In Vitro Evaluation of the Inhibitory Action of PGI₂ to Vasoconstrictions Induced by Various Prostaglandins, Serotonin and Hemoglobin Using the Canine Basilar Artery

Takao ASANO, Tomio SASAKI, Chikayuki OCHIAI
and Kintomo TAKAKURA

Department of Neurosurgery, Faculty of Medicine University of Tokyo, Tokyo

Abstract

Current investigations have indicated that prolonged vasoconstriction in vasospasm following subarachnoid hemorrhage due to rupture of intracranial aneurysms is the result of a combined action of various vasoconstrictive agents such as serotonin, oxyhemoglobin, thrombin and prostaglandins. PGI₂ (prostacyclin), on the other hand, has been known to possess antagonistic actions to some of the above agents.

To elucidate the role of PGI₂ as a natural protecting factor against the occurrence of vasospasm, its inhibitory action against vasoconstrictions induced by serotonin, oxyhemoglobin, PGA₂, PGD₂, PGE₂ and PGF_{2α} was studied *in vitro* using the canine basilar artery. PGI₂ in concentration of 10⁻⁶ M caused vasodilation ranging between 20 to 70% of the basal contraction developed by each agonist. Thus it was revealed that PGI₂ caused a significant dilation of the artery which had been constricted by any of the above agents. Since the progressive diminution in PGI₂ synthesis in experimental SAH has been previously demonstrated, the result of the present study indicates a possible benefit of the use of PGI₂ as well as the need for preservation of PGI₂ synthesis in any pharmacological treatment of vasospasm.

Key words: vasospasm, prostaglandins, PGI₂ (prostacyclin), oxyhemoglobin, serotonin

Introduction

It has been suggested that the presence of a subarachnoid clot is closely related to the occurrence of vasospasm following subarachnoid hemorrhage due to rupture of intracranial aneurysms (SAH).⁸⁾ As a possible mechanism by which such clots induce vasospasm, liberation of oxyhemoglobin (oxyHb), thrombin, and enhanced production of vasoconstrictive prostaglandins such as PGF_{2α} has been proposed.^{15,18,20)} On the other hand, there is evidence suggesting that the endothelial cells of the main cerebral artery are damaged in a relatively early period of SAH.^{3,11,17)} The damaged intima induces adhesion and aggregation of platelets with subsequent release of serotonin, norepinephrine, ADP, and various products of the arachidonate cascade, among which thromboxane A₂ is the most potent vasoconstrictive agent.⁶⁾ Thus the cerebral artery is exposed to a

combined effect of these vasoconstrictive agents following SAH and this is currently deemed as the most likely cause of the prolonged arterial constriction in vasospasm.

Against the actions of multiple vasoconstrictive agents, PGI₂ (prostacyclin) is the single substance hitherto known as a naturally occurring autacoid that dilates the cerebral artery.^{4,13)} The main actions of PGI₂ are vasodilation and inhibition of platelet aggregation, which are both thought to protect the artery against vasospasm. In this regard, we have previously demonstrated that a significant diminution in PGI₂ synthesis in the canine basilar artery takes place following an experimental SAH.¹⁶⁾ This finding indicates that the diminished synthesis of PGI₂ due to endothelial injury may have an equal importance in the pathogenesis of vasospasm to the production of vasoconstrictive agents following SAH.

To further clarify the role of PGI₂ in the genesis of vasospasm, we evaluated the vasodilatory action of PGI₂ *per se* and its action against the vasoconstriction induced by serotonin, various prostaglandins such as

PGA₂, D₂, E₂, F_{2α} and oxyHb using the canine basilar artery. The present paper reports that PGI₂ significantly dilates the artery which has been constricted by any of the above agents.

Materials and Methods

Mongrel dogs of both sexes, weighing 7–13 kg were anesthetized with intravenous injection of sodium pentobarbital and sacrificed by exsanguination via a catheter inserted into the femoral artery. The brain was rapidly removed and the basilar artery was resected and immediately placed in a nutrient solution at 37°C. Under an operating microscope, the arterial lumen was washed with a nutrient solution and segments 4 mm in length were cut from the artery. The arterial segments were then mounted on the parallel prongs of an apparatus.¹⁸⁾ The bath was filled with 10 ml of a nutrient solution that was maintained at $37 \pm 0.5^\circ\text{C}$ and saturated with 95% O₂ and 5% CO₂. The composition of the nutrient solution was (mM): NaCl, 120; KCl, 4.5; CaCl₂, 2.5; MgSO₄, 1.0; NaHCO₃, 27.0; KH₂PO₄, 1.0; and glucose, 10.0. The pH of the solution ranged from 7.4 to 7.5.

