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REVIEW ARTICLE

Skeletal muscle as an endocrine organ: Role of $[Na^+]_i/[K^+]_i$-mediated excitation-transcription coupling

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Abstract During the last two decades numerous research teams demonstrated that skeletal muscles function as an exercise-dependent endocrine organ secreting dozens of myokines. Variety of physiological and pathophysiological implications of skeletal muscle myokines secretion has been described; however, upstream signals and sensing mechanisms underlying this phenomenon remain poorly understood. It is well documented that in skeletal muscles intensive exercise triggers dissipation of transmembrane gradient of monovalent cations caused by permanent activation of voltage-gated Na^+ and K^+ channels. Recently, we demonstrated that sustained elevation of the $[Na^+]_i/[K^+]_i$ ratio triggers expression of dozens ubiquitous genes including several canonical myokines, such as interleukin-6 and cyclooxygenase 2, in the presence of intra- and extracellular Ca^{2+} chelators. These data allowed us to suggest a novel $[Na^+]_i/[K^+]_i$-sensitive, Ca_i^{2+}-independent mechanism of excitation-transcription coupling which triggers myokine production. This pathway exists in parallel with canonical signaling mediated by Ca_i^{2+}, AMP-activated protein kinase and hypoxia-inducible factor 1α (HIF-1α). In our mini-review we briefly summarize data supporting this hypothesis as well as unresolved issues aiming to forthcoming studies.

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Skeletal muscles represent up to 40% of the total body mass and contain 50—75% of all body proteins. As a part of the musculoskeletal system it maintains posture and provides locomotion.[1] During the last two decades it was shown that skeletal muscles also function as an exercise-dependent endocrine organ secreting dozens of cytokines, regulatory glycoproteins with molecular weights of 15—30 kDa[2] called myokines by analogy with adipokines and hepatokines, i.e. proteins secreted by adipocytes and hepatocytes, respectively.[3] Myokines exert auto-, para- or endocrine effects communicating with other organs, such as adipose tissue, liver, bone, and immune system.[4,5]

In 1990th—2000th several investigations demonstrated that during exercise plasma interleukin-6 (IL-6) was transiently increased up to 100-fold.[6—9] Importantly, unlike sepsis-induced production of this cytokine the sharp increment of IL-6 evoked by exercise was not preceded by elevation of circulating tumor necrosis factor TNF-α. Pedersen and co-workers were the first who found that exercise does not affect IL-6 mRNA content in monocytes thus ruling out the possible implication of immune system cells.[6] Keller and co-workers reported that both IL-6 mRNA and immunoreactive protein content are increased in human contracting skeletal muscle. They also found augmented IL-6 transcription rate in nuclei isolated from human muscle biopsies after the onset of exercise.[10] Viewed collectively, these experiments demonstrated that myoblasts rather than other type of cells presented in skeletal muscle and neighboring tissues are the major source of IL-6.

Recent proteomics studies identified more than 500 proteins secreted by human and rodent skeletal muscle cells.[11—13] Along with IL-6, the highest exercise-dependent up-regulation of transcription and secretion exhibited IL-7, IL-8, murine chemokine CXC ligand-1 (CXCL-1), leukemia inhibitory factor (LIF). Irisin, a recently discovered myokine, is suggested to mediate beneficial effect of exercise by inducing browning in adipose tissue.[14] Contrary to above-listed myokines, sustained training resulted in attenuation of expression of few other peptides including myostatin.[4,5]

Side-by-side with above-listed peptides, numerous research teams observed exercise-dependent production of prostaglandins (PGEs). Thus, *in situ* microdialysis of human skeletal muscle detected ~5-fold increment of interstitial PGE_2 concentration after 60 min of dynamic exercise.[15] Importantly, exercise-induced PGE_2 production was suppressed by cyclooxygenase (COX) inhibitors[16] suggesting activation and/or *de novo* expression of this enzyme. At least two isoforms of COX have been identified, COX-1 and COX-2. COX-1 is considered as a constitutively expressed enzyme while COX-2 is induced by diverse cell type-specific stimuli.[17] In humans exercise increases activity of both isoforms and selectively increases COX-2 mRNA and protein content in contracting skeletal muscle of humans.[18—20]

