SYMPOSIUM REVIEW / SYNTHÈSE DE SYMPOSIUM

Regulation of vascular tone: cross-talk between sarcoplasmic reticulum and plasmalemma[1]

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Abstract: Selected topics on the roles of sarcoplasmic reticulum (SR) in the control of vascular smooth muscle (VSM) tone are briefly reviewed with particular reference to the regulation of cytosolic concentration of free calcium ions, $[Ca^{2+}]_i$. Although morphological evidence and subcellular membrane studies indicate a relatively meager quantity of SR in VSM and of endoplasmic reticulum (ER) in endothelial cells (ECs) compared with skeletal muscle and cardiac muscle, contractility studies suggest that vascular tone is, to a large extent, regulated by the intracellular Ca^{2+} stores in smooth muscle and endothelial cells. Cytosolic Ca^{2+} levels control myosin light chain phosphorylation and contraction in VSM and activation of NO synthase and phospholipase A_2 in ECs to regulate nitric oxide (NO) and prostaglandin I_2 formation. Understanding of the importance of SR or ER in modulating the $[Ca^{2+}]_i$ in VSM and ECs has been further advanced as a result of the new development and refinement of biophysical techniques in the measurement of cellular Ca^{2+} concentrations and ion currents, such as fluorescent Ca^{2+} indicators and patch-clamp techniques. Experimental evidence has accumulated in support of the existence of cross-talk between SR−ER and the plasma membrane (PM). Novel pharmacological tool drugs selective for the SR−ER Ca^{2+} pump, such as thapsigargin and cyclopiazonic acid, as well as for SR−ER Ca^{2+} channels, such as ryanodine (for the Ca^{2+}-induced Ca^{2+} release channel) and inositol polyphosphates and heparin (for the inositol-1,4,5-trisphosphate activated Ca^{2+} channel), together with the use of blockers for selective PM Ca^{2+} channels have enabled better formulation and elucidation of the mechanisms of cross-talk between SR−ER and PM. It appears that SR has multiple roles in maintaining the homeostasis of cytosolic Ca^{2+}, which controls the VSM tone directly or indirectly via ECs. These roles include (*i*) activation of phasic contraction of VSM by opening of the Ca^{2+} release channels, (*ii*) acting as a sink for entering Ca^{2+} when the Ca^{2+} stores are depleted, extruding Ca^{2+} when the Ca^{2+} stores are refilled, and (*iii*) signalling and regulating the amount of Ca^{2+} entry in relation to the degree of filling of the store. This symposium concensus paper reflects current thoughts on the above roles of SR−ER in the control of cytosolic Ca^{2+} concentration, and thus the regulation of vascular tone.

Key words: calcium channels, calcium pumps, sarcoplasmic reticulum, plasma membranes, vascular smooth muscle, endothelial cell.

Résumé : On révise brièvement quelques thèmes relatifs aux rôles du réticulum sarcoplasmique (RS) dans le contrôle du tonus du muscle lisse vasculaire (MLV), en portant une attention particulière à la

