Calcium microdomains and gene expression in neurons and skeletal muscle cells

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Abstract

Neurons generate particular calcium microdomains in response to different stimuli. Calcium microdomains have a central role in a variety of neuronal functions. In particular, calcium microdomains participate in long-lasting synaptic plasticity—a neuronal response presumably correlated with cognitive brain functions that requires expression of new gene products. Stimulation of skeletal muscle generates – with few milliseconds delay – calcium microdomains that have a central role in the ensuing muscle contraction. In addition, recent evidence indicates that sustained stimulation of skeletal muscle cells in culture generates calcium microdomains, which stimulate gene expression but not muscle contraction. The mechanisms whereby calcium microdomains activate signaling cascades that lead to the transcription of genes known to participate in specific cellular responses are the central topic of this review. Thus, we will discuss here the signaling pathways and molecular mechanisms, which via activation of particular calcium-dependent transcription factors regulate the expression of specific genes or set of genes in neurons or skeletal muscle cells.

Keywords: Calcium channels; Transcription factors; Synaptic plasticity; Muscle contraction; Calcium release; Nuclear calcium signals; Calcium-dependent kinases; Calcineurin

1. Introduction

In this review we will use the definition of calcium microdomains generally understood by cell biologists and recently defined by Rizzuto and Pozzan [1] as "*all the increases in cellular Ca^{2+} that do not involve the generality of the cell cytoplasm, but remain localized to part of the cell*". Therefore, we will consider here as calcium microdomains all localized calcium signals, including those generated just below the plasma membrane, within the nucleus or in its close proximity, and in the immediate vicinity of mitochondria or the sarcoplasmic/endoplasmic reticulum (SR/ER). We will analyze the particular calcium microdomains known to control gene expression in skeletal muscle cells or neurons. Other recent reviews have dealt with this topic in cardiac muscle [2] or smooth muscle cells [3,4].

Skeletal muscle cells and neurons possess unique structural features; their highly specialized morphology is suited to carry out their distinctive functions. In particular, several calcium-sensitive biochemical pathways, including those that participate in the regulation of gene expression, are not distributed evenly in muscle or neuronal cells [5]. In consequence, calcium microdomains generated in different cell locations are bound to produce dissimilar responses.

Of special relevance to this article is the role of calcium microdomains as the main mediators of activity-dependent gene expression in neurons, and presumably in skeletal muscle cells as well, as recent results seem to indicate. Here, we will analyze the known molecular mechanisms underlying

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calcium regulation of gene expression in these cells, including the participation of transcription factors activated by the generation of calcium microdomains.

2. Calcium microdomains and gene expression in neuronal cells

Calcium signals initiate many neuronal responses, including axonal growth, secretion of neurotransmitters and synaptic plasticity [6–8]. Calcium microdomains can initially arise as a consequence of calcium influx through plasma membrane voltage- or neurotransmitter-activated calcium channels or through store-operated calcium channels; activation of calcium release channels present in intracellular stores can also generate calcium microdomains [9–12]. Through calcium-induced calcium release (CICR), small and localized calcium microdomains can be amplified and propagated to other neuronal regions, including the nucleus [6,10]. Calcium activation of ryanodine receptors (RyR) or IP3 receptors (IP3R), the two best-characterized release channels, provides the molecular basis for CICR. Yet the three mammalian RyR or IP3R isoforms do not always present the same calcium activation response. Other variables, such as cellular redox state or IP3 concentration (in the case of IP3R), determine if and how these channels will respond to calcium microdomains [13,14]. Nuclear calcium microdomains are especially important, since calcium at the nucleus can directly modulate gene expression. At the nucleus, calcium microdomains can arise from propagated cytoplasmic calcium signals or by other mechanisms, including calcium release directly at the nucleus; mitochondria can also take up calcium from primary cytoplasmic microdomains, to release it later giving rise to new microdomains [15]. In theory, mitochondria-generated microdomains could also affect gene expression; yet, to our knowledge studies on this particular aspect have not been reported.