It should be stressed that the increment of plasma content of some myokines triggered by exercises might be caused by their release from cells distinct of skeletal muscle. Thus, along with skeletal muscle cells intense exercise augmented IL-8 production by peripheral blood mononuclear cells.[21] Cocks and co-workers using quantitative immunofluorescence demonstrated that elevation of the content of endothelial nitric oxide synthase (eNOS) in the human *vastus lateralis* biopsy evoked by endurance and sprint interval training is caused by its elevation in microvasculature endothelial cells.[22] Considering this, mouse skeletal muscle cell lines, C_2C_{12} myoblasts, and primary human myotubes subjected to electrical pulse stimulation (EPS) are widely employed as an *in vitro* exercise model for the study of myokine production.[23—25] Using this approach it was shown that 24 h exposure of human myotubes to EPS resulted in 183 differentially expressed transcripts with the highest secretion level of IL-6, IL-8, CXCL-1, and LIF.[26]

Here, we briefly summarized the data on the upstream intermediates of intracellular signaling involved in the exercise-dependent regulation of myokine production with emphasis on a novel mechanism of excitation-transcription triggered by elevation of intracellular $[Na^+]_i/[K^+]_i$ ratio.[27] Physiological and pathophysiological implications of myokines were considered in several comprehensive reviews.[2,28—32]

Search for upstream intermediates of exercise-dependent myokine transcription

Intracellular Ca^{2+}

Contraction of skeletal muscle is induced by propagation of action potential along T-tubule evoked by opening of voltage-sensitive Na^+ channels (Na_v) and sarcolemma depolarization from the resting potential (E_m) of −80 mV to +30 mV. Conformation transition of the skeletal muscle isoform of voltage-sensitive L-type Ca^{2+} channels (Ca_v), also known as dihydropyridine receptors (DHPR), leads to physical interaction with the skeletal muscle isoform of the ryanodine receptor Ca^{2+} release channels (RyR) (Fig. 1). Activation of RyR triggers Ca^{2+} release from the sarcoplasmic reticulum, elevation of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), Ca_i^{2+} binding to troponin that, in turn, results in activation of myosin ATPase and shortening of sarcomeres.[33,34]

Besides triggering muscle contraction, elevation of $[Ca^{2+}]_i$ from ~0.1 to 1 μM affects the expression of hundreds of genes, i.e. phenomenon termed excitation-transcription coupling.[35—37] It was shown that Ca_i^{2+} affects transcription via at least three signaling pathways. ***i***) Elevation of $[Ca^{2+}]_i$ promotes translocation of nuclear factor kappa-light-chain enhancer of activated B cells (NFκB) from cytosol to the nucleus. NFκB translocation is triggered by activation of Ca^{2+}/calmodulin-sensitive protein kinase (CaMKI, II or III), leading to phosphorylation of the inhibitor of κB (IκB) by phosphorylated IκB kinase. Phosphorylated IκB dissociates from NFκB, which evokes its translocation into the nucleus. ***ii***) $[Ca^{2+}]_i$ elevation also leads to translocation of activated T-cells nuclear factor (NFAT) from cytosol to the nucleus. However, in contrast to NFκB, NFAT translocation is evoked by its dephosphorylation by the (Ca^{2+}/calmodulin)-dependent phosphatase calcineurin.[38] ***iii***) The rise of cytosolic and nucleoplasmic Ca^{2+} concentrations lead to phosphorylation of cAMP response element-binding protein (CREB) by CaMKII and CaMKIV, respectively. Phosphorylated CREB and its co-activator CREB-binding

