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REVIEWS

Evolution of Mechanisms of Ca^{2+}-Signalization. Role of Ca^{2+} in Regulation of Specialized Cell Functions

I. V. Shemarova and V. P. Nesterov

Sechenov Institute of Evolutionary Physiology and Biochemistry, Russian Academy of Sciences, St. Petersburg, Russia
E-mail: shem@iephb.ru

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Abstract—The review describes peculiarities of Ca^{2+}-signalization in electro-excitable cells of higher eukaryotes. The light has been shed on problems of Ca^{2+}-dependent mechanisms of regulation of muscle contractility and of neuronal synaptic plasticity in the higher vertebrate animals. A particular attention has been paid to analysis of contribution of such poorly studied components of Ca^{2+}-signalization as non-selective TRPC-channels, Orai channels, sensory STIM1 proteins, Ca^{2+}-controlled K^+-channels of large and small conductance, and neuronal Ca^{2+}-sensors (NCS).

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INTRODUCTION

The present work continues a series of our articles focused on examination of mechanisms and major stages of evolutionary development of intracellular Ca^{2+} signalization. Earlier, we showed that the main structural elements and functional blocks of the Ca^{2+} signaling system existed in lower eukaryotes. In unicellular eukaryotes it fulfills not only the function of regulation of cell homeostasis like in prokaryotes, but also provides control for the general biological functions, such as growth, cytodifferentiation, and apoptosis [1, 2].

Specialization of cells in multicellular organisms has led to the appearance of distinct mechanisms of intracellular regulation allowing them to provide synchronization and performance of their specific functions. Electro-excitable cells acquired populations of unspecific ion channels activated by the time-dependent alterations of potential, the channels of large and small conductance activated by Ca^{2+}, and widely distributed became mechanisms of regulation of potential-controlled channels with participation of signaling systems dependent on intracellular Ca^{2+} concentration $[Ca^{2+}]i$.

In this work we will examine the poorly studied Ca^{2+}-dependent mechanisms of regulation of muscle contraction and neuronal synaptic plasticity in the higher vertebrate animals.

ROLE OF CA^{2+} IN MUSCLE CONTRACTILITY

The fundamental role of Ca^{2+} in the process of muscle contraction was established in the second half of the XIX century by Sydney Ringer in experiments on frog heart. These works proved for the first time that the heart loses contractile capacity in the absence of Ca^{2+} [3]. Since then, numerous investigations have been performed with emphasis on the role of Ca^{2+} in regulation of heart muscle func-

tioning. The majority of works focused on determination of the place of Ca^{2+} in the system of electro-mechanical coupling of muscle cells. The data obtained in these works have revealed the specificity of functional role of extra- and intracellular calcium ions in muscles. The extracellular Ca^{2+} was shown to be important for induction of contraction, while intracellular Ca^{2+}—predominantly for its realization. Transmembrane transport of extracellular Ca^{2+} inside myocytes plays the most important role in induction of contraction of myocardium and smooth muscles occurring by the principle of "Ca^{2+}-induced Ca^{2+}-release." This mechanism of triggering contractions is realized as a result of functional interaction of dihydropyridine receptors of plasma membrane (PM) with ryanodine receptors (RyR) of sarcoplasmic reticulum (SR). In skeletal muscles, dihydropyridine receptors and RyR directly interact with each other due to conformational alterations of PM induced by depolarization, thus providing a rapid electromechanical coupling and phase muscle contraction [4, 5].

The major elements of Ca^{2+}-regulated contraction processes in muscle cells are potential-operated Ca^{2+}-channels of L-type (Ca(v)1.2) providing input of extracellular Ca^{2+} functionally coupled with RyR receptors to inositol-1,4,5-triphosphate (IP_3R) whose activation stimulates the release of intracellular Ca^{2+} from the stores, intracellular Ca^{2+} sensors of the calmodulin (CaM) type, Ca^{2+} ions themselves directly involved in contraction, and clusters of signaling proteins—"plasmerosomes" including Na^+–K^+-ATPases and Na^+–Ca^{2+}-exchangers localized in sites of tight junction of PM and SR and responsible for changes in local $[Ca^{2+}]_i$ [6].

The synchronization and successful functioning of these components of Ca^{2+}-signalization in turn is under control of numerous modulating proteins interacting with their targets at the distinct stages of transduction of triggering signal. In this part of review we consider important to examine the contribution to the regulation of contraction of such poorly studied components of Ca^{2+}-signalization as the non-selective TRPC-channels, Orai channels, sensor proteins STIM1, Ca^{2+}-dependent K^+-channels of large and small conductivity, as well as proteins belonging to the family of neuronal Ca^{2+}-sensors (NCS).

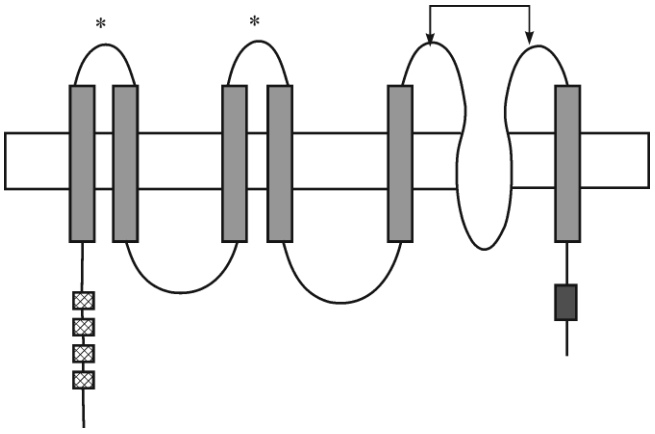

Fig. 1. Scheme of structure of TRPC-channels. Sensor TRPC domain is presented as black rectangle, ankyrin repeats—as patterned rectangles, transmembrane segments of TM-domain—as gray rectangles, tentative pore-forming area is indicated with arrows. *Asterisks* show the sites of glycosylation. Channels TRPC3 have one such site, TRPC6—two sites.