2.1. General aspects

Cytoplasmic as well as nuclear calcium microdomains can both induce changes in calcium-sensing pathways that either stimulate or inhibit gene transcription in neuronal cells [16,17]. In general, calcium microdomains enhance gene expression by inducing different nuclear events according to the nature of the calcium-activated transcription factor involved [12,18]. A case in point is the activation of cytoplasmic or nuclear calcium-dependent kinases and phosphatases, which through phosphorylation or dephosphorylation of transcription factors modify their transactivating properties.

Neuronal activity generates calcium microdomains, which in turn stimulate the expression of genes that are essential for dendritic development, synaptic plasticity and neuronal survival. Several steps in the process of activity-dependent gene expression are calcium-dependent [16,17], and engage nuclear and cytoplasmic factors that decode the spatial and temporal properties of particular calcium microdomains [6,12,18,19]. Calcium microdomains play a central role in the maintenance of many forms of long-term potentiation (LTP) or long-term depression (LTD). Both LTP and LTD represent experimental models to study activity-dependent synaptic modifications, which through the expression of new gene products entail long-term structural and functional neuronal changes [20,21]. In particular, LTP – a reversible increase in synaptic transmission inducible in several brain regions in response to tetanic stimulation of afferent fibers – is considered a cellular model of synaptic plasticity. All these features give special relevance to the functional connections among neuronal activity, calcium microdomains and gene expression.

Increasing evidence points to a role for calcium released from intracellular stores as a source of the calcium increase required for activation of most calcium-regulated transcription factors [6]. In particular, a role of CICR as an amplification mechanism of the initial calcium signal generated by calcium entry, and in relaying the amplified signals to the nucleus, has been postulated (see [6,10], and references therein). Both IP3R and RyR present in the ER can generate calcium microdomains via CICR or through alternative mechanisms, and may thus contribute to calcium-dependent gene expression in neurons. For example, in hippocampal spines activity-dependent calcium influx via the NMDA receptors is significantly amplified by RyR-mediated CICR, suggesting an essential contribution of calcium arising from intracellular stores to the postsynaptic calcium microdomain [22]. New findings providing a link between neuronal activity and calcium release from intracellular stores were reported recently in nerve terminals isolated from hypothalamic neurons [23]. These authors showed that, in the absence of extracellular calcium, spontaneous short-lived cytoplasmic calcium transients due to RyR-mediated calcium release increase in frequency with depolarization. It remains to be determined whether these calcium signals produce an increase in nuclear calcium that may directly affect the activity of nuclear transcription factors.

2.2. Calcium-dependent transcription factors

We will summarize here current knowledge on the molecular mechanisms underlying activation by calcium microdomains of the transcription factors cAMP/calcium response element binding protein (CREB) and nuclear factor of activated T cells (NFAT). We will also include a brief description of some other transcription factors, such as NFκB, which are also stimulated by the generation of calcium microdomains.

2.2.1. CREB

Phosphorylation of the nuclear transcription factor CREB is a necessary condition for the transcription of genes containing cAMP/calcium response element (CRE) sequences in their promoters, as do a number of genes activated by

neuronal electrical activity, including c-fos and brain derived nerve factor (BDNF). As a result, the calcium-dependent activation (phosphorylation) of the nuclear transcription factor CREB and the ensuing gene transcription have been extensively investigated in neurons [17,24–26]. It is now widely accepted that activity-dependent CREB phosphorylation is crucial for several neuronal functions, including synaptic plasticity—a cellular response considered essential for learning and memory [20,27–29].

