The Ca\(^{2+}\) hypothesis of transcription suggests that increments in the cytosolic Ca\(^{2+}\) concentration \([\text{Ca}^{2+}]_c\), can activate transcription. The Ca\(^{2+}\) hypothesis has emerged in the field of neurosciences\(^{1-2}\) and was translated to vascular smooth muscle cells (VSMC) by the Burlington group.\(^3\)-\(^5\) The article by Pulver et al in this issue\(^6\) demonstrates a close correlation between the increase in \([\text{Ca}^{2+}]_c\), phosphorylation of nuclear CREB, and the expression of c-fos. Pulver et al introduce the idea that transcriptional activation may be regulated by store-operated Ca\(^{2+}\) entry (SOCE)\(^7\)-\(^9\) as it is activated when thapsigargin blocks the Ca\(^{2+}\)-ATPase SERCA, thereby depleting the SR Ca\(^{2+}\) content. The authors demonstrate this thapsigargin effect not only in cultured VSMC but also in VSMC of intact arteries. They conclude that SOCE may activate transcription and may therefore control the differentiated (contractile) or proliferative (secretory) phenotype of VSMC.\(^10\)

Ca\(^{2+}\)-Mediated Phosphorylation of CREB

The Ca\(^{2+}/\text{cAMP} \) response element binding protein CREB is a transcription factor.\(^1\) CREB transcriptional activation occurs through its binding to the CREB-binding protein, CBP, a co-activator protein that links to many factors of the general transcriptional machinery. Phosphorylation of CREB at Ser-133 increases its affinity for CBP, and it can be observed with phospho-specific antibodies. CREB can be phosphorylated not only at SER-133 but also at S-142. Phosphorylation is mediated by a variety of kinases, with the cAMP/PKA pathway often being the principal modulator.\(^12\) In the case of Ca\(^{2+}\)-dependent activation, CREB phosphorylation is thought to result from the following signaling cascade:\(^13\) Ca\(^{2+}\) binds to calmodulin, Ca\(^{2+}/\text{CaM}\) enters the nucleus and activates calmodulin-dependent kinase IV (CaMKIV), with Ca\(^{2+}\)/CaMKIV phosphorylating CREB. In addition, Ca\(^{2+}/\text{CaM}\) activates CaMKIV kinases that substantially enhance Ca\(^{2+}\)/CaMKIV activity. Further, Ca\(^{2+}\)/CaMKIV can regulate CREB binding protein by direct phosphorylation.\(^13\) Whether and to what extent these cascades apply to VSMC have not yet been clarified.

Ca\(^{2+}\) Controls Transcription Beyond CREB

Ca\(^{2+}/\text{CaM}\) modulates transcriptional activation via signaling cascades in addition to the CREB cascade. For example, Ca\(^{2+}/\text{CaM}\) activates the phosphatase calcineurin (CaN) that activates NFAT.\(^14\) Dephosphorylated NFAT can enter the nucleus, where it binds to and activates CBP in synergy with MEF2. Diverse transcription-dependent forms of neuronal plasticity suggest that the Ca\(^{2+}\) concentration in the cytosol, \([\text{Ca}^{2+}]_c\), and in the nuclear matrix, \([\text{Ca}^{2+}]_\text{nu}\), can modulate transcription differentially.\(^15\) For example, \([\text{Ca}^{2+}]_\text{nu}\) is known to activate the retinoic acid orphan receptor via Ca\(^{2+}/\text{CaMKIV}\). \([\text{Ca}^{2+}]_\text{nu}\) activates members of the MEF2 family of transcription factors via Ca\(^{2+}/\text{CaMKIV}\) phosphorylation. Independently of CaM, elevated \([\text{Ca}^{2+}]_\text{nu}\) can activate p38, which in turn phosphorylates and activates MEF2.\(^13\) Increments in \([\text{Ca}^{2+}]_\text{nu}\) and Ca\(^{2+}/\text{CaM}\) have been reported to inhibit transcription when they activate Cabin-1, which competes with CBP for binding to MEF2. Because Cabin-1 associates with histone deacetylases 1 and 2, its dissociation from MEF2 may replace a repressing complex with an activating complex. Thus, Ca\(^{2+}/\text{CaMKIV}\) could enhance MEF2 transcription in an indirect way.\(^16\)

