Cooperative Type of Platelet Hypersensitivity to ADP

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We found that gestosis is associated with platelet hypersensitivity to ADP. Cell P2X1 receptors exhibited a positive cooperative response to ADP (EC_{50}=10.88±3.70 nM, Hill constant *n*=2.59±0.50 rel. units). Cooperative binding of ADP to platelet P2X1 receptors was also observed during incubation of cells from pregnant women with isosorbide dinitrate.

Key Words: *cooperativeness; hypersensitivity; purine receptors; gestosis*

The effects of cyclic nucleotides are mediated by two groups of purine receptors (P2 receptors) on platelets. Group 1 includes P2X1 receptors that belong to the family of ligand-regulated ion channels and are responsible for rapid agonist-induced Ca^{2+} entry into cells. Group 2 comprises G protein-coupled P2Y receptors. Metabotropic P2Y1 and P2Y12 receptors ($P2Y_{ADP}$) are coupled to Gq and Gi proteins that mediate activation of phospholipase Cβ and inhibition of adenylate cyclase, respectively [5].

Under normal conditions ADP-induced platelet activation via P2X1 receptors is achieved at EC_{50} 18.8± 4.5 nM. $P2X_1$ receptors play an important role in the rise in intracellular Ca^{2+} concentration and transition of platelets to a spherical state. The value of ADP EC_{50} during activation and aggregation reflects sensitivity of P2 receptors on platelets to this purine and increases in the following order: $EC_{50}(P2X_1) < EC_{50}(P2Y_1) < EC_{50}(P2Y_{ADP})$. EC_{50} for P2Y1 and P2Y12 are 93.4± 6.6 and 152.8±48.3 nM, respectively [2].

Pilot study of platelet function in pregnant women showed high sensitivity of these cells to ADP (EC_{50}= 55.96±1.89 nM), which suggest that platelet aggregation is realized via P2X1 and P2Y1 receptors (data not published).

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Here we studied the dependence of platelet aggregation in pregnant women with gestosis on agonist concentration (ADP). It was interesting to evaluate whether isosorbide dinitrate can be used for correction of hypersensitivity of the platelet component of hemostasis.

MATERIALS AND METHODS

Platelets were isolated from 15 pregnant women with gestosis. The severity of gestosis was comparable to that of stage I nephropathy. Blood samples were taken from the cubital vein and stabilized with sodium citrate (3.2%, 9:1, pH 6.0). The citrate blood was divided into 2 samples (control sample 1, 5 ml; test sample 2, 5 ml). Isoket (120 μM, 0.1% isosorbide dinitrate) was added to the test sample. Platelet-rich plasma (PRP) was obtained by centrifugation of blood samples 1 and 2 at 100*g* for 7 min. The total period of platelet incubation was 20 min.

Functional activity of platelets from pregnant women was estimated by the method of low-angle light scattering. This approach allows us to study all stages of platelet transformation. As distinct from the standard Born method, our experiments were performed in a saline medium containing 140 mM NaCl, 10 mM Tris-HCl buffer, and 1 mM $CaCl_2$ (pH 7.8). PRP was diluted to a final platelet concentration not exceeding 10^7 cells/ml. Aggregation was induced by 0.5-1.0 mM [Ca^{2+}]. Low-angle light scattering was recorded on a Laska device (Lyumeks). The light from a laser source

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was directed to a cuvette at an angle of 90°. The intensity of light scattering was recorded with photodiodes (angles 2 and 12°). Signals from photodiodes were transformed and fed into a PC.

Experiments were performed in a thermostatic cuvette containing 5 ml medium and PRP. PRP was diluted by 50-250 times, which depended on the initial concentration of platelets. The study was conducted at a platelet concentration of 10^6-10^7 cells/ml [1].

RESULTS

Each sample of PRP was subjected to 5-10 independent measurements. The aggregation rate was estimated in the presence of ADP in concentrations of 2-40 nM. Cell response to the agonist in very low concentration (2-5 nM) was revealed 30-50 min after isolation of PRP. Coagulation of the plasma was detected 40-60 min after the start of recording. Therefore, platelet suspension was consecutively treated with ADP in decreasing concentrations (40, 30, 25, 20, 15, 10, 5, and 2 nM).