Activity-dependent calcium entry through L-type calcium channels and/or *N*-methyl D-aspartate (NMDA) receptors is the initial signal for the activation of cytoplasmic signaling cascades that culminate in nuclear CREB phosphorylation and neuronal gene expression [18,30]. In the hippocampus, cytoplasmic or nuclear calcium microdomains can promote CREB phosphorylation, either via the Ras/ERK (extracellular signal-regulated kinase) pathway or via pathways involving kinases calcium/calmodulin-dependent (CaM) kinases or cAMP-dependent protein kinase (PKA), albeit the latter seems to be much less important than the preceding two. CREB phosphorylation by the Ras/ERK1/2 pathway or by CaM kinases occurs with different kinetics [31–33]. In addition, whereas CaM kinase-dependent CREB activation requires nuclear calcium to activate the nuclear CaMKIV [34], an increase in nuclear calcium is not necessary for ERK-mediated CREB phosphorylation [32]. Yet nuclear calcium microdomains seem to be necessary to activate phosphorylation by CaMKIV of CREB binding protein (CBP), a co-activator protein of CRE-mediated transcription [34–36].

2.2.2. *CREB phosphorylation induced by calcium entry through NMDA receptors*

The most extensively studied LTP and LTD at the CA1 hippocampal region depend on postsynaptic calcium microdomains generated by calcium influx through NMDA receptors [20,37,38]; calcium entry through these receptors promotes ERKs and CREB phosphorylation [32,39,40]. Several reports indicate that CREB-dependent transcription of genes involved in synaptic plasticity entails long-term CREB phosphorylation by the ERK pathway [20,31,33,41–43]. Enhanced ERK phosphorylation occurs downstream of Ras activation by calcium, which is caused primarily by calcium activation of Ras-specific exchange factors (Ras-GRF) [43,44]. Several reports indicate that ERK activation is required for several forms of LTP and in some types of long-term spatial memory; as an example, the hippocampus of rats trained in spatial learning tasks exhibits ERK activation whereas ERK inhibition impairs this form of learning (see [43]). Interestingly, different Ras-GRF act as calcium sensors for different classes of NMDA receptors and, through activation of particular MAP kinase (MAPK) family members, generate opposing forms of synaptic plasticity. Thus, expression of Ras-GRF-2 in post-pubescent mice KO for Ras-GRF mediates activation of ERKs via NMDA receptor NR2A subunits and contributes primarily to the induction of LTP, whereas Ras-GRF-1 mediates p38 MAPK activation via NR2B-containing NMDA receptors and contributes predominantly to the induction of LTD [45].

Inhibition of GABA A receptors with bicuculline induces bursts of action potentials in cultured hippocampal neurons, which are associated with global, synaptic NMDA receptor-induced calcium transients [32,46]. Restriction of the intracellular calcium microdomain to the immediate submembrane region with EGTA-AM, which allows the calcium concentration to increase only near the mouth of the calcium entry channel and effectively prevents spreading of free calcium into the dendritic shaft and the cell soma, produces ERK and CREB phosphorylation [39]. These results strongly suggest that calcium microdomains just below the postsynaptic membrane, generated by calcium entry via NMDA receptors, can effectively activate the transcription regulators ERK and CREB. The use of a similar model to study NFκB activity (see below) also indicated that submembranous calcium microdomains were required as a switch for this transcription factor [46].

2.2.3. *CREB phosphorylation induced by calcium entry through L-type calcium channels*

In cortical neurons calcium entry through L-type calcium channels generates calcium microdomains that also enhance ERK and CREB phosphorylation [30]. This activation is specific for L-type calcium channels over other voltage-dependent calcium channels (P-, N-, P/Q), which also induce cytoplasmic and nuclear calcium microdomains, indicating that in cortical neurons the route of calcium entry plays a central role in transcription regulation. The α1 subunit of L-type channels has a CaM-binding IQ motif that is required for signaling to ERK and for the subsequent induction of CREB activation [30]. Other voltage-dependent calcium channels, however, also possess this motif suggesting that some other features of L-type channels are responsible for their specificity.

