Early Stages in the Mechanism of Action of Glucocorticoids on Human Platelets. Effect of Hydrocortisone on Platelet Aggregation

P. V. Sergeev, A. S. Dukhanin, and F. R. Gubaeva

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 123, No. 1, pp. 54-57, January, 1997
Original article submitted November 1, 1995

Hydrocortisone (1-10 μM) has no effect on spontaneous platelet aggregation and induces a 5-10-sec latency after platelet activation with 1 μM ADP. Hydrocortisone inhibits collagen-induced platelet aggregation; this effect is blocked by excess of progesterone. Hydrocortisone potentiates the effect of adenosine on disaggregation: in the absence of the hormone IC_{50} for adenosine is 2.5 μM, while in the presence of 3 and 10 μM hydrocortisone it drops to 1.7 and 0.4 μM, respectively.

Key Words: *platelets; inhibitors of aggregation; hydrocortisone; ADP; collagen*

According to modern concepts, the effects of steroid hormones are mediated by two mechanisms: genomic (slow or transnuclear) and extragenetic (fast).

The genomic effects of glucocorticoids (GC) are linked to the interaction between the hormones with intracellular (cytosolic or nuclear) type II GC receptors [7]. The nuclear effects are characterized by a considerable latency (1-2 hours) and depend on the *de novo* protein synthesis.

Extragenetic effects of GC (modulation of the second messenger synthesis, transmembrane glucose and amino acid transport, and activity of membrane-bound enzymes) are realized at the plasma membrane level, develop within a 30-min period, and are insensitive to the inhibitors of RNA and protein synthesis. Specific binding sites for GC on the plasma membrane are similar to type III GC-receptors: they equally bind different natural corticosteroids (hydrocortisone, corticosterone, aldosterone), have a lower affinity for GC analogs (dexamethasone, prednisolone), and their equilibrium dissociation constant (K_d) ranges within micromolar concentrations [5].

Department of Molecular Pharmacology and Radiobiology, Russian State Medical University, Moscow

The contribution of both mechanisms to the integral biological response of the target cell can be evaluated with the use of polymeric steroid derivatives, since they do not cross the plasma membrane [4,6]. Anucleate erythrocytes and platelets bearing specific hydrocortisone binding sites are a convenient model for the investigation of molecular and cellular mechanisms of the early GC effect [2].

Our objective was to examine the effect of hydrocortisone on the activation and inhibition of platelet aggregation in platelet-rich human plasma.

MATERIALS AND METHODS

The following reagents were used: cortisol (Merck), EDTA (Sigma), ADP, adenosine, sodium nitroprusside (Serva), and Russian-manufactured salts, acids, and alkalies of high grade purity.

Preparation of platelet-rich plasma and evaluation of platelet aggregation in an Aggregation Analyzer were described previously [1].

The effect of hydrocortisone on platelet aggregation was estimated as a function of hormone concentration (1, 3, 5, 10, and 25 μM) and time of preincubation with cells (0, 10, 20, and 30 min). Final concentration of the hormone was calculated as-

0007-4888/97/0001-0046$18.00

suming that the hydrocortisone-binding capacity of the plasma, which depends on transcortin and albumin, is 0.7 μmol/liter [3]. The final concentration of ethanol was 0.005%. The aggregation curves were analyzed using the following parameters: amplitude and maximum rate of aggregation and the time during which the maximum aggregation amplitude was reached.

Confidence intervals of experimental values and significance of the differences were evaluated by the Student's tests at p=0.05 using standard software.

RESULTS

At 1-10 μM hydrocortisone had no effect on platelet aggregation, while at 25 μM (the maximum concentration) it decreased the amplitude of aggregation by 20%, the changes being significant on the 3th-5th min after the start of recording. Previously, we showed that hydrophobic GC molecules are incorporated into the plasma membrane lipid bilayer, which increases the membrane microviscosity [2]. Microviscosity of the plasma membrane is an important regulating factor of spontaneous platelet aggregation. At a concentration of 25 μM, there are 15×10^6 GC molecules per platelet. For comparison, the plasma membrane of a medium-size animal cell contains $100\text{-}200\times10^6$ cholesterol molecules. Since the effect was observed at high concentrations of GC, it can be suggested that it is determined by nonspecific (receptor-independent) hormone-platelet interactions.

