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IP_3-dependent intracellular Ca^{2+} release is required for cAMP-induced *c-fos* expression in hippocampal neurons

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ABSTRACT

Ca^{2+} and cAMP are widely used in concert by neurons to relay signals from the synapse to the nucleus, where synaptic activity modulates gene expression required for synaptic plasticity. Neurons utilize different transcriptional regulators to integrate information encoded in the spatiotemporal dynamics and magnitude of Ca^{2+} and cAMP signals, including some that are Ca^{2+}-responsive, some that are cAMP-responsive and some that detect coincident Ca^{2+} and cAMP signals. Because Ca^{2+} and cAMP can influence each other's amplitude and spatiotemporal characteristics, we investigated how cAMP acts to regulate gene expression when increases in intracellular Ca^{2+} are buffered. We show here that cAMP-mobilizing stimuli are unable to induce expression of the immediate early gene *c-fos* in hippocampal neurons in the presence of the intracellular Ca^{2+} buffer BAPTA-AM. Expression of enzymes that attenuate intracellular IP_3 levels also inhibited cAMP-dependent *c-fos* induction. Synaptic activity induces *c-fos* transcription through two *cis* regulatory DNA elements – the CRE and the SRE. We show here that in response to cAMP both CRE-mediated and SRE-mediated induction of a luciferase reporter gene is attenuated by IP_3 metabolizing enzymes. Furthermore, cAMP-induced nuclear translocation of the CREB coactivator TORC1 was inhibited by depletion of intracellular Ca^{2+} stores. Our data indicate that Ca^{2+} release from IP_3-sensitive pools is required for cAMP-induced transcription in hippocampal neurons.

1. Introduction

In neurons, synaptic activity-induced alterations in cellular Ca^{2+} and cAMP levels elicits changes in gene expression that are required for long-lasting synaptic plasticity. The importance of transcriptional changes induced by these two signaling molecules for synaptic plasticity is exemplified by studies on the protein synthesis-dependent late phase of long-term potentiation (LTP) in the mammalian hippocampus. LTP is a much-studied form of synaptic plasticity that is induced by high frequency electrical stimulation of a group of axons that cause depolarization of the postsynaptic membrane leading to an increase in postsynaptic Ca^{2+}. At hippocampal synapses between the CA3 Schaffer collaterals and CA1 commissural neurons the increase in postsynaptic Ca^{2+} is mediated by the activation of the NMDA receptor and triggers signaling pathways leading to the genomic response needed to enhance synaptic transmission for long periods [1]. In addition to an increase in postsynaptic Ca^{2+}, LTP induced by electrical stimulations of CA3 axons also requires adenylyl cyclase (AC) activity highlighting the importance of cAMP signaling [2]. In fact, LTP can also be induced by bath application of pharmacological compounds that elevate intracellular cAMP – this chemically-induced form of LTP was initially thought to be a result of direct activation of transcription by cAMP [3] but is in fact dependent on NMDA receptor activation [4]. Thus, whatever the LTP inducing stimulus, electrical or chemical, it mobilizes both Ca^{2+} and cAMP signaling cascades. This is not surprising given that neurons have a variety of mechanisms by which changes in the concentration of Ca^{2+} affect cellular cAMP levels and vice versa. For example, adenylyl cyclases and phosphodiesterases that are activated or inhibited by Ca^{2+} can influence cellular cAMP levels [5,6]. Similarly, cAMP can affect cellular Ca^{2+} transients

Abbreviations: CRE, cAMP response element; CREB, cAMP response element binding protein; IP_3, inositol 1,4,5 triphosphate; SRE, serum response element; BAPTA-AM, 1,2-bis(2-aminophenoxy) ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid acetoxymethyl ester; EGTA-AM, ethyleneglycol-bis(β-aminoethyl)-*N*,*N*,*N*′,*N*′-tetra acetoxymethyl ester; MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; TORC, transducers of regulated CREB activity; NMDA, *N*-methyl-D-aspartate; AC, adenylyl cyclase; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.