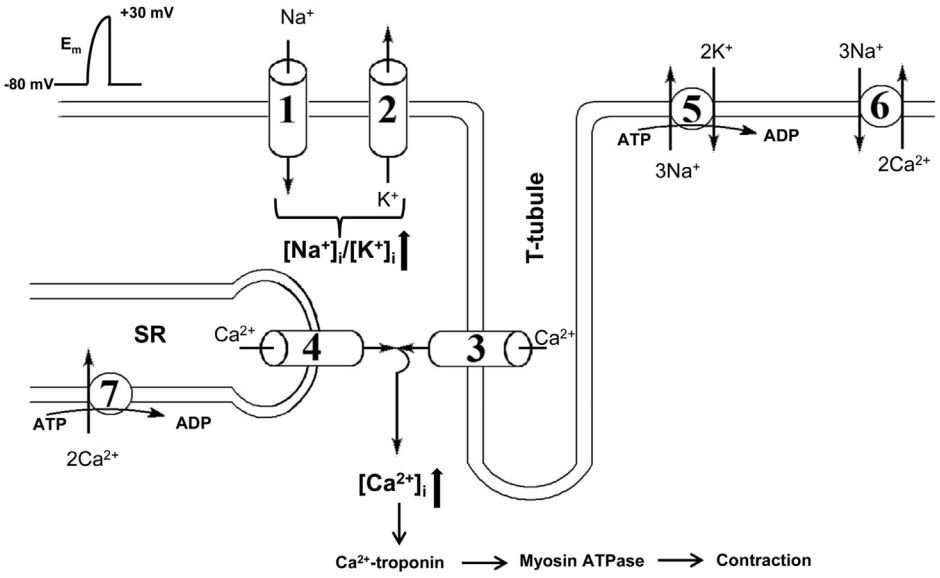

Fig. 1 Major ion transporters involved in skeletal muscle excitation-contraction coupling. **1** — voltage-gated Na^+ channels; **2** — K^+ channels; **3** — voltage-sensitive L-type Ca^{2+} channels; **4** — Ca^{2+} release channels; **5** — Na^+,K^+-ATPase, 6 — Na^+/Ca^{2+} exchanger; **7** — Ca^{2+}-ATPase; **SR** — sarcoplasmic reticulum; $\mathbf{E_m}$ — electrical membrane potential. For more details, see text.

protein regulate transcription via their binding to the (Ca^{2+}+cAMP)-response element (CRE) sequences of DNA (for comprehensive review, see Ref. 35,39).

Both CRE and NFκB response elements were found in several myokines, including IL-6[2,40] suggesting implication of Ca_i^{2+}-mediated signaling in exercise-induced myokine production. It should be noted, however, that NFκB signaling pathway is activated by contraction in rodents[41] but not in humans.[42,43] In mice, IκB kinase had no effect on IL-6 transcription[44] whereas in human skeletal muscle exercise did not affect the nuclear abundance with NFAT.[42] Treatment of rat soleus muscle with Ca^{2+} ionophore ionomycin for one hour resulted in 5-fold elevation of IL-6 mRNA content.[45] Later on, Whitham and co-workers detected that exposure of C_2C_{12} myotubes to less selective Ca^{2+} ionophore A23187 sharply increased IL-6 transcription that was not affected by inhibitors of NFκB signaling.[46] Using the same *in vitro* exercise model it was shown that extracellular Ca^{2+} chelator EGTA diminishes by 2-fold EPS-induced accumulation of CXL chemokines. It should be noted, however, that calcineurin inhibitor cyclosporine A did not affect the increment of these cytokines production.[47] Recently, we reported that extracellular Ca^{2+} chelators sharply increase permeability of the plasma membrane for monovalent ions (for more details, see below).[48] Thus, additional experiments should be performed to clarify the relative impact of Ca_i^{2+}-mediated signaling in the transcription of myokines as well as mechanisms of its modulation by Ca^{2+} ionophores and chelators.

Partial oxygen pressure

The drop of partial oxygen pressure (P_{O2}) results in elevation of local blood flow via several mechanisms including NO-dependent relaxation of vascular smooth muscle cells triggered by ATP release from erythrocytes.[49,50] Because of this, oxygen delivery to skeletal muscle is subjected to strong feed-back regulation thus buffering the decrease of intracellular partial oxygen pressure (P_{O2i}) caused by augmented exercise-induced increment of oxygen consumption and providing the tight linkage of oxygen demand and supply during exercise.[51] Using ^1H magnetic resonance spectrometry of myoglobin it was shown that in spite of this regulatory feedback P_{O2i} is decreased during intensive exercise up to 5 fold[52] with the prevalence in fast/glycolytic fibers as compared to slow/oxidative ones.[53]

Hypoxia-inducible factor 1alpha (HIF-1α), considered to be a major oxygen sensor, regulating gene expression in hypoxic conditions via interaction of HIF-1α/HIF-1β heterodimer with hypoxia response elements (HREs) in promoter/enhancer regions of the target genes. In normoxia, HIF-1α is hydroxylated by oxygen-dependent prolyl hydrolase that elicits its proteasomal degradation. In contrast, under hypoxic conditions, HIF-1α is translocated to the nucleus, where it forms HIF-1α/HIF-1β complex. The list of HIF-1-sensitive genes comprises *Hif-1α per se*, and others related to vasomotor control (nitric oxide synthase-2, adrenomedulin, endothelin-1), angiogenesis (vascular endothelial growth factor (*VEGF*) and its receptor *FLT1*), erythropoiesis and iron metabolism (erythropoietin, transferrin, transferrin receptor, ceruloplasmin), cell proliferation (*IGF1*, *IGFBP1*, *TGFb*), energy metabolism (glucose transporters *GLUT1-GLUT3*, phosphoenolpyruvate carboxylase, lactate dehydrogenase A, aldose, phosphoglucokinase-1, -L and -C, endolase, tyrosine hydroxylase and plasminogen activator inhibitor-1) (for review see,[54–58]).

