Biochemistry (Moscow), Vol. 70, No. 1, 2005, pp. 24-39. Translated from Biokhimiya, Vol. 70, No. 1, 2005, pp. 33-50.
Original Russian Text Copyright © 2005 by Kulinsky, Kolesnichenko.

REVIEW

Molecular Mechanisms of Hormonal Activity. I. Receptors. Neuromediators. Systems with Second Messengers

V. I. Kulinsky[1]* and L. S. Kolesnichenko[2]

[1]Department of Biochemistry and [2]Department of Bioinorganic and Bioorganic Chemistry, Irkutsk State Medical University, ul. Krasnogo Vosstaniya 1, 664003 Irkutsk, Russia; fax: (3952) 24-0826; E-mail: kulinsky@pp.irkutsk.ru

Received February 16, 2004
Revision received April 15, 2004

Abstract—Hormone signal transfer along all of the cell compartments including nucleus is powered by signal transduction systems. Characteristics and importance of hormone receptors, principal components, functional mechanisms, and biological role of different systems with second messengers are described. Considerable examples of the importance of these systems for medicine are adduced. The drugs modifying these systems comprise more than 65% of contemporary medicines.

Key words: mechanism of hormone action, signal transduction system, receptor, second messenger, protein kinase

Studies on the molecular mechanisms of hormonal activity are in the forefront of biochemistry. Nobel Prizes have been awarded for nine eminent discoveries in this field. Many new journals, including daughters of *Nature* and *Science*, are devoted to this problem. Novel ideas and discoveries are enriching modern biology and benefiting medicine. Unfortunately, no reviews analyzing all general signal transduction systems (STSs) have been published in Russian. The review we present here is mainly devoted to those functional facets of the problem that are essential for understanding of the general facts and principles. The data reported during the last four years (until the middle of November, 2003) are analyzed in this review.

Since 1992, we have defined the term "hormone" (in a broad sense) as a specialized and mobile intercellular regulator of receptor action [1-3]. The phrase "specialized regulator" indicates the regulatory (rather than metabolic or energy) function as the major if not exclusive function of hormones; the word "intercellular" implies that hormones are produced by cells and influence the cells from outside; the "receptor action" is the initial step of the mechanism by which any hormone expresses its activity; and "mobile" (newly added) excludes immobilized regulators, such as proteins associated with either plasma membrane (PM) or intracellular matrix. This definition encompasses all three classes of regulators called hormones: circulating hormones (CH), this term being far broader than the old one "endocrine gland secreta"; neurohormones/neurotransmitters including neuropeptides; and tissue hormones, such as growth factors (GFs), cytokines, eicosanoids, and amines. Similarity in their main properties is much more prominent than difference that is not biochemical. The definition presented differentiates hormones from both nonspecialized (glucose and fatty acids) and intracellular regulators (effectors, second messengers, and transcription factors (TFs)), as well as from non-receptor substances such as enzymes and their activators and inhibitors.

After publication in 1997 in *Biochemistry (Moscow)* [3], our definition for hormones is accepted and used (with small variations) in reviews [4] and manuals of histology [5] and pathophysiology [6]. The worth and necessity of the changes in old definition of the term hormones and usage of the common term for all classes of intercellular regulators is recognized in many reviews [7, 8] and manuals [9-11] published in Russia and other countries as well. This allows regarding molecular mechanisms of circulating hormones as well as neurotransmitters and tissue hormones (GFs, cytokines, eicosanoids, and amines) in a unified group.

Abbreviations: GABA) γ-aminobutyric acid; PM) plasma membrane; STS) signal transduction system (pathway); TF) transcription factor; GF) growth factor; CH) circulating hormones; CBP) CREB-binding protein; GPCR) G-protein-coupled receptor; HRE) hormone-reactive element.

* To whom correspondence should be addressed.

1. HORMONE RECEPTORS

Molecular receptors are specific cellular proteins transmitting any external signal (physical, chemical, or biological) into the cell. This is a universal mechanism

0006-2979/05/7001-0024 ©2005 Pleiades Publishing, Inc.

providing cellular responses to the changing environment owing to the involvement of STSs and the cytoskeleton. Specific receptors exist to mediate different external stimuli, such as light, smell, taste, hormones, intercellular matrix, other cells, and bacterial and viral substances.

Interaction of a hormone with its recognizing receptor is the first step, which is more obligatory and specific than the following ones. All effects of the hormones are realized via their receptors. It is this item that is included into our definition of the term "hormone". No intercellular regulator can be considered as a hormone until its receptor mechanism is proven. A hormone receptor is a protein specifically binding a hormone, which eventually results in a specific response. Receptor couples the hormone-binding process with signal transduction (receptor–effector coupling). As a rule, receptors are composed of 300-1200 aminoacyl residues [12]. Active modulators can be divided into two classes: agonists (hormone and its analogs), which stimulate the given receptor, and antagonists (blockers) prevented the effect of agonists. Reverse agonists also exist. They change the receptor conformation so that the reverse effect is observed also in the absence of agonists [13]. In terms of protein chemistry, all substances that bind to a special active site are receptor ligands:

$$H + R \leftrightarrow HR \rightarrow \text{effects},$$

where HR is a hormone–receptor complex. This equation is similar to that describing an interaction between enzyme and its ligands (substrates and modulators).

Hormone and receptor are equally essential for expression of hormonal effect. In particular, there are two distinct types of diabetes: diabetes mellitus type I characterized by insulin deficiency, and type II by malfunction of receptor-mediated mechanisms or subsequent components of the STS. Sterility can result from deficiency of not only of sex hormones, but also of functional receptors. When the latter are absent administration of high doses of hormones is of no avail, but it can cause some side (or toxic) effects. Innate lack of testosterone receptors leads to the testicular feminization, in which karyotypical male (XY) develops into phenotypical female because testosterone turns into estrogens in peripheral cells. At present, such persons are excluded from woman's sports. V. P. Efroimson gave arguments that Maid of Orleans had had this syndrome [14]. The same hereditary receptor deficiency with primary hormone resistance is known for somatotropin (Laron Syndrome, a form of dwarfism), gonadotropin (ovarian resistance syndrome), antidiuretic hormone (familial nephrogenic diabetes insipidus), parathyrin (pseudohypoparathyroidism), calcitriol (calcitriol-resistant rickets), leptin (obesity), and dopamine (hypertension caused by mutation of D_1-receptor in kidney). Receptor deficiency commonly causes (by a feedback loop mechanism) high hormone concentration in blood followed by side effects. Hereditary deficiency of functional receptors is usually caused by loss-of-function mutations in their genes, as it was proven for at least ten hormones [9, 15].

The contrary effect is characteristic of gain-of-function mutations, which enhance hormonal effects (for instance, in familial non-immune hyperthyroidism) and, along with alternative splicing, can result in appearance of constitutively active (that is, hormone-independent) receptors, whose spontaneous activity can lead to the development of endocrine tumor [15, 16].

In 1988, Sir James W. Black was awarded a Nobel Prize for the discovery of β-adrenoceptors and H_2-receptors and their antagonists. This discovery has led to the development of highly effective drugs that are now widely used in therapy of cardiovascular disorders, bronchial asthma, and gastric ulcer.

Receptors are usually highly specific to their hormones. At near-physiological concentration, hormones have no activity towards alien receptors. However, excessively secreted hormone can stimulate receptors of allied hormones. In particular, fetal overweight characteristic of diabetes mellitus type II in pregnancy results from the effect of a high level of insulin on insulin-like GF 1-receptors; a similar phenomenon was found in hormone-active tumor [9]. Administration of increasing doses of dopamine results in stimulation of not only dopamine D-receptors, but also β_1- and then α_1-adrenoceptors to maintain arterial tension in different phases of shock or collapse [17]. Nonetheless, some notable exceptions have been found from receptor specificity. In the family of melanocorticoid hormones, the only corticotropin has its own receptor-2 (MCR-2), whereas the receptors MCR-1, MCR-3, MCR-4, and MCR-5 are common for both corticotropins and melanocyte-stimulating hormones α-γ [18]. Effects of cholecystokinin and gastrin are expressed via a shared receptor [19]. Hormones belonging to the transforming GF-β superfamily express their activity via two shared receptors. Shared receptors of two to eight hormones are particularly definitive of cytokines and chemokines according to their overlapping functions. At present, only 20-23 receptors have been found for 53 known chemokines [12, 20]. Four receptors activate the functions of ten distinct fibroblast GFs; these GFs and epidermal GF express their activities not only via their own receptors, but also via ephrin (Eph) receptors; and four members of the glia-derived neurotrophic factor (GDNF) family act via a single receptor, Ret [21]. It is obvious that receptor (as well as enzyme) specificity can be not only absolute, but also relative. However, the hormone–receptor interaction is the most specific step in hormone signal transduction into the cell and then into the genome.

The number of different receptor types and subtypes for an individual hormone varies over a wide range. A few of hormones, such as liberins, tropins, oxytocin, calci-

tonin, parathyrin, glucagon, secretin, prostacyclin, insulin, cortisol, aldosterone, and testosterone, have one receptor each; angiotensin II, bradykinin, endothelins, leukotrienes B_4 and C_4-D_4, cholecystokinin, estradiol, and iodothyronines have two receptors each; the antidiuretic hormone, lysophosphatidate, galanin, and GABA have three receptors each; prostaglandin E_2, histamine, and adenosine have four receptors each; somatostatin, melanocortin, dopamine, neuropeptide Y, and sphingosine 1-phosphate have five receptors each; and the champions among hormones are serotonin with 13, glutamate and ATP with 11, and catecholamines with 9 receptors [12, 22]. It is worth noting that CHs usually have one receptor each, whereas neurotransmitters and neuropeptides have several (if not many) receptors. Hormone can express similar effects via distinct receptors, in particular, glutamate excites and GABA inhibits neuronal activity, somatostatin expresses inhibitory activity via all of its five receptors, and catecholamines stimulate cells via all of six α_1- and β-adrenoceptors subtypes. Nevertheless, different receptors usually mediate different (antidiuretic hormone, histamine, serotonin, and dopamine) and even contrary effects of individual hormone (angiotensin II, vessel endothelium GF, estradiol, angiopoietin; catecholamines express inhibitory activities via α_2-receptors).

The existence of several receptors to the same hormone and a link of receptors with various STS apparently facilitate pleiotropy (and often reversibility and duality) of hormonal effects and realization of negative feedbacks. One cell may be sensitive to 4-23 various hormones (which is especially typical of chemokines and cytokines), effects of which complementarily modulate and interfere with each other [12].

