Smooth Muscle Cell Cycle and Proliferation

RELATIONSHIP BETWEEN CALCIUM INFLUX AND SARCO-ENDOPLASMIC RETICULUM 
Ca\(^{2+}\) ATPase REGULATION

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From the ‡Institut National de la Santé et de la Recherche Médicale Unité 348, IFR Circulation Lariboisière, Hôpital Lariboisière, 8 rue Guy Patin 75010 Paris, France, §SANOFI Recherche, Centre de Toulouse, 195 Route d’Espagne BP 1169, 31036 Toulouse Cedex, France, and †Laboratorium voor Fysiologie, Katholieke Universiteit, Campus Gasthuisberg, 3000 Leuven, Belgium

The role of Ca\(^{2+}\) influx in the regulation of the sarcoplasmic reticulum Ca\(^{2+}\) ATPases (SERCA) associated with intracellular Ca\(^{2+}\) pools was investigated during smooth muscle cell (SMC) proliferation induced by platelet-derived growth factor (PDGF). We first defined that the previously described up-regulation of the SERCA2a isoform found in vascular SMC after a 24-h stimulation with PDGF (Magnier, C., Papp, B., Corvazier, E., Bredoux, R., Wuytack, F., Eggermont, F., Maclouf, J., and Enouf, J. (1992) J. Biol. Chem. 267, 15808–15815) was precisely associated with SMC entry into S phase as it appeared linked with \(^{3}H\)thymidine incorporation. This was further confirmed by testing the effect of transforming growth factor-β\(_{1}\), which inhibited both aortic SMC proliferation associated with G\(_{i}\) cell cycle arrest and PDGF-induced SERCA2a up-stimulation. Then, we tested the role of Ca\(^{2+}\) influx by using SR 33805, a new Ca\(^{2+}\) channel blocker, which was characterized with regard to the voltage Ca\(^{2+}\) channel blocker nifedipine and the capacitative entry Ca\(^{2+}\) blocker SKF 96365. SR 33805 was found to be the most potent inhibitor of both PDGF-induced SMC proliferation and the associated rise in intracellular Ca\(^{2+}\) concentration with IC\(_{50}\) values of 0.2 ± 0.1 and 0.31 ± 0.04 μM, respectively. Finally, by examining in parallel both SERCA2a and SERCA2b isoforms, in terms of activity and expression, we could determine that PDGF-induced stimulation of total SERCA activity (detected by formation of the phosphorylated intermediate, E–P) and of SERCA2a expression (Western blotting) were abolished when extracellular Ca\(^{2+}\) entry was prevented by SR 33805. This study demonstrates that SERCA2a up-regulation is: 1) related to the G\(_{i}/S\) transition step of cell cycle and 2) dependent on Ca\(^{2+}\) entry during PDGF-induced SMC proliferation.

Calcium is an essential regulator of living cells, and it controls numerous aspects of the cellular physiology, including cell proliferation (1). An intracellular rise in cytosolic Ca\(^{2+}\) concentration occurs upon cell activation due to both Ca\(^{2+}\) influx from the extracellular medium and Ca\(^{2+}\) release from intracellular storage Ca\(^{2+}\) pools (for reviews, see Refs. 2 and 3). Ca\(^{2+}\) channels in the plasma membrane allow Ca\(^{2+}\) influx following a stimulation due to membrane depolarization (voltage-operated channels) (3, 4) or binding of a ligand to its receptor (receptor-operated channels) (5). Intracellular Ca\(^{2+}\) pools are also strongly implicated in the increase in cytosolic Ca\(^{2+}\) concentration by liberating their Ca\(^{2+}\) content into the cytosol through intracellular Ca\(^{2+}\) channels: the inositol 1,4,5-trisphosphate (IP\(_{3}\)) receptor (6) and the ryanodine receptor (7).

This increase in cytosolic Ca\(^{2+}\) concentration is retro-controlled by the activation of Ca\(^{2+}\) transport ATPases which decrease the Ca\(^{2+}\) concentration of the cytosol. Plasma membrane Ca\(^{2+}\) ATPases (PMCA) (8) eliminate Ca\(^{2+}\) from the cell by trans-plasma membrane Ca\(^{2+}\) transport, and sarco-endoplasmic reticulum Ca\(^{2+}\) ATPases (SERCA) (9) enzymes reaccumulate cytosolic Ca\(^{2+}\) in intracellular Ca\(^{2+}\) storage pools. A close relationship between Ca\(^{2+}\) influx and efflux mechanisms involved in the regulation of cytosolic Ca\(^{2+}\) concentration has been formulated: indeed, according to the capacitative Ca\(^{2+}\) entry model, the gating of Ca\(^{2+}\) entry across the plasma membrane may be controlled by the depletion of intracellular stores (10–12).

