Levels and Disappearance of Prostaglandin F₂α in Cerebral Spinal Fluid: A Clinical and Experimental Study

A. A. HAGEN, PH.D., J. N. GERBER, PH.D., C. C. SWEELEY, PH.D., R. P. WHITE, PH.D., AND J. T. ROBERTSON, M.D.

SUMMARY The concentration of prostaglandin F₂α (PGF₂α) was measured in cerebrospinal fluid (CSF) obtained by lumbar puncture in patients with subarachnoid hemorrhage and compared to control values. The level of this prostaglandin was elevated at some time in most patients during the course of their illness. However, this could not be correlated with the severity of neurological deficits observed. The possibility that the concentration of PGF₂α in lumbar fluid may not reflect that present intracranially was tested experimentally in anesthetized dogs. In these experiments only a small fraction of the radioactive PGF₂α, injected into the cisterna magna, appeared in lumbar CSF. Prostaglandin F₂α rapidly disappeared from the cisterna magna, half time of 8 minutes, and radioactivity was present in blood from the jugular vein indicating that normally this prostaglandin rapidly egresses from the CSF into blood. These findings suggest that PGF₂α can be rapidly transported away from the brain. This could explain the low concentrations of PGF₂α in CSF of normal individuals and in some patients who have severe cerebral vasospasm. Conversely, the elevation of PGF₂α in lumbar CSF noted in some patients might be due, in part, to an impairment of transport caused by the size and location of the hemorrhage.

FOR MANY YEARS considerable effort has been made to identify the factor(s) responsible for the pathogenesis of cerebral vasospasm. Included are the prostaglandins (PGs), primarily prostaglandin F₂α (PGF₂α) and prostaglandin E₂ (PGE₂) since these two are potent constrictors of cerebral vessels,¹ ⁴ when applied topically. Moreover, several studies⁶⁻⁷ have demonstrated that such prostaglandins are elevated in cerebral spinal fluid (CSF) in patients suffering brain trauma or subarachnoid hemorrhage (SAH). However, these levels varied markedly from day to day in the same patient as well as between patients and correlated poorly with the presence or absence of cerebral vasospasm. Also among control patients the samples of CSF were obtained by lumbar puncture so it is possible that the concentrations found are not indicative of what is occurring intracranially at the site of hemorrhage or trauma since little is known of normal transport of PGs in CSF. The present study agrees with earlier reports that CSF levels of PGs can rise following SAH⁹⁻⁷ and, in addition, experimental data are presented which help explain the variability in PG levels noted clinically.

Methods

A. Patient Studies

Lumbar CSF samples were obtained from 5 patients undergoing pneumoencephalography and served as controls. These patients were free of signs of intracranial hemorrhage and abnormal neurological signs. The second group of patients were those who had suffered SAH. In these, samples were collected before and following surgery whenever possible. The CSF was added immediately to glass tubes containing 1.9 mg EDTA and stored at −40 C until analyzed. Some of the samples were xanthochromic but none (except possibly one) contained fresh blood. No relationship between the color of CSF and PGF₂α level was noted.

B. Animal Studies

Ten mongrel dogs of both sexes, weighing from 14 to 21 kg, were anesthetized with sodium pentobarbital (30 mg/kg) administered intravenously. A laminectomy was performed and a small catheter placed in the lumbar region for collection of CSF. The dura was sutured so that no leakage would occur. A branch of the right external jugular vein was cannulated for collection of venous blood draining from the head. By means of an 18 gauge needle, 2.5 ml of CSF was withdrawn from the cisterna magna, capping the needle afterwards. In five animals the CSF was added to a test tube containing 1 μC ³H-PGF₂α (New England Nuclear) plus 200 μg PGF₂α, an amount which will consistently produce spasm when injected intracisternally.² After mixing thoroughly, the sample was reinjected via the same needle into the cisterna magna. Only trace amounts of radioactivity remained in the tube. Subsequently, samples of CSF were obtained simultaneously from the cisterna magna and lumbar catheter and blood was obtained from the jugular vein. These samples were taken at 1, 2, 4, 8, 15, 30 and 60 minute intervals and hourly thereafter for a period of 6 hours. One-tenth ml of CSF and blood were added to liquid scintillation vials, digested with perchloric acid and hydrogen peroxide, and counted in a liquid scintillation spectrometer (Nuclear Chicago Unilux II).