The calcitonin family receptors are peculiar. Calcitonin itself acts via its own receptor, whereas other members of this family can only interact with calcitonin- or calcitonin-like (CLR) receptors when these receptors form heterodimeric complexes with one of the transmembrane receptor-activity-modifying proteins (RAMP). The CLR–RAMP-1 dimer plays the role of receptor for the calcitonin gene-related peptide (CGRP); CLR–RAMP-2 and CLR–RAMP-3 are the receptors of adrenomedullin, and a dimer of calcitonin receptor with any of the RAMPs is the receptor of amylin. All of those receptors are coupled with GTP-activated proteins (G-proteins) [23].

Receptor decoys, truncated or soluble, which are localized outside the cells, were found to interact with a variety of hormones (particularly, with cytokines and chemokines). This kind of receptors binds hormones with no resulting action. So, the portion of hormone interacting with active cell receptors decreases resulting in reduction of its biological effect [24]. Chemokine receptors are important for immune response and infections. In particular, human immunodeficiency virus (HIV) penetrates lymphocytes due to its binding with two receptor types, namely T-cellular and chemokine coreceptors CCR5 (this receptor has two adjacent cysteines) and CXCR4 (in which two cysteine residues are separated by an aminoacyl group); cells are resistant to HIV in the absence of the latter receptors, and antagonists of chemokine receptors are being tested as drugs in therapy of AIDS [25] and other infections and inflammations as well [20]. Large DNA-viruses (herpes- and poxviruses) were recently found to simulate chemokines or their cell-associated or soluble receptors, thus interfering with chemokine functions [26].

Five types of hormone receptors can be differentiated (table). Addressing of protein-receptor into the cell plasma membrane (PM) is a common feature for the first four types (Fig. 1). They penetrate the membrane either once (No. 1, 3, 4), or seven times (7-transmembrane receptors, No. 2). Hydrophilic hormone, which poorly penetrates the cell, cannot do so because the hormone–receptor complex is formed on the plasmatic membrane, and not hormone itself, but a hormonal signal arising from hormone binding to receptor enters the cell. Lipophilic hormone (No. 5) easily penetrates the proteolipid PM and enters inside the cell in which it interacts with its receptor. Distinct receptor types tabulated (table) and displayed in the Fig. 1 will be considered together with the mechanisms of further signal transduction. STSs with protein kinases without second messengers are rarely seen (see part II of this review).

Three major variants of hormonal effects can be defined.

1. *Fast effects* (milliseconds) are the changes in membrane charge arising from nerve impulses, which are realized only by ion channels (that is, channel receptors, No. 1). These receptors are called ionotropic.

2. *Slow effects* (usually from seconds to minutes) are typical of metabolism and function regulation and occur mainly due to receptors with seven transmembrane domains; they are commonly called G-protein coupled receptors (GPCR, No. 2). Slow effects are mediated by guanylyl cyclase receptors as well (receptor guanylyl cyclases, No. 4A in the table). These receptor types are called metabotropic.

3. *Genomic effects* (tens of minutes, hours, perhaps days) are especially typical of lipophilic hormones and their intracellular receptors (No. 5), but also of receptors coupled with non-receptor kinases (No. 3), such as tyrosine kinases (TK; nRTK, No. 3A) and protein kinases (PK; nRPK, No. 3B), receptor TK (RTK) or tyrosine kinase receptors (No. 4B), and receptor PK (RPK) or protein kinase receptors (No. 4C).

However, this description is simplified. It has become clear during the last two or three years that the situation is much more complex. For instance, lipophilic hormones have been proven to possess non-genomic effects, and hormones acting via 7-transmembrane receptors do regulate genomic processes as well (see part II of this review).

Hormone receptors and effects

No.	Hormone	Receptor type	Major STSs	Basic effects
1	neuromediators	ion channels (channel receptors)	Na^+ and Cl^- ions – changing of membrane charge	transmission of nerve impulse
2	circulating hormones, neuromodulators, and chemokines	membrane-associated, coupled with G-proteins (7-transmembrane)	G-proteins – enzymes – second messengers – protein kinases and/or ion channels	metabolic and functional alterations, genomic effects
3A	cytokines, somatotropin, prolactin, leptin	membrane-associated, coupled with non-receptor tyrosine kinases (cellular proteins)	non-receptor tyrosine kinases – STAT + 4B	genomic effects, functional and metabolic alterations
3B	tumor necrosis factor-α, interleukins 1 and 18	membrane-associated, coupled with non-receptor protein kinases	non-receptor protein kinases – NF-κB or stress-activated protein kinases	«
4		membrane-associated enzymes (enzymatic receptors):		
4A	NP, guanylin	guanylyl cyclase	cGMP – protein kinase G	metabolic and functional alterations
4B	insulin, growth factors	receptor tyrosine kinases	Ras – MAPK, PI3K – Akt, phospholipase C	metabolic and functional alterations, genomic effects
4C	transforming growth factor-β, BMP	receptor protein kinases	transcription factor Smad	«
5	lipophilic (steroids, iodothyronines, retinoate, calcitriol)	intracellular (in the nucleus or hyaloplasm)	hormone–receptor complex (TF, produced in the nucleus or in cytosol with subsequent entry into the nucleus)	genomic effects (expression, proliferation, differentiation, survival, apoptosis, malignant transformation)

Note: STS) signal-transduction system(s); G-protein) GTP-activated protein; STAT) signal transducers and transcription activators; NF-κB) nuclear factor kappa B; NP) natriuretic peptide; Ras) small GTPase Ras; MAPK) mitogen-activated protein kinase; PI3K) phosphatidylinositol-3 kinase; Akt) protein kinase B; BMP) bone morphogenetic protein; TF) transcription factor.

2. REALIZATION OF NEUROMEDIATOR EFFECTS

This is probably the simplest of the various mechanisms (see table, No. 1). Receptors of neuromediators are regulatory subunits/sites of fast ion channels in the PM, so they are commonly named ionotropic or channel receptors. When opened by neuromediators, those ligand-dependent channels (distinct from the nerve impulse-driven potential-dependent ones) evoke a fast (in milliseconds) entering of Na^+ or Cl^- ions (Fig. 1), whose extracellular concentration is 14- and 33-fold, respectively, higher than their intracellular concentration. So, they enter the cell with no energy consumption and alter the fast postsynaptic potential.

Excitatory neuromediators, such as glutamate (via NMDA (N-methyl-D-aspartate), AMPA (2-amino-3-(3-hydroxy-5-methylisoxazol-4-yl)-propionate)), and kainate receptors and acetylcholine (via N-cholinoreceptors), open fast Na^+-channels resulting in entering of Na^+ into the cell, PM depolarization, and cell excitation. Calcium ions also enter the cell via NMDA-receptors [12, 27, 28]. N-Cholinoreceptors possess modulatory sites for neurosteroids, and NMDA-receptors have analogous sites for glycine, which are also necessary for expression of glutamate effects. The excitation process is required for all of the general functions of the brain, such as physical and psychic activities, purposeful behavior, learning ability, memory, and perception of sensory and pain impulses. However, in epilepsy an excessive release of glutamate into synapses leads to extra excitation followed by the development of seizure. In brain ischemia, excessive release of glutamate with following accumulation of Ca^{2+} (glutamate–calcium

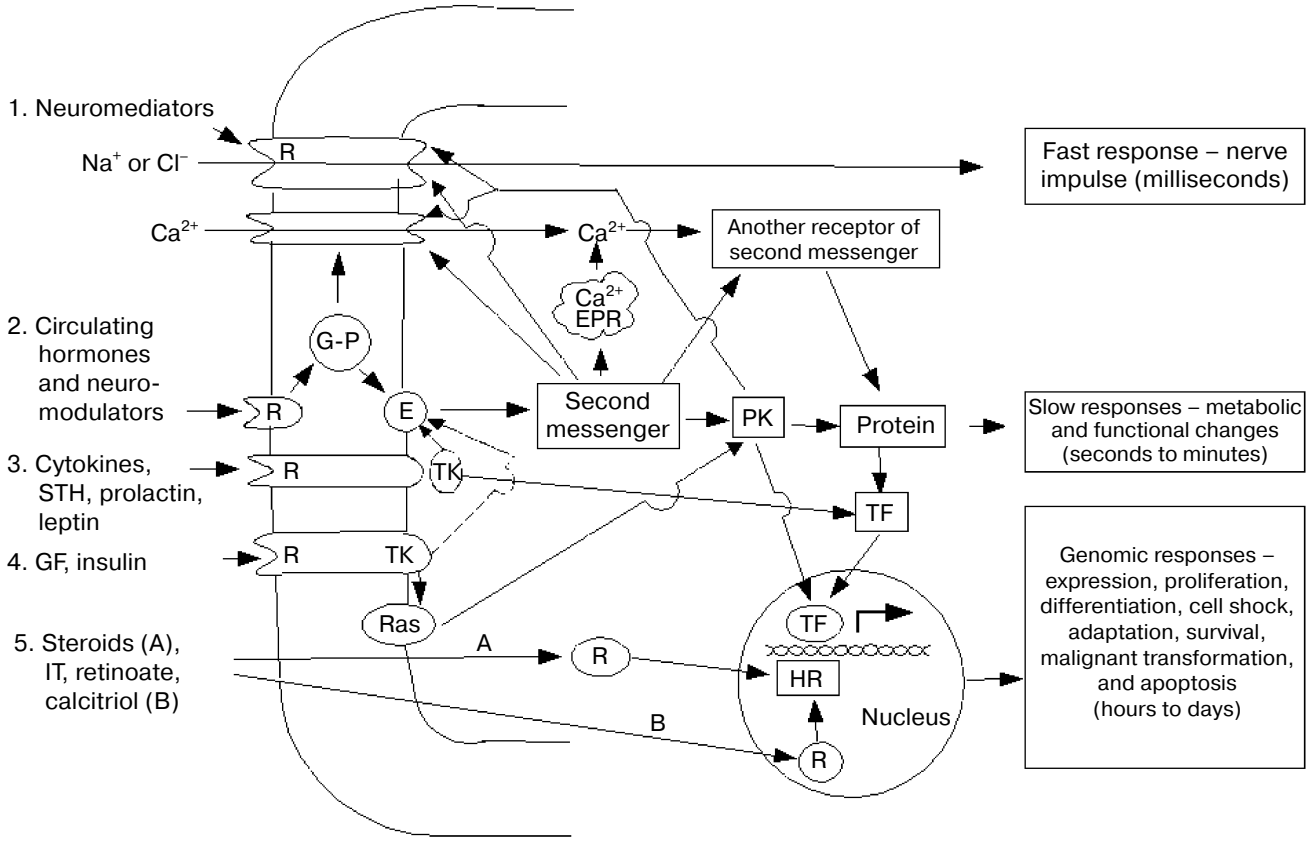

Fig. 1. Signal-transduction systems of the cell (general scheme). Designations: STH) somatotropic hormone; GF) growth factor; IT) iodothyronine; R) receptor; G-P) G-protein; E) enzyme; TK) tyrosine kinase; Ras) small GTPase Ras; EPR) endoplasmic reticulum; PK) protein kinase; TF) transcription factor; HR) hormone–receptor complex.

cascade) is an important mechanism of neuronal injury and death.