It was shown that eccentric exercise increased the content of VEGF and endothelial nitric oxide synthase (eNOS) mRNA and protein in rat skeletal muscle as well as prompted the binding of HIF-1α to promoters of VEGF and eNOS genes thus indicating HIF-1α-mediated mechanism of this phenomenon.[59] It should be noted, however, that hypoxic microvasculature rather than skeletal muscle *per*

se might be the source of over-expression of these genes.[22] Indeed, exercise-induced production of VEGF and eNOS seen in muscle biopsy was accompanied by elevation of capillary density.[59] Importantly, our recent studies demonstrated that in vascular smooth muscle cells hypoxia-induced transcriptomic changes are at least partially triggered by HIF-1α-independent, $[Na^+]_i/[K^+]_i$-mediated, excitation-transcription coupling.[60] The role of this novel mechanism of excitation-transcription coupling in myokine production by contracting skeletal muscle is considered below.

AMP-activated protein kinase

Independent of HIF-1α, hypoxia can affect gene expression via decline of intracellular ATP content that, in turn, leads to accumulation of AMP and activation of AMP-sensitive protein kinase (AMPK). AMPK is a phylogenetically conserved αβγ heterodimeric enzyme activated by phosphorylation the α subunit under elevation of AMP/ATP ratio. AMPK acts as a "metabolic master switch" and a cellular energy sensor whose activation results in increased catabolism and augmented ATP production.[61] It might be proposed that intensive exercise is accompanied by elevation of intracellular AMP content due to high activity myosin ATPase, Na^+,K^+-ATPase and Ca^{2+}-ATPase (Fig. 1) which together account for 90% of ATP use.[62]

The role of AMPK in exercise-induced myokine expression is supported by several observations: *i*) the increment of IL-6 mRNA during contraction is sharply attenuated in skeletal muscle with high content of glycogen as well as by glucose ingestion during exercise (for review, Ref. 2) suggesting the role of energy metabolism in myokine transcription regulation; *ii*) administration of AMPK agonist activated expression of dozens of metabolic genes in skeletal muscle and enhanced running endurance by almost 2-fold[63]; *iii*) both in human and experimental animals, exercises evoked fiber type specific activation of AMPK[64–66]; *iv*) exercise-induced IL-15 production was decreased in mice lacking both β1 and β2 AMPK subunits in skeletal muscle.[67]

It should be noted, however, that in contrast to myokines mentioned above contraction-mediated IL-6 expression was normal in muscle-specific AMPK α2 knock out mice.[68] Importantly, because effective feedback regulation of metabolic and ATP consuming pathways ATP content in skeletal muscle during intensive exercise is decreasing by only 20–25%.[62] Considering this it might be assumed that AMPK activation is caused by distal stimuli such as augmented production of NO[69,70] rather than by elevated AMP/ATP ratio *per se*. More recently, Benziane and co-workers demonstrated that AMPK stimulates rather than inhibits Na^+,K^+-ATPase activity[71] thus providing negative regulation of Na_i^+/K_i^+-sensitive mechanism of excitation-transcription coupling considered in the next section.

Intracellular $[Na^+]_i/[K^+]_i$ ratio

Sustained excitation of skeletal muscle results in dissipation of transmembrane gradient of monovalent cations due to Na^+ influx via Na_v that, in turn, leads to depolarization and K^+ efflux via voltage-gated K^+ channels (K_v), Ca^{2+}-activated K^+ channels (K_{Ca}) and voltage-insensitive inwardly rectifying K^+ channels (Fig. 1).[33] Using distinct experimental approaches it was shown that both in humans and in experimental animals intensive exercise contribute to increases of $[Na^+]_i$ by 3–4-fold and decreases of $[K^+]_i$ by up to 50% in skeletal muscles through activation of ion channels as well as through partial inactivation of the Na^+,K^+-ATPase. It was also demonstrated that K^+ efflux from myotubes during exercise resulted in elevation of $[K^+]$ in skeletal muscle interstitial fluid from 4 to 5 to 11–15 mM. In humans, intensive dynamic and static exercises lead to up to 2-fold elevation of venous $[K^+]$ due to its release from skeletal muscle, i.e. a major source of intracellular K^+ (for comprehensive reviews, Ref. 72–76).

