ARTICLE

Signal transduction and gene expression regulated by calcium release from internal stores in excitable cells

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ABSTRACT

Calcium regulation of several transcription factors involves different calcium-dependent signaling cascades and engages cytoplasmic as well as nuclear calcium signals. The study of the specific sources of calcium signals involved in regulation of gene expression in skeletal muscle has been addressed only recently. In this tissue, most cytoplasmic and nuclear calcium signals originate from calcium release from internal stores, mediated either by ryanodine receptor (RyR) or IP3 receptor (IP3R) channels. The latter are located both in the sarcoplasmic reticulum (SR) and in the nuclear membrane, and their activation results in long-lasting nuclear calcium increase. The calcium signals mediated by RyR and IP3R are very different in kinetics, amplitude and subcellular localization; an open question is whether these differences are differentially sensed by transcription factors. In neurons, it is well established that calcium entry through L-type calcium channels and NMDA receptors plays a role in the regulation of gene expression. Increasing evidence, however, points to a role for calcium release from intracellular stores in this process. In this article, we discuss how RyR-mediated calcium release contributes to the activation of the calcium-dependent transcription factor CREB and the subsequent LTP generation. We present novel results from our laboratory showing ERK-mediated CREB activation by hydrogen peroxide. This activation takes place in the absence of extracellular calcium and is blocked by inhibitory ryanodine concentrations, suggesting it is caused by redox activation of RyR-mediated calcium release.

Key words: Calcium signals; intracellular stores; transcription; oxidation; neurons; skeletal muscle

INTRODUCTION

Calcium plays an important role in the regulation of gene expression due to the fact that several steps of this process are calcium-sensitive. In particular, a number of studies have focused on the direct activation of transcription factors by calcium-activated signaling pathways (West et al., 2002; Dolmetsch, 2003, Deisseroth et al., 2003). Transcriptional activity requires both cytoplasmic and nuclear calcium signals. A cytoplasmic calcium rise is known to stimulate calcium-dependent cascades that activate different transcription factors, whereas a nuclear calcium increase can modify the transcription factors themselves as well as other elements that play a role in the general transcription machinery (Mellstrom and Naranjo, 2001). Calcium signals differing in amplitude, spatial and temporal properties can trigger different transcriptional responses (Dolmetsch et al., 1997, 1998; Chawla and Bading, 2001). These selective responses are accomplished by intracellular signaling pathways laid down in a sophisticated manner to enable cells to distinguish between calcium signals of differing origins and spatio-temporal properties.

CALCIUM REGULATION OF TRANSCRIPTION IN SKELETAL MUSCLE

Recent studies have focused on the signaling pathways, transcription factors, and gene expression patterns induced by exercise or depolarization of skeletal muscle, and in particular, on the resulting hypertrophy and fiber type changes (Sakamoto and Goodyear, 2002; Schiaffino and Serrano, 2002). In some cases, the role of calcium has been assessed increasing intracellular calcium by long-term exposure to a calcium ionophore (Kubis et al., 1997; Freyssenet et al., 1999, 2004; Friday and Pavlath, 2000; Allen and Leinwald, 2002), but the specific source of calcium has also been studied with inhibitors or activators of specialized calcium channels (Adams and Goldman, 1998; Vali et al., 2000; Powell et al., 2001; Ojuka et al., 2002, 2003; Carrasco et al., 2003a).

During an action potential, calcium entry in skeletal muscle is insignificant because the L-type skeletal muscle calcium channels act as voltage sensors, which through voltage-driven conformational changes stimulate RyR-mediated calcium release from the neighboring sarcoplasmic reticulum (SR) and not as calcium channels (Rios and Pizarro, 1991). Thus, extracellular calcium is not required for single contractions, which are due entirely to calcium release from the SR following depolarization-induced opening of RyR calcium release channels (Fill and Coppello, 2002). Nevertheless, significant calcium entry occurs during sustained stimulation of muscle fibers, such as during tetanic stimulation (Cairns et al., 1998).

Transcription regulation induced by calcium entry

Stimulation of a muscle cell by nerve activity controls fiber type. The AChR, which mediates synaptic transmission at the motor endplate, has a central role in this process. Calcium entry through L-type calcium channels affects the expression of nicotinic AChR subunits, and thus may be relevant to determine fiber type. In rat skeletal muscle primary cultures, the L-type channel agonist BayK 8644 decreases significantly the expression of the e subunit of the AChR, sparing the other subunits (Adams and Goldman, 1998). In a spontaneously contracting rat skeletal muscle cell line, the expression of the AChR α subunit increases following channel blockade with nifedipine (Vali et al., 2000).

