Prostaglandin Release From Isolated Rabbit Cerebral Cortex Micro-Vessels — Comparison of 6-Keto PGF\textsubscript{1\alpha} and PGE\textsubscript{2} Release From Micro-Vessels Incubated in 100% O\textsubscript{2}, Room Air and 95% N\textsubscript{2}:5% CO\textsubscript{2}

ASTRIDE M. RODRIGUES, B.S., AND MARY E. GERRITSEN, PH.D.

SUMMARY  Prostaglandin release from microvessels isolated from the rabbit cerebral cortex was determined under three different atmospheric conditions: 100% O\textsubscript{2} ("O\textsubscript{2}") room air, and 95% N\textsubscript{2}:5% CO\textsubscript{2} ("N\textsubscript{2}-CO\textsubscript{2}"). Initial studies with homogenates prepared from rabbit cerebral microvessels (RCMV) indicated two pathways of enzymatic PGH\textsubscript{2} transformation, namely PGI\textsubscript{2} synthase and GSH-dependent PGH-PGE isomerase. We measured the release of the principal products of these pathways, 6-keto PGF\textsubscript{1\alpha} and PGE\textsubscript{2} from freshly prepared RCMV. The release of 6-keto PGF\textsubscript{1\alpha} exceeded that of PGE\textsubscript{2} in all three protocols. RCMV incubated in "N\textsubscript{2}-CO\textsubscript{2}" exhibited a reduction in the release of 6-keto PGF\textsubscript{1\alpha} compared to room air or "O\textsubscript{2}" incubated RCMV, evident at 30-60 min of incubation. No significant differences in the release of PGE\textsubscript{2} were observed among the three incubation protocols. In all three incubation protocols the ratio of 6-keto PGF\textsubscript{1\alpha} to PGE\textsubscript{2} did not differ during the initial 10 minutes of each incubation. After 30 to 60 min of incubation, this ratio did not change from the "O\textsubscript{2}" or room air treated RCMV, but decreased significantly for the "N\textsubscript{2}-CO\textsubscript{2}" treated group. To determine the reversibility of the apparent "N\textsubscript{2}-CO\textsubscript{2}" induced decline in 6-keto PGF\textsubscript{1\alpha} release, microvessels were removed from the nitrogen atmosphere and incubated in room air. Release was measured during the initial 10 min following reintroduction to room air and was compared to room air pretreated controls treated in an identical manner. Complete recovery of 6-keto PGF\textsubscript{1\alpha} production was observed, and an enhanced ratio of 6-keto PGF\textsubscript{1\alpha} to PGE\textsubscript{2} observed in the "N\textsubscript{2}-CO\textsubscript{2}" treated RCMV. In view of the opposing actions of PGI\textsubscript{2} (vasodilator) and PGE\textsubscript{2} (mild vasoconstrictor) on cerebrovascular tone, this study suggests that release of prostaglandins by the microvasculature may participate in the cerebrovascular response to ischemia. In addition, this study suggests that the release of 6-keto PGF\textsubscript{1\alpha} and PGE\textsubscript{2} by cerebral microvessels may be regulated independently.

THE SYNTHESIS OF PROSTAGLANDINS (PGs) and related substances in the cerebral tissues of several species has been well documented. Among the products identified are PGE\textsubscript{2}, PGG\textsubscript{2}, PGD\textsubscript{2}, as well as TxB\textsubscript{2} and 6-keto PGF\textsubscript{1\alpha}. Several groups have focused in the cerebral vasculature and microvasculature, where the major product formed from endogenous substrate appears to be PGI\textsubscript{2}.\textsuperscript{1,2} Studies with exogenous PGH\textsubscript{2} and arachidonic acid have indicated that cerebral microvessels may also synthesize PGE\textsubscript{2} (cat, rabbit, cow) or PGD\textsubscript{2} (rat).\textsuperscript{3,4}

The physiological and pathophysiological roles of PGs in the cerebral circulation are poorly understood. These substances have powerful actions on cerebrovascular tone\textsuperscript{5} and have been implicated in cerebral vasospasm and cerebral ischemia.\textsuperscript{6,7} Siess\textsuperscript{o} and Nilsson\textsuperscript{4} have recently suggested that PG or PG-like substances may be direct coupling agents in the regulation of cerebral blood flow (CBF).

