77. Decreased Activity of Prostaglandin Endoperoxide Synthetase in Prostaglandin I_2 (Prostacyclin) Biosynthesis from Arachidonic Acid in Isolated Rat Aortae

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Prostaglandin (PG) I_2 has potent activities in inhibition of platelet aggregation and in relaxation of vascular smooth muscle, and is considered to be released physiologically from vascular endothelial cells.¹⁾⁻⁵⁾

It has been reported that isolated blood vessel segments spontaneously release PGI_2 from endogenous arachidonic acid into the incubation medium, but the release became smaller with each successive incubation.³⁾ Addition of arachidonic acid to the exhausted vessel segments in the incubation medium increased the PGI_2 formation, which was again decreased with a lapse of time.

This paper describes that the later decrease of PGI_2 formation from arachidonic acid is due to decreased activity of PG endoperoxide synthetase, particularly PG hydroperoxidase, and not to that of PGI synthetase.

Materials and methods. Aortic strips (about 4 cm, 30–40 mg wet weight), isolated from male rats (SD strain, 8–10 weeks) and cut longitudinally, was incubated for 10 min at 22°C in 1 ml of the medium composed of one fourth volume of 150 mM Tris-HCl buffer (pH 7.4) in 0.9% NaCl solution. The incubation medium was changed every 10 min, and PGI_2 content, produced for 10 min, was immediately bioassayed as an anti-aggregatory activity using rabbit platelet aggregation induced by ADP (1, 3 and 10 μ M).

Platelet rich plasma was prepared by centrifugation at 200×g for 15 min from rabbit whole blood containing one tenth volume of 3.8% sodium citrate. Platelet aggregation was measured photometrically at 37°C.⁶⁾ The anti-aggregatory activity in the incubation medium was compared with that of authentic PGI_2 . At the day of use, 50 μ g of $PGI_2 \cdot Na$ in an ampoule was dissolved in absolute ethanol (0.25 ml) and then was serially diluted with 50 mM glycine-NaOH buffer (pH 10.1). The solutions were stored at 0°C and further diluted to fifty fold and neutralized with the incubation medium im-

mediately before use.

ADP and tryptophan (Kyowa Hakko Co., Ltd., Tokyo) and arachidonic acid (Applied Sci. Lab. Inc., Penn.) were purchased. PGH_2 , $\text{PGI}_2 \cdot \text{Na}$ and 15-hydroperoxy-5,8,11,13-eicosatetraenoic acid (15-HPETE) were kindly supplied by Ono Pharmaceut. Co., Ltd., Osaka. Indomethacin was a gift from Merck, Sharp and Dohme Res. Lab., Penn.

Results and discussions. When a rat aorta was excised out and incubated in the medium, the anti-aggregatory activity was released into the medium, as reported by others.^{3),7)} This activity was considered to be due to PGI_2 , since the generation was inhibited by indomethacin ($10 \mu\text{M}$) and 15-HPETE ($5 \mu\text{M}$). Furthermore, a half life of the activity was 11–14 min in the incubation medium (pH 7.4, 22°C) and agreed practically with that of authentic PGI_2 (13 min).

The production of the anti-aggregatory activity for 10 min was highest immediately after excision of the aortae (0.1–0.16 nmoles equivalent to PGI_2), but became gradually smaller when the medium was changed every 10 min. When arachidonic acid ($10 \mu\text{M}$) was added to the medium, the PGI_2 biosynthesis for 10 min was increased approximately 5 times as high as that immediately before (3 experiments). The successive incubation with arachidonic acid, however, did not keep the PGI_2 generation at the same level, even in the presence of the same amounts of arachidonic acid in the medium (Fig. 1A). This suggests that enzyme inactivation may be involved at least partly in the decline of the PGI_2 formation from arachidonic acid.

On the contrary, when PGH_2 ($1.5 \mu\text{M}$) was used as a substrate, the PGI_2 formation for 10 min was increased 4–5 times and was kept constant (3 experiments), so that the reduction of the formation was not observed, as shown in Fig. 1B. This indicates that PGI synthetase itself may be intact.

It is known that PGI synthetase can be inactivated by 15-HPETE and other hydroperoxy fatty acid.⁸⁾ Thus, a nascent oxidizing agent, derived from enzymatic reduction of PGG_2 to PGH_2 , might inactivate PGI synthetase in PGI_2 biosynthesis from arachidonic acid.⁹⁾ Fig. 2 indicates that this is not the case.

When PGH_2 was added to the medium, PGI_2 formation was observed as in Fig. 1. Then, arachidonic acid ($10 \mu\text{M}$) was superimposed to PGH_2 ($1.5 \mu\text{M}$). If the inactivation of PGI synthetase by the nascent oxidizing agent from PGG_2 is expected, the PGI_2 production from PGH_2 should be inhibited after addition of arachidonic acid. Even after PGI_2 biosynthesis from arachidonic acid has waned off, the level of PGI_2 production from PGH_2 was still kept exactly at the same level before the addition of arachidonic acid (Fig. 2A). This

Fig. 1. PGI₂ formation by isolated rat aortae from arachidonic acid and PGH₂. Arachidonic acid (AA) (A) or PGH₂ (B) was added to the incubation medium, when spontaneous PGI₂ formation was markedly reduced after standing for 80 min at room temperature following excision. Each point indicates the amount of PGI₂ released into the medium for 10 min. Abscissa shows the time of incubation.

result clearly shows that PGI synthetase in the rat aorta was not inactivated by the oxidizing agents, which was supposed to be generated in conversion of PGG₂ to PGH₂. PGI synthetase was reported to be inhibited by oxidizing agents from PGG₁ and PGG₂ as well as 15-HPETE, when ram seminal vesicle microsomes were used as an enzyme source.⁹⁾ These results were not supported by the present experiment using living endothelial cells of rat aortae, which might have an excellent mechanism to reduce the nascent oxidizing agents and may quite different from microsomes.

Accordingly, later decrease of PGI₂ generation from arachidonic acid could be suspected to be due to inactivation of the enzyme, PG endoperoxide synthetases, which convert either arachidonic acid to PGG₂ or PGG₂ to PGH₂; fatty acid cyclooxygenase or PG hydroperoxidase. PG hydroperoxidase in ram seminal vesicle microsomes is also reported to be inactivated by the oxidizing agent from PGG₂¹⁰⁾ and

Fig. 2. PGI₂ formation by isolated rat aortae from PGH₂ superimposed by arachidonic acid and from arachidonic acid with tryptophan. A: PGH₂ was added to the incubation medium, and then arachidonic acid (AA) was superimposed. B: Arachidonic acid was added with tryptophan (Trp). Each point indicates the amount of PGI₂ released into the medium for 10 min. Abscissa shows the time of incubation.

the inactivation was protected so-called radical-scavengers.^{10),11)} Studies with the purified enzymes from bovine seminal vesicles also revealed that PG hydroperoxidase requires various aromatic compounds such as tryptophan with heme compounds as cofactors in conversion of PGG₂ to PGH₂, although cyclooxygenase requires heme compounds alone.¹²⁾⁻¹⁴⁾

As shown in Fig. 2B, addition of tryptophan (10 mM) to the incubation medium prevented markedly the decrease of PGI₂ production from arachidonic acid. Thus, the later decrease in PGI₂ biosynthesis from exogenous arachidonic acid in the isolated rat aortae may be explained by gradual deterioration of PG hydroperoxidase. This is supported by the fact that purified PG hydroperoxidase from bovine

vesicular gland microsomes is activated by heme, and then inactivated by the same cofactor, and this inactivation is protected by tryptophan or various other aromatic compounds.¹³⁾

From these results, it could be concluded that PG endoperoxide synthetases, particularly PG hydroperoxidase, may be inactivated during PGI₂ biosynthesis from exogenous arachidonic acid in rat aortae and PGI synthetase is intact in this system.

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