Synthesis of Prostaglandins and Thromboxane B₂ by Cerebral Arteries

A. AINSWORTH HAGEN, PH.D., RICHARD P. WHITE, PH.D., AND JAMES T. ROBERTSON, M.D.

SUMMARY The capacity of cerebral arteries to synthesize prostaglandins was studied by 2 procedures. In one, bovine cerebral arteries were incubated with 0.1 μCi (1-¹⁴C)-arachidonic acid for 3 hours. Using thin layer chromatography, 5 products of this arachidonic acid were isolated: prostaglandin E₃ (PGE₃), prostaglandin F₃₀ (PGF₃₀), 6-keto prostaglandin F₁α (6-keto PGF₁α), prostaglandin D₂ (PGD₂), and thromboxane B₂ (TXB₂). In the second group of experiments the biosynthesis of these lipids from endogenous substrate was confirmed, except for PGD₂, by means of gas liquid chromatography-mass spectrometry. In addition, the production of PGE₃ and PGF₃₀ was quantified. An average of 196 ng PGF₂₀ and 172 ng PGF₁₀ were synthesized per gram tissue in one hour. Meclofenamate inhibited the formation of these 2 prostaglandins while serotonin stimulated synthesis approximately 20-25%. The present finding demonstrates that cerebral arteries form several prostaglandins and, likely, thromboxane B₂. It is hypothesized that these lipids may play a role in the vasomotion of cerebral blood vessels in health and disease. The relative rates of synthesis of these lipids may be important for maintaining normal cerebral circulation. However, in cerebrovascular disease the normal balance between the rate of synthesis of those prostaglandins which constrict and those which dilate may be disturbed.

CEREBRAL ARTERIAL VASOSPASM may be a consequence of subarachnoid hemorrhage or trauma and may lead to cerebral ischemia. The mechanism(s) for this vasospasm, however, is not well understood. It has been postulated that contraction is due to endogenous substances whose synthesis and/or release is stimulated as a result of hemorrhage or mechanical stimuli. Among the many potential mediators which have been studied are the prostaglandins.¹⁻⁴ Both prostaglandin E₂ (PGE₂) and prostaglandin F₂₀ (PGF₂₀) are potent constrictors of cerebral vessels and produce prolonged vasospasm when given intracisternally.²⁻³ Other prostaglandins are also spasmodic but have weaker activity. Recently, thromboxane A₂ (TXA₂), another product of arachidonic acid metabolism, has been shown to be a potent spasmodic agent when applied topically to cerebral vessels.⁵ The first indication that cerebral arteries may synthesize prostaglandins was by Pickard and co-workers⁶ who reported the release of a substance from bovine cerebral arteries which produced an effect similar to prostaglandins when applied to rat fundal strips. Moreover, treatment with indomethacin, a prostaglandin synthetase inhibitor, abolished its formation. Although identification was not made, this evidence suggested that the material released was most likely a prostaglandin. White et al.⁷ confirmed that prostaglandin-like material is released from cerebral arteries and further demonstrated by bioassay and chromatography that 2 such substances are released, having identical Rf values to authentic PGF₂₀ and PGE₂ on thin-layer chromatography. In order to obtain a better basis for considering the role prostaglandins may play in cerebrovascular phenomena the present study was performed to establish by chemical analysis whether cerebral arteries synthesize these vasoactive lipids and whether this synthesis may be modified by drugs.