The role of prostaglandins in the observed actions of indomethacin on the cerebral circulation have been investigated to be a limited extent. Pickard and Mackenzie\textsuperscript{8} have shown that injection of indomethacin in baboon induces a decrease in CBF, proposed by these authors to be due to inhibition of prostacyclin synthesis by vascular endothelium. Pickard and Mackenzie\textsuperscript{8} also demonstrated a reduction in the CBF response under hypercapnic conditions when indomethacin is administered. However, a recent study by Wennmalm and co-workers\textsuperscript{9} indicates the effects of indomethacin on basal and hypercapnic CBF (in man) is not due to the effects of indomethacin on PGI\textsubscript{2} (or other prostaglandins) synthesis and release in the cerebral circulation. In this study, these authors compared the effects of indomethacin, naproxen and aspirin on CBF under basal and hypercapnic conditions. Administration of all three drugs eliminated arachidonic acid induced platelet aggregation in platelets obtained from the treated patients. Only indomethacin, and only when given acutely, elicited a decrease in CBF under basal or hypercapnic conditions. Additionally, these authors did not detect an increase in the release of PGI\textsubscript{2} metabolites (6-keto PGF\textsubscript{1\alpha}, 6,15-diketo, 13,14-dihydro PGE\textsubscript{2}), arachidonic acid or other free fatty acids during hypercapnia. The results of this study do not support the hypothesis that inhibition of cyclooxygenase impairs basal cerebral blood flow or cerebral blood flow during hypercapnia.

Prostaglandins may have some function in the circulatory response to normal and pathological states of oxygen tension in the brain. Numerous studies have documented a massive accumulation of arachidonic
acid and other free fatty acids in the brain during or following ischemia, hypoglycemia and epileptic episodes. In ischemia, the depletion of brain oxygen levels may reduce the metabolism of arachidonic acid to PGs and related substances. However, a burst of PG synthesis has been observed following post-ischemic recirculation. Ellis et al found that hypoxia was not associated with any significant alteration in brain-tissue levels of PGE₂, PGF₂α or 6-keto-PGF₁α in the cat, although there was a trend for decrease in PGE₂ levels during hypoxia that neared statistical significance. In the same study, arterial hypercapnia was associated with a substantial decrease in tissue levels of PGE₂ and PGF₂α, with a tendency toward a decline in 6-keto-PGF₁α, suggesting that more severe hypoxia, perhaps combined with hypercapnia, may be required to bring about increased arachidonate release followed by subsequent increases in PG formation. In the study by Ellis et al, levels of PGE₂ and PGF₂α were five times higher than 6-keto-PGF₁α. The levels of 6-keto-PGF₁α did not alter significantly during hypoxia or hypercapnia. It has been suggested that 6-keto-PGF₁α formation in the brain is vascular in origin, although the vasculature constitutes a relatively small percentage of the cerebral cortex. It is possible, therefore, that altered release of PGI₂(6-keto PGF₁α) might be difficult to detect in whole cerebral preparations.

In the present study, we report on our studies investigating the effects of various atmospheric conditions on the release of prostaglandins from isolated microvessels prepared from the rabbit cerebral cortex. Microvessels were incubated in three different atmospheric conditions (100% O₂ (“O₂”), room air, and 95% N₂:5% CO₂ (“N₂-CO₂”)) at 37°C. Release of 6-keto-PGF₁α and PGE₂ from the microvessels was measured at various time intervals (1 min to 60 min) of incubation under these conditions. The results from these studies indicated that the synthesis and release of 6-keto-PGF₁α in isolated microvessels can be significantly altered with variations in the atmospheric conditions. Compared with microvessels incubated in room air, release of 6-keto-PGF₁α was depressed at later time points during the incubation with “N₂-CO₂”, but did not differ from microvessels incubated with “O₂”.