GABA and glycine are the major **inhibitory neuromediators** in brain and spinal cord, respectively (and glycine is also active in the brainstem) [12, 27, 28]. They open the fast Cl^--channels (GABA acts via $GABA_A$-receptors) with subsequent Cl^--induced hyperpolarization of the membrane resulting in inhibition. Glycine receptors are also targets of β-alanine, β-aminobutyrate, and taurine. Inhibition is equally necessary for the cell as excitation. But most important is a balance between excitatory and inhibitory mediators. Both tetanus toxin and strychnine impair this balance: the former turns off the $GABA_A$-receptors and the latter glycine receptors, so that glutamate is not counteracted and causes seizures. Barbiturates, benzodiazepines, and general anesthetics activate the $GABA_A$-receptor due to the interaction with specific sites on its molecule, thus potentiating the effect of endogenous GABA. It is the reason for their use as tranquillizers, somnifacient, and general anesthetics. Stimulators of the GABA system with different mechanisms of action have proven themselves in therapy of epilepsy and even stroke (experiments described in [29]). Both $GABA_A$- and glycine receptors realize the inhibitory effects of neuroactive steroids and participate in alcohol-evoked inhibition of neuronal functions.

In recent years, evidence has been obtained for protein kinase- and TF-mediated regulatory effects of nerve impulses and neuromediators on genomic processes. In turn, three ionotropic glutamate receptor types were found to function in non-nerve tissues, such as bone, skin, and pancreas, in which glutamate plays a role of tissue hormone (the authors of [30] gave it the name "cytokine").

Neuromediators exhibit their activity not only via ionotropic, but also metabotropic receptors as well: the former for glutamate are NMDA-, AMPA-, and kainate receptors, and the metabotropic ones are designated as mGlu: for GABA, $GABA_A$ and $GABA_B$-receptors; for acetylcholine, N- and M-choline receptors. However, metabotropic receptors are involved in realization of slow (from seconds to minutes) but not fast (millisecond) modulatory effects, that is, the mentioned substances combine two different roles, mediatory and modulatory. "Pure" neurotransmitters-modulators only act via metabotropic receptors (see below).

3. THE MAIN SIGNAL TRANSDUCTION SYSTEMS (STSs) WITH SECOND MESSENGERS

3.1. General Characteristics

Receptors play an important role, but hormonal signal transduction is not limited by the receptors. The common principle of any cellular information-transfer process, whether hereditary or operative regulatory, is its obligate transformation at each step [3]. In mechanisms of hormonal regulation, this function is fulfilled by the signal transduction systems (STSs), the ordered sequences of proteins and some small molecules, which perceive, transform, drastically amplify, and transmit hormonal and other extracellular signals to all of the cell compartments. The main components of STSs are receptors, G-proteins, effector enzymes, second messengers, ion channels, tyrosine kinases, and protein kinases; translation factors are involved in signal transduction into the cell, and TFs into the nucleus (Fig. 1).

Hormone concentration in blood serum and intercellular fluid is commonly within 10^{-15}-10^{-6} M [10, 31], which is too low to exert any direct control on the cell. One of the main STS functions is amplification of hormonal signal. This occurs at each step: the concentration of second messengers is already 10^{-7}-10^{-6} M, and that of metabolites and ions is generally 10^{-4}-10^{-3} M, i.e., the total amplification factor is 10^5-10^{11}. This phenomenon is known as a cascade mechanism of signal amplification. It is also typical of STS with tyrosine kinases and protein kinases without second messengers, because the kinase cascade involves still larger amounts of phosphorylated proteins at each step.

Receptors and protein kinases are the most important components of STSs. More than 60% of contemporary medicines act through GPCRs [32], and protein kinases comprise about 30% of all drug targets under study [33]. The former are recognized as drugs of XXth century, and the latter may be recognized as drugs of XXIth century [34]. According to the PubMed/Medline database, 2458 reviews on STSs have been published on average per year during the last three years, wherein protein kinases have been considered in one-half of them.

Protein kinases are enzymes phosphorylating proteins at serine, threonine, or tyrosine residues. Protein phosphatases catalyze protein dephosphorylation: there are protein phosphatases catalyzing phosphate cleavage from serine/threonine residues, bispecific phosphatases and protein tyrosine phosphatases dephosphorylating tyrosine residues. All of these can be also subdivided into intracellular and receptor phosphatases. The latter are distinct in being in the extracellular domain, whose functional role in hormone action has only been demonstrated in limited cases (somatostatin is one of the instances):

$$\text{protein} + \text{ATP} \rightarrow \text{protein-P} + \text{ADP},$$

$$\text{protein-P} + H_2O \rightarrow \text{protein} + H_3PO_4.$$

It has long been considered that protein kinases are characteristic of only eukaryotes [35], but protein kinases and phosphatases are found in a variety of prokaryotes as well [36]. The set of all kinases is now called the "kinome". In the human proteome, 518 protein kinases have been found, and these comprise ~2% of the proteins encoded by the whole genome. By its frequency, this is the third most common protein domain in the human genome. About 92% of protein kinases belong to a superfamily in which seven main families can be distinguished. Protein kinases phosphorylating serine and threonine residues are commonly called protein kinases (427 in number, or 82%), and those phosphorylating tyrosine are called tyrosine kinases (91 in number, or 18%) [33, 37]. Far fewer tyrosine kinases are observed because they are involved in perception and following primary transduction from PM into the cell of a limited number of hormonal signals. The percentage of phosphorylated tyrosine residues is very small, comprising 0.03% of total tyrosine residues [10]. Serine-threonine protein kinases are regulated by second messengers, such as cAMP, cGMP, diacylglycerol, Ca^{2+}-calmodulin, PtdIns(3,4,5)P_3 (PIP_3), and by tyrosine kinases as well (either directly or mediated by the minor GTPase Ras). Protein kinases are necessary for the action of the majority of hormones, including ones that do not need tyrosine kinases, and for further signal transduction from tyrosine kinases to all cell compartments including the nucleus. Protein kinases regulate almost all intracellular processes and are important components of cascade signal amplification. Protein kinases play a particularly important role in coordination of complex functions, such as metabolic pathways, cell cycle, morphogenesis, and other genomic processes.

Enzymes, ion channels, translation factors, transcription factors, and structural and other protein are substrates for protein kinases. Phosphorylation of proteins is the most common mechanism of their post-translation modification and the regulation of their activity. The use of this mechanism is wider than hormone effects: some protein kinases, such as pyruvate dehydrogenase kinase and AMP-activated kinase, are hormone-independent. The latter modulates activity and induction of key metabolic enzymes, thus decreasing synthetic processes and enhancing catabolic ones [35, 38].

3.2. G-Protein Dependent Signal Transduction Systems

Major components (see the table, No. 2, and Fig. 2). The first components of these systems are *GPCRs*, or 7-transmembrane receptors. They mediate the effects of the majority of hormones. Estimations for the number of GPCR genes vary from 1000 to 1500, which comprises about 3-10% of the human genome [22, 39]. The func-

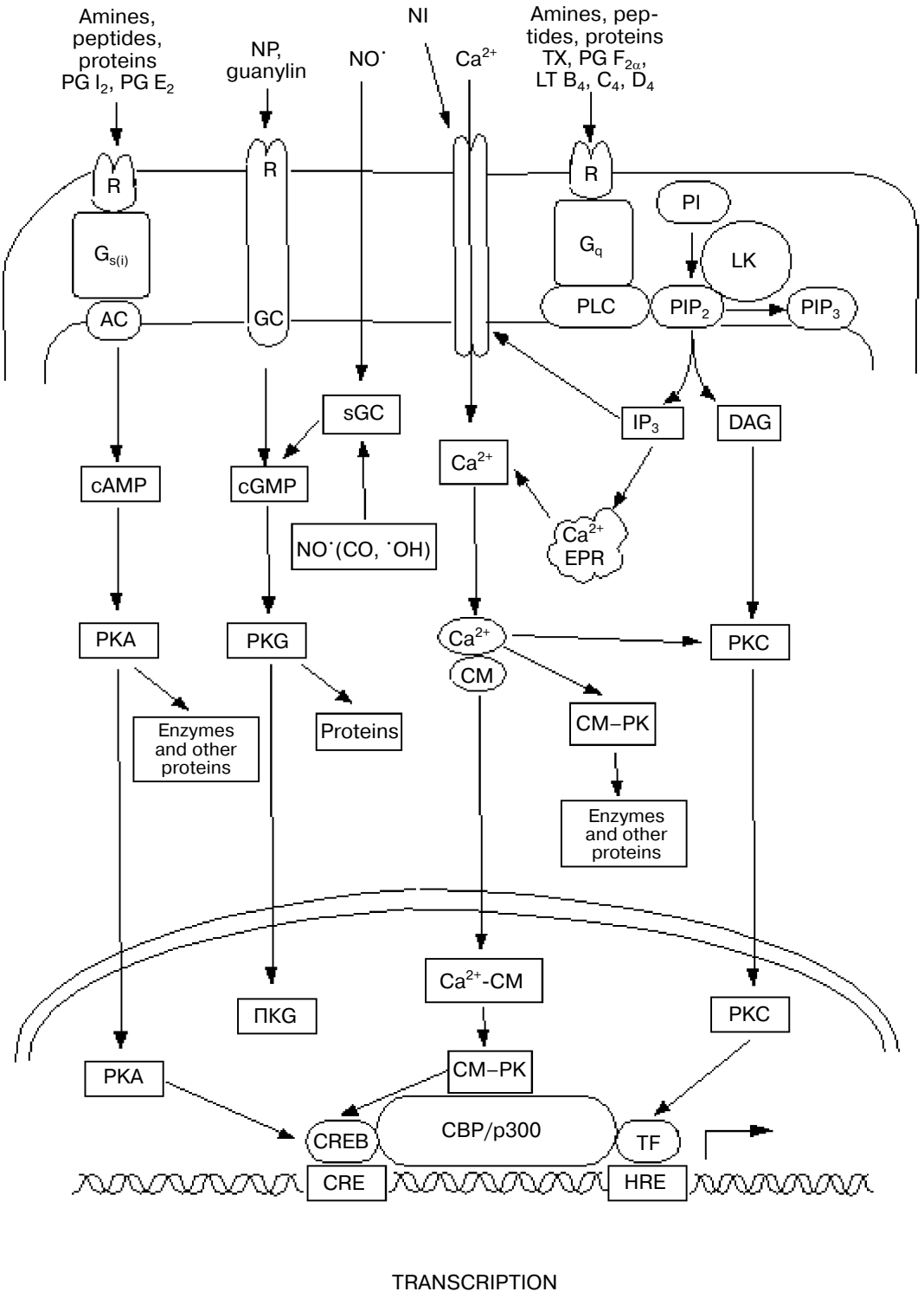

Fig. 2. Signal transduction systems with second messengers. Designations: PG) prostaglandin; NP) natriuretic peptide; NI) nerve impulse; TX) thromboxane; LT) leukotriene; R) receptor; G) G-protein; AC) adenylyl cyclase; GC) guanylyl cyclase; PLC) phospholipase C; PI) phosphatidylinositol; PIP_2) phosphatidylinositol-4,5-bisphosphate; PIP_3) phosphatidylinositol-3,4,5-trisphosphate; LK) lipid kinase (including PI-3K); sGC) soluble guanylyl cyclase; IP_3) inositol trisphosphate; DAG) diacylglycerol; EPR) endoplasmic reticulum; PK) protein kinase; CM) calmodulin; CBP) CREB-binding protein; p300) cointegral 300-kD-protein; TF) transcription factor; CRE and HRE) cAMP-reactive and hormone-reactive DNA elements; ┌→) transcription.

tions of almost 200 GPCRs are established and studied. Structural aspects of GPCRs have been recently described in detail [40].