Under physiological conditions, platelet activation is triggered primarily by adenosine diphosphate (ADP), which at low concentrations (>1 mM) stimulates changes in platelet shape (spherulation) and adhesion [8]. Under our experimental conditions ADP (0.25 μM) induced the formation of microaggregates consisting of 3-20 platelets. This reaction is completely reversible due to the disaggregation process; after 1-3 min, the aggregation parameters returned to the initial values. This reaction of platelets to low ADP doses is termed as the first wave (phase) of aggregation. The use of weak inducers such as ADP, vasopressin, serotonin, and norepinephrine makes it possible to assess the effect of pharmacological agents on the first phase of aggregation.

Analysis of the GC effect on ADP-induced platelet aggregation showed that in a concentration of 1-3 μM GC does not change the number and size of aggregates. At 5 μM and higher, GC markedly inhibited platelet spherulation and caused a 5-15-sec latency in cell response to ADP. Changes in other quantitative parameters of aggregation were inconsiderable; the decrease in amplitude and rate of aggregation varied from animal to animal in the 10-20% range. The weak effect of GC on the reversible phase of platelet aggregation is probably due to some peculiarities of the ADP-stimulated cell aggregation. Activation of platelets with ADP (<1 μM) is mediated though activation of P_{2x}-purine receptors, which act as the plasma membrane calcium channels (similarly to nicotine cholinoreceptors). Thus, the effect of low ADP concentrations on platelets is not mediated by second messengers and is not regulated

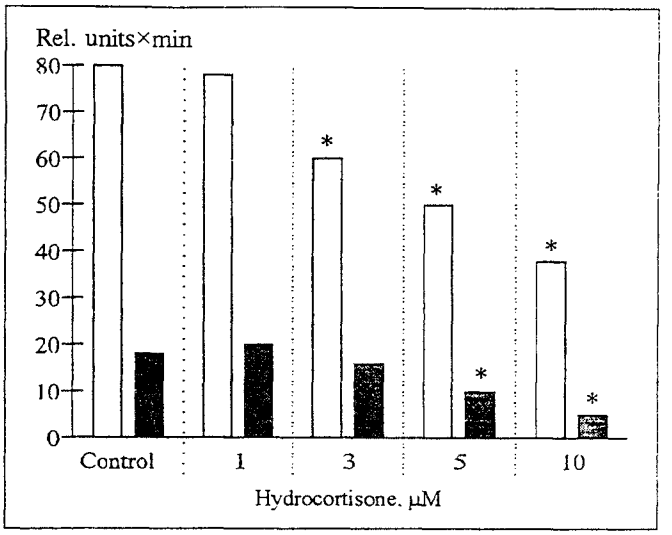

Fig. 1. The maximum rate of changes in light transmission (A, light bars) and maximum rate of changes in the mean aggregate radius (R, dark bars) in the platelet-rich plasma as a function of hydrocortisone concentration. Asterisk indicates statistically significant differences.

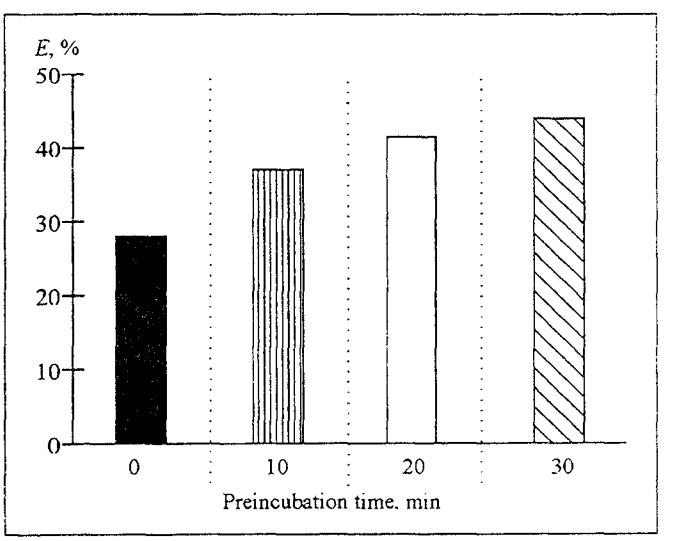

Fig. 2. Inhibiting effect of hydrocortisone (5 μM) as a function of the preincubation time. Ordinate: magnitude of the effect calculated by the formula: $E=(A_c\text{-}A_{exp})/A_c\times100\%$, where A_c and A_{exp} are the maximum rates of changes in light absorbance in control and experimental samples, respectively.

by any known G-proteins, i.e., systems and structures regarded as the targets of GC.