Methods

Cerebral Microvessel Isolation Procedure

Adult male white New Zealand rabbits (1–2 kg) were sacrificed by cervical dislocation (6 in each experiment). The cortex and midbrain were removed quickly and placed into cold PBS on ice. All subsequent procedures were done at 4°C. The cerebellum and midbrain were removed and the remaining cortical mantle was freed of the pia membranes, arachnoid matter and any obvious large vessels. Microvessels were isolated by the method of Selivonchick and Root. The resulting tissue was homogenized in 10 volumes of ice cold phosphate buffered saline (PBS), pH 7.4, using a Dounce homogenizer and 25 up and down strokes. The homogenate was poured over an 88 μm nylon sieve. The retentate was resuspended in ice cold PBS in 50 ml (Sorvall) centrifuge tubes. The suspension was centrifuged at 1500 × g for 10 min, the pellet resuspended in 0.25 M sucrose and layered over a discontinuous gradient, 12 ml each, of 1.0 M and 1.5 M sucrose. This was centrifuged at 58,000 × g for 45 minutes (4°C) in a Beckman ultracentrifuge using a Beckman SW 25.1 rotor. The microvessel pellet was resuspended in 4°C PBS and filtered over a 20 μm sieve. The retentate was thoroughly rinsed with PBS, gently removed from the sieve and used immediately.

In another study, homogenates of microvessels were prepared by mincing with razor blades followed by sonication (Branson sonifier) in 100 mM Tris-HCl buffer, pH 8.1. Homogenates were used immediately for PGI₂ incubations.

Incubation Protocol

The isolated microvessels were divided into three groups and weighed. Each group was placed into nylon sieve (20 μm) baskets, which were subsequently put into individual 25 ml erlenmeyer flasks, stopped with rubber stoppers. Each stopper was fitted with three 23 gauge needles. Two needles were used for the inflow and outflow of gas. The third needle was fitted with a tuberculin syringe for sample removal and a three-way stopcock, enabling buffer removal without disturbance of the atmospheric conditions or the microvessels. Each flask contained 6 ml of PBS at 37°C and had been pre-equilibrated with one of I. room air II. 100% O₂ or III. 95% N₂:5% CO₂ for 30 min prior to addition of the microvessels. The microvessel basket was rapidly inserted at time 0, the stoppers immediately replaced, and incubated in a 37°C shaking water bath with constant flow of the appropriate gas to the flasks to maintain initial conditions. A 500 μl aliquot was removed at times ranging from 1–60 minutes. In trials 10–14, following the 60 minute time point, groups I and III were transferred to another 25 ml flask containing 6 ml of fresh PBS (37°C) and incubated in room air only. A 500 μl aliquot was taken at 1, 5 and 10 minutes.

Each aliquot was frozen and subsequently assayed for 6-keto-PGF₁α and PGE₂ by radioimmunoassay.

[14C] PGH₂ Metabolism

Homogenates prepared from rabbit cerebral microvessels were incubated with [1,14C] PGH₂, prepared from [1,14C] arachidonic acid (52 mCi/mmole) following the protocols outlined in earlier publications. Incubations were performed in the presence or absence of 2 mM glutathione, and boiled homogenates and rabbit serum albumin were controls. Products were extracted with 3.5 volumes of ethyl acetate: methanol:0.2M citric acid (15:2:1 v/v), the organic phase applied to silica gel GHL thin-layer chromatography (TLC) plates along with authentic standards (kindly provided by Upjohn Co., Kalamazoo, MI). Chromatograms were developed in the organic phase of ethyl acetate-hexane, acetic acid:water (56:24:12:60) and quantification achieved by radiochromatogram scanning (Packard 7201) and scraping and counting appropriate zones of the TLC plates.
Radioimmunoassay of PGE\(_2\) and 6-keto PGF\(_{10}\)