Quantitation of PGF₂α was accomplished by gas chromatography/mass spectroscopy (GC/MS) using our method reported previously.⁴ Accordingly, deuterated PGF₂α was added as a carrier and internal standard. The procedure involves solvent extraction, methylation, purification on a silicic acid column and quantitation by GC/MS after formation of the corresponding tris trimethyl silyl derivative. Areas of the peak, as well as peak heights, for ions at 423 and 427 were computer integrated. Using a standard curve with reference samples, the prostaglandin was quantified. If the volume of CSF was 20 ml the lower limit of sensitivity was approximately 50–60 pg/ml. With 10 ml CSF the lower limit was 100 pg/ml.

FROM THE DEPARTMENT OF PHARMACOLOGY AND NEUROSURGERY, 800 MADISON AVE., UNIVERSITY OF TENNESSEE CENTER FOR THE HEALTH SCIENCES, MEMPHIS, TN, AND DEPARTMENT OF BIOCHEMISTRY, MICHIGAN STATE UNIVERSITY, EAST LANSING, MI.
TABLE 1  Concentrations of PGF$_{2a}$ in Lumbar CSF Samples from Patients with Subarachnoid Hemorrhage

<table>
<thead>
<tr>
<th>Case No.</th>
<th>Day from Surgery</th>
<th>PGF$_{2a}$ (cpm/0.1 ml)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>+1</td>
<td>116</td>
<td>Minimal neurological deficit</td>
</tr>
<tr>
<td>2.</td>
<td>+1</td>
<td>ND*</td>
<td>Severe neurological deficit (died)</td>
</tr>
<tr>
<td>3.</td>
<td>+1</td>
<td>1300</td>
<td>Severe neurological deficit</td>
</tr>
<tr>
<td>4.</td>
<td>+4</td>
<td>1327</td>
<td>High spinal RBC count at time of elevated</td>
</tr>
<tr>
<td>5.</td>
<td>+7</td>
<td>2187</td>
<td>Severe neurological deficit</td>
</tr>
<tr>
<td>6.</td>
<td>-1</td>
<td>ND</td>
<td>Mild neurological deficit</td>
</tr>
<tr>
<td>7.</td>
<td>-4</td>
<td>ND</td>
<td>No neurological deficit</td>
</tr>
<tr>
<td>8.</td>
<td>+3</td>
<td>ND</td>
<td>No neurological deficit</td>
</tr>
<tr>
<td>9.</td>
<td>+6, +8</td>
<td>ND</td>
<td>Died +6 days</td>
</tr>
<tr>
<td>10.</td>
<td>+1, +3</td>
<td>ND</td>
<td>Had pneumonia</td>
</tr>
<tr>
<td>11.</td>
<td>+5, +8</td>
<td>ND</td>
<td>Severe neurological deficit</td>
</tr>
</tbody>
</table>

Control Range = ND-73 pg/ml (N = 5)

*ND = non-detectable.

Results

Human Studies

As summarized in table 1, PGF$_{2a}$ is usually elevated in lumbar CSF in patients who have suffered SAH. However, it is not possible to finitely associate the clinical status of the patient with these levels even when serial samples were taken. Nevertheless, the data indicate that at some time during the course of the disease most of those patients (6 of 7) manifesting mild to severe neurological deficits contained markedly elevated levels of this prostaglandin in the lumbar fluid.