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through its direct action on the IP_3 receptor [7] and indirect effects on L-type Ca^{2+} channels, Ryanodine receptors (RyR), IP_3 receptors, PMCA and phospholamban all of which are targets of the cAMP-effector PKA [8]. In addition, there is also considerable crosstalk in the downstream signaling pathways that are activated by Ca^{2+} and cAMP. For example, Ca^{2+} and cAMP elevation can lead to activation of the mitogen-activated protein kinases, ERK1/2 albeit by different mechanisms [9,10].

Given the crosstalk between Ca^{2+} and cAMP, both at the level of the messengers themselves and at the level of downstream effectors, here we investigated how cAMP acts to regulate hippocampal gene expression in the absence of appreciable changes in intracellular Ca^{2+}. We used the intracellular Ca^{2+} buffer BAPTA-AM to attenuate Ca^{2+} in cells that were exposed to cAMP mobilizing stimuli and found that the expression of the immediate early gene *c-fos* was inhibited. We compared the inhibitory effects of BAPTA-AM on cAMP-induced *c-fos* expression to the effects of depleting intracellular Ca^{2+} stores and suppressing IP_3-induced Ca^{2+} release by overexpression of IP_3 metabolizing enzymes. Since *c-fos* transcription is regulated by two *cis* regulatory DNA elements – the SRE and the CRE, we examined the effect of attenuating IP_3 on cAMP-induced SRE-mediated and CRE-mediated gene expression.

2. Materials and methods

2.1. Hippocampal cell culture and transfection

Primary hippocampal neurons from neonatal Wistar rats were cultured and transfected as described previously [11]. All animal use was in accordance with UK Home Office regulations. Neurons were transfected after 8–9 days in culture with plasmids expressing IP_3 metabolizing enzymes using Lipofectamine 2000 (Invitrogen). Expression plasmid tdTomato-IP_3 5-phosphatase contains the open reading frame of human Type 1 IP_3 5-phosphatase with tdTomato tagged at the N-terminus. IP_3kinase A-tdTomato encodes full length IP_3kinase A with tdTomato fluorescent protein fused to its C-terminus. Transfected cells were stimulated as indicated 24–48 h after transfection. To increase intracellular cAMP neurons were treated with 10 μM forskolin or 250 μM 8-(4-chlorophenylthio) adenosine-3′,5′-cyclic monophosphate (CPT-cAMP). The membrane of hippocampal neurons was depolarized by increasing the extracellular KCl concentration to 50 mM. Expression of c-Fos protein was analyzed 2 h after stimulation by immunofluorescence. For hippocampal neurons that were subjected to luciferase assays, stimulations were performed for 4 h.

2.2. Immunofluorescence and Western blotting

Neurons grown on glass coverslips were processed for immunocytochemistry as described previously [11]. Immunofluorescence images were acquired on an inverted Zeiss Laser scanning confocal system (Carl Zeiss, Germany) and analyzed using ImageJ software (National Institute of Health, Bethesda, MD). More than 100 cells were analyzed for each pharmacological maneuver from three independent experiments. In experiments that involved transfection of IP_3 metabolizing enzymes, around 25–40 transfected cells were analyzed. Five untransfected cells surrounding each transfected cell were selected. Western blotting and analysis of CREB phosphorylation was done as described [11].

2.3. Luciferase assay

Hippocampal neurons cultured in 24-well plates were co-transfected with either 0.25 μg td-tomato or 0.25 μg td-tomato-IP_3 5-phosphatase (tdTomato IP5P), or 0.25 μg IP_3kinase A-tdTomato along with 0.25 μg of a luciferase reporter plasmid (either $pCRE_4$-Luc, driven by four copies of the *c-fos* CRE, or $pSRE_5$-Luc, driven by five copies of the *c-fos* SRE). The plasmid pRL-TK (Promega, Madison, WI), which expresses a *Renilla luciferase* under the constitutively active thymidine kinase promoter, was included in all transfections to control for variations in transfection efficiency. Luciferase assays were performed using the Dual Luciferase Reporter Assay System (Promega) using a TD-20e Turner luminometer. Firefly luciferase activity was normalized to the R. luciferase signal and all measurements were made in duplicate. The results are presented as mean ± SEM. Statistical analysis was performed using a Student's *t*-test.

