EFFECT OF PROSTAGLANDINS E_1, E_2, and $F_{2\alpha}$ AND PROSTACYCLIN ON LOCAL THROMBOSIS FOLLOWING LASER INJURY TO THE MICROVASCULAR WALL

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UDC 616.16-001-02:615.849.19]-07:
616-005.6-02:615.35:577.175.859

KEY WORDS: microthrombogenesis; embolization; prostaglandin; prostacyclin.

After slight injury to the vascular wall local thrombi consisting mainly of platelets are formed. The functions of platelets, connected with their activation, aggregation, and adhesion, are determined by dynamic equilibrium between prostaglandin compounds formed both in the vessel wall and in the platelets themselves [1, 5]. Disturbance of the balance between the components of the prostacyclin-thromboxane system in cardiovascular diseases, and also changes in the sensitivity of platelets to these compounds are considered to be among the fundamental mechanisms of regulation of local microthrombus formation [4, 6]. The investigation of this process in the vascular wall after injury to it by a laser beam has demonstrated the great dependence of microthrombosis on inhibitors inhibiting the synthesis of prostaglandins (PG) with pro- or antiadhesive action [3]. This investigation was continued in the present study, the aim of which was to examine the effects of PG E_1, E_2, and

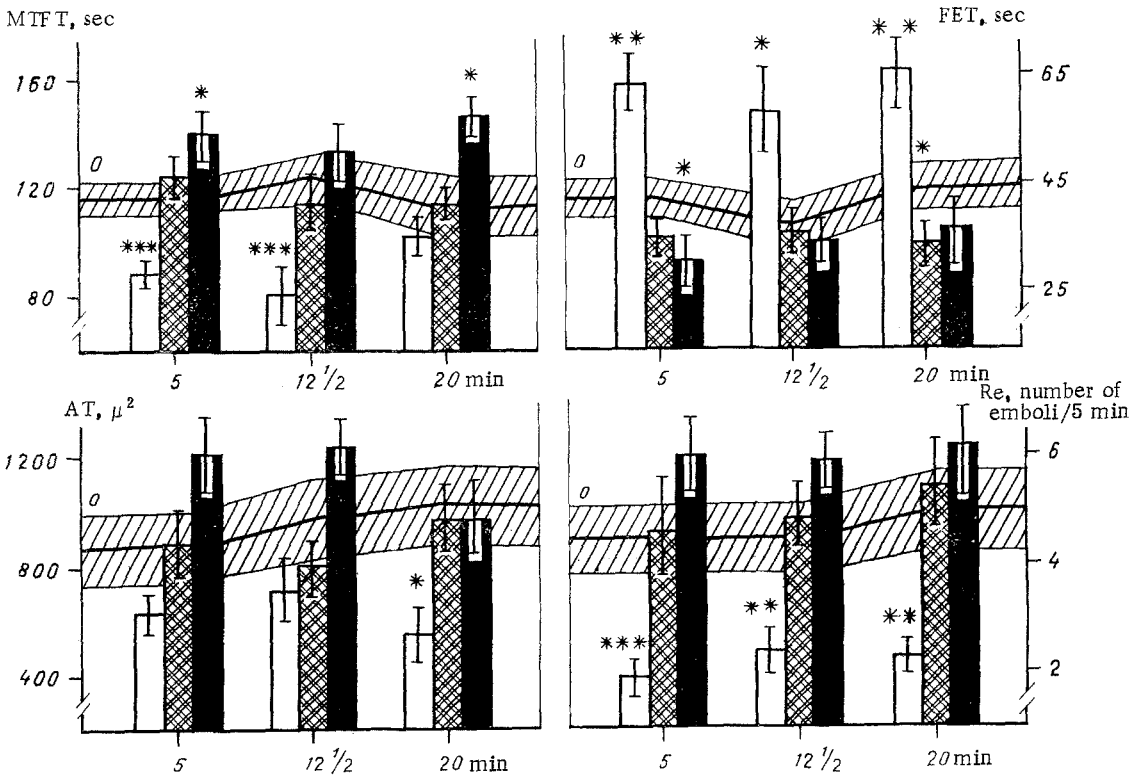

Fig. 1. Changes in parameters of microthrombosis in vessel wall injured by laser beam, under the influence of PG infusion. 0) Control (n = 10); unshaded columns — PG E_1 (n = 7); cross-hatched columns PG E_2 (n = 8); black columns — PG $F_{2\alpha}$ (n = 7). *P < 0.05; **P < 0.01; ***P < 0.001.