The concentration of immunoreactive 6-keto PGF\(_{10}\) (the stable hydrolytic product of PGI\(_2\)) and immunoreactive PGE\(_2\) were determined in nonextracted samples of PBS that had been incubated with isolated rabbit cerebral microvessels. Antisera was kindly supplied by Dr. Lawrence Levine (Brandeis University, Waltham, MA). The radioimmunoassay procedure was performed as detailed by Granstrom and Kindahl. The detection limit of both the 6-keto PGF\(_{10}\) and PGE\(_2\) antisera was 10 pg/tube and intra- and interassay variation was routinely less than 10%. Values are expressed as the amount of prostaglandin released/mg wet weight. The anti-6-keto PGF\(_{10}\) antisera demonstrated some cross-reactivity with 6-keto PGE\(_2\) (3.1%), 6,15-diketo PGF\(_{10}\) (1.0%) and PGF\(_{5\alpha}\) (0.3%). The remaining prostanoids tested (13,14-dihydro, 6,15-diketo-PGF\(_{10}\), PGE\(_2\), TXB\(_2\), PGA\(_2\) and arachidonic acid) exhibited cross-reactivity of less than 0.03%. The antisera directed against PGF\(_{5\alpha}\) was not as specific, exhibiting complete cross-reactivity with PGA2 (150%), and detectable cross-reactivity with PGE\(_2\) (25.6%), PGD\(_2\) (0.4%), PGE\(_{5\alpha}\) (0.3%), 6-keto PGE\(_2\) (10.1%), 15-keto PGE\(_2\) (2.6%). The cross-reactivity with other metabolites of the arachidonic acid cascade (i.e. TXB\(_2\), 6-keto PGF\(_{10}\), 13,14-dihydro, 6,15-diketo PGF\(_{10}\) and arachidonic acid was less than 0.01%.

Statistical Analysis

The time-course of prostaglandin release during incubation in "O\(_2\)" or "N\(_2\)-CO\(_2\)" were compared to room air controls using a two-way analysis of variance. Significance was accepted at \(p < 0.05\). In other experiments, data was compared to room air controls using a Student’s t-test (non-paired values).

Results

Metabolism of [1-\(^{14}\)C] PGH\(_2\) by Rabbit Cerebral Microvessel Homogenates

Homogenates (equivalent to 100 \(\mu\)g protein) prepared from rabbit cerebral microvessels were incubated with [1-\(^{14}\)C] PGH\(_2\) (25,000 dpm, 0.2 nmol) in the presence and absence of reduced glutathione (GSH). Control incubations used boiled homogenates or rabbit serum albumin. Two enzymatic pathways of PGH\(_2\) metabolism were observed: PGI\(_2\) synthase, resulting in the formation of 6-keto PGF\(_{10}\), and a PGH-E isomerase, as indicated by the GSH-enhanced formation of PGE\(_2\). Neither activity was detected in boiled homogenate or serum albumin incubations (table 1). The GSH-enhanced PGE\(_2\) forming activity was GSH-specific, and was inhibited by preincubation with 5mM N-ethylmaleimide or 5mM p-hydroxymercuribenzoate (data not shown).

<table>
<thead>
<tr>
<th>Preparation</th>
<th>2 mM GSH</th>
<th>6-keto PGF(_{10})</th>
<th>PGE(_2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microvessel homogenate</td>
<td>–</td>
<td>6 ± 1</td>
<td>44 ± 2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>8 ± 1</td>
<td>62 ± 1</td>
</tr>
<tr>
<td>Microvessel homogenate boiled</td>
<td>–</td>
<td>0</td>
<td>42 ± 2</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>42 ± 2</td>
</tr>
<tr>
<td>Rabbit Serum Albumin</td>
<td>–</td>
<td>0</td>
<td>41 ± 1</td>
</tr>
<tr>
<td></td>
<td>+</td>
<td>0</td>
<td>42 ± 3</td>
</tr>
</tbody>
</table>

Homogenates equivalent to 100 \(\mu\)g of protein, boiled controls, and rabbit serum albumin (100 \(\mu\)g protein) were incubated with 0.2 nmol [1-\(^{14}\)C] PGH\(_2\) for 5 min in the absence and presence of reduced glutathione (GSH). Values are expressed as pmol product formed (\(\bar{X} \pm \text{S.E.M.}, n = 4\)). (*) denotes a significant change (\(p < 0.05\), Student’s t-test, non-paired values).

