Tohoku J. exp. Med., 1982, **137**, 65–71

Effects of Prostaglandins on Thrombus Formation in Mesenteric Arterioles of Rats

TAKEHIKO SUZUKI, the Late ATSUKO HAYASHI and SHIZUKO TAKANO

Department of Pharmacology, Fukushima Medical College, Fukushima 960

SUZUKI, T., the late HAYASHI, A. and TAKANO, S. *Effects of Prostaglandins on Thrombus Formation in Mesenteric Arterioles of Rats.* Tohoku J. exp. Med., 1982, **137** (1), 65–71 —— Effects of locally applied prostaglandins on platelet thrombus formation in the mesenteric arterioles of rats were studied in vivo. Prostaglandin (PG) I_2 and PGE_1, which were reported to be powerful inhibitors of blood platelet aggregation, prevented the thrombus formation, whereas PGE_2, which was reported, in low concentrations, to enhance platelet aggregation, showed a tendency to promote the thrombus formation. PGA_1, PGA_2 or $PGF_{2\alpha}$ had no effect. PGD_2 had a very weak inhibitory effect. As prostaglandins tested had no effect on the diameter of mesenteric arterioles, it is assumed that the effectiveness of PGI_2 and PGE_1 in preventing thrombus formation is due mainly to their powerful inhibiting effects on platelet aggregation. ———— prostaglandins; PGI_2; thrombus formation; mesenteric arterioles

Inhibiting effects of prostaglandin (PG) I_2 and PGE_1 on blood platelet aggregation in human and experimental animals are reported by many authors. Gorman et al. (1977) have reported that prostacyclin (PGI_2) can dilate the vessel and stimulate platelet adenyl cyclase which will retard the aggregation and platelet thrombus formation that have been induced by the endoperoxide-TXA_2 system. Such findings suggest that PGE_1 and PGI_2 may be of use clinically in the treatment of thrombic disease. Westwick (1977) studied the thrombus-preventing effects of prostaglandins (PGE_1, PGE_2, PGD_2 and PGG_2) using hamster cheek pouch preparation and found that PGE_2 appeared to be a potent inhibitor of thrombus formation in vivo as first described by Emmons et al. (1967). Also PGI_2 applied locally in low concentrations inhibited thrombus formation caused by ADP in the microcirculation of the hamster cheek pouch (Higgs et al. 1978). Thrombus formation in rat mesenteric arterioles has been described by Reber (1966), Gordon and Gresham (1970) and Potvliege and Bourgain (1976), but the effects of PGs in these preparations have not been studied. Therefore, we attempted to study the effects of PGE_1, PGE_2, PGD_2, PGA_1, PGA_2, $PGF_{2\alpha}$ and PGI_2 on thrombus formation using mesenteric arterioles of rats. A preliminary report was published in Proceedings of the 52nd General Meeting of the Japanese Pharmacological Society (Suzuki et al. 1979).

Received for publication, July 2, 1981.

Materials and Methods

Thrombus formation

White Wistar male rats weighing from 180 to 300 g were used. Rats were anesthetized with sodium pentobarbital (40 mg/kg, i.m.) and laid on a glass tray. The technique used to study platelet thrombi in the mesenteric arterioles of rats was a modification of the method of Gordon and Gresham (1970) by using an inverted microscope (Nicon Ltd.).

A platelet thrombus was produced in one of the vessles (about 50 μm in diameter) by delivering an electrical pulse to a point on the vessel wall through a microelectrode made of stainless steel, 150 μm diameter. An indifferent electrode made of silver plate (2 cm$\times$ 4 cm) having a thickness of 0.25 mm was placed under the mesentery forming the anode. A square-wave stimulator (Nihon Kohden MSE 3R), set at a voltage of 50 V or 150 V, was used to deliver a single electrical pulse. The current was about 3 mA in the latter and about 1 mA in the former. The experiment was carried out at constant room temperature of 25°C. The mesentery was kept moist by a drip of Tyrode solution (8 drops/min). At a voltage of 150 V a pulse of about 1 msec duration was necessary and sufficient to produce a "threshold response," adhesion of a few platelets to the injury site, while at a voltage of 50 V a pulse longer than 10 msec was necessary to produce the same effect.

Increasing the duration of electrical stimulation produced a greater platelet thrombus build-up at the site, and the response was then classified as follows. Grade I: White body formation covering up to 20% of the vessel lumen. Grade II: White body formation covering up to 40% of the lumen. Grade III: White body formation covering over 40% of the lumen. About 30 min were necessary to reach the grade III response after the first electrical stimulation. Electrical stimulation was begun 5 min after application of PG or control solution.