Fig. 1, *a* illustrates aggregation of platelets from pregnant women induced by single treatment with ADP. The curve for the dependence of the aggregation rate on ADP concentration was constructed from the intensity of light scattering (Fig. 1, *b*). The saturation curve had a sigmoid shape. For calculation of the concentration of substrate (ADP) corresponding to half-maximum reaction rate (sigmoid kinetic curve) we used modified Hill equation:

$$V_n = V_{\max} \frac{[\mathrm{ADP}]^h}{\mathrm{EC}_{50} + [\mathrm{ADP}]^h},$$

where h is the Hill coefficient; EC_{50} is half-maximum concentration of the agonist; [ADP] is ADP concen-

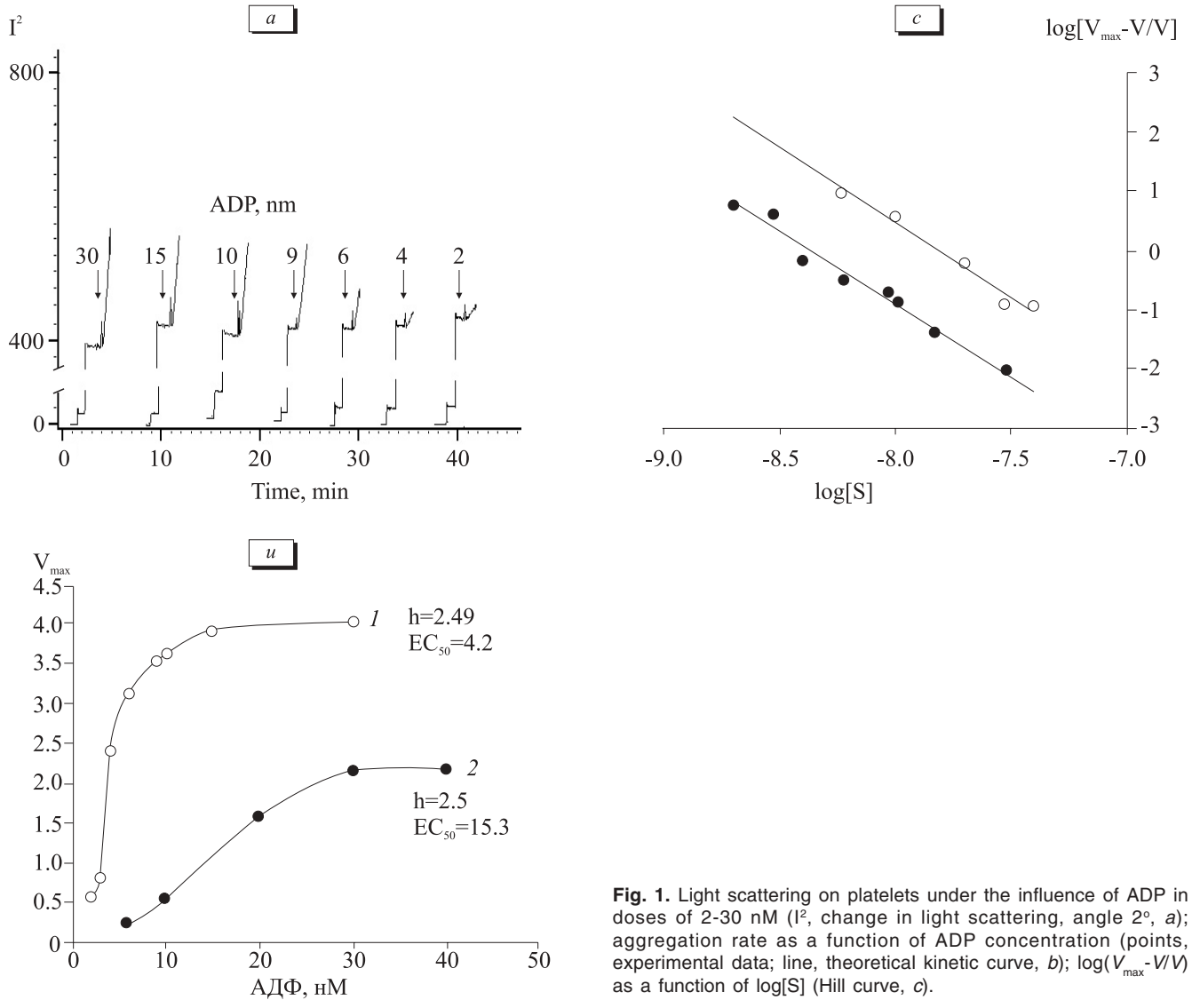

Fig. 1. Light scattering on platelets under the influence of ADP in doses of 2-30 nM (I^2, change in light scattering, angle 2°, *a*); aggregation rate as a function of ADP concentration (points, experimental data; line, theoretical kinetic curve, *b*); $\log(V_{\max}\text{-}V/V)$ as a function of log[S] (Hill curve, *c*).