Transcription regulation induced by RyR-mediated calcium release

Several studies show that RyR-mediated calcium release controls the expression of certain skeletal muscle proteins, including the nicotinic AChR subunits (Adams and Goldman, 1998; Vali et al., 2000). Stimulation of SR calcium release by thapsigargin or low ryanodine concentrations decrease the RNA content of all nAChR subunits, including the e subunit (Adams and Goldman, 1998). The inhibitory effects of calcium may be mediated at least partially by CaMKII (Macpherson et al., 2002; Tang et al., 2004). Incubation of a spontaneously contracting rat skeletal muscle cell line with 200 μM ryanodine (a concentration that inhibits RyR-mediated calcium release) does not affect AChR α subunit expression, but partially decreases the expression levels of glycogen phosphorylase (Vali et al., 2000). Dantrolene, an inhibitor of ryanodine-sensitive calcium release, and depletion of intracellular stores with thapsigargin produced similar effects (Vali et al., 2002).

A role for RyR-mediated calcium release in gene expression has also been described in the non-contracting skeletal muscle cell line L6 (Ojuka et al., 2002, 2003). Addition of caffeine increases the

expression of several mitochondrial enzymes and transcription factors involved in their regulation, and this effect is antagonized by dantrolene. The increase in expression of several mitochondrial proteins induced by caffeine is antagonized by an inhibitor of CaMKs (Ojuka et al., 2003).

Transcription regulation induced by calcium release from IP3-sensitive stores

Calcium release mediated by IP3 is responsible for a slow calcium increase induced by skeletal muscle depolarization (Jaimovich et al., 2000; Estrada et al., 2001; Araya et al., 2003). In addition to the fast calcium transient mediated by RyR channels, which drives muscle contraction, IP3R-mediated calcium release generates slower calcium transients (see Jaimovich and Espinosa, this issue). This IP3-induced calcium signal, which appears in the nuclei and also in the cytoplasm surrounding the nuclei and is not associated with muscle contraction, regulates several transcription-related events that follow membrane depolarization (Powell et al., 2001; Jaimovich and Carrasco, 2002; Carrasco et al., 2003a; Araya et al., 2003). In primary rat skeletal muscle cells, depolarization induces transient activation of ERK MAP kinases and the transcription factor CREB, as well as an increase in the early genes c-fos, c-jun and egr-1 mRNAs (Powell et al., 2001; Jaimovich and Carrasco, 2002; Carrasco et al., 2003a). The activation of these factors occurs in the absence of extracellular calcium or in the presence of high concentrations of ryanodine, but is significantly reduced by inhibitors of the IP3 system that block the slow calcium transient (Powell et al., 2001; Carrasco et al., 2003a; Araya et al., 2003). Both ERKs and the a isoform of the PKC family are necessary for CREB phosphorylation in these primary cells, and the activation of both ERK and PKCα specifically requires the calcium transient induced by the IP3 system (Carrasco et al., 2003a; Cárdenas et al., 2004).

The IP3R-mediated calcium increase in the nuclear region of skeletal muscle cells depolarized by K^+ lasts several seconds (Jaimovich et al., 2000). Yet, when myotubes are depolarized by electrical stimulation this slow calcium increase takes much longer (minutes) to fade away from the nuclei (Eltit et al., 2004). Since several transcription factors require a nuclear calcium increase to activate transcription, there might be a regulating role for the IP3-induced calcium signals at this level.

Our results, showing a specific involvement of calcium signals arising from IP3R-mediated calcium release from intracellular stores, certainly do not exclude the participation of RyR-induced calcium signals in the regulation of muscle gene expression. Considering the complexity of the mechanisms involved, to attribute this role solely to IP3R-induced calcium signals would be an oversimplification. As shown in other cellular systems, in skeletal muscle calcium signals of different spatial and temporal properties may activate transcription factors and the signaling cascades involved in their regulation. In particular, we have recently found that H_2O_2 induces intracellular calcium transients in skeletal muscle cells in culture even in Ca^{2+}-free medium; these signals are not affected by inhibitors of IP_3-mediated pathways but are blocked by 25 μM ryanodine (Leiva et al., 2004). In addition, H_2O_2 transiently increases ERK 1/2 and CREB phosphorylation and c-fos and c-jun mRNA levels; inhibitory ryanodine concentrations decreased the effect of H_2O_2 on early gene levels, suggesting involvement of RyR-mediated calcium release in their activation (Leiva et al., 2004).