Drugs

Prostaglandins (Ono Pharmaceutical Co., Ltd.) used were PGE_1, PGE_2, PGA_1, PGA_2, $PGF_{2\alpha}$, PGD_2 and PGI_2. Except when using PGI_2 1 mg of prostaglandins was dissolved in alcohol and stored in a deep freezer at –20°C until use. This original preparation was diluted with nine parts of Tyrode solution before use. Aliquots of 0.02 ml of the diluted solution were dropped on the vessel. Tyrode solution containing 10% alcohol was used as the control. Sodium salt of PGI_2 was stored in a deep freezer at –80°C and dissolved in alcohol and then diluted with a 0.1 M phosphate buffer pH 9.0 before use. As its corresponding control, phosphate buffer solution was used.

Statistical analysis was made by Student's *t*-test.

Results

Effects of control Tyrode solution on the mesenteric arterioles. In the present experiments, it was impossible to compare the effects of test drugs with that of the control solution by using the same vessel. Therefore, effects of the control solution on the thrombus formation in two arterioles from the same animal and having almost the same diameter were compared first. The responses to electrical stimulation set at a voltage of 150 V or 50 V on the first and the second vessel were quite similar. In the following experiments, the first vessel was chosen for the control and the second for prostaglandins. Fig. 1 shows platelet thrombus in the mesenteric arterioles caused by electrical stimulation set at 50 V.

Effects of 2 μg of PGE_1 and PGE_2. The effects of PGE_1 and PGE_2 on the thrombus formation produced by electrical stimulation set at 150 V are shown in

Fig. 2. PGE_1 significantly inhibited the thrombus formation at Grade I and Grade II, while PGE_2 has a tendency to promote the thrombus formation and significantly facilitated the thrombus formation at Grade I. Similar results were obtained by electrical stimulation set at 50 V (Fig. 3). In the following experiments electrical stimulation set at 50 V was used.

Effects of 2 μg of PGA_1, PGA_2, $PGF_{2\alpha}$, PGD_2 and PGI_2. PGA_1, PGA_2, or $PGF_{2\alpha}$ had almost no effect on the thrombus formation produced by electrical stimulation. The thrombus formation was potently inhibited by PGI_2, but only slightly by PGD_2 (Fig. 4). The inhibiting effect of PGI_2 on the thrombus formation produced by electrical stimulation was significant at Grades I, II and III.

Effects of 0.2 μg of PGI_2 and PGE_1. Among the prostaglandins tested PGI_2 and PGE_1 had remarkable inhibiting effect on the thrombus formation. Therefore,

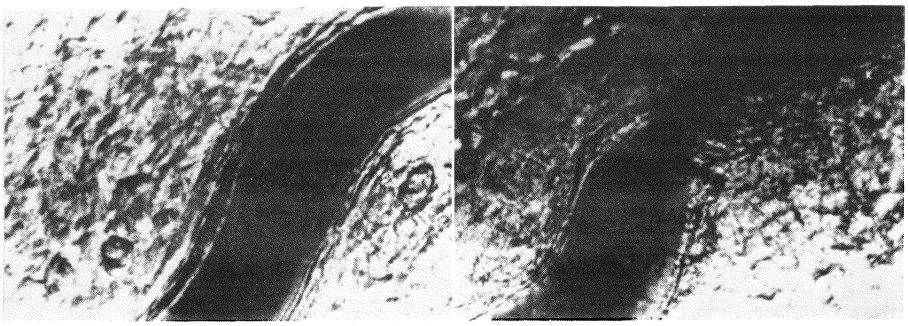

Fig. 1. Platelet thrombus produced by electrical stimulation.
Left: Mesenteric arteriole of rat before stimulation. Right: Resulting platelet thrombus covering about 50% of the vessel lumen.

