REVIEWS

Role of Membranotropic Effects of Glucocorticoids in the Realization of Their Pharmacological Activity

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In the 1970s studies of molecular and physicochemical characteristics of cytosolic receptors for steroid hormones (SH) resulted in the concept of steroid-induced changes. According to this concept, the effects of steroids are realized via 2 stages. Steroid molecules diffuse into the competent cell and bind to cytosolic receptors. Hormone-receptor complexes are activated, undergo translocation into the nucleus, initiate gene expression, and induce synthesis of specific proteins that determine the cell response to hormones. The plasma membrane (PM) was suggested to have a passive role in this process.

In the past 10-15 years the involvement of new experimental methods and development of the theory for selective effects of medicinal preparations extended the notion about changes produced by steroids. PM plays a role in the realization of biological activity of SH [11,20,26,39,45,49]. Phospholipids and regulatory and functional proteins of PM serve as the target for membranotropic activity of SH. Various SH, including androgens, estrogens, gestagens, active vitamin D metabolites, and mineralocorticoids, regulate activity of serotonin, NMDA, δ-opiate, and $GABA_A$ receptors, modulate enzymatic functions of tyrosine kinase, Na,K-ATPase, adenylate cyclase, phospholipase C, and coupled secondary messengers, and affect membrane permeability for calcium, chlorine, potassium, and sodium ions [21,24,28,42,48,51].

Studies performed in the past 5-7 years showed that SH produce rapid extragenomic membrane effects [25,30,33,41,46,50]. For example, progesterone affects calcium concentration and activities of tyrosine kinase and phospholipase C in gametes. Aldosterone produces a nongenomic effect on transmembrane sodium transport in epithelial cells of renal tubules. The early effects of vitamin D_3 include modulation of Ca^{2+} and cAMP contents in bone cells. Membrane receptors for estrogens and progestins regulate activity of membrane-bound enzymes adenylate cyclase, protein kinase C, and 5'-nucleotidase under normal conditions and during tumor growth.

The role of membrane effects produced by glucocorticoids (GC) in the realization of their physiological and pharmacological activity received little attention, which explains the absence of general criteria for the extranuclear mechanisms of cell reactions. There are 3 methods for studying genomic and extragenomic responses to hormones. The first method is based on the dependence of nuclear effects of GC on *de novo* protein synthesis. This method uses preparations blocking the formation of RNA, DNA, and proteins (actinomycin D, puromycin, and cycloheximide). The second method suggests the use of polymeric steroid preparations. In these preparations hormones are covalently immobilized on high-molecular-weight carriers and cannot cross the membrane [13]. Non-nucleated cells erythrocytes and platelets are used as the object in studies by the third method. To determine the mechanisms of membranotropic effects produced by GC, we used a complex approach that combines the advantages of these methods.

Here we review the results of our previous experiments, published data, and perspectives of studying this problem.

Studies performed by Yu. P. Denisov, S. Sh. Suleimanov, G. V. Shutko, T. G. Pukhal'skaya, and T. A. Tikhonova at the Department of Molecular Pharmacology and Radiobiology of the Russian State Me-

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dical University in the 1970-1980s provided a theoretical and experimental background for our work. These authors for the first time developed the concept of multistage effects produced by SH. The concept describes the main stages of interaction between steroids and target cells, provides methodical recommendations for studying SH-binding sites on membranes, and determines the principles of hormone binding under pathological conditions [12]. Various methodological approaches demonstrated that PM plays the major role in recognition of steroids. From published data the authors drew a conclusion that the membrane contains specific sites for binding of SH.

In the second stage, studies of the extragenomic pathway focused on the effects of SH that are triggered on PM (nongenomic membranotropic effects). It was shown that the effect of aldosterone, progesterone, vitamin D_3, and neurosteroids is realized by the extragenomic pathway [24,48,50]. We performed high-priority studies of nongenomic changes produced by GC.

