Cerebral Arterial Smooth Muscle Contraction by Thromboxane A2

EARL F. ELLIS, PH.D., ALAN S. NIES, M.D., AND JOHN A. OATES, M.D.

SUMMARY The contractile effects of thromboxane A2 (TXA2), a labile arachidonic acid metabolite, were studied in arterial smooth muscle strips. TXA2 was generated upon the addition of 255 mM prostaglandin cyclic endoperoxide H2 to human platelet particles in the muscle bath. Using the isometric contraction produced by 40 mM K+ in isotonic saline as the reference contraction, bovine middle cerebral artery strips contracted to 153 ± 14% of the reference response while bovine coronary and porcine coronary, renal and common carotid strips contracted to 47 ± 3, 26 ± 5, 43 ± 2 and 2 ± 1% of reference, respectively. The cerebral arterial response to the TXA2 generating system was as great as the maximum response to prostaglandin F2α and two times the maximum response to 5-hydroxytryptamine. Because TXA2 is formed by brain tissue and released from aggregating platelets, it may be important in the pathogenesis of spasm associated with injured brain tissue or pathologic changes leading to platelet aggregation.

THE EXACT CAUSE of cerebral arterial spasm associated with subarachnoid hemorrhage is unknown; however, substances released from or by blood have been implicated.1-4 Prostaglandin F2α (PGF2α) and 5-hydroxytryptamine (5HT), which are released from brain tissue and aggregating platelets, have been suggested as possible chemical mediators of spasm.5-6

Recent advances in prostaglandin research have suggested that arachidonic acid metabolites other than prostaglandin F2α may be involved in the genesis of vasospasm. Prostaglandin cyclic endoperoxides G2 and H2 (PGG2, PGH2), which are intermediates in the formation of prostaglandin E2, F2α and D2 (PGE2, PGF2α, PGD2), have also been shown to be the precursors of the very labile thromboxane A2 (TXA2) and its stable metabolite thromboxane B2 (TXB2) (fig. 1).7 Importantly, the conversion of PGG2 and PGH2 to the thromboxanes is now known to be the preferential metabolic pathway in platelets, brain, and lung with PGF2α, PGE2 and PGD2 being minor metabolic products.8-10 PGG2, PGH2 and TXA2 are not only released during platelet aggregation, but can induce platelet aggregation.11-14 PGG2 and PGH2 have a 5 minute half-life in aqueous medium while that of TXA2 is only 32 seconds.11

PGG2, PGH2 and TXA2 have been shown to cause contraction of isolated rabbit aortic smooth muscle.5,11,18 Recently, we have shown that TXA2 released from aggregating human platelets is capable of inducing contraction of isolated, porcine coronary artery strips.14 The vasoactivity of platelet released TXA2, coupled with the fact that TXA2 is produced by brain tissue, has led us to investigate the effects of TXA2 on cerebral arterial smooth muscle. It was felt that TXA2 induced vasoconstriction might be relevant to the genesis of cerebral transient ischemic attacks or the vasospasm of subarachnoid hemorrhage, where platelet aggregation or brain injury has occurred.

Methods

Fresh bovine and porcine tissue from a local slaughter house was transported in 4°C Krebs-Ringer bicarbonate solution.14 The arteries used were taken from the following anatomic locations: 1) the left coronary artery and proximal two thirds of the left anterior descending artery, 2) the distal renal artery and its first intra-renal branches, 3) the distal one-half of the common carotid, and 4) the middle cerebral. Arteries were isolated and spirally cut to produce strips approximately 2 cm long and 1.5 mm wide. The strips were then placed in 3 ml baths containing 37°C Krebs-Ringer bicarbonate buffer saturated with 95% O2 - 5% CO2 and containing 8 μM indomethacin, which inhibited endogenous prostaglandin synthesis. After 3 hours of equilibration, changes in isometric tension were measured in strips which had been pre-loaded with 1.25-1.50 grams of tension. Before the experiment, all arteries were tested with 5-HT or norepinephrine to insure normal drug reactivity. Experimental contractile responses are expressed relative to the maximum tension developed by 40 mM K+ substituted for an equivalent concentration of Na+ in Krebs-Ringer bicarbonate solution. After obtaining dose response curves for 5-HT, the artery strips were treated with the 5-HT antagonist methysgeride (2.83 μM) and tested with 5-HT (5.7 μM) to insure completeness of blockade. This was done to prevent action of any 5-HT released from platelet particles used in the TXA2 generating system. A concentrated stock solution of PGF2α, tromethamine salt (Upjohn Co.) was made by dissolving PGF2α in ethanol. Care was taken that the concentration of ethanol in the artery baths was less than 1 μl per ml.