The list of cascades involved in the Ca\(^{2+}\) modulation of transcription could be extended. It has been previously reported that elevated Ca\(^{2+}\) modulates transcription not through a single pathway, but via multiple cascades. Extrapolation of the present knowledge from neuronal to vascular tissue requires a re-analysis of the existence and function of these components. The use of transgenic mice will likely further our understanding of which one of the cascades described is important for VSMC. Future experiments should extend global measurements of \([\text{Ca}^{2+}]_\text{nu}\), through Ca\(^{2+}\) imaging to provide information on whether the Ca\(^{2+}\) concentration increases close to the sarcolemma, either in the central parts of the cytosol or within the nuclear matrix.

Elevated \([\text{Ca}^{2+}]_\text{nu}\) Results From Multiple Ca\(^{2+}\) Fluxes

The cellular distribution of Ca\(^{2+}\) ions is tightly controlled. In VSMC, \([\text{Ca}^{2+}]_\text{nu}\), increases as consequences of passive Ca\(^{2+}\) influx through the sarcolemma (Ca\(^{2+}\) channels and nonselective cation channels) and Ca\(^{2+}\) release from the sarcoplasmic reticulum SR (ryanodine and IP\(_3\) receptors). \([\text{Ca}^{2+}]_\text{nu}\), decreases because of ATPases that transport Ca\(^{2+}\) ions into the extracellular space (PMCA) and into the SR Ca\(^{2+}\) store (SERCA). In addition, \([\text{Ca}^{2+}]_\text{nu}\), decreases when Ca\(^{2+}\) ions bind to the
mobile and fixed buffers in the cytosol and translocate into cell compartments such as the mitochondria (Ca\(^{2+}\) uniporter in the inner mitochondrial membrane) or the nucleus (pathways are controversial at present).

Ca\(^{2+}\) influx through dihydropyridine-sensitive L-type Ca\(^{2+}\) channels generates the Ca\(^{2+}\) current \(I_{\text{L-\text{Ca}}}\). In VSMC, \(I_{\text{L-\text{Ca}}}\) has been thoroughly studied. The results help to explain how \(I_{\text{L-\text{Ca}}}\) increases [Ca\(^{2+}\)], and activates contraction, and how drugs relax VSMC (eg, dihydropyridines blocking Ca\(^{2+}\) channels; K\(^+\) channel openers gating Ca\(^{2+}\) channels). Whereas L-type Ca\(^{2+}\) channels are voltage-gated, a small (+10 mV) change in membrane potential has been shown to increase [Ca\(^{2+}\)], by 75 nM.\(^{17}\) The influence of voltage-gated \(I_{\text{L-\text{Ca}}}\) is often demonstrated by measuring increments in [Ca\(^{2+}\)], or in contractile force by superfusing solutions with elevated [K\(^{+}\)].

The article by Pulver et al\(^{16}\) asks whether transcriptional activation can be activated by additional Ca\(^{2+}\) influx pathways. The authors demonstrate that SOCE can both increase [Ca\(^{2+}\)], and activate CREB phosphorylation and c-fos expression. They also defined the components of the cascade by means of “specific” inhibitors: dihydropyridines for \(I_{\text{L-\text{Ca}}}\), calmidazolium for CaM, and KN-93 for inhibition of calmodulin-dependent kinases.

Although these results are easy to accept in context with membrane physiology, it becomes problematic to apply these results to transcriptional control in vivo, where SERCA is active. Sun et al compared the increments in [Ca\(^{2+}\)], caused by thapsigargin with those caused by administration of serum, an intervention known to stimulate IP\(_3\)-mediated Ca\(^{2+}\) release.\(^{20}\) Serum transiently increased [Ca\(^{2+}\)], and the comparison between increments in [Ca\(^{2+}\)], caused by to serum and thapsigargin suggests that [Ca\(^{2+}\)], can be reliably measured with chameleon Ca\(^{2+}\) indicator. The comparison, however, does not address the point of whether the “physiological” serum-induced increment in [Ca\(^{2+}\)], can activate CREB phosphorylation at all or to an extent similar to post-thapsigargin application.