NON-SELECTIVE TRPC-CHANNELS

The family of classical, or canonical, TRP cation channels (TRPC) is composed of proteins closely related to the proteins of transient receptor potential of Drosophila implicated in photoreception [7]. This family is composed of seven subfamilies (TRPC1–TRPC7), of which the proteins of TRPS1 and TRPC2 subfamilies have almost unique structure, while TRPC4 and TRPC5 are identical by 65%. TRPC3, -6 and -7 form a subfamily whose members have 70–89% of identity by amino acid residues. The peculiarity of all TRPC proteins is their capacity to be activated by diacylglycerol (DAG), the product of degradation of phosphatidyl-4,5-biphosphate or inositol-1,4,5-triphosphate (IP_3). The common structural features of these channels are: four N-terminal ankyrin repeats, six short transmembrane (TM) domains and the existence of the pore-forming site located between transmembrane domains 5 and 6 (Fig. 1).

Proteins of the TRPC family (with exception of TRPC2) can form the functional homo- and heterodimers (TRPC1/4/5 and TRPC3/6/7) [8]. The aggregation of TRPC1 with TRPC4 or -5 alongside with TRPC3, -6 or -7 in tetramer complexes with representatives of different TRPC subfamilies were identified in cardiomyocytes (CM) and

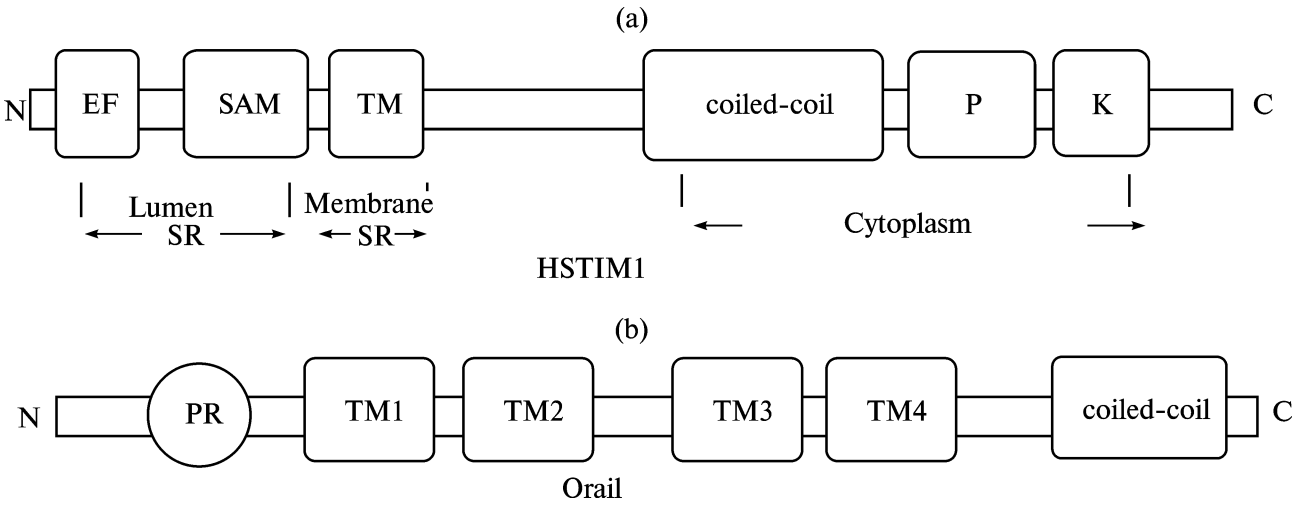

Fig. 2. Structural peculiarities of the proteins HSTIM1 (a) and Orai1 (b). Rectangles include functional domains of the proteins: EF—Ca^{2+}-binding motif of "EF-hand"; SAM—the so called "sterile"-α-motif; TM—transmembrane domain; coiled-coil—the "coiled coil"-domain; P—domain enriched with serines and prolines; K—domain enriched with lysines; PR—domain enriched in prolines and arginines; TM1–4—transmembrane domains; N and C—NH_2 and COOH-terminal parts of molecule, respectively; SR—sarcoplasmic reticulum.

smooth muscle cells (SMC) [9, 10]. The functional significance of heteromeric TRPC complexes is they provide the specificity and narrow direction of Ca^{2+} signal, since each such complex interacts with "their own" variant of Ca^{2+}-channels [11].

All TRPC proteins depend on the receptors coupled with phospholipase C (PLC), as they directly or indirectly are activated by the phospholipid products generated due to activation of this enzyme and induction of hydrolysis of membrane phospholipids. However, the mechanisms of activation of the channels of the TRPC3, -6 and -7 subfamily differ from the mechanisms of activation of the TRPC1, -2 and -5 subfamily. The channels of the first group are established to be activated by DAG in response to physiological stimuli associated with depletion of Ca^{2+}-depot, and are responsible for receptor-operated Ca^{2+} entry (ROCE) [12, 13]. They form non-selective ROCE-cation cannels and participate in the receptor-mediated mechanism of modulation of the strength of muscle contractions. At the same time, the TRPC1, -2 and -5 channels are activated by the binding of IP_3 with corresponding receptors and are responsible for store-operated Ca^{2+} entry (SOCE). In this case an interaction of TRPC with Orai proteins and STIM1 is observed, what apparently underlies the formation of store-operated Ca^{2+}-channel (SOC), which functional peculiarity is an ability to be activated upon depletion of Ca^{2+}-store by Ca^{2+}-chelators or inhibitor of Ca^{2+}-pumps [14]. There is still no a clear conception how the TRPC1, -2 and -5 function as the modulators of muscle contraction, but presently one can convincingly say that these proteins represent only a part of SOCE mechanism, for which two variants of channel-forming proteins, STIM1 and Orai, serve as obligatory components.