Prostaglandin cyclic endoperoxide G2 and H2 were synthesized from sheep seminal vesicular gland microsomes, isolated and structurally confirmed as reported by Hamberg et al.11 We used the particulate fraction from human platelets to convert cyclic endoperoxides into TXA2. Blood was drawn from normal volunteers who had not had aspirin or other drugs for at least one week. Washed platelets were prepared11 and subsequently frozen and thawed 3 times, homogenized and centrifuged at 2,000 x g for 15 minutes. The 2000 x g supernatant fluid was then spun at 100,000 x g for 1 hour, and the resulting pellet resuspended in a volume of Krebs-Ringer bicarbonate equivalent to one-fifth the washed platelet volume. Aliquots (0.1 ml) of the suspension of platelet particles were added to 2.9 mls of medium and preincubated at 37°C for 3 minutes before being transferred to the artery baths. The TXA2 generating system was started by the addition of 25.5 or 255 mM PGH2 to the baths containing the platelet particles. We have previously shown that this system generates TXA2 by measuring the formation of its stable metabolite, TXB2, in the artery baths.14

From the Departments of Medicine and Pharmacology, Vanderbilt University, Nashville, Tenn. Reprint requests: Earl Ellis, Ph.D., Department of Pharmacology, Vanderbilt University, Nashville, Tenn. 37232
CONTRACTILE EFFECTS OF TxA₂/Ellis et al.

Arachidonic Acid → Cyclo-oxygenase → Prostaglandin G₂, H₂ → (cyclic endoperoxides) → Prostaglandin E₂, F₂α, D₂ → Thromboxane A₂ → Thromboxane B₂

FIGURE 1. A simplified scheme of prostaglandin and thromboxane biosynthesis in platelets. Aspirin and indomethacin block the formation of cyclic endoperoxides by inhibiting cyclo-oxygenase. The major products formed from cyclic endoperoxides are Thromboxane B₂ and HHT (not shown, see ref. 8) while PGE₂, D₂, and F₂α are minor products. Arachidonic acid is also metabolized to HETE by the enzyme lipoxynenase which is not inhibited by aspirin or indomethacin (see ref. 8).

Attempts to generate greater concentrations of TxA₂ by using more cyclic endoperoxide substrate or platelet particle enzymes were technically unsuccessful for two reasons. First, higher substrate concentrations have a substantial effect on the vascular smooth muscle. Cyclic endoperoxide itself induces contraction of porcine arterial strips and so can PGE₂ which is spontaneously formed by isomerization of cyclic endoperoxide. Secondly, oxygen bubbling in the baths, which is needed to insure mixing as well as oxygenation, causes foaming when higher concentrations of platelet protein are added. Future studies are being directed toward isolating TxA₂ and purifying the enzyme necessary for conversion of cyclic endoperoxide to TxA₂.

Initial studies on vascular smooth muscle showed that contraction of the bovine middle cerebral artery strip produced by the TxA₂ generating system (platelet particles plus 255 nM PGH₂) was at least three times stronger than that of bovine coronary or porcine coronary, renal, or common carotid artery (fig. 2). The contraction produced upon addition of PGH₂ to the platelets in the bath reached a maximum in .9 to 1.5 minutes.

Because the cerebral artery response to TxA₂ was particularly strong, we compared the cerebral artery response to TxA₂ with that of PGF₂α and 5-HT, which have been suggested to be mediators of cerebral vasospasm. The cerebral contraction produced by the TxA₂ generating system, consisting of platelet particles plus 255 nM PGH₂, was twice that produced by 255 nM PGF₂α or 5-HT and six times that produced by 255 nM PGH₂ alone (fig. 3). TxA₂ generated by adding 255 nM PGH₂ to platelet particles, induced a contraction which was almost twice as great as the maximum attainable 5-HT (2.5 μM) contraction and was as great as the maximum PGF₂α (2.5 μM) induced contraction (fig. 4).