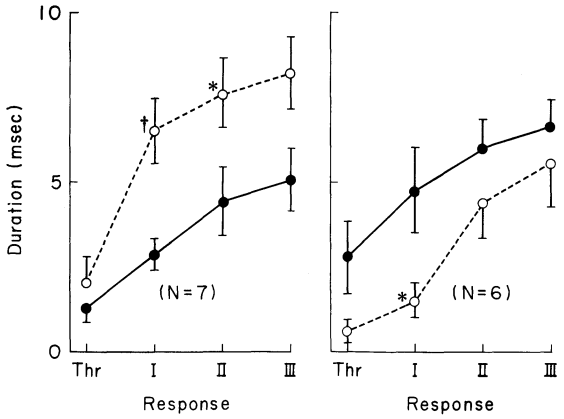

Fig. 2. Effects of PGE_1 (2 μg) (left) and PGE_2 (2 μg) (right) on the thrombus formation. Electrical stimulation set at 150 V (about 3 mA). Thr, threshold. Grades I, II and III (see text). Means±S.E. * $p<0.05$, † $p<0.01$. ●—●, control; ○---○, PG.

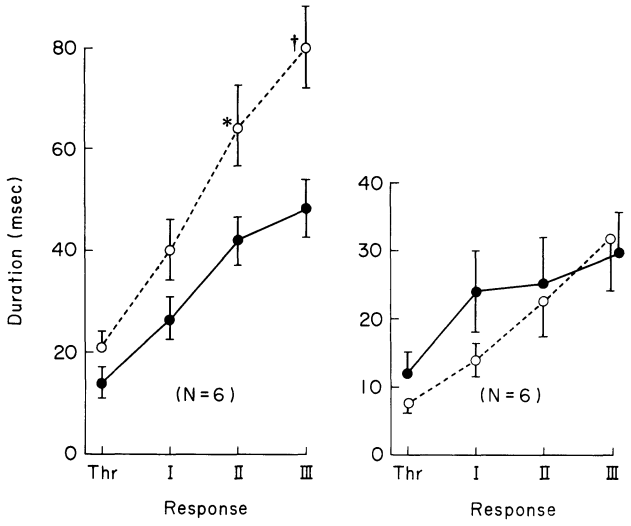

Fig. 3. Effects of PGE_1 (2 μg) (left) and PGE_2 (2 μg) (right) on the thrombus formation. Electrical stimulation set at 50 V (about 1 mA). ●—●, control; ○---○, PG. Mean± S.E. * $p<0.05$, † $p<0.01$.

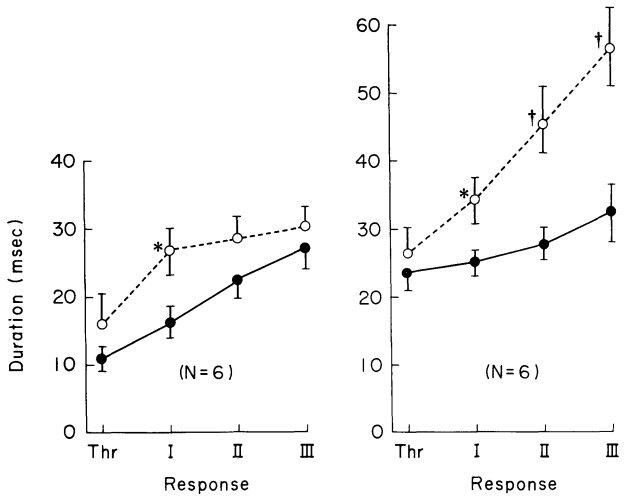

Fig. 4. Effects of PGD_2 (2 μg) (left) and PGI_2 (2 μg) (right) on the thrombus formation. Electrical stimulation set at 50 V. ●—●, control; ○---○, PG. Mean±S.E. * $p<0.05$, † $p<0.01$.

effects of a small dose of these prostaglandins were tested. As shown in Fig. 5, PGI_2 significantly inhibited the thrombus formation at Grades II and III, while PGE_1 had no inhibiting effect at Grade II or III, though significantly inhibiting effect was found at Grade I.

Effects of prostaglandins on the mesenteric arterioles. Effects of locally applied prostaglandins on the mesenteric arterioles without electrical stimulation were

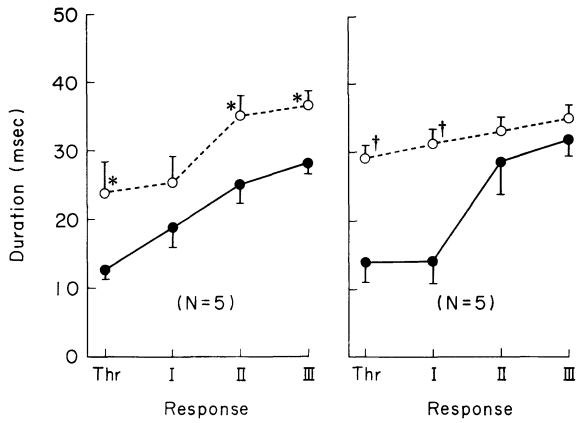

Fig. 5. Effects of PGI_2 (0.2 μg) (left) and PGE_1 (0.2 μg) (right) on the thrombus formation. Electrical stimulation set at 50 V. Mean±S.E. * $p<0.05$, † $p<0.01$. ●—●, control; ○---○, PG.