A recent study shows that xestospongin, an inhibitor of the IP3R, fails to inhibit calcium signals necessary for the activation of calcineurin (CaN)-NFAT pathway involved in the initiation of fast-to-slow transformation in primary skeletal muscle cells (Kubis et al., 2003). In these studies, NFAT translocates to the nucleus following cyclic electrical stimulation at 1 Hz that produces short-lived intracellular calcium transients (with a 75% decay time of about 250 ms). The lack of effect of xestospongin makes unlikely the participation of IP3-generated calcium signals in NFAT activation. In fact, more than 200 pulses (each pulse of 1 ms) at a frequency of at least 10 Hz is required to evoke IP3-dependent slow calcium transients in primary skeletal muscle cells (Eltit et al., 2004). NFAT translocation to the nucleus is finely tuned to the pattern of electrical stimulation, as has been determined in adult skeletal muscle fibers (Liu et al., 2001). Further studies are required to establish whether the slow calcium transient modulates CaN-NFAT activation in primary skeletal muscle cells.

CALCIUM SIGNALING AND REGULATION OF TRANSCRIPTION IN NEURONS

Calcium is the main mediator of electrical activity-dependent gene expression in neurons. Neuronal calcium signals involved in gene expression and plasticity are initiated by calcium entry, which activates cytoplasmic signaling pathways that affect nuclear events, leading to the expression of genes that are essential for dendritic development, neuronal survival, and synaptic plasticity.

Increasing evidence points to a role for calcium released from intracellular stores as a source of the nuclear calcium increase required for activation of most calcium-regulated transcription factors (Berridge, 1998). In particular, a role of calcium-induced calcium release (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 Berridge, 1998; Meldolesi, 2001, and references therein). Both the IP3R and RyR present in the endoplasmic reticulum (ER) are calcium-sensitive release channels that exhibit CICR (see Foskett and Mak, Verhkrasty, and Friel, in this issue) and which may thus contribute to calcium-dependent gene expression in neurons. New findings providing a link between neuronal activity and calcium release from intracellular stores were reported recently in nerve terminals isolated from hypothalamic neurons (De Crescenzo et al., 2004). 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 calcium-regulated nuclear transcription factors. Depolarization-induced calcium release (DICR) in neurons is further discussed by Hidalgo et al., in this issue.

Calcium signals and CREB activation

Activation of the nuclear transcription factor CREB is crucial for several neuronal functions, including activity-dependent synaptic plasticity_a requisite for learning and memory (Silva et al., 1998; Lonze and Ginty, 2002; Lynch, 2004). Activation of CREB and the ensuing CRE-mediated transcription are required for LTP (long-term potentiation), a reversible increase in synaptic transmission inducible in several brain regions in response to tetanic stimulation of afferent fibers. LTP is considered a cellular model of synaptic plasticity and thus may underlie learning and memory. The calcium-dependence and regulation of CREB activation has been the subject of many studies because a number of genes activated by neuronal electrical activity, such as c-fos and BDNF, have functional CRE sequences in their promoters (Sheng et al., 1990; Tao et al., 1998).

CREB and ERK activation by calcium entry

Following neuronal depolarization, calcium entry through L-type calcium channels and NMDA receptors (NMDAR) initiates signaling cascades that result in nuclear CREB activation (Dolmetsch et al., 2001; Deisseroth et al., 2003). Nuclear CREB activation (phosphorylation) in the hippocampus is mediated by MAP kinases-specifically by the ERK1/2 pathway-and by the CaM kinase pathway, which phosphorylate CREB with different kinetics (Hardingham et al., 1999, 2001; Wu et al., 2001). These two pathways exhibit different requirements in terms of the localization of the activating calcium signal. The ERK signaling proteins are mainly cytoplasmic, and when activated by cytoplasmic calcium signals, they translocate to the nucleus together with RSK, their downstream CREB kinase (Xing et al., 1996). An increase in nuclear calcium is not necessary for ERK-mediated CREB activation (Hardingham et al., 2001). In contrast, CREB activation via the CaM kinase pathway requires an increase in nuclear calcium in order to activate nuclear CaMKIV (Chawla et al., 1998). In addition, CaMKIV and nuclear calcium may be necessary for CRE-mediated transcription, presumably via activation of the co-activator CBP (Chawla et al., 1998; Hu et al., 1999; Impey et al., 2002). These combined results indicate that both cytoplasmic or nuclear calcium signals can produce CREB activation.