Isometric tension was measured using a force-displacement transducer, an amplifier, and an ink-writing recorder (Transducer; model TB-611T, and Amplifier; Model RP5, manufactured by Nihon-Koden Co. Ltd., Tokyo, Japan. Recorder; Model FU-81A, manufactured by TOA Denpa Kogyo Co. Ltd., Tokyo, Japan). An initial basal tension of about 1.5 gm was applied and the segments were allowed to stabilize for 90 minutes. Before starting the experiment, the basal tension was readjusted to 2.0 gm. Then the response of the segment to 40 mM-KCl was determined. This was repeated three to four times until stabilized responses were obtained. Then the response to serotonin (5-HT, Sigma) in concentration of 10^{-8} to 10^{-5} M was obtained. The arterial response to 40 mM-KCl and 10^{-6} M serotonin were used as reference contractions for subsequent determinations.

The following drugs were used in the present study. Prostaglandins A₂, D₂, E₂, F_{2α} and I₂-Na were the kind gifts of Ono Pharmc. Co. Ltd. A stock solution of each drug was made with ethanol every week and kept in a refrigerator at -20°C . Test solutions of desired concentrations were made by dilution of the stock solution with the nutrient solution just prior to the experiment. Since the vasoconstrictive capacities of crude and purified oxyHb differed little in the preceding experiment,¹⁸⁾ only a crude preparation of oxyHb as follows was carried out in the present study. The arterial blood was obtained from the femoral artery of a dog, centrifuged at 1,500 G for 10 minutes and the

plasma was discarded. The cells were washed five times with three to four volumes of ice-cold 0.9% sodium chloride, and the buffy coat was removed. An equal volume of distilled water was added to the packed cells and the mixture was agitated for five minutes. The lysed red cells were then centrifuged at 15,000 G for 20 minutes to remove stroma. The resulting hemolysate usually contained *ca.* 10 gm/dl of oxyHb. The test solution was prepared by dilution with 0.9% sodium chloride. The volume of test agent added to the bath was 0.1 ml except oxyHb of 10^{-4} M, in which up to 0.3 ml was used, depending on the original concentration of oxyHb in the hemolysate.

In the present study, the concentration of each agent was expressed as its final concentration in the bath. Results shown in the text, figures and table represent mean values $\pm$ standard error of the mean.

Results

I. Responses of the arterial segments to test agents

The actual tension developed by 40 mM-KCl ranged from 3 to 7 gm, being around 5 gm in the majority of segments. Addition of a maximum of 0.1 ml ethanol in the bath did not affect the basal tension. The addition of PGA₂, D₂, E₂ and F_{2α} in concentrations ranging from 10^{-7} to 10^{-5} M caused a dose-dependent contraction of the artery (Fig. 1). There were also dose-dependent contractions by serotonin (10^{-8} to 10^{-6} M) and oxyHb (10^{-6} to 10^{-4} M). PGI₂ (10^{-8} to 10^{-6} M) caused a slight but consistent dilation of the artery. In the concentration of 10^{-5} M, however, PGI₂ always caused a prominent contraction.

II. The mode of action of PGI₂ in the bath

The mode of action of PGI₂ when added to the bath was first studied in a preliminary experiment. The representative pattern of PGI₂ action is shown in Fig. 2. PGI₂ (10^{-7} M) caused an immediate dilation of the artery within several seconds, which then gradually diminished. A successive addition of PGI₂ (10^{-6} M) caused a greater dilation than the preceding one, from which the occurrence of tachyphylaxis could be denied. In most specimens examined, the dilation by 10^{-6} M-PGI₂ became more pronounced within several minutes and gradually diminished. When added in a cumulative fashion, PGI₂ caused a dose-dependent dilation (Fig. 3). Addition of 10^{-5} M-PGI₂ always caused a contraction which was greater than the original basal contraction. Since studying the contractile activity of PGI₂ was not the aim of the present study, only the concentration range from 10^{-8} to 10^{-6} M of PGI₂ was used.

As shown in Fig. 3, there was a gradual increase in

Fig. 1 The response of canine basilar artery segments to serotonin, PGE₂, PGD₂, PGE₂, PGF_{2α}, PGI₂, and oxyhemoglobin are shown. *abscissa*: Concentrations of each agonist in the bath. *ordinate*: The magnitude of contractions expressed as the percent of the contraction by 40 mM-KCl in each arterial segment. The numbers of arterial segments used are shown in the parenthesis.

