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ANTIAGGREGATING ACTIVITY OF SOME SYNTHETIC PROSTAGLANDINS

S. A. Kudryashov, N. G. Geling,
V. I. Mel'nikova, and K. K. Pivnitskii

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Prostaglandins (PG) play an important role in the regulation of the circulation. Some of them are able to inhibit platelet aggregation and to cause dissociation of aggregated platelets, thereby regulating thrombus formation and the lumen of blood vessels. The most effective inhibitors of platelet aggregation are prostacycline (PGI_2) and prostaglandin E_1 (PGE_1) [12]. Natural PG are very labile (especially PGI_2) and they quickly lose their activity *in vivo*. Since some synthetic analogs of PG are more stable and have a prolonged action, the preparation of these compounds and the study of their biological properties have recently acquired great importance.

The 11-deoxyprostaglandins are among the simplest and most accessible analogs of natural PG. Absence of the 11-hydroxyl group in these compounds usually causes no change in the direction of their biological action [4]. For instance, the 11-deoxyprostaglandins largely preserve their ability to stimulate contraction of smooth muscles [6], and the antisecretory [10] and bronchodilator [7] activity of the PG. However, there are no data in the literature on their effect on platelet aggregation. In addition, in the case of several PG cases are known when the two opposite poles of the same PG are biologically active [11]. Since only the antiaggregating activity of PG of the natural stereochemical series is known, it was interesting to obtain data on the activity of their analogs not found in nature. In the investigation described below the antiaggregating activity of certain synthetic 11-deoxyprostaglandins and of racemic PGE_1 was studied.

EXPERIMENTAL METHOD

Samples of racemic PG synthesized previously at the Institute of Experimental Endocrinology and Hormone Chemistry, Academy of Sciences of the USSR — rac-11-deoxy PGE_1 (m.p. 83-85°C) [1], rac-15-methyl-11-deoxy-PGE_1 (m.p. 97.5-98.5°C), rac-PGE_1 (m.p. 110-111°C) [2], rac-11-deoxy-PGE_2 (m.p. 48.5-50°C) [3], the sodium salt of rac-11-deoxy-PGI_2 (aqueous solution; obtained from rac-11-deoxy-$PGE_{2\alpha}$ [3] by analogy with [9]), and optically active PG — the sodium salt of nat-PGI_2 (from Upjohn, USA), nat-PGE_1 (Institute of Chemistry, Academy of Sciences of the Estonian SSR, Tallin), and nat-PGE_2 (from Upjohn, USA) were investigated. Immediately before the work the substances were dissolved in 50 mM of Tris-buffer (pH 10.0) and several dilutions of these solutions were prepared. Blood was taken from the left ventrical of a male rabbit under pentobarbital anesthesia. Sodium citrate (one part of a 3.8% solution of sodium citrate to nine parts of blood) was used as anticoagulant. To obtain platelet-enriched plasma blood was centrifuged at 160g for 10 min at room temperature. Platelet-deprived plasma was obtained by centrifugation of the lower layer at 4000g for 6 min. Platelets were counted under the microscope in a Goryaev's chamber. Platelet-enriched plasma was diluted with platelet-deprived plasma to a concentration of 600,000 platelets/μl. Platelet

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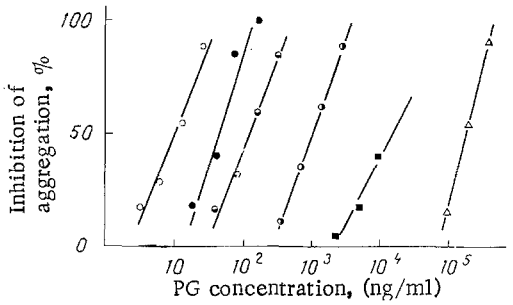

Fig. 1. Dependence of inhibition of ADP-induced platelet aggregation on dose of PG. Legend: empty circles — PGI_2 (r = 0.979); filled circles — PGE_1 (r = 0.983); horizontal semicircles — $+PGE_2$ (r = 0.994); vertical semicircles — +11-deoxy-PGI_2 (r = 0.999); filled squares — PGE_2 (r = 0.987); triangles — +11-deoxy-PGE_1 (r = 0.999); r — coefficient of correlation with calculated regression line.