In hippocampal pyramidal neurons that express predominantly the Cav1.2 L-type calcium channel (and only rather low levels of the Cav1.3 isoform), a specific PDZ domain-binding carboxy-terminal motif, which has been shown to associate with NIL-16 PDZ domains, plays an important role in coupling L-type calcium channel activity to CREB phosphorylation [47,48]. Knockout mice for Cav1.2 have impaired CREB activation, defective NMDA receptor-independent LTP in the CA1 region, and spatial learning deficits, while NMDA-dependent LTP is not affected [48]. Albeit gene inactivation also occurs in the cerebral cortex, the principal impairment originates from defective hippocampal-associated learning and memory. These findings indicate that Cav1.2 L-type calcium channels exert a critical function in hippocampus-dependent spatial memory, coupling NMDA receptor-independent synaptic activity to transcriptional events, which as already discussed above, are thought to be molecular prerequisites for persistent LTP and learning.

The Cav1.3 L-type calcium channels are the main mediators of enhanced CREB phosphorylation in striatal neurons;

these channels also contain a PDZ domain, which in this case associates with a PDZ domain of the Shank adaptor protein [49,50]. These combined results indicate that the association of L-type calcium channels to complexes of signaling proteins that promote CREB phosphorylation can vary according to L channel subtype and neuronal origin.

There are other examples of specificity of the calcium entry channels that convey signals to the nucleus. CREB activation or inactivation by synaptic or extrasynaptic NMDARs, respectively, has been reported in hippocampal cultures [40]. The P/Q-type channels are involved in the activation of syntaxin A in transfected cells, and signaling from P/Q channels to the syntaxin A gene requires the amplification of the initial calcium entry signal by calcium release (RyR-mediated or IP3R-mediated) from intracellular stores [51].

2.2.4. The role of calcium release from intracellular stores in CREB phosphorylation

In hippocampal neurons, calcium release from intracellular stores generates calcium microdomains that also activate ERK, CREB and gene expression. Thus, the activation of several receptors by their respective neurotransmitters, including metabotropic glutamate receptors, dopamine receptors and muscarinic acetylcholine (ACh) receptors, stimulates ERKs and CREB phosphorylation in the hippocampus [52]. Presumably, receptor activation promotes ERK and CREB phosphorylation via IP3-induced calcium microdomains, but experiments to test this mechanism have not been reported. Enhanced CREB phosphorylation and c-fos expression takes place following nicotine-induced activation of ionotropic ACh receptors in hippocampal cells; noteworthy, thapsigargin or selective RyR inhibition with ryanodine reduced these effects to background levels, implicating RyR-mediated calcium release in CREB stimulation [53]. Likewise, RyR blockade significantly reduces tetanically induced LTP and the concomitant increase in phosphorylated CREB in postsynaptic neurons of the CA1 hippocampal region [54]. Induction of action potentials by an antagonist of GABA receptors produces NMDA receptor-mediated CREB activation in hippocampal neurons [32]; in this case, the initial calcium increase in the dendrites is followed by a nuclear calcium increase, which is modulated by changing the frequency of the action potential bursts. Two important observations are that CREB phosphorylation correlates with the nuclear calcium transients and that depletion of intracellular calcium stores with cyclopiazonic acid or thapsigargin decrease synaptically evoked nuclear calcium transients and virtually cause a complete block of CREB phosphorylation and CREB-mediated gene transcription, assessed by measuring c-fos expression [32].

The above studies indicate that cytoplasmic calcium microdomains, generated initially by activity-dependent calcium entry, are presumably propagated via RyR-mediated CICR to the nucleus; the resulting nuclear calcium microdomains can then promote CREB-mediated gene transcription. Yet, several reports indicate that RyR activity, and hence RyR-mediated CICR, is significantly inhibited if a few crucial RyR cysteines are in the free sulfhydryl state (see [13]). Since neuronal activity significantly enhances the production of reactive oxygen species (ROS) [55], we are currently testing the hypothesis that neuronal activity promotes RyR redox modifications, facilitating as a result CICR and calcium-induced ERK and CREB phosphorylation. In support of this hypothesis, we have recently reported that calcium release mediated by RyR redox-modified with hydrogen peroxide stimulates ERK and CREB phosphorylation in hippocampal neurons in culture and in hippocampal slices [56], and also enhances the expression of the immediate early genes c-fos and egr-1 [57].