Institute of General Pathology and Physiology, Academy of Medical Sciences of the USSR, Moscow. Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 92, No. 12, pp. 677-680, December, 1981. Original article submitted January 14, 1981.

0007-4888/81/9212-1647$07.50

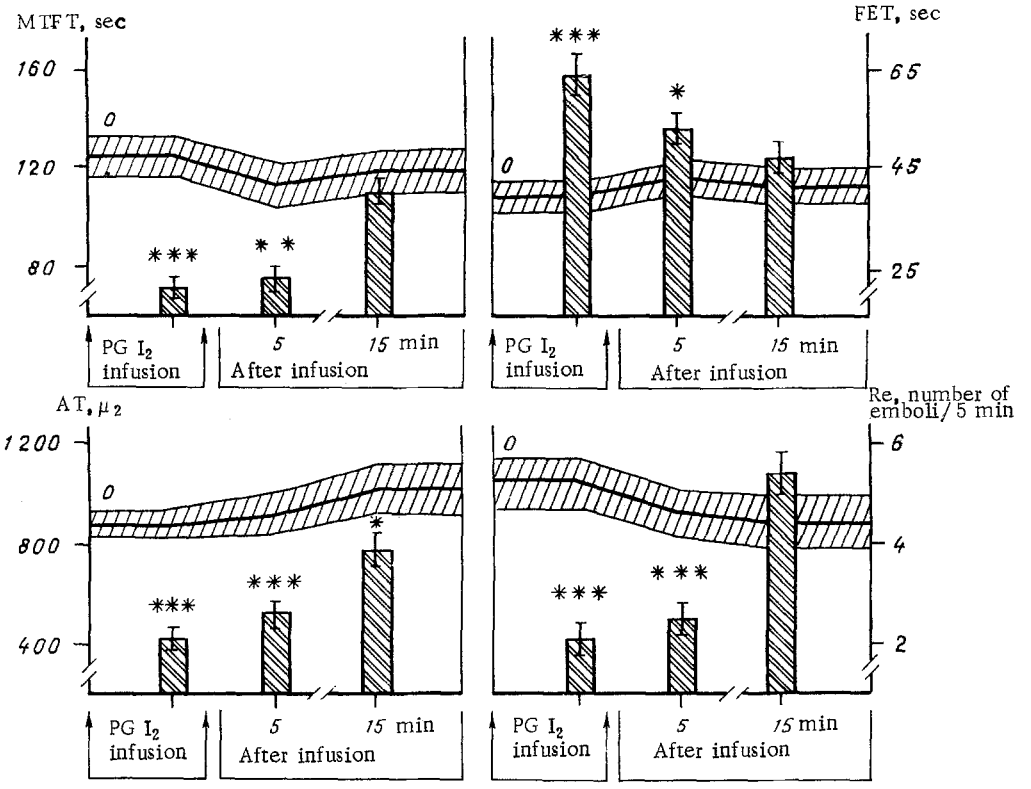

Fig. 2. Changes in parameters of thrombogenesis during and after infusion of prostacyclin (n = 15). 0) Control (n = 16). Remainder of legend as to Fig. 1.

$F_{2\alpha}$ and of prostacyclin (PG I_2) on microthrombosis evoked by injury to a mesenteric venule by the laser beam.

EXPERIMENTAL METHODS

Experiments were carried out on 63 male Wistar rats weighing 180-210 g, anesthetized with pentobarbital. A set of equipment consisting of an MBI-6 microscope, coupled with a UV-laser [2], was used. The substances were injected by means of an infusion pump at the rate of 0.01-0.04 ml/min through a catheter inserted into the peripheral end of the caudal artery. Changes in arterial pressure were monitored by an electromanometer, the transducer of which was introduced into the left carotid artery. The test objects were 189 mesenteric venules with an internal diameter of 34.4 ± 0.3 μ, the walls of which were injured on the endothelial side by a laser beam (to some extent the blood cells in the lumen of the vessel were also affected by the beam). Exposure to the laser continued until the appearance of microhemorrhages and adhesion of the first platelets at the site of injury. In the course of all experiments this time remained unchanged at 15.6 ± 0.4 sec. In preliminary experiments the doses of PG were chosen so that the arterial pressure changed by not more than 15-20% of its initial value during infusion. The diameter of the test venules and the character of the blood flow in them were unchanged under these circumstances. In the experiments of series I, II, and III the action of PG E_1 (4.1 μg/kg/min), PG E_2 (8.6 μg/kg/min), and PG $F_{2\alpha}$ (15.6 μg/kg/min) was studied. Injuries to the chosen venules were inflicted 5, 12.5, and 20 min respectively after the beginning of infusion. In the experiments of series IV, PG I_2 was infused in a dose of 0.23 μg/kg/min for 8 min. Considering the extreme instability of prostacyclin in solution, the sodium salt of PG I_2 was dissolved immediately before the experiment in 0.05 M Tris-HCl buffer, pH 9.4; isotonic solutions of the substance were adjusted to a final pH of 8.2. The first injury was inflicted 3 min after the beginning of infusion, the second and third injuries 5 and 15 min respectively after the end of infusion. In each series control tests were carried out with physiological saline (or Tris buffer). The substances used in the experiments were synthesized in the Laboratory of Pure Substances (Director, J. Lille), Institute of Chemistry, Academy of Sciences of the Estonian SSR. In separate series for comparison analogous PG preparations obtained from Upjohn (USA) were used.