3. Results

3.1. IP_3-dependent intracellular Ca^{2+} release is required for cAMP-induced c-fos expression

Hippocampal neurons in dissociated culture form synapses and show spontaneous action potential firing after 8–9 days in culture [12]. We treated such hippocampal neurons with the adenylyl cyclase activator forskolin to elevate neuronal cAMP levels and examined the effect of BAPTA-AM on cAMP-induced c-Fos protein expression by immunofluorescence. Fig. 1A shows examples of hippocampal neurons displaying increased c-Fos immunofluorescence in response to forskolin. Quantitative analysis of the immunofluorescence (Fig. 1B) revealed that forskolin induces a 3.36 ± 0.58-fold increase in c-Fos protein expression. The increase in forskolin-induced c-Fos immunoreactivity was completely abolished when neurons were preloaded with BAPTA-AM prior to forskolin stimulation (Fig. 1). BAPTA-AM also attenuated c-Fos induction when the cell-permeant cAMP analogue CPT-cAMP was used to elevate intracellular cAMP levels independently of adenylyl cyclases (data not shown). The Ca^{2+} chelator BAPTA is able to interfere with both global and submembraneous Ca^{2+} transients generated locally near Ca^{2+} influx or Ca^{2+} release sites. We therefore compared the effects of BAPTA with another Ca^{2+} chelator EGTA that has a similar equilibrium affinity for binding Ca^{2+} but an on-rate that is 150 times lower than that of BAPTA and is consequently effective at chelating global Ca^{2+} while allowing signals that occur very locally around Ca^{2+} channels to persist [13]. While BAPTA completely abolished forskolin-induced c-Fos protein expression, EGTA had a very small effect on forskolin-induced c-Fos levels reducing it by 23%. These results suggest that in hippocampal neurons cAMP requires a submembraneous Ca^{2+} signal to induce c-fos gene expression. In neurons, Ca^{2+} influx through L-type voltage-sensitive Ca^{2+} channels (VSCCs) and NMDA receptors activates signaling cascades that are triggered by a local Ca^{2+} pool near the mouth of the channel to induce gene expression [14,15]. We therefore investigated the effects of the L-type VSCC antagonist nifedipine and the NMDA receptor blocker APV on cAMP-induced c-fos expression. Fig. 1B shows that compared to BAPTA (74% reduction), APV and nifedipine when applied together had only a small effect on cAMP-induced gene expression (25% reduction).

We next tested whether the submembraneous Ca^{2+} signal required for cAMP-induced *c-fos* expression in hippocampal neurons is provided by Ca^{2+} release from intracellular stores. We used the SERCA inhibitor thapsigargin to deplete the endoplasmic reticulum (ER) Ca^{2+} store. Fig. 2A shows that thapsigargin pretreatment reduced forskolin-induced c-Fos levels in hippocampal neurons by 48% while c-Fos protein levels in KCl stimulated cells was reduced by 74%. This suggests that similar to KCl, forskolin-induced c-Fos expression requires Ca^{2+} release from intracellular Ca^{2+} stores. In neurons, RyR and IP_3 receptors (IP_3R) both contribute to Ca^{2+} release from stores and while the contribution of RyR-mediated