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régulation de la concentration cytosolique d'ions calcium libres ($[Ca^{2+}]_i$). Des données morphologiques et des études membranaires subcellulaires indiquent que, comparativement aux muscles squelettiques et cardiaques, le MLV est pauvre en RS, et les cellules endothéliales (CE), pauvre en réticulum endoplasmique (RE). Toutefois, des études de contractilité suggèrent que, dans le muscle lisse et dans les cellules endothéliales, le tonus vasculaire est en grande partie régulé par les réserves de Ca^{2+} intracellulaire. Les taux de Ca^{2+} cytosolique contrôlent la phosphorylation des chaînes légères de myosine et la contraction dans le MLV, ainsi que l'activation de NO synthase et de phospholipase A_2 dans les CE, afin de réguler la formation de prostaglandine I_2 et de monoxyde d'azote. L'élaboration et l'amélioration des techniques biophysiques permettant de mesurer les concentrations de Ca^{2+} cellulaires et les courants ioniques, par exemple, les indicateurs Ca^{2+} fluorescents et les techniques de « patch clamp », ont contribué à mieux saisir l'importance du RS ou du RE dans la modulation de la $[Ca^{2+}]_i$ dans le MLV et les CE. De nombreux résultats expérimentaux soutiennent l'existence d'une diaphonie entre RS−RE et la membrane plasmique (MP). De nouvelles drogues sélectives de la pompe à Ca^{2+} RS−RE, telles que la thapsigargine et l'acide cyclopiazonique, et des canaux Ca^2 RS−RE, telles que la ryanodine (pour le canal de libération du Ca^{2+} induit par le Ca^{2+}) et les inositol polyphosphates et l'héparine (pour le canal Ca^{2+} activé par l'inositol-1,4,5-trisphosphate), ainsi que l'utilisation d'inhibiteurs des canaux Ca^{2+} sélectifs de la MP ont permis de mieux comprendre les mécanismes de la diaphonie entre SR−RE et la MP. Il semble que le RS participe de multiples façon au maintien de l'homéostasie du Ca^{2+} cytosolique qui contrôle le tonus du MLV directement ou indirectement par l'intermédiaire des CE. Ainsi, (*i*) il active la contraction phasique du MLV par l'ouverture des canaux de libération du Ca^{2+}, (*ii*) il agit comme un puits pour l'entrée du Ca^{2+} lorsque les réserves de Ca^{2+} sont appauvries, et expulse le Ca^{2+} lorsque les réserves de Ca^{2+} sont refaites, et (*iii*) il indique et régule la quantité de Ca^{2+} en fonction du degré de remplissage de la réserve. Ce résumé des thèmes discutés au cours du symposium reflète la pensée actuelle relativement aux rôles joués par le RS et le RE dans le contrôle de la concentration de Ca^{2+} cytosolique, et par conséquent de la régulation du tonus vasculaire.

Mots clés : canaux Ca, pompes Ca, réticulum sarcoplasmique, membranes plasmatiques, muscle lisse vasculaire, cellule endothéliale.
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Introduction

Under physiological conditions, agonist activation of vascular smooth muscle (VSM) frequently results in biphasic force development: an initial phasic contraction as a result of inositol-1,4,5-trisphosphate (IP_3) mediated Ca^{2+} release from the intracellular stores, predominantly sarcoplasmic reticulum, followed by a tonic contraction as a result of Ca^{2+} entry across the plasma membrane (PM). The close spatial arrangement between SR−ER (sarcoplasmic reticulum − endoplasmic reticulum) and PM and the temporal relationship between the phasic and tonic force generation in VSM suggest that an effective communication exists between SR−ER and PM. Such a cross-talk between SR−ER and PM in VSM indeed occurs at different levels. Similarly in endothelial cells (ECs), a number of agonists (classically acetylcholine, histamine, or bradykinin) initiate a similar chain of events, causing transient $[Ca^{2+}]_i$ elevation followed by sustained Ca^{2+} entry and production of NO from L-arginine and prostaglandin I_2 (PGI_2) from arachidonate.

Occupation of PM receptors by agonists leads to the activation of SR−ER Ca^{2+} release channels via the generation of cellular second messengers at or near the PM surfaces: one such message is carried by IP_3 to activate Ca^{2+} channels (IACCs), which are inhibited by heparin, and the other such message is carried by Ca^{2+} itself to activate channels (CACCs), which are affected by ryanodine. Secondly, activation of PM receptors by agonists, under certain conditions, may be associated with membrane depolarization, resulting in the opening of voltage-operated Ca^{2+} channels (VOCCs) or direct activation of the receptor-operated Ca^{2+} channels (ROCCs) on the PM. If the SR−ER is partially or wholly empty, Ca^{2+} entering through these channels is rapidly taken up by SR−ER (the refilling process) driven by the Ca^{2+} pump and (or) other unidentified pathways. When the Ca^{2+} stores are overloaded, entering Ca^{2+} is not buffered by the superficial SR−ER and produces a faster and larger contractile response. Under resting conditions the SR is filled to half of its capacity. The regulated buffering of Ca^{2+} entry by the SR is the essence of the superficial buffer barrier hypothesis (van Breemen 1977). Alternatively, Ca^{2+} movement across the PM may also be signalled and controlled by the extent of filling of Ca^{2+} stores; i.e., when stores are empty, Ca^{2+} entry is enhanced. In 1981, Casteels and Droogmans proposed that the combined effects of SR Ca^{2+} release and direct SR filling from the extracellular space constitute an agonist-activated Ca^{2+} pathway. This mechanism was termed capacitative entry (CCE) by Putney (1986), who subsequently elaborated it to the present concept of signalling from the empty ER to activate the plasmalemmal Ca^{2+} channels in nonexcitable cells. The superficial buffer barrier (SBB) model goes beyond the notion that the degree of emptying of the stores determines their buffering capacity. It suggests that when the stores are full, Ca^{2+} is pumped vectorially toward the compartment bounded by the PM, from which Na^+/Ca^{2+} exchange as well as the PM Ca^{2+} pump contribute to extrusion. Both models suggest possible heterogeneity of the $[Ca^{2+}]_i$ distribution, the superficial buffer barrier model functioning both to lower the global $[Ca^{2+}]_i$ and to elevate