Striatal neurons represent another example of CREB activation by calcium released from intracellular stores [58]. In these neurons, thapsigargin blocks the increase in CREB phosphorylation produced by forskolin-induced activation of PKA, suggesting that calcium release from intracellular stores contributes to this response. A role for calcium release from intracellular stores in stimulating CREB phosphorylation in sensory neurons, through the activation of the metabotropic P2Y2 receptors by ATP or UTP, has also been described [59]. In this cellular model, the activation of P2Y2 receptors induces sustained trains of action potentials; the resulting calcium entry also contributes to CREB phosphorylation and only the concerted inhibition of both calcium sources blocks CREB activation [59].

2.2.5. NFAT

The activity of the cytoplasmic transcription factor NFAT is highly dependent on calcium via calcineurin (CaN), a calcium/calmodulin-dependent serine/threonine phosphatase that by removing phosphorylation of specific serine residues allows NFAT translocation to the nucleus [60]. In comparison to CREB, much less is known regarding neuronal gene expression induced by calcium activation of NFAT. The NFATc4 isoform has been identified in the hippocampus where it initiates gene expression following periods of heightened synaptic activity; this NFAT-mediated response may be important for long-term changes in cell excitability [61,62]. The IP3R type-1, an NFAT target gene, presents increased expression in response to depolarization [61] as well as following BDNF addition to induce NFAT-dependent transcription [62]. Activation of NFAT by depolarization requires calcium entry through L-type calcium channels, while the effect of BDNF on NFAT is independent of calcium entry and involves IP3-dependent calcium release from stores [61,62]. The study of the downstream effects of BDNF is an attractive area, since the highest levels of BDNF are found within the hippocampus and the induction of LTP is impaired in BDNF knockout mice [63]. In terms of calcium microdomains, a significant difference between the activation of CREB and NFAT is that only calcium entry via L-type calcium channels and not NMDA receptors mediate NFAT activation in the hippocampus [61].

2.2.6. NFκB

This transcription factor, which is activated by basal synaptic activity in hippocampal neurons, can be further activated by exogenous glutamate; significantly, mice lacking p65 NFκB show a selective deficit in hippocampal-dependent spatial learning [46]. Using a similar approach as described above for CREB, where the calcium microdomain was restricted to the immediate sub-membrane region with EGTA-AM [39], revealed that calcium microdomains localized just below the membrane are also required to stimulate NFκB activity [46]. Furthermore, synaptic activity induces the nuclear import of a reporter gene for NFAT, which can be inhibited by blockade of globally elevated calcium levels [46]. Direct nuclear translocation of NFκB induced by locally restricted cytoplasmic calcium microdomains in response to synaptic inputs can be contrasted to CREB stimulation, which requires both nuclear and restricted postsynaptic calcium microdomains. Yet, the possibility that nuclear calcium microdomains are required for maximal NFκB-dependent transcription cannot be excluded [12].

3. Calcium microdomains and gene expression in skeletal muscle cells

Skeletal muscle cells are capable of modifying both their structure and function in response to changes in contractile activity. Calcium signals have a crucial role in this adaptation process, and knowledge about how the properties of calcium microdomains originated through different sources regulate the transcription of genes involved in these changes is recently emerging [64]. Additionally, skeletal muscle cells represent a unique environment, where regulation of transcription must occur within the background of the strong calcium signals originated prior to each contraction.

Work by Carrasco, Jaimovich and co-workers has revealed that depolarization of cultured muscle cells elicits a complex pattern of calcium microdomains, which couple excitation to contraction or to transcription signaling. These authors have shown that depolarization produces a new type of calcium microdomain, other than the fast RyR-dependent calcium release transients that drive muscle contraction and that may also stimulate transcription (see below). Thus, depolarization produces an increase in IP3 mass, which via IP3R-mediated calcium release generates slower and long-lasting calcium microdomains of smaller amplitude that do not induce contraction and are especially prominent in cell nuclei [65–69].