In view of the central role intracellular Ca\(^{2+}\) pools play in cell signaling, the activity of the associated SERCA pumps could conceivably control cell proliferation. Indeed, a study which made use of the SERCA inhibitor thapsigargin, suggested that SERCA proteins may act as regulators of cell growth, by controlling growth- and transformation-related genes (c-fos and c-jun) (13). Subsequent studies suggested that profound alterations of the proliferative system of the DDT, MF-2 smooth muscle cell line were caused by the emptying of the intracellular thapsigargin-sensitive Ca\(^{2+}\) pools and demonstrated that SERCA expression and Ca\(^{2+}\) pool function are closely associated with growth and proliferation of these cells (14–16). In the meanwhile, we could demonstrate the implication of the SERCA in a physiopathological model of cell proliferation by showing the specific up-regulation of the SERCA2a isoform during platelet-derived growth factor (PDGF)-induced smooth muscle cell (SMC) proliferation (17).

The abbreviations used are: IP\(_{3}\), inositol 1,4,5-trisphosphate; SERCA, sarco-endoplasmic reticulum Ca\(^{2+}\) ATPase; PMCA, plasma membrane Ca\(^{2+}\) ATPase; PDGF, platelet-derived growth factor; SMC, smooth muscle cell(s); TGF-β\(_{1}\), transforming growth factor-β\(_{1}\); DMEM, Dulbecco’s modified Eagle’s medium; FCS, fetal calf serum; E–P, phosphorylated intermediate; DTT, dithiothreitol; PAGE, polyacrylamide gel electrophoresis.
The aim of the present study was first to further precise the time course of this up-regulation of the SERCA2a isoform. Since it has been shown earlier that the different phases of the cell cycle exhibit each a different sensitivity to Ca\textsuperscript{2+} ions (18), we looked for a relationship between SMC cycle and SERCA expression during PDGF-induced aortic SMC proliferation. We further tested the effect of the transforming growth factor-\(\beta_1\) (TGF-\(\beta_1\)), an inhibitor of SMC entry into S phase (19). In addition, we attempted to change the loading state of the SMC Ca\textsuperscript{2+} pools during cell proliferation by interfering with the Ca\textsuperscript{2+} influx. We used a new analog of fantofarone, SR 33805, a potent Ca\textsuperscript{2+} antagonist as shown by its binding to the \(\alpha_1\)-subunit of the t-type voltage channel, because it: (i) presented a vascular selectivity, (ii) exhibited a severalfold more efficiency than other Ca\textsuperscript{2+} blockers in PDGF-induced SMC proliferation, and (iii) was previously shown to strongly reduce myointimal thickening following endothelial injury (20–22). We characterized this Ca\textsuperscript{2+} channel antagonist with regard to its effects on PDGF-induced SMC proliferation and Ca\textsuperscript{2+} influx, compared it to the hydroxyindole Ca\textsuperscript{2+} blocker, nifedipine, it well as to the capacitative entry blocker, SKF 96365, and SR 33805. After 24 h or 3 days in culture, cells were detached from triplicate wells by trypsin treatment (0.05% trypsin and 0.02% EDTA) and counted in a Coulter counter (Coultronics, France).

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We previously established a specific up-regulation of SERCA2a isoform after a 24 h-stimulation of porcine aortic SMC with PDGF-BB both by measuring SERCA activity and expression studies (17). To further point out the possible role of this PDGF-induced SERCA2a expression in cell proliferation, we looked for a correlation between the SERCA expression and a specific step of cell cycle.

**RESULTS**

**SERCA2a Expression and the Cell Cycle**

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followed by a 1-h pulse of \[^3H\]thymidine (Fig. 1). Between 0 and 16 h of PDGF stimulation, no incorporation of \[^3H\]thymidine was observed. PDGF-induced \[^3H\]thymidine incorporation began to increase only after 20 h, corresponding to the cell entry into S phase. This rise in \[^3H\]thymidine incorporation continued and reached a maximal value after 34 h of stimulation by PDGF. \[^3H\]Thymidine incorporation subsequently decreased to the basal level for periods of over 48 h of stimulation.

