Mechanism of CO\textsubscript{2} Response in Cerebral Arteries of the Newborn Pig: Role of Phospholipase, Cyclooxygenase, and Lipoxygenase Pathways

L. Craig Wagerle and Om P. Mishra

The role of phospholipase, lipoxygenase, and cyclooxygenase pathways in the mechanism of the cerebrovascular response to CO\textsubscript{2} and H\textsuperscript{+} was investigated in newborn piglets. Responsiveness of pial arterioles, 48–206 \textmu m diameter, to inhalation of 6\% CO\textsubscript{2} and to suffusion of acidic cerebrospinal fluid (CSF, pH = 6.84), adenosine (10\textsuperscript{-4} M), or theophylline (10\textsuperscript{-2} M) was studied using a closed cranial window. Pial arteriolar diameter was measured using intravital microscopy. Phospholipase inhibitors quinacrine hydrochloride (10\textsuperscript{-4} M in CSF) and p-bromophenacyl bromide (10\textsuperscript{-4} M in CSF) abolished the CO\textsubscript{2} vasodilation from \textDelta diameter = 27 \pm 5\% and 28 \pm 3\% during baseline to 0 \pm 4\% and −1 \pm 1\% following the respective inhibitors. Following administration of the cyclooxygenase inhibitor indomethacin (5 mg/kg i.v.), the CO\textsubscript{2} response was converted from vasodilation, 31 \pm 6\%, to constriction, −4 \pm 1\% (p<0.001), while the lipoxygenase inhibitor nordihydroguaiaretic acid (2 mg/kg i.v. or 10\textsuperscript{-4} M in CSF) augmented the pial arteriolar response to CO\textsubscript{2} from 21 \pm 4\% to 34 \pm 7\% (p<0.005). Topical application of superoxide dismutase (40 units/ml CSF) plus catalase (40 units/ml CSF) also appeared to augment the CO\textsubscript{2} response. Suffusion of the cortical surface with acidic CSF at constant P\textsubscript{co2} increased pial arteriolar diameter by 11 \pm 2\% that was also abolished by indomethacin. Vasodilatory responses to topical adenosine and theophylline were not affected by indomethacin, suggesting specificity for H\textsuperscript{+} ion-related vasodilation. The data support a role for arachidonic acid metabolism and production of vasoactive prostaglandins mediating the cerebrovascular response to hypercapnia in the newborn piglet. A mechanism by which extracellular H\textsuperscript{+} may influence cell membrane function and activate phospholipase to release arachidonic acid is suggested.

(Circulation Research 1988; 62:1019–1026)
expired CO₂ was maintained at 30–35 mm Hg and sufficient O₂ was given to maintain PaO₂ between 80–100 mm Hg. The animal was wrapped in a warming blanket, and core body temperature was continuously monitored and maintained at 38° C. Arterial blood pressure was measured with a Statham P23Db pressure transducer (Gould, Cleveland, Ohio) connected to the femoral arterial catheter. Arterial blood samples were collected and analyzed for pH, PCO₂, and PO₂ at appropriate times during the experimental protocols. Blood gas tensions and pH were measured with conventional electrodes (Radiometer America, Westlake, Ohio). Hemoglobin concentration and saturation were measured on a co-oximeter (Instrumentation Laboratories, Lexington, Massachusetts). Hematocrit was measured by micromethod.

The piglets were equipped with a closed cranial window placed over the left parietal lobe as previously described for newborn piglets. The space under the window was filled with artificial CSF of the following composition (mM): KC1 2.9, MgCl₂ 1.4, CaCl₂ 1.2, NaCl 132, NaHCO₃ 24.6, urea 6.7, and glucose 3.7. The CSF was warmed to 39° C and blood samples were collected and analyzed for pH, PCO₂, and PO₂ at appropriate times during the experimental protocols. Blood gas tensions and pH were measured with conventional electrodes (Radiometer America, Westlake, Ohio). Hemoglobin concentration and saturation were measured on a co-oximeter (Instrumentation Laboratories, Lexington, Massachusetts). Hematocrit was measured by micromethod.

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Pial arterioles ranging in diameter from 48 to 206 μm were visualized with a trinocular stereomicroscope (model MTS, Wild, Heerbrugg, Switzerland). One to three arterioles were studied in each animal. Vessel width was measured using a Panasonic television camera (model WV3030, Yokohama, Japan) mounted on the microscope, NEC video monitor (model CT1901A, Tokyo, Japan), and video microscaler (model VPA-1000, For-A-Corp, Los Angeles, California). The video images were recorded on a Sony video cassette recorder (model DL-HF900, Tokyo, Japan).