Skeletal muscle cells express all three IP3R isoforms; each isoform exhibits a particular intracellular distribution [70]. The IP3R type-1 is particularly interesting, since apart from its SR localization at the I band, it is the only IP3R isoform present in the nuclear envelope region. This particular location suggests that IP3R type-1 may bestow on the nuclear envelope and its associated membrane structures the ability to generate calcium microdomains [70,71]. Type-3 IP3R is mainly distributed inside the nucleus, and type-2 IP3R is distributed more or less homogeneously in the cytoplasm, in a reticular pattern that in undifferentiated myotubes probably corresponds to SR membranes [70]. The slow calcium signal travels through the cytoplasm as a wave and seems to trigger the emergence of higher intensity calcium microdomains inside the nuclei. In fact, the slow calcium wave can be envisaged as having two components: a faster but diffuse component of low fluorescence intensity that always precedes a localized wave of higher fluorescence that propagates from one nucleus to another [65]. The type-1 IP3R present in the I-band SR region could originate the diffuse, low intensity cytoplasmic calcium microdomains that are always associated with the generation of localized nuclear calcium microdomains of higher intensity. Since the cell does not contract in response to these signals, the overall cytosolic calcium concentration must remain below the muscle contraction threshold; likewise, to avoid contraction the higher intensity calcium microdomains must be highly compartmentalized, as should be the case for nuclear calcium microdomains [65,66]. Both calcium and IP3 are required to activate IP3R channels [14], the IP3R type-1 located in the I-band SR region may propagate calcium waves to the nuclear region, where they can contribute to generate higher intensity calcium signals without causing contraction.

The finding that the C2C12 skeletal muscle cell line expresses functional RyR type-1 on intranuclear SR extensions provides an additional mechanism whereby cells expressing RyR can regulate calcium signals in discrete regions within the nucleus [72]. In fact, the fast RyR-mediated calcium signal, which is initially highly localized to the triads, can also be visualized at the nuclei at a later time [65].

As described above, the transcriptional responses that arise from neuronal calcium microdomains may have different outcomes according to the amplitude, kinetics and subcellular localization of these domains [73–76]. In skeletal muscle, both the kinetics of the calcium signals and their subcellular location are bound to give rise to calcium microdomains with different spatial and temporal properties, which are likely to exert differential effects on transcriptional responses. The role of calcium arising from RyR and IP3R activity in the induction of specific transcriptional responses in skeletal muscle is a current subject of study in our as well as in other laboratories [64,71,77–82].

We have reported that the slow IP3R-dependent calcium microdomains, induced by membrane depolarization with K^+, regulate several transcription-related events [67,68,70,71,77,79]. In primary cultures of rat skeletal muscle cells, K^+ depolarization induces transient ERK and CREB activation and increases the mRNAs of the early genes c-fos, c-jun and egr-1 [64,67,71,77,79]. Activation of these transcriptional regulators occurs in the absence of extracellular calcium or in the presence of high concentrations of ryanodine that inhibit RyR; in contrast, inhibitors of the IP3R, which block the generation of the slow calcium transients, significantly reduce the activation of these transcriptional regulators [68,71,77,79]. In primary cultures of rat skeletal

muscle cells, CREB phosphorylation depends on the activation of both ERKs and PKCα, which are most likely driven by the slow IP3R-dependent calcium microdomains [77,79]. Recent experiments, carried out with nuclei isolated from skeletal muscle cells in culture, have provided direct evidence of increased CREB phosphorylation by IP3-induced nuclear calcium microdomains [70]. These results indicate that nuclear IP3R are part of a distinct calcium release mechanism that originates calcium microdomains in the nucleus. As indicated earlier in this review article, nuclear calcium microdomains contribute to stimulate CREB-mediated gene expression in neuronal cells [32].