Animal Experiments

Prostaglandin F$_{2a}$ injected intracisternally rapidly disappeared from the site of injection having a half-time of approximately 8 minutes, (fig. 1-A). Some transport of radioactivity to the lumbar region occurred, although the levels in most remained low (approximately 150-300 dpm per 0.1 ml CSF throughout the period of observation, fig. 1-B). In one animal a rise in lumbar PGF$_{2a}$ of greater magnitude was noted. In this animal the rise began at approximately 8 minutes and reached a plateau of approximately 5000-6000 dpm per 0.1 ml. This rise lasted approximately 30 minutes to one hour. Concurrently, PGF$_{2a}$ rapidly disappeared from the injection site. When 2.0 ml of blood was substituted for CSF and injected simultaneously with the prostaglandin, much the same data were obtained. However, the presence of blood reduced the radioactivity present in samples from the cisterna magna even though the same concentration was injected, (fig. 2-A). Again the appearance of radioactivity in the lumbar CSF was low, 200 to 800 dpm per 0.1 ml, although it was slightly higher than when blood was omitted, (fig. 2-B). A trend was also noted in most of the animals injected with blood in that a rise in radioactivity in lumbar CSF occurred with time.

Samples of blood taken from the jugular vein at the same time the CSF samples were obtained revealed the presence of radioactivity, indicating that PGF$_{2a}$ or metabolites are transported from the CSF to the peripheral circulation. This radioactivity appeared within minutes after the intracisternal injection and ranged from 50 to 250 dpm per 0.1 ml of blood during the first 4 hours. Between the fourth and sixth hour, the dpm in blood rose gradually to reach levels of 100 to 1,000 per 0.1 ml. Although these dpm values are relatively minuscule, the early appearance of this radioactivity, coupled with the volume of venous return occurring throughout the experimental period, suggests that the amount of the radioactive substance transported by this route must be biologically significant.

Discussion

Whether prostaglandins play a role in the subarachnoid hemorrhage complex is still not established. Many of the reported actions of prostaglandins suggest a contribution to the symptoms of SAH; hypertension, fever, cerebral edema, and vasospasm. These vasoactive lipids are synthesized by cells in the area of hemorrhage; e.g., platelets, brain, and cerebral vessels. Furthermore, synthesis of prostaglandins is enhanced by a variety of specific blood born substances which can be released during hemorrhage such as thrombin, catecholamines, serotonin, and bradykinin. This could explain why the onset of the more severe symptoms associated with SAH is delayed. Likewise, degradation of prostaglandins by cerebral tissue also appears to be limited which could contribute to a prolongation of activity.

The present study confirms earlier reports of an elevation of PGF$_{2a}$ in CSF of patients having SAH. The degree of elevation, however, varied day to day within the same patient and some patients did not manifest this rise. Six of seven patients with mild to severe neurological deficits had elevated PGF$_{2a}$ values whereas 3 of 4 with minimal or no deficit had low or normal values. More samples and a better knowledge of factors which affect production and distribution of prostaglandins throughout the CSF may strengthen this correlation. Likewise, peak levels may occur only at the time of neurological damage. Moreover, there are numerous prostaglandins, and related substances, which also may contribute to these symptoms. In this regard, CSF levels of PGE$_{2}$, which is also spasmogenic, can vary independently with PGF$_{2a}$ and in some patients may be the only one of
these prostaglandins detected.\textsuperscript{7} Also, thromboxane A\textsubscript{2} may be continuously synthesized and, in spite of its short half-life, could cause spasm of the cerebral arteries.\textsuperscript{16}

Thus, it may be crucial to measure a whole profile of these lipid substances in order to fully understand the disease process. Their effects may be additive and certain ones may better reflect the clinical status of the patient. One finding which is consistent in all studies is that CSF levels of PGF\textsubscript{2\alpha} obtained from normal individuals is less than 100 pg/ml.