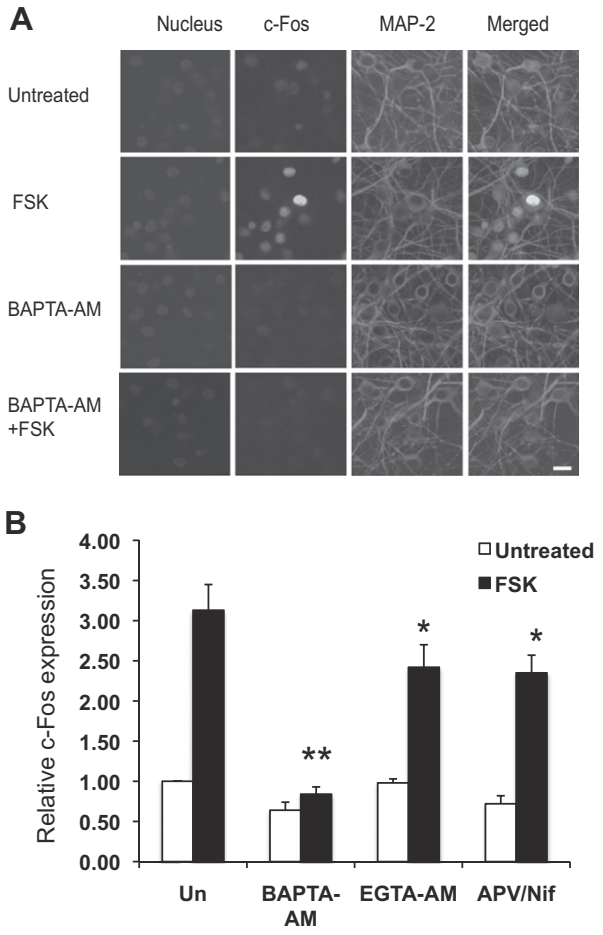

Fig. 1. Intracellular Ca^{2+} is required for cAMP-induced *c-fos* expression in hippocampal neurons. (A) Representative images of c-Fos immunofluorescence in hippocampal neurons after 50 μM BAPTA pretreatment for 30 min followed by stimulation with 10 μM forskolin (FSK) for 2 h. MAP2 staining identifies neurons and Hoechst 33342 stain was used to identify nuclei. Scale bar is 10 μm. (B) A graph showing quantitative analysis of c-Fos immunoreactivity in neurons left untreated or stimulated with 10 μM forskolin (FSK) with or without a 30 min pretreatment with 50 μM BAPTA-AM, or 50 μM EGTA-AM or APV and nifedipine. Values are shown as mean ± SEM. and are from three independent experiments, **indicates significant reduction of c-Fos expression in BAPTA-AM treated cells when compared to levels in FSK stimulated cells $p < 0.001$ (Student's *t*-test). *Indicates significant reduction in c-Fos protein levels compared to levels in FSK stimulated cells $p < 0.05$ (Student's *t*-test).