TABLE 1. *Effects of prostaglandins on the mesenteric arterioles*

	Control $N=5$ 10% alcohol in Tyrode	PGE_1 $N=4$	PGE_2 $N=4$	$PGF_{2\alpha}$ $N=5$	PGA_1 $N=5$	PGA_2 $N=4$	PGD_2 $N=5$	0.1 M Phosphate buffer $N=4$	PGI_2 in phosphate buffer $N=4$
Before	100%	100	100	100	100	100	100	100	100
1 min	100	97.8	99.0	98.6	100	100	100	98.7	100
5	102.3	98.9	99.0	96.7	100	100	100	98.0	100.2
10	101.2	101	100	96.7	92	99.3	97.2	97.7	95.5
15	100	101.5	100	94.6	86.3	100	98.8	98.0	97.6
20	100	102	100	95.5	94.1	100	98.8	97.4	101.8
25	100	100	100	95.8	99.2	100	95.9	98.5	103.6
30	100	100	100	95.8	100	100	100	97.4	102

Diameter of the vessels was measured at various times and expressed as percentage of the value before the drug application.
PGs were given in a dose of 2 μg.

studied for 30 min after the application of the drugs. Prostaglandins tested had almost no vasodilator nor vasoconstrictor effects on these vessels (Table 1).

DISCUSSION

Gordon and Gresham (1970) reported the in vivo production of platelet thrombi in rat mesenteric arterioles. Our method is a modification of that of Gordon and Gresham, who used the inverted microscope which had a ring attached to the condenser which held a fine tungsten microelectrode (a tip diameter 2 to 5 μm). We used a stainless steel microelectrode (a tip diameter 150 μm), which was not attached to the condenser. We also did tests using a fine tungsten microelectrode, but the results were not constant. Further study using a fine tungsten microelectrode attached to a suitable micromanipulator was carried out and the results will be reported by Takano and Suzuki.

An indifferent electrode, a plate of silver, was placed under the loop of the intestine thus forming an anode as described by Reber (1966), who used a platinum wire electrode of 50 μm diameter and always as a cathode. Gordon and Gresham (1976) and Reber (1966) used electrical stimulation set at 150 V. We found that stimulation at 50 V is great enough to produce thrombus formation, though about ten times longer stimulation was necessary to produce the same effect as that produced by 150 V. As described by Gordon and Gresham, electric gas bubbles appeared at the microelectrode tip, but they disappeared shortly after the stimulation and we were able to observe thrombus formation after repeated experiments.

Westwick (1977) studied the effect of prostaglandins (PGE_1, PGD_2, PGE_2 and PGG_2) on arteriolar thrombus by using hamster cheek pouch preparations and found that PGE_1 inhibited thrombus formation, while PGE_2 or PGD_2 did not. Higgs et al. (1978) reported that thrombus formation in venules and in arterioles of the hamster cheek pouch was abolished by 500 ng/ml prostacyclin (PGI_2) and that PGI_2 was appoximately twenty times more potent than PGE_1 in preventing thrombus formation in the circulation. Also in our experiments on the mesenteric arterioles of the rat, PGI_2 and PGE_1 prevented the thrombus formation, and the effect of GPI_2 was greater than that of PGE_1. PGD_2, which was shown to be a potent inhibitor of human platelet aggregation but ineffective on rat platelets (Whittle et al. 1978), had only a slight inhibiting effect on thrombus formation in our experiments. Prostaglandins from A, B and F series are shown to be relatively inactive in preventing platelet aggregation (Higgs et al. 1978). In our experiments PGA_1, PGA_2 or $PGF_{2\alpha}$ had no effect. PGE_2, which was shown to enhance platelet aggregation in low concentrations (Higgs et al. 1978), showed a tendency to enhance thrombus formation.

