We studied the ex vivo production of prostaglandin D$_2$, prostaglandin E$_2$, 6-ketoprostaglandin F$_{1a}$, and leukotriene C$_4$ in the brain tissue of rats subjected to experimental subarachnoid hemorrhage. The ex vivo method allows the study of arachidonic acid metabolites released from brain slices at different times after subarachnoid hemorrhage induction and reflects the residual capacity for arachidonic acid metabolism after the pathologic event. The rats were sacrificed 30 minutes, 1 and 6 hours, and 2 days after subarachnoid hemorrhage was induced by the injection of 0.30 ml autologous arterial blood into the cisterna magna. Concentration of prostaglandin D$_2$ and 6-ketoprostaglandin F$_{1a}$ was increased significantly relative to control 2 days after induction. The concentration of prostaglandin E$_2$ was increased significantly 6 hours after induction, while ex vivo production of leukotriene C$_4$ was increased significantly at 1 and 6 hours and 2 days. The correlation between these results and the occurrence of vasospasm after subarachnoid hemorrhage is discussed. The results obtained from the ex vivo incubation of brain tissue slices after experimental subarachnoid hemorrhage suggest that after the hemorrhage there is a significant modification of brain eicosanoid metabolism, which could be of great importance in interpreting the pathogenesis of subarachnoid hemorrhage-related neuronal impairment. (Stroke 1990;21:328–332)

Most experimental studies of subarachnoid hemorrhage (SAH) have provided data about pathophysiologic aspects of arterial vasospasm, but neurochemical patterns of neuronal damage caused by the direct effect of bleeding and local modifications in cerebral blood flow (CBF) have not been investigated thoroughly. Experimental and clinical studies have demonstrated that, after aneurysm rupture, modifications of CBF and cerebral metabolism are evident and that neurologic deterioration due to arterial vasospasm complicates the clinical course in 20–30% of cases. From previous studies of AA metabolism in experimental cerebral ischemia and reperfusion have shown that the accumulation of fatty acids, mainly AA, due to the breakdown of structural membrane lipids leads to significant variations in the concentrations of prostaglandins (PGs) in brain tissue. In the instance of aneurysmal SAH, an active AA metabolism via the lipoxygenase and cyclooxygenase pathways has been suggested from data obtained in human cisternal cerebrospinal fluid (CSF), but only the vascular effects of these compounds, with special consideration of the pathogenesis of cerebral vasospasm have been considered. Furthermore, each eicosanoid could derive from a different source, but previous studies have mainly investigated the synthesis and release of AA metabolites from the arterial wall and subarachnoid blood clots, without a complete view of brain biosynthesis. Some important questions arise from the available data: 1) Could the elevated concentration of AA metabolites in the CSF be related...
to a temporary accumulation of arachidonate or to an in vivo enhanced synthesis in the brain? 2) Is the brain cortex a possible source of selected eicosanoids in response to the hemorrhagic insult, and are the eicosanoids involved in some pathophysiologic mechanisms of this response? and 3) Is the enhancement of AA metabolism well correlated with time-dependent CBF variations, with delayed vasospasm occurrence, and with brain hypoxic damage? Kempski et al.12 have recently addressed a similar question, studying the postischemic production of PGs in experimental cerebral ischemia, using a new ex vivo method that provides information about the residual capacity of brain tissue to synthesize AA metabolites in vitro, reducing the effect of all components and of any initial accumulation of AA. We try to answer these questions by investigating the ex vivo production of four selected metabolites: PGD2, PGE2, 6-keto-PGF1α (the stable metabolite of prostacyclin), and leukotriene C4 (LTC4) (an AA metabolite formed via the lipoxygenase pathway) after experimental SAH in rats.