INTRACELLULAR CA^{2+}-SENSOR STIM1

For long time, the question how the coupling between Ca^{2+} entrance and intracellular reservoirs can occur remained completely unclear. The mechanism of Ca^{2+} transport across plasma membrane (PM) induced by depletion of Ca^{2+}-depot was also unknown. Recently, using the method of RNAi-screening, it was shown that the depletion of Ca^{2+} in SR is initially perceived by specialized proteins STIM1, which serves as connecting link between SR and PM [15]. In response to decreased Ca^{2+} concentration in SR these proteins form homo-oligomers and translocate to the sites of SR–PM contacts, where co-localized with calcium ATPase—SERCA, IP_3R and membrane proteins forming SOC pore [15]. However, it is

still unclear how such close protein localization is occurred.

STIM1 proteins contain the specific sequences, located in the transmembrane and cytoplasmic domains responsible for module inter-protein interactions, as well as two Ca^{2+}-binding motifs of "EF-hand" (Fig. 2a) looking into SR lumen. Those Ca^{2+}-binding sites are the primary detectors of Ca^{2+} level. It is interesting to note that the link of Ca^{2+} with "EF-hand" motif is unstable, what is confirmed by low (few hundreds μM order) Kd of Ca^{2+}-binding complex EF-SAM, isolated from STIM1 protein, and its stoichiometry 1 : 1 [16].

Low affinity of Ca^{2+} to "EF-hand" motif is in accordance with the idea that binding of Ca^{2+} occurs when SR-stores are filled, whereas its release, initiating the processes leading to activation of selective capacity channels CRAC (Ca^{2+} release-activated Ca^{2+})—upon SR depletion [17].

ORAI CHANNELS

Orai channels were initially identified as the pore-forming units of capacitative channels in T-cells of patients with various forms of immune deficit, but lately in electro-excitable cells including myocytes [18]. Orai proteins (Orai1-3) have four conservative transmembrane domains and intracellular C- and N-terminals. Orai1 also contains the domain enriched with prolines, the site of glycosylation located between TM2- and TM3-segments, as well as super-helical "coiled-coil"-domain responsible for interaction with STIM1 protein, formation of ORAI1-STIM1 complex and activation of capacity channels CRAC (Fig. 2b) [19].

It was established that in inactive state CRAC channels existed in the form of dimers or tetramers [20], while in active one—in the form of functional tetramer of four Orai1 subunits [21]. Such shape of complex provides formation of selective pore for Ca^{2+}. It was shown that the main role in the maintenance of selective permeability for Ca^{2+} through CRAC channels was played by conservative glutamate residues in the transmembrane segments TM1 (E106) and TM3 (E190).

Orai1 channels not only can aggregate into multimeric complexes for formation of pore-forming CRAC units, but also interact with TRPC proteins for formation of less selective for Ca^{2+} store-operated channels SOC [22]. It was observed that co-expression of these proteins increased 8–100 times input of extracellular Ca^{2+} [17, 23]. Orai1 protein physically interacts with N- and C-terminals of TRPC3 and TRPC6, thereby activating their channel function. Thus, for example, it was observed that Orai1, -2 and -3 increased the thapsigargin-induced Ca^{2+}-influx by 50–150% in the cells stably over-expressing TRPC3 or TRPC6 [22]. The authors suggest that Orai proteins, by aggregation with TRPC, act as regulatory subunits of SOC channels and enhance their sensitivity to decreased Ca^{2+} concentration in Ca^{2+}-store.

At present the precise mechanisms of interaction of TRPC and Orai proteins have not been established, but there are quite a few data that formation of Ca^{2+}-channel mediating the store-operated Ca^{2+} input in the cell has a complex character and includes not only direct interaction of these proteins, but also their aggregation with IP_3R, as well as with receptor-operated proteins RACK1 (receptor for activated C-kinase-1) and STIM1 [15, 24, 25]. The formed signaling complex can be involved both in the agonist-induced release of Ca^{2+} from depot and in mechanism of its input across PM (Fig. 3).

ROLE OF SINGLE REPRESENTATIVES OF THE FAMILY OF CANONICAL TRPC IN REGULATION OF CONTRACTION OF MUSCLES OF DIFFERENT TYPES

In the smooth muscle cells, five out of seven known subtypes of TRPC channels were identified: TRPC1, TRPC3, TRPC4, TRPC5 and TRPC6, with the predominance of TRPC1 and TRPC3 [26, 27, 28]. The main attention in investigations of these channel proteins was paid to their involvement in regulation of the resting potential and $[Ca^{2+}]_i$ [27]. Using the technique of blockage of the functional protein units with monoclonal antibodies, it was established that TRPC3 were important molecular components of non-selective cation channels and have a great contribution to the maintenance of resting membrane potential (MP) and regulation of calcium homeostasis in SMC. In sensitized myocytes, these proteins, alongside with