Release of 6-keto PGF\(_{10}\) and PGE\(_2\) by Isolated Rabbit Cerebral Microvessels

Release of 6-keto PGF\(_{10}\) and PGE\(_2\) was measured in 500 \(\mu\)l aliquots removed from the incubations at 1, 5, 10, 30 and 60 minutes. Aliquots were removed carefully with the tuberculin syringe as described in the methods. In all three incubation conditions release of both 6-keto PGF\(_{10}\) and PGE\(_2\) occurred at a similar rate for the initial 10 minutes of incubation. This may be explained, in part, by the disturbance of the equilibrated atmosphere during the insertion of the microvessels. At 30 and 60 min, microvessels incubated in "N\(_2\)-CO\(_2\)" exhibited a significant reduction in release of 6-keto PGF\(_{10}\) compared to microvessels incubated in room air or "O\(_2\)" (fig. 1). The release of PGE did not differ significantly from one incubation condition to another (fig. 2). Microvessels incubated in "O\(_2\)" tended to release somewhat larger amounts of 6-keto PGF\(_{10}\) at 30 min, although the differences were not significant. The ratio of 6-keto PGF\(_{10}\) to PGE\(_2\) release at the

![Graph showing the release of 6-keto PGF\(_{10}\) and PGE\(_2\) by isolated rabbit cerebral microvessels.](https://example.com/graph.png)
different time points was also determined (table 2). No significant difference in 6-keto PGF$_{1α}$/PGE$_2$ was observed between microvessels incubated in room air versus "O$_2". However, microvessels incubated in "N$_2$-CO$_2" demonstrated an initial elevation in the ratio of 6-keto PGF$_{1α}$/PGE$_2$, followed by a decline evident at 30 and 60 min. In another series of experiments, microvessels that had been incubated in room air or "N$_2$-CO$_2" for 60 min were removed, placed in a new incubation flask containing PBS and incubated for an additional 10 min. Five hundred μl aliquots were removed at 1, 5, and 10 min. The results from this experiment are shown in figure 3. Upon reintroduction to room air after incubation in "N$_2$-CO$_2" microvessels that had previously demonstrated depressed 6-keto PGF release (fig. 1) exhibited a "rebound" increase in release. Although all of the individual experiments indicated that "N$_2$-CO$_2" pretreated microvessels apparently released greater amounts of 6-keto PGF$_{1α}$ than room-air pretreated controls, the variation from one experiment to another was such that "N$_2$-CO$_2" versus room-air pretreated groups did not differ significantly. However, in view of the significant decrease in 6-keto PGF$_{1α}$ release after 60 min of incubation in "N$_2$-CO$_2" recovery to room air control levels upon reintroduction to room air indicates that the hypoxia-induced depression in 6-keto PGF$_{1α}$ release was reversible. Release of PGE by room air versus "N$_2$-CO$_2" pretreated groups did not differ after reintroduction to room air. The ratio of 6-keto PGF$_{1α}$/PGE$_2$ declined from the first to the tenth minute of reintroduction to room air for the "N$_2$-CO$_2" pretreated group. The ratio of 6-keto PGF$_{1α}$/PGE$_2$ was significantly higher in the "N$_2$-CO$_2" pretreated microvessels versus the room air control pretreated preparations. Table 3, at the first time point, but did not differ significantly at 5 and 10 min.