The inhibiting effects of PGI_2 and PGE_1 on blood platelet aggregation are well known. Dusting et al. (1978) observed that close intra-arterial injections of PGI_2 ($0.02 \sim 2$ μg) and PGE_2 ($0.05 \sim 1$ μg) in the mesenteric vascular bed of dogs caused vasodilatation. However, PGE_1, PGE_2, PGA_1, PGA_2, and $PGF_{2\alpha}$ ($10 \sim 10^5$ pg/ml) perfused through a rat mesenteric vascular preparation caused little or no effect except PGA_1 and $PGF_{2\alpha}$. PGA_1 and $PGF_{2\alpha}$ elevated the baseline pressure (Manku et al. 1977). On the other hand, Weiner and Kaley (1969) reported topical administration of PGE_1 ($0.1 \sim 1.0$ μg/0.1 ml) on the rat mesenteric microvasculature caused vasodilatation. The onset of vasodilatation was visible within $5 \sim 10$ sec, and the dilatation reached its peak in $25 \sim 30$ sec and was no longer manifest at the end of 1 min (Weiner and Kaley 1969).

In our experiments, the diameter of the vessel was measured from 1 min to 30 min after the application of the drug (Table 1) owing to our experimental conditions. Although we thus could not observe the effects of prostaglandins just after the application of the drugs, it is clear that in the present study locally applied prostaglandins tested had neither vasodilator nor vasoconstrictor action during the period necessary for the observation of the thrombus formation.

Therefore, the effectiveness of PGI_2 and PGE_1 in preventing thrombus formation seems to be due mainly to their inhibiting effects on blood platelet aggregation. At the same time we are not forgetting the inhibiting effects of these prostaglandins on platelet adhesion to the damaged vessel wall.

Acknowledgments

Prostaglandins were kindly provided by Ono Pharmaceutical Co., Ltd.

References

1) Dusting, G.J., Moncada, S. & Vane, J.R. (1978) Vascular actions of arachidonic acid and its metabolites in perfused mesenteric and femoral beds of the dog. *Europ. J. Pharmacol.*, **49**, 65–72.
2) Emmons, P.R., Hampton, J.R., Harrison, M.J.G., Honour, A.J. & Mitchell, J.R.A. (1967) Effect of prostaglandin E_1 on platelet behaviour in vitro and in vivo. *Brit. med. J.*, **2**, 468–472.
3) Gordon, J.L. & Gresham, G.A. (1970) The in vivo production of thrombosis in rat mesenteric vessels. In: *Platelets and the Vessel Wall-Fibrin Deposition. Symposium of the European Atherosclerosis Group, June 15–17, 1969*, edited by G. Schettler, Georg Thieme Verlag, Stuttgart, and Nankodo Company, Tokyo, pp. 107–114.
4) Gorman, R.R., Bunting, S. & Miller, O.V. (1977) Modulation of human platelet adenylate cyclase by prostacyclin (PGX). *Prostaglandins*, **13**, 377–388.
5) Higgs, E.A., Higgs, G.A., Moncada, S. & Vane, J.R. (1978) Prostacyclin (PGI_2) inhibits the formation of platelet thrombi in arterioles and venules of the hamster cheek pouch. *Brit. J. Pharmacol.*, **63**, 535–539.
6) Manku, M.S., Mtabaji, J.P. & Horrobin, D.F. (1977) Effects of prostaglandins on baseline pressure and responses to noradrenaline in a perfused rat mesenteric artery preparation: PGE_1 as an antagonist of PGE_2. *Prostaglandins*, **13**, 701–709.
7) Potvliege, P.R. & Bourgain, R.H. (1976) Thrombosis induced in vivo in the mesenteric artery of rats. An electron microscopic study of the initial phases. *Brit. J. exp. Path.*, **57**, 722–732.
8) Reber, K. (1966) A device for the production of well-defined lesions of mesenteric blood vessels with resulting platelet thrombi. *Thrombos. Diathes. Haemorrh.*, **15**, 471–475.
9) Suzuki, T., Takano, S. & Hayashi, A. (1979) Effects of prostaglandins on thrombus. *Jap. J. Phamacol.*, **29**, Suppl, 19P.
10) Weiner, R. & Kaley, G. (1969) Influence of prostaglandin E_1 on the terminal vascular bed. *Amer. J. Physiol.*, **217**, 563–566.
11) Westwick, J. (1977) Modulation of thrombus formation in vivo by prostaglandins. *Brit. J. Pharmacol.*, **61**, 138p–139p.
12) Whittle, B.J.R., Moncada, S. & Vane, J.R. (1978) Comparison of the effects of prostacyclin (PGI_2), prostaglandin E_1 and D_2 on platelet aggregation in different species. *Prostaglandins*, **16**, 373–388.