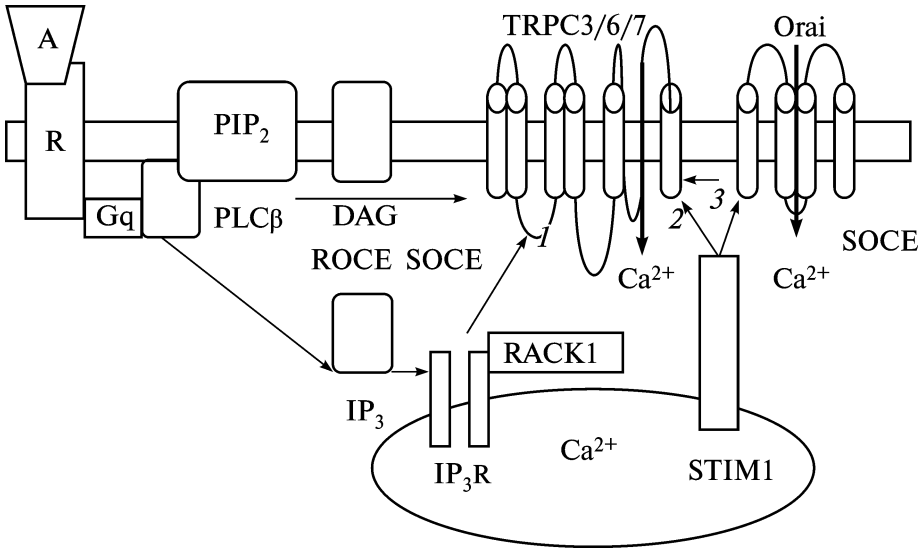

Fig. 3. Tentative regulatory mechanism of TRPC3/6/7 activation. Three possible variants of regulation of the store-operated Ca^{2+} entry with involvement of TRPC proteins are indicated with the numbers: (*1*) Conformational binding of activated IP_3 receptors with the channels protein; (*2*) interaction of STIM1 in SR with TRP-channels, and (*3*) interaction of the store-operated Orai channels with TRP-channels in heteromeric complexes. A—Agonist, R—receptor, PLCβ—G protein (Gq)-activated phospholipase C (isoform β), PIP_2—phosphatidylinositol-4,5-biphosphate, IP_3—inositol-1,4,5-triphosphate, IP_3R—receptor to inositol-1,4,5-triphosphate, DAG—diacylglycerol, TRPC3/6/7—channel proteins of corresponding types (-3, -6, -7), RACK1—receptor for activated kinase C1, ROCE—receptor-operated Ca^{2+} entry, SOCE—store-operated Ca^{2+} entry.

TRPC1, provide the secondary influx of extracellular Ca^{2+} and participate in the mechanism of development of hypersensitivity to allergens [27].

In the plasma membrane of arterial smooth muscle cells, predominant are the TRPC6 channels that are responsible for alterations in MP, activation of receptors and depletion of Ca^{2+}-store [26]. The majority of them do not exhibit selectivity in respect to actions, and, as it is typical for channels of this group, are activated by DAG generated due to the receptor-induced PLC activation. Excitation of receptors leads to input of potential-generating cations, including Na^+, through the TRPC6 channels, depolarization of PM and subsequent activation of slow Ca(v)-channels. Such coupling can play a certain role in modulation of tonus of blood vessels [26]. The similar intracellular processes also induce opening in SMC of TRPC3 channels activated by the binding of IP_3R with agonist [27].

To date, numerous data on existence of canonical TRPC channels in different vessels of blood stream have been accumulated, but little is known on the role of these channels in the mechanism of store-operated Ca^{2+} influx and induction of CRAC current via eponymous channels [28]. Nevertheless, by using inhibitory antibodies and the method of delivery of small interfering RNA (siRNA), the proofs were obtained that TRPC channels were composing elements of SOC in myocytes of aorta, cerebral and mesenteric arteries, coronary artery, as well as vena cava [28, 29]. TRPC1 and TRPC5 were found to be co-localized and associated with each other in the SMC of rabbit pial arterioles [30], and antibodies against these proteins inhibit both CRAC current in myocytes of mesenteric artery [29]. The obtained data allow an assumption that in smooth muscle cells TRPC1 and TRPC5 may form hetero-tetrameric complexes functioning as SOC subunits. In SMC of rabbit coronary artery, such complexes form the channels TRPC1, TRPC5, and TRPC6, whereas in the vena cava—the channels TRPC1, TRPC5, and TRPC7 [29]. However, in spite of value of obtained experimental data, precise identification of molecular markers of the endogenous SOC requires the comparison of hetero-tetrameric structures, revealed in expressing systems, with the native SOCs. To obtain comparative results is a goal of the near future.

In cardiomyocytes, several subtypes of TRPC channels, expressed differently depending on age and heart loading, were identified [31]. Thus, for example, in rats the content of TRPC4 and TRPC5 in cardiomyocytes increases by the 10-week age. It is interesting that expression of these channels in adult animals correlates with elevation of blood pressure, which might indicate their important role at the initial period of hypertension and participation in development of ventricular systolic dysfunction [31].

In adult rat ventricular cardiomyocytes, mRNA of the TRPC1, -3, -4, and -7 are expressed [10]. Their induction is associated with the rise of production of purine nucleotides ATP and UTP and opening of non-selective cation channels. Basal activity of these channels is low, but ATP considerably increases their permeability to divalent cations after an increase in the functional loading of heart. From the medical point of view, the obtained results are important for understanding pathogenesis of the heart diseases whose development is associated with hyper-production of purine nucleotides and participation of compensatory Ca^{2+}-dependent mechanisms in regulation of myocardium contractility [10].

If the mechanisms of activation of canonical TRPC channels at present are relatively well studied, the mechanisms of their inactivation are still a "black box". Only recently the data have appeared that the natural inhibitor of TRPC in SMC is nitrogen oxide (NO) that performs its action via the PKG-dependent mechanism. This is indicated by two facts. First, the TRPC activity induced by 8-bromo-cGMP, a PM-permeable cGMP analog, is blocked by nitrogen oxide. Second, the activating action of 8-bromo-cGMP is suppressed by compound KT5823, an inhibitor of PKG. Application of electrophysiological methods also showed that NO contributed to vasorelaxation by suppressing the non-selective La^{3+}-sensitive channels coupled with TRPC1 and TRPC3 [32].

In the skeletal muscles the activity of TRPC1, TRPC3 and TRPC4 is found [33, 34], among which the most representative is the population of TRPC1 [33]. The nature and properties of these channels in skeletal myocytes have been studied poorly. Apparently, like analogous channels in cells of other types, TRPC1 are located in PM, while their activity has the PLC-dependent character. It was suggested that in the skeletal muscles the TRPC1 channels are also implicated in the plasmalemmal Ca^{2+} entry and are stimulated by depletion of Ca^{2+}-store or muscle stretching. Indeed, using patch-clump, it was shown that TRPC1 channels were involved in the mechanism of influx of exogenous Ca^{2+} [33]. However, Ca^{2+} entry through these channels represents only a minor part of the total Ca^{2+} amount entering myofibrils via PM. It was observed that the absence of TRPC1-channels in TRPC1(-/-)-mice had no effect on the value of Ca^{2+} spikes appearing in the process of single isometric contraction [33]. However, in comparison with control, the muscles of mice knock-out by TRPC1, contain less myofibril proteins and develop lesser muscle strength per area unit. These results obtained on isolated muscles agree well with the data of in vivo experiments, in which an enhanced fatigue of TRPC1(-/-) mice is revealed as compared with control. Based on the obtained data, the authors conclude that TRPC1 channels play a modulating role in the mechanism of Ca^{2+} input during repeating contractions and thus help the muscles to develop the strength necessary for realization of isomeric contractions.