The importance of *G-proteins*, the second components of these systems, is evident from their gene mutations leading to diseases. In particular, one of the pseudohypoparathyrodism types arises from mutation in a receptor-coupled G_s-protein rather than from a mutation in parathyrin receptor itself. Mutations are known endowing G_s-protein constitutively active: in ovary they result in sexual precocity, and in some other cases in somatotropin excreting cell tumors with acromegaly, tumor of Leydig's cells, or hyperthyroid adenoma [15, 16, 41].

Generally, conformational changes first take place, followed by dimerization of these receptors [42] and binding to *G-protein heterotrimers* (Alfred G. Gilman and Martin Rodbell, Nobel Prize winners, 1994) transmitting the hormone signal within the boundaries of membrane: from the hormone–receptor complex to effector proteins of PM (enzymes or ion channels). The most important subfamilies of G-proteins are G_s, $G_{q/11}$, and $G_{i/o}$ (they are classified by α-subunits, which differ much more than βγ-dimers). The first two G-proteins transmit activating signals, and $G_{i/o}$ inhibits adenylyl cyclase and more often than others regulate ion channels. Interaction between hormone–receptor complex and GDP-G-protein induces first GDP/GTP exchange to form GTP-G-protein and then its dissociation to GTP-α-subunit and βγ-dimer. Both these complexes bind to effector proteins on PM and alter their activity [12, 22, 40]. Most of the 172 studied GPCRs (89%) interact with one distinct G-protein, particularly 43% with $G_{i/o}$, 33% with $G_{q/11}$, and 25% with G_s; rarely, a receptor couples with two or (for thyrotropic and luteinizing hormones, prostaglandin E_2 (EP_3- receptor), and α_{2A}-adrenoceptor even with three G-proteins [22].

Desensitization of GPCR is realized by two different mechanisms: the first is fast phosphorylation of receptors by a specific protein kinase GRK (GPCR-kinase) or protein kinases A or C → interaction of phosphorylated receptors with β-arrestin proteins → receptor decoupling from G-protein; the second is slow endocytosis of receptors followed by their sequestration and degradation with decrease in their expression [22, 40, 43]. G-proteins are ADP-ribosylated by several toxins: cholera toxin irreversibly activates G_s, and pertussis toxin inhibits G_i-protein; in both cases, activating signal is transmitted along.

The third components of these systems are *effector proteins*, such as PM enzymes (adenylyl cyclase, phospholipase C, phosphatidylinositol-3-kinase) and ion channels. Potential-dependent Ca^{2+}-channels are activated by $G_{\alpha s}$ and inhibited by $G_{\alpha i/o}$ and $G_{\beta\gamma}$-subunits, and G-protein-dependent inwardly rectifying potassium channels (GIRK) are activated by $G_{\alpha i/o}$ and dimeric $G_{\beta\gamma}$ and inhibited by $G_{\alpha q/11}$ [12, 44, 45]. Activated enzymes and channels transmit signals inside the cell.

These signals are the fourth components: second messengers (see paper [3] for the critical assessment of the term "secondary" messenger instead of second messenger) and change in cation concentration. *Second messengers* are low molecular weight substances, such as nucleotides (cAMP, cGMP) and lipid metabolism products (inositol trisphosphate, diacylglycerol, PtdIns(4,5)P_2 (PIP_2), and PtdIns(3,4,5)P_3 (PIP_3)) formed by the mentioned enzymes. They are often formed on the inner side of PM and enter the cytoplasm (cAMP, cGMP, inositol trisphosphate) or remain PM-associated (diacylglycerol, PIP_2, and PIP_3). Free Ca^{2+} is also considered as a second messenger. The discovery of the first one, cAMP, and the concept of two messengers (Earl W. Sutherland, Jr., 1958, Nobel Prize winner in 1971) has become one of the most important events of the revolution started in biology in the 1950s and 1960s. Second messengers (cAMP) have arisen in prokaryotes as the most important intracellular regulators. In multicellular organisms, they preserve this function and gained another one: intercellular (most often hormonal) and extracorporeal signal transduction. Concentration of second messengers in the cell is determined by a balance of enzymes realizing their synthesis and catabolism; the latter is mediated by specific hydrolases.

Opening of Ca^{2+}-channels and influx of free Ca^{2+} into the cell (the concentration in cytoplasm is four orders of magnitude lower than the intercellular one) result in cell activation (see below). Opening of K^+-channels induces the efflux of K^+ from the cell (intercellular concentration is 38-fold lower), hyperpolarization of PM, and inhibition.

The fifth STS components are *receptor proteins for second messengers*. More often, they are STS-regulated protein kinases (A, G, C, and calmodulin kinase) phosphorylating serine or threonine residues. This significantly alters activity of protein targets and, as a result, regulates all cell functions (Edmond H. Fisher and Edwin G. Krebs, Nobel Prize 1992). Other proteins may serve as receptors for second messengers: for cAMP, Na^+-channels (olfactory and gustatory reception); for inositol trisphosphate, its receptor in reticulum membrane and slow Ca^{2+}-channels in PM; and for Ca^{2+}, calmodulin.

Thus, a G-protein-dependent STS generally includes the following components: hormone receptor coupled with G-protein (GPCR) → G-protein → effector protein in PM (enzyme or channel) → second messenger → protein kinase or other receptor. In addition, those STS regulate functions of Ca^{2+}- and K^+-channels at three different levels: via direct action of G-proteins, second messengers, and protein kinases [46]. Erwin Neher and Bert Sakmann (1991) and Peter Agre and Roderick MacKinnon (2003) won Nobel Prizes for the discovery of channels and their structures. Possible excessive activity of these systems is prevented by enzymes metabolizing second messengers, by the balance of counteracting G_s-

and G_i-proteins, by regulators of G-protein signaling (RGS) inhibiting G_α-subunit, and by protein phosphatases [12, 21, 40]. The most important distinct STS acting according this mechanism will be considered below, but note that hormones and neurotransmitters can transduce their signals via STS without G-proteins or second messengers (see part II of this review).

cAMP system. The main components of the system are GPCR, G_s- and G_i-proteins, cAMP, two metabolic enzymes, and protein kinase A (Fig. 2). Adenylyl cyclase produces cAMP, and phosphodiesterase catalyzes its hydrolysis:

$$ATP \rightarrow cAMP + H_4P_2O_7,$$

$$cAMP + H_2O \rightarrow 5'\text{-AMP}.$$

The system mediates effects of a large number of hormones: 30 hormones through 45 receptors activate G_s and elevate cAMP concentration and 31 hormones through 76 receptors activate G_i-proteins and decrease cAMP. The first group consists of somato- and corticoliberins, corticotropin, glucagon, parathyrin, calcitonin, prostacyclins, secretin, antidiuretic hormone (V_2-receptors), glutamate ($mGlu_{2\text{-}4,6\text{-}8}$-receptors), dopamine ($D_{1,5}$-receptors), catecholamines (β-receptors), serotonin ($5\text{-}HT_{4,6,7}$-receptors), histamine (H_2-receptors), adenosine ($A_{2A,2B}$-receptors), etc. The group of G_i-protein activators consists of somatostatin, melatonin, opioids, neuropeptide Y, chemokines, platelet-activating factor, lysolipids (sphingosine-1-phosphate and lysophosphatidate), glutamate ($mGlu_{1,5}$-receptors), acetylcholine ($M_{2,4}$-receptors), GABA ($GABA_B$-receptors), dopamine ($D_{2\text{-}4}$-receptors), catecholamines (α_2-receptors), serotonin ($5\text{-}HT_{1,5}$-receptors), histamine ($H_{3,4}$-receptors), adenosine ($A_{1,3}$-receptor), angiotensin II (receptor type 2), etc. Follicle-stimulating, luteinizing, and thyrotrophic hormones activate both G-proteins [22]. Adenylate cyclase is inhibited by oxidants and protected by thiols.

Protein kinase A is the major cAMP receptor. There are protein kinase AI localized mainly in hyaloplasm and protein kinase AII, which is membrane-associated and also found in cytoskeleton and some organelles including the nucleus; the latter may be attached to receptors and ion channels by anchor proteins. In the absence of a stimulus, the regulatory subunits of protein kinase A inhibit its catalytic subunits [12]. When cAMP is bound, the protein kinase A dissociates, and its free active catalytic subunits phosphorylate target proteins, such as enzymes (phosphorylase kinase, glycogen synthase, L-pyruvate kinase, triglyceride lipase, cholesterol desmolase, tyrosine hydroxylase, carboanhydrase, and glutathione metabolism enzymes), structural (phospholamban), and matrix synthesis components. cAMP is called stress signal. It participates in glycogen and fat decay to develop hyperglycemia, hyperlactatemia, and accumulation of fatty acids in blood plasma, increases heart rate, and activates mitochondrial processes [47]. This is an evolutional attainment because potato phosphorylase is not regulated by phosphorylation [48]. However, the regulatory functions of the cAMP system are not limited to the above processes: it is involved in smooth muscle relaxation, platelet disaggregation, H^+ secretion in stomach, secretion of bicarbonate and water in duodenum, and of Cl^- from epithelium, water reabsorption, maintenance of Ca^{2+} level in blood, inhibition of lymphocyte proliferation, inflammation and immunity; learning, and so on. In general, this is regulation of metabolism and functions. In 2000, Arvid Carlsson and Paul Greengard were awarded the Nobel Prize for the discovery of the role of dopamine and the cAMP system in regulation of brain functions.