These findings allow us to hypothesize that elevation of the $[Na^+]_i/[K^+]_i$ ratio *per se* is sufficient to trigger myokine production. This hypothesis is based on several observations. *First*, employing Affymetrix-based technology, we detected up to 60-fold changes in the expression levels of 684, 737 and 1839 transcripts in HeLa cells, human umbilical vein endothelial cells (HUVEC) and rat aorta smooth muscle cells (RASMC), respectively, that were highly correlated in cells subjected to 3 h Na^+,K^+-ATPase inhibition with ouabain or K^+-free medium. Among these Na_i^+/K_i^+-sensitive genes, 80 transcripts were common (ubiquitous) for all three of cell types.[77] Importantly, almost half of ubiquitous Na_i^+,K_i^+-sensitive transcripts was represented by immediate response genes (IRG) and other genes involved in the regulation of transcription/translation which was ~7-fold higher than in the total human genome. *Second*, we demonstrated that several myokines, including IL-6, as well as prostaglandin producing COX-2 are among the ubiquitous genes whose expression is strongly increased by elevation of the $[Na^+]_i/[K^+]_i$ ratio.[77] Recently, Broholm and co-workers reported that side-by-side with canonical myokines resistance exercise triggers profound accumulation of several IRG in human skeletal muscle biopsies including ~4-fold elevation of JUNB.[78] We noted that this gene is also subjected to sharp up-regulation by sustained elevation of the $[Na^+]_i/[K^+]_i$ ratio in all cell types being under investigation.[77] *Third*, several research teams reported that myokine secretion is accompanied by upstream activation of ERK1/2-, JNK- and NF-κB-dependent pathways.[26,46] These signaling pathways might be also activated by elevation of the $[Na^+]_i/[K^+]_i$ ratio triggered by Na^+,K^+-ATPase inhibition.[79,80]

In RASMC and HeLa cell lines inhibition of the Na^+,K^+-ATPase by ouabain resulted in expression of several IRG including 10- and 4-fold increment of immunoreactive c-Fos and c-Jun.[81,82] A 4-fold increment of c-Fos mRNA was detected in 30 min after ouabain addition. Within this time interval, $[Na^+]_i$ was increased by ~5-fold whereas $[K^+]_i$ was decreased by only 10–15%. These results show that $[Na^+]_i$ augmentation rather than $[K^+]_i$ attenuation generates a signal that leads to c-Fos expression. Uddin and co-workers demonstrated that in human cytotrophoblasts IL-6 secretion might be triggered by ouabain and marinobufagenin,[83] i.e. potent Na^+,K^+-ATPase inhibitors causing different structural changes in its a1-subunit.[84] Viewed collectively, these data strongly suggest that these cardiotonic steroids trigger IL-6 expression via elevation the $[Na^+]_i/[K^+]_i$ ratio rather than Na_i^+/K_i^+-independent signaling pathways.