Addition of only platelet particles to the artery baths did not cause an increase in artery tension. TxB₂, the stable metabolite of TxA₂, was also tested on the cerebral strips. Unpurified TxB₂, formed by allowing platelet particles to react with 255 nM PGH₂ for 3 minutes, produced no cerebral artery contraction. However, TxB₂ extracted from this generating system and purified by high performance liquid chromatography induced a small, slowly developed increase in cerebral artery tension.

Results

Initial studies on vascular smooth muscle showed that contraction of the bovine middle cerebral artery strip produced by the TxA₂ generating system (platelet particles plus 255 nM PGH₂) was at least three times stronger than that of bovine coronary or porcine coronary, renal, or common carotid artery (fig. 2). The contraction produced upon addition of PGH₂ to the platelets in the bath reached a maximum in .9 to 1.5 minutes.

Because the cerebral artery response to TxA₂ was particularly strong, we compared the cerebral artery response to TxA₂ with that of PGF₂α and 5-HT, which have been suggested to be mediators of cerebral vasospasm. The cerebral contraction produced by the TxA₂ generating system, consisting of platelet particles plus 255 nM PGH₂, was twice that produced by 255 nM PGF₂α or 5-HT and six times that produced by 255 nM PGH₂ alone (fig. 3). TxA₂ generated by adding 255 nM PGH₂ to platelet particles, induced a contraction which was almost twice as great as the maximum attainable 5-HT (2.5 μM) contraction and was as great as the maximum PGF₂α (2.5 μM) induced contraction (fig. 4).

Addition of only platelet particles to the artery baths did not cause an increase in artery tension. TxB₂, the stable metabolite of TxA₂, was also tested on the cerebral strips. Unpurified TxB₂, formed by allowing platelet particles to react with 255 nM PGH₂ for 3 minutes, produced no cerebral artery contraction. However, TxB₂ extracted from this generating system and purified by high performance liquid chromatography induced a small, slowly developed increase in cerebral artery tension.

FIGURE 2. Contraction of arterial smooth muscle strips by the thromboxane A₂ generating system (platelet particles plus 255 nM PGH₂). The bars represent the mean response ± S.E. The number under the bar represents the number of artery strips.

FIGURE 3. Contraction of cerebral arterial smooth strips by 255 nM 5-HT, PGF₂α, PGH₂ and TxA₂. The thromboxane A₂ generating system consisted of platelet particles and 255 nM PGH₂. The bars represent the mean response ± S.E. The number under the bar represents the number of artery strips.
Discussion

Numerous studies of the physiology and pharmacology of the cerebral vasculature have been reported, yet controversy continues over factors which control normal and pathologic brain blood flow. It is likely that several chemical factors from blood and neural tissue, including 5-HT, prostaglandins and others, act together to control pathologic cerebral vascular smooth muscle tension. Our data show that thromboxane A₂ should be added to the list of potential chemical mediators of spasm associated with subarachnoid hemorrhage and brain injury.

While 5-HT can cause contraction of cerebral vascular smooth muscle both in vitro and in vivo, it is of particular interest since it is released by aggregating platelets. White et al. have shown 5-HT to cause cerebral vasospasm when injected intracisternally in relatively low doses. Our data show that at low doses bovine cerebral artery strips are more sensitive to 5-HT than PGF₂α. Similarly, Allen et al. have shown human cerebral strips to be more responsive to low concentrations of 5-HT than equimolar concentrations of PGF₂α. Also, this study demonstrates that the maximum bovine cerebral artery response to PGF₂α is two times stronger than the maximum response to 5-HT. Somewhat analogously, White et al. have reported that intracisternal injections of PGF₂α induced a more prolonged and more severe cerebral vasospasm than equimolar doses of 5-HT.

Our results show TxA₂ is an effective contractor of vascular smooth muscle from several major organs in different animal species. While it is effective on coronary and renal arteries, the extraordinarily powerful cerebral response make it of particular interest since TxA₂ is released from aggregating platelets and TxB₂ is the most abundant arachidonic acid metabolite synthesized by the brain. Continuous formation of TxA₂ might induce a prolonged spasm whereas acute TxA₂ formation and severe vasoconstriction could lead to intravascular thrombosis.