the peripheral $[Ca^{2+}]_i$, with the capacitative entry model functioning also to raise the peripheral $[Ca^{2+}]_i$ when the SR–ER stores are empty. Heterogeneity of $[Ca^{2+}]_i$ leading to its elevation in superficial regions allows Ca^{2+}-activated PM processes (ion channels, enzymes, etc.) to operate, even though the global $[Ca^{2+}]_i$ may be 100 nM or less.

Since the vascular tone is also under fine regulation by the release of vasoactive substances, including NO and PGI_2, which are also Ca^{2+}-dependent processes, similar considerations apply to the roles of ER in the regulation of $[Ca^{2+}]_i$ in vascular endothelial cells. This communication represents a position paper as well as a concensus report on the basis of the experimental evidence presented and discussed on August 3, 1994, at the VIIIth International Symposium on Vascular Neuroeffector Mechanisms held in Kananaskis, Alta., on the proposed roles of SR in the regulation of vascular tone, with a special emphasis on the mechanistic issues of cross-talk between SR and PM. First we will summarize areas of concensus.

SR – ER has multiple Ca^{2+} release channels

Two major Ca^{2+} release channels (CRCs) have been suggested to be present in VSM by functional studies: one is activated by IP_3 and sensitive to heparin (IACC) and the other is activated by Ca^{2+} and sensitive to ryanodine (CACC). So far the modulation of CACCs in smooth muscle by ryanodine or endogenous agents other than Ca^{2+} has not been demonstrated directly. Binding sites for $[^3H]IP_3$ and [^{3}H]ryanodine have recently been demonstrated in SR-enriched membranes from many smooth muscle preparations (reviewed by Zhang et al. 1994*a*). IP_3 receptors in VSM have been characterized, isolated, purified, and incorporated in planar bilayer to demonstrate the channel activities (Watras et al. 1989; Chadwick et al. 1990; Mayrleitner et al. 1991). Ryanodine receptors have also been characterized using SR-enriched membrane preparation of VSM (Zhang et al. 1994*b*). The isolated VSM ryanodine receptor protein, when incorporated into lipid bilayer, showed ion channel activity that could be activated by micromolar Ca^{2+} and millimolar caffeine and modulated by millimolar ATP and micromolar ruthenium red. Ryanodine at 1 mM completely closed the channel, while heparin had no effect (Herrmann-Frank et al. 1991). Zhang et al. (1993*a*) and Z.D. Zhang, E.E. Daniel, and C.-Y. Kwan (unpublished) observed that up to 1 mM ryanodine did not release or prevent release of Ca^{2+} from SR-enriched smooth muscle microsomes, but it substantially inhibited oxalate-stimulated ATP-driven Ca^{2+} uptake. These findings raise the possibility that the functional effect of ryanodine on $^{45}Ca^{2+}$ movement and contractile function in VSM may be, in part, mediated by its action on the SR Ca^{2+} pump. Indeed, the vascular effects of ryanodine were very similar to that of SR Ca^{2+} pump inhibitors, such as thapsigargin (TSG) (Low et al. 1993), cyclopiazonic acid (CPA) (Deng and Kwan 1991; Darby et al. 1993), and 2,5-di-(*tert*-butyl)-1,4-benzohydroquinone (Shimamoto et al. 1992). Some homology of IP_3 and ryanodine receptors has been noted at the molecular level, but they appear to be two separate proteins. Although both channels are believed to be involved in regulating $[Ca^{2+}]_i$ in VSM, their relative importance in different smooth muscles is still not clear.