Lympholytic Effect of Glucocorticoids: Role of Genomic and Nongenomic Mechanisms

Our experiments with GC-induced apoptosis in thymic lymphocytes showed that the lympholytic effect of these hormones is realized via the extranuclear mechanisms. Studies of pharmacological and biochemical markers of the initial stages of apoptosis included:

— evaluation of changes in mitochondrial ($\Delta\varphi_M$) and cell ($\Delta\varphi_P$) membrane potential in the initial stage of GC-induced apoptosis in lymphocytes using the amphiphilic cationic probe 4-*p*-dimethylaminostyryl-1-methylpyridinium [5];
— analysis of the effect of GC on intracellular pH using fluorescent probe BCECF and comparison of changes in intracellular pH during apoptosis and necrosis in thymocytes [6];
— analysis of the dynamics of changes in intracellular Ca^{2+} concentration during GC-induced apoptosis [17].

Our results and published data allowed us to propose the scheme of GC-induced apoptosis. In the early stage of apoptosis (1.0-1.5 h after induction) GC produce mainly genomic and nongenomic membranotropic effects. In thymocytes the transmembrane potential decreases in and calcium metabolism is enhanced. This is manifested in increase in cytoplasmic Ca^{2+} concentration and initial rate of Ca^{2+} accumulation [47]. Irreversible Ca^{2+} consumption is not initiated in the initial period. Excessive accumulation of Ca^{2+} in the intracellular space (mitochondria, nucleus, *etc.*) can produce a direct damaging effect on various cell structures, *e.g.*, DNA. Nongenomic membranotropic effects of GC determined by the direct influence of hormones on PM include activation of K^+ channels and inhibition of Na^+/K^+ metabolism.

Genomic intracellular effects of GC develop at the late stage of apoptosis and are determined by the receptor-mediated activation of *Fas*, *c-myc*, *E1A*, *p53*, and *bcl-2* genes and synthesis of oncoproteins. These changes are followed by activation of cytoplasmic Ca^{2+}/Mg^{2+}-dependent endonuclease, serine and cysteine proteinases, and cyclin-dependent protein kinases. The product of protooncogene *bcl-2* is incorporated into the mitochondrial membrane, which increases its permeability.

Redistribution of membrane phospholipids is followed by exposition of determinant groups on the cell surface (*e.g.*, phosphatidylserine). These groups are recognized by phagocytes, which promotes rapid absorption of apoptotic cells.

These changes are accompanied by cell volume reduction, energy starvation associated with exhaustion of ATP reserves, fragmentation of DNA, and formation of apoptotic bodies. Apoptotic bodies are phagocytized by macrophages or histiocytes. This process is not followed by the development of inflammatory reactions.

These data show that the initial stages of apoptosis are mediated by the genomic and nongenomic effects of GC and include changes in Ca^{2+} and K^+ transport, variations in the intensity of Na^+/H^+ metabolism, and decrease in the transmembrane potential. The initial stage of apoptosis in thymic lymphocytes is phenotypically manifested in a decrease in cell volume. Intracellular pH, Ca^{2+} concentration, and mitochondrial and plasma potentials serve as pharmacobiochemical markers for the initial stage of GC-induced apoptosis.

Comparison of the Effects of GC on Signal Transduction in Purinergic Receptors on Lymphoid Cells

We studied the mechanisms of coupling between GC receptors and membrane signal systems. Published data indicate that receptor systems are not isolated, but work cooperatively. The term "cross-talk" suggests "conversation" between various receptor systems [32]. In this respect, it is important to determine cross-points in mechanisms underlying the effect of compounds with immunosuppressive activity. In addition to GC, the purinergic system is involved in immunoregulation.

Endogenous physiologically active substances (adenosine, ATP, and AMP) and pharmacological preparations acting as ligands of purine receptors (theophylline and 8-chloroadenosine) affect lymphopoiesis. A_1 and A_2 receptors for adenosine present in various

cells and coupled to adenylate cyclase play a role in the regulation of proliferation, differentiation, and maturation. Intracellular adenosine concentration sharply increases under stress conditions (*e.g.*, hypoxia). Adenosine crosses PM, and its content in the plasma increases to 1-3 μM. In this concentration adenosine produces an immunosuppressive effect. The genetically determined decrease in adenosine deaminase activity (key enzyme of purine metabolism) is followed by a rise in adenosine concentration, which contributes to the development of congenital immunodeficiency.

Therefore, GC and purine receptor agonists produce an immunosuppressive effect on lymphoid cells. However, the relationship between purinergic and glucocorticoid receptors is poorly understood.