A mechanism for ERK activation close to the plasma membrane mediated by calcium entry through L-type calcium channels has been described in cortical neurons (Dolmetsch et al., 2001). Selective ERK activation by these channels over other voltage-dependent calcium channels (P-, N-, P/Q), which also induce cytoplasmic and nuclear calcium signals, indicates that the route of calcium entry plays a central role in the regulation of transcription. A CaM-binding IQ motif present in the $\alpha 1$ subunit of L-type channels is necessary for signaling to ERK and for the induction of CREB activation (Dolmetsch et al., 2001). Other voltage-dependent calcium channels, however, also

present this motif, suggesting that other L-type channel features are responsible for their specificity. The α1c subunit terminal domain contains a PDZ binding domain, which also is critical for CREB activation, suggesting that a second requirement for the specificity of L-type channels may be the interaction between α1c and PDZ domain proteins (Weick et al, 2003).

There are other examples of specificity of the calcium entry channels that convey signals to the nucleus. The P/Q-type channels are involved in the activation of syntaxin A in transfected cells (Sutton et al., 1999), and CREB activation or inactivation by synaptic or extrasynaptic NMDARs in hippocampal cultures, respectively, has been reported (Hardingham et al., 2002). 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 (Sutton et al., 1999).

A role for calcium stores in synaptic plasticity

Participation of calcium release from intracellular stores in LTP induction has been described (Auerbach and Segal, 1994; Wang et al., 1996; Reyes-Harde et al., 1999; Lu and Hawkins, 2002; Lauri et al., 2003; Matias et al., 2003; Lynch, 2004). LTP induction in most hippocampal synapses requires a rise in intracellular postsynaptic calcium mediated by NMDAR, with contributions from L-type channel activation and intracellular calcium stores (Matias et al., 2003; Lynch 2004). In the CA1 hippocampal region, RyR blockade significantly reduces tetanic LTP as well as NO- and cGMP-induced LTP (Lu and Hawkins, 2002). These three types of potentiation (tetanic, NO, cGMP) are accompanied by an increase in P-CREB immunofluorescence in postsynaptic neurons in hippocampal slices, which is significantly reduced following RyR inhibition. These results point to a well-defined postsynaptic location of the RyR channels involved. A presynaptic effect of NO resulting in induction of long-term depression (LTD) as well as LTP in the same hippocampal region has also been described (Reyes-Harde et al., 1999). A contribution of RyR, in this case presynaptic, has been suggested by a markedly reduction of LTD by ryanodine inhibitory concentrations. With respect to LTD, interestingly, a switch from LTD to LTP was induced at the hippocampal dentate gyrus by a low stimulatory ryanodine concentration, suggesting that RyR-mediated calcium release plays a significant role in LTP induction (Wang et al., 1996).

Presynaptic RyR also contribute to LTP generation in mossy hippocampal fibers (Lauri et al., 2003). This form of LTP is independent of NMDARs; instead, calcium entry is mediated by presynaptic kainate glutamate receptors, which initiate a cascade involving RyR-mediated calcium release and which is abolished by inhibitory ryanodine concentrations. In addition, caffeine (10 mM) can evoke LTP in CA1 hippocampal neurons, which is different from the classical LTP since it does not require post-synaptic receptor activation or calcium increase in this area (Martin and Buño, 2004). This new form of LTP is generated presynaptically, through the interaction of caffeine with presynaptic purinergic receptors and RyR channels. The resulting increased calcium signals enhance neurotransmitter release.

The contribution of IP3Rs to LTP induction has been studied as well. RyRs are present in greater abundance than IP3Rs in the spines of CA1 hippocampal cells, while IP3Rs are more abundant in the dendritic shafts of these cells (Sharp et al., 1993). The possible role of glutamatergic metabotropic receptors has been particularly assessed, with dissimilar results (Lynch, 2004). Cholinergic receptors have been also considered as an additional trigger for LTP induction (reviewed in Lynch, 2004). Carbachol can induce LTP in the hippocampus in the absence of extracellular calcium, also indicating a possible role for IP3-dependent stores in this response (Auerbach and Segal, 1994). The role of IP3 receptors in LTP and LTD induction in the hippocampal CA1 region was studied in mutant mice lacking the IP3R1 isoform (Nagase et al., 2003). There was no essential requirement of IP3R1 activity for the induction of LTP or LTD. Mutant mice, however, exhibited higher magnitude LTP when compared to the wild type, suggesting a physiological suppressive effect of IP3R1 on LTP that needs further study.

