Pleocytosis and Elevation of Prostaglandins \(F_{2a}\) and \(E_2\) in Cerebrospinal Fluid Following Intracisternal Injection of Thrombin

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SUMMARY A marked pleocytosis and increase in the levels of prostaglandin \(F_{2a}\) (PGF\(_{2a}\)) and prostaglandin \(E_2\) (PGE\(_2\)) were noted in cerebrospinal fluid of dogs within two hours following the intracisternal injection of thrombin. Quantitation of the prostaglandins (PG's) was done by gas chromatography-mass spectroscopy using deuterated PGF\(_{2a}\) and PGE\(_2\) as internal standards. Whereas the levels of these prostaglandins were below the sensitivity of the method in control animals, a marked increase was noted following thrombin. PGF\(_{2a}\) levels were 15-21 ng/ml and the PGE\(_2\) levels were 55-72 ng/ml. This concentration of the PG's is adequate to cause spasm of the cerebral vessels and could explain the spasm which occurs following the intracisternal injection of thrombin.

These two effects, a pleocytosis and elevation of PG levels, may be specific to thrombin.

A NUMBER OF STUDIES indicate that the genesis of cerebral vasospasm is complex. Many substances derived from brain tissue\(^1\) or from blood\(^4\) produce cerebral vasospasm experimentally. The fact that thrombin\(^6\) is among these seems of special interest because of its use in neurosurgery.

The spasm generated by thrombin is delayed in onset, suggesting that thrombin might be acting indirectly, possibly by stimulating the synthesis and/or release of some spasmodogen.\(^6\) The present study was undertaken to elucidate the mechanism(s) by which thrombin might induce cerebrovascular spasm.

The results clearly show that two hours following the intrathecal injection of thrombin there is a marked pleocytosis suggesting an inflammatory response as well as a significant increase in the concentrations of prostaglandins \(F_{2a}\) (PGF\(_{2a}\)) and \(E_2\) (PGE\(_2\)) in cerebrospinal fluid (CSF).

These findings are of special significance because inflammation and cerebral vasospasm are clinical observations in patients with subarachnoid hemorrhage (SAH). Both PGF\(_{2a}\) and PGE\(_2\) are spasmodogenic on cerebral vessels\(^4\) and may be involved in the inflammatory process.\(^3\)\(^4\)

Materials and Methods

**Animals** — Mongrel dogs of both sexes weighing from 16–31 kg were anesthetized with pentobarbital sodium (30 mg/kg) intravenously.

**Thrombin Injection and Collection of CSF** — In the animals treated with thrombin four ml of CSF were removed from the cisterna magna via an 18 ga. spinal needle. One-half ml of the CSF was taken for cell counts and the remainder was mixed with 1,000 units of Thrombin N. F. With the needle left in place the CSF containing the thrombin was reinjected into the cisterna magna and the spinal needle removed. At the end of two hours a second cisternal puncture was made and 10–19 ml of CSF withdrawn. The first and last 0.5 ml fraction collected was taken for cell counts. The remainder was added to glass tubes containing 1.9 mg EDTA and stored at \(-40^\circ\) C until analyzed for PGE\(_2\) and PGF\(_{2a}\).

**Saline, PGE\(_2\) or Air Injection** — The procedure used was similar to that described above except that 0.5–1.0 ml of CSF was replaced with an equal volume of either 0.9% saline, 5.0% saline or 7.2 \(\mu\)g PGE\(_2\) dissolved in 1.0 ml of saline. In the dogs receiving an air embolus a total of 5 ml air was injected.

**Extraction of Prostaglandins** — Deuterated PGF\(_{2a}\) and PGE\(_2\) were added to all samples of CSF and the pH adjusted to 9.0 with 1N NaOH. The samples were initially extracted with hexane, 10 ml \(\times\) 2, discarding the hexane. After adjusting the samples to pH 3.5 with 1N HCl, extraction was carried out with chloroform, 10 ml \(\times\) 3. The chloroform was evaporated to dryness and the samples were methylated with a diethyl ether-diazomethane solution. Further purification was accomplished with a silicic acid column. A solution of the methylated derivative in benzene/ethyl acetate (9:1) was placed on the column and nonpolar contaminants were eluted with 25 ml benzene/ethyl acetate (9:1). The prostaglandins were then eluted from the column with 25 ml ethyl acetate/methanol (9:1). Prior to quantitation the samples were dried and treated with a solution of benzene/piperidine/N-trimethylsilylimidazole (TSIM) (1:1:1). Under these conditions the PGF\(_{2a}\)-ME is converted into the corresponding tri-TMS derivative while PGE\(_2\)-ME is converted into mono-TMS derivative of prostaglandin B\(_2\)-ME.\(^4\)

**Quantitation of Prostaglandins** — The prostaglandins were quantitated by selected ion monitoring (SIM) using an LKB-9000 gas chromatograph/mass spectrometer which was interfaced to a PDP-8/E computer for data acquisition and reduction. Deuterated PGF\(_{2a}\) and PGE\(_2\) were used as the internal standards and carrier for these analyses.

The prostaglandin derivatives were separated by gas chromatography on 3% OV-210 (6' \(\times\) 3 mm glass coiled column) at 215\(^\circ\) C. The retention time for the mixture of PGF\(_{2a}\) and deuterated PGF\(_{2a}\) was approximately 3 minutes. The PGB\(_2\) derivatives derived from PGE\(_2\) and deuterated PGE\(_2\) had a retention time of approximately 9 minutes.