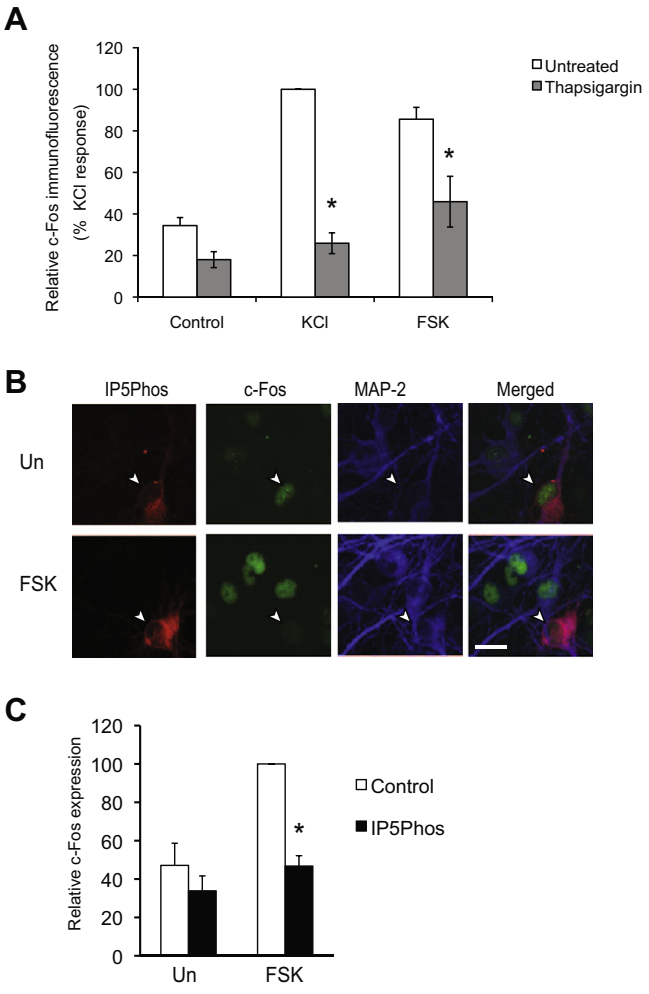

Fig. 2. IP_3-induced Ca^{2+} release from the endoplasmic reticulum is required for forskolin-induced c-Fos expression. (A) c-Fos immunofluorescence was quantified in unstimulated neurons (control) and neurons stimulated for 2 h with either 50 mM KCl (KCl) or 10 μM forskolin (FSK) with or without (untreated) a 30 min pretreatment with 10 μM thapsigargin. Data are from three independent experiments and shown as mean ± SEM. *Indicates significant reduction in thapsigargin treated neurons compared to untreated neurons in the corresponding stimulation. $p < 0.05$ (Student's *t*-test). (B) Representative examples of tdTomato tagged Type I IP_3 5-phosphatase transfected hippocampal neurons showing c-Fos immunofluorescence (green) in neurons (MAP2 shown in blue) transfected with 1 μg tdTomato-IP_3 5-phosphatase (IP5Phos shown in red) compared to surrounding non-transfected cells. Cells were left untreated or stimulated for 2 h with 10 μM forskolin (FSK) 36 h post-transfection. Scale bar is 10 μm. (C) Graph showing average c-Fos immunofluorescence in tdTomato-IP_3 5-phosphatase (IP5Phos) transfected and surrounding untransfected (Control) cells. Cells were stimulated 36 h post-transfection for 2 h with 10 μM forskolin (FSK) or left untreated (U). Data are from three independent transfection experiments and is shown as mean ± SEM. *Indicates significant decrease in c-Fos immunofluorescence in IP5Phos transfected cells when compared to c-Fos expression in surrounding untransfected cells. $p < 0.05$ (students *t*-test).

Ca^{2+} release to neuronal gene expression is well documented [16] the role of IP_3R-dependent Ca^{2+} release is less well studied. We therefore examined the effect of expressing the IP_3 metabolizing enzyme, Type I IP_3 5-phosphatase on the induction of *c-fos* by cAMP. Expression of IP_3 5-phosphatase has previously been shown to blunt histamine-induced Ca^{2+} transients in HeLa cells [17]. Hippocampal neurons were transfected with tdTomato tagged Type I IP_3 5-phosphatase and the relative level of c-Fos protein was compared with the surrounding untransfected cells. We found that Type I IP_3 5-phosphatase activity decreased c-Fos protein expression by 62.57% in forskolin treated cells (Fig. 2B and C).

3.2. IP_3-induced Ca^{2+} release contributes to both CRE-mediated and SRE-mediated gene expression

The *c-fos* gene contains two key *cis* regulatory sequences the cAMP response element (CRE) and the serum response element (SRE) that mediate its transcriptional induction. We therefore investigated how expression of Type I IP_3 5-phosphatase affects expression of a luciferase reporter gene expression driven by either the CRE or the SRE. In hippocampal neurons expressing the empty vector td-tomato, CRE-driven luciferase reporter gene was induced 6.5-fold by forskolin while SRE-mediated gene expression was induced 4-fold. In neurons transfected with the Type I IP_3-5 phosphatse forskolin-induced CRE reporter gene expression decreased by 38% (Fig. 3A) and forskolin-stimulated SRE-luciferase reporter gene was reduced to a lesser extent (by 29%). We also examined the effect of another IP_3 metabolizing enzyme-IP_3kinase A, which inhibits IP_3-induced Ca^{2+} signals [17], on the ability of cAMP to induce CRE-mediated gene expression. Similar to the effect of the IP_3

