PHARMACOLOGY AND TOXICOLOGY

Human Platelets as the Object of Investigation of the Molecular Mechanisms Underlying Nongenomic Effects of Glucocorticoid Hormones

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

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The effect of hydrocortisone (100 nM-10 μM) on the major biochemical parameters of platelet activity (intracellular free calcium concentration, thromboxane B_2 content, and baseline and stimulated levels of cAMP and cGMP) is examined. The results obtained indicate that the inhibitory effect of glucocorticoids on platelet aggregation is mediated by activation of the adenylate cyclase system and suppression of the calcium response. Presumably, neither guanylate cyclase nor phospholipase A_2-dependent systems are the targets of nongenomic actions of glucocorticoid hormones. Platelets can serve as a convenient tool for the investigation of nongenomic effects of glucocorticoid hormones.

Key Words: *hydrocortisone; calcium; cGMP; cAMP; thromboxane; inhibitors and activators of platelets*

Nongenomic effects contribute considerably to the physiological and pharmacological activity of steroid hormones. Molecular targets of steroid hormones are usually located in the cytoplasma membrane. The following trigger mechanisms of nongenomic effect of steroid hormones have been demonstrated: 1) stimulation of Na^+/K^+-ATPase in the kidney tubular epithelium [12,13]; 2) activation of rapid calcium entry by progesterone, testosterone, and 1α,25-dihydroxyvitamin D_3 [3-5,9,14]; 3) modification of the metabolism of second messengers in normal and transformed cells [6,7,11]; 4) modulation of the activity of neuronal Cl-channels coupled to $GABA_A$-receptors [8]. The nongenomic effects of steroid hormones are characterized by latency, duration of their action (within 30 min), and tolerance to the blockers of RNA and protein syntheses. Steroids induce a membranotropic effect without penetrating the plasma membrane, which has been used to distinguish between genomic (mediated by intracellular receptors) and nongenomic effects. For example, the membrane stage in the action of steroid hormones can be identified with the use of these hormones immobilized on a high-molecular-weight carrier [1]. Anuclear cells (erythrocytes and platelets) provide another approach to the investigation of the mechanisms underlying the membrane-mediated effects of steroids.

The aim of the present study was to develop a theoretical and experimental basis for the evaluation of the nongenomic effect of the glucocorticoid hydrocortisone (cortisol) on platelet activity.

MATERIALS AND METHODS

The procedures of platelet isolation, loading with the fluorescent probe Fura 2-AM, and calculation of the intracellular free calcium content were described elsewhere [2]. The content of cAMP, cGMP, and thromboxane B_2 was measured by the radioligand

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

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method with Amersham kits. The following platelet activators were employed: ADP (3 μM), thrombin (0.2 U/ml), epinephrine (5 μM), collagen (1 mg/ml), platelet activation factor (1 μM), and serotonin (10 μM).

Data were processed using Pharmacological Basic Statistics software. Confidence intervals for experimental parameters and significance of differences were evaluated by the Student's t test at $p=0.05$.

RESULTS

The effects of various compounds on platelet activity can be examined by aggregometry, which permits evaluation of the effects on spontaneous and stimulated cell aggregation [10]. The effects of numerous platelet activators and inhibitors are realized by modulation of four main transmembrane signaling systems. **The adenylate cyclase** and **guanylate cyclase systems** mediate the inhibitory effect of various endogenous biologically active substances (prostacyclin, adenosine, prostaglandin E_1, and nitric oxide) and pharmacological agents (forskolin, nitroglycerin, sodium nitroprusside, and cAMP phosphodiesterase inhibitors such as dipiridamole, theophylline, and aminophylline). Modifiers of the plasma membrane phospholipases A_2 (PLA_2) and C (PLC) produce a platelet-activating effect. Serotonin, vasopressin, and thrombin activate PLA_2 and trigger arachidonic acid conversion via the receptor-mediated mechanism, thus stimulating the synthesis of thromboxane A_2, a potent activator of platelet aggregation. Receptor-mediated stimulation of PLC followed by elevation of the intracellular calcium concentration ($[Ca^{2+}]_i$) is linked to the effects of platelet activators such as collagen, thromboxane, epinephrine, and platelet activating factor. Inhibitors of **PLC** and **PLA_2-dependent regulation** of platelet activity exhibit antiaggregation activity. These inhibitors are nonsteroid anti-inflammatory agents, peptide inhibitors of PLA_2, and competitive antagonists of thromboxane, fibrinogen, thrombin, and serotonin receptors, and α-adrenoreceptors.

Hydrocortisone (HC) suppresses platelet activity. It inhibits collagen-induced aggregation and potentiates adenosine-induced disaggregation of platelets. The effect reaches the maximum after a 30-min preincubation.