Fig. 2 A tracing of a representative pattern of the action of PGI₂ in the bath. The initial contraction was induced by PGD₂(10⁻⁵ M).

dilation after addition of PGI₂(10⁻⁶ M). After the maximal dilation was reached within several minutes,

Fig. 3 *upper*: The responses to a cumulative addition of PGI₂ (10⁻⁸ to 10⁻⁶ M). The initial contraction was induced by PGF_{2α} (10⁻⁵ M). Note the secondary decay in tension following the immediate vasodilation by PGI₂ (10⁻⁶ M). *lower*: The response of the arterial segment obtained from the same artery as above to PGF_{2α} (10⁻⁵ M). Note the spontaneous decay in tension.

the artery began to contract again, reaching a plateau within about 20 minutes. The level of contraction finally reached was lower than that of the initial contraction in most specimens. In seven arterial segments contracted by PGF_{2α} (10⁻⁵ M), the time after the addition of PGI₂ (10⁻⁶ M) until the maximal dilation was 5.8±0.7 minutes, and the time until the resumption of contraction reached a plateau was 18.3±2.9 minutes. The tension finally resumed was 92.7±4.0% of the initial contraction. The above results indicate that PGI₂ added in the bath was almost completely catabolized within *ca.* 20 minutes. On the other hand, since PGI₂ in concentrations greater than 10⁻⁶ M caused contraction of the artery, it is possible that the concentration which causes the maximal dilation lies between 10⁻⁷ and 10⁻⁶ M. Therefore, the late component of dilation by PGI₂ (10⁻⁶ M) may in part reflect the vasodilatory action of PGI₂ which parallels the spontaneous alteration of its concentration in the bath.

Fig. 4 The vasodilations caused by PGI_2 in the arteries undergoing active contraction by each agonist in different concentrations are shown. The initially developed tension by each agonist and the magnitude of the immediate drop in tension by PGI_2 at each concentration was expressed as the percent of the tension developed by serotonin (10^{-6} M) in each arterial segment. The number of segments used are shown in the parenthesis.

III. Quantitative evaluation of the vasodilatory action of PGI_2

The magnitude of PGI_2 action on various test agents were evaluated in two concentrations of each agonist which caused different initial contractions (Fig. 4). As shown in the figure, PGI_2 (10^{-6} M) caused a considerable vasodilation irrespective of the kind of the agonist. Also the vasodilatory action of PGI_2 was far greater in the presence of active vasoconstriction

Table 1 The responses of the arterial segments with and without the pretreatment by PGI_2 (0.5×10^{-6} M) to serotonin and $\text{PGF}_{2\alpha}$ in low concentrations (10^{-8} and 10^{-7} M). The statistical evaluation was carried out by the paired student t-test.

Number of pairs	PGI_2 0.5×10^{-6}	Serotonin 10^{-8}	10^{-7} (M)
12	$\begin{cases} -3.0 \pm 0.9 \\ 0 \end{cases}$	$\begin{cases} 11.1 \pm 6.2 \\ 19.0 \pm 6.2 \end{cases}$	$\begin{cases} 44.3 \pm 8.1 \\ 69.2 \pm 5.4 \end{cases}$
		*P < 0.05	
	PGI_2 0.5×10^{-6}	$\text{PGF}_{2\alpha}$ 10^{-8}	10^{-7} (M)
10	$\begin{cases} -5.1 \pm 1 \\ 0 \end{cases}$	$\begin{cases} -2.1 \pm 1.4 \\ 0.4 \pm 0.2 \end{cases}$	$\begin{cases} 5.4 \pm 1.7 \\ 15.0 \pm 3.6 \end{cases}$
		*P < 0.05	

*P < 0.05, **P < 0.01

than that by PGI_2 alone. This action of PGI_2 was particularly prominent with PGD_2 , in which the dilation reached about 70% of the initial contraction.

To see the effect of PGI_2 on agonists in lower concentrations, the following experiment was carried out. A pair of arterial segments was obtained from the same canine basilar artery. One received PGI_2 (0.5×10^{-6} M) just prior to the addition of the agonist and the other not. The responses of each of the pair to serotonin (10^{-8} and 10^{-7} M) and $\text{PGF}_{2\alpha}$ (10^{-8} and 10^{-7} M) were statistically evaluated by the paired t-test (Table 1). In a very low concentration of agonists (10^{-8} M), only small contractions were obtained, yet the PGI_2 -treated group showed a statistically significant reduction in tension compared to the other.

