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Biochemistry

Synthesis and biological properties of a 9,11-azo-prostanoid: Highly active biochemical mimic of prostaglandin endoperoxides*

(9,11-peroxido-prostanoid/platelet aggregation/serotonin release/muscle contraction)

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ABSTRACT **The 9,11-azo-prostanoid III [(5Z, 9α, 11α, 13E, 15S)-9, 11-azo-15-hydroxyprosta-5,13-dienoic acid] has been obtained by synthesis and tested for biological activity in systems which are responsive to the prostaglandin endoperoxides PGH_2 (I) and PGG_2 (II). The azo analog III is a powerful mimic of these endoperoxides with reference to platelet aggregation and release of serotonin when added to human platelet-rich plasma. The analog III is substantially more active (about 7 fold) than PGG_2 in stimulating muscle contraction in the isolated rabbit aorta strip. The very great stability of III relative to PGH_2 and PGG_2 and its potency as a mimic of these important substances suggest that this azo analog will be of considerable value in future studies of the prostaglandin endoperoxides.**

The two prostaglandin (PG) endoperoxides, PGH_2 (I, Fig. 1) and PGG_2 (II), derived *in vivo* from arachidonic acid, are very potent in inducing rapid and irreversible aggregation of human platelets through release of ADP and serotonin (1–3). The endoperoxides are rapidly metabolized to a hemiacetal derivative 8-(1-hydroxy-3-oxopropyl)-9, 12L-dihydroxy-5,10-heptadecadienoic acid (PHD) in platelets, and this metabolite is released in large amounts during aggregation induced by various agents (4, 5). A physiological role of the endoperoxide system in human platelets has been established through studies demonstrating that a hemostatic defect involving an abnormal platelet release mechanism was due to deficiency of the cyclo-oxygenase responsible for endoperoxide synthesis (3). The endoperoxides are also potent stimulants of vascular (rabbit aorta and umbilical artery) and air-way smooth muscle (6).

The endoperoxides PGH_2 and PGG_2 are intrinsically highly unstable substances (half-life, $t_{1/2}$, about 5 min in aqueous solution at 37° and pH 7.4). Further, they are transformed with great rapidity enzymically [e.g., $t_{1/2}$ about 10 sec in platelet-rich plasma (PRP)]. Because of the extraordinary lability of these endoperoxides and the consequent difficulties and limitations in experimental use, the design and synthesis of a stable and active analog was deemed important. The most interesting analog for initial study appeared to be the azo compound represented by formula III in Fig. 1. It was anticipated that this substance would possess very nearly the same molecular geometry as PGH_2 (I), but would be indefinitely stable at pH 7 and 37°. Although there was no assurance *a priori* that III would behave as a close mimic of PGH_2 (or PGG_2) rather than as an antagonist, the experimental resolution of this question itself seemed worthwhile as a step toward a better understanding of the biochemical mode of action of PGH_2.

MATERIALS AND METHODS

The preparation of the endoperoxides PGG_2 and PGH_2 has been described previously (2). Blood from healthy donors, who had not taken any drugs for at least 1 week, was collected from the antecubital vein with 0.13 volume of 0.1 M trisodium citrate. After centrifugation at 200 × *g* for 15 min at room temperature, PRP was removed. Calcium chloride (10 μl 0.25 M solution per ml of PRP) was added.

Platelet aggregation was measured by continuous recording of light transmission (Aggregometer, Chrono-log Corp., Broomall, Pa.). Samples were preincubated for 2 min at 37° before adding the compounds to be investigated.