Electrically induced depolarization at tetanic frequencies or K^+-induced depolarization both produce dual phase calcium transients in skeletal muscle cells in primary culture, which exhibit ryanodine-sensitive and IP3-sensitive components [65,69]. We have extended the study of transcriptional regulation by calcium microdomains to skeletal muscle cells in primary culture and to the C2C12 cell line. After depolarization of both cell types with K^+ or by electrical stimulation, which represents a more physiological stimulation paradigm that allows control over the number of pulses delivered as well as the use of a wide range of frequencies, we have determined the response of the transcription factors NFκB and NFAT. The calcium response of C2C12 cells to K^+ or electrical stimulation follows the same pattern displayed by primary myotubes [66,102]. The activation of both transcription factors requires cytoplasmic and nuclear calcium microdomains [12].

We have chosen to study NFκB based on previous reports describing its activation in skeletal muscle by physiological and pathological stimuli [83–86] and its calcium regulation, described in several systems [46,87–89]. As described above, calcineurin is responsible for NFAT calcium-dependent activity. NFAT has the ability to sense dynamic changes in intracellular calcium in several cell types, including lymphocytes [73,74,90] and striated muscle [78,82,91,92]. NFAT activation has been linked to striated muscle growth and development [93]; it has also been linked to the regulation of muscle hypertrophy [94,95] and to fiber type specification [96,97] albeit these two latter roles remain controversial [60,98].

We have found that both NFκB and NFAT are activated by high extracellular K^+ (brief sustained depolarization) and by electrical stimulation (fluctuating depolarization); both RyR- and IP3R-mediated release of calcium from intracellular stores contribute to NFκB activation but only RyR-mediated calcium release is involved in NFAT activation (Valdes et al., manuscript in preparation) [102]. These findings presumably indicate that activation of these two release channels produces different calcium microdomains. Furthermore, NFAT requires extracellular calcium while NFκB activation does not. In this regard, NFκB shares with CREB, c-fos and c-jun the capacity to be activated by depolarization even in the absence of extracellular calcium [77,102]. Additionally, we have found that while NFAT activation senses electrical stimulation frequency independently of the number of stimuli, NFκB activation does not discriminate among frequencies but varies according to the number of stimuli applied. These results strongly suggest that in skeletal muscle cells in culture different electrical stimulation patterns activate differently these two transcription factors. It remains to be tested if these differences are due to a very fine control of gene transcription produced by specific calcium microdomains.

Our data corroborate previous reports showing that in electrically stimulated rabbit primary skeletal muscle cultures, RyR-mediated calcium release causes NFAT activation [78]. The same result was obtained in K^+-depolarized C2C12 cells, together with an important role for extracellular calcium [82,99]. Regulation of NFAT-dependent transcription by calcium release, mediated by IP3R and RyR, has also been studied in adult avian skeletal muscle; in these fibers, transcriptional regulation of the slow myosin heavy chain 2 MyHC2 promoter was assayed [80,81,100]. The main findings on these studies are that the expression of the slow MyHC2 in fast PM muscle fibers is repressed by the endogenous RyR and IP3R activity [80,81]. These results might reflect the existence in adult muscle of additional complex mechanisms of NFAT regulation, as well as differences arising from the particular stimulatory patterns used.

We have also found recently that activity-dependent ROS generation is likely to stimulate, via generation of calcium microdomains, calcium-dependent intracellular signaling pathways in skeletal muscle cells. Thus, ROS generation increased after electrical field stimulation or K^+-induced depolarization of rat skeletal muscle cells in primary culture; additionally, myotubes exposed to hydrogen peroxide displayed RyR-mediated transient intracellular calcium signals, enhanced ERK1/2 and CREB phosphorylation and increased mRNA levels for the early genes c-fos and c-jun [101].

4. Concluding remarks

The results from many different laboratories presented in this review indicate unambiguously that both neurons and skeletal muscle cells generate specific calcium microdomains in response to stimulation. Depending on their intensity, time course and location, these calcium microdomains have the potential to engage specific signaling pathways, which by stimulating different calcium-dependent transcription factors can induce the expression of a particular gene, or set of genes, as required for specific cellular responses.

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