The computer automatically maintained optimal accelerating voltage during analyses, so the intensities of m/e 423 and 427, representing the proton and deuterium-labeled species of PGF\(_{2a}\) respectively, and intensities for...
**Results**

Two hours following the intracisternal injection of 1,000 units thrombin, the levels of PGF\(_2\alpha\) and PGE\(_2\) in CSF were markedly elevated (table 1). PGF\(_2\alpha\) rose from non-detectable levels (<0.1 ng/ml) in control animals to levels of 15–21 ng/ml in thrombin treated animals. PGE\(_2\) likewise showed a marked increase rising from non-detectable levels (<0.5 ng/ml) to levels of 55–72 ng/ml, approximately three times higher than PGF\(_2\alpha\).

In addition to the increase in prostaglandins, a marked pleocytosis was noted in dogs treated with thrombin (table 2). Prior to the treatment with thrombin the cell counts were in the normal range\(^1\) but there was a significant increase in cells after thrombin and a change in cell type with polymorphonuclear cells becoming more prominent.

When assayed for prostaglandins, only CSF from thrombin treated animals had elevated levels. Thus the action of thrombin is somewhat specific since no other treatment, including a bolus injection of PGE\(_2\) or air, caused a pleocytosis.

**Discussion**

Thrombin, when present in cerebral spinal fluid, is capable of initiating two effects: a marked pleocytosis suggesting an inflammatory response and an elevation in the levels of PGF\(_2\alpha\) and PGE\(_2\). These two observations are believed to be related.

Thrombin may be acting on cerebral tissues in a manner similar to an irritant leading to inflammation and subsequent release of PG's. Since prostaglandins are known to be released during inflammation\(^2\) and by polymorphonuclear leucocytes,\(^3\) it is possible that the increased levels were the result of this process. Such an indirect effect would explain the delay in onset of spasm that one sees following thrombin in contrast to the prompt spasm induced by PG's injected intrathecally.\(^4\) Thrombin has been demonstrated to cause severe endothelial injury to isolated strips of rabbit aorta with marked histological changes noted after 15 minutes of exposure.\(^5\) Similar changes could potentially occur in cerebral vessels.

It is also possible that thrombin is directly stimulating the synthesis of PGF\(_2\alpha\) and PGE\(_2\) by brain tissue or cerebral vessels since brain tissue has been shown to synthesize these two prostaglandins\(^6\) as has bovine mesenteric blood vessels.\(^7\) Thrombin may be a potent stimulator of PG synthesis in many tissues since it is known that thrombin stimulates PG synthesis by platelets\(^8\) and recently a similar action was demonstrated in fibroblasts maintained in tissue culture.\(^9\) This action of thrombin may be specific since 0.9% saline, hypertonic saline, or air embolism does not elicit a similar action in our experiments.

It is unlikely that the elevation of PG's was due to their presence in the thrombin preparation since these should be removed during purification. Moreover, preliminary experiments show that exogenous PG's rapidly disappear from the site of intrathecal injection, having a half time of approximately 8 minutes. Therefore, the elevated levels observed 2 hours after the injection of thrombin is most likely due to endogenous synthesis.

Whether these experimental findings are applicable to what is seen in patients with subarachnoid hemorrhage is problematic. It has been reported\(^10\) that 1 ml of blood generates 300–360 units of thrombin so 3 ml of blood released during hemorrhage could generate approximately as much thrombin as used in these studies, 1,000 U. Experimentally, 2 ml of blood or as little as 100 units thrombin injected intrathecally are capable of eliciting spasm.\(^6\) Thus, it seems reasonable that a similar process could occur in patients as demonstrated in these experiments. The inflammation and cerebral vasospasm which occurs in patients following subarachnoid hemorrhage could be explained, in part at least, by the release of thrombin and subsequent production of PG's.

In spite of the mechanism(s) involved, the results suggest that the use of thrombin during intracerebral surgery should be done with caution.

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Experimental Model for Systematic Study of Impaired Microvascular Reperfusion

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SUMMARY A technique is described for reliably producing quantifiable impairment of microvascular reperfusion of the brain after ischemia in dogs. The technique is derived from an analysis of the cerebrospinal fluid compression reperfusion model as a Starling resistor. It is proposed that this model would be useful in a systematic study of post-ischemic impairment of reperfusion.

IN DEATHS due to dysbaric cerebral air embolism observed by the authors, a course suggesting progressive impairment of microcirculatory reperfusion has repeatedly occurred. Recompression to a simulated 165 feet in a chamber has usually been delayed for at least 5 minutes in these cases but, when instituted, should have eliminated the mechanical obstruction of cerebral vessels by bubbles. During the 15 to 30 minute interval at 165 feet called for by standard Navy treatment tables, these patients characteristically regained consciousness and demonstrated varying degrees of recovery. However, beginning about 30 minutes to 2 hours after the initial ictus, the clinical state progressively deteriorated, often with the patient still under pressure breathing hyperbaric oxygen. The deterioration was refractory to further recompression.

It follows that an experimental model permitting study of the factors that influence the adequacy of post-ischemic microcirculatory reperfusion would have potential therapeutic relevance. Models of cerebral ischemia in animals abound. Most of these models involve preliminary surgery which ranges from major to mutilating. The consequent tissue damage introduces the probability of alteration in the organism which could theoretically affect the experimental outcome, a circumstance for which the term "physiologic reactance" has been used. Indeed, Bergent: has demonstrated circulating platelet aggregates in the hamster cheek-pouch minutes after crush injury to the thigh and has classified into 4 phases the serial response of blood to tissue injury. Borst: has noted intravascular aggregation of red cells 1-3 hours after blunt leg trauma in rabbits. Three to six hours after the same trauma an increase tendency for thrombus formation in ligated veins occurred. The latency for all of these changes was much shorter in the vicinity of the tissue damage.

Most of the surgical trauma can be avoided in animals. Several studies have in...
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