BIOPHYSICS AND BIOCHEMISTRY

Effects of Oxytocin and Prostaglandin $F_{2\alpha}$ (Enzaprost) on Platelet Aggregation

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The effects of uterotonic agents (oxytocin and enzaprost) on platelet aggregation in pregnant and nonpregnant women were studied by low-angle light scattering. In nonpregnant women oxytocin produced different effects on ADP-induced platelet aggregation: potentiation at low (<200 nM) and inhibition at high (>400 nM) ADP concentrations. In pregnant women oxytocin did not modulate ADP-induced platelet aggregation or this effect was negligible. Enzaprost competitively inhibited ADP-induced platelet aggregation in all examined women (inhibition constant 84.8±25.7 nM).

Key Words: *platelet aggregation; oxytocin; prostaglandin F_{2a}; pregnancy; labor*

Abnormalities of uterine contractility (UC) are still a frequent labor complication. Some types of this labor abnormality are associated with serious threats to the life and health for both the mother and fetus. The most severe consequence of UC abnormalities is bleeding occurring in 2.7-8.0% labors [4]. Uterotonic drugs (oxytocin, prostaglandins) are the leading means of UC regulation.

The majority of drugs used in obstetrics affect the hemostasis system, particularly, its vascular and platelet components.

Non-activated platelets circulate as individual cells and do not interact with intact endothelium and other blood cells. Their adhesive properties appear only after induction. Collagen, thrombin, catecholamines, ADP, thromboxane, platelet activation factor, Willebrandt factor, vasopressin, *etc.* are well known agonists of platelet activation and subsequent aggregation [1,6].

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Uterotonic drugs used for UC regulation are characterized by a wide spectrum of pharmacological activity and produce some side effects, *e.g.* affect the vascular and platelet components of the hemostasis system. Understanding of the mechanisms of their effects will help to prevent thrombohemorrhagic complications, particularly in patients at risk of hemorrhages during the placental and early postpartum periods.

In the present study the effects of oxytocin and enzaprost (prostaglandin $F_{2\alpha}$) on platelet aggregation in nonpregnant and pregnant women and women in labor were studied by the low-angle light scattering method. This method allows kinetic evaluation of cell activation and initial stages of aggregation (formation of dimers) in a medium containing physiological concentration of Ca^{2+}, which considerably increases platelet sensitivity to agonists.

MATERIALS AND METHODS

Venous blood was collected from the cubital veins of nonpregnant and pregnant women and women in labor

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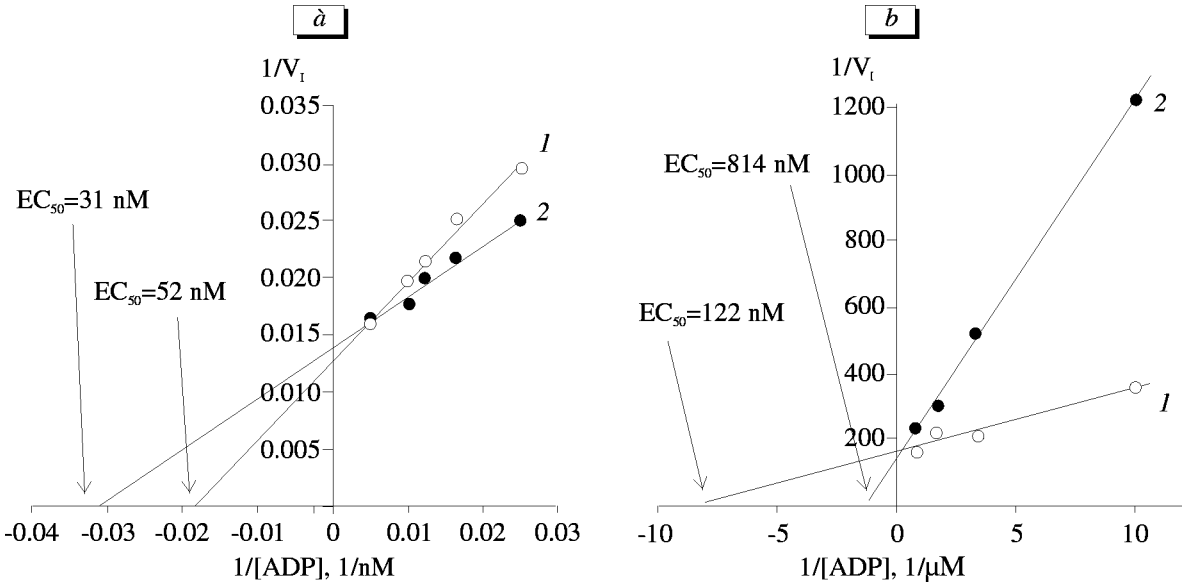

Fig. 1. Relationship between the initial aggregation velocity (V_I) and ADP concentration ([ADP]) in double inverse coordinates. Graphic method for determining EC_{50} is shown. *1*) control; *2*) experiment: preincubation with oxytocin (0.002 pg/ml, 20 min, *a*) or enzaprost (543 nM, 2 min, *b*).

and stabilized with 3.2% sodium citrate (pH 6.0). Platelet-rich plasma (PRP) was prepared by blood centrifugation at 1000 rpm for 10 min (final pH 7.3-7.5).