These unanswered questions should not exclude SOCE as a potentially important player in the Ca\(^{2+}\) control of transcription. Typically, SOCE caused by IP\(_3\)-induced SR Ca\(^{2+}\) release can be measured only if SERCA was inhibited (thapsigargin), or if Ca\(^{2+}\) was heavily buffered by millimolar concentrations of EGTA or BAPTA.\(^{8,9}\) Recent work on white blood cells (RBL-1 cells) demonstrates that IP\(_3\)-induced Ca\(^{2+}\) transients can activate SOCE at physiological conditions, providing that the mitochondria take-up Ca\(^{2+}\) from SOCE.\(^{21}\) In other words, in the absence of exogenous buffers and without competition from noninhibited SERCA, the Ca\(^{2+}\) ions released from SR are rapidly transferred into mitochondria. Mitochondria may be suitable for just such a job, because they colocalize with the domains of Ca\(^{2+}\) entry via SOCE and with the SERCA in the ER. In this way, mitochondria could reduce the rate and amount of refilling SR with Ca\(^{2+}\) and can therefore reduce the inactivation of SOCE. These results suggest that SOCE can significantly contribute to the physiological Ca\(^{2+}\) balance of leukocytes.\(^{9}\) Whether similar arguments apply to SOCE in VSMC has yet to be analyzed.

**Ca\(^{2+}\) Control of Transcription in VSMC: Where Do We Stay?**

Neuroscience suggests that the Ca\(^{2+}\) control of transcription can adapt cell function to a wide variety of demands, from synaptic plasticity to cell metabolism. The diversity of the effects is attributed to the distribution of the Ca\(^{2+}\) signals over time and space. In neurons, the time domain is the interval between the groups of action potentials.\(^{22}\) The space domain depends on the place where the Ca\(^{2+}\)-permeable neuronal synapses localize. With regard to long-term potentiation, Ca\(^{2+}\) influx through L-type Ca\(^{2+}\) channels has a higher efficacy than Ca\(^{2+}\) influx via NMDA receptors.\(^{23}\) The results suggest that the colocalization of the Ca\(^{2+}\)-permeable membrane channel with submembranous proteins could be a key factor. The information changes, for example, after Ca\(^{2+}\) ions have bound to diffusible proteins (such as CaM)—it can slowly spread over longer distances than can the Ca\(^{2+}\) ions, whose diffusion is fast but limited in space by Ca\(^{2+}\) binding. The effects of increments in cytosolic [Ca\(^{2+}\)], have been separated from those of elevated nuclear [Ca\(^{2+}\)]\(_{\text{nuc}}\), ie, these 2 events seem to modulate different Ca\(^{2+}\)-dependent cascades.\(^{24}\) Understanding how [Ca\(^{2+}\)], and [Ca\(^{2+}\)]\(_{\text{nuc}}\) operate together is only the beginning.
In comparison with neuroscience, vascular biology analyzes the “Ca\(^{2+}\)” control of transcription” with fewer groups and over a shorter time period. The article by Pulver et al. is an important step in furthering our understanding. However, it is also an example that much work is still needed to provide information on specific signaling cascades, to differentiate effects caused by cytosolic from those caused by nuclear Ca\(^{2+}\), and to find out if the state of mitochondrial respiration modulates the efficacy by which SOCE can contribute to increments in [Ca\(^{2+}\)], or in transcription. Expanding on the work of the Burlington group will surely further our understanding of VSMC, their plasticity between differentiated contractile phenotype, and the proliferating phenotype involved in the development of vascular pathologies. Any new developments will likely profit from the neurosciences, because any new studies will initially apply similar questions, models, and methods as those already used in the neurosciences field. Pulver et al. have introduced the idea that transcription may be activated when the Ca\(^{2+}\) stores are emptied. Whether this hypothesis holds true when the store is emptied by more physiological, agonist-induced SR Ca\(^{2+}\) release remains to be tested. Regardless, ideas and results presented by Pulver et al. will certainly stimulate the community of cardiovascular biologists to further test and study the Ca\(^{2+}\) hypothesis in future experiments.

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