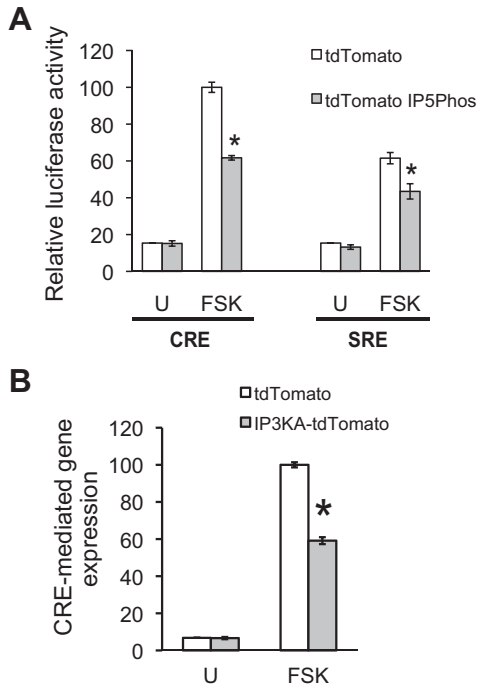

Fig. 3. IP_3 metabolizing enzymes attenuate cAMP-induced SRE and CRE-mediated gene expression. (A) Effect of Type I IP_3 5-phosphatase on CRE-luciferase and SRE-luciferase reporter gene expression. Hippocampal neurons were co-transfected with tdTomato vector or the tdTomato tagged Type I IP_3 5-phosphatase (tdTomato IP5Phos) along with pRL-TK and the indicated luciferase reporter plasmid ($pCRE_4$-Luc or $pSRE_5$-Luc). Cells were stimulated 48 h post-transfection with 10 μM forskolin (FSK) for 4 h or left untreated (U). Data are from six independent experiments and the mean ± SEM is shown here. *Indicates a significant decrease in luciferase activity by tdTomato IP5Phos expression when compared to the corresponding stimulation in tdTomato transfected cells ($p < 0.05$, Student's t-test). (B) Effect of IP_3kinase A on CRE-luciferase reporter gene expression. Neurons were co-transfected with tdTomato vector or the tdTomato tagged IP_3kinase A (IP_3KA-tdTomato) along with pRL-TK and $pCRE_4$-Luc. Cells were stimulated 48 h post-transfection with 10 μM forskolin (FSK) for 4 h or left untreated (U). Data are from three independent experiments and shown as mean ± SEM. *Indicates significant reduction in CRE-mediated expression compared to neurons transfected with vector tdTomato. $p < 0.05$ (Student's t-test).