SR – ER serves as a superficial buffer barrier

Contractile responses of many VSM preparations are dependent on extracellular Ca^{2+}. Once Ca^{2+} has traversed the PM, it is either sequestered into the superficial SR or it diffuses into the myoplasm. Accordingly, myofilament activation depends on the relationship between the rates of Ca^{2+} entry and sequestration. When Ca^{2+} entry is rapid through VOCCs or ROCCs and the stores are full or have Ca^{2+} sequestration inhibited by selective inhibitors of the SR Ca^{2+} pump, Ca^{2+} entry into the cytosol near the contractile apparatus is rapid and higher levels are achieved (van Breemen 1977; Loutzenhiser and van Breemen 1983). When SR Ca^{2+} stores are empty and the pumps are active, with a similar extent of Ca^{2+} entry, more Ca^{2+} is sequestered and the global $[Ca^{2+}]_i$ rises to a lesser extent (Loutzenhiser and van Breemen 1983). Since most techniques to estimate $[Ca^{2+}]_i$ measure the global Ca^{2+} concentrations in the cytosol, these considerations predict a dissociation between global $[Ca^{2+}]_i$ and calcium entry in VSM. Similarly, there may be a dissociation in ECs between calcium entry and global $[Ca^{2+}]_i$ as well as NO release.

When Ca^{2+} stores are full and then emptied by an agonist, acting through either IP_3 or Ca^{2+} messengers, most of this Ca^{2+} goes to raise global $[Ca^{2+}]_i$ and initiate responses, aided by Ca^{2+} entry induced secondarily according to the capacitative entry model. In the steady state, when the Ca^{2+} stores of VSM are loaded, the SBB hypothesis (Chen et al. 1992) suggests that the SR stores contribute to Ca^{2+} efflux by vectorial transport of Ca^{2+} out of the SR toward the PM, raising $[Ca^{2+}]_i$ in a superficial compartment to levels at which the low affinity, high capacity Na^+/Ca^{2+} exchanger of PM operates to extrude the Ca^{2+}. No clear evidence of Na^+/Ca^{2+} participation in Ca^{2+} efflux exists in ECs. This SBB hypothesis leads to three predictions, which follow.

(*i*) In the steady state, the heterogeneity of $[Ca^{2+}]_i$ distribution, with a higher Ca^{2+} concentration near PM, serves to enhance extrusion of Ca^{2+} and activate K^+ channels, nonspecific cation channels, and Cl^- channels without concomitant activation of contraction. Dr. van Breemen at the symposium presented evidence to demonstrate the Ca^{2+} heterogeneity and localization of Na^+/Ca^{2+} exchangers near the superficial sites of high $[Ca^{2+}]_i$ (Williams et al. 1985; Bond et al. 1984). Much evidence (e.g., depolarization by selective channel blockers) has also been published consistent with activation of Ca^{2+}-activated K^+ channels in relaxed smooth muscle cells (when the global $[Ca^{2+}]_i$ is expected to be at the resting level). Also, continuous Ca^{2+}-dependent activation of NO release from ECs without activation by agonist (e.g., during shear stress) has been suggested. All these considerations raise the question of whether there are special biochemical properties of PM and the SR–ER that is located in superficial regions of the cell. Space considerations do not allow a complete review of these issues. However, some areas of importance are elaborated below.

(*ii*) The Ca^{2+} concentration in the subsarcolemmal barrier region increases to reach levels that allow for an alternative avenue for refilling of SR–ER when the SR–ER Ca^{2+} pump is inhibited. Rembold (1989) showed that in the pig carotid artery $[Ca^{2+}]_i$ measured with aequorin rises during

the refilling period, but contraction does not occur. Similar findings were also reported in the rabbit vena cava, using fura 2 (Chen et al. 1992). These findings indicate that refilling takes place via the peripheral cytoplasm. Chen et al. (1992) also found that the dissociation between force and global $[Ca^{2+}]_i$ is much more pronounced if Ca^{2+} entry is mediated by the Na^+/Ca^{2+} exchanger after depletion of the SR. Prevention of Ca^{2+} accumulation in SR with caffeine abolished the delay between the rise in $[Ca^{2+}]_i$ and force development and changed the nature of the $[Ca^{2+}]_i$ increase from biphasic to monophasic. However, Missian et al. (1990), Low et al. (1992), and Bourreau et al. (1991, 1993) have also demonstrated that the refilling of SR need not necessarily involve the SR Ca^{2+} pump (i.e., it can be CPA insensitive) and Ca^{2+} may enter directly to SR from the extracellular space via a "privileged" pathway, which involves plasmalemmal VOCCs in association with an unidentified channel of SR.