Discussion

The actions of various prostaglandins on cerebral vessels have been extensively surveyed. The vasoconstrictive actions of $\text{PGF}_{2\alpha}$ and PGE_2 are well known, whereas PGA_2 and PGD_2 have received relatively little attention. Nakano *et al.* have shown that the intraarterial injection of PGA_2 and PGE_2 caused dilation of the vascular bed of the carotid artery.¹⁴⁾ In the recent study by Ellis *et al.*, external application of

PGD₂ and PGE₂ caused dilation of the cerebral precapillary vessels in cats.⁷⁾

In the present study, PGA₂, PGD₂, PGE₂ and PGF_{2α} all caused a similar magnitude of arterial constriction, among which PGF_{2α} was the most potent. The discrepancy in the experimental results between the present and the preceding studies may be due to the differences in species, the method of drug administration, and the kind of the artery examined. In this respect, Handa *et al.* have shown that PGE₂ and other prostaglandins (PGA₁, PGE₁) constrict the feline basilar artery on topical application and that they dilate the artery on intravenous administration.¹⁰⁾ It is also possible that the response to prostaglandins differ between the smooth muscle of the arteriole and that of the main trunk of the cerebral artery. This point seems of importance when the role of prostaglandins in the regulation of cerebral circulation is studied.

OxyHb, although crude in preparation, caused a significant vasoconstriction in the present study which compared well to preceding reports.^{18,19)} Since the production of PGF_{2α} and PGE₂ in the brain and the cerebral vessels has been confirmed and that of PGD₂ and PGA₂ seems likely,^{1,2,9,11)} it seems a plausible hypothesis that the sustained contraction of major cerebral arteries following SAH is at least due in part to a combined effect of these vasoconstrictive prostaglandins, serotonin, oxyHb, and possible other substances.

PGI₂, on the other hand, caused a small but consistent vasodilation when applied solely in the bath in the concentration range from 10⁻⁸ to 10⁻⁶ M. In the concentration of 10⁻⁵ M, PGI₂ invariably caused a prominent constriction. PGI₂ rapidly catabolizes in a neutral medium at 37°C, but the rate of its hydrolysis is significantly slower in an aqueous medium at 4°C, even at pH 7.48.⁵⁾ Test solutions of PGI₂ were made from the stock solution (10⁻⁵ M PGI₂ in ethanol) by dilution with ice-cold phosphate buffer at pH 7.5. Each test solution of PGI₂ was used within several minutes after preparation. Although these precautions were carried out, it cannot be denied that certain amounts of PGI₂ underwent hydrolysis during preparation. As to the effect of the PGI₂ catabolite, namely PGF_{1α}, on the cerebral artery, there has been no report in the literature to our knowledge. Judging from the result shown in Fig. 2, PGF_{1α} does not seem to possess any significant effect on the cerebral artery. More importantly, the above results were in good agreement with a preceding report by Chapleau and White.⁴⁾ Similar to their study, the immediate drop in tension at each concentration of PGI₂ was taken as the index of the vasodilatory action of PGI₂ in the

present study. Thus the present study revealed that PGI₂ significantly inhibits contractions induced by various agents such as PGA₂, PGD₂, PGE₂, PGF_{2α}, serotonin and oxyHb (Fig. 4). Since PGI₂ is also synthesized in the cerebral artery,^{2,16)} it may be argued that PGI₂ constitutes a natural defense mechanism against overt vasoconstrictions induced by the above agents.

Regarding the mechanism of action of PGI₂, little can be said from the present study. That PGI₂ did not completely abolish the arterial response to serotonin and PGF_{2α} (Table 1) together with other evidence⁴⁾ indicates that PGI₂ exhibits a physiological type of antagonism which is probably of a non-competitive character. In any case, this unique property of PGI₂ would point to the importance of its physiological role in the regulation of the caliber of major cerebral vessels. In addition, in pathological conditions which are accompanied by endothelial injury, the synthesis of PGI₂ might bear a critical importance to the maintenance of normal peripheral circulation, since PGI₂ prevents platelet adhesion and aggregation in the injured vessel. In this context, we have previously shown that there was an actual diminution in PGI₂ synthesis in the canine basilar artery following an experimental SAH.¹⁶⁾ There is ample evidence showing the early occurrence of endothelial injury in the cerebral artery in experimental as well as human SAH.^{3,11,17)} Therefore, it seems likely that the diminution in PGI₂ synthesis in the cerebral artery is equally as important in the pathogenesis of vasospasm following SAH as the production of various vasoconstricting substances.

