GeneNet-BASED MODEL OF TWO-STAGE ALDOSTERONE EFFECT ON PRINCIPAL CELLS OF CORTICAL COLLECTING DUCTS

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Resume

Motivation:
GeneNet-based accumulation and visualization of data and their application for describing gene networks of molecular genetic mechanisms underlying regulation of physiological functions is an important background for further theoretical analysis of these data. Study of the gene networks mediating the effect of hormones on target cells is of special interest.

Results:
We have systematized the available experimental data on immediate (nongenomic) and slow (genomic) patterns of aldosterone effect on the target cells and we are suggesting a model of two-stage aldosterone effect on the Na⁺,K⁺-ATPase function in the principal cells of rat kidney cortical collecting ducts (CCD). A GeneNet-based formalization and representation of these data as a model allowed us to reconstruct the gene network comprising both the classic (genomic) mechanism of aldosterone action in cortical collecting ducts and immediate (nongenomic) realization of aldosterone function.

Availability:
The system GeneNet is Internet-accessible at http://wwmgs.bionet.nsc.ru/systems/mgl/genenet/. The diagram of the model described is titled Principle Cell of CCD.

Introduction
The computer technology GeneNet, developed at the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences, accumulate and visualize the data on molecular genetic mechanisms underlying the regulation of organism physiological functions [Kolpakov,F.A. et al., 1998]. The format of GeneNet provides for formalized accumulation of the information on major components of gene networks—objects (genes, proteins, low-molecular-weight substances, inducers, etc.); their interactions and interrelations; and organs, cells, and cell compartments wherein these objects are located and their interactions are realized. Java viewer allows the entire GeneNet-filed graphical and textual information on the objects within a diagram and their interactions to be accessed. Current release of GeneNet contains a number of gene networks [Kolchanov,N.A. et al., 2000]. Analysis of the GeneNet information and the available published data allow several major types of gene networks to be separated: (a) gene networks providing for cyclic processes, e.g. cell cycle, heart muscle constriction cycle, etc.; (b) providing for cell growth and differentiation, tissue and organ morphogeneses, organism growth and development (erythropoiesis, seed germination, etc.); (c) providing for homeostasis of organism biochemical and physiological parameters; and (d) providing for organism reactions to changes of the environment (heat shock, antiviral response, etc.).

The goal of this work was to develop a GeneNet-based model of gene network providing for the effect of hormone in its target cells. The molecular mechanism of aldosterone effect on Na⁺,K⁺-ATPase of kidney cortical collecting duct cells was used as the object.

Brief characteristics of biological objects
Na⁺,K⁺-ATPase, or the so-called sodium pump, is present in all the eukaryotic cells. It is a membrane protein consisted of α- and β-subunits, assembled at a 1 : 1 ratio to form a stable heterodimer. This enzyme is capable of drawing three Na⁺ ions out and bringing two K⁺ ions into the cell against their gradients per each ATP molecule used [Skou J.Ch. and Esmann M., 1992]. It is involved in forming transmembrane potential; controlling cell volume; and regulating Na⁺-dependent transport of protons and other ions, sugars, and amino acids. Hormones and neurotransmitters regulate the activities of Na⁺,K⁺-ATPase in different tissues [Ewart H.S. and Klip A., 1995].
Principal cells of kidney cortical collecting ducts (CCD) of higher animals are the main targets of aldosterone, thus providing for a hormone-dependent regulation of sodium reabsorption.

Aldosterone is the key steroid hormone regulating the activity of Na⁺,K⁺-ATPase in the principal cells of cortical collecting ducts.

Reconstruction of gene network

The data on Na⁺,K⁺-ATPase activity in principal CCD cells are dispersed in numerous publications, each describing results of studying certain aspects or stages of this regulation. To integrate the experimental information on mechanisms of aldosterone effect on CCD cells, we have reconstructed the gene network using the system GeneNet. The information was inputted into GeneNet via the Internet using the interactive data input system [Kolpakov F.A. and Ananko E.A., 1999]. Each component of the gene network has its own image reflecting its specific features (Fig. 1a). For example, a monomeric protein is represented by a circle; homodimeric protein, by twin oval, etc. The overall diagram was constructed by uniting the available data on elementary molecular events and processes from the relevant papers. Graphic representations of elementary events and processes are exemplified in Fig 1 (b-f).