The free catalytic subunit of protein kinase A enters the nucleus (Fig. 2), where it phosphorylates nuclear TF CREB (CRE-binding protein). It binds to the important HRE (hormone reactive element) CRE (cAMP-reactive element TGACGTCA [10]), the regulatory DNA site of which is present in cAMP-sensitive genes, and with the activating integrator, CREB-binding protein (CBP), and stimulates transcription (see part II of this review and Fig. 4 in the review [17]). As a result, cAMP system regulates genomic processes, such as gene expression, development of endocrine cells, syntheses of catecholamines and hypophysial and steroid hormones, adaptation, memory and learning, remodeling of blood vessels, heart, and bones, and spermatogenesis. Knockout of regulatory subunit of protein kinase AI provokes early embryonal death [49].

Receptors for cAMP and cGMP are also nonspecific cation channels in sense organ cells, which release not only Na^+ but also Ca^{2+} into the cell. This is necessary for smell and taste perception [12, 50]. The third cAMP receptor is an exchange protein, which is directly activated by cAMP (EPAC). It stimulates minor GTPase Rap [51, 52], which interacts with mitogen-activated protein kinase (MAPK) [21]. Activity of the cAMP system is limited by cAMP-hydrolyzing phosphodiesterase localized in both hyaloplasm and membranes and by protein phosphatases dephosphorylating proteins.

This system is important for medicine. In 1982, Sune K. Bergström, Bengt I. Samuelsson, and John R. Vane won a Nobel Prize for the discovery of prostaglandins stimulating the cAMP system and elucidation of mechanisms of non-steroid anti-inflammatory drug activity including the inhibition of prostaglandin synthesis. The cAMP system is involved in pathogenesis of cholera, pertussis, and mucoviscidosis (cystic fibrosis). Cholera toxin induces irreversible stimulation of G_s-protein → activation of adenylyl cyclase → cAMP accumulation → protein kinase activation → phosphorylation and stimulation of cystic fibrosis transmembrane regulator (CFTR-protein providing Cl^- and water efflux into intestinal space) → secretory diarrhea → dehydration and NaCl loss from the

organism. A recessive mutation excluding phosphorylation of CFTR by protein kinase A enhances resistance to cholera in heterozygotes and, hence, is not eliminated. However, it results in a common hereditary disease, cystic fibrosis, in homozygotes [31]. Pertussis and anthrax toxins contain soluble adenylate cyclase (edema factor) [53]. HCO_3^--activated soluble adenylyl cyclase is found in testes and sperm. Desensitization of β-adrenoceptors in myocardial hypertrophy is combined with enhanced G_i-protein expression. Inhibitors of cAMP-phosphodiesterase 4 (piclamilast, cilomilast) are potent anti-inflammatory agents beneficial in rheumatoid arthritis, bronchial asthma, and chronic obstructive chest disease [54, 55]. Protein kinase A activity is increased in liver in hepatoectomia (2-3-fold) and especially in colorectal cancer (10-30-fold). Overexpresion of protein kinase AI regulatory subunit occurs in cancer; and specific antisense oligonucleotides and protein kinase A inhibitors slowed down experimental tumor growth rate [56].

The phosphatidylinositol system is the most complex system involving GPCR, $G_{q/11}$-proteins, four second messengers (PtdIns(4,5)P_2 (PIP_2), inositol trisphosphate, diacylglycerol, and Ca^{2+}), enzymes of their synthesis and degradation, and two protein kinases (calmodulin- and C kinase, Fig. 2). Thirty-eight hormones via 59 receptors activate $G_{q/11}$-proteins. These hormones include thyro-, gonado-, and prolactoliberins, cholecystokinin, bradykinin, endothelin, thromboxane A_2, prostaglandins F_{2a}, leukotrienes C_4 and D_4, lysolipids, oxytocin, neuromedins, neurotensins, thrombin, purine nucleotides (P_{2Y}-receptors), antidiuretic hormone (V_1-receptors), angiotensin II (type 1 receptors), catecholamines (α_1-receptors), serotonin (5-HT_2-receptors), histamine (H_1-receptors), acetylcholine ($M_{1,3,5}$-receptors), and glutamate ($mGlu_{1,5}$-receptors). We have already mentioned above that effects of thyrotropic and luteinizing hormones are mediated by all three G-proteins [22].

Lipid kinases phosphorylate an initial substance, the minor phospholipid PtdIns localized in PM, twice on the inositol residue to form PtdIns(4,5)P_2. Lipid kinases are the enzymes phosphorylating lipids. The functions of lipid kinases and protein kinases are different: the former participate in formation of lipid second messengers, and the latter transduce their signals onto protein targets. Hormones via their receptors and G_q-protein activate phospholipase C-β resulting in PtdIns(4,5)P_2 hydrolysis to two lipid second messengers, inositol trisphosphate and diacylglycerol [12, 57, 58]. The first one and PtdIns are also found in the nucleus, and the last is involved in regulation of transcription [59].

Inositol trisphosphate opens Ca^{2+}-channels in reticulum and PM resulting in input of Ca^{2+} into cytosol (its concentration in cytosol is 10^{-7} M, two orders of magnitude higher in reticulum, and four orders of magnitude higher in intercellular fluid). Calcium ions enter the cytosol through glutamate NMDA-channels as well. Other mechanisms of Ca^{2+} efflux from reticulum involve other second messengers: cADP-ribose, which acts via ryanodine receptors, and nicotinic acid-ADP (NAADP) (they are formed from coenzymes NAD and NADP, respectively) [60]. The first mechanism is used by acetylcholine in motor nerves, and the second one by cholecystokinin in pancreas [61]. A phenomenon of Ca^{2+}-induced Ca^{2+} release (CICR) is known: initial entry of small amounts of Ca^{2+} induces a much larger Ca^{2+} efflux from reticulum into cytosol, in which its concentration grows to 10^{-6}-10^{-5} M [57, 62], that is, positive feedback is realized. Calcium ion binds to its main receptor, the protein calmodulin (CM), and via Ca^{2+}/CM-dependent protein kinases (CM-PKs) induces a diversity of effects. "Dedicated" (specific) CM-PKs phosphorylate and activate phosphorylase and light myosin chain kinases (inducing glycogen phosphorolysis and smooth muscle contraction, respectively) and phosphorylate the elongation factor-2 kinase (eEF2-kinase), thus inhibiting translation [12]. CM-PK II is more active in the brain, in which it stimulates synaptic and behavioral memory [63]. CM-PK kinase (CM-PKK) phosphorylates and activates multifunctional CM-PKs I and IV [10, 63], which, in turn, induce multiple effects in hyaloplasm (I) and nucleus (IV), respectively [12, 64]. Another mechanism of the Ca^{2+}–CM complex activity is CM-dependent activation of calcineurin (protein phosphatase 2B), which dephosphorylates NFAT (nuclear factor of activated T-lymphocytes), thus enabling it to penetrate the nucleus and activate the cells. This pathway is more important in the brain, wherein the calcineurin concentration is maximal: it participates in memory and synaptic plasticity development. Calcineurin is involved in ion homeostasis and cell cycle control [65, 66].

Calcium ion is one of the most flexible and universal second messengers. It is required for elementary (membrane excitation, mitosis, synaptic plasticity, hormone release), global intracellular (fertilization, metabolism regulation, muscle contraction, gene transcription, proliferation), and intercellular (endothelium, vessel, gland, and brain functions) events [67]. The Ca^{2+}–CM complex activates many enzymes, such as kinases, phosphatases, phospholipase A_2, some proteinases, endothelial $NO^{\cdot}$-synthase, mitochondrial enzymes, and cyclic nucleotide metabolism enzymes, as well as structural proteins of the cell. This stimulates glycogen and phospholipid degradation, gluconeogenesis, mitochondrial functions, hormone and neurotransmitter release, synaptic plasticity, myocardium and smooth muscle contraction, gland secretion, and platelet aggregation. The Ca^{2+}–CM complex penetrates the nucleus and activates nuclear CM-PK IV (which also can enter being already active), which phosphorylates and activates the TFs CREB and CBP and protein kinase B/Akt (PKB). TFs bind to corresponding HREs resulting in transcription of many genes, mitosis and angiogenesis stimulation, modulation of

inflammation and immunity, and other genomic processes [12, 63]. Actually, Ca^{2+} stimulates all cell types, it provides the coupling of muscle contraction with its energy supply and cell division with gene transcription. Upon completion of hormonal action, Ca^{2+} is removed from the cell by Ca^{2+}-ATPases of PM and reticulum, thus returning the cell into the resting condition. Phosphatidylinositol STS-induced Ca^{2+} accumulation in the cell is limited by the G_i-protein-dependent decrease in the ion level induced by other hormones (see below). Over accumulation of Ca^{2+} damages the cell and leads to apoptosis, which is probably linked to the activation of Ca^{2+}–CM-dependent cell death-associated protein kinases (DAPKs) [12, 68].

The medical importance of this STS is well known. The blockers of receptors of Ca^{2+}-dependent hormones, such as angiotensin (sartans) and α_1-receptors (zosins), as well as Ca^{2+}-channel antagonists (dipines) are widely used in therapy of hypertension. The Ca^{2+} sensitizer levosimendan is effective in therapy of cardiac failure and was recognized as one of the best pharmaceuticals in 2002 [69].

Diacylglycerol, which is also formed by phospholipase C-β from PtdIns(4,5)P_2, remains in PM and recruits protein kinase C which is then activated in the presence of phosphatidyl serine and Ca^{2+} (conventional isoenzymes α, β, γ), or in the presence of phosphatidyl serine alone (novel isoenzymes δ, ε, η, θ, μ). Atypical isoenzymes (ζ, λ, ι) are activated by phosphatidylinositol-dependent kinase (PDK) [12]. Diacylglycerol is formed in the nucleus as well, resulting in translocation of protein kinase C into the nucleus and activation. In PM, protein kinase C phosphorylates and alters activity of some ion channels and cytoplasmic enzymes, and in the nucleus it phosphorylates histones and some TFs and stimulates genomic processes. The first results in opening of potential-dependent Ca^{2+}-channels and receptor glutamate channels and closing of inward rectifying K^+-channels, suggesting neurotransmitter release, long-term potentiation in hippocampus, and vasoconstriction. Subarachnoid hemorrhage of the brain is accompanied by prolonged vasospasm mediated by protein kinase C [70]. Via this enzyme, adenosine opens K_{ATP}-channels in PM and mitochondria with the development of myocardium preconditioning (elevation of resistance to ischemia and hypoxia) [71]. Protein kinase C participates in stimulation of digestive enzyme secretion by cholecystokinin. It phosphorylates and activates MAPK-cascades, kinases SYK–JNK (Jun N-terminal kinase), PI3-K (phosphatidylinositol 3-kinase)–Akt (PKB), and NF-κB (nuclear factor kappa B) [21, 72].