**Effect of PDGF on the Cell Cycle of Porcine SMC**

We first defined the SMC cycle by following the incorporation of \[^3H\]thymidine into SMC for time periods between 0 and 48 h stimulation with PDGF. After a 96-h depletion of serum, quiescent SMC were stimulated for 0–48 h by 5 ng/ml PDGF-BB followed by a 1-h pulse of \[^3H\]thymidine (Fig. 1). Between 0 and 16 h of PDGF stimulation, no incorporation of \[^3H\]thymidine was observed. PDGF-induced \[^3H\]thymidine incorporation began to increase only after 20 h, corresponding to the cell entry into S phase. This rise in \[^3H\]thymidine incorporation continued and reached a maximal value after 34 h of stimulation by PDGF. \[^3H\]Thymidine incorporation subsequently decreased to the basal level for periods of over 48 h of stimulation.

**Effect of TGF-\(\beta\)_1 on PDGF-induced Cell Cycle in Porcine SMC**

To further establish the S phase step, we tested the effect of TGF-\(\beta\)_1, known to block SMC cycle at the G1/S transition (19). From preliminary studies, we determined that 1 ng/ml TGF-\(\beta\)_1 initiated a significant inhibition (80%) of PDGF-induced SMC mitogenic activity (data not shown). Then, we tested the effect of TGF-\(\beta\)_1 on PDGF-stimulated SMC cycle progression. Quiescent SMC were incubated from 0 to 48 h in the presence of 5 ng/ml PDGF-BB and 1 ng/ml TGF-\(\beta\)_1, followed by a 1-h pulse of \[^3H\]thymidine (Fig. 1). TGF-\(\beta\)_1 inhibited 80% of cell entry into S phase. Only a very slight increase in \[^3H\]thymidine incorporation occurred after 20–40 h of PDGF stimulation in the presence of TGF-\(\beta\)_1, probably corresponding to entry into S phase of very few SMC.

**Expression of SERCA2a and SERCA2b throughout the Cell Cycle**

To study the effect of PDGF-induced SMC proliferation on the expression of SERCA2a and SERCA2b isoforms, SMC were stimulated by 5 ng/ml PDGF for 0–34 h and lysed, and proteins were separated by 8% SDS-PAGE and analyzed by Western blotting. We used isoform-specific anti-SERCA2a and anti-SERCA2b antibodies, previously shown to react specifically in porcine SMC with the 100 kDa SERCA2a and SERCA2b proteins, respectively (17, 29, 30). As shown in Fig. 2A, the SERCA2a expression level was very low for times up to 14 h of stimulation with PDGF, but an increase could be detected after 16 h of stimulation. This up-regulation still persisted after 34 h in the presence of PDGF, although at that time a decline began to appear, as expected for a cell cycle dependent regulation. In sharp contrast with the modulation of the expression of SERCA2a isoform, no variation of the expression of the SERCA2b isoform occurred all along the cell cycle, between 0 and 34 h (Fig. 2B).

As PDGF-induced SERCA2a expression seemed to coincide with the entry into the S phase, we checked the effect of TGF-\(\beta\)_1 on SERCA expression. Quiescent SMC were incubated for increasing time intervals up to the entry into S phase, i.e., ranging from 0 to 20 h, in the presence of both 5 ng/ml PDGF and 1 ng/ml TGF-\(\beta\)_1, lysed, and analyzed by Western blotting as described above. Fig. 3, A and B, shows the expression of SERCA2a and SERCA2b in these conditions. They clearly demonstrate that the PDGF-induced up-regulation of SERCA2a was totally inhibited by TGF-\(\beta\)_1, as a weak and identical expression of this protein was detected for each incubation time (see Fig. 3A). The expression level of SERCA2b was no more affected with TGF-\(\beta\)_1 and PDGF than it was with PDGF alone (Fig. 3B). As an additional control, the effect of TGF-\(\beta\)_1 alone (1 ng/ml) was investigated. Results demonstrated that SERCA2a and SERCA2b proteins were expressed at the same level between 0 and 20 h of incubation with TGF-\(\beta\)_1, showing the specificity of its effect in the presence of PDGF (data not shown).