The results obtained in the dog clearly show that the concentration of PGs in the lumbar area does not accurately reflect those present intracranially and further indicate that prostaglandins, or their metabolites, ultimately egress from the CSF into the systemic circulation. Somewhat analogous to this observation is the report that procaine injected into the lumbar region may not appear in the cisterna magna while its metabolite, p-aminobenzoic acid, appears in blood,\textsuperscript{17} indicating local absorption. Consequently, unless the transport system for eliminating some substances from the CSF were impaired throughout the neuraxis, concentrations found at opposite ends of the subarachnoid space may differ markedly. Variations in the degree of such impairment may explain, in part, the variations in PG levels reported by various groups of investigators.\textsuperscript{4-7} Nevertheless, results obtained from the patient study show that lumbar levels of PGF\textsubscript{2\alpha} are likely to be inordinately high when neurological impairment is present.

Since the brain has limited capacity to absorb\textsuperscript{13,15} or metabolize\textsuperscript{16} prostaglandins but manufactures these substances in considerable quantities,\textsuperscript{11-19,20} the finding of PGF\textsubscript{2\alpha} or its metabolites in jugular blood supports the concept of others\textsuperscript{21} that prostaglandins are removed from the extracellular fluid of brain by an active process. The rapid egress from the CSF obtained, half time disappearance of 8 minutes, helps, therefore, to explain why lumbar levels of PGs in normal patients are consistently low\textsuperscript{5-7} (also see table 1). In subarachnoid hemorrhage, however, the normal flow of CSF could be impaired by the coagulum which might inhibit the efflux of prostaglandins and other spasmogens from the CSF. This, coupled with the aforementioned increase in

---

**Figure 1.** A. Disappearance of \(^{13}H\)-PGF\textsubscript{2\alpha} following injection into the cisterna magna. B. Appearance of radioactivity in lumbar CSF following the injection of \(^{13}H\)-PGF\textsubscript{2\alpha} into the cisterna magna.

**Figure 2.** A. Disappearance of \(^{13}H\)-PGF\textsubscript{2\alpha} following injection into the cisterna magna in the presence of 2.0 ml of blood. B. Appearance of radioactivity in lumbar CSF following the injection of \(^{13}H\)-PGF\textsubscript{2\alpha} into the cisterna magna in the presence of 2.0 ml of blood.
PG synthesis, could ultimately elevate levels of PGs in the lumbar fluid. In most patients lumbar levels are significantly elevated\textsuperscript{5-7} (also table 1). Further studies may indicate which prostaglandin best reflects the pathophysiological condition of the patient and what variables in treatment and pathology alter spinal levels of these substances. The present study indicates prostaglandins are removed by the systemic circulation and shows that the concentration of these substances can differ markedly throughout the subarachnoid space, a finding which helps explain the variation in PG levels reported clinically.

Acknowledgment

We would like to thank Dr. A. B. Sisco for his surgical assistance, Ms. Betty Hammett and Ms. Marion Johnson for their technical assistance and Jack Harten for his help with gas chromatographic-mass spectrometry measurements. PG\textsubscript{FK\textsubscript{16}}, (3, 3, 4, 4-\textsuperscript{2H\textsubscript{2}}) was generously supplied by Dr. Udo Axen of the Upjohn Company. This study was supported in part by USPHS Gr. NS06826 and RR00480.

References

5. La Torre E, Patrono C, Fortuna A and Grossi-Belloni D: Role of prostaglandin F\textsubscript{2\alpha} in human cerebral vasospasm. J Neurosurg 41: 293-299, 1974
7. Hagen AA, Gerber JN, Sweeney CC, White RP, Robertson JT: Plocecytosis and elevation of prostaglandins F\textsubscript{2\alpha} and E\textsubscript{2} in cerebrospinal fluid following intracranial injection of thrombin. Stroke 8: 236-238, 1977
Levels and disappearance of prostaglandin F2alpha in cerebral spinal fluid: a clinical and experimental study.
A A Hagen, J N Gerber, C C Sweeley, R P White and J T Robertson

*Stroke*. 1977;8:672-675
doi: 10.1161/01.STR.8.6.672

*Stroke* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1977 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/8/6/672

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/