Various genomic effects of protein kinase C are known: stimulation of gene expression, proliferation, cell differentiation, embryogenesis, angiogenesis, cytoskeleton alterations, extracellular matrix protein synthesis, survival. Protein kinase C activity is elevated in hepatitis B, C, and E and in tumors, and phorbol esters, the enzyme activators, are carcinogenic. Improvement in results of breast and lung cancer treatment was demonstrated when affinitac (ISIS 3521), a protein kinase C inhibitor, was added to conventional chemotherapy [73]. Tocopherol, retinoids, and selenium moderately inhibit the enzyme (one of multiple examples of redox regulation).

PtdIns(4,5)P_2 is not only an intermediate of PtdIns metabolism, but also a second messenger having its own functions. It regulates the work of a number of channels and ion transporters. In particular, it activates calcium channels in PM and ryanodine channels in reticulum, all inward rectifier K^+-channels, and Na^+-channels in epithelium and inhibits Ca^{2+}-channels unlocked by inositol trisphosphate and channels opened by cAMP and cGMP in retinal rods [74, 75]. PtdIns(4,5)P_2 enhances 300-fold the sensitivity of K_{ATP}-channels to the inhibitory effect of ATP [44]. Moreover, PtdIns(4,5)P_2 regulates cytoskeleton structure, secretory vesicle motion, and exocytosis. Pathology of PtdIns(4,5)P_2 dephosphorylation is found in oculocerebrorenal syndrome and invasion of *Listeria*, *Shigella dysenteriae*, and *Salmonella* [58]. Conversion of PtdIns(4,5)P_2 into PtdIns(3,4,5)P_3 and the role of the latter are considered in part II of the present review.

Interaction between cAMP and PtdIns systems is complex. Calcium ions activate two of eight adenylyl cyclase isoenzymes (AC 1 and 8) and inhibit one (AC 3) [76], elevate cAMP level and protein kinase A activity [77], but stimulate one of the phosphodiesterase isoenzymes. Besides, the final result of the interaction depends largely on the cell type. In heart and liver effects of cAMP-dependent and Ca^{2+}-mobilizing hormones are similar and synergic in general (stimulation of function), but in blood vessels (vasodilatation and vasoconstriction) and platelets (aggregation and disaggregation) they are antagonistic, and in fat tissue lipolysis is influenced by cAMP only. For the first two groups of tissues these characteristics are connected with the influence of cAMP system on cytoplasmic Ca^{2+} level. In cardiomyocytes, hepatocytes, hypophysis, and adrenal secretory cells cAMP-dependent hormones induce or potentiate elevation of cytoplasmic Ca^{2+} concentration and/or enhance its effect. Influx of Ca^{2+} into cardiomyocytes resulting from protein kinase A activation by catecholamines may serve as an example. In contrast, the same hormones inhibit Ca^{2+} elevation in blood cells, smooth muscle cells in vessels, and in astrocytes [78].

For *inhibitory neuromodulators* (Fig. 3), such as acetylcholine ($M_{2,4}$-receptors), catecholamines (α_2-receptors), dopamine (D_2-receptors), GABA ($GABA_B$-receptors), adenosine ($A_{1,3}$-receptors), and opioids (μ-receptors), the usage of various G_i-protein-dependent mechanisms is typical: decrease in adenylate cyclase activity and cAMP level, decrease in Ca^{2+}-channel activ-

ity with the reduction of Ca^{2+} concentration in cytoplasm, and opening of K^+-channels inducing K^+ efflux from the cell with its hyperpolarization. All these mechanisms reduce cellular activity [31].

Since second messengers are formed in cytosol, the question of the mechanisms of their influence on cellular organelles is important. In mitochondria, Ca^{2+} and cAMP act on the external surface of the inner membrane and via transport into the matrix. In this case (unlike cytosol) Ca^{2+} acts directly (without calmodulin) on enzymes, and cAMP acts either via protein kinase A, which is present in all mitochondrial subcompartments, or on receptor protein on the outer side of the inner membrane. As a result, two groups of hormones: Ca^{2+}-

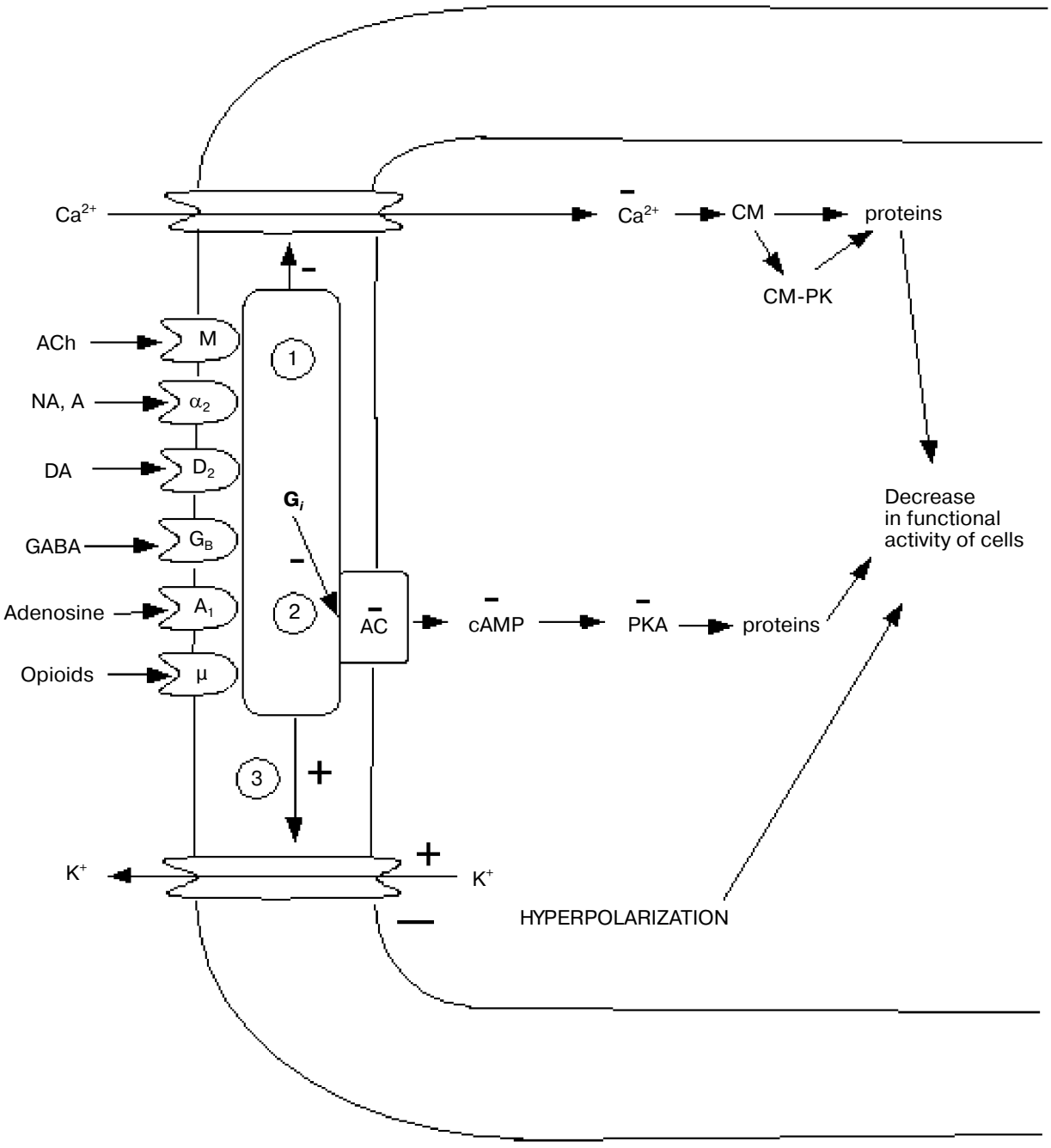

Fig. 3. Mechanism of inhibitory neuromodulator action. Designations: ACh) acetylcholine; M) $M_{2,4}$-choline receptors; NA) noradrenaline; A) adrenaline; α_2) α_2-adrenoreceptors; DA) dopamine; D_2) $D_{2\text{-}4}$-dopamine receptors; GABA) gamma-aminobutyric acid; G_B) $GABA_B$-receptors; A_1) adenosine A_1-receptors; μ) opioid μ-receptors; G_i) G_i-protein; AC) adenylyl cyclase; PKA) protein kinase A; CM) calmodulin; CM-PK) calmodulin protein kinase.

mobilizing (catecholamines through α_1-receptors, antidiuretic hormone via V_1-receptors, and angiotensin II via type 1 receptors) and cAMP-dependent (catecholamines via β-receptors, glucagon) activate all mitochondrial oxidative functions [47]. Signal transduction of hormones into the nucleus (Fig. 2) occurs through penetration of the protein kinase A catalytic subunit into the nucleus, Ca^{2+}–CM complex, activating nuclear CM-PK, transport of activated CM-PK and protein kinase C. These protein kinases phosphorylate nuclear TF (the first two, CREB), which activate CRE and other hormone-reactive DNA elements (HRE). GPCR transduces signals on kinase STSs as well (see part II of the present review).

Interestingly, the existence of extracellular GPCR that crosses the PM seven times and functions as a sensor of Ca^{2+} concentration in blood plasma is proven for parathyroid, bones, intestine, and kidney [79, 80]. Analogous cAMP receptor has been revealed even in bacteria and single-celled eucaryotes, but it is not yet identified in mammals. However, glucagon is known to stimulate cAMP excretion into blood plasma mediating natriuretic and phosphaturetic effect of glucagon [81]. Antidiuretic hormone and parathyrin induce cAMP excretion in blood flow from the kidney. Hence, these two second messengers fulfill functions of first messengers as well. At first glance, this seems illogical, but as Albert Szent-Györgyi said, nature has its own logic. Nature virtually does not recognize any inflexible barriers between phenomena and concepts (we underlined earlier the lack of such barriers between CHs, neurotransmitters, GFs, cytokines, and eicosanoids).