**TABLE 2** Ratio of 6-keto PGF$_{1α}$ Release to PGE$_2$ Release from Rabbit Cerebral Microvessels. Microvessels were Incubated at 37°C in Phosphate Buffered Saline under one of Three Atmospheric Conditions

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Room air</th>
<th>100% O$_2$</th>
<th>95% N$_2$/5% CO$_2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4.6 ± 2.2</td>
<td>3.6 ± 1.7</td>
<td>8.3 ± 3.0</td>
</tr>
<tr>
<td>5</td>
<td>1.8 ± 0.9</td>
<td>3.4 ± 1.9</td>
<td>7.9 ± 5.7</td>
</tr>
<tr>
<td>10</td>
<td>2.3 ± 0.9</td>
<td>2.8 ± 1.4</td>
<td>3.2 ± 1.6</td>
</tr>
<tr>
<td>30</td>
<td>3.8 ± 2.0</td>
<td>2.8 ± 1.3</td>
<td>2.4 ± 0.9</td>
</tr>
<tr>
<td>60</td>
<td>3.1 ± 1.9</td>
<td>1.8 ± 0.9</td>
<td>1.5 ± 0.7*</td>
</tr>
</tbody>
</table>

*Significantly different (p < 0.05) from ratio at 1 min for that atmospheric condition (S.E.M. n = 14).

**TABLE 3** Ratio of 6-keto PGF$_{1α}$ Release to PGE$_2$ Release from Rabbit Cerebral Microvessels. Microvessels were Preincubated in PBS, 37°C in Room Air or 95% N$_2$/5% CO$_2$ 60 min, then Introduced to Fresh PBS (37°C) and Incubated in Room Air

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>Room air pretreated</th>
<th>95% N$_2$/5% CO$_2$ pretreated</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3.9 ± 2.0</td>
<td>13.5 ± 5.7*</td>
</tr>
<tr>
<td>5</td>
<td>6.2 ± 4.6</td>
<td>7.4 ± 3.2</td>
</tr>
<tr>
<td>10</td>
<td>1.7 ± 0.9</td>
<td>5.5 ± 2.0†</td>
</tr>
</tbody>
</table>

Significantly different (p < 0.05) from room-air pretreated control at the same time point. †Significantly different (p < 0.05) from ratio at 1 min observed with preparations pretreated with 95% N$_2$/5% CO$_2$ for 60 min followed by reintroduction to room air (S.E.M. n = 5).
Discussion

Altered blood flow following periods of ischemia is a well-recognized phenomenon with respect to both experimental transient cerebral ischemia. Both "no-reflow phenomenon" and hyperemia have been observed, although the mechanisms for these divergent observations remain unclear.

It is conceivable that regional blood flow in the ischemic brain can be altered by the synthesis and release of prostaglandins. These vasoactive substances may derive from the cerebral cortical tissue (i.e., the neural and glial elements) and/or from the cerebral vasculature. Furthermore, it is possible that ischemia may affect neuronal/glial tissue in a different manner than the cerebral vasculature. Release of a vasoconstrictor principle from one element might be balanced by the release of vasodilators from another. Thus, the net effect on regional blood flow following ischemia would be the result of these opposing influences.

The present study addressed the effects of altered atmospheric conditions on prostaglandin release by isolated cerebral microvessels. Incubations with 100% O2 tended to release somewhat larger amounts of 6-keto PGF1α and PGE2 compared to the ratio at one minute. Microvessels incubated in room air at 30 and 60 minutes. These observations suggest that the release of PG1 by the cerebral microvasculature of the rabbit may be more sensitive to physiological stimulation, and furthermore, that the release of PGI2 and PGE2 from RCMV are not directly coupled. These RIA results may be somewhat complicated by the observed non-enzymatic isomerization of PGH2 to PGE2, as indicated in table 1. Thus, PGE2 release may not reflect enzymatic production of the prostaglandin, but rather the sum of enzymatic and spontaneous isomerization of PGH2.

The results from this study do not support the general theory that hypoxia is a stimulus to enhance prosta-glandin release. The results from this study suggest that the rate of release of 6-keto PGF1α may be related to O2 tension, as would be predicted by the O2 requirement of the cyclooxygenase enzyme. Reintroduction of RCMV from a low O2 environment to room air indicated that the 95% N2:5% CO2 induced depression of 6-keto PGF1α release was reversible. One possible explanation for the lack of a significant difference between 100% O2 versus room air incubated preparations might be that O2 concentrations in room air are not rate-limiting.