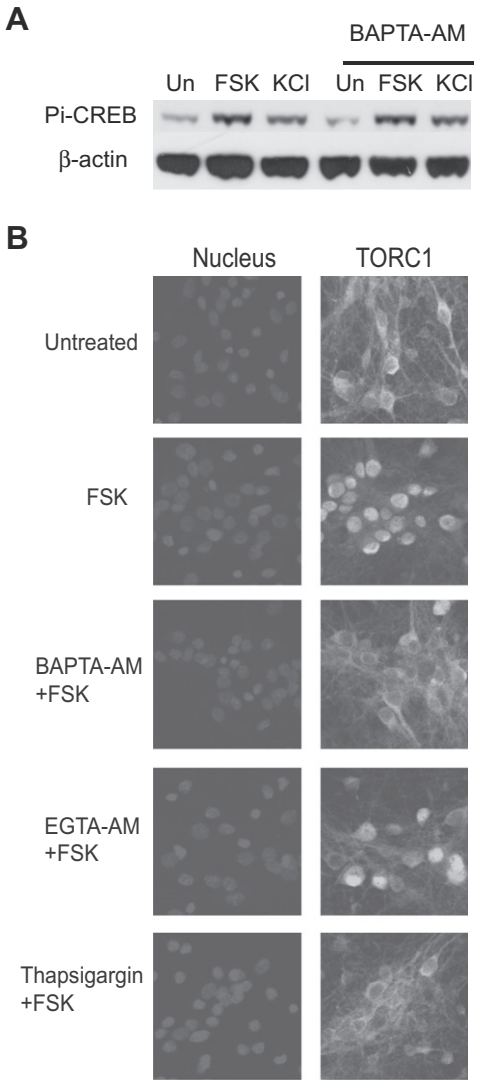

Fig. 4. A submembraneous Ca^{2+} pool is required for cAMP-induced TORC1 nuclear translocation but not for CREB phosphorylation. (A) Representative Western blot showing CREB phosphorylation induced by forskolin and KCl (top panel). BAPTA-AM pretreatment for 30 min did not affect CREB phosphorylation. Hippocampal neurons cultured in 35 mm dishes were treated with 10 μM forskolin (FSK) or 50 mM KCl or left untreated (Un), with or without a 30 min pretreatment with 50 μM BAPTA-AM. Cells were lysed for protein harvest 30 min after treatment. CREB phosphorylation was detected using a rabbit antibody specific for CREB phosphorylation on serine 133 and anti-rabbit secondary antibody. Blots were stripped and reprobed using an antibody for β-actin to normalize for protein loading in each well. (B) Representative images of hippocampal neurons showing the effects of BAPTA-AM, EGTA-M and thapsigargin on cAMP-induced nuclear translocation of TORC1. Hippocampal neurons were stimulated with 10 μM forskolin (FSK) for 1 h after a 30 min pretreatment with 50 μM BAPTA-AM or 50 μM EGTA-AM or 10 μM thapsigargin. TORC1 localization was assessed using a rabbit antibody specific for TORC1. Hoechst 33342 stain was used to identify nuclei. Scale bar is 10 μm.

5-phosphatase, expression of IP_3KA-tdTomato in hippocampal neurons led to a decrease in the forskolin-induced CRE-driven luciferase induction by 40.9% (Fig. 3B). Taken together, our data implicate a contribution of IP_3-induced Ca^{2+} release to CRE-dependent gene expression in response to cAMP signals in hippocampal neurons.

3.3. Intracellular Ca^{2+} release is required for cAMP-induced TORC1 nuclear translocation

In hippocampal neurons CRE-dependent gene expression in response to cAMP is mediated by the transcription factor CREB. Activation of CREB by cAMP requires its phosphorylation on serine 133 by PKA [18]. We therefore assessed the ability of cAMP to induced CREB phosphorylation in hippocampal neurons loaded with BAPTA-AM using an antibody that recognizes serine 133 phosphorylated CREB. Fig. 4A shows that forskolin treatment induces a robust increase in CREB phosphorylation at serine 133 and this is unaffected by BAPTA-AM. Thus, cAMP is able to trigger CREB phosphorylation when Ca^{2+} signals are buffered but is unable to induce expression of the CREB-dependent gene *c-fos*. The activation of CREB-mediated gene expression can also occur independently of CREB phosphorylation on serine 133 via recruitment of the CREB coactivators called transducers of regulated CREB activity (TORC). TORCs interact with the basic leucine zipper domain of CREB and accumulate in the nucleus in response to Ca^{2+} and cAMP signals [18–20]. Of the three TORC family members that have been identified (TORC1, TORC2 and TORC3), TORC1 is most abundant in the mammalian hippocampus where it has been shown to be involved in the late phase of LTP [21,22]. Ca^{2+} or cAMP induce nuclear translocation of TORCs and this involves their dephosphorylation. TORC1 dephosphorylation in response to cAMP occurs due to cAMP-mediated inhibition of salt-inducible kinases (SIK1 and SIK2) that phosphorylate TORCs [23] and the effects of Ca^{2+} are

mediated by the phosphatase calcineurin. Here we examined the effects of BAPTA-AM on TORC1 nuclear translocation by cAMP in hippocampal neurons. Fig. 4B shows that forskolin potently induces nuclear translocation of TORC1 (Fig. 4B compare first and second row) The forskolin-induced nuclear accumulation of TORC1 is abolished in BAPTA-AM loaded neurons but not in EGTA-AM loaded cells (Fig. 4B). Depletion of intracellular Ca^{2+} stores with thapsigargin prior to forskolin treatment also inhibited nuclear import of TORC1 (Fig. 4B). This indicates that similar to *c-fos* gene expression, TORC1 nuclear import in response to cAMP requires Ca^{2+} release from intracellular stores.

4. Discussion

During synaptic activity Ca^{2+} influx through L-type VSCCs and NMDA receptors and increases in intracellular cAMP levels couple electrical activity to biochemical signals that regulate neuronal gene expression. In hippocampal neurons, Ca^{2+} release from intracellular stores has been shown to be important for gene induction triggered by Ca^{2+} influx through L-type VSCCs and NMDA receptors. Here, we show that intracellular Ca^{2+} stores, via IP_3-induced Ca^{2+} release, also contribute to hippocampal gene expression mediated by cAMP activated pathways.