Synthesis of (5Z, 9α, 11α, 13E, 15S)-9,11-azo-15-hydroxyprosta-5,13-dienoic Acid (III). (For prostaglandin nomenclature see ref. 7.) The chemical synthesis of the azo analog III of the PG endoperoxides was carried out as outlined in Fig. 1 starting from PGA_2 methyl ester acetate (IV) which is available either by total synthesis (8–10) or by extraction of the soft coral *Plexaura homomalla* (11). The methyl ester acetate IV was converted to a mixture of α- and β-10,11-epoxides (V) by reaction at −20° for 1.5 hr in methanol (18 ml/g of IV) with 0.5 eq of sodium hydroxide and 10 eq of 30% aqueous hydrogen peroxide, following a known method (12). The reaction was quenched at −20° with solid ammonium chloride and after isolation the mixture of α and β epoxides V (ratio about 1.25:1) was freed of minor contaminants by chromatography on silica gel (ether elution, yield 82%). Treatment of the mixture of epoxides V in tetrahydrofuran–water (100:1, 70 ml/g of V) at 0° using 7 eq of amalgamated aluminum foil with stirring for 2 hr afforded after isolation a mixture of 11α- and 11β-hydroxy-9-keto compounds (12) from which the 11β-isomer (VI) was obtained in pure form (29% yield) by chromatography on silica gel (the R_F values observed for the 11β- and 11α-hydroxy compounds were 0.40 and 0.30, respectively, on silica gel plates with ether as eluent).§ Reduction of the 9-keto group of VI was accomplished stereoselectively using zinc borohydride (1.5 eq) in dimethoxyethane (15 ml/g of VI) at 0° for 20 hr or 25° for 4 hr to form VII (90%) containing a small amount (about 10%) of the 9α-epimer. It was advantageous to use the mixture of alcohols for the next step, since purification of the mesylate VIII was straightforward. Mesylation was

Abbreviations: PG, prostaglandin; PHD, 8-(1-hydroxy-3-oxopropyl)-9,12L-dihydroxy-5,10-heptadecadienoic acid; PRP, platelet-rich plasma; eq, molar or atomic equivalents.

* This is paper no. 9 in a series on prostaglandin endoperoxides; paper no. 8 is ref. 3.

§ The structures assigned herein to intermediates VI–IX and to the azo-PG III were confirmed by infrared, nuclear magnetic resonance, and mass spectrometric data obtained using chromatographically homogeneous samples.