Quinacrine hydrochloride, nortidroguaiartic acid (NDGA), p-bromophenacyl bromide (BPB), adenosine, theophylline, superoxide dismutase (SOD) from bovine blood (3,200 units/mg protein), and catalase (CAT) from bovine liver (34,000 units/mg protein) were obtained from Sigma Chemical, St. Louis, Missouri. Sodium indomethacin trihydrate was a gift from Mr. William Henckler, Merck Sharp & Dohme Research Laboratories, Rahway, New Jersey.

Experimental Protocols

Effect of indomethacin, NDGA, and superoxide dismutase plus catalase on response to CO₂. The effect of indomethacin, NDGA, and SOD + CAT on the pial arteriolar response to CO₂ was examined in 16 piglets. Following baseline measurements, 6% CO₂ was added to the inspiratory gas mixture for 10 minutes. For comparison to baseline, measurements of pial arteriolar diameter were averaged over the final 4 minutes of CO₂ inhalation where a steady-state change in arteriolar diameter had been achieved. Arterial blood was also sampled during this time. CO₂ was then withdrawn from the inspiratory gas and all parameters were allowed to return to baseline levels. Five of the piglets were given 5 mg/kg i.v. indomethacin and repeated measurements of the response to 6% CO₂ inhalation were made. This dose of indomethacin has been shown to cross the blood–brain barrier and decrease to one tenth the synthesis of cortical 6-keto-prostaglandin F₁₆, prostaglandin E₂, and thromboxane B₂ measured in the subarachnoid CSF of newborn piglets. To assess the effect of lipoxygenase activity on the CO₂ response in four piglets, the CO₂ response was repeated after treatment with NDGA, a lipoxygenase inhibitor, 2 mg/kg i.v. dissolved in 50% ethyl alcohol. When it became apparent that NDGA did not inhibit the CO₂ response, in two of the animals the response to CO₂ was determined a third time after NDGA, 10⁻⁴ M in CSF, was placed directly onto the pial surface in an attempt to eliminate the possibility that NDGA administered intravenously had not crossed the blood–brain barrier.

Seven piglets received equal volume of saline and served as time controls. To determine the possible influence of superoxide radicals released into the extracellular space, three of these piglets were pretreated with SOD + CAT to scavenge oxygen free radicals. We chose to administer SOD + CAT prior to the first CO₂ treatment rather than afterward because it was possible that tissue damage caused by oxygen free radicals released during CO₂ inhalation would influence vessel responsiveness in a nonreversible manner. In these animals, SOD (40 units/ml) + CAT (40 units/ml) in CSF was placed over the cortical surface under the window for 30 minutes prior to and during inhalation of CO₂.

Effect of quinacrine and BPB on the response to CO₂. Quinacrine at concentrations of 500 μM severely inhibits bee venom and porcine pancreatic phospholipases. The mechanism, however, appears to be via some indirect effect on the cell membrane. BPB seems to act specifically on the active site of phospholipase by irreversible alkylation of histidine. To assess the role of phospholipase activity on the pial arteriolar response to CO₂, nine piglets were divided into two groups. Following initial measurements of the pial vascular response to inhalation of 6% CO₂, as described above, quinacrine HCl, 10⁻⁴ M in CSF, was flushed onto the cortical surface in four animals for 30 minutes. The quinacrine was then washed out of the window with normal CSF, and the response to CO₂ was determined again. The other five piglets were treated similarly with 10⁻⁴ M BPB in CSF. BPB was dissolved in ethyl alcohol and diluted to the final concentration in CSF. The final concentration of ethyl alcohol in CSF was 0.5%. This concentration of ethyl alcohol had no effect on pial arteriolar diameter.

Effect of indomethacin on response to H⁺. In a second series of four piglets, we examined whether the cyclooxygenase inhibitor, indomethacin, altered the pial arteriolar response to decreased pH of the extra-
cellular fluid at constant CO\textsubscript{2} tension. In addition to the normal artificial CSF, acidic CSF was prepared in which NaHCO\textsubscript{3} concentration was reduced from 24.6 mM to 5 mM. To maintain osmolarity, NaCl concentration was increased from 132 mM to 152 mM. When equilibrated at 6.5% CO\textsubscript{2}, the pH calculated for the baseline and acidic solution was approximately 7.36 and 6.67, respectively. In these experiments, the space under the window was perfused at a constant rate of 0.64 ml/min. At this rate, the volume of CSF under the window (0.3 ml) was turned over approximately two times per minute. Perfusion with the normal CSF was continued for 10 minutes and pial arteriolar diameter recorded. This was followed by 10 minutes of perfusion with acidic CSF. Pial arteriolar responses to the acidic CSF perfusion achieved a steady state by 10 minutes. Then normal CSF was perfused and pial arterioles allowed to return to baseline diameter. Indomethacin (5 mg/kg i.v.) was administered, and after 20 minutes, the procedure was repeated. We chose to set up a continuous infusion system because preliminary experiments where acidic CSF was placed statically under the window resulted in vasodilation that was transient, and its magnitude was inconsistent. The response to continuous infusion of acidic CSF was found to be more stable and reproducible. To assess the acid-base status of the CSF that had suffused the cortical surface during CSF infusion, we collected 1 ml of CSF effusate anaerobically via the outflow port under the window over the final 2 minutes of perfusion. The pH and Pco\textsubscript{2} achieved in the perivascular space during perfusion were more accurately reflect the pH and Pco\textsubscript{2} achieved in the perivascular space during perfusion.

Effect of indomethacin on response to adenosine and theophylline. In seven animals, the response to topical adenosine and theophylline in CSF was examined. The space under the window was flushed with normal CSF and pial arteriolar diameter recorded for 4 minutes to provide baseline measurements. Flushing the space with normal CSF had no significant effect on pial arterioles. This was followed by CSF containing 10\textsuperscript{-4} M adenosine for an additional 4 minutes. The pial vascular response to adenosine typically was maximal by 2–3 minutes. Adenosine was then washed off the cortical surface by two to three successive flushes with normal CSF and pial arteriolar diameter allowed to return to baseline levels. Indomethacin (5 mg/kg i.v.) was then administered, and after 20 minutes, the response to adenosine was determined again. In two additional animals, the response to 10\textsuperscript{-2} M theophylline was examined using a protocol identical to that for adenosine.

Statistical Analysis

All values are presented as mean ± SEM. Comparisons among treatments were made with two-way analysis of variance with repeated measures followed by pairwise test with Bonferroni correction when appropriate. Analysis of pial diameter was made following square root transformation and percent change was analyzed following arcsine transformation. Differences were considered significant when p<0.05.

Results

Effect of Vehicle and Superoxide Radical Scavengers on CO\textsubscript{2} Response

The effect of inhalation of 6% CO\textsubscript{2} on pial arteriolar diameter, mean arterial blood pressure (MABP), and arterial blood gases (Paco\textsubscript{2} and Paco\textsubscript{2}) before and after vehicle (saline) treatment are presented in Table 1. Inhalation of 6% CO\textsubscript{2} increased Paco\textsubscript{2} from 34 ± 3 to 58 ± 2 mm Hg during control and from 36 ± 3 to 60 ± 4 mm Hg following vehicle treatment. There was a concomitant increase in pial arteriolar diameter from 96 ± 13 to 120 ± 7 μm (25 ± 7%) during control and from 97 ± 15 to 130 ± 21 μm (33 ± 4%) following vehicle treatment. There was no statistical difference in pial arteriolar responses between the first and second CO\textsubscript{2} challenge, demonstrating the reproducibility of the CO\textsubscript{2} response.

The effect of CO\textsubscript{2} administration in the presence of free radical scavenging system, SOD + CAT, is shown at the bottom of Table 1. SOD + CAT (40 units/ml CSF of each) was flushed onto the cortical surface 30 minutes prior to the first CO\textsubscript{2} response determination. SOD + CAT alone during normocapnia had no effect

| TABLE 1. Effect of Repeated Hypercapnia on Pial Arteriolar Diameter: Time Controls and Effect of Superoxide Dismutase + Catalase |
|--------------------------------------------------|----------------|----------------|----------------|----------------|----------------|----------------|
| Arteriolar diameter (μm)  | MABP (mm Hg) | Paco\textsubscript{2} (mm Hg) | Paco\textsubscript{2} (mm Hg) | PACO\textsubscript{2} (mm Hg) |
| Control  | Vehicle | Control  | Vehicle | Control  | Vehicle | Control  | Vehicle | Control  | Vehicle |
| Baseline | 96 ± 13 | 97 ± 15 | 81 ± 4 | 78 ± 7 | 34 ± 3 | 36 ± 3 | 93 ± 12 | 76 ± 5 |
| 6% CO\textsubscript{2} | 120 ± 17* | 130 ± 21* | 84 ± 3 | 77 ± 8 | 58 ± 2* | 60 ± 4* | 95 ± 9 | 82 ± 8 |

Effect of pretreatment with SOD + CAT

| Baseline | 88 ± 12 | 87 ± 10 | 81 ± 1 | 78 ± 4 | 35 ± 2 | 37 ± 2 | 91 ± 4 | 97 ± 13 |
| 6% CO\textsubscript{2} | 132 ± 17* | 138 ± 16* | 90 ± 5 | 85 ± 3 | 59 ± 1* | 59 ± 1* | 101 ± 12 | 86 ± 7 |

Data are mean ± SEM. Time control group: 8 arterioles, 4 animals; SOD + CAT group: 6 arterioles, 3 animals. MABP, mean arterial blood pressure; SOD + CAT, superoxide dismutase + catalase 40 μg/ml in cerebrospinal fluid placed on cortical surface. Vehicle for indomethacin (saline), 1 ml/kg i.v.

*Significantly different from baseline, p<0.05.
on arteriolar diameter (data not shown). However, SOD + CAT augmented the response to CO₂ (compare top of Table 1 control with bottom control) where pial arteriolar diameter increased from 88 ± 12 to 132 ± 17 μm; this 53% increase in diameter was significantly greater than the 26–33% increase seen without SOD + CAT. Following vehicle administration, the second CO₂ response was not different from the first (59% increase in diameter).

**Effect of Indomethacin and NDGA on CO₂ Response**

The changes in Paco₂, MABP, and pial arteriolar diameter during inhalation of 6% CO₂ during control and again following administration of either cyclooxygenase inhibitor, indomethacin, or lipoxygenase inhibitor, NDGA, are shown in Table 2 and Figure 1. CO₂ inhalation resulted in elevated Paco₂, however, this effect of indomethacin became statistically significant only if data from all 18 animals that received indomethacin were combined from all of the experimental protocols. Although changes in Paco₂ were comparable, indomethacin totally abolished the vasoconstriction where pial arteriolar diameter decreased significantly by 4 ± 1% from 94 ± 13 to 91 ± 13 μm.

Nordihydroguaiaretic acid had no effect on pial diameter during normocapnia but caused a statistically significant augmentation in the percent increase in diameter during CO₂ inhalation from 21 ± 4% to 34 ± 7% (Table 2, Figure 1). In two animals, further topical application of 10⁻⁴ M NDGA to the cortical surface for 30 minutes also had no effect on pial arteriolar diameter and failed to inhibit the CO₂ vasodilation.

**Effects of Quinacrine and BPB on CO₂ Response**

The effects of the phospholipase A₂ inhibitors quinacrine and BPB on the pial arteriolar response to CO₂ inhalation are presented in Table 3. Topical application of 10⁻⁴ M quinacrine onto the cortical surface caused a 28 ± 14% increase in arterial diameter approximately 15–20 minutes after application. When quinacrine was washed off the cortical surface, pial arterioles returned toward baseline levels but remained elevated (129 ± 14 μm) compared with control (116 ± 14 μm). The response to 6% CO₂ inhalation prior to quinacrine was a 27 ± 5% increase in pial arteriolar diameter that was abolished (0 ± 4%) by topical quinacrine administration (Figure 1).

Topical application of site-specific phospholipase inhibitor BPB also caused a substantial increase in diameter from 103 ± 18 to 133 ± 17 μm that was sustained when BPB was flushed from the cortical surface. The 28 ± 3% vasodilatory response to 6% CO₂, however, was lost following BPB administration (Table 3). Since BPB alone resulted in significant dilation, a test to compare the response to CO₂ at equivalent vascular dimensions was performed.

In two animals, control measurements were made in the presence of 5 x 10⁻⁴ M adenosine, which initially dilated the vessels by 27 ± 7%. Inhalation of 6% CO₂ caused further pial arteriolar dilation by 18 ± 3% from
The effect of indomethacin on the pial arteriolar response to topical infusion of acidic CSF is shown in Table 4 and Figure 2. CSF pH and Pco2 of the effusate sampled during the final 2 minutes of each infusion period are also presented.

During infusion of acidic CSF, pH of the effusate CSF decreased from 7.39 to 6.84; effusate CSF Pco2 was unchanged. There was an 11 ± 2% increase in pial arteriolar diameter from 117 ± 8 to 130 ± 10 µm during infusion of acidic CSF. Following indomethacin, pial arterioles did not respond to infusion of acidic CSF.

Topical application of 10⁻⁴ M adenosine increased pial arteriolar diameter by 17 ± 3% from 109 ± 10 to 127 ± 12 µm (Table 5, Figure 2). Following indomethacin, adenosine induced a 25 ± 5% increase in diameter. Similarly, the 37 ± 5% increase in diameter following topical theophylline (10⁻² M) appeared to be augmented to 55 ± 4% following administration of indomethacin but was not statistically significant.

Discussion

The present study has shown that in the newborn piglet, 1) indomethacin administration abolished the pial arteriolar response to hypercapnia and to superfusion of acidic CSF at normal Pco2; 2) phospholipase A₂ inhibitors quinacrine and BPB also abolished the CO₂ induced vasodilation while lipoxygenase inhibitor, NDGA, augmented the response; 3) pretreatment of the cortical surface with free radical scavengers SOD + CAT augmented the response to CO₂ and 4) indomethacin, BPB, and quinacrine had no effect on the vasodilatory response to topical adenosine or theophylline. The data support a role for a prostaglandin product of the cyclooxygenase pathway in the cerebrovascular response to CO₂ in the newborn piglet.

In adult animals, some studies have shown that indomethacin reduces CBF and impairs the CBF response to an increase in arterial CO₂ in baboons, cats, rats, and gerbils. 14-19 Other laboratories, however, have found that indomethacin, in doses that inhibit the cerebrovascular response to exogenous arachidonic acid, did not alter the CBF or pial arteriolar response to CO₂ in adult cats, rabbits, or dogs. 20-23 In addition, other cyclooxygenase inhibitors, such as meclofenamate and AHR-5850, appear to have little effect on CBF hyperemia to CO₂. 24 These data, coupled with the fact that cortical levels of prostaglandin E₂, prostaglandin F₂α, and 6-keto-prostaglandin F₁α did not increase during CO₂ inhalation in adult cats, have led some investigators to speculate that indomethacin may act via some process other than inhibition of prostaglandin production. 24-25 To evaluate this possibility in

137 ± 24 to 161 ± 24 µm. Normal CO₂ was then restored, and the adenosine was washed off the cortical surface and replaced with BPB for 30 minutes. Pial diameter following BPB returned to near the "control + adenosine" value, 144 ± 23 µm, and subsequent CO₂ administration had no further effect (147 ± 25 µm). The effect of 10⁻⁴ M adenosine was determined on pial arteries that were unresponsive to CO₂ following indomethacin but was not statistically significant.

### Table 3. Effect of Quinacrine and p-Bromophenacyl Bromide on the Pial Arteriolar Response to Hypercapnia

<table>
<thead>
<tr>
<th>Articulardiameter (µm)</th>
<th>Baseline</th>
<th>Quin</th>
<th>Effect of BPB</th>
<th>Baseline</th>
<th>Quin</th>
<th>6% CO₂</th>
<th>Baseline</th>
<th>Quin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>130 ± 12</td>
<td>130 ± 12</td>
<td>130 ± 18</td>
<td>130 ± 18</td>
<td>130 ± 22*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quin</td>
<td>108 ± 10</td>
<td>108 ± 10</td>
<td>108 ± 10</td>
<td>108 ± 10</td>
<td>108 ± 10</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BPB</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indo</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td>103 ± 16</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

Data are mean ± SEM. Quinacrine group: 8 arterioles, 4 animals; BPB group: 9 arterioles, 5 animals. MABP, mean arterial blood pressure; Indo, sodium indomethacin trihydrate, 5 mg/kg i.v. Measurements were taken after 10 minutes of constant infusion of cerebrospinal fluid (CSF) equilibrated with 6.6% CO₂ and 6.0% O₂ and containing either 24.6 mM HCO₃⁻ (baseline CSF) or 5 mM HCO₃⁻ (acidic CSF). Values reported CSF Pco₂ and pH are of CSF collected from the outflow port after having perfused the subarachnoid space.