On the other hand, by using the methods of immune staining and protein hybridization, it was established that TRPC1-channels in the skeletal myofibrils were the channels of "passive leakage" of Ca^{2+} from SR and participate, above all, in formation of resting potential [35]. This is indicated by the higher $[Ca^{2+}]_i$ level after application of cyclopiazonic acid (a blocker of Ca^{2+}-pump in SR), by the absence of alterations in MP and entry of divalent ions, as well as by the slower fall of $[Ca^{2+}]_i$ induced by 200-ms pulses of changing current in the cells expressing hybrid protein TRPC1-yellow fluorescent protein (YFP) in comparison with control or the TRPC1-YFP-negative cells [35].

CA^{2+}-DEPENDENT MECHANISMS OF REGULATION OF PERMEABILITY OF ELECTRO-EXCITABLE CELLS

It is well known that excitation process is based on PM depolarization, which results in alterations of its permeability to the main potential-forming ions and in the appearance of rapid action poten-

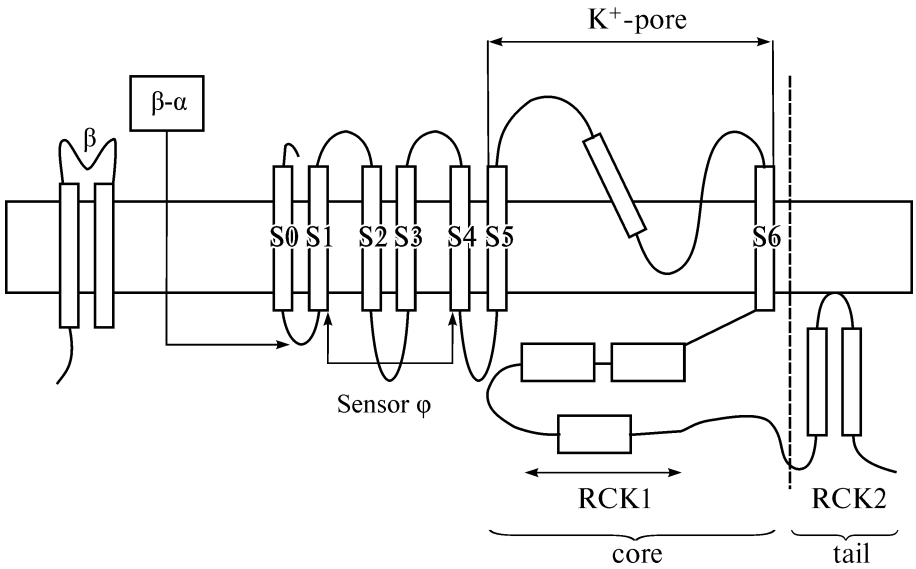

Fig. 4. Scheme of structure of BK-channels. β—β-subunit of BK-channel, S0–S6—transmembrane segments of transmembrane domain, sensor φ—site of transmembrane domain identifying shifts of MP, RCK1, and RCK2—calcium-binding domains being the regulators of potassium conductivity. RCK1 is included in the structure of central part of protein (core), RCK2—in the tail part, β–α—the site of coupling with α-subunit.

tial (AP) spreading to other excitable cells. The nature of distributed AP in excitable cells is first of all due to peculiarities of molecular organization of their PM and mechanisms of transduction of electric signals to the biochemical ones. In contrast to non-excitable cells, the membranes of myocytes and neurons have a series of molecular "instruments" providing this specialized function. Among such "instruments", it is to mention the following: Na^+–Ca^{2+}- and Na^+–K^+-exchangers, Ca^{2+}-uniporters, as well as RyR, which, alongside with Ca(v), are implicated in processes of exocytosis and endocytosis of synaptic vesicles [36, 37]. Studying their structure and specialized function is the subject of numerous works of Russian and foreign researchers. For the last few years, the major attention of investigators has been focused on analysis of mechanisms of thin direct and retrograde regulation of membrane permeability of electro-excitable cells with involvement of specialized components of intracellular Ca^{2+}-signalization and endo-cannabiods released upon increase in $[Ca^{2+}]_i$ in post-synaptic neurons [38]. Among them, less studies are Ca^{2+}-dependent K^+-channels of large and small conductivity (BK and SK), as well as the proteins related to the family of neuronal Ca^{2+}-sensors. We will consider in greater detail each of the aforementioned components.

BK-CHANNELS

BK-channels ($K_{ca}1.1$) are the channels of large conductivity (more than 200 pS). They are composed of regulatory α- and modulating -subunits, of which α-subunits are studied in the greater degree. The only gene *KCNMA1*, responsible for expression of BK-channels, encodes four pore-forming α-subunits, which has the extracellular N-terminal domain, seven TM segments (S0–S6), and large intracellular C-terminal site organized into sensitive to Ca^{2+} and other intracellular ligand domains RCK1 and RCK2 (regulator of conductance for K^+) (Fig. 4) [39].

Transmembrane domains of BK-channels S1–S6 are structurally and functionally homologous to those in other potential-operated K^+-channels (K(v)). They contain pore-forming segments S5–P–S6 and segment S4 being the sensor of potential [40]. Similarly to other K(v), BK-channels are tetramers [39], however, in contrast to classical channels composing the family of potential-operated K^+-channels, they contains not 6, but 7 transmembrane segments (S0–S6), of which S6 is connected to intracellular N-terminus (Fig. 4). Long C-terminal domain has four hydrophobic segments (S7–S10) and two adjacent sites (S0–S8 as "nucleus" and S9–S10 as "tail"), called

"Salkov's group" by the name of who was the first to describe them.