We proposed a scheme that reflects the influence of GC on signal transduction in purinergic receptors on lymphoid cells (Fig. 1).

A_1 receptors for adenosine inhibiting adenylate cyclase are absent on PM of bone marrow lymphoblasts (Fig. 1, *a*). There are 2 subtypes of A_2 receptors: high-affinity A_{2A} receptors and low-affinity A_{2B} receptors. These receptors display selective affinity for the cytostatic 6-mercaptopurine [3]. Stimulation of both subtypes of receptors is followed by an increase in intracellular cAMP concentration and mediates the cytostatic effect of A_2 receptor ligands on lymphoblasts [8]. The regulatory influence of GC on adenosine receptors is a typical genomic effect of steroids. GC induces *de novo* synthesis of protein molecules of A_2 adenosine receptors via the intracellular system. The transcriptional inhibitor actinomycin D abolishes this effect of GC. These changes are followed by an increase in the number of high-affinity A_{2A} receptors and low-affinity A_{2B} receptors for adenosine [18]. These data suggest that 6-mercaptopurine and prednisolone potentiate the effects of each other during combination polychemotherapy. GC also produce an indirect effect on purinergic receptors on lymphoblasts. In PM, GC activate the key enzyme of purine metabolism 5'-nucleotidase that catalyzes conversion of AMP into adenosine. Therefore, local adenosine concentration in the premembrane space increases.

As differentiated from bone marrow lymphoblasts, PM of peripheral blood lymphocytes has 2 types of adenosine receptors (A_1 and A_2) [19]. Coupling of A_1 and A_2 receptors for adenosine to the effector molecule adenylate cyclase is realized via various regulatory G proteins. The inhibitory effect of A_1 receptors on adenylate cyclase is mediated by pertussis toxin-sensitive G_i protein. Stimulation of adenylate cyclase via A_2 receptors is mediated by G_S protein. Basal cAMP level in lymphocytes depends on the ratio between functional activities of A_1 and A_2 receptors. The genomic effect of GC in lymphocytes is manifested in the initiation of transcription of genes encoding A_2 receptors. In our experiments GC had no effect on the number of A_1 receptors on the lymphocyte membrane [16]. The membranotropic effect of GC on lymphocytes is manifested in uncoupling of A_1 receptors and adenylate cyclase at the level of G_i proteins. These changes develop rapidly (over 30 min) and are not associated with the genomic effect. Transduction of transmembrane signals in A_1 receptors is blocked. The functional relationship between A_1 and A_2 adenosine receptors is shifted toward A_2 receptors. GC potentiate the adenosine-induced increase in cAMP concentration and its immunotoxic effect on lymphocytes.

Theoretical and experimental data regarding the membrane stage of steroid-induced changes provided the basis for construction of test systems for evaluation of individual resistance of patients with bronchial asthma and acute lymphoblast leukemia to hormonotherapy [3,4].

Optimization of Indications for Pharmacotherapy in Patients with Bronchial Asthma

GC normalize activity of purine receptors, which determines their therapeutic effect in patients with bronchial asthma [1]. On the one hand, GC block G_i proteins at the membrane level and suppress A_1 receptors. On the other hand, GC contribute to the appearance of A_2 receptors, which is mediated by their late genomic effects. This dual action prevents the imbalance between A_1 and A_2 purine receptors and normalizes the cAMP response of cells to adenosine.

Preparations of theophylline are widely used for the therapy and prevention of bronchial asthma attacks. Recent studies showed that the bronchodilator effect of theophylline is realized via competitive antagonism with adenosine. The use of theophylline (preparation with prolonged action) for the therapy of patients with bronchial asthma for 3 weeks decreased the number of A_1 receptors and increased the count of A_2 receptors, which correlated with the clinical efficiency of treatment [9]. The duration of treatment was 3 weeks because the minimum period necessary for the development of changes in the receptor system is 14-21 days. The purine hypothesis of bronchial asthma suggests that the endogenous purine compound adenosine plays an important role in the pathogenesis of this disease in 15-20% patients. Primary stimulation of A_1 receptors determines hyperreactivity of the bronchi in these patients. The antagonist of purine receptors theophylline produces a strong positive effect in these patients [10].

We proposed that the count and ratio between purine receptors on lymphocytes should be evaluated

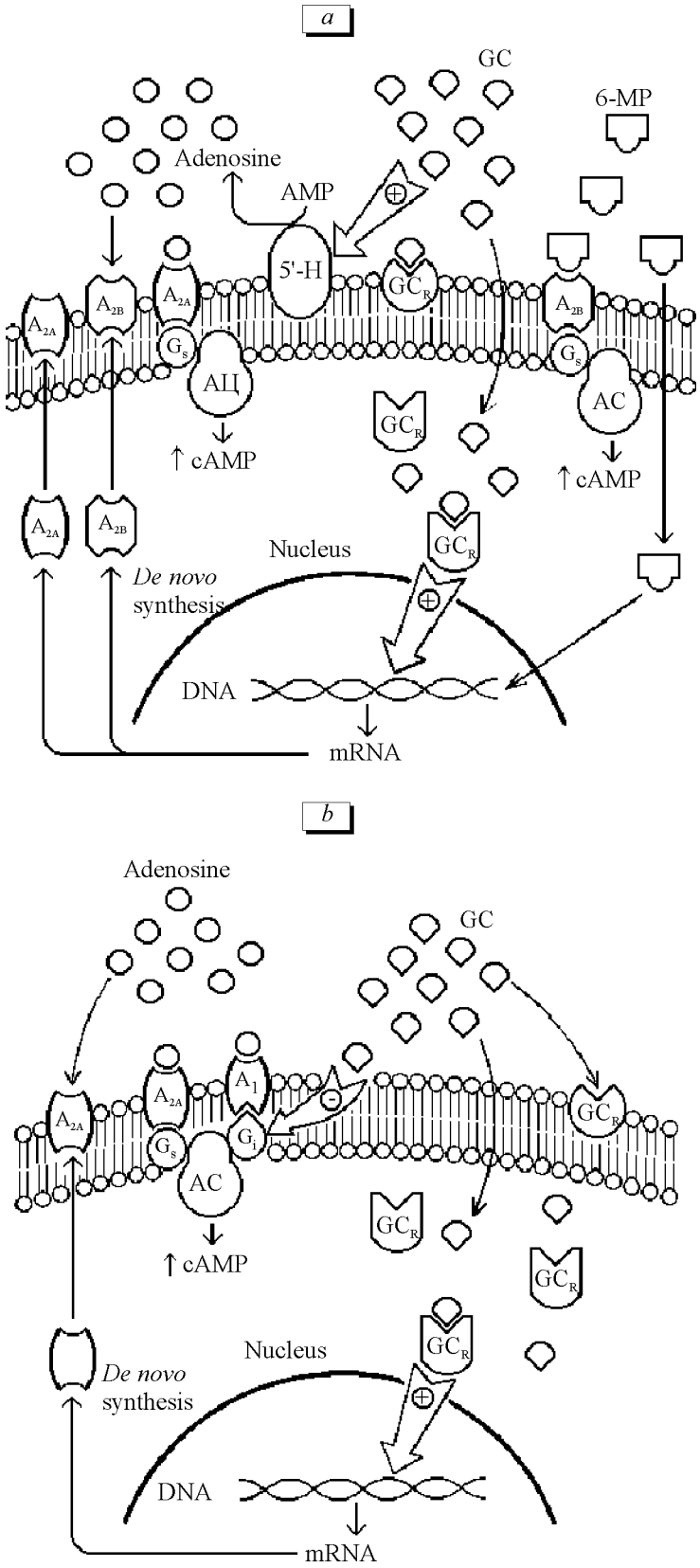

Fig. 1. Pathways of effects of glucocorticoids (GC) on the purinergic system of bone marrow lymphoblasts (*a*) and peripheral blood lymphocytes (*b*). AC: adenylate cyclase; 6-MP: 6-mercaptopurine; GC_R: receptors for GC; A_{2A} and A_{2B}: subtypes of adenosine receptors. Arrow with "+": activating effect of GC. Arrow with "—": inhibitory effect of GC.

to optimize pharmacotherapy of patients with bronchial asthma. The method is based on studies of the ratio between A_1 and A_2 adenosine receptors on peripheral blood lymphocytes from children with bronchoobstructive syndrome not receiving pharmacotherapy.

Pharmacobiochemical Assay of the Interaction between GC and Human Platelets. Platelets as the Experimental Model for Studying Nongenomic Effects of SH

Studies of membranotropic activity of GC were followed by experiments with anucleated cells, platelets. The molecular mechanisms that underlie the nongenomic influence of GC on platelet activity were evaluated using various experimental approaches [15].

The method of aggregometry is widely used for the primary analysis of changes in platelet activity produced by various compounds. This method estimates the effect of preparations on spontaneous and induced aggregation of cells. The influence of various inductors and inhibitors of platelets is realized via changes in activity of 4 major transmembrane signaling systems. Adenylate cyclase and guanylate cyclase mediate the inhibitory effect of endogenous biologically active substances (prostacyclin, adenosine, prostaglandin E_1, and nitric oxide) and pharmacological preparations (forskolin, nitroglycerine, sodium nitroprusside, and cAMP phosphodiesterase inhibitors dipyridamole, theophylline, and aminophylline) on activity of platelets. If membrane phospholipases A_2 (PLA_2) and C (PLC) serve as the effector molecule for a chemical compound, its effect is associated with platelet activation. Serotonin, vasopressin, and thrombin activate PLA_2 and initiate metabolism of arachidonic acid by the receptor-dependent mechanism. These changes activate the synthesis of thromboxane A_2, which acts as a potent physiological inductor of platelet aggregation. The receptor-mediated stimulation of PLC and increase in intracellular Ca^{2+} concentration underlie the effects of collagen, thromboxane, epinephrine, and platelet-activating factor that act as inductors of aggregation. Pharmacological preparations inhibiting PLC- and PLA_2-dependent pathways that regulate platelet activity possess antithrombotic activity. These preparations are presented by nonsteroid antiinflammatory agents, peptide inhibitors of PLA_2, and competitive antagonists of receptors for thromboxane, fibrinogen, thrombin, and serotonin and α-adrenoceptors.

We revealed that GC affect platelet aggregation by inhibiting their activity. GC in concentrations of 1-25 μM dose-dependently suppressed collagen-induced aggregation of cells and potentiated platelet disaggregation produced by adenosine [14].

The effects of GC on basal and induced Ca^{2+} level and intracellular concentrations of cyclic nucleotides cAMP and cGMP were studied to estimate the mechanisms of changes produced by these hormones [7]. The final concentration of GC varied from 100 nM to 10 μM, which corresponded to circadian changes in their content in human plasma and average therapeutic doses of hormones.

GC in concentrations of 1-10 μM dose-dependently inhibited the induced increase in intracellular Ca^{2+} concentration, but had no effect on basal Ca^{2+} level in platelets. The ability of GC to inhibit the increase in Ca^{2+} content produced by various inductors decreased in the following order: epinephrine—collagen—ADP—platelet-activating factor—thrombin—serotonin.

The comparison of calcium-blocking effects of GC in a medium containing 1 mM $CaCl_2$ and in calcium-free buffer showed that steroids primarily inhibited the influx of exogenous Ca^{2+}, but did not modulate its mobilization from intracellular stores. The calcium-blocking effects of GC developed without latency.

GC in concentrations from 100 nm to 1 μM potentiated the increase in cAMP content produced by 1 μM adenosine, but had no effect on changes in cAMP level induced by forskolin (direct activator of a catalytic unit in the adenylate cyclase complex), sodium fluoride, or AlF_4^- (stimulators of G_S protein in the adenylate cyclase complex).

In pharmacological concentrations (1-10 μM) GC produced a significant increase in cAMP content in platelets. cAMP level underwent dose- and time-dependent changes. Specific GC antagonists mifepristone (30 μM) and progesterone (50 μM) abolished changes in cAMP concentration.

In these doses GC had no effect on basal and induced contents of cAMP (after treatment with 0.1 μM sodium nitroprusside).

These data indicate that the inhibitory effect of GC on platelet aggregation is mediated by activation of adenylate cyclase and suppression of the Ca^{2+} response in cells. Probably, the regulation of platelet activity by guanylate cyclase and PLA_2-dependent systems is not mediated by the nongenomic effect of GC.