3.3. cGMP-Dependent Signal Transduction Systems

The cGMP system (No. 4A in the table) consists of guanylyl cyclase receptor with single transmembrane domain (receptor, or membrane guanylyl cyclase, mGC), cGMP, phosphodiesterase, and protein kinase G (Fig. 2). The cGMP and cAMP systems are similar in general: the two cyclic nucleotides are very similar in their structures, both are formed of purine NTP and are hydrolyzed by phosphodiesterase to ordinary linear 5′-NMP, and the mechanism of second messenger–protein kinase is used. However, major differences exist: cGMP system is switched on by a narrow hormone spectrum; in PM, cGMP is formed by the catalytic domain, but not by a separate cyclase, and G-protein is not involved. This system is activated by natriuretic peptides (NPs), such as atrial natriuretic peptide (ANP), "brain" natriuretic peptide (BNP) (which is formed mainly in cardiac ventricles), endothelial C-type natriuretic peptide (CNP) (all three NP types are formed in the brain); kidney urodilatin, intestinal guanylin, and uroguanylin. These peptides regulate water and ion homeostasis in kidney, intestine, and lungs, provoke natri- and diuresis, and act as hypotensers [82-84]. Peptide attractants for spermatozoids secreted by ovum, speract and resact, activate guanylyl cyclase as well [28].

The monoxides–cGMP system is entirely localized in cytoplasm. Its important feature is the usage of soluble guanylyl cyclase (sGC), activated by NO$^{\bullet}$ and CO, and also $^{\bullet}$OH (Fig. 4). The reactive nitrogen species NO$^{\bullet}$ (nitric oxide) is formed by NO$^{\bullet}$-synthase (NOS) from arginine, and this process is activated by acetylcholine, adrenomedullin, bradykinin, and CO_2 in vessel endothelium, by a number of cytokines in macrophages, and glutamate in neurons (endothelial, inducible, and neuronal NOS—eNOS, iNOS, nNOS, respectively). Low concentrations of the formed NO$^{\bullet}$ influence the heme of sGC and induce cGMP accumulation [85, 86]. In 1998, Robert F. Furchgott, Louis J. Ignarro, and Ferid Murad were awarded a Nobel Prize for the discovery of NO$^{\bullet}$ and its role as vasodilator. NO$^{\bullet}$ is a special regulator: it appears under the action of hormones, but it is not an ordinary second messenger, because like a hormone it acts not only at intra-, but also at intercellular level. The latter is because NO$^{\bullet}$ is a lipophilic gas easily penetrating membranes. However, it differs from ordinary intercellular regulators in the lack of depots in the cell producing the gas (it is like steroid hormones in this respect), and in non-directed signal transmission. Since NO$^{\bullet}$ effects all surrounding cells, it is named a volume regulator [87]. In brain, NO$^{\bullet}$ participates in feedback of post-to-presynaptic neuron [85]. CO is formed by heme oxygenase in the first reaction of hemoglobin decay, activates sGC, and is similar to NO$^{\bullet}$ in its properties and biological activity [88].

cGMP formed either by membrane GC (mGC) or sGC activates its receptor proteins, most often protein kinase G. This event results in a decrease of Ca^{2+} level in all cells, weakens its effects [78, 89], induces smooth muscle relaxation with vasodilatation (which is manifested as improvement of blood flow, hypotension, and erection) and decrease in intestine tonus, platelet disaggregation and decrease of platelet adhesion, increase in natriuresis/diuresis, and neuronal stimulation [86, 89]. These effects are typical of both NO$^{\bullet}$ and CO. Protein kinase G, like protein kinase A, activates CFTR and enhances Cl^- and water secretion into the intestine. Other cGMP targets are cyclic nucleotide phosphodiesterase and ion channels. In retina, light activates phosphodiesterase through rhodopsin and G_t-protein (transducin), and a decrease in cGMP concentration induces nonspecific Na^+, Ca^{2+}-channel closing. The arising nerve impulse is perceived as light. The cGMP system has been shown recently to regulate gene expression at transcriptional and post-transcriptional levels [90].

However, mechanisms of NO$^{\bullet}$ effects are much broader. They include S-nitrosylation, ADP-ribosylation, peroxynitrite formation [85, 89, 91, 92], expression of a number of proteins (stress, anti-oxidant defense),

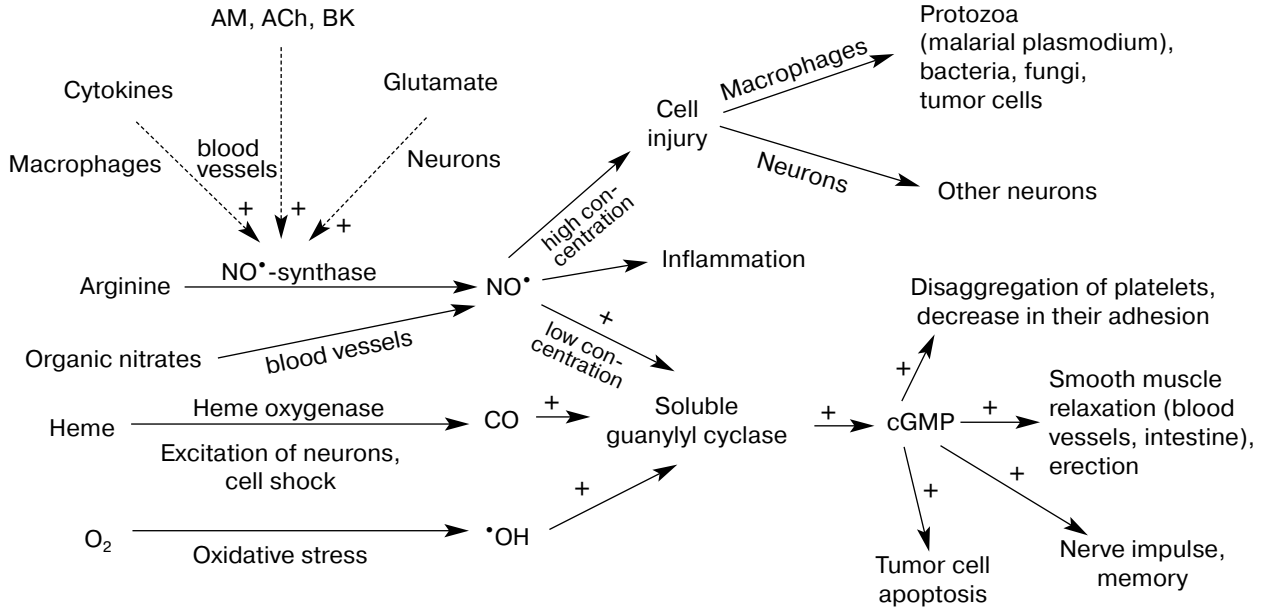

Fig. 4. Monoxides activating guanylyl cyclase. Designations: AM) adrenomedullin; ACh) acetylcholine; BK) bradykinin.

activation of MAPK cascades and protein kinase C, modulation of other enzymes, and development of immune responses [85].

The thermostable *E. coli* endotoxin (more potent guanylin homolog) as a result of intestinal guanylyl cyclase activation drastically enhances secretion in pathology, thus resulting in excretory diarrhea (diarrhea of voyagers). cGMP participates in protection against hypertension, myocardial hypertrophy, arteriosclerosis, and damage to vessels/restenosis [90]. NO• protects endothelium, decreases proliferation and migration of smooth muscle cells, possesses anti-sclerosis properties [93], and accelerates wound healing. CO enhances blood flow, improves cardiac function, decreases proliferation of smooth muscle cells and platelet aggregation, and protects against septic shock [88]. Nesiritide, a recombinant analog of B-type natriuretic peptide, is successfully used in acute cardiac failure and it was recognized as one of the best medicines in 2002 [69]. Sildenafil, an inhibitor of phosphodiesterase (Viagra, Pfizer, USA), induces cGMP accumulation, dilation of penis vessels, and its erection. Exisulind, a new phosphodiesterase inhibitor, induces an increase in cGMP concentration, activates protein kinase G, and induces apoptosis in cancer, but not normal, cells [94]. NO• donors (nitroglycerol, isosorbide nitrates) have been used as vasodilators in cardiac ischemia for a long time.

High NO• concentrations inhibit heme- and ferric-sulfur proteins including Krebs cycle and respiratory enzymes, induce apoptosis and necrosis in cells of a macroorganism (neurons, cancer cells) and in invasive agents, such as protozoa (malarial plasmodium, etc.), bacteria, and fungi, i.e., NO• is a species of important biochemical defense of macrophages which is functionally like active oxygen species [85].

In general, broad and productive usage of ideas and achievements in the field of mechanisms of hormone action has had a very positive effect in medicine.

REFERENCES

1. Kulinsky, V. I., and Ol'khovskii, I. A. (1992) *Usp. Sovrem. Biol.*, **112**, 697-714.
2. Kulinsky, V. I. (1994) *Vopr. Med. Khim.*, **40**, 14-18.
3. Kulinsky, V. I., and Kolesnichenko, L. S. (1997) *Biochemistry (Moscow)*, **62**, 1171-1173.
4. Pankov, Yu. A. (1998) *Biochemistry (Moscow)*, **63**, 1361-1373.
5. Ulumbekov, E. G., and Chelyshev, Yu. A. (eds.) (2001) *Histology* [in Russian], GEOTAR-MED, Moscow, p. 266.
6. Litvitskii, P. F. (2002) *Patophysiology* [in Russian], Vol. 2, GEOTAR-MED, Moscow, p. 316.
7. Petrovsky, N. (2001) *Immunol. Cell Biol.*, **79**, 350-357.
8. Wilson, J. D. (2000) *Ann. Rev. Physiol.*, **62**, 947-950.
9. Lavin, N. (2002) *Manual of Endocrinology and Metabolism*, 3rd Edn., Lippincott Williams & Wilkins.
10. Murray, R. K., Granner, D. K., Mayes, P. A., and Rodwell, W. W. (2003) *Harper's Illustrated Biochemistry*, *Int. edition*, 26 Edn., McGrow-Hill Co., Boston-NY., et al., pp. 434-473.
11. Nikolaev, A. Ya. (2004) *Biological Chemistry* [in Russian], 3rd Edn., Meditsinskoe Informatsionnoe Agentstvo, Moscow.
12. Watling, K. J. (ed.) (2001) *Sigma-RBI Handbook of Receptor Classification and Signal Transduction*, 4th Edn., Natick, USA.