TABLE 1. Platelet Aggregation during Gestosis

Sample	*h*	EC_{50}	V_{max}
Control	2.60±0.55	10.88±3.69	4.2±1.0
Test	2.0±0.7	52.68±20.70	4.15±1.67

tration; V_{max} is the maximum rate; and V_n is the initial rate.

The study of platelet aggregation in pregnant women with gestosis (n=15) yielded the following parameters for functional activity of platelets: h=2.59±0.50, EC_{50}=10.88±3.70, V_{max}=4.2±0.4. Values of EC_{50} and V_{max} illustrate functional activity of platelets. Hill coefficient (h) reflects the number of substrate-binding receptor sites. The Hill equation is applied to describe cooperative binding of the receptor and ligand by several interacting sites. The higher is h, the more abrupt is the transition from the absence of cell response to maximum reaction. In our experiments binding of the agonist to the receptor occurred more than by two substrate-binding sites (h=2.59±0.50, Fig. 1, *b*).

Previous studies showed [2] that under normal conditions activation of platelets is realized via P2X1 receptors (EC_{50} for ADP is 5-30 nM) and is not accompanied by aggregation. Under normal conditions ADP-induced aggregation (110-160 nM) is mediated by P2Y1 and P2Y12 receptors. In our experiments the concentration zone for aggregation of platelets from pregnant women corresponded to EC_{50} 7-15 nM. It can be hypothesized that aggregation of these cells involves only P2X1 receptors. There are no data on a cooperative type of the ligand-receptor interaction for P2X receptors. However, previous studies [6] revealed a cooperative type of desensitization of recombinant P2X1 receptors in the presence of ATP in nanomolar concentrations. P2X1 receptors on platelets from pregnant women exhibited a positive cooperative response to ADP (EC_{50}=7-15 nM). These platelets were characterized by hypersensitivity to ADP in concentrations that did not cause desensitization of P2X1 receptors. In some experiments we observed a decrease in aggregation induced by ADP in doses >20 nM. It was probably related to desensitization of these receptors.

The molecular mechanisms for hypersensitivity of platelets from pregnant women remain unclear. It is clear that platelet function should be a subject for pharmacological correction. Nitric oxide (NO) plays an important role in the pathogenesis of gestosis [1,4,7]. Published data show that aggregation activity of platelets significantly decreases after treatment with organic nitrates [3]. Antiaggregant activity of nitrovasodilating agents should be considered during combination therapy of gestosis.

Aggregation of platelets preincubated with NO was induced by the agonist (ADP) in concentrations of 2-40 nM (Table 1). Isoket (0.1%, 120 mM) served as a donor of NO.

The value of EC_{50} for ADP differed from that observed in control samples. No significant differences were found in h. Cooperative binding of P2X1 receptors to agonist was revealed during 20-min incubation of platelets with dinitrate. Isoket increased EC_{50} ADP for platelets. These data indicate that the sensitivity of platelets to ADP decreased, but did not return to normal.

The study of the dependence of platelet aggregation in women with gestosis on agonist concentration (ADP) showed that EC_{50} corresponds to 7-15 nM. We conclude that aggregation is realized only via P2X1 receptors. Isosorbide dinitrate tended to stabilize hypersensitivity of platelets from this group of pregnant women.

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REFERENCES

1. E. F. Derkachev, I. V. Mindukshev, A. I. Krivchenko, and A. A. Krashennikov, *Byull. Izobretenii*, No. 10, Pt. II, 298.
2. M. R. Sakaev, I. V. Mindukshev, E. E. Lesiovskaya, *et al*., *Eksp. Klin. Farmakol*., **63**, No. 3, 65-69 (2000).
3. P. M. Bath, R. Pathansali, R. Iddenden, and F. J. Bath, *Cerebrovasc. Dis*., **11**, No. 3, 265-272 (2001).
4. J. Neri, F. Piccinini, M. Marietta, *et al*., *Hypertens. Pregnancy*, **19**, No. 3, 323-330 (2000).
5. V. Ralevic and G. Burnstock, *Pharmacol. Rev*., **50**, No. 3, 415-475 (1998).
6. J. Rettinger and G. Schmalzing, *J. Gen. Physiol*., **121**, 451-461 (2003).
7. M. G. Signorello, R. Pascale, and G. Leoncini, *Eur. J. Biochem*., **270**, No. 9, 2005-2012 (2003).