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Maintenance of intracellular Ca^{2+} basal concentration in airway smooth muscle (Review)

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Abstract. In airway smooth muscle, the intracellular basal Ca^{2+} concentration $[_b(Ca^{2+})_i]$ must be tightly regulated by several mechanisms in order to maintain a proper airway patency. The $_b[Ca^{2+}]_i$ is efficiently regulated by sarcoplasmic reticulum Ca^{2+}-ATPase 2b, plasma membrane Ca^{2+}-ATPase 1 or 4 and by the Na^+/Ca^{2+} exchanger. Membranal Ca^{2+} channels, including the L-type voltage dependent Ca^{2+} channel (L-VDCC), T-type voltage dependent Ca^{2+} channel (T-VDCC) and transient receptor potential canonical 3 (TRPC3), appear to be constitutively active under basal conditions via the action of different signaling pathways, and are responsible for Ca^{2+} influx to maintain $_b[Ca^{2+}]_i$. The two types of voltage-dependent Ca^{2+} channels (L- and T-type) are modulated by phosphorylation processes mediated by mitogen-activated protein kinase kinase (MEK) and extracellular-signal-regulated kinase 1 and 2 (ERK1/2). The MEK/ERK signaling pathway can be activated by G-protein-coupled receptors through the α_q subunit when the endogenous ligand (i.e., acetylcholine, histamine, leukotrienes, etc.) is present under basal conditions. It may also be stimulated when receptor tyrosine kinases are occupied by the appropriate ligand (cytokines, growth factors, etc.). ERK1/2 phosphorylates L-VDCC on Ser^{496} of the β_2 subunit and Ser^{1928} of the α_1 subunit, decreasing or increasing the channel activity, respectively, and enabling it to switch between an open and closed state. T-VDCC is also probably phosphorylated by ERK1/2, although further research is required to identify the phosphorylation sites. TRPC3 is directly activated by diacylglycerol produced by phospholipase C (PLC_β or $_\gamma$). Constitutive inositol 1,4,5-trisphosphate production induces the release of Ca^{2+} from the sarcoplasmic reticulum through inositol triphosphate receptor 1. This ion induces Ca^{2+}-induced Ca^{2+} release through the ryanodine receptor 2 (designated as Ca^{2+} 'sparks'). Therefore, several Ca^{2+} handling mechanisms are finely tuned to regulate basal intracellular Ca^{2+} concentrations. It is conceivable that alterations in any of these processes may render airway smooth muscle susceptible to develop hyperresponsiveness that is observed in ailments such as asthma.

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1. Introduction

In unstimulated tissues, numerous cellular mechanisms contribute to the influx and efflux of Ca^{2+} to and from the cytoplasm in order to maintain homeostasis of intracellular basal Ca^{2+} concentrations $[_b(Ca^{2+})_i]$, a phenomenon that occurs in almost all cells (1-7). In smooth muscle at rest, $_b[Ca^{2+}]_i$ must be kept tightly within the range of 100 and 150 nM (8-15) to maintain an equilibrium between contraction and relaxation. In these cells, the processes of Ca^{2+} influx and efflux preserve the myogenic tone, resting membrane potential and sarcoplasmic reticulum (SR) Ca^{2+} refilling (1,10,16-18). It has been proposed that the influx process involves entry of extracellular Ca^{2+} through L-type voltage dependent Ca^{2+} channels (L-VDCCs) (10,19-22), receptor-operated

Ca^{2+} channels (ROCCs) activated by agonists (23-28) and store-operated Ca^{2+} channels (SOCCs, capacitative Ca^{2+} entry) activated by SR-Ca^{2+} depletion (10,29-33). An additional cytosolic Ca^{2+} source is the SR, that is the main intracellular Ca^{2+} store, activated via inositol 1,4,5-trisphosphate (IP_3) receptor channels (30,34-36) and ryanodine-receptor (RyR) channels (35,37-40). Ca^{2+} extrusion from the cytoplasm is accomplished via the action of membrane and sarcoplasmic Ca^{2+} ATPases and Na^+/Ca^{2+} exchanger (NCX) in its forward mode (41-49).

Pivotal work on basal Ca^{2+} influx performed in aortic vascular smooth muscle cells using a pharmacological approach, demonstrated two predominant mechanisms of basal Ca^{2+} entry: One associated with L-VDCCs, accounting for ~23-43% of the total Ca^{2+} entry, and another associated with SOCCs, which contributed ~30% of the total (50).

In a recent study on airway smooth muscle (ASM), the present authors observed that the basal Ca^{2+} entry was mediated by L-VDCCs and probably also a constitutively active transient receptor potential canonical 3 (TRPC3) channel (18), which is described below. However, the mechanisms that maintain their permeability to Ca^{2+} have yet to be elucidated.

In the present review, current knowledge regarding different structures that maintain the $_b[Ca^{2+}]_i$ in ASM, including those involving L- and T-VDCCs, TRPC3, membrane and sarcoplasmic Ca^{2+}-ATPases, NCX in its forward mode, IP_3 and RyRs, is discussed, including the most recent findings associated with the phosphorylation of L- and T-VDCCs and the dependence of TRPC3 on diacylglycerol (DAG).

For a better understanding of the participation of each of these proteins in the $_b[Ca^{2+}]_i$ regulation of ASM, novel unpublished data from studies by our group have been included. Firstly, Fig. 1A shows the maximal reduction of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) produced under Ca^{2+} free medium. This maneuver allowed determination of the proportional effect of each protein in the handling of $_b[Ca^{2+}]_i$.

2. VDCCs

L- and T-VDCCs have been described in different types of smooth muscle (19,51,52); in particular, L-VDCC expression has been abundantly reported in the ASM of different species, including human (20,21,53-56). Opening of both types of channel is dependent on membrane depolarization, allowing the entry of Ca^{2+}, which subsequently contributes to contraction and SR Ca^{2+} refilling (9,10,19,20,57).

Several subunits for L-VDCC have been described: $Ca_V1.1$, $Ca_V1.2$, $Ca_V1.3$ and $Ca_V1.4$ (58). In ASM, L-VDCC had generally been characterized by pharmacological and electrophysiological methods (19). However, the presence of all the subunits of this channel was recently reported in rat bronchial smooth muscle (59). Nevertheless, in bovine and guinea-pig tracheal myocytes, only $Ca_V1.2$ and $Ca_V1.2$-$Ca_V1.3$, respectively, were observed (21,60). As identified recently by the present authors and shown in Fig. 1B and E, in guinea-pig ASM, D-600 (methoxyverapamil hydrochloride), a blocker of L-VDCC, significantly decreased the $_b[Ca^{2+}]_i$, corroborating that this channel is constitutively active and contributes towards maintaining the $_b[Ca^{2+}]_i$ (18). It is well known that this channel is greatly dependent on the membrane voltage, and in canine ASM our group observed that its membrane potential at rest is approximately-59 mV, and is held steady. Furthermore, when the tissue was stimulated with carbachol, a cholinergic agonist, its membrane was depolarized, and when the depolarization reached-45 mV, it started oscillating (20). These oscillations are nifedipine-sensitive, and therefore corresponded to the opening and closing of the L-VDCC (61). Since the membrane potential at rest is unchanging, it was highly improbable that the voltage was influencing its opening at this stage.

Recently, a study in rat cardiomyocytes demonstrated that extracellular signal-regulated kinases 1 and 2 (ERK1/2), the mitogