Endogenous Platelet Activating Factor Does Not Modulate Blood Flow and Metabolism in Normal Rat Brain

Patrick M. Kochanek, MD, John A. Melick, BS, Rebecca J. Schoettle, MD, Mary Jo Magargee, BS, Rhobert W. Evans, PhD, and Edwin M. Nemoto, PhD

Both platelet activating factor and eicosanoids participate in the cerebrovascular response to ischemia. Eicosanoids also modulate cerebrovascular tone under normal physiologic circumstances, but a similar role for platelet activating factor has not been investigated. Therefore, using 16 rats, we studied the effects of the platelet activating factor receptor blockers BN 52021 (10 mg/kg, n=4 or 30 mg/kg, n=2) and WEB 2086 (5 mg/kg, n=6) on global cerebral blood flow and the cerebral metabolic rate for oxygen and compared them with the effect of indomethacin (10 mg/kg, n=4). Neither antagonist altered cerebral blood flow (112±16 and 107±14 ml/100 g/min at baseline versus 108±16 and 105±18 ml/100 g/min after BN 52021 and WEB 2086, respectively). In contrast, indomethacin significantly (p<0.05) decreased cerebral blood flow from 106±8 to 69±4 ml/100 g/min. No treatment altered the cerebral metabolic rate for oxygen compared with baseline. These data suggest that in normal rat brain, concentrations of platelet activating factor, unlike those of eicosanoids, are subthreshold and do not modulate cerebral blood flow or the cerebral metabolic rate for oxygen. (Stroke 1990;21:459-462)

Substantial evidence supports the notion that platelet activating factor (PAF) is involved in the cerebrovascular response to ischemia. PAF (1-\(\text{O-alkyl-2-acetyl-sn-glyceryl-3-phosphorylcholine}\)) is an endogenous lipid that potently constricts blood vessels, increases microvascular permeability, activates granulocytes, and stimulates arachidonate-independent platelet aggregation. Intracarotid infusion of PAF in rats produces cerebral hypoperfusion and hypermetabolism, a pattern similar to that observed during reperfusion following cerebral ischemia. PAF receptor blockade attenuates the development of hypoperfusion after cerebral air embolism in rats and dogs and after carotid occlusion in gerbils. Thus, it is clear that PAF plays a role in the pathophysiologic response of the cerebrovascular system.
abolish rate for oxygen (CMRO₂) in normal rat brain. PAF and the eicosanoids can be derived from a common precursor phospholipid 1-O-alkyl-2-arachidonoylglycerol-phosphorylcholine via phospholipase A₂. Since endogenous eicosanoids modulate both normal and postischemic CBF, our second aim was to compare the effects of PAF receptor blockade on CBF and CMRO₂ with the effect obtained with cyclooxygenase inhibition by indomethacin.

Materials and Methods
The experimental protocol was approved by the Animal Care and Use Committee of the University of Pittsburgh. Anesthesia was induced in 16 male Wistar rats weighing 300-450 g with 5% halothane in O₂. Each rat was intubated and mechanically ventilated with 1% halothane/66% N₂O/33% O₂ and immobilized with 0.1 mg/kg/hr pancuronium. Femoral arterial and venous catheters were surgically inserted. The dorsal calvaria was exposed by a midline incision, and the rat's head was fixed in a stereotactic device (David Kopf Instruments, Tujunga, California). Rectal temperature was maintained at 38±0.2°C with a heated water blanket. Femoral arterial blood was collected into a cooximeter (Instrumentation Laboratories, Inc., Lexington, Massachusetts). Rectal temperature was maintained at 38±0.2°C with a heated water blanket. An arterial blood sample was obtained to verify that blood gases, pH, and hemoglobin concentration (Hb) were within normal limits. All blood samples were replaced with an equal volume of heparinized whole blood from donor Wistar rats. Under ×20 magnification with an operating microscope and using an air dental drill, a burr hole was made over the superior sagittal sinus and a platinum microelectrode (50 µm tip diameter) was advanced into the sagittal sinus. After saturation with 5-6% H₂ in the inspired gas, H₂ washout was effected and CBF measured as the difference (arterial minus cerebral venous) in O₂. Mean arterial blood pressure (MABP) was monitored on a Honeywell recorder (Denver, Colorado). An arterial blood sample was obtained to verify that blood gases, pH, and hemoglobin concentration (Hb) were within normal limits. All blood samples were replaced with an equal volume of heparinized whole blood from donor Wistar rats. Under ×20 magnification with an operating microscope and using an air dental drill, a burr hole was made over the superior sagittal sinus and a platinum microelectrode (50 µm tip diameter) was advanced into the sagittal sinus. After saturation with 5-6% H₂ in the inspired gas, H₂ washout was effected and CBF measured as the difference (arterial minus cerebral venous) in O₂.

BN 52021 (a 2 mg/ml solution of the lyophilized form in the accompanying aqueous diluent; IHB Laboratories, Le Plessis-Robinson, France) was administered intravenously at two doses. WEB 2086 (Boehringer Ingelheim, Ingelheim am Rhein, FRG) was administered as a 5 mg/ml solution in 0.1N HC1 and 0.9% saline (1:20 by volume). Indomethacin (Sigma Chemical Co., St. Louis, Missouri) was prepared as a 4 mg/ml solution in distilled water with 2.5 mg NaHCO₃/ml. In pilot studies, neither the WEB 2086 vehicle containing HC1 nor the indomethacin vehicle containing NaHCO₃ significantly affected CBF or CMRO₂.

After surgical preparation, each rat was allowed to stabilize for 60 minutes while being ventilated with 0.4% halothane in N₂O/O₂ (2:1 by volume). Baseline measurements of MABP, CBF, and CMRO₂ were obtained. The physiologic variables blood pH, Pao₂, Paco₂, and HBO₂ were maintained at 7.40±0.05, 125±25 torr, 35±5 torr, 0.0±5.0 mmol/l, and 13±1.5%, respectively. After baseline measurements were obtained, BN 52021 (10 mg/kg, n=4; 30 mg/kg, n=2), WEB 2086 (5 mg/kg, n=6), or indomethacin (10 mg/kg, n=4) was administered intravenously over 1 minute. MABP, CBF, and CMRO₂ were again measured in each rat 15 and 60 minutes after drug administration. Subsequently, the rats were killed with an intravenous injection of saturated KCl.

Results from the two doses of BN 52021 were combined into a single group since both doses produced a similar lack of response. All data are given as mean±SEM. Comparisons within groups were made using one-way repeated-measures analysis of variance followed by the Student-Newman-Keuls test. Comparisons among groups were made using one-way analysis of variance followed by the Student-Newman-Keuls test. The level of significance was taken as p<0.05.

Discussion
Our finding that PAF receptor blockade with BN 52021 or WEB 2086 had no effect on CBF in normal Wistar rats is consistent with the observation of Armstead et al. who found that resting pial artery diameter in piglets was unaffected by PAF receptor blockade with U66985. The threshold concentration at which PAF causes vasoconstriction in the pial artery is approximately 20 nmol/l. The data suggest that PAF levels in normal rat brain are less than this value, more consistent with the levels of 0.25 nmol/l reported by Kumar et al. that with that of approximately 50 nmol/l reported by Tokumura et al. We confirmed the report by Dahlgren et al. that the cyclooxygenase inhibitor indomethacin decreases CBF in normal rat brain without affecting CMRO₂. This suggests that in normal brain, PAF levels, unlike those of the eicosanoids, are less than threshold and exert no influence on global CBF.
Neither PAF receptor antagonist significantly altered normal CMRO₂ in this model; however, an increase in CMRO₂ 1 hour after WEB 2086 administration cannot be completely ruled out because of the large variability in CMRO₂ and thus the inadequate power (0.5) of the statistical analysis for WEB 2086 at this time. Previous studies in this model have demonstrated an increase in CMRO₂ during infusion of 67 pmol/min PAF. No effect of endogenous PAF on CMRO₂, however, has been described in normal or pathologic states.

The doses of BN 52021 and WEB 2086 used in this study inhibit PAF-related effects in rats, including hypotension and increased vascular permeability. Consistent with complete inhibition of any PAF effects, unpublished results from our laboratory using Bligh-Dyer extraction and vapor phase chromatography indicate that a 10 mg/kg dose of WEB 2086 in rats results in a plasma concentration of 42 ±17 μmol/l after 15 minutes. This is well above the IC₅₀ value of 0.17 μmol/l reported for WEB 2086 inhibition of platelet aggregation induced by 50 nmol/l PAF. WEB 2086 is structurally similar to the benzodiazepines, but it has no anticonvulsant or sedative action.

We conclude that PAF receptor antagonism failed to alter normal global CBF and CMRO₂ in rats, suggesting that in normal brain PAF levels are subthreshold and modulate neither CBF nor CMRO₂.

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**Table 1.** MABP, Controlled Physiologic Variables, and CMRO₂ in Rats During Intravenous Infusion of PAF Receptor Blocker or Cyclooxygenase Inhibitor

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Variable</th>
<th>Baseline</th>
<th>15</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PAF receptor blocker</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BN 52021 (n=6)</td>
<td>MABP (torr)</td>
<td>123±5</td>
<td>124±5</td>
<td>119±5</td>
</tr>
<tr>
<td></td>
<td>PacO₂ (torr)</td>
<td>34±1</td>
<td>37±1</td>
<td>38±2</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>7.37±0.01</td>
<td>7.36±0.01</td>
<td>7.36±0.01</td>
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<tr>
<td></td>
<td>PacO₂ (torr)</td>
<td>140±4</td>
<td>147±3</td>
<td>141±4</td>
</tr>
<tr>
<td></td>
<td>Temp (° C)</td>
<td>38.0±0.2</td>
<td>38.1±0.3</td>
<td>37.9±0.2</td>
</tr>
<tr>
<td></td>
<td>Hb (%)</td>
<td>14.3±0.4</td>
<td>14.2±0.6</td>
<td>13.5±0.5</td>
</tr>
<tr>
<td></td>
<td>CMRO₂ (ml/100 g/min)</td>
<td>6.22±1.19</td>
<td>6.77±0.89</td>
<td>6.59±0.70</td>
</tr>
<tr>
<td>WEB 2086 (n=6)</td>
<td>MABP (torr)</td>
<td>133±6</td>
<td>129±8</td>
<td>125±8</td>
</tr>
<tr>
<td></td>
<td>PacO₂ (torr)</td>
<td>36±2</td>
<td>38±2</td>
<td>33±2</td>
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<tr>
<td></td>
<td>pH</td>
<td>7.38±0.03</td>
<td>7.37±0.03</td>
<td>7.40±0.03</td>
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<td>PacO₂ (torr)</td>
<td>144±11</td>
<td>135±9</td>
<td>145±11</td>
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<tr>
<td></td>
<td>Temp (° C)</td>
<td>38.1±0.1</td>
<td>38.2±0.1</td>
<td>37.9±0.1</td>
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<tr>
<td></td>
<td>Hb (%)</td>
<td>13.7±1.2</td>
<td>13.5±1.2</td>
<td>13.0±1.0</td>
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<tr>
<td></td>
<td>CMRO₂ (ml/100 g/min)</td>
<td>6.69±0.69</td>
<td>7.42±1.10</td>
<td>8.81±0.91</td>
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<td><strong>Cyclooxygenase inhibitor</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Indomethacin (n=4)</td>
<td>MABP (torr)</td>
<td>117±4</td>
<td>106±5</td>
<td>111±4</td>
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<tr>
<td></td>
<td>PacO₂ (torr)</td>
<td>40±1</td>
<td>39±2</td>
<td>39±1</td>
</tr>
<tr>
<td></td>
<td>pH</td>
<td>7.35±0.07</td>
<td>7.36±0.02</td>
<td>7.36±0.02</td>
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<tr>
<td></td>
<td>PacO₂ (torr)</td>
<td>135±6</td>
<td>150±16</td>
<td>148±17</td>
</tr>
<tr>
<td></td>
<td>Temp (° C)</td>
<td>38.2±0.1</td>
<td>38.1±0.2</td>
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<td></td>
<td>Hb (%)</td>
<td>12.5±0.3</td>
<td>12.1±0.2</td>
<td>11.9±0.2</td>
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<tr>
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<td>CMRO₂ (ml/100 g/min)</td>
<td>6.48±0.58</td>
<td>6.18±0.47</td>
<td>7.22±0.30</td>
</tr>
</tbody>
</table>

MABP, mean arterial blood pressure; PAF, platelet activating factor; Temp, rectal temperature; Hb, hemoglobin concentration; CMRO₂, cerebral metabolic rate for oxygen. Data are mean±SEM.
their generous supplies of BN 52021 and WEB 2086, respectively. We thank Lisa Cohn for editing and Janet Santucci for preparing the manuscript.

References

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