Biomicroscopic observations of local thrombus formation at the site of injury to the venule enabled the successive stages of this process to be examined: adhesion of the first platelets, their gradual conversion into a platelet conglomerate, detachment of microemboli, and further enlargement of the thrombus. The ever-increasing number of detached emboli gradually led to complete disappearance of the thrombus. This process could be considerably modified by the use of various procedures. The processes studied were evaluated quantitatively by the following parameters: the maximal thrombus formation time (MTFT) — the time from switching on the laser to the time when the thrombus reached its maximal height; the first embolization time (FET), the time from switching on the laser until detachment of the first embolus (part of the thrombus) from the main conglomerate of platelets; the area of the thrombus (AT) before the first embolization; the rate of embolization (RE) — the number of emboli detached from the main conglomerate in the course of 5 min. The parameters were counted on an automatic TAS television analyzing system (from Leitz, West Germany), by a program elaborated by V. S. Shinkarenko. The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Analysis of the data (Fig. 1) shows that PG E_1 considerably depressed the process of microthrombus formation in the injured vascular wall. The platelet conglomerate was formed more slowly (MTFT), its area was smaller (at the 20th minute of infusion AT was reduced by 46%), FET was increased, but the velocity of this process was reduced on average by 47-57%. On the whole the changes in these parameters depended only a little on the duration of PG E_1 infusion. Infusion of PG E_2 had virtually no effect on the process of microthrombosis in the injured vessel walls. PG $F_{2\alpha}$, on the other hand, had a prothrombogenic action, but only to a slight degree. Significant changes in MTFT and FET were found at the 5th minute of infusion; in the other cases the increase in the parameters of thrombogenesis did not amount to more than a tendency. All three prostaglandins, incidentally, were used in doses producing minimal shifts of arterial pressure.

Considering the different vasomotor activity of PG E_1, PG E_2, and PG $F_{2\alpha}$, the quantity of the substance entering the blood stream differed for each preparation. Nevertheless, analysis of the results revealed a significant qualitative difference in the action of the substance. In addition, the smallest dose of PG E_1 had the strongest action.

Experiments with PG I_2, considering its instability in the blood stream, were carried out rather differently. The results (Fig. 2) enabled not only the highest antithrombogenic activity of PG I_2, the working concentratin of which was only 1/18th that of PG E_1, but also the duration of the after-effect to be estimated. Whereas during infusion of PG I_2 for 7 min the values of MTFT, FET, AT, and RE changed by 42.1, 65.7, 51.8, and 59.6% respectively, 15 min after the end of infusion only the value of AT differed significantly from the control. The short duration of this effect can be contrasted with the long after-effect of aspirin and indomethacin [3], drugs which inhibit the synthesis of prostaglandin endoperoxides, which lasts many hours.

Comparison of the chosen parameters of microthrombosis, namely temporal (MTFT, FET), linear (AT), and dynamic (RE), enables the process of microhemostasis to be split up into separate components, so that the role of the various types of PG can be evaluated. On the whole it can be concluded that FET and RE, which characterized the intensity of destructive (antiadhesive) processes, are the most variable parameters. Meanwhile the area and height (not considered in this paper) of the thrombus undergo much smaller changes. These data draw attention to the potential benefit to be obtained from studying other (not only PG-dependent) components of microhemostasis, which combined to form the complex dynamic picture of interaction during the repair of injuries in the microvascular wall.

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