PRP was 30-80-fold diluted with a medium containing 140 mM NaCl, 1 mM $CaCl_2$, 5 mM Tris-Cl (pH 7.8) to a final platelet concentration of 7×10^6 ml. Low-angle light scattering was recorded on a Light Scan device (Lumeks Firm), laser beam was directed at an angle of 90° to the cuvette and intensity of scattered light was recorded at 2° and 12° with two photodiodes. The signals from photodiodes were transformed and in-put into a PC. The cuvette contained 5 ml medium with PRP.

It was shown previously that changes in signal intensity at angles <4° are determined by aggregation processes (aggregation and disaggregation) [3]. Platelet activation can be recorded at angles of 6-15° with 20-60% useful signal.

In our experiments (*i.e.* in certain salty medium at certain stirring rate and platelet concentration) the initial aggregation velocity (V_I) can be described by the following equation:

$$V_I=V_{max}\times[ADP]/(EC_{50}+[ADP]),$$

where [ADP] is ADP concentration, EC_{50} is the concentration of ADP ensuring half-maximum initial aggregation velocity, and V_{max} is the maximum initial velocity.

EC_{50} determines platelet sensitivity to ADP and reflects activity of intracellular signaling systems (*e.g.* Ca^{2+}/cAMP). If the experiment is methodologically standard (standard cell concentration and medium conditions), V_{max} depends on the efficiency of platelet collisions and reflects the number of aggregation binding sites on the cell.

The effect of oxytocin (0.002-0.040 pg/ml) on ADP-induced platelet aggregation *in vitro* was studied at ADP concentrations of 40-200 nM. The effect of enzaprost was studied at ADP concentrations of 100-1200 nM. Preincubation with enzaprost (543 nM) was carried out for 2 min.

RESULTS

Oxytocin in doses of 0.002-0.040 pg/ml did not induce activation and aggregation of platelets isolated by plasma stabilization with sodium citrate.

In nonpregnant women oxytocin increased V_I of aggregation induced by ADP in concentrations of 40-100 nM. Increasing the concentration of ADP to 200 and 400 nM did not promoted aggregation of platelets preincubated with oxytocin in comparison with the control and even decreased it (particularly at ADP dose of 400 nM). Preincubation with 0.02 pg/ml oxytocin for 20-min produced the same effect.

For elucidation of the effect of oxytocin and evaluation of EC_{50}, the data were presented graphically in inverse coordinates (Fig. 1, *a*). The nanopeptide reduced EC_{50} for ADP, *i.e.* produced a potentiating effect. The effect of oxytocin on ADP-induced platelet aggregation was complex and modulates both EC_{50} and V_{max} (the cross-point of the linear trends does not lie on axes).

The effect of oxytocin on platelets from pregnant women was unstable (in contrast to the control): we observed both the potentiating (similar to the effect on platelets from nonpregnant women) and inhibitory effects. The decrease in EC_{50} in the group of pregnant women indicated increased platelet sensitivity to immediate inductor of aggregation (ADP). The effect of oxytocin of ADP-induced platelet aggregation was unstable, which could be due to increased sensitivity to ADP during pregnancy and to receptor desensitization.

Hence, oxytocin alone did not induce platelet activation and aggregation. This suggests that oxytocin activates intracellular kinases through a Ca^{2+}-independent pathway, in contrast to other nanopeptide, vasopressin, which induces mobilization of intracellular calcium and activates platelets by itself [8,9].

Enzaprost *per se* also did not induce platelet activation, but inhibited ADP-induced platelet aggregation in all groups of examinees.

Enzaprost suppressed platelet aggregation without changing V_{max} and increasing EC_{50} (Fig. 1, *b*). This mechanism corresponds to the competitive inhibition type, which was described for substances activating the adenylate cyclase system (adenosine, forskolin) [2]. Under our conditions the inhibition constant (K_I) was estimated by the following formula:

$$K_I = \frac{[I]}{EC^O_{50}/EC^K_{50}-1}$$

For the selected group K_I=84.8±25.7 nM. Hence, enzaprost is a weak inhibitor of ADP-induced platelet aggregation (for comparison: K_I for adenosine is 2 μM, for forskolin 0.1 μM).

We revealed no differences in the effects of enzaprost on platelets of nonpregnant and pregnant women and women in labor.

Our results indicate that the studied uterotonics do not directly induce platelet aggregation, but modulate ADP-induced platelet aggregation.

A dose-dependent effect of oxytocin was detected in nonpregnant women (potentiating effect at low ADP concentrations and inhibitory effect on the rate of platelet aggregation at higher ADP doses). In pregnant women with elevated blood clotting capacity the effect of oxytocin on platelet aggregation was unstable. Hence, our study showed mixed effects of oxytocin on ADP-induced platelet aggregation.

The mechanism of the effect of enzaprost on ADP-induced platelet aggregation corresponded to competitive inhibition. Activation of the adenylate cyclase system with subsequent increase of cAMP content in the cells is a defense mechanism of autoregulation of platelet functions by the feedback pathway, which can prevent the development of thrombotic complications in intravenous infusion of enzaprost.

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