Materials and Methods

We conducted experiments on male Sprague-Dawley rats (Charles River strain, Calco, Como, Italy) weighing 375–425 g using the experimental SAH procedure according to Solomon et al,23 with a few modifications.24 General anesthesia was induced with 3% halothane in 70% N2O and 30% O2 and was maintained with 0.75% halothane in the same gas mixture. A burr hole at the interparietal/occipital suture connection was made using a refrigerated twist-drill. A small catheter (PB-10, Clay-Adams, Parsippany, New Jersey) was inserted into the cisterna magna and its placement assessed by testing (under magnification) the lower distal part of the catheter through the atlanto/occipital membrane. A femoral artery was cannulated for anaerobic sampling of blood to measure arterial pH, PaCO2, and PaO2 while the arterial blood pressure was monitored with an indirect blood pressure sensor (446420, Bel-Art Products, Pequannock, New Jersey). Body temperature, monitored by a rectal thermometer, was adjusted and maintained near 37°C by external heating. When the rats were in a steady respiratory state (PaO2 and PaCO2 90 and 35–40 mm Hg, respectively), 0.35 ml autologous arterial blood was collected from the femoral artery, and an aliquot of 0.30 ml was injected into the cisterna magna via the catheter within approximately 2 minutes. Before SAH induction, a CSF sample of approximately 0.01–0.03 ml was gently drawn to limit changes in intracranial pressure. The rats were held in a 20° head-down position.

We divided the rats into experimental groups containing six to eight animals each. Sham-operated rats were submitted to the surgical procedure and injected with 0.30 ml saline at 37°C, and SAH rats were subjected to the surgical procedure and injected with 0.30 ml autologous arterial blood; biochemical evaluations were performed 30 minutes, 1 and 6 hours, and 2 days later. The rats were decapitated, and the brains were carefully removed and immediately frozen in dry ice and maintained in a prerefrigerated glove box at −22°C. Cortex slices weighing approximately 10–15 mg were bound-cut, weighed, and placed immediately in tubes containing 1 ml oxygenated (95% O2 and 5% CO2) Krebs’ solution, pH 7.4, of the following millimolar composition: NaCl 118, KCl 4.7, MgSO4 7, H2O 1.2, KH2PO4 1.2, NaHCO3 25, and 1 g/l glucose. The tubes were tightly closed. The interval between decapitation and the beginning of incubation was approximately 3 minutes.

The slices were incubated at 37°C in a shaking water bath for up to 1 hour. At the end of the incubation, the supernatant was decanted and centrifuged at 3,000 rpm and 0°C. Three aliquots of the supernatant were kept at −80°C until analysis. Levels of AA metabolites were determined using radioimmunoassay (RIA) techniques for PGD2 as previously described in detail25,26, and RIA kits for PGE2 (NEK-020) and 6-keto-PGF1α (NEK-008) (New England Nuclear Chemicals GmbH, Dreieich, F.R.G.). The radioactive labels were iodine-125 for PGE2 and tritium for 6-keto-PGF1α; antiserum for these two metabolites had a cross-reactivity of <2.5% with other PGs. Immunoreactive LTC4-like activity (LTC4) was detected with the RIA technique according to Levine et al,27 using an antiserum (New England Nuclear Chemicals) to LTC4 that has a cross-reactivity of 10.1% with LTD4, 2.3% with LTE4, 0.07% with hydroxyeicosatetraenoic acid, and 0.006% with LTD4. Ten milliliters of Atomlight High Capacity Sample Scintillation Solution (NEF-968) (United Technologies Packard, Packard Instruments, Downers Grove, Illinois) was added to each sample. Radioactivity was measured using a liquid scintillation spectrometer (Model 3320, Packard Instruments) as previously described.17,28 Results are expressed as nanograms per milligram protein. Protein content was determined according to the method of Lowry et al.29 The assay sensitivity was 15 pg/mg protein.

Statistical analysis was performed using analysis of variance and Tukey's test for multiple comparisons. Statistical significance was accepted for p<0.05.

Results

We injected saline into our sham-operated control rats to mimic the changes in intracranial pressure due to the injection of autologous blood in the SAH rats. In a separate experiment (F. Marzatico, unpublished data) we showed that the ex vivo release of AA metabolites in rats without manipulation does not differ significantly from that in sham-operated rats.

Figure 1 presents the time-dependent ex vivo release of PGD2 at four times after SAH induction. The increase in PGD2 concentration was significant (p<0.01) compared with control only 2 days after the procedure.