**Relationship between Ca\(^{2+}\) Influx and SERCA Regulation in PDGF-induced SMC Proliferation**

The first events following the binding of PDGF to its receptor at the surface of SMC membrane are changes in cytosolic Ca\(^{2+}\)
FIG. 3. SERCA2α and SERCA2β expression throughout the cell cycle: effect of PDGF + TGF-β1. Quiescent SMC were stimulated with 5 ng/ml PDGF-BB and 1 ng/ml TGF-β1 from 0 to 20 h and lysed, and each protein sample (100 μg) was submitted to 8% SDS-PAGE and analyzed by Western blotting using the anti-SERCA2α (A) or the anti-SERCA2β (B) antibody as described under “Experimental Procedures.” Number on the left side of the figure gives the molecular masses of the proteins detected, estimated with standard markers. Results (A and B) are typical of three different experiments.

Characterization of SR 33805

Comparative Effects of Nifedipine, SR 33805, and SKF 96365 on PDGF-induced [Ca2+]i increase in human SMC—First, in preliminary experiments, we found that 50 ng/ml PDGF-BB maximally induced human SMC proliferation and that it was associated with a rapid rise in cytosolic free Ca2+. This elevation in [Ca2+]i was characterized by an EC50 value of 15.3 ± 4.8 ng/ml, and the half-time of the Ca2+ accumulation was 1.0 ± 0.4 min (data not shown). To look for the effect of SR 33805 on cytosolic Ca2+ concentration, quiescent cells were incubated with various concentrations of SR 33805 for 1 h, treated for cytosolic free Ca2+ measurements using fluorescent probes as described under “Experimental Procedures,” and then stimulated by PDGF-BB. Moreover, because SR 33805 was suggested to possess a different profile of activity than other Ca2+ channel antagonists in PDGF-induced SMC proliferation (20), we investigated the activity of SR 33805 as a capacitative entry blocker by comparing its effect on PDGF-induced Ca2+ increase with those of the dihydropyridine Ca2+ blocker nifedipine and capacitative entry blocker SKF 96365. Fig. 4 shows these comparative effects of nifedipine, SR 33805, and SKF 96365 on PDGF-BB-mediated rise of Ca2+ in SMC which demonstrated a dose-dependent inhibition of Ca2+ increase by the three Ca2+ blockers. With regard to their relative potencies, the higher inhibitory effect was observed by SR 33805 with an IC50 value of 0.31 ± 0.04 μM. Indeed, this value was 7-fold higher than the IC50 of 2.3 ± 0.8 μM obtained using SKF 96365 and 30-fold higher than that of nifedipine which reached 10.1 ± 1.5 μM. This meant that the SR 33805 inhibitory effect appeared in agreement with its suggested different mechanism of action than that of typical Ca2+ channel blocker in SMC.

Effect of SR 33805 on Human SMC Proliferation—Quiescent human SMC were treated for 24 h or 3 days with 50 ng/ml PDGF-BB in the absence or in the presence of 10 μM SR 33805, trypsinized, and counted. No cytotoxicity could be detected under these experimental conditions and up to 100 μM SR 33805 as shown either by lactate dehydrogenase release or trypan blue exclusion test. Fig. 5A shows these results of PDGF-induced SMC proliferation after 24 h (lanes 1 and 2) or 3 days (lanes 3 and 4) of culture in the absence (lanes 1 and 3) or in the presence (lanes 2 and 4) of SR 33805. The control experiments (lanes 1 and 3) stated a very good proliferation of SMC after either 24 h or 3 days in culture. This figure clearly demonstrates an inhibition of cell proliferation after either 24 h (lane 2) or 3 days (lane 4) of culture in the presence of SR 33805 suggesting a long term effect of the drug.

Comparative Effects of Nifedipine, SR 33805, and SKF 96365 on SMC Proliferation—Quiescent human SMC were allowed to grow for 3 days with 50 ng/ml PDGF-BB in the absence or in the presence of various concentrations of the different Ca2+ channel blockers. Fig. 5B shows their dose-response inhibitory effects on PDGF-induced SMC proliferation. No cytotoxicity was detected using either 10 μM SKF 96365 or nifedipine as checked by lactate dehydrogenase release (data not shown). An inhibition was detected using the different Ca2+ blockers, al-
though differences in their potencies were observed. Again SR 33805 was the most potent inhibitor of PDGF-induced SMC proliferation, with an inhibitory effect already obtained with 0.1 μM SR 33805 and an IC_{50} value of 0.2 ± 0.1 μM, compared with the IC_{50} values of 1.1 ± 0.2 and 3.6 ± 0.5 μM for SKF 96365 and nifedipine, respectively. Hence, a good correlation was found between the dose-response curves for the inhibitory effect of SR 33805 on the PDGF-induced SMC Ca^{2+} increase and cell proliferation.