Gene network of two-stage aldosterone effect on cortical collecting duct cells

The model with the GeneNet database comprises two mechanisms of aldosterone effect on Na⁺,K⁺-ATPase on principal CCD cells of animal kidneys (Fig. 2). First is a classic genomic mechanism, the so-called long pattern (Long in Fig. 2). It requires hours and days for manifesting the aldosterone effect on the sodium pump [Verrey,F. et al., 1996]. Intracellular transduction of the hormone signal commences from aldosterone–mineralocorticoid receptor (MR) interaction [Funder,J.W., 1992; Marver,D., 1992]. Once aldosterone binds to receptor, the complex formed is activated accompanied by dissociation of heat shock proteins (not shown in diagram). The activated hormone–receptor complex is translocated into the nucleus, interacts with binding sites of mineralocorticoid receptors located in the regulatory regions of the genes coding for α1- and β1-subunits of Na⁺,K⁺-ATPase (NAKA alpha 1 and NAKA beta 1 genes), and increases the syntheses of corresponding mRNAs (NAKA alpha 1 and NAKA beta 1 mRNAs) [Verrey et al., 1989; Logvinenko et al., 1991a; Logvinenko et al., 1991b; Farman et al., 1992]. This increases the number of new pumps localized into the basolateral membrane of principal cells (SodPump) and elevates consequently the sodium reabsorption and potassium secretion. Thus, this part of the model describes production of new additional Na⁺,K⁺-ATPase molecules and represents the traditional area of aldosterone action.

The nongenomic pattern of aldosterone action (Fast in Fig. 2) is represented by immediate aldosterone effect on Na⁺,K⁺-ATPase and mediated by secondary intermediates [Wehling,M., 1995]. This action takes minutes and seconds for its manifestation. Aldosterone binds to the membrane receptor (MRm), thereby stimulating phospholipase C activity (PLCi, inactive form; PLC, active form). Phospholipase C hydrolyzes membrane phosphoinositides (Inositol Phospholipoid) to form two secondary mediators—inositol triphosphate (IP3), increasing intracellular Ca²⁺ content, and diacylglycerol [Schneider.M. et al., 1997]. Diacylglycerol and Ca²⁺ activate Ca²⁺-dependent protein kinase C (PKC_i, inactive form; PKC_a, active form) and switch on the cascade of phosphorylation of pump preexisting molecules [Bertorello,A.M. et al., 1991; Beguin P. et al., 1994]. Protein kinase C activation (PKC_a) leads to phosphorylation in serine at position 23 of the N-terminal part of Na⁺,K⁺-ATPase α1-subunit and to a inhibition of the pump hydrolytic activity under in vitro conditions (SodPump_1P_23) [Feschenko M.S. and Sweadner K.J., 1995, Logvinenko,N.S. et al., 1996]. This pattern of
Na⁺,K⁺-ATPase regulation may be modulated by a preliminary phosphorylation of serine at position 943 in α₁-subunit (the phosphorylated protein is designated as SodPump_1P_943) by cAMP-dependent protein kinase A [Belusa R. et al., 1997]. The preliminary phosphorylation of serine at position 943 by protein kinase A significantly attenuates the PKC phosphorylation effect and results in appearance of the additional phosphorylation sites located on the big intracellular loop of the α₁-subunit (this form of Na⁺,K⁺-ATPase, phosphorylated at several positions, is designated SodPump_>2P). Note that the inverse phosphorylation order fails to exhibit such attenuation effect. The functional effect of kinase-dependent Na⁺,K⁺-ATPase phosphorylation depends linearly on the intracellular concentration of Ca²⁺ ions [Cheng,S.X.J. et al., 1999] (not shown on diagram). PKA and PKC stimulations at low Ca²⁺ concentration (125 nM) decrease the sodium pump activity, whereas at high concentration (450 nM) phosphorylation results in the increase of the activity.

Conclusion

The new gene network with the GeneNet database characterized describes the mechanisms underlying realization of the hormone effect on its target cell. Descriptions of both the classic genomic and nongenomic patterns of aldosterone effect are the specific feature of the developed model of Na⁺,K⁺-ATPase activity regulation in principal CCD cells. Of interest is the fact that cell membrane–located proteins—aldosterone membrane receptor and phospholipase C—play the key role in realization of the immediate, nongenomic regulation pattern. According to the model, aldosterone can act through both routes of intracellular hormonal signal transduction simultaneously; interaction of these two mechanisms might provide for a wide range of cellular reactions. Insight into these processes is of considerable value from physiological standpoint, as it clarifies a variety of controversial data on intracellular aldosterone effects. Computer-based representation of the model makes it open to further development on acquisition of new experimental data. In future, we believe extremely purposeful to systematize the data on intercrossing of aldosterone effect realization routes with those

Figure 2. Graphical representation of information from the section "Principal cell of CCD" of the GeneNet database.
of other hormones (in particular, vasopressin) of CCD cells in the framework of this model. In addition to the advantages noted, this GeneNet-based computer model, to the knowledge of authors, is the first computer image of the molecular mechanisms underlying aldosterone effect transduction in the target cell.

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References