Downloaded from http://stroke.ahajournals.org/ by guest on April 14, 2017
Figure 1. Bar graph of mean±SEM time-dependent ex vivo release of prostaglandin D\(_2\) (ng/mg of protein) in sham-operated control rats (open bars) and rats subjected to experimental subarachnoid hemorrhage (filled bars). ***p<0.01 different from sham-operated by Tukey's test.

Figure 2 shows a significantly increased (p<0.01) concentration of PGE\(_2\) 6 hours after SAH induction, while at 2 days the concentration had returned to control levels.

Concentration of the stable metabolite of prostacyclin 6-keto-PGF\(_{1\alpha}\) was significantly increased (p<0.05) only 2 days after SAH induction (Figure 3).

Formed via the lipoxygenase pathway, LTC\(_4\) showed an increase in ex vivo release early after SAH (p<0.01); concentrations remained significantly elevated at 6 hours and 2 days (Figure 4).

Discussion
The pathogenesis of arterial vasospasm and neuronal damage is considered to be multifactorial, and the enhancement of AA metabolism seems to have a crucial role.\(^3\)\(^0\) We evaluated the ex vivo release of four selected AA metabolites from the brain cortex of rats subjected to experimental SAH. This method makes it possible to limit the accumulation of AA in the brain cortex because most is washed out by the initial procedure before incubation of the tissue samples.\(^1\)\(^2\) We measured the ex vivo concentration of the metabolites 30 minutes 1 hour 6 hours, and 2 days after SAH to study their ex vivo release from brain cortex over time and to correlate the results with the occurrence of vasospasm and modifications in CBF. Delgado et al\(^3\)\(^1\) have evidence of a biphasic pattern of arterial vasospasm after experimental SAH in rats. The acute phase is evident 10—15 minutes after SAH and is followed in 2 days by the so-called late phase of vasospasm, which is more important from a metabolic point of view.\(^3\)\(^2\) Otherwise, our results demonstrate that AA metabolism is significantly enhanced in the brain after SAH and that the cyclooxygenase and lipoxygenase pathways are both activated. In previous studies, we had measured the level of eicosanoids in samples of cisternal CSF from patients operated on for cerebral aneurysm (not earlier than 10 days after the last SAH) to verify the importance of subarachnoid blood clot as a possible source of spasmogens during the degradation of blood components, we found that the level of eicosanoids in the cisternal CSF was significantly higher than that in the lumbar CSF and that the levels of LTC\(_4\) and PGD\(_2\) in the CSF correlated well with the incidence of vasospasm.\(^1\)\(^7\),\(^2\)\(^6\),\(^2\)\(^8\) In our present study, the biosynthesis and release of PGD\(_2\) and LTC\(_4\) were significantly higher 2 days after SAH induction because of the enhancing effect of subarachnoid blood on AA metabolism in the brain cortex. Moreover, at this time there was greater availability of AA due to
vasospasm and modifications in CBF. The results of our study suggest that, aside from degradation of subarachnoid blood clot, the hemorrhagic event could enhance AA metabolism even in the cerebral cortex. Furthermore, the biosynthesis and release of specific eicosanoids from the cerebral cortex has a prominent role; the release of PGD₂, which is largely derived from brain parenchyma³³ and has a vasoconstrictor effect on cerebral arteries, is significantly higher 2 days after SAH. Thus, the significantly higher concentrations of PGD₂ in the cisternal CSF of patients developing vasospasm²⁶,²⁸ and the late phase of vasospasm in experimental SAH seem to be correlated with the significantly higher ex vivo release of this metabolite late after SAH (Figure 1). The results of LTC₄ release deserve some adjunctive comments.