*Significantly different from baseline, p<0.05.

**Significantly different from control, p<0.05.

### Table 4. Effect of Indomethacin on Pial Arteriolar Response to Topical Infusion of Acidic Cerebrospinal Fluid

<table>
<thead>
<tr>
<th>Articulardiameter (µm)</th>
<th>Baseline</th>
<th>Indo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>117 ± 8</td>
<td>107 ± 6</td>
</tr>
<tr>
<td>Acicid</td>
<td>130 ± 10*</td>
<td>105 ± 5</td>
</tr>
</tbody>
</table>

Data are means ± SEM of 8 arterioles in 4 animals. MABP, mean arterial blood pressure; Indo, sodium indomethacin trihydrate, 5 mg/kg i.v. Measurements were taken after 10 minutes of constant infusion of cerebrospinal fluid (CSF) equilibrated with 6.6% CO₂ and 6.0% O₂ and containing either 24.6 mM HCO₃⁻ (baseline CSF) or 5 mM HCO₃⁻ (acidic CSF). Values reported CSF Pco₂ and pH are of CSF collected from the outflow port after having perfused the subarachnoid space.

*Significantly different from baseline, p<0.05.
TABLE 5. Effect of Indomethacin on the Pial Arteriolar Response to Topical Application of Adenosine and Theophylline

<table>
<thead>
<tr>
<th>Arteriolar diameter (μm)</th>
<th>MABP (mm Hg)</th>
<th>PaCO₂ (mm Hg)</th>
<th>PaO₂ (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Indo</td>
<td>Control</td>
<td>Indo</td>
</tr>
<tr>
<td>Baseline</td>
<td>Baseline</td>
<td>Baseline</td>
<td>Baseline</td>
</tr>
<tr>
<td>109 ± 10</td>
<td>95 ± 9†</td>
<td>74 ± 6</td>
<td>76 ± 5</td>
</tr>
<tr>
<td>Adenosine</td>
<td>Adenosine</td>
<td>Adenosine</td>
<td>Adenosine</td>
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<tr>
<td>127 ± 12*</td>
<td>118 ± 12*</td>
<td>72 ± 6</td>
<td>76 ± 5</td>
</tr>
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</table>

Effect of indomethacin on response to adenosine (10⁻⁴ M)

Baseline 109 ± 10  Adenosine 127 ± 12*

Effect of indomethacin on response to theophylline (10⁻² M)

Baseline 124 ± 24  Theophylline 168 ± 28*

Data are mean ± SEM. Adenosine group: 15 arterioles, 7 animals; theophylline group: 4 arterioles, 2 animals. MABP, mean arterial blood pressure; Indo, sodium indomethacin trihydrate, 5 mg/kg i.v.

⁎Significantly different from baseline, p<0.05.

†Significantly different from control, p<0.05.

Indomethacin has been shown to inhibit cyclic AMP–dependent protein kinase in intestinal mucosa. The apoenzyme myosin light chain kinase is phosphorylated by cyclic AMP–dependent protein kinase and this phosphorylation may interfere with activation of myosin resulting in relaxation. Thus, indomethacin may facilitate vasoconstrictor stimuli via this enzymatic system. Adenosine, via activation of adenylyl cyclase, and theophylline, via inhibition of phosphodiesterase, invoke relaxation of vascular smooth muscle by increasing cyclic AMP levels. The present study has shown that indomethacin did not impair pial arteriolar responsiveness to these compounds, providing evidence against this nonspecific action of indomethacin in the newborn piglet cerebral circulation.

Indomethacin may interfere with the lipoxygenase pathway and therefore inhibit production of metabolites of arachidonate metabolism other than prostanoids. Recent studies, however, have shown that superoxide anion generation by cyclooxygenase, but not lipoxygenase, was inhibited by indomethacin at concentrations similar to that used in the present study. In the present study, the lipoxygenase inhibitor, NDGA, did not decrease the vasodilatory response to CO₂, but rather enhanced it from 21 ± 4% to 34 ± 7%. Since products of the lipoxygenase pathway, leukotrienes C₄, D₄, and E₄, cause vasoconstriction in the piglet pial circulation, these findings suggest that lipoxygenase products do not mediate the vasodilation to CO₂.