From the NFATc family of transcription factors, the NFATc4 isoform has been identified in the hippocampus and shown to initiate gene expression following periods of heightened synaptic activity, which may have importance in long-term changes in cell excitability (Graef et al., 1999; Groth and Mermelstein, 2003). NFAT-mediated transcription is dependent on the activity of the calcium-calmodulin phosphatase calcineurin (Crabtree, 2001). One NFAT target, IP3R1, presents

increased expression in response to both depolarization (Graef et al., 1999) and to BDNF-induced NFAT-dependent transcription (Groth and Mermelstein, 2003). Activation of NFAT by depolarization requires calcium entry through L-type calcium channels, while the effect of BDNF is independent of calcium entry and requires IP3-dependent calcium release from stores (Graef et al., 1999; Groth and Mermelstein, 2003). The study of BDNF downstream effects 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 (Pozzo-Miller et al., 1999).

CREB and ERK activation by calcium release from intracellular stores

The activation of several neurotransmitter responsive receptors in the hippocampus-including metabotropic glutamate receptors, dopamine receptors and muscarinic ACh receptors-stimulates ERKs and CREB (Roberson et al., 1999). Presumably, receptor activation generates IP3 and produces IP3-mediated calcium release signals that cause ERK and CREB stimulation, but this point has not been tested experimentally. Another study in hippocampus cells in culture describes enhanced CREB phosphorylation and c-fos expression following nicotine-induced activation of ionotropic ACh receptors (Hu et al., 2002). Thapsigargin or 10 μM ryanodine reduced these effects to background levels. A role for calcium release from intracellular stores in CREB phosphorylation, through the activation of the metabotropic P2Y2 receptors by ATP or UTP, has also been described in sensory neurons (Molliver et al., 2002). In this cellular model, the activation of P2Y2 receptors induces sustained trains of action potentials; the resulting calcium entry also contributes to CREB phosphorylation. Only the concerted inhibition of both sources of calcium signals blocked CREB activation (Molliver et al., 2002). Striatal neurons represent another example of CREB activation by calcium released from intracellular stores (Zanassi et al., 2001). 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.

In hippocampal neurons, the induction of action potentials by an antagonist of GABA receptors results in NMDAR-mediated CREB activation (Hardingham et al, 2001). In this model, 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 (Hardingham et al, 2001).