The biosynthesis and release of LTC₄ after experimental SAH have been demonstrated in the gray matter,³⁵ in the hypothalamus,³⁶ from subarachnoid blood clot, and from granulocytes, mainly during hypoperfusion.³⁷ Recently Yokota et al³⁸ demonstrated that the increased biosynthesis and release of LTs from subarachnoid blood clot is related to the development of arterial spasm in a canine model of SAH. We investigated the response of rat brain cortex to subarachnoid blood and we found that LTC₄ biosynthesis and release are long-lasting, with an early activation at 1 hour after SAH and a steady state until 2 days after SAH. Asano et al³⁹ demonstrated in a different experiment that lipoxygenase activity in the basilar artery is significantly enhanced after SAH, with a characteristic peak corresponding to the biphasic course of vasospasm. Data indicating biosynthesis and release of LT in the cerebral cortex for a long time after SAH are quite different from the observations in experimental brain ischemia; cortical synthesis of LT occurs within few minutes after reperfusion.³⁷ These data support the hypothesis of Asano et al³⁰ of an early activation of nonenzymatic lipid peroxidation (from subarachnoid blood clot degradation products) and the subsequent activation and stimulation of the lipoxygenase pathway in the vascular and cerebral compartments. Moreover, the chemotactic action of LTC₄ could be considered an important factor in the development of an inflammatory response of the arterial wall, leading to subintimal edema, cell infiltration of the subarachnoid space, and the subintimal proliferation of leukocytes. Moreover, when leukocyte adhesion and leakage occur in the subarachnoid space, the synthesis and release of lipoxygenase products are activated with a self-maintaining mechanism; therefore, LT could exert a long-lasting vasoconstrictor effect and an inhibitory effect on some vasodilating compounds such as prostacyclin.⁸

Changes in 6-keto-PGF₁α biosynthesis and release have been studied only in experimental cerebral ischemia²³; the variation of its synthesis has been reported to be region-specific (in the hippocampus) and closely time-dependent (increased 30 minutes after reflow, and decreased to 60% of control at 24 hours). Prostacyclin is involved in regulation of the microcirculation, is synthesized by endothelial cells, has a marked vasodilating effect,¹⁸,³⁶ and prevents blood-brain barrier derangement³⁹ and impairment due to postischemic brain reperfusion.⁸,⁴⁰ In our present study, the release of 6-keto-PGF₁α was significantly reduced 1 and 6 hours after SAH; we hypothesize that at this time the endothelial damage caused by the SAH exerted an inhibitory effect on cyclooxygenase enzyme in the arterial wall.

PGF₂α is considered a potent vasoconstrictive agent,⁸,²⁰,⁴¹ and its production in the arteries is enhanced after experimental SAH.¹⁵,⁴¹ This metabolite has also been related to the presence of macrophages in the subarachnoid space⁴² and to brain edema.¹²,⁴⁳ The peak of PGF₂α concentration 6 hours after SAH that we observed could reflect increased biosynthesis of the metabolite during the acute phase.

In conclusion, our results show that 1) AA metabolism is enhanced after experimental SAH, via both the cyclooxygenase and the lipoxygenase pathways; the changes in the release of AA metabolites are time-dependent; 2) the biosynthesis and release of AA metabolites from brain tissue should be considered an important neurochemical pattern of brain response to SAH together with release of eicosanoids from blood vessels and blood clot; 3) the activation of the lipoxygenase pathway seems to play a primary role in the local brain response to SAH, showing an early activation followed by a long-lasting release of LTC₄; 4) the synthesis and release of PGD₂ derives mainly from the brain cortex and peaks late (2 days) after SAH, which relates well with the occurrence of arterial spasm. Aside from the problem of prevention and reversal of vasospasm, management strategies for patients suffering from SAH must also take into account the characteristic neurochemical patterns of brain response to the hemorrhage to provide the necessary brain protection. The effects of brain protective agents (calcium channel blockers and high-dose methylprednisolone) on lipid peroxidation and AA metabolism is the target of ongoing studies using this model of SAH.

Acknowledgment

The authors gratefully recognize the help of Ms. I. Fugaccia in preparing the manuscript.

References

Arachidonic acid metabolism and pathophysiologic aspects of subarachnoid hemorrhage in rats.

P Gaetani, F Marzatico, R Rodriguez y Baena, L Pacchiarini, T Viganò, G Grignani, M T Crivellari and G Benzi

Stroke. 1990;21:328-332
doi: 10.1161/01.STR.21.2.328

Stroke is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 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/21/2/328

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/