(*iii*) Ca^{2+} can be recycled from SR to subsarcolemmal space, including SR–PM junctional space and perhaps caveolae, and re-enters through Ca^{2+} channels to maintain contraction. Steady-state maintenance of the SBB function requires continuous SR–ER unloading to the extracellular space (van Breemen et al. 1986), implying that Ca^{2+} extrusion from the cytoplasm proceeds partly via the coordination with SR–ER Ca^{2+} pump. Inhibition of SR Ca^{2+} accumulation in VSM by modulators, such as caffeine, ryanodine, and TSG, indeed raised the $[Ca^{2+}]_i$ without increasing divalent cation permeability (Nishimura et al. 1986; Chen and van Breemen 1993). More recently, Q. Chen, W. Haynes, and C. van Breemen (unpublished results) found that abolition of SR Ca^{2+} sequestration by the above SR modulators caused a 60% inhibition of the rate of Ca^{2+} extrusion measured as the rate of decline of $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. In the vena cava, removal of external Na^+ caused a similar inhibition, which was not additive to the inhibition induced by TSG. Therefore Ca^{2+} extrusion, at least in vena cava smooth muscle preparation, proceeds via two separate pathways; i.e., the PM Ca^{2+} pump accounts for 40% of the extrusion into the global extracellular space or caveolae and 60% is first taken up into the SR, from where it is released into the SR–PM junctional space to be extruded by the Na^+/Ca^{2+} exchanger. Ca^{2+} imaging is a very important new tool that with sufficient resolution may further clarify the nature of the subplasmalemmal region of SBB and the heterogenity of $[Ca^{2+}]_i$ within it. Recently L. Montano, C. Barajas-Lopez, and E.E. Daniel (unpublished) showed that bronchial smooth muscle can undergo sustained contractions to agonists such as carbachol that release SR calcium despite being in a Ca^{2+}-free medium, provided the SR Ca^{2+} pump and L Ca channels are functional. Apparently a small amount of calcium recycling from a superficial, extracellular binding site to the SR can provide for sustained contraction, possibly by activating protein kinase C.

SR – ER modulates capacitative Ca^{2+} entry

Putney (1986) proposed a different model for agonist-induced Ca^{2+} entry in nonexcitable cells based on an earlier model postulated by Casteels and Droogmans in smooth muscle (1981). This model proposes that in cells that are regulated through membrane receptors, Ca^{2+} entry is enhanced when the Ca^{2+} stores are emptied. Therefore the degree of filling or the emptiness of the Ca^{2+} stores signals and regulates the movement of Ca^{2+} into the cells to refill the ER via a direct access pathway. This model was later modified and refined to accommodate the TSG-sensitive cytosolic pathway for the refilling of ER (Putney 1992); i.e., emptying of stores by way of an intrinsic leak after its Ca^{2+} pump was inhibited also increased Ca^{2+} entry. This CCE model appears to be operative in smooth muscle (Deng and Kwan 1991; Low et al. 1991, 1992; Shimamoto et al. 1992; Bourreau et al. 1991, 1993) and other nonexcitable cells, including ECs (Schilling et al. 1992; Jacob 1990; Luckhoff and Clapham 1992). In these studies, elevation of $[Ca^{2+}]_i$ as a result of Ca^{2+} entry following restoration of extracellular Ca^{2+}, measured directly with fura 2 fluorescence or indirectly as contractile responses, was enhanced when the Ca^{2+} stores were previously depleted by agonists, by prolonged Ca^{2+}-free exposure, by intracellular perfusion with Ca^{2+} chelators, or by SR Ca^{2+} pump inhibition. It has been difficult to demonstrate a channel opened by depletion of Ca^{2+} stores, but recently cell-attached patches of A431 cells derived from a human epidermal cell line held at low (0.5 μM) external Ca^{2+} exhibited transient channel activity, lasting for 1–2 min, after store depletion by dialysis of the cytosol with 10 mM BAPTA solution (Luckhoff and Clapham 1994). TSG also induced channel activation in cells kept in 1 mM external Ca^{2+} after BAPTA dialysis. This report describes the first single-channel records of a Ca^{2+} depletion current.