BK-channels were cloned in 1992 [41], which to the significant degree facilitates studying their molecular, electro-physiological characteristics, kinetic and pharmacological properties. These channels are supposed to be able to form clusters (plasmerosomes) in microdomains of membranes of endoplasmic reticulum and function as important modulating component of Ca^{2+} signalization in the cells of nervous system [42]. The mechanisms of activation of these channels have been elucidated only recently. It was found that this necessitated combination of two trigger factors—depolarization of PM and an increase in $[Ca^{2+}]_i$. The rise of cytosolic Ca^{2+} concentration is reached with involvement of IP_3R, which mediated effects of several growth factors, neurotransmitters, and hormones on the cell functions via generation of local Ca^{2+} spikes.

The currents appearing due to opening of BK-channels are the basis of post-hyperpolarization, at which the membrane potential for a short time becomes more negative than before the start of action potential. Controlling the value and duration of post-hyperpolarization, these channels play fundamental role in the regulation of AP activity patterns in the primary sensor neurons [43]. Moreover, activation of BK-channels, leading to increase of inflow Ca^{2+} current, prolongs repolarization phase of action potential, which is of significance for increased activity of presynaptic axons [44]. Thus, by the modulation (sculpting) of AP duration, as well as the level and duration of post-hyperpolarization the channels of large conductivity can to the considerable degree change excitation of sensor neurons.

In the heart muscle, AP is generated and distributed as a result of synchronous activation of Na^+-, Ca^{2+}-, and K^+-channels. Among them the key role in regulation of the heart cycle is played by various types of K^+ channels [45]. Until now, it was considered that BK-channels did not participate in regulation of functional activity of myocardium. Only recently the channels of high large have been established as important modulators of the heart contraction rhythm. The work of Imlach et al. [45] has shown that paxillin and lollitrem B, the compounds specifically inhibiting the activity of BK-channels, slow down the rhythm of heart contractions (HC) in mice of the wild type by 30% and 42%, respectively. In contrast, in knock-out (Kcnma1(-/-))-mice HC is not disturbed, which assumes involvement of BK-channels in mediation of the stimulating action of Ca^{2+} on excitation of heart muscle.

Analysis of mechanism of the BK-channel regulation in the vascular smooth muscles has shown that their activity is under control of processes of tyrosine phosphorylation [46]. Particularly, it was found that interaction of $\alpha5\beta1$-integrin with fibronectin induced an increase of K^+ influx occurring through K^+-channels of inner rectifier (KIR) and Ca^{2+}-activated channels, with the consequent PM hyperpolarization [47]. The revealing and clustering of $\alpha5\beta1$-integrin by using monoclonal antibodies resulted in a rapid, for a few minutes, increase in the amplitude of BK-current by approximately 30–50%. The current potentiation was blocked both by the compound IBTX, an inhibitor of BK-channels, and by the compound PP2, an inhibitor of c-Src-tyrosine kinases.

The confirmation of direct connection between activation of $\alpha5\beta1$-integrin on the surface of smooth muscle cells and potentiation of BK-channels is the reproducible result obtained on HEK293 cells expressing α-subunit of BK-channels [48]. It is to be noted that earlier the similar results were obtained by studying properties of BK-channels in arterioles of the rat skeletal muscle by using peptide RGD [49], which can indicate universality of mechanisms of modulation of BK-channels with peptide regulators. Overall, the results of these experiments prove an involvement of proteins of the extracellular matrix and processes of tyrosine phosphorylation in modulation of BK-channels, which can have significance for understanding of the complicated Ca^{2+}-dependent mechanisms of regulation of the tone of blood vessels.

SK-CHANNELS

SK-channels are found in all types of excitable cells. Subunits $K_{ca}2.1$–$K_{ca}2.3$, encoded by the genes *KCNN1*, *KCNN2*, and *KCNN3*, respectively, were the first to be isolated from brain cells and cloned by Kochler et al. in 1996 [56]. All these channel subpopulations are the channels of

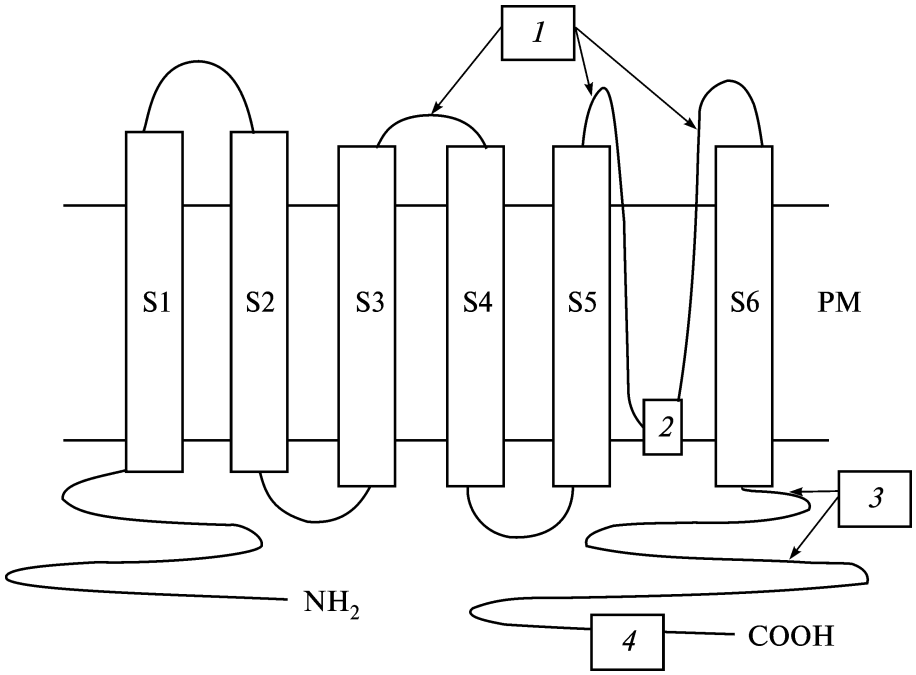

Fig. 5. Scheme of structure of SK-channels. S1–S6—transmembrane domains, NH_2–NH_2-terminal domain, COOH—COOH-terminal domain, (*1*) site of apamin binding, (*2*) P-loop, (*3*) CaM-binding domain, (*4*) site of PKA phosphorylation. PM—Plasma membrane.

low conductivity (2–20 pS) and structurally resemble other types of K^+-channels that have six transmembrane domains and helical P-site homologous to the pore area of potential-operated K^+-channels (S5 and S6), as well as to the C- and N-terminal domains (Fig. 5).