**Role of Ca^{2+} Influx on SERCA2 Proteins**

**Effect of SR 33805 on Total SMC SERCA Activity**—The effect of SR 33805 on the combined activity of SERCA2a and SERCA2b was followed on isolated membranes by means of their phosphorylated intermediate (E−P) complex (Fig. 6). This E−P corresponds to a transient step in the catalytic cycle of the two isoforms. We also verified that in the presence of 0.1 mM EGTA, no phosphorylation was detected in human SMC membranes (data not shown) thus showing the Ca^{2+} specificity of the E−P formation. The combined activity of the 100-kDa Ca^{2+} ATPase (SERCA2a and SERCA2b) was checked by phosphorylating human SMC membranes in the presence of 0.05 mM CaCl_2, with 0.1 mM EGTA, or without EGTA, as described under “Experimental Procedures.” After trichloroacetic acid precipitation, the phosphorylated proteins were processed by electrophoresis on 7.5% acidic SDS-PAGE, electroblotted onto nitrocellulose, and autoradiographed at −80 °C for 48 h. Lane 1, control mixed human SMC membranes; lanes 2 and 3, PDGF-stimulated SMC for 24 h (2) or 3 days (3); lanes 4 and 5, SMC treated with PDGF and SR 33805 for 24 h (4) or 3 days (5); lanes 6 and 7, SR 33805-treated SMC for 24 h (6) or 3 days (7). Number on the left side of the figure gives the molecular mass of the Ca^{2+} ATPases, estimated with standard molecular weight markers. The results are typical of three experiments.

**Effect of SR 33805 on SERCA2a and SERCA2b Protein Expressions**—To check whether this effect of SR 33805 on PDGF-induced stimulation of SERCA activity could be explained by changes in the expression level of SERCA2a and/or SERCA2b, or to modifications in the catalytic cycle of these enzymes, we performed Western blottings on isolated membranes from SMC using the same isoform-specific anti-SERCA2a and anti-SERCA2b antibodies as those used in Figs. 2 and 3. Fig. 7 shows the results of Western blottings using the anti-SERCA2a antibody (Fig. 7A) or the anti-SERCA2b antibody (Fig. 7B). On both blots, only one band at 100 kDa was detected corresponding to the recognition of the respective SERCA isoforms. Again,
This work demonstrates the involvement of specific Ca\(^{2+}\) pools, which accumulate Ca\(^{2+}\) through the action of the SERCA2 isoforms of Ca\(^{2+}\) pumps, and shows the importance of Ca\(^{2+}\) influx in the regulation of proliferation of arterial SMC stimulated by PDGF. First, by exploring the expression of the two smooth muscle SERCA isoforms, SERCA2a and SERCA2b, throughout the cell cycle, we could determine that the previously observed PDGF-induced SERCA2a up-regulation coincided precisely with the entry into S phase of the SMC cycle (Figs. 1 and 2A). This result was confirmed by the action of TGF-\(\beta_1\), which is associated with the arrest of SMC at the G\(_1\)/S boundary of the cycle and with the concomitant abolishment of SERCA2a expression (Fig. 3A). Second, in an attempt to investigate the role of Ca\(^{2+}\) entry in both PDGF-stimulated SMC proliferation and SERCA expression, using the Ca\(^{2+}\) channel blocker SR 33805, we demonstrated the abolishment of (i) the PDGF-induced SMC proliferation (Fig. 5, A and B); (ii) the associated increase in cytosolic Ca\(^{2+}\) concentration (Fig. 4); and (iii) the SERCA2a up-regulation both in terms of activity and protein levels (Figs. 6 and 7).