Materials and Methods

Bovine cerebral arteries were obtained from the abattoir following exsanguination and placed on ice. These were frozen and stored at −7° C. At the time of incubation the arteries were cut into small fragments 1-2 mm in length and 1 gm tissue placed into a flask containing 10 ml Krebs bicarbonate buffer, pH 7.4. The samples were preincubated for 10 minutes at 37 C and the supernatant was discarded. Ten ml fresh buffer was added to the flask as well as 0.1 μC (1-¹⁴C)-arachidonic acid (Sp. Act. 55 mCi/mMol) and in selected cases, either serotonin (12.5 μM) or meclofenamate (1.0 mM). Following a 3 hour incubation period the aqueous phase, which was colorless, was removed, acidified to pH 3.5, and extracted 3 times with equal volumes of chloroform. The chloroform was dried under nitrogen and separation of the prostaglandins was accomplished by thin-layer chromatography (TLC). The first system used was chloroform; methanol:acetic acid:water (90:9:1:0.65) which permitted partial separation, especially of PGF₁₀ and prostaglandin D₂ (PGD₂). The zone containing PG₁₀ was eluted and rechromatographed on TLC in the system ethyl acetate:isoctane:acetic acid:water (11:5:2:10) which permitted separation of 6-keto prostaglandin F₁α (6-keto PGF₁α) from PGE₂ and thromboxane B₂ (TXB₂). Separation of these latter 2 lipids was completed by TLC in the system ethyl acetate:acetic acid (99:1). The radioactivity in these areas was determined by lipid scintillation counting. The values are expressed as percent distribution in the recovered radioactivity. The largest percent of radioactivity in the extract was arachidonic acid. No

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attempt was made to identify the radioactive products within the tissue.

With another group of arteries a similar incubation procedure was used except no exogenous arachidonic acid was added and incubation was for only one hour. Following the incubation the aqueous phase was transferred to tubes to which deuterated PGE₂ and deuterated PGE₆ had been added. The use of the deuterated PGs permitted quantitation of PGE₂ and PGF₂α which was synthesized endogenously. Extraction, methylation, and purification on silicic acid columns were done as reported previously. Quantitative analysis of PGE₂ and PGF₂α was accomplished using selected ion monitoring (m/e 423 and 427 for PGE₂α and m/e 321 and 325 for PGE₂) on a Hewlett-Packard Model 5930 A mass spectrometer connected through a silicone membrane separator to a Hewlett-Packard Model 5700 A gas chromatograph. A 6 × 4 mm ID silanized glass column was packed with 3% OV-210, 100/200 mesh, on Gas Chrom Q and the temperature was 220°C. Helium flow through the column was 30 ml/min. Ionization voltage was 35 eV, emission was 0.1 mA, and the ion source temperature was 180°C.

In addition to the above prostaglandins, several major single ion peaks were monitored for the presence of thromboxane B₂ (TxB₂) and 6-keto-prostaglandin F₁α (6-keto PGF₁α). Two ions selected for thromboxane B₂ were m/e 256 and 366 and under the above conditions the retention time was approximately 2.3 minutes. Three ions were monitored for 6-keto-prostaglandin F₁α, m/e 324, 349, and 420. Retention time of this compound was approximately 4.5 minutes. Several ions were also monitored for the presence of prostaglandin D₂ but the results were inconclusive.

**Results**

That bovine cerebral arteries have the capacity to synthesize several prostaglandins from (1-³¹C)-arachidonic acid in vitro, is shown in table 1. Based on chromatographic mobility, 4 major products were initially isolated: PGE₂, PGF₂α, 6-keto PGF₁α, and PGD₂. The first 3 of these were approximately of equal importance quantitatively. The TLC systems used did not permit separation of TxB₂ from PGE₂ so several samples were rechromatographed in a TLC system which permitted separation. Two peaks were observed with the major peak retaining a mobility similar to authentic PGE₂ but a small peak migrated with an RF similar to authentic TxB₂. This small peak accounted for 15–20% of the radioactivity in the original PGE₂ zone. Incubation of cerebral arteries in the presence of meclofenamate markedly inhibited the synthetic capacity.