To examine relative contribution of Ca_i^{2+}-mediated and -independent signaling, we compared transcriptomic changes triggered by elevation of the $[Na^+]_i/[K^+]_i$ ratio in control and Ca^{2+}-depleted cells. Surprisingly, Ca^{2+}-depletion increased rather than decreased the number of ubiquitous and cell-type specific Na_i^+/K_i^+-sensitive genes.[77] Among the ubiquitous Na_i^+/K_i^+-sensitive genes up-regulated independently of the presence of Ca^{2+} chelators, we found canonical myokine IL-6 as well as JUNB and COX-2. To further examine the role of Ca_i^{2+}, we studied action of Ca^{2+} chelators on intracellular monovalent ion handling. In vascular smooth muscle cells, addition of 50 μM EGTA to Ca^{2+}-free medium led to ~3-fold elevation of $[Na^+]_i$ and 2-fold attenuation of $[K^+]_i$. Ca^{2+}-depletion resulted in almost 3-fold elevation of the rate of ^{22}Na and ^{86}Rb influx measured in the presence of inhibitors of Na^+,K^+-ATPase and $Na^+,K^+,2Cl^-$ cotransport.[48,85] The augmented permeability for monovalent cations seen in Ca^{2+}-depleted cells is probably caused by attenuation of extra-rather than intracellular Ca^{2+}. Indeed, in contrast to extracellular Ca^{2+} chelator EGTA, neither the $[Na^+]_i/[K^+]_i$ ratio nor permeability of VSMC for Na^+ were affected by Ca^{2+}-free medium lacking Ca^{2+} chelators as well as by addition of intracellular Ca^{2+} chelator BAPTA-AM alone.[48] Importantly, the list of genes up regulated in Ca^{2+}-depleted cells by more than 4-fold was abundant with genes whose expression was also affected by inhibition of the Na^+,K^+-ATPase in K^+-free medium. In additional experiments, we found that dissipation of transmembrane gradients of Na^+ and K^+ in high-K^+, low-Na^+-medium abolished the increment of the $[Na^+]_i/[K^+]_i$ ratio as well as sharp elevation of *Atf3, Nr4a1* and *Erg3* mRNA content triggered by 3-hr incubation of VSMC in Ca^{2+}-free, EGTA containing medium.[48] Thus, alternative approaches should be developed to clarify relative impact of Ca_i^{2+}-independent and Ca_i^{2+}-mediated mechanisms of excitation-transcription coupling in transcriptomic changes triggered by elevation of the $[Na^+]_i/[K^+]_i$ ratio.

Does exercise affect myokine translation?

Comparative analysis of exercise-sensitive secretome of skeletal muscle cells revealed little correlation between mRNA and protein levels,[12,86] indicating pronounced modulation of myokine translation and/or secretion. Data considered below strongly suggest that besides of transcription elevation of the $[Na^+]_i/[K^+]_i$ ratio seen in contracting skeletal muscles affects myokine translation. Almost 50 years ago, it was demonstrated that protein synthesis in prokaryotes is sharply inhibited in the absence of K^+.[87] Later on, the requirement of K^+ for protein synthesis was detected in animal cells of different origins (for review, Ref. 88). In human fibroblasts, sustained Na^+/K^+-ATPase inhibition suppresses translation without any impact on transcription, ATP content and amino acid transport,[89] indicating a direct influence of $[K^+]_i$ on the protein synthesis machinery. In reticulocytes, globin contributes to more than 90% of total protein synthesis. In these cells, it was found that K_i^+ depletion inhibits the elongation step without any impact on ribosome subunit assembly. Half-maximal activation of globin synthesis by reticulocyte lysate in medium containing 60, 90 and 125 mM Na^+ was observed at $[K^+]$ of 15, 25 and 40 mM, respectively.[90] These data indicate that elevation of $[Na^+]_i$ diminishes the efficiency of protein synthesis regulation by K_i^+ via attenuation of K^+ interaction with its hypothetical sensor (Fig. 2). As alternative hypothesis it might be proposed that elevation of $[Na^+]_i$ diminishes the transcription of elongation factors.[91–93] This hypothesis is currently being examined in our laboratory.

Does exercise affect myokine secretion?

It is generally accepted that myokine secretion is mediated by exocytosis.[35] Exocytosis consists of multiple kinetically defined stages such as recruitment, targeting, tethering and docking of secretory vesicles with the sarcolemma, priming the fusion machinery and finally membrane fusion. The final stage is triggered by Ca^{2+} and involves several secretory vesicle proteins including Ca^{2+}-sensing protein synaptotagmin 1 (SYT1).[94] These data suggest that elevation of $[Ca^{2+}]_i$ in contracting muscle may affect myokine secretion independently on regulation of their transcription and translation (Fig. 2). Indeed, using confocal and green fluorescent protein to visualize intracellular targets, Lauritzen and co-workers found that contraction stimulates IL-6 vesicle depletion from mouse muscle fibers *in vivo*.[68]

In addition to Ca^{2+}, exocytosis may be regulated by intermediates of intracellular signaling such as cAMP-binding protein EPAC, guanine-exchange factors (Rap1-CEFs).[95] Importantly, in vascular smooth muscle and endothelial cells, sustained inhibition of the Na^+,K^+-ATPase affected expression of dozen proteins involved in these signaling cascades (data prepared for publication). The role of elevated $[Na^+]_i/[K^+]_i$ ratio in regulation of myokine secretion by altered expression of $[Na^+]_i/[K^+]_i$-sensitive genes involved in the secretory machinery remains unknown.