We first demonstrated that in hippocampal neurons, *c-fos* expression in response to cAMP is attenuated by BAPTA-AM but not by EGTA-AM implicating a spatially restricted submembraneous Ca^{2+} pool in the cAMP induction of *c-fos*. We investigated the identity of the BAPTA-sensitive Ca^{2+} pool. The Ca^{2+} signal in the immediate vicinity of synaptic NMDA receptors [15] and L-type VSCCs [14] activates the extracellular signal-regulated kinase (ERK1/2) and this might explain why cAMP-induced *c-fos* gene expression is compromised by BAPTA-AM but not EGTA-AM. However, we found that inhibition of NMDA receptors and L-type VSCCs by coapplication of APV and nifedipine had a very small effect on cAMP-induced *c-fos* gene expression. This is in contrast to a previous report [24] that identified a role for NMDA receptors in cAMP-induced gene expression in young cortical neuron cultures in which synaptogenesis had not yet occurred. Instead, we find that the BAPTA-sensitive Ca^{2+} pool that is essential for cAMP-induced *c-fos* expression in synaptically active hippocampal neurons derives from intracellular Ca^{2+} stores–depletion of the ER store by pharmacological means with the SERCA inhibitor thapsigargin attenuates cAMP-induced *c-fos* expression. To complement our findings with the pharmacological inhibition of SERCA, we used genetic approaches that suppressed Ca^{2+} release specifically from IP_3 receptors. We overexpressed IP_3 dephosphorylating enzyme Type I IP_3 5-phosphatase and IP_3kinase A, which converts IP_3 to the metabolite IP_4, that effectively blunt IP_3-dependent Ca^{2+} release [17]. We show here that in both type I IP_3 5-phosphatase and IP_3-kinase A transfected neurons the induction of *c-fos* by cAMP was markedly reduced. One possible explanation for this finding lies in the ability of the cAMP effector PKA to potentiate Ca^{2+} release from intracellular stores – such potentiation could be required for cAMP to induce gene expression. PKA has been reported to phosphorylate both RyR and IP_3 receptors increasing their open probability and Ca^{2+}conductance [8]. More recently, cAMP has been shown to directly activate Ca^{2+} release from the IP_3 receptor [7].

Our findings raise questions about the signaling events that make intracellular Ca^{2+} release an obligatory step in cAMP-induced *c-fos* expression in hippocampal neurons. We show here that Ca^{2+} released from IP_3 receptors in response to intracellular increase in cAMP levels is important for both CRE-mediated and also, albeit to a lesser extent, for SRE-mediated gene expression. In cultured striatal neurons, intracellular Ca^{2+} release induced by PKA leads to CREB phosphorylation via the ERK pathway, by activating the Ras homologue Rap1 and its effector Raf-like kinase B-Raf [25]. It is possible that in hippocampal neurons such crosstalk between cAMP/PKA and IP_3 receptors potentiates Ca^{2+} release from IP_3 receptors, which then activates CREB phosphorylation and the SRE-interacting transcription factors SRF and Elk-1 via the ERK pathway. However, we found that cAMP is able to induce robust CREB phosphorylation in the presence of BAPTA-AM. This led us to investigate how cAMP-induced nuclear translocation of the CREB coactivator TORC1 is affected and we show that intracellular Ca^{2+} release is required for TORC1 nuclear translocation by cAMP. While it has been reported that in hippocampal neurons TORC1 nuclear shuttling in response to coincident cAMP and Ca^{2+} signals requires calcineurin activation [22], a role for intracellular Ca^{2+} release in cAMP-induced TORC1 translocation has not been previously been identified in neuronal cells. IP_3-induced Ca^{2+} release has however very recently been implicated in TORC2 activation by the hormone glucagon, which acts by mobilizing cAMP production, in hepatocytes [26]. Taken together, our data indicate that IP_3-dependent intracellular Ca^{2+} release is necessary for cAMP to induce gene expression in hippocampal neurons.

Acknowledgments

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