SK-channels do not depend on membrane potential, their activity is regulated exclusively by Ca^{2+} and mediated by calmodulin (CaM) constitutively bound to the C-terminal domain. Binding of CaM results in conformational alterations of SK-channels, induces their opening and K^+ entry [51]. The selective inhibitors of these channels are such natural and synthetic toxins as bee venom apamin, tubocurarine, quaternary salts of bicuculline, scillatoxin and some others [52]. It is interesting that different subtypes of SK-channels have different sensitivity to these inhibitors. In particular, the $K_{ca}2.2$ channels (IC_{50}0.03–0.14 nM) are the most sensitive to apamin, $K_{ca}2.3$ channels (IC_{50} 0.6–4 nM)—to the lesser degree, while the $K_{ca}2.1$ channels (IC_{50} 0.1–12 nM) are the least sensitive [45], which seems to be explained by type differences in structure of two sites of the apamin binding, which are located at the pore area between transmembrane segments S5 and S6 and in the extracellular area of transmembrane site between segments S3 and S4 (Fig. 5).

SK-channels are expressed in different tissues including brain, peripheral nervous system, heart, smooth and skeletal muscles [53, 54], being the components of Ca^{2+}-signalization and as the regulators of "reversible link" participate in control for formation of patterns of the nerve impulse transduction, synaptic plasticity, pacemaker activity, and excitability [51]. Moreover, these channels greatly contribute to development of all phases of post-hyperpolarization [51, 55]. In neurons of different brain sections, SK-channels induce M- and H-currents, which are the most important for development of medium, rapid after-hyperpolarization (mAHP) and terminal, slow after-hyperpolarization (sAHP) phases, modulating the behavior of excitation. The physiological meaning of this function of the small conductivity channels is protection of neurons from excessive loading induced by repeated AC.

In the heart myocytes, SK-channels were revealed and cloned in 2005 [53]. As neuronal subtypes of SK-channels, they show the functional

differences that can be explained by peculiarities of their molecular organization. Thus, for example, SK3 channels differ from closely related isoforms by the existence in NH_2-terminal domain of inserted sequence localized between additional poly-glutamine repeats. For SK1 channels to the greater degree than for other isoforms, typical are modifications in the COOH-terminal domain leading to alterations in the sixth transmembrane segment (S6) and the CaM-binding domain (CaMBD). The SK2 channels are apparently closer to the classical channels of low small conductivity. They do not have pronounced structural peculiarities, but the degree of their binding to apamin is higher than that of other isoforms [56], which might indicate the higher specificity of this protein in comparison with its other molecular forms. The quantitative analysis of SK1, SK2, and SK3 transcripts has shown that SK1- and SK2-channels are predominant in the atria, whereas in the ventricles the most representative is the population of SK3-channels. These differences are probably mediated by the functional specialization and differentiated contribution of the SK-channel isoforms to terminal phases of re-polarization. The contribution of the channels of low small conductivity in modulation of re-polarization is confirmed by their blockage by apamin, which results in distortion of excitation of membrane of atrial CM and development of arrhythmia [56]. The fact that the channels of small conductivity (SK2 isoform) are expressed in atria, but not in ventricles, is important, above all, from the therapeutic point of view, as it provides a unique possibility for the search for medical preparations (ligands of SK-channels) able to correct function of atrial CM, but not affecting the contractile function of atrial myocytes.

In smooth muscle cells, all three subtypes of SK-channels were revealed [57]. The subunits SK1, SK2, and SK3 were initially described in the proximal section of mouse intestine [57]. As in the heart, in intestine these isoforms are expressed differentially. The level of their expression in the colon is as follows: SK2 > SK3 > SK1, which also might be determined by unequal requirement of intestine myocytes for Ca^{2+}. Like in other types of excitable cells, in SMC the activity of SK-channels does not depend on extracellular Ca^{2+}, but is regulated by intracellular Ca^{2+} released from the stores due to interaction of purinergic receptors with ligand (ATP). In cells of stomach and intestine, ATP is released from inhibitory motoneurons performing the major inhibiting control for contractive function of myocytes of the digestive tract. In these cells, binding of receptors to ligand leads to an increase in $[Ca^{2+}]_i$ followed by differential activation of SK-channels. As a result, the membrane potential of postsynaptic neurons changes with development of membrane hyperpolarization. The duration of hyperpolarization in SMC can be shortened by application of apamin, which proves contribution of SK-channels to development of inhibitory postsynaptic potentials and induction of currents resulting in hyperpolarization [58]. The biological significance of this function of GMC SK-channels is regulation of excitability of myocyte membranes and providing the relaxation of muscle symplast after contraction.

In the skeletal muscles only $K_{ca}2.3$ of three subtypes of SK-channels are found to date [59]. They are expressed after denervation of muscle fibers, where are implicated in post-hyperpolarization and hyper-excitability of muscle membranes. In denervated muscle SK-channels are localized extra-synaptically, as well as on presynaptic membrane of nervous–muscle synapse. In the fibers of innervated skeletal muscles SK3-channels are not revealed, but exist in presynaptic membranes of motor nervous terminals, what also may indicate their important role in the regulation of synaptic transduction [59].

