing. Results of this study suggest that PGE_2 and PGI_2 release CSF opioids during strong stimulatory treatments, but probably do not do so during resting conditions. With respect to hypoxia, these data suggest that elevated prostaglandin concentrations during severe, but not moderate hypoxia, release opioids which in turn contribute to hypoxic pial dilation. Therefore, opioids and prostaglandins contribute to hypoxic pial dilation by themselves, as well as by interacting with one another. Because indomethacin did not block hypoxia-induced release of opioids, these data finally suggest that other, as yet unknown, vasoactive systems may also serve to cause the release of opioids during hypoxia.

Several different mechanisms could account for the release of opioids by prostaglandins. Recently, it was observed that PGE_2 and PGI_2 increase CSF cGMP concentration and that LNA, a nitric oxide synthase inhibitor, attenuated pial dilation in response to topical application of these two prostaglandins, suggesting a role for nitric oxide in that dilation (17). Additionally, it has also been observed that the cGMP analog, 8 Bromo-cGMP, increases CSF methionine enkephalin and leucine enkephalin concentration, indicating that this cyclic nucleotide releases opioids into CSF (18). These data suggest that prostaglandin-associated increases in CSF cGMP could mediate the release of opioids observed in the present study. Alternatively, previous *in vitro* studies have shown that isoproterenol or 8 Bromo-cAMP causes the release of opioids from glial cells, adrenal chromaffin cells, and ventricular cardiac muscle cells, suggesting the involvement of cAMP in such release (19–21). Since PGE_2 and PGI_2 activate adenylate cyclase and increase CSF cAMP (17), the results of the present study also suggest the involvement of cAMP in prostaglandin-induced CSF opioid release *in vivo*. Possible sources of these opioids include cortical vessels, nerves associated with these vessels, neurons, or glia. However, the origin of the opioids cannot be determined from the present experiments.

Table II. Blood Gases and Mean Arterial Blood Pressure

	Normoxia 1	Normoxia 2
pH	7.44 ± 0.03	7.43 ± 0.3
P_{CO_2} (mm Hg)	31 ± 1	32 ± 1
P_{O_2} (mm Hg)	96 ± 3	96 ± 3
MABP (mm Hg)	68 ± 2	63 ± 3
$n = 12$		
	Normoxia	**Moderate hypoxia**
pH	7.42 ± 0.02	7.41 ± 0.02
P_{CO_2} (mm Hg)	33 ± 1	34 ± 1
P_{O_2} (mm Hg)	95 ± 3	35 ± 1[a]
MABP (mm Hg)	64 ± 2	61 ± 2
$n = 23$		
	Normoxia	**Severe hypoxia**
pH	7.43 ± 0.02	7.44 ± 0.02
P_{CO_2} (mm Hg)	32 ± 1	32 ± 1
P_{O_2} (mm Hg)	88 ± 3	25 ± 1[a]
MABP (mm Hg)	68 ± 2	62 ± 4
$n = 23$		

Note. Values are expressed as mean ± SEM;
[a] $P < 0.05$ compared with corresponding normoxia value.

The role of prostaglandins in hypoxic cerebrovasodilation has been investigated previously. For example, Wei *et al.* (22) showed that indomethacin did not alter the hypoxic vasodilation of pial arteries in the adult cat, and Pearce *et al.* (23) showed that vasodilation was cycloxygenase independent in isolated carotid vessels of the adult rabbit. Moreover, indomethacin also did not alter hypoxic vasodilation in the adult rat (24). However, indomethacin has also been observed to attenuate hypoxic hyperemia in the newborn pig (14). The results of the present study confirm and extend observations in the latter study and indicate that prostaglandins themselves directly contribute to hypoxic pial dilation as well as indirectly *via* the release of opioids.

Opioids contribute to the regulation of cerebral hemodynamics (1, 7). Previous studies have shown that methionine enkephalin and leucine enkephalin produce vasodilation (1). More recently, these two dilator opioids have been observed to contribute to hypoxic pial artery dilation via activation of ATP dependent K^+ channels in the newborn pig (25). Methionine enkephalin– and leucine enkephalin–induced dilation has also been observed to be associated with increased CSF prostaglandin concentration and indomethacin blunted that dilation (26). Taken together, these studies finally suggest that PGE_2, PGI_2, and opioids could interact in a positive feedback mechanism whereby prostaglandins released during hypoxia cause the release of opioids which in turn cause the further release of prostaglandins.

In conclusion, the results of the present study indicate that prostaglandins contribute to hypoxic pial artery dilation. These data also show that prostaglandins release CSF methionine enkephalin and leucine enkephalin. Finally, these data suggest that elevated CSF prostaglandin concentrations during severe hypoxia release opioids which in turn contribute to hypoxic pial dilation.

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