Previously work by Dr. Schilling using ECs showed that emptying of Ca^{2+} stores by agonists such as bradykinin or by Ca^{2+}-pump inhibitors, such as TSG and CPA, caused in sequence a transient $[Ca^{2+}]_i$ elevation due to loss of Ca^{2+} from ER followed by a sustained $[Ca^{2+}]_i$ elevation due to Ca^{2+} entry (Schilling et al. 1989, 1992). Doler et al. (1992) showed that agonists acting through IP_3 to deplete Ca^{2+} stores or such depletion by three different Ca^{2+}-pump inhibitors without effect on IP_3 formation opened a Ca^{2+} entry pathway which was not affected by inhibitors of VOCCs but was inhibited by a putative inhibitor of ROCCs (SKF96365). ECs apparently lack VOCCs (see Dolor et al. 1992). Numerous studies since then have confirmed the main tenets of these findings. Moreover, in native ECs, Ca^{2+} pump inhibitors such as CPA cause NO release and relaxation of arteries (Zheng et al. 1994). Further work on cultured ECs has suggested a common pattern of electrophysiological responses to agonists and to Ca^{2+}-pump inhibitors that may help explain their action; i.e., inhibition of any inwardly rectifying K^+ channels, activation of Ca^{2+}-activated K^+ channels and of nonspecific cation channels (Inazu et al. 1993; Zhang et al. 1994*a*, 1994*b*; Pasyk et al. 1995). These changes would open a Ca^{2+} entry pathway through nonspecific cation channels and maintain the driving force for Ca^{2+} entry by keeping the cell membrane polarized. This route of Ca^{2+} entry via high and low conductance nonspecific cation channels seems to differ from the channel described above, suggesting that not all cells may open the same Ca^{2+} entry pathway (Inazu et al. 1993).

Authors such as Grinstein et al. (1987) and Gosink and Forsberg (1993) also suggest that Ca^{2+} entry does not appear to be triggered by depletion of intracellular Ca^{2+} stores per se,

but instead propose a Ca^{2+}-induced Ca^{2+} entry model; i.e., Ca^{2+} elevation near the PM that initiates Ca^{2+} entry. In this connection, it bears some conceptual resemblance to van Breemen's SBB model with respect to entrance through a Ca^{2+}-activated nonspecific cation channel. Therefore, despite general acceptance, Putney's CCE model may not be generally applicable.

The mechanism by which the surface membrane Ca^{2+} influx pathway responds to depletion of the internal Ca^{2+} store and, indeed, the molecular identity of the Ca^{2+} influx pathway remain unknown. A clue to the possible identity of the Ca^{2+} influx channel comes from studies on *Drosophila* phototransduction. Stimulation of the photoreceptor cells of *Drosophila* by light initiates a sequence of events that results in the opening of cation-selective channels in the surface membrane and depolarization of the receptor potential. As in vertebrate phototransduction, photons activate rhodopsin, which in turn catalyses the dissociation of a heterotrimeric GTP-binding protein into α and $\beta\gamma$ subunits. However, in *Drosophila* G-protein activation stimulates phospholipase C, causing an increase in cytosolic IP_3, a cellular second messenger that releases Ca^{2+} from ER, and activation of Ca^{2+} influx. This gives rise to prolonged depolarization of the membrane potential as a result of activation of inward membrane current. In the *transient receptor potential* mutant, or *trp*, light stimulation causes only a transient change in the receptor potential, and it has been suggested that the *trp* protein is a Ca^{2+}-permeable cation channel activated by an IP_3-dependent mechanism. Both *trp* and another *Drosophila* gene designated *trp*-like, or *trpl*, have been cloned and shown to be homologous to voltage-gated Ca^{2+} and Na^+ channels. Recent studies have attempted to answer three questions: (*i*) do *trp* and *trpl* form Ca^{2+}-permeable cation channels, (*ii*) can these channels be activated by a receptor-mediated mechanism, and (*iii*) can these channels be activated by depletion of the internal Ca^{2+} stores? To address these questions, Dr. Schilling examined the functional expression of *trp* and *trpl* in Sf9 insect cells using the baculovirus expression vector. The results presented showed that *trp* and *trpl* encode Ca^{2+}-permeable cation channels. The *trp* channels can be activated by depletion of the internal Ca^{2+} stores by TSG, appear to be slightly more permeable to Ca^{2+} versus Na^+, and have some permeability for Mg^{2+} but not Ba^{2+}. In contrast, *trpl* channels are constitutively active under basal, nonstimulated conditions and can be activated by a receptor-dependent mechanism, but not by TSG. The *trpl* channnels are nonselective with respect to Ca^{2+}, Na^+, and Ba^{2+} and have a low permeability to Mg^{2+}. These results suggest that the C-terminal domain of *trp*, which is absent in *trpl*, may be necessary for capacitative Ca^{2+} entry. Because of the similarity between insect phototransduction and Ca^{2+} signalling mechanisms in mammalian nonexcitable cells. The channels responsible for receptor-mediated Ca^{2+} influx may be structurally homologous to *trp* and (or) *trpl*. Homology screening and functional expression using the baculovirus – Sf9 insect cell system may be useful for identification of the mammalian channel clones.