TABLE 1. Antiaggregating Activity of PG

PG	ED_{50} ± standard deviation, ng/ml	Relative activity ± standard deviation, %
nat-PGI_2	9,6±2,2 (n=4)	450±140
nat-PGE_1	42,8±8,7 (n=4)	100
rac-PGE_1	120±15 (n=4)	36±8
rac-11-deoxy-PGI_2	1052±27 (n=4)	4,1±0,9
nat-PGE_2	(15,8±3,5)·10^3 (n=3)	0,27±0,07
rac-11-deoxy-PGE_1	(183,6±1,5)·10^3 (n=3)	0,023±0,005

aggregation was tested by the method in [5] on a "Chrono-Log" Model 330 aggregometer (USA), with constant stirring of the plasma at 37°C. The sodium salt of ADP was used as aggregating agent, the lowest concentration (4-6 μM) capable of inducing irreversible platelet aggregation being chosen. Aliquots (10 μl) of PG solutions were added to the cuvette of the aggregometer, containing 0.45 ml plasma, 1 min before addition of the ADP. The degree of inhibition of aggregation was calculated from the change in amplitude of the aggregation curve compared with the control response of plasma to ADP.

EXPERIMENTAL RESULTS

Typical dose-dependent curves of inhibition of ADP-induced aggregation by the various PG are illustrated in Fig. 1. On the linear part of the effect — log concentration curve parameters of regression curves were calculated by the method of least squares, and hence the values of ED_{50} (the concentration of PG giving 50% inhibition of aggregation). Relative activities were calculated as ratios of ED_{50} of nat-PGE_1, chosen as the standard (its activity was taken to be 100%) and ED_{50} of each PG. The values of ED_{50} and relative activities of the PG are given in Table 1.

The PGs studied in the presence of ADP mainly exhibited antiaggregating activity. rac-PGE_1 preserved 36 ± 8% of the activity of nat-PGE_1. Since rac-PGE_1 is a mixture of the natural and synthetic isomers, it can be concluded that the synthetic isomer of PGE_1 (enantio-PGE_1) does not behave as an antiaggregating agent, but at the same time it does not stimulate aggregation (within the limits of experimental error). 11-Deoxyprostaglandins exhibit weak activity. Rac-11-deoxy-PGE_1 has only 0.027% of the activity of nat-PGE_1, whereas rac-11-deoxy-PGI_2, the analog of prostacycline, has 0.9% of the activity of nat-PGI_2. Even allowing for the fact that the rac-11-deoxy-PGE_1 and rac-11-deoxy-PGI_2 used contain 50% of potentially inactive (by analogy with rac-PGE_1) synthetic isomer, the results show that removal of the 11-hydroxyl group from PG leads to a reduction of their antiaggregating activity by 50-2000 times. For comparison, with the same modification of the PG molecule, antisecretory activity [10] and ability to stimulate contraction of smooth muscles [6] are reduced only by a factor of 10.

Rac-15-methyl-11-deoxy-PGE_1, in a concentration of 21-82 μg/ml, on the other hand, stimulated ADP-induced aggregation. Rac-11-deoxy-PGE_2 in concentrations under 50 μg/ml does not exhibit antiaggregating activity, but in a concentration of 100 μg/ml it induces aggregation. PG of series 2 under certain conditions possess aggregating activity [8].

The results are evidence that a change in the absolute configuration of the molecule, namely introduction of an additional double bond or removal of the 11-hydroxyl group, leads to a sharp decrease in antiaggregating activity or its complete disappearance. Just as in the series of natural PG, in the series of 11-deoxy-analogs it is the analog of prostacycline (rac-11-deoxy-PGI_2) which possesses the highest absolute activity; its activity, moreover, is quite commensurate with the activity of PGE_1.

On the basis of these findings the search for active and selective analogs of prostacycline for practical use in medicine by further modification of the strucure of the 11-deoxy prostaglandins would seem promising.

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