It is difficult to extrapolate from this reintroduction experiment to the "reflow" in ischemic tissue following hypoxia. However, the net increase in 6-keto PGF1α release in the room air following the 95% N2:5% CO2 incubation is suggestive of an enhanced release of PGI2 following "hypoxia". RCMV incubated in room air for 60 min had released 3.5 ± 0.9 ng/mg wet wt 6-keto PGF1α at 60 min; a 10 min reintroduction to fresh buffer under identical conditions resulted in further release of 0.9 ± 0.4 ng/mg wet wt of 6-keto PGF1α after 10 min. In contrast, microvessels incubated in 95% N2:5% CO2 for 60 min had released 1.3 ± 0.5 ng/mg wet wt 6-keto PGF1α.

Throughout all of incubations, the release of 6-keto PGF1α exceeded that of PGE2. This ratio was depressed following 30–60 min incubations in "N2-CO2" compared to the ratio at one minute.

The ratio of 6-keto PGF1α/PGE2 was enhanced when microvessel preparations under hypoxic conditions were transferred to normal room air conditions after the 60 minute time point (table 3), indicating that the stimulation of PG products upon restoration of oxygen appears to favor PGI2. The levels of PGE2 released from the oxygen deprived microvessels did not differ from the room air controls when the preparations were reintroduced to room air.

A known physiological cerebral vasodilator, PGI2, has been implicated in the hyperemia observed during reperfusion following transient brain ischemia. Several investigators report that PGI2 reverses contractions of cerebral vessels and produces enhancement of post-ischemic reperfusion. It has also been revealed that a combination of indomethacin and PGI2 treatment during reperfusion will increase CBF,
possibly due to an imbalance in PG pathways at the endothelial interface. In light of these findings, a high ratio of PGI₂ to PGE₂ production in the cerebral circulation, and further elevation of PGI₂ levels upon oxygen restoration to tissue might have some protective value. Our results show such a ratio of cyclooxygenase products occurs in the isolated cerebral microvasculature in response to the "post-ischemic" incubation. Prolonged incubation in "N₂-CO₂" suggested that some imbalance in PG synthesis, i.e., a reduced ratio of PGI₂ to PGE₂, production, may occur. If such an imbalance does occur in vivo, it is likely that vasoconstrictor prostaglandin synthesis by other cell types (e.g., platelets, neural or glial elements) could play important roles. Various lines of reasoning may be advanced to account for the differential effects of the atmospheric manipulations of 6-keto PGF₁₀ and PGE₂ release. If there were more than one mechanism of arachidonic acid release perhaps regulated by different phospholipases, different patterns of PG production might occur. A recent report by Lysz and Needleman indicated two distinct forms of cyclooxygenase in brain tissue. These authors observed differential rate of and termination of PGE₂ production in rabbit medul- lar microsomes incubated with [¹⁴C] arachidonic acid. PGE₂ levels rose rapidly and abruptly plateaued while PGF₂α levels continued to rise for longer periods. The elimination of several possibilities to explain the differential rate (i.e., deficiency of substrate or cofactor etc.) supported the existence of two distinct cyclooxygenases. The data from this series of experiments indicates that oxygen tension may differentially affect prostaglandin synthesizing activities leading to PGI₂ versus PGE₂ production. A possible means whereby such regulation could occur might be selective activation of a phospholipase.

Acknowledgments

The authors express their sincere appreciation to Ms. Linda Capobianco for her secretarial skills and to Ms. Carol Cheli for her technical assistance.

References


Prostaglandin release from isolated rabbit cerebral cortex micro-vessels--comparison of 6-keto PGF1 alpha and PGE2 release from micro-vessels incubated in 100% O2, room air and 95% N2:5% CO2.

A M Rodrigues and M E Gerritsen

doi: 10.1161/01.STR.15.4.717

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1984 American Heart Association, Inc. All rights reserved.
Print ISSN: 0039-2499. Online ISSN: 1524-4628

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://stroke.ahajournals.org/content/15/4/717

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in *Stroke* can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to *Stroke* is online at:
http://stroke.ahajournals.org//subscriptions/