Our comparison of the cerebral response to TxA₂, PGF₂α, and 5-HT is somewhat hampered by the fact that the effective TxA₂ concentration existing in the artery baths at any particular time is unknown. While we use 25.5 or 255 nM cyclic endoperoxide as the substrate for the platelet particles, only a small fraction of the added cyclic endoperoxide is converted to and exists as TxA₂ at any particular instant. Therefore, less than 25.5 or 255 nM TxA₂ exists in the bath at any time, and, on a molar basis, TxA₂ is at least 10 times more powerful than 5-HT or PGF₂α in our test system.

We conclude that TxA₂ can contract cerebral arterial smooth muscle in vitro. These findings provide the basis for the following hypothesis: brain trauma or platelet aggregation in areas of damaged endothelium can release TxA₂ and thus cause constriction of cerebral arteries. This hypothesis may merit testing in conditions where cerebral spasm is known or postulated such as in subarachnoid hemorrhage and transient ischemic attacks.

Acknowledgments

This work was supported in part by grant GM-15431 from The National Institutes of Health. Prostaglandins and sheep seminal vesicles were generously provided by J. Pike of The Upjohn Company. Methysergide maleate was provided by Sandoz Pharmaceutical Company.

References

Relationship of Transient Ischemic Attacks and Angiographically Demonstrable Lesions of Carotid Artery

RONALD L. EISENBERG, M.D., WILLIAM R. NEMZEK, M.D., WESLEY S. MOORE, M.D., AND RICHARD L. MANI, M.D.

SUMMARY Eighty-eight percent of arteries in patients with amaurosis fugax or hemispheric transient ischemic attacks had angiographically demonstrable lesions at the carotid bifurcation. Eighty-one percent had stenoses or occlusions at the carotid bifurcation; 7 percent had ulcerative lesions without stenoses at this site. Forty-nine percent of arteries in these patients demonstrated ulcerative lesions with or without stenosis at the carotid bifurcation.

There was no significant difference in the incidence or types of ulcerations between those patients with amaurosis fugax and those with hemispheric transient ischemic attacks. Eighty-eight percent of arteries examined in this series were amenable to surgical reconstruction. Amaurosis fugax and hemispheric transient ischemic attacks were of equal value in predicting the possibility of a surgically treatable lesion at the carotid bifurcation.

Hemispheric transient ischemic attacks and amaurosis fugax have been related to ulcerative and occlusive lesions of both the extracranial and intracranial carotid arterial systems. Recent publications suggested that there is a relatively high occurrence of normal findings on arteriography in patients with well-documented transient ischemic attacks. Lemak and Fields, reviewing the data from the cooperative study on extracranial arterial occlusive disease, demonstrated angiographically normal carotid arteries in 22% of patients with amaurosis fugax (with or without hemispheric transient ischemic attacks) and in 43% of patients with hemispheric transient ischemic attacks alone. Since these data were originally collected when the importance of the non-stenotic but ulcerative lesion of the carotid artery had not yet been recognized, we felt that the reported incidence of normal angiographic findings in these patients might be erroneously high.

The purpose of this communication is to review our series of patients with hemispheric transient ischemic attacks and amaurosis fugax with regard to (a) the incidence and types of arteriographically-identifiable lesions of the appropriate carotid artery and, as a corollary, (b) the incidence of lesions potentially correctable by surgical intervention.

Methods

From August 1974 to August 1976, 123 patients underwent carotid angiography at the San Francisco Veterans Administration Hospital for evaluation of hemispheric transient ischemic attacks (93) or amaurosis fugax (with or without hemispheric transient ischemic attacks) (30). Fifteen of 123 had bilateral symptoms, bringing the total number of individual carotid arteries for study to 138.

From the Departments of Radiology (RLE, WRN, RLM) and Vascular Surgery (WSM), University of California School of Medicine, San Francisco, and the Veterans Administration Hospital, San Francisco, Calif. Reprint requests to Ronald L. Eisenberg, M.D., Radiology Service, Veterans Administration Hospital, 4100 Clement St., San Francisco, Calif. 94121.
Cerebral arterial smooth muscle contraction by thromboxane A2.
E F Ellis, A S Nies and J A Oates

Stroke. 1977;8:480-483
doi: 10.1161/01.STR.8.4.480

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/8/4/480

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/