13. Strange, Ph. G. (2002) *Trends Pharmacol. Sci.*, **23**, 85-95.
14. Efroimsom, V. P. (1998) *Genius and Genetics* [in Russian], Russkii Mir, Moscow, pp. 210-214.
15. Szkudlinski, M. W., Fremont, V., Ronin, C., and Weintraub, B. D. (2002) *Physiol. Rev.*, **82**, 473-502.
16. Kandror, V. I. (2001) *Probl. Endokrinol.*, **47**, 3-10.
17. Kulinsky, V. I., and Kolesnichenko, L. S. (2002) *Vopr. Med. Khim.*, **48**, 44-67.
18. Makarova, E. N. (2002) *Usp. Sovrem. Biol.*, **122**, 365-375.
19. Rosengurt, L., and Walsh, J. H. (2001) *Ann. Rev. Physiol.*, **63**, 49-76.
20. Grainger, D. J., and Rackless, J. (2003) *Biochem. Pharmacol.*, **65**, 1027-1034.
21. *Innovative Discovery Tools for Signal Transduction Research* (2003), Cell Signaling Technology, Beverly, USA.
22. Wong, S. K-F. (2003) *Neurosignals*, **12**, 1-12.
23. Poyner, D. R., Sexton, P. H., Marshall, J., Smith, D. M., Quirion, R., Born, W., Muff, P., Fischer, J. A., and Foord, S. M. (2002) *Pharmacol. Rev.*, **54**, 233-246.
24. Mantovani, A., Bonecchi, R., Martinez, F. O., Galliera, E., Perrier, P., Allavena, P., and Locati, M. (2003) *Int. Arch. Allergy Immunol.*, **132**, 109-115.
25. Fujii, N., Nakashima, H., and Tamamura, H. (2003) *Expert Opin. Investig. Drugs*, **12**, 185-195.
26. Beet, B. T., and McFadden, G. (2002) *J. Leukoc. Biol.*, **72**, 24-34.
27. Sergeev, P. V., Shimanovskii, N. L., and Petrov, V. I. (1999) *Receptors of Physiologically Active Substances* [in Russian], 2nd Edn., Sem' Vetrov, Moscow-Volgograd.
28. Garrett, R. H., and Grisham, Ch. M. (1995) *Molecular Aspects of Cell Biology*, Saunders College Publishers, Fort Worth, Philadelphia, pp. 1180-1254.
29. Kulinsky, V. I. (2000) *Vestn. Ross. Akad. Med. Nauk*, No. 9, 39-43.
30. Skerry, T. M., and Genever, P. G. (2001) *Trends Pharmacol. Sci.*, **32**, 174-181.
31. Kulinsky, V. I. (2004) *Lecture Charts on Biochemistry* [in Russian], 5th Issue, 6th Edn., ISMU, Irkutsk.
32. *G-Protein Coupled Receptors* (2003) IBC Life Sci., London.
33. Biondi, R. M., and Nebreda, A. R. (2003) *Biochem. J.*, **372**, 1-13.
34. Cohen, P. (2002) *Nature Rev. Drug Discov.*, **1**, 309-315.
35. Berg, J. M., Timoczko, J. L., and Stryer, L. (2002) *Biochemistry*, 5th Edn., W. H. Freeman Co., N. Y.
36. Kennely, P. J. (2002) *FEMS Microbiol.*, **206**, 1-8.
37. Manning, G., Whyte, D. V., Martinez, R., Hunter, T., and Sudarsanan, S. (2002) *Science*, **298**, 1912-1934.
38. Winder, W. W., and Hardie, D. G. (1999) *Am. J. Physiol.*, **277**, E1-E10.
39. Malbon, C. C. (2004) *Front. Biosci.*, **9**, 1048-1058.
40. Avakyan, A. E., and Tkachuk, V. A. (2003) *Ross. Fiziol. Zh. im. I. M. Sechenova*, **89**, 219-239.
41. Weinstein, L. S., Chen, M., and Liu, J. (2002) *Ann. N. Y. Acad. Sci.*, **168**, 173-197.
42. Franko, R., Canals, M., Marcellino, D., Ferre, S., Agnati, L., Mallol, J., Casado, V., Ciruela, F., Fuxe, K., and Llius, C. (2003) *Trends Biochem. Sci.*, **28**, 237-243.
43. Brzostowski, J. A., and Kimmel, A. R. (2001) *Trends Biochem. Sci.*, **26**, 291-297.
44. Ashcroft, F. M. (1998) *Science*, **282**, 1059-1060.
45. Descal, N. (2001) *Trends Endocrinol. Metab.*, **12**, 391-398.
46. Trimmer, J. S. (2002) *Science Signal Transduction Knowledge Environment*, 114, PE2.
47. Kulinsky, V. I. (1997) *Usp. Biol. Khim.*, **37**, 171-209.
48. Elliot, W. H., and Elliot, D. C. (2005) *Biochemistry and Molecular Biology*, 3rd Edn., Oxford University Press.
49. Amieux, P. S., and McKnight, G. S. (2002) *Ann. N. Y. Acad. Sci.*, **968**, 75-95.
50. Kaupp, U. B., and Seifert, R. (2002) *Physiol. Rev.*, **82**, 769-824.
51. Dremier, S., Kopperud, R., Dosceland, S. O., Dumont, J. E., and Saenhaut, M. (2003) *FEBS Lett.*, **546**, 73-80.
52. Kopperud, R., Krakstad, C., Selheim, F., and Dosceland, S. O. (2003) *FEBS Lett.*, **546**, 121-126.
53. Ascemi, P., Visca, P., Ippolito, G., Spallarosa, A., Bolognosi, M., and Montecucco, C. (2002) *FEBS Lett.*, **531**, 384-388.
54. Torphi, Th. J., and Page, C. (2000) *Trends Pharmacol. Sci.*, **21**, 157-159.
55. Houslay, M. D., and Adams, D. R. (2003) *Biochem. J.*, **370**, 1-18.
56. Cho-Chung, Y. S., Nesterova, M., Becker, K. G., Srivastava, R., Park, Y. G., Lee, Y. N., Cho, Y. S., Kim, M. K., and Neary, C. (2002) *Ann. N. Y. Acad. Sci.*, **468**, 22-36.
57. Tkachuk, V. A. (1998) *Biochemistry (Moscow)*, **63**, 38-46.
58. Pendaries, C., Trochere, H., Plantavid, M., and Payrastre, B. (2003) *FEBS Lett.*, **546**, 25-31.
59. Hammond, G., Thomas, C. L., and Schiavo, G. (2004) *Curr. Top. Microbiol. Immunol.*, **282**, 177-206.
60. Lee, H. C. (2000) *Science Signal Transduction Knowledge Environment*, 40, PE1.
61. Petersen, O. H., and Cancela, J. N. (1999) *Trends Neurosci.*, **22**, 488-494.
62. Barbara, J. G. (2002) *Biochim. Biophys. Acta*, **1600**, 12-18.
63. Soderling, Th. R. (1999) *Trends Biochem. Sci.*, **24**, 232-236.
64. Lisman, J., Schulman, H., and Cline, H. (2002) *Nature Rev. Neurosci.*, **3**, 178-190.
65. Sugiura, R., Siu, S. O., Shunton, H., and Kuno, T. (2001) *Cell Mol. Life Sci.*, **58**, 278-283.
66. Shibasaki, F., Hallin, U., and Uchino, H. (2002) *J. Biochem. (Tokyo)*, **131**, 1-15.
67. Berridge, M. J., Bootman, M. D., and Lipp, P. (1998) *Nature*, **395**, 645-648.
68. Shohat, G., Shani, G., Elzanstein, M., and Kimchi, A. (2002) *Biochim. Biophys. Acta*, **1600**, 45-50.
69. Andrejak, M., Gayet, J. L., and Ambrosi, P. (2002) *Arch. Mol. Coeur. Vaiss.*, **96**, Spec. No. 1, 44-50.
70. Laher, I., and Zhang, H. (2001) *J. Cerebr. Blood Flow Metab.*, **21**, 887-906.
71. Mubagwa, K., and Flameng, W. (2001) *Cardiovasc. Res.*, **52**, 25-39.
72. Williams, J. A., Sans, M. D., Tashiro, M., Schafer, C., Bragado, H. J., and Dabrowski, A. (2002) *Pharmacol. Toxicol.*, **91**, 297-303.
73. Roychowdhury, D., and Lahn, M. (2003) *Semin. Oncol.*, **30**, No. 2, Suppl. 3, 30-33.
74. Hilgemann, D. W., Feng, S., and Nasuhoglu, C. (2001) *Science Signal Transduction Knowledge Environment*, 11, RE 19.
75. Zefirov, A. L., and Sitdikova, G. F. (2002) *Usp. Fiziol. Nauk*, **33**, 3-33.
76. Wong, H., and Storm, D. R. (2003) *Mol. Pharmacol.*, **63**, 463-468.

77. Waltereit, R., and Weller, M. (2003) *Mol. Neurobiol.*, **27**, 99-106.
78. Tkachuk, V. A. (1998) *Ross. Fiziol. Zh. im. I. M. Sechenova*, **84**, 1006-1014.
79. Brown, E. M. (2000) *Rev. Endocrinol. Metab. Disord.*, **1**, 307-315.
80. Hofer, A. M., and Brown, E. M. (2003) *Nature Rev. Mol. Cell. Biol.*, **4**, 530-538.
81. Bankir, L., Ahloulay, M., Devreotes, P. N., and Parent, C. A. (2002) *Am. J. Physiol.*, **282**, F376-F392.
82. Michels, P., and Tarnow, J. (2001) *Anesthesiol. Intensivmed. Noftallmed. Schmerzther*, **36**, 406-416.
83. Beltowski, J. J. (2001) *J. Physiol. Pharmacol.*, **52**, 351-375.
84. Shailubhai, K. (2002) *Curr. Opin. Drug Discov. Dev.*, **5**, 261-268.
85. Turpaev, K. T. (1998) *Mol. Biol. (Moscow)*, **32**, 581-591.
86. Lucas, K. A., Pitary, G. M., Kazerounian, Sh., Ruiz-Stewart, I., Park, J., Schulz, S., Chepenik, K. P., and Waldman, S. A. (2000) *Pharmacol. Rev.*, **52**, 375-414.
87. Okhotin, V. E., Kalinichenko, S. G., and Dudina, Yu. V. (2002) *Usp. Fiziol. Nauk*, **33**, 41-55.
88. Chen, Y. H., Yet, S. F., and Perrella, M. A. (2003) *Exp. Biol. Med. (Maywood)*, **228**, 447-453.
89. Schlossmann, J., Feil, R., and Hoffman, F. (2003) *Annals Med.*, **35**, 21-27.
90. Pilz, R. B., and Casteel, D. E. (2003) *Circ. Res.*, **93**, 1034-1046.
91. Estevez, A. G., and Gordan, G. (2002) *Ann. N. Y. Acad. Sci.*, **962**, 207-211.
92. Hogg, N. (2002) *Ann. Rev. Pharmacol. Toxicol.*, **42**, 585-600.
93. Behrendt, D., and Glanz, P. (2002) *Am. J. Cardiol.*, **90**, 40L-48L.
94. Deguchi, A., Boh, J. W., Pamucou, R., Thompson, W. J., and Weinstein, I. B. (2002) *Mol. Cancer Ther.*, **1**, 803-809.