It would appear, therefore, that in the cerebral circulation of the newborn pig, the effect of indomethacin is due to inhibition of cyclooxygenase and reduced release of vasoactive prostanoids. The following findings lend further support to this conclusion. First, Leffler and Busija have demonstrated a threefold to fivefold increase in prostaglandin E₂ and 6-keto-prostaglandin F₁α and a smaller increase in thromboxane B₂ in the cortical CSF during combined hypoxia and hypercapnia, suggesting that in this species respiratory acidosis is associated with in-
creased metabolism of arachidonic acid. While separate effects of hypoxia and hypercapnia were not selectively examined in those studies, the degree of hypoxia was mild (Pao₂ = 40 mm Hg); therefore the predominant stimulus for prostanooid release was likely due to the large increase in Paco₂ (Paco₂ = 93 mm Hg). Also in that study, indomethacin in the dose used in the present study greatly decreased prostanooid levels in the cortical CSF. Second, the fact that lipoxygenase inhibition augmented the CO₂ vasodilation is consistent with the concept of increased substrate availability for the cyclooxygenase pathway. Such an explanation can also be applied to the observed constriction to CO₂ following indomethacin, where increased production of vasoconstrictor leukotrienes may have occurred. No effect of CO₂ was noted in the presence of the phospholipase inhibitors BPP or quinacrine. Third is the observation that animals pretreated with the free radical scavenging system, SOD + CAT, showed increased dilation to CO₂ (53% compared with 31% in untreated animals). The increase in CO₂ response with SOD + CAT might be due to one of several factors that remain unresolved. One possible explanation resides with the fact that oxygen free radicals released during the enzymatic conversion of prostaglandin G₂ to prostaglandin H₂ were shown to be destructive toward prostaglandin cyclooxygenase, and free radical scavengers prevent this destruction. Thus, SOD + CAT may have enhanced production of vasodilator prostaglandins through this mechanism. While alternate explanations for these effects can be made, taken in combination, all of the above experimental observations are consistent with prostaglandin mediation of the vasodilatory response to CO₂.

Studies from adult animals show that, in the cerebral circulation, CO₂-induced vasodilation is mediated via a direct effect of H⁺ on vascular smooth muscle. In adult cats, the vasodilatory response was observed whether pH was altered by changing CO₂ at constant HCO₃⁻ concentration or by changing HCO₃⁻ concentration at constant Pco₂. No effect was noted when Pco₂ or HCO₃⁻ concentration was varied and pH was not changed. The dependence of the response on pH of the perivascular space suggests that effects on the cell membrane are important. The findings of the present study, that indomethacin blocks the response to increased Paco₂ or to decreased extracellular pH at constant Pco₂, lends further support for H⁺ as mediating the vasodilation to the two experimental conditions. In addition, it would appear that one effect of H⁺ on cell membrane is activation of membrane phospholipase A₂ and release of vasodilator prostaglandins.

The mechanism by which H⁺ influences phospholipase activity is not known; nor is it clear from these studies whether release of prostanooids occurs in the arterioles or from neuronal or glial cells. A direct effect of pH on the enzyme seems unlikely, however, since optimal pH for membrane phospholipase A₂ activity in smooth muscle was over a wide pH range from 6 to 7.5. Phospholipase A₂ activity is influenced by Ca⁺², and membrane fluidity changes or membrane perturbation can affect phospholipase A₂ activity by altering the accessibility of the substrate to the active site or by altering the enzyme conformation. Thus, H⁺ may exert its effect indirectly, increasing phospholipase activity by one of various mechanisms, including alteration in availability of Ca⁺², changes in the ionic environment, or changes in membrane fluidity or structure. Paradoxically, in adult cats, increased perivascular Ca⁺² concentration inhibits pial arteriolar dilation caused by acidosis. The interaction between Ca⁺² and H⁺ induced vasodilation has yet to be determined in the newborn animal.

Acknowledgments

The authors wish to express their gratitude to Russell Roth, Christopher Cameron, and Paula Mokay for their fine technical assistance, and to Dorothy Zurlo for typing the manuscript.

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Key Words: cerebrovascular • newborn • prostanoids • phospholipase • lipoxygenase • cyclooxygenase • CO2 • hydrogen ion • adenosine
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Circ Res. 1988;62:1019-1026
doi: 10.1161/01.RES.62.5.1019

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