In order to identify potential primary targets of HC in platelets we studied the effects of the hormone on basal and stimulated Ca^{2+} levels, concentration of thromboxane B_2 (a stable metabolite of thromboxane A_2), and the intracellular content of cyclic AMP and GMP. The final concentration of HC in the samples varied from 100 nM to 10 μM, which covers both the circadian physiological variations of HC levels and its therapeutic concentrations in human blood.

1. In the concentration range 1-10 μM HC inhibits the induced rise of $[Ca^{2+}]_i$ in a dose-dependent manner and has no effect on the basal calcium level. The ability of HC to inhibit the rise of Ca^{2+} induced by agents differing in effectiveness and mechanism of action decreases in the following order: norepinephrine>collagen>ADP>platelet activation factor>thrombin>serotonin.

Comparison of Ca-blocking effects of HC in the presence of 1 μM $CaCl_2$ and in a calcium-free medium shows that the hormone inhibits primarily calcium entry and has no effect on calcium mobilization from intracellular depots. It should be noted that the Ca-blocking effect of HC develops without latency.

2. In the studied concentrations HC does not affect thromboxane production and cell response to this inducer of PLA_2-dependent platelet activation, judging from the content of thromboxane B_2.

3. In the concentration range 100 nM-1 μM HC potentiates elevation of cAMP induced by 1 μM adenosine but not by forskolin (an activator of the catalytic subunit of the adenylate cyclase complex), sodium fluoride, or AlF_4 (stimulators of G_s regulatory protein in the adenylate cyclase complex).

Pharmacological concentrations (1-10 μM) of HC significantly increase the platelet content of cAMP in a dose- and time-dependent manner. The specific glucocorticoid antagonists progesterone (30 μM) and deoxycorticosterone (50 μM) abolishes the cAMP-response.

4. In the studied concentrations HC has no effect on the basal and sodium nitroprusside (0.1 μM)-induced levels of cGMP.

Our findings indicate that the inhibiting effect of HC on platelet aggregation is mediated by activation of the adenylate cyclase system and suppression of Ca response. Presumably, the regulatory mechanisms of platelet activity involving the guanylate cyclase and PLA_2-dependent systems are not associated with the nongenomic effect of HC.

Thus, human platelets can serve as a convenient model for determination and investigation of the mechanisms responsible for the nongenomic effects of glucocorticoid hormones. This model has the following advantages: 1) Platelets can be obtained in the clinic by a simple isolation procedure. 2) Their response can be rapidly analyzed by assessing the successive stages of platelet aggregation: shape changes, adhesion, aggregation, secretion, and degranulation. 3) The platelet plasma membrane has a wide spectrum of receptors to endogenous biologically active substances and various pharmacological agents. 4) There are verified biochemical and pharmacological approaches to the analysis of the function of major systems involved in the transmembrane signal trans-

duction. 5) Platelets can be regarded as the target cells for HC, since it was proved that they participate in the inflammation response.

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Abnormal Sleep Pattern and Impaired Learning Capacity in Rats with MPTP-Induced Parkinsonian Syndrome

L. N. Nerobkova, N. V. Markina, T. A. Voronina, V. A. Kraineva, T. L. Garibova, G. M. Molodavkin, and L. M. Sharkova

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Rats with the Parkinsonian syndrome induced by systemic injection of the neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) develop extrapyramidal disorders (oligokinesia, tremor, and rigidity) which depend on the dose and duration of action of MPTP. At 15 and 30 mg/kg MPTP impairs the learning of the conditioned passive avoidance response, shortens both stages of sleep, particularly the paradoxical (REM) stage, and prolongs the period of wakefulness. The mnestic function disturbance is not associated with extrapyramidal disorders, since it develops in their absence. In MPTP-treated rats memory and sleep disorders are interrelated.

Key Words: *extrapyramidal disorders; Parkinsonian syndrome; sleep pattern*

In addition to extrapyramidal symptoms (tremor, oligokinesia, and rigidity) and characteristic autonomic disturbances, patients with parkinsonism have impaired intellectual and mnestic functions and develop sleep disorders [5-8]. The causes of these abnormalities and their relation to pathogenesis and therapy of Parkinson's disease so far remain unclear. The Parkinsonian syndrome has been modeled with the use of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a neurotoxin which damages dopaminergic neurons of the striatum thus inducing extrapyramidal disturbances [2,9]. Chronic administration of MPTP in low doses to monkeys impairs the learning of conditioned responses, the effect being reversed by dopaminergic agonists [10].

In this study we examined mnestic functions and sleep disorders in rats with the Parkinsonian syndrome induced by MPTP.

Laboratory of Psychopharmacology, Institute of Pharmacology, Russian Academy of Medical Sciences, Moscow

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