These results show that human platelets can be used for evaluation of the mechanisms of nongenomic effects produced by GC. Selection of platelets as the experimental object is related to their availability and simplicity of isolation during clinical tests. The cell response can be rapidly determined by consecutive stages of platelet aggregation: modification of the shape, adhesion, formation of reversible aggregates, secretion and degranulation, and irreversible aggregation. The platelet membrane has a variety of receptors for endogenous biologically active substances and pharmacological preparations. There are many biochemical and pharmacological methods for studying functional activity of transmembrane systems of signal transduction. Platelets are involved in the inflammatory response and, therefore, may be considered as the target for GC.

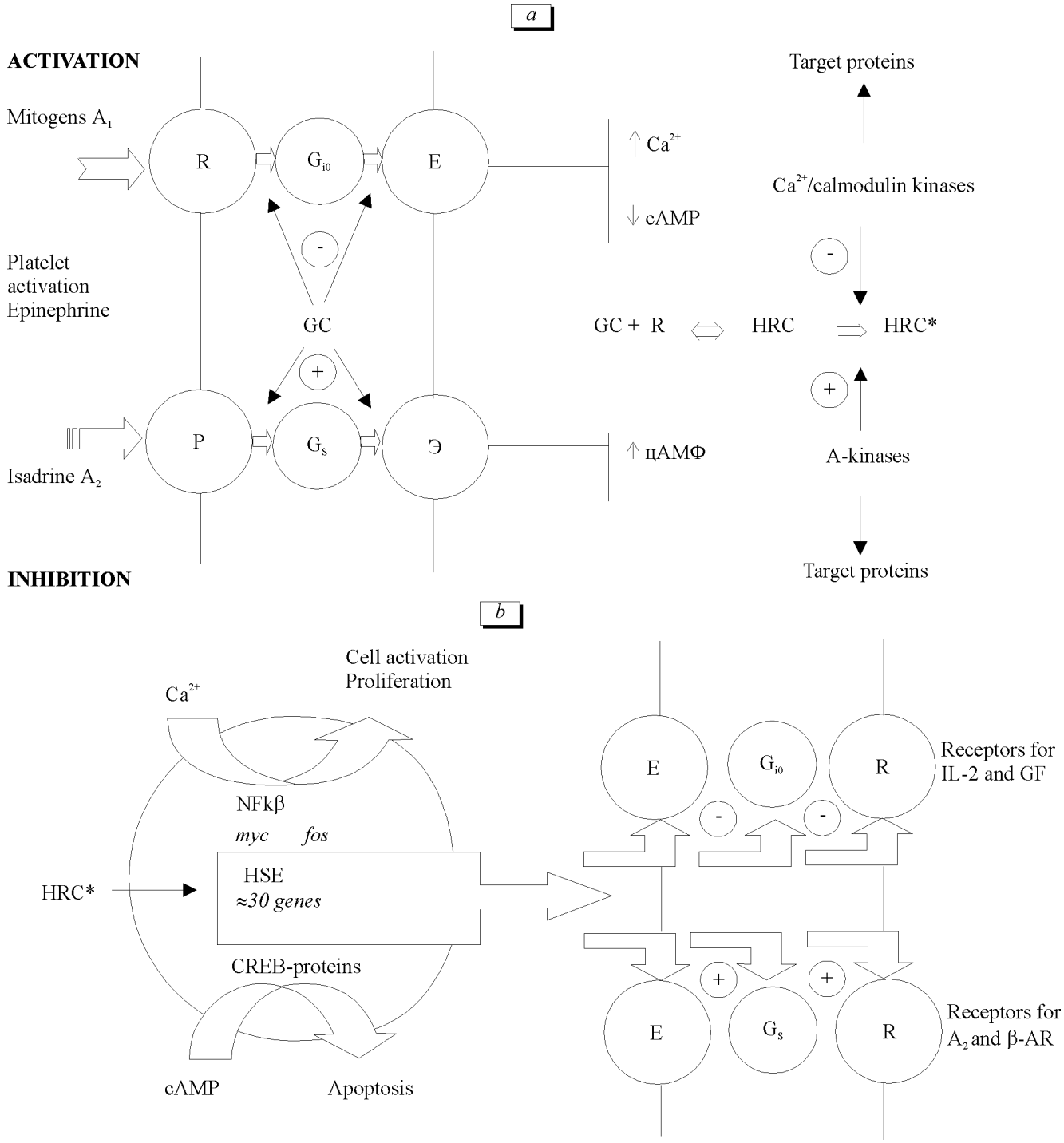

Fig. 2. Nongenomic (*a*) and genomic (*b*) membranotropic effects of glucocorticoids (GC). R: membrane receptor; E: effector molecule; HRC and HRC*: hormone-receptor complex and its activated form, respectively; HSE: hormone-sensitive element; IL-2, interleukin-2; β-AR: β-adrenoceptors; GF: growth factors; A: adenosine; AP-1: transcription-activating protein; CREB: cAMP-binding proteins; "+" and "—": activating and inhibitory effects, respectively.

We classified antagonists of GC by their ability to block membrane and/or intracellular mechanisms of hormone-induced changes.

Antagonists with membrane activity (progesterone and 11-deoxycorticosterone) block nongenomic membranotropic effects of GC, whose trigger mechanisms are localized on PM of target cells. These effects include modulation of cAMP level, Ca^{2+} concentration, pH, and Na^+/H^+ exchange.

Antagonists of intracellular receptors for GC (dexamethasone 21-mesylate) abolish the genomic effect of CS (modulation of RNA and protein synthesis by GC).

Total antagonists of GC (mifepristone) inhibit the effects of hormones mediated by membrane and intracellular receptors, including the combined action of GC.

Classification and Schematic Representation of Membranotropic Effects Produced by GC

Studies performed in Russia and other countries evaluated the distinctive features of membranotropic activity in GC that modulate functional and regulatory properties of PM. Depending on the mechanism, the effects of GC are divided into genomic and nongenomic.