To gain insight into the capacity of cerebral arteries to synthesize prostaglandins from endogenous precursors additional arteries were incubated for one hour and PGE₂ and PGF₂α were quantitated. Results of these studies are summarized in table 2. Synthesis of these two prostaglandins was similar but in all cases the concentration of PGE₂ was highest. As expected, meclofenamate inhibited the synthesis of these 2 prostaglandins. Of interest was the ability of serotonin to stimulate synthesis by 20–25%. This increase was significant statistically (Student's t-test, unmatched pairs) for PGF₂α (P < 0.05) but was of questionable significance for PGE₂ (P < 0.10). An analysis of these 2 values by combining probabilities (Snedecor's) indicated that the synthesis of both of these lipids had been enhanced by serotonin significantly (P < 0.05). When several of the major mass ions for 6-keto PGF₁α, TxB₂, and PGD₂ were monitored it was apparent both 6-keto PGF₁α and TxB₂ were present. While these 2 lipids were not quantitated, the areas of the peaks demonstrated that 6-keto PGF₁α was a major metabolite whereas TxB₂ was of lesser importance. The peak areas of these 2 compounds were diminished in those samples which contained meclofenamate. These data complement the results from the incubations with (1-³¹C)-arachidonic acid in regard to the synthesis of these lipids.

**Discussion**

Prostaglandins have been postulated to play an important role in the regulation of cerebral circulation. Alterations in cerebrovascular tone can be demonstrated following the intracarotid injection of several prostaglandins. Topical application of prostaglandins F₂α and F₁α to cat pial arteries elicited profound vasoconstriction as did the application of prostaglandins B₁, B₂, and F₂α to pial vessels of mice. Topical administration of several prostaglandins also

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**Table 1. Percent Distribution of Prostaglandins Following Incubation of Bovine Cerebral Arteries with (1-³¹C)-Arachidonic Acid**

<table>
<thead>
<tr>
<th>Prostaglandin</th>
<th>Control N = 4</th>
<th>Meclofenamate N = 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGE₂</td>
<td>5.5 (3.5-8)</td>
<td>1.8 (0-3)</td>
</tr>
<tr>
<td>PGF₂α</td>
<td>4.8 (2-9)</td>
<td>1.8 (1-4)</td>
</tr>
<tr>
<td>6-keto PGF₁α</td>
<td>5.0 (3-7)</td>
<td>2.0 (1-3.5)</td>
</tr>
<tr>
<td>PGD₂</td>
<td>2.8 (2-4)</td>
<td>-</td>
</tr>
</tbody>
</table>

*Values are expressed as percent distribution in the recovered radioactivity.

**Table 2. Quantitation of Prostaglandins E₂ and F₂α Synthesized by Bovine Cerebral Arteries**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>PGE₂</th>
<th>PGF₂α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>4</td>
<td>196 (182–210)</td>
</tr>
<tr>
<td>Meclofenamate</td>
<td>4</td>
<td>116 (110–125)</td>
</tr>
<tr>
<td>Serotonin</td>
<td>3</td>
<td>234 (206–268)</td>
</tr>
</tbody>
</table>
constricts the basilar artery when applied either in vitro or in vivo.\textsuperscript{1, 2, 18-20} and enhanced the chances of blood to cause cerebral vasospasm experimentally.\textsuperscript{3} Such findings have led to the hypothesis that these lipids play a major role in cerebral vasospasm seen following subarachnoid hemorrhage (SAH).

The present results support the hypothesis advanced by Pickard et al.\textsuperscript{4} that the formation of prostaglandins within the arterial wall could be more important in the pathogenesis of vasospasm than the level of prostaglandins present in the CSF. That this endogenous synthesis may be appreciable for PGE\textsubscript{2} and PGF\textsubscript{2\alpha} is shown in table 2 and agrees favorably with values reported by others\textsuperscript{5-9} using bioassay to determine PG-like material. The enhanced synthesis of prostaglandins by cerebral arteries caused by serotonin, from platelets and from brain tissue, strengthens this view and may explain, in part, the mode of action of serotonin as a constrictor of cerebral arteries. Although the concentration of serotonin in this study may not have been optimal, it is of interest that synthesis was stimulated approximately 20-25\% whereas the contractile response of cerebral arteries to serotonin is reportedly diminished by 18.7\% in the presence of indomethacin, a prostaglandin synthetase inhibitor.\textsuperscript{6} Several blood borne substances are known to stimulate prostaglandin synthesis by brain,\textsuperscript{21} platelets,\textsuperscript{22} as well as various arteries and veins.\textsuperscript{23-26} These may do likewise with cerebral arteries. Therefore, serotonin, norepinephrine, hematin, and other agents could be important in SAH because of their intrinsic activity and/or their ability to stimulate prostaglandin synthesis.