Potent platelet activators (thrombin, collagen, thromboxane A_2, platelet activating factor, and ADP at concentrations higher than 2 μM) induce irreversible platelet aggregation, which is always accompanied by release of endogenous bioactive substances from the platelets and is characterized by a lag-period [8]. In our experiments irreversible platelet aggregation was induced with 0.5 μg/ml collagen.

Hydrocortisone produced a dose-dependent effect on the collagen-induced aggregation (Fig. 1). A significant decrease in the amplitude and rate of aggregation was observed at a hormone concentration of 5 μM and higher. A 30-min preincubation potentiated the inhibiting effect of GC on platelet aggregation: 44% *vs.* 28% without preincubation (Fig. 2). Since the effect of hydrocortisone was abolished by the natural GC antagonist progesterone, it can be suggested that this effect is receptor-dependent.

Collagen-induced aggregation triggers various effector systems in platelets. Theoretically, the hormone may affect *initial stages of platelet activation*: 1) interaction of collagen with receptors and stimulation of phospholipase A_2; 2) production of active arachidonic acid metabolites (thromboxane A_2 and platelet activating factor) in platelets, *intermediate stages*: 3) activation of phospholipase C by thromboxane and platelet activating factor and formation of the second messengers inositol tris-phosphate and diacylglycerol; 4) elevation of intracellular free calcium and activation of protein kinase C; 5) platelet degranulation (release of Ca^{2+}, ADP, serotonin), and *final stages:* 6) ADP-induced exposure of glycoprotein IIb/IIIa serving as fibrinogen-binding sites; 7) irreversible aggregation. It is likely that hydrocortisone affects the 1st, 3rd, and 5th stages since they are directly related to the structural and functional activity of the platelet membrane.

Endothelium-dependent relaxing factor, namely, nitric oxide (NO) and agents elevating cAMP concentration (prostacyclin, adenosine, and prostaglandins E_1, E_2, D_2,) are physiological inhibitors of platelet aggregation.

We have studied the effect of hydrocortisone on platelet disaggregation induced by adenosine (2.5 μM) and sodium nitroprusside (10 μM), an exogenous imitator of endothelium-dependent relaxation factor spontaneously generating NO in aqueous solutions. These concentrations were determined in preliminary experiments so that the amplitude of ADP-induced aggregation constituted 50% of the control (IC_{50}). The hormone was added together with one of the aggregation inhibitors after platelet aggregation had reached a plateau (usually 1 min after the addition of ADP).

Hydrocortisone (1-25 μM) exhibited no disaggregation activity and did not affect cell response to sodium nitroprusside. In combination with adenosine this hormone markedly potentiated disaggregation. In the presence of 3 μM and 10 μM hydrocortisone, IC_{50} of adenosine dropped to 1.7 μM and 0.4 μM, respectively. In contrast to adenosine that acts through the adenylate cyclase system, NO acts by activating guanylate cyclase with subsequent elevation of intracellular cGMP. Thus, our findings imply that the effect of hydrocortisone on disaggregation of human platelets is realized predominantly via the adenylate cyclase system.

Further investigations of the early stages of the hydrocortisone effect on platelets will focus on the regulation of intracellular calcium and cAMP concentrations.

REFERENCES

1. I. Yu. Gavrilov, E. G. Popov, and Z. A. Gabbasov, *Klin. Lab. Diagnostika*, No. 3-4, 40-43 (1992).
2. P. P. Golikov, *Gematol. Tranfusiol.*, **33**, No. 3, 35-39 (1988).
3. P. P. Golikov, in: *Receptor Mechanisms of the Glucocorticoid Effect* [in Russian], Moscow (1988), p. 21.
4. P. V. Sergeev, A. S. Dukhanin, and A. V. Semeikin, *Byull. Eksp. Biol. Med.*, **104**, No. 12, 678-681 (1987).
5. P. V. Sergeev, A. S. Dukhanin, and N. L. Shimanovskii, *Ibid.*, **120**, No. 10, 342-348 (1995).
6. S. L. Timofeevskii, E. F. Panarin, V. E. Baikov, and V. D. Pautov, *Vysokomolek. Soed.*, **36**, No. 1, 15-19 (1994).
7. M. Beato, *Cell*, **56**, 335-344 (1989).
8. H. Michael, K. I. Schafer, and A. I. Schafer, *Blood*, **74**, No. 4, 1181-1194 (1989).