Genomic membranotropic effects are mediated by the interaction of GC with intracellular receptors and changes in gene transcription. These effects are delayed in time, characterized by a latency of 30-120 min, develop at GC concentrations of 10-100 nM are irreversible, sensitive to inhibitors of RNA and protein synthesis and antagonists of intracellular GC receptors, and positively correlate with affinity for intracellular binding sites.

The genomic effects include the increase in the number of β-adrenoceptors, decrease in the concentration of membrane-bound inducible PLA_2 and PLC, and inhibition of expression of receptors for cytokines (interleukin-2, interleukin-4, and interferon-γ) [27, 34,37,43].

Nongenomic membranotropic effects are associated with the direct interaction between GC and PM. These effects are divided into specific and nonspecific.

Specific membranotropic effects are realized through membrane receptors for GC (acceptors of action). These effects develop rapidly (within 30 min) at GC concentrations of 0.1-5 μM, are reversible (require the presence of GC), can be abolished by antagonists of GC with membrane activity (progesterone), and are insensitive to antagonists of intracellular GC receptors and blockers of RNA and protein synthesis. A positive correlation was revealed between the ability of GC to produce specific membranotropic effects and their affinity for binding sites on membranes. These effects include the increase in basal cAMP level, inhibition of the induced rise in Ca^{2+} concentration and cytoplasmic pH, and suppression of Na^+/H^+ metabolism [31,36, 38,40,44].

Nonspecific membranotropic effects of GC are related to their intercalation into cell membrane and changes in its physicochemical characteristics (*e.g.*, microviscosity). These effects develop without latency, are observed at steroid concentrations of above 5 μM, reversible, do not correlate with affinity for membrane and intracellular binding sites, and nonselective (typical of synthetic and natural GC). These effects include changes in basal intracellular Ca^{2+} concentration, pH, and basal activity of membrane-bound enzymes (PLC and PLA_2) [22,23,35].

The mechanisms of genomic and nongenomic membranotropic effects produced by GC are shown in Fig. 2.

GC modulate transmembrane transduction of extracellular signals by affecting coupling of membrane receptors to G proteins and effector molecules. GC inhibit transduction of activating signals on PM (effect of mitogens and activators on lymphoid cells and platelets, respectively). Ca^{2+} act as the secondary messenger of activating signals. Intracellular Ca^{2+} concentration increases by 3-5 times compared to the basal content (≈100 nM). In the presence of GC, Ca^{2+} concentration increases no more than to 200 nM.