Thromboxane B\textsubscript{2} was also present in both experiments and apparently reflects the capacity of cerebral arteries to synthesize TxA\textsubscript{2}, which is very unstable. Special effort was made to remove all sources of platelets but since platelets may have been adhering to the arteries they cannot be ruled out as a source of TxA\textsubscript{2} in our experiments. However, thromboxanes are reported to be synthesized by brain tissue,\textsuperscript{27} human umbilical artery,\textsuperscript{28} and kidney\textsuperscript{29} as well as by platelets.\textsuperscript{30, 31} Although this metabolite appeared, herein, to be of lesser importance quantitatively, its physiological or pathophysiological role may be very important. Ellis and co-workers\textsuperscript{32} have studied the constrictor activity of TxA\textsubscript{2} on cerebral vessels and found that the contraction produced, \textit{in vitro}, was as great as that produced by PGF\textsubscript{2\alpha} and approximately twice that produced by serotonin. Likewise, the importance of TxA\textsubscript{2} to experimentally induce stroke or heart attack has been suggested by Shimamoto.\textsuperscript{33}

Another prostaglandin of major importance in this study is 6-keto-PGF\textsubscript{1\alpha}. This is a stable metabolite and reflects the synthesis of PGI\textsubscript{2}. Prostaglandin I\textsubscript{2}, because of its vasodilating activity and ability to inhibit platelet aggregation, is most important under physiological conditions.\textsuperscript{34} Boullin and Blaso\textsuperscript{35} have suggested that cerebral vasospasm may be a result of the decreased synthesis of this lipid. The fact that cerebral vessels produce both vasodilator and vasoconstrictor prostaglandins suggests that an imbalance in rate of synthesis could contribute to cerebrovascular disease such as vasospasm and migraine. The finding in other vessels that prostaglandins may be differentially synthesized throughout the vascular wall supports this suggestion.\textsuperscript{36} When damaged, arteries may predominantly form vasoconstrictor prostaglandins.

While the present results support the hypothesis that cerebral vasospasm could result from an inordinate generation of prostaglandins or thromboxane within the vessel wall, other sources of vasoactive lipids such as brain tissue\textsuperscript{37-39} and platelets\textsuperscript{40-42} also could be important in the occurrence of this phenomenon. Consequently, the severity of the spasm following SAH could be due to contributions from all of these sources. The presence of blood might be expected to impede the efflux of prostaglandins from the CSF, which is normally very rapid,\textsuperscript{43} so that the level of these lipids could be abnormally elevated in the area of hemorrhage. The synthesis and level of prostaglandins could increase with time as a result of the release of substances such as norepinephrine and hematin and contribute to the chronic phase of spasm seen following SAH. If so, then the administration of a prostaglandin synthetase inhibitor with the specificity to affect synthesis in the tissues involved\textsuperscript{44} should benefit patients with SAH. In this regard, meclofenamate may be such an agent and should be tested experimentally in an \textit{in vivo} model. The Canadian Cooperative Study Group report\textsuperscript{45} indicates that aspirin is beneficial in one form of cerebrovascular disease and further study may reveal that other forms are amenable to treatment by similar drugs, each of which have a different pharmacologic profile.\textsuperscript{46} In any case, this demonstration that cerebral arteries manufacture prostaglandins provides a rational basis for considering the role these vasoactive lipids may play in health and disease.

Acknowledgment

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