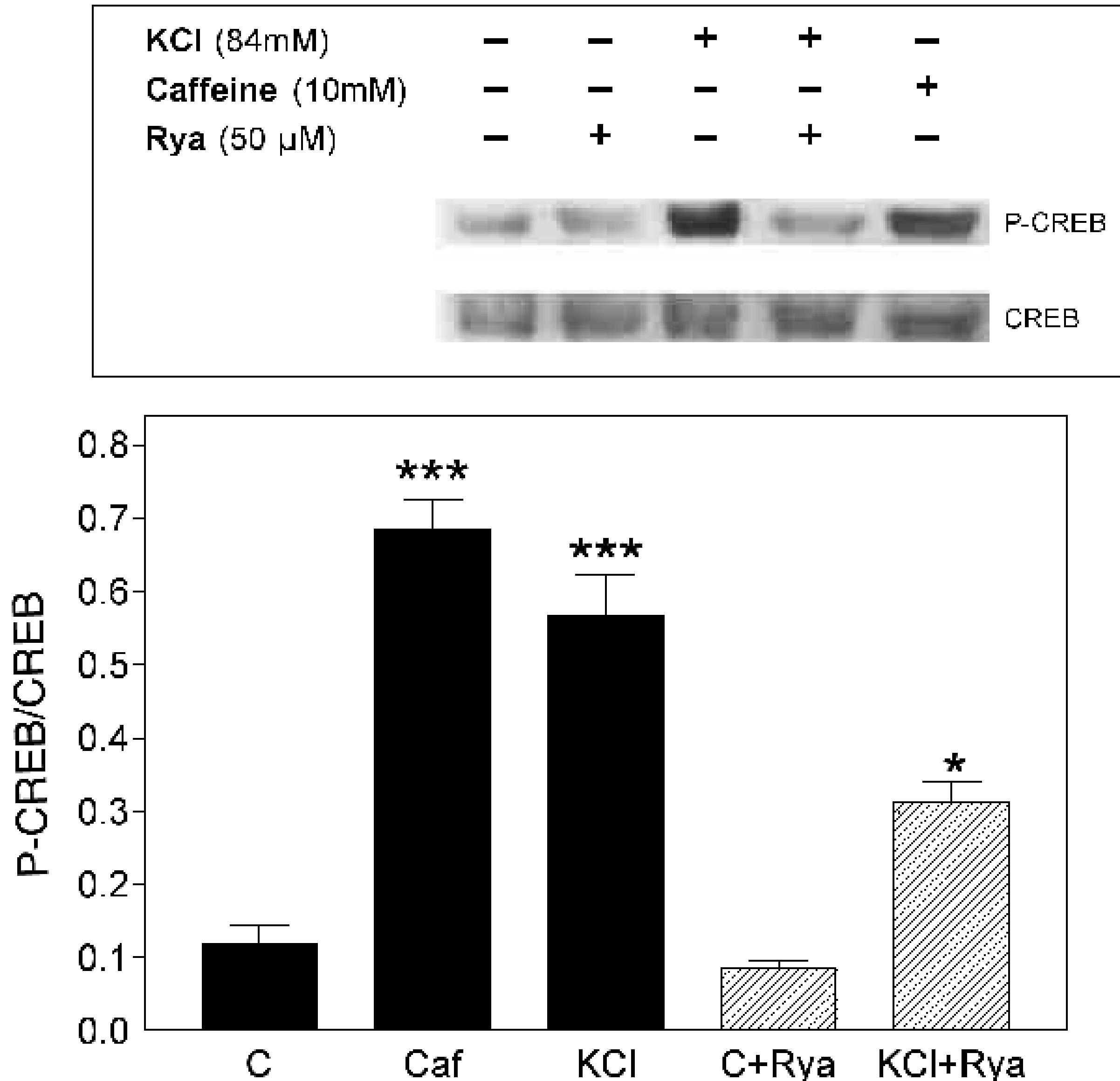
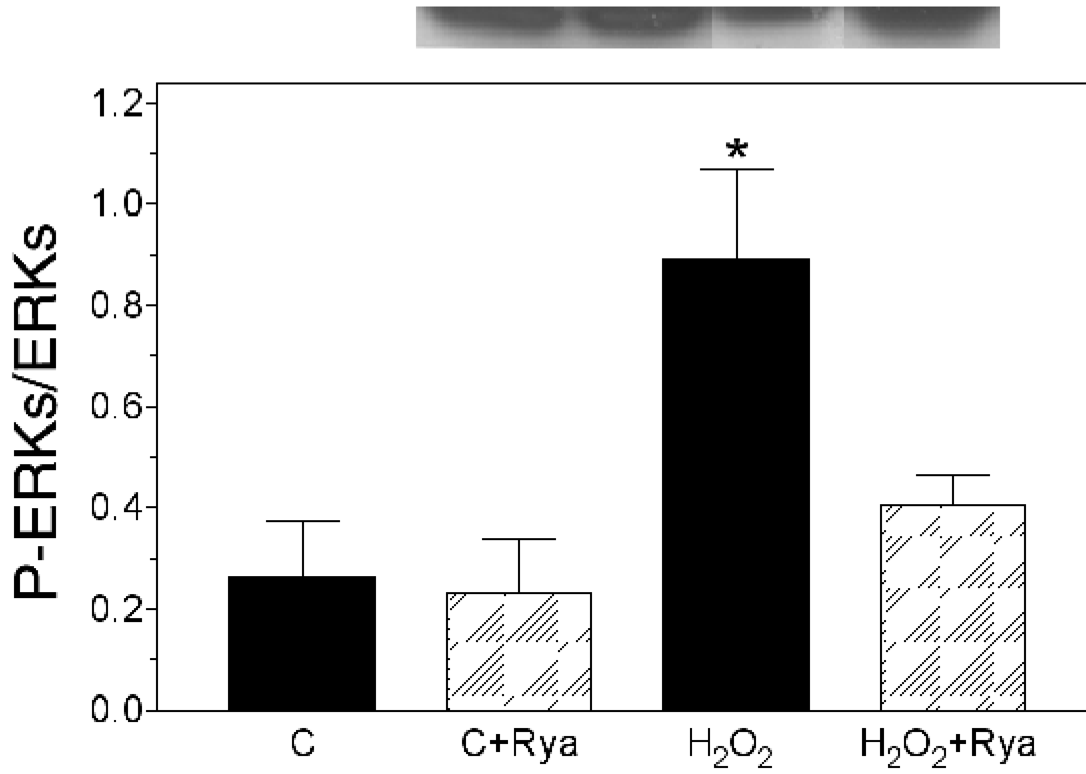


Figure 1: CREB phosphorylation in neuronal N2a cells. Cells were washed with PBS and maintained for 60 min under resting conditions in Krebs-Ringer medium. After the preincubation period, cells were exposed to 10 mM caffeine for one min, or depolarized in the absence or presence of 50 μM ryanodine. In the latter case, ryanodine was also present during the preincubation period. Depolarization was induced by changing cells to a medium containing 84 mM KCl for 10 min. After stimulation, cells were solubilized and analyzed by Western blotting with specific antibodies against P-CREB and CREB.