Conclusions and unresolved issues

It is beyond any reasonable doubt that vascular muscle tone is finely regulated by effective communications between VSM and ECs and integrated interactions between the intracellular Ca^{2+} compartments and the extracellular space in these cells via ion channels and Ca^{2+} pumps in SR–ER and PM, and Na^+/Ca^{2+} exchanger in PM. Activation at the PM results in the release of Ca^{2+} from the stores via CACCs or IACCs. Inhibition of the SR–ER Ca^{2+} pump also results in the release of Ca^{2+} via an IP_3-independent pathway. Supporting evidence in recent years has rapidly accumulated to allow formulation of two major hypotheses for the investigation of the cross-talk between the Ca^{2+} stores and PM: the superficial buffer barrier model and the capacitative entry model. Both hypotheses postulated a special subsarcolemmal zone between the superficial Ca^{2+} stores and the PM for the regulation of Ca^{2+} entry and (or) Ca^{2+} extrusion. These hypotheses have facilitated the understanding of a large number of functional and electrophysiological observations. However, there are still many unresolved issues, for example, the following. (*i*) What are the relative roles of CACCs and IACCs of the Ca^{2+} stores in the regulation of vascular muscle tone? Do they differ in different vasculature? (*ii*) How do the Ca^{2+} stores communicate with the PM to initiate Ca^{2+} entry? The molecular identity of the chemical messenger(s) that communicates from the SR–ER to the PM to initiate Ca^{2+} entry has recently been under active investigation and briefly reviewed by Putney (1994). So far no reports confirm the generality of the existence of novel chemical messengers proposed by Randraimampita and Tsien (1993) and Parekh et al. (1993). Signalling of Ca^{2+} entry by store depletion is probably mediated by different messengers and different mechanisms. (*iii*) What is the nature of the Ca^{2+} depletion regulated channels? How many different Ca^{2+} depletion regulated channels exist? How are these channels regulated by the messengers derived from Ca^{2+}-store depletion? In some cells, it appears to be a very small conductance channel, not resolved by single-channel analysis (Zweifach and Lewis 1993; Hoth and Penner 1993), while in an epidermal cell line, it had a slope conductance of 2 pS with 200 mM Ca^{2+} in the pipette or 16 pS with 60 mM Ba^{2+} in the pipette (Luckhoff and Clapham 1994). In cultured endothelial cells, nonspecific cation channels open in response to Ca^{2+} store depletion (Zhang et al. 1994*a*, 1994*b*; Pasyk et al. 1994), and a similar mechanism seems to allow Ca^{2+} entry in response to agonists (Inazu et al. 1993, 1994). It seems likely that different channels may be involved. (*iv*) Is there direct evidence for the subsarcolemmal regions in the superficial buffer barrier model which serve to regulate Ca^{2+} entry and extrusion? What are the morphological characteristics and properties of these regions? Digital imaging studies of $[Ca^{2+}]_i$ in single smooth muscle cells (Williams et al. 1985; Himpens et al. 1994) suggest the possible existence of the subsarcolemmal barrier, but studies of its properties are still limited by the spatial resolution of current methods.

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