At the same time, GC potentiate the exogenous inhibitory signal (influence of adenylate cyclase stimulators). These hormones enhance coupling of G_S regulatory proteins to the corresponding membrane receptors. The secondary messenger of this process is cAMP, whose concentration increases by 2-3 times in the presence of GC.

At the cellular level, Ca^{2+} and cAMP act as antagonists and trigger opposite cascade reactions that involve Ca^{2+}/calmodulin and A-kinases.

Activation and proliferation of lymphoid cells, the final cell response to stimulation with mitogens, are recorded by incorporation of labeled precursors of RNA (uridine) and DNA (thymidine).

The lympholytic effect of adenylate cyclase stimulators and GC is realized via apoptosis. Genomic membranotropic effects include inhibition of expression of membrane receptors and G_i inhibitory proteins that are involved in transduction of activation signals.

By contrast, the synthesis of receptors that mediate the lympholytic effect of biologically active substances on lymphocytes is intensified. These changes are followed by an increase in the number of surface receptors (β-adrenoceptors and A_2 adenosine receptors).

Perspectives of Synthesizing Glucocorticoid Preparations with Membranotropic Activity

The discovery of the membrane stage in GC-induced changes provided a theoretical background for the search and synthesis of new glucocorticoid preparations producing selective effects on membrane receptors. Two methodical approaches were used. The original method for immobilization of GC on hydrophobic polymers was developed at the Department of Pharmacology (St. Petersburg Institute of Pediatrics) and Institute of High-Molecular-Weight Compounds.

GC bound to high-molecular-weight carriers cannot enter the cell and affect only PM of target cells. Anti-inflammatory activity of GC polyesters is comparable well with that of the initial compounds (dexamethasone and hydrocortisone). Moreover, GC polyesters are more potent than initial compounds in producing the antishock effect during traumatic shock. These effects are related not only to the interaction of modified hormones with membrane receptors on target cells, but also to the increase in local steroid concentration. It should be emphasized that polyesters of GC produce only insignificant undesirable effects (growth inhibition and thymus hypotrophy).

Another approach to the synthesis of membranotropic GC preparations was developed at the Center for Chemistry of Medicinal Compounds (Chemical and Pharmaceutical Institute, Moscow). G. S. Grinenko, M. E. Kaminka, and collaborators studied 16α-methyl-pregnan derivatives producing local antiinflammatory and antiallergic effects. During local application these substances were superior to dexamethasone, Sinaflan, and triamcinolone acetonide by the therapeutic effect. These compounds produced no systemic side effects (general catabolic activity, disturbances in metabolism of carbohydrates and electrolytes, and hormonal dysfunction in the thymus and adrenals). Radioligand assay showed that these preparations display high affinity for membrane GC receptors; their binding to cytosolic receptors for GC in the liver and thymus is less efficient.

Thus, PM is involved in recognition of GC and transformation of chemical signals into the biological response of target cells. These data indicate that the membrane stage is a specific phase of realization of hormonal and pharmacological activity in GC. This phase complements the general two-stage model of steroid-induced changes. GC preparations that possess primarily membrane activity hold much promise for adequate hormonotherapy and diagnostics of diseases.

It should be emphasized that not all effects of GC can be explained from the viewpoint of the general "hormone—gene—protein" scheme. Apart from the classic receptor-mediated genomic pathway, GC produce the direct effect on target cells, which is realized via secondary messengers and changes in ion permeability of membranes. However, the contribution and activity of these regulatory mechanisms in the regulation of individual target cells require detailed investigations.

With respect to the membrane-receptor theory, the urgent problems of molecular pharmacology and use of GC in practical medicine are:

— development of new experimental approaches to studying the nongenomic effect of GC and interaction between intracellular and membrane receptors for these hormones;
— evaluation of the role of each system in transformation of hormonal signals into the biochemical response of target cells;
— creation of a scientific basis for the directed search for new highly efficient and safe glucocorticoid preparations possessing primarily membranotropic activity;
— construction of new test systems for the diagnostics of patient's individual sensitivity to glucocorticoid preparations (with regard to the membrane stage of GC-induced changes).

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