Investigation of cultivated myocytes of H9c2 line, originated from embryonic myoblasts of rat ventricles, established that additional depolarizing inflow calcium current, appearing due to PM depolarization, induced slow efflux K^+ current. It is observed that the stationary conductivity significantly increases upon administration of Ca^{2+} to the cell. The dependence of these currents on Ca^{2+} is indicated by their inactivation in calcium-free medium. The blockage of currents by apamin (IC50 = 6.2 nM) and d-tubocurarine (IC50 = 49.4 μM) confirms that these channels are SK-channels. The RT-PCR analysis of mRNA found that in the H9c2 cells the SK3 channels are expressed. Neither SK1 nor SK2 in the cultivated myocytes were revealed, which might serve a confirmation of the narrow type-specificity of

SK-channels and their presence in the skeletal myofibrils at least at the early stages of postnatal ontogenesis (in myoblasts their existence is not confirmed) [60].

Apart from the channel Ca^{2+}-dependent proteins, the Ca^{2+}-sensors able to react to alterations in $[Ca^{2+}]_i$ are involved in structure of signaling pathways modifying processes of excitation and muscle contraction. One of such sensors is, for example, CaM. Currently, the nature of this protein is established and its role in the regulation of specialized cellular functions is well studied. Considerably less is known on the structure and regulatory functions of recently identified sensor protein NCS-1, belonging to the family of neuronal calcium sensors. Presently, it is established that NCS-1 protein is an effective regulator of synaptic plasticity of contraction of heart muscle [60].

NEURONAL CA^{2+}-SENSOR

Neuronal Ca^{2+}-sensor, or neuronal Ca^{2+}-sensory protein 1 (neuronal Ca^{2+}-sensor protein-1 (NCS-1)) is a member of the superfamily of Ca^{2+}-binding proteins with structural resemblance with CaM (20% of identity). Its structure is composed of four calcium-binding motifs of EF-hand, of which only three are functional sites of Ca^{2+} binding [61]. The N-terminus of molecules contains the site of myristoylation that facilitates protein binding to cell membrane and allows the protein to function as a "molecular switch" [62].

NCS-1 is expressed in different brain parts, where it interacts with receptors and other regulatory proteins [61]. Mainly, interaction of NCS-1 with proteins-targets has the Ca^{2+}-dependent character, like, for example, with dopamine receptor D_2, but with some molecules, in particular, with receptor $InsP_3$, NCS-1 interacts indirectly, without direct involvement of Ca^{2+}. Modifications of the proteins induced by NCS-1 lead to changes in synaptic plasticity—long-term potentiation (LTP) and depression (long-term depression, LTD). The molecular mechanisms of these processes include different signaling pathways whose investigation is still fragmental. Thus, recent studies have shown that LTD mechanisms include at least two independent signaling pathways, one of which is initiated from NMDA receptors, while the second—from metabotropic glutamate receptor mGluR [63]. The resemblance of these pathways is based on their dependence on intracellular Ca^{2+}, the difference—on intracellular Ca^{2+}-sensors. In the first case, this is calmodulin, in the second—NCS-1. Different affinity of these Ca^{2+}-binding proteins to Ca^{2+} is apparently is the basis of specificity of LTD mechanisms [63]. In vivo mechanisms of LTD are a part of transcriptional response of neurons and is associated with LTP induction.

Investigations of the last years have shown that NCS-1 as additional subunit of Kv4-channels can also be expressed in the heart muscle [64]. It was found that in myocytes this protein was co-immunoprecipitated with K^+-channels and modulates their funnel function [65]. The ground of physiological action of NCS-1 on myocytes is its ability to change the amplitude of transient efflux K^+-current due to an increase in its density and a decrease in the rate of inactivation of Kv4-channels. Immunoblotting analysis has shown that NCS-1 is expressed in the heart ventricles of adult animals, with the level of expression of this protein being comparable with the level of its expression in different brain areas. However, in the heart of adult animals the content of NCS-1 is considerably lower than in the heart of embryos and newborns, which might indicate both an important role of NCS-1 in regulation of contractile function of underdeveloped myocardium and its possible involvement in regulation of growth and proliferation of cardiac myocytes in prenatal and neonatal periods of organism development [64].

The mechanisms of action of CNS-1 directly on the Ca^{2+}-channels are still not completely understood. It is supposed that NCS-1 differentially modulates α- and β-subunits of potential-operated Ca^{2+}-channels. This is indicated by the data of inhibitory analysis, according to which the amplitude of Ca^{2+}-currents in the presence of NCS-1 decreases in the cells expressing α-subunits of Cav1.2, Cav2.1, and Cav2.2-channels, and remains unaltered in the cells not expressing β-subunits or expressing only β3-subunits [66].

CONCLUSION

The course of progressive evolution of organisms

was accompanied by the development of specialized types of cells and, above all, of those of them whose functions are directed to the maintenance of fundamental cellular requirements—protection and rapid adaptive response to external stimuli. According to this, the role of Ca^{2+} signaling system has considerably increased. After the appearance of the nerve–muscle apparatus in the multicellular organisms, Ca^{2+} has become a participant of processes of excitation, transduction, and contractility.

In the higher eukaryotes, all Ca^{2+}-dependent mechanisms of regulation of specialized cell functions, originated as early as in the primitive Metazoa, got further development. The cells of nervous and muscle tissues, responsible for rapid transduction of triggering signal, have formed additional molecular structures that allowed modulating the strength of signal and the rate of its distribution. Such structures include the Ca^{2+}-dependent K^+-channels of large and small conductivity described in this work, the contact proteins providing the link between channels in SR and PM, the channels activated by shifts of membrane potential, as well as the proteins-sensors of extra- and intracellular Ca^{2+}. Currently, the structure of these components of Ca^{2+}-signalization have been described, although their functional peculiarities and coordinating links with other signaling proteins in specialized cells have not yet been studied sufficiently. It is to be noted that the majority of proteins examined in the work are targets of action of therapeutic preparations, therefore the study of their functional properties is the corner stone in solving medical aspects of the problem of Ca^{2+}-signalization in electro-excitable cells.

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