Therapeutically, the present study implicates that the use of PGI₂ may be beneficial in the treatment of vasospasm. However, considerable practical problems still exist as to the method of its administration, the maintenance of a proper concentration in the blood, and the avoidance of overt systemic reactions. Considering that there may be an imbalance of production of PGI₂ and TXA₂ in the artery undergoing vasospasm, the use of a specific inhibitor of thromboxane A₂ synthetase may be more practical. It may be finally stated that the use of PGI₂ against vasospasm is worthy of further investigation and in any pharmacological handling of vasospasm, interference to PGI₂ production should be avoided.

Acknowledgements

The authors wish to thank Yoshimi Ohtsuka for expert technical assistance.

References

- 1) Abdel-Halim MS, Hamberg M, Sjöquist B, Änggård E: Identification of prostaglandin D₂ as a major prostaglandin in homogenates of rat brain. *Prostaglandins* 14: 633–643, 1977
- 2) Abdel-Halim MS, von Holst H, Meyerson B, Sachs C, Änggård E: Prostaglandin profiles in tissue and blood vessels from human brain. *J Neurochem* 34: 1331–1333, 1980
- 3) Alksne JF, Bronson PJ: Prevention of experimental subarachnoid hemorrhage-intracranial arterial vasonecrosis with phosphodiesterase inhibitor Phthalazinol (EG 626). *Stroke* 10: 638–643, 1979
- 4) Chappleau CE, White RP: Effects of prostacyclin on the canine isolated basilar artery. *Prostaglandins* 17: 573–580, 1979
- 5) Cho MJ, Allen MA: Chemical stability of prostacyclin (PGI₂) in aqueous solutions. *Prostaglandins* 15: 943–955, 1978
- 6) Ellis EF, Nies AS, Oates JA: Cerebral arterial smooth muscle contraction by thromboxane A₂. *Stroke* 8: 480–483, 1977
- 7) Ellis EF, Wei EP, Kontos HA: Vasodilation of cat cerebral arterioles by prostaglandins D₂, E₂, G₂ and I₂. *Am J Physiol* 237(3): H381–H385, 1979
- 8) Fisher CM, Kistler JP, Davis JM: Relation of vasospasm to SAH visualized by computerized tomographic scanning. *Neurosurgery* 6: 1–9, 1980
- 9) Hagen AA, White RP, Robertson JT: Synthesis of prostaglandins and thromboxane B₂ by cerebral arteries. *Stroke* 10: 306–309, 1979
- 10) Handa J, Yoneda S, Matsuda M, Handa H: Effects of prostaglandins A₁, E₁, E₂ and F_{2α} on the basilar artery of cats. *Surg Neurol* 2: 251–255, 1974
- 11) Hughes JT, Schianthi PM: Cerebral artery spasm; a histological study at necropsy of the blood vessels in cases of subarachnoid hemorrhage. *J Neurosurg* 48: 515–525, 1978
- 12) Kraemer RJ, Phernetton TM, Folts JD: Prostaglandin-like substances in coronary venous blood following myocardial ischemia. *J Pharmacol Exp Ther* 199: 611–619, 1976
- 13) Moncada S, Vane JR: The role of prostacyclin in vascular tissue. *Fed Proc* 38: 66–71, 1979
- 14) Nakano J, Change ACK, Fisher R: Effects of prostaglandins E₁, E₂, A₁, A₂ and F_{2α} on canine carotid arterial blood flow, cerebrospinal fluid pressure, and intraocular pressure. *J Neurosurg* 38: 32–39, 1973
- 15) Pickard JD, Vinall PE, Simeone FA: Prostaglandins and cerebral vasospasm; a problem of interpretation. *Surg Forum* 26: 496–498, 1975
- 16) Sasaki T, Asano T, Sano K, Murota S: Evaluation of prostacyclin biosynthetic activity in canine basilar artery following subarachnoid hemorrhage. *J Neurosurg* 55: 771–778, 1981
- 17) Tanabe Y, Sakata K, Yamada H, Ito T, Takada M: Cerebral vasospasm and ultra structural changes in cerebral arterial wall. *J Neurosurg* 49: 229–238, 1978
- 18) Tanishima T: Cerebral vasospasm; contractile activity of hemoglobin in isolated canine basilar arteries. *J Neurosurg* 53: 787–793, 1980
- 19) Wellum GR, Irvine TW, Zervas NT: Dose responses of cerebral arteries of the dog, rabbit, and man to human hemoglobin in vitro. *J Neurosurg* 53: 486–490, 1980
- 20) White RP: Multiplex origin of cerebral vasospasm, in Price TR, Nelson E (eds): *Cerebrovascular Diseases*. New York, Raven, 1979, pp 307–319

Request reprints to: T. Asano, M.D., D.M.Sc., Department of Neurosurgery, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113, Japan.