This work stresses the role of Ca\(^{2+}\) ions in mitogenesis and suggests a specific function of the SERCA2a isoform, during the cell cycle, and consequently of its associated Ca\(^{2+}\) pool in the regulation of intracellular Ca\(^{2+}\) concentration during the critical period of S phase of the cell cycle. Although an increase in cytosolic Ca\(^{2+}\) acts as a physiological trigger in cell proliferation, the different phases of the cell cycle present different requirements for Ca\(^{2+}\). Kobayashi et al. (35) showed that pre-treatment of aorta in primary culture with ryanodine and NiCl\(_2\) abolished cytosolic Ca\(^{2+}\) concentration transients but did not prevent PDGF-stimulated entry of G\(_0\) into G\(_1\) phase. Conversely, the progression of cells into the S phase and the further mitosis were abolished, suggesting that these latter steps depend on Ca\(^{2+}\) (36) and that growth factors action is governed by Ca\(^{2+}\)-regulated events. Accordingly, cells at G\(_1\)/S boundary enter quiescence in the absence of Ca\(^{2+}\) (37). Besides, these data confirmed several previous studies showing that when Ca\(^{2+}\) concentration could not be raised in the cytosol, the mitogenesis was prevented (38, 39). However, on the other hand, SERCA inhibitors such as 2,5-di-(\(t\)-butyl)-1,4-benzohydroquinone and thapsigargin, which induce an irreversible increase in cytosolic Ca\(^{2+}\) concentration by emptying Ca\(^{2+}\) pools (40, 41), cause a cell growth arrest (14–16). This depletion of Ca\(^{2+}\) pools would affect G\(_1\)/G\(_0\) and S, but not the subsequent G\(_2\) and M cell cycle phases (18). A possible explanation for this apparent discrepancy might be that although an increase in cytosolic Ca\(^{2+}\) concentration is required for a proliferative state, PDGF-induced smooth muscle cell proliferation also requires the action of SERCA pumps which refill Ca\(^{2+}\) pools at the moment of entry into the S phase, as suggested for proliferation of DTT,MF-2 cells (15, 16). We effectively demonstrated here a close relationship between the entry into S phase and an increase in functional SERCA2a-associated Ca\(^{2+}\) pool, which can result in an oscillatory Ca\(^{2+}\) response or in a locally lower Ca\(^{2+}\) concentration as a specific cell cycle event necessary for the G\(_1\) phase to S phase transition. The SERCA2a Ca\(^{2+}\) pool may be close to, or included in the nuclear envelop (42, 43) and thereby be proximal to the genetic machinery and nuclear events likely to be important for cell cycle control.

Another essential feature of this work concerns a differentiation between the SERCA2a and SERCA2b associated Ca\(^{2+}\) pools in PDGF-induced SMC proliferation. Indeed, because the up-regulation of SERCA2a was strictly correlated with the S phase of the SMC cycle and was found to be specific, whereas the SERCA2b expression was found to be identical along the cell cycle, one can formulate the hypothesis according to which the two SERCA2a and SERCA2b associated Ca\(^{2+}\) pools play distinct roles in the regulation of cytosolic Ca\(^{2+}\) concentration, both in temporal and functional terms. The first events following the binding of PDGF to its receptor at the surface of SMC membrane are changes in cytosolic Ca\(^{2+}\) concentration including an initial and acute transient increase in Ca\(^{2+}\) concentration, due to Ca\(^{2+}\) release from Ca\(^{2+}\) pools.

**Discussion**

This work demonstrates the involvement of specific Ca\(^{2+}\) pools, which accumulate Ca\(^{2+}\) through the action of the SERCA2 isoforms of Ca\(^{2+}\) pumps, and shows the importance of Ca\(^{2+}\) influx in the regulation of proliferation of arterial SMC stimulated by PDGF. First, by exploring the expression of the two smooth muscle SERCA isoforms, SERCA2a and SERCA2b,
depletion-induced Ca\(^{2+}\) leads to SMC proliferation. Now, such a relationship between Ca\(^{2+}\) depletion of intracellular Ca\(^{2+}\) pools (without IP\(_3\) formation and as a result, mimic the ability of surface membrane, IP\(_3\) agonists, to activate Ca\(^{2+}\) entry) (50). Now, we might suggest the physiological role of SERCAs in the Ca\(^{2+}\) signaling cascade by their function of refilling Ca\(^{2+}\) stores following store depletion and Ca\(^{2+}\) influx, as the further essential step in Ca\(^{2+}\) movements associated with PDGF-induced SMC proliferation.

To conclude, this study brings new basic data regarding the involvement of SERCA proteins in Ca\(^{2+}\) homeostasis with their regulatory role as a crucial event through the SMC cycle and proliferation. Their target step has been defined as well as a way to get the regulation of their expression by drugs interacting with Ca\(^{2+}\) channels. Whether this restored basal SERCA expression is a direct or indirect consequence of this treatment remains to be established, it is of significant importance with regard to the numerous cardiovascular pathologies with abnormal SMC proliferation.

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