Upper panel: Western blot analysis of P-CREB and total CREB as loading control.

Lower panel: P-CREB over total CREB levels of cells exposed to caffeine or 84 mM KCl in the absence (solid bars) or in the presence of ryanodine (hatched bars). Results are expressed as mean ± SE of 3 to 5 independent experiments. The significance of differences was evaluated using ANOVA followed by Newman-Keuls's multiple comparison post test. (***): $P< 0.001$ compared with untreated controls. The difference between cells exposed to KCl and to KCl plus ryanodine was significant, with $P< 0.01$. Values obtained in cells exposed to KCl plus ryanodine were significantly different to values obtained in cells exposed only to ryanodine, with $P < 0.05$ (*). Differences between control cells and cells exposed to ryanodine were not statistically significant.

We have found that calcium release from intracellular stores contributes to depolarization-induced CREB activation in neurons in culture. In the neuroblastoma cell line N2a, depolarization induced by high K^+ concentration or caffeine stimulate CREB phosphorylation (Carrasco et al., 2003b; Kemmerling et al., 2004). As illustrated in Figure 1, caffeine stimulated CREB phosphorylation 5.7-fold, whereas K^+-induced depolarization stimulated CREB phosphorylation 4.7-fold. Inhibitory concentrations of ryanodine reduced by a third depolarization-induced CREB phosphorylation, suggesting that RyR-mediated calcium release is partly responsible for the observed stimulation. We have also studied CREB and ERK activation in N2a cells (Figure 2) and in hippocampal primary culture cells exposed to the oxidant agent H_2O_2 (Kemmerling et al., 2004). In both cell types, exogenous H_2O_2 activates CREB and ERKs; these effects are independent of extracellular calcium and are inhibited significantly by ryanodine concentrations that completely inhibit RyR channel-mediated calcium release (Figure 2). Furthermore, thapsigargin antagonizes the increase in P-CREB levels induced by H_2O_2. These results strongly suggest that RyR activation by oxidation, which has been demonstrated by several groups (see Hidalgo et al., this issue), enhances calcium release from the ER and activates CREB and ERK phosphorylation.



H_2O_2 (200 μM) – – + +

Rya (50 μM) – + – +

Figure 2: H_2O_2 stimulation of ERK and CREB phosphorylation in N2a cells; Effect of ryanodine. Cells were washed with PBS and maintained for 60 min under resting conditions in Krebs-Ringer medium in the presence or absence of 50 mM ryanodine. After the preincubation period, cells were incubated for 20 min in 200 μM H_2O_2 in the presence or absence of ryanodine.

Upper panel: Western blot of phosphorylated ERK1/2 and ERK as loading control. Bar graphs represent the ratio between P-ERK1/2 over total ERK expressed as the average-fold increase (mean ± SE, n= 3). (*): $P < 0.05$ compared with the untreated control.

Lower Panel: Western blot analysis of P-CREB and total CREB as loading control. Bar graphs represent mean ± SE (n=4) of P-CREB over total CREB levels of cells exposed to H_2O_2 in the absence or in the presence of ryanodine. The increase in CREB phosphorylation was significant with respect to the control group; (***) $P < 0.001$.

In both the upper and the lower panel, values obtained in cells incubated with H_2O_2 plus ryanodine were not significantly different from control values or from the values obtained in cells incubated only with ryanodine.

CONCLUSIONS

A summary scheme illustrating the routes involved in CREB activation in skeletal muscle cells or neurons is illustrated in Figure 3. In skeletal muscle cells, we have determined that depolarization generates slow calcium signals due to IP3-induced calcium release, which activate CREB and transcription of the early genes *c-fos*, *c-jun* and *egr-1*. This activity-dependent gene transcription may underlie the plasticity of skeletal muscle toward exercise or to the lack of exercise that leads to atrophy. In neurons, we propose that redox-activated RyR-mediated calcium release makes a significant contribution to the generation of intracellular calcium signals that enhance CREB phosphorylation at the nucleus. This redox-dependent stimulation of neuronal calcium release signals may have a significant role in controlling the expression of the genes involved in activity-dependent, long-term neuronal responses.