Search for intracellular monovalent ion sensors

Our model suggests that elevation of the $[Na^+]_i/[K^+]_i$ ratio affects myokine transcription and translation independently via interaction of Na_i^+ and K_i^+ with their hypothetical sensors: NaS and KS, respectively (Fig. 2). The molecular origin of monovalent cation sensors distinct from ion transporters is still a mystery. This uncertainty is in contrast with rapid progress in the identification of Ca_i^{2+} sensors. It should be noted, however, that high-affinity binding sites, initially detected in parvalbumins and calmodulin, are formed by a highly conservative linear amino acid sequence consisting of 14 amino acid residues (the so-called "EF-hand" domain). This knowledge led to the rapid identification of more than 30 other Ca_i^{2+} sensors by the screening of cDNA libraries.[96] In contrast, monovalent ion sensors are probably formed by 3D protein structures and recruit space-separated amino acid residues.[88] In addition, high-affinity Ca_i^{2+} sensors are almost completely saturated at $[Ca^{2+}]_i$ of 1 μM. This feature led to the identification of amino acid residues by ^{45}Ca binding assay. Unlike Ca^{2+}, monovalent

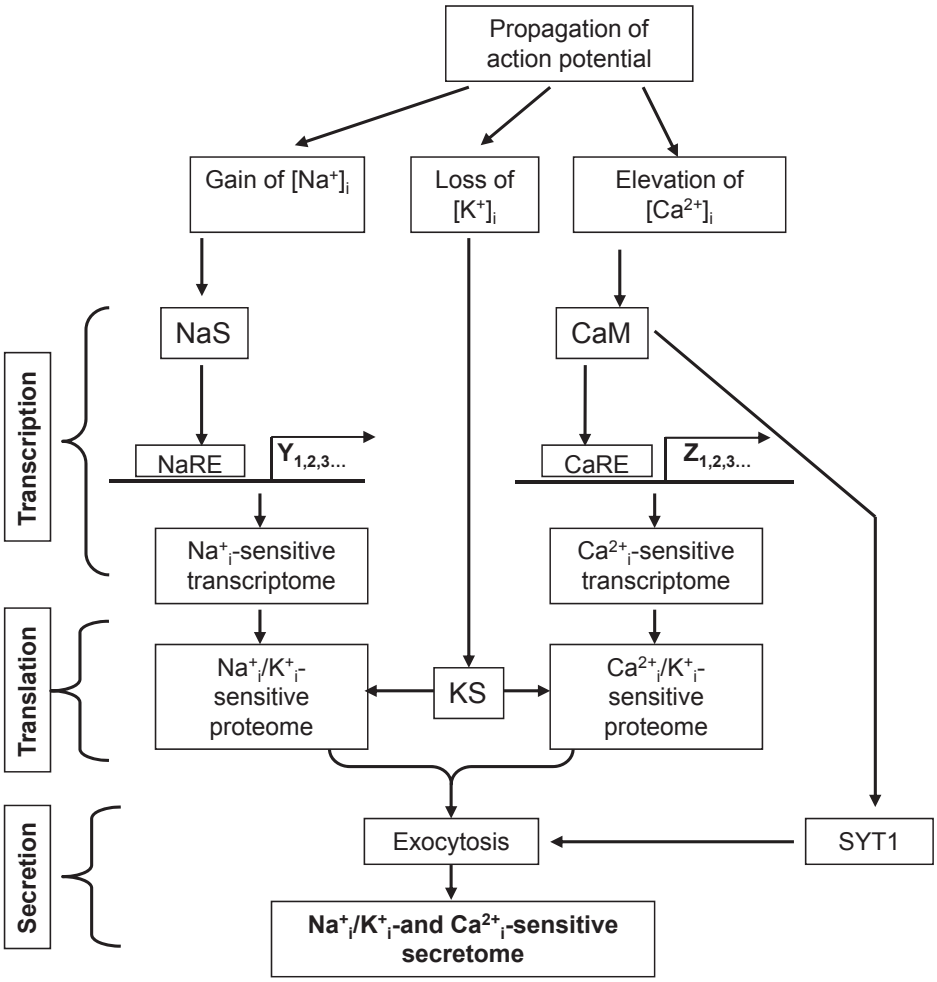

Fig. 2 Ionic mechanisms of excitation-contraction, excitation-transcription, excitation-translation and excitation-secretion coupling involved in exercise-induced myokine production by skeletal muscles. **CaM** — calmodulin and other intracellular Ca^{2+}-sensors; **CaRE** — Ca^{2+}-sensitive response elements in gene promoters; **KS** and **NaS** — intracellular K^+ and Na^+ sensors, respectively; **NaRE** — Na^+-sensitive response elements in gene promoters; **SYT1** — synaptotagmin 1. For more details, see text.

cations affect cellular function in the millimolar range that complicates their identification by screening with radioisotopes.