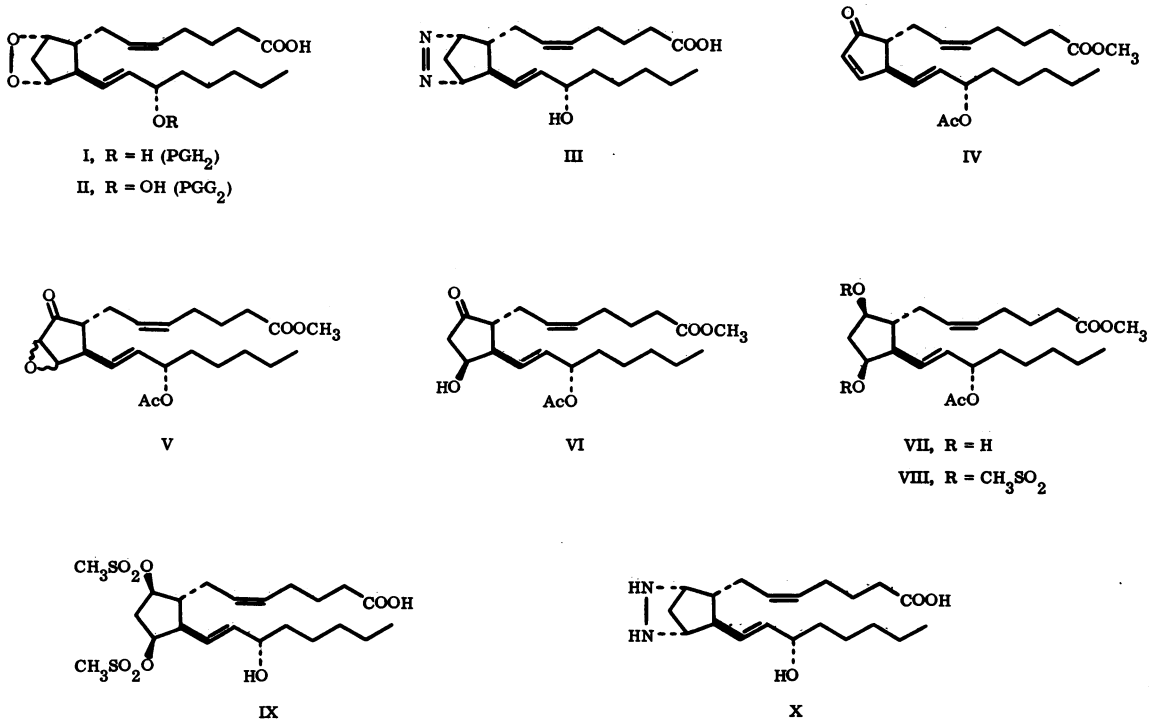

FIG. 1.

carried out using mesyl chloride (3 eq) and triethylamine (3 eq) in methylene chloride (40 ml/g of VII) at −20° for 1 hr using the standard method (13). The mesylate (80% yield) was purified by preparative layer chromatography on silica gel using 0.75% methanol in methylene chloride and multiple development which separated VIII from the faster moving 9α-epimer (found for pure VIII, mp 63–64°; $[\alpha]^{22}_D$ +6.40° in chloroform). Saponification of VIII to form the oily hydroxy acid IX (85% yield) was effected by treatment with lithium hydroxide (5 eq) in 1:1 water–methanol (60 ml/g of VIII) at 25° for 20 hr (found for IX, $[\alpha]^{22}_D$ +16.75° in chloroform). Treatment of IX in *t*-butyl alcohol–ethanol (3:1, 30 ml/g of IX) with a large excess of 95% hydrazine at reflux under argon for 12 hr and standard isolation led to the cyclic hydrazine derivative X, which without purification was dissolved in methanol–ether and stirred in air after addition of 1 mg of cupric acetate (to catalyze oxidation) to afford the azo compound III. Pure III was obtained by preparative layer chromatography on silica gel (development with 4% methanol in ether) as a colorless crystalline solid (from pentane–ether) in 60% yield; mp 74–75°; $[\alpha]^{22}_D$ −17.7° in chloroform; ultraviolet absorption 342 nm (ε 90); infrared absorption due to N=N at 1495 cm^{-1}, COOH at 1710 cm^{-1}, OH at 3300 cm^{-1}. The azo-PG III is stable indefinitely at 0° in aqueous solution or as the pure solid; further, no appreciable decomposition has been observed after several days at 25°.

Release of [^{14}C]Serotonin. The PRP was incubated at room temperature for 45 min with 5×10^{-7} M [2-^{14}C]serotonin creatinine sulfate (58 mCi/mmol, The Radiochemical Centre, Amersham, England). One-milliliter samples were

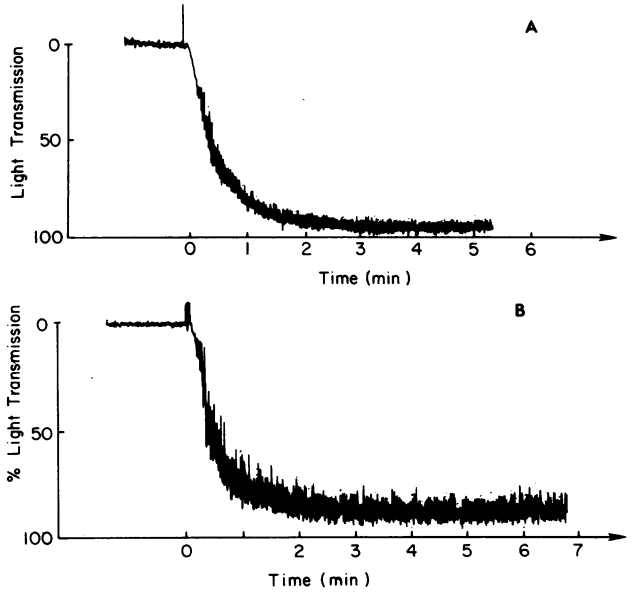

FIG. 2. Platelet aggregation in PRP from the same donor induced by (A) 1 μM PGG_2 and (B) 0.1 μM azo analog III. The compounds were added at 0 time.

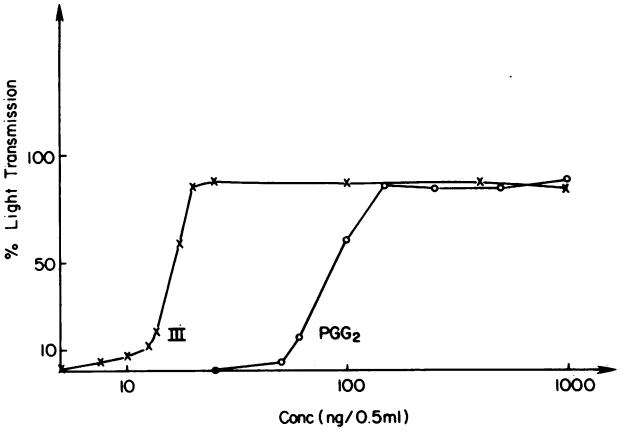

FIG. 3. Dose (log scale)–response in platelet aggregation induced with PGG_2 (O) and azo analog III (X).

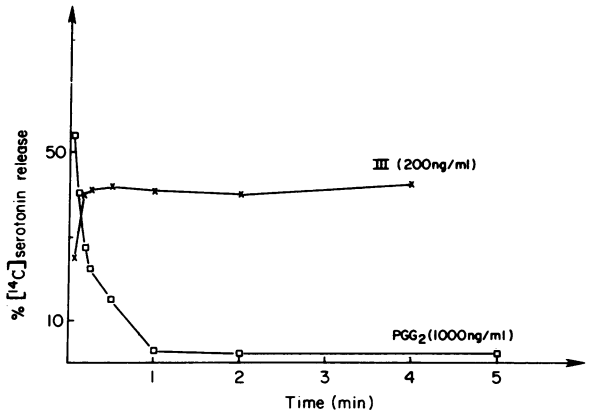

FIG. 4. [^{14}C]Serotonin release from prelabeled platelets by PGG_2 (□) and azo analog III (×).

subsequently preincubated for 2 min at 37° before adding the test compound. The release reaction was stopped by adding 0.2 ml of ice-cold 0.1 M EDTA, pH 7.4, with rapid mixing. Platelets and aggregates were removed by centrifugation at 650 × *g* for 15 min. Aliquots of the supernatant were removed for determination of radioactivity (14).

Contraction of Rabbit Aorta Strips. Strips, about 3 cm long and 0.5 cm wide, were prepared from spirally cut thoracal aortas from rabbits weighing about 2 kg. The strips were suspended and superfused (6, 15) with 3 ml/min of Krebs-Henseleit bicarbonate medium (16) saturated with 96.5% O_2–3.5% CO_2. The contractions (isometric) were recorded by a Grass model FT 03C force-displacement transducer connected with a Grass model 79C polygraph. The endoperoxide or the azo analog III was dissolved in 0.5 ml of Krebs medium and immediately (within 15 sec) added to the strip.

RESULTS

Platelet aggregation

Like the endoperoxides, the azo analog III caused rapid and irreversible aggregation of platelets in human PRP (Fig. 2). However, the azo compound III was 7.9 ± 2.3 (mean ± SD) times more potent than PGG_2 (studied with four different donors of PRP). A dose–response curve is shown in Fig. 3. Higher doses of PGG_2 inhibited the aggregation. This was not observed with the azo analog.

Release of [^{14}C]serotonin

The effects of PGG_2 and the azo analog III on the release of [^{14}C]serotonin from prelabeled platelets are shown in Fig. 4.

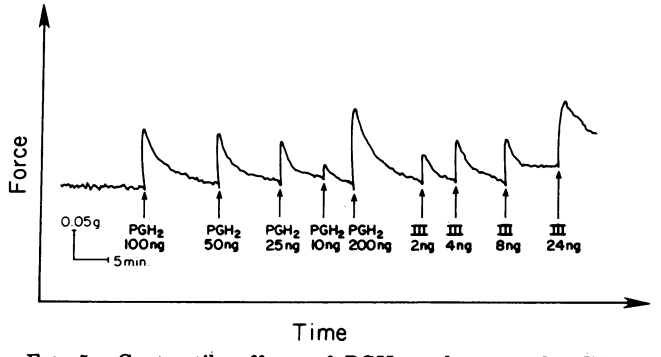

FIG. 5. Contractile effects of PGH_2 and azo analog III on strips of rabbit aorta.

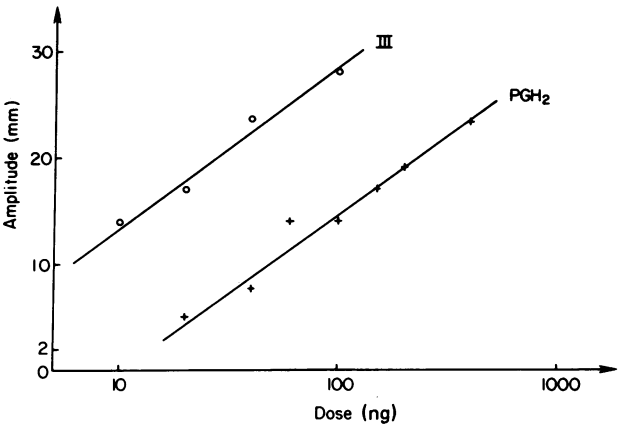

FIG. 6. Dose (log scale)–response of the contractile effect of PGH_2 and azo analog III on strips of rabbit aorta.

Both compounds caused rapid release. However, with PGG_2 there was re-uptake of [^{14}C]serotonin. The azo analog was 6.0 ± 1.4 (mean ± SD, n = 4) times more potent than PGG_2 in inducing [^{14}C]serotonin release. The concentration of the azo analog required for 25% release of [^{14}C]serotonin was 79 ± 21 ng/ml.

Contraction of strips from rabbit aorta

Earlier studies showed that PGG_2 was 80 ± 19 and PGH_2 was 210 ± 41 times more active than PGE_2 in contracting the aorta strips. In the present experiments the azo analog III was 6.9 ± 3.3 times more active than PGH_2 using four differrent strips, i.e., about 1450 times more active than PGE_2. The contractile effects and a dose-response curve are shown in Figs. 5 and 6, respectively.

DISCUSSION

As discussed in the introduction, the endoperoxide PGG_2 has a physiological role in human blood platelets. The azo analog III, now readily available by chemical synthesis, shows remarkable biological potency and clearly functions as a mimic rather than an antagonist of the PG endoperoxides.

Like the endoperoxide PGG_2, the analog III caused rapid and irreversible aggregation of platelets in PRP and released [^{14}C]serotonin from prelabeled platelets. It was six times more active than PGG_2 in the release reaction and 7.9 times more active with respect to the aggregation. The release reaction was almost as fast with the azo analog as with PGG_2. Both compounds caused a much faster release than other aggregating agents, e.g., ADP, epinephrine, collagen, and thrombin. A significant difference between PGG_2 and the azo analog III was the continued release with the azo analog as opposed to the rapid re-uptake with PGG_2. This might be due to increased metabolic stability of the azo analog.

Another test system, the isolated rabbit aorta strip, which is very sensitive to the endoperoxides PGG_2 and PGH_2, also responded to the azo analog III. The analog was about seven times more active than PGH_2 and about 1450 times more active than PGE_2.

The properties of the azo analog III in the systems studied indicate that it should be of considerable value in future studies of the mechanism of action of endoperoxides in various biological systems. A more general study of the biological activity of III is clearly appropriate.

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