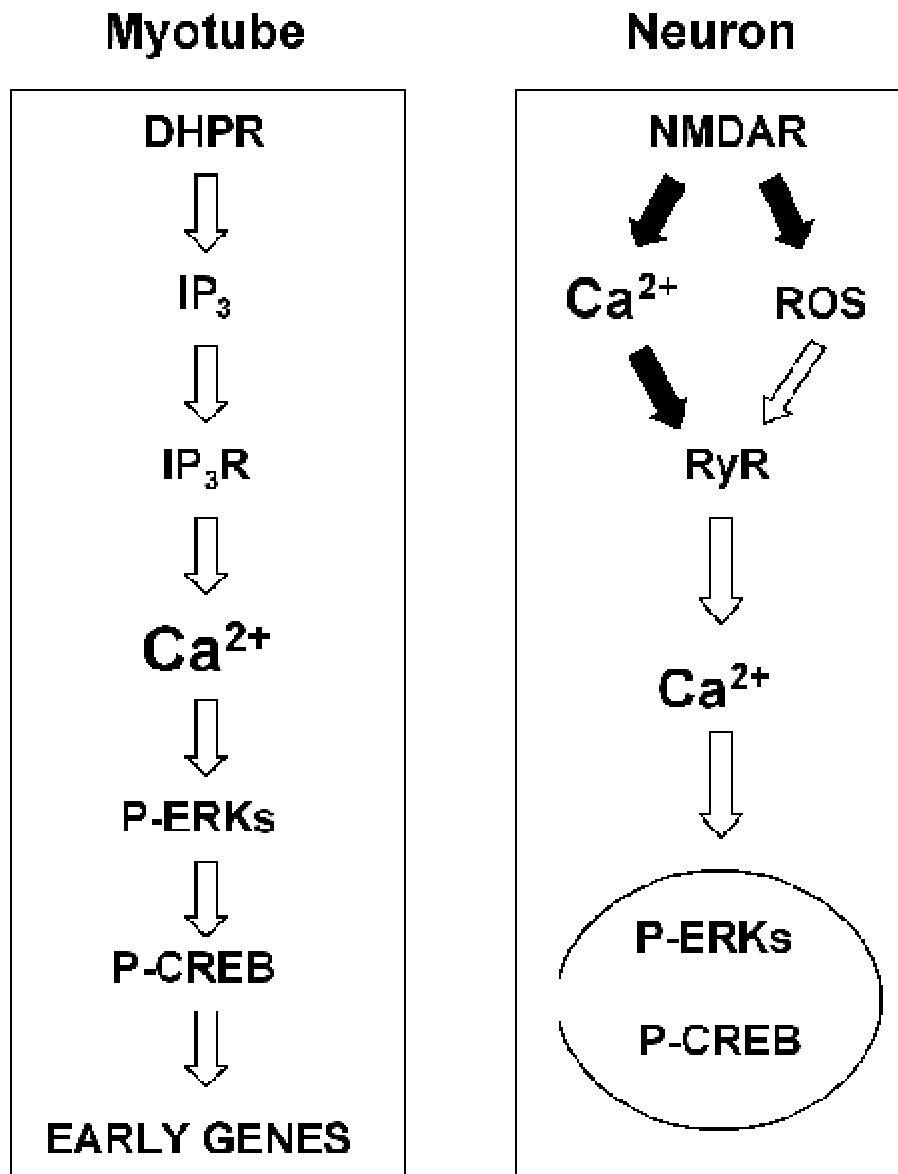


Figure 3: Scheme depicting the pathways that link calcium increase to ERK and CREB activation in skeletal muscle and neurons. The scheme for skeletal muscle cells shows published results obtained in our laboratory and described in detail in the text. Membrane depolarization, through activation of DHPR results in IP3-induced calcium release; the ensuing activation of ERK, CREB and early genes depends on the slow calcium transients evoked by IP3R stimulation. In neurons, activation of NMDAR allows calcium entry and ROS/RNS generation (solid arrows), as described in the text. We have found H2O2-induced activation of ERK and CREB that is antagonized by ryanodine, suggesting redox-induced calcium release mediated by RyR may contribute to activation of calcium-dependent transcription factors in neurons.

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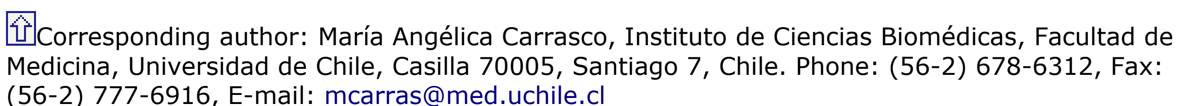

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