It is generally accepted that transcription is under the control of transcription factors interacting with specific response elements. Considering this, we tried to find Na^+ response element (NaRE) within c-Fos promoter. With the construct containing CRE and all other known transcription elements of the c-Fos promoter, we failed to detect any significant elevation of luciferase expression in HeLa cells subjected to 6-hr inhibition of Na^+/K^+-ATPase that contrasted with massive accumulation of endogenous c-Fos mRNA and immunoreactive protein in ouabain-treated HeLa cells.[82]

Several hypotheses could be proposed to explain negative results obtained in this study. (***i***) NaRE is located within introns or/and the c-Fos 3′-UTR. (***ii***) $[Na^+]_i/[K^+]_i$ ratio elevation affects gene expression via epigenetic modification of the DNA, histones or nucleosome remodeling, i.e. regulatory mechanism having a major impact on diverse cellular functions.[97] Importantly, the epigenetic mechanism of gene expression does not contribute to the regulation of L-luc transcription in the plasmid employed in our experiments.[82] (***iii***) Increasing evidence indicates that gene activation or silencing is under the complex control of three-dimensional (3D) positioning of genetic materials and chromatin in the nuclear space (for review, Ref. 98). It may be proposed that augmented $[Na^+]_i/[K^+]_i$ ratio affects gene transcription by changing the chromatin structure. (***iv***) Ono and co-workers reported that at the baseline level of $[Ca^{2+}]_i$ (~100 nM), Na^+ interacts with calpain Ca^{2+}-binding sites, and this enzyme functions as Na_i^+-dependent protease with $K_{0.5}$ of 15 mM for Na^+.[99] Additional experiments should be performed to examine the role of Ca^{2+}-binding proteins as potential monovalent cation sensors involved in transcriptomic and proteomic changes triggered by elevation of the $[Na^+]_i/[K^+]_i$ ratio.

It should be underlined that side-by-side with transcription, translation and secretion stages myokines can affects their production by autocrine receptor-mediated mechanisms.[100] Thus, IL-15 augments expression of peroxisome proliferator-activated receptor δ (PPARδ) and silent

regulator of transcription-1 (SIRT1) via interaction with its receptor IL-15Rα,[101] PGE_2 triggers accumulation of IL-6[40,102] whereas CXL-1 expression is regulated by IL-6.[103]

Conclusion and unresolved issues

During the last two decades it was shown that skeletal muscles function as an exercise-dependent endocrine organ secreting numerous myokines. In spite of diverse physiological and pathophysiological implications, upstream signals and sensing mechanisms underlying this phenomenon remain poorly understood. Data summarized in our mini-review show that side-by-side with canonical Ca_i^{2+}-AMPK- and HIF-1α-mediated signaling pathways, myokine production by contracting skeletal muscle may be mediated by the novel $[Na^+]_i/[K^+]_i$-sensitive, Ca_i^{2+}-independent mechanism of excitation-transcription coupling. Comparative analysis of HUVEC, RASMC and HeLa cells demonstrated that elevation of the $[Na^+]_i/[K^+]_i$ ratio triggers cell type-specific transcriptomic changes via Ca_i^{2+}-mediated and -independent signaling.[77] What is the relative impact of $[Na^+]_i/[K^+]_i$-sensitive genes in overall exercise-induced transcriptomic changes in fast and slow skeletal muscles? What is the relative impact on myokine production of $[Na^+]_i/[K^+]_i$-sensitive, Ca_i^{2+}-mediated and -independent mechanisms of excitation-transcription, excitation-translation and excitation-secretion coupling? What is the molecular origin of $[Na^+]_i$ and $[K^+]_i$ sensors involved in Ca_i^{2+}-independent regulation of gene transcription and translation? We address these questions to forthcoming studies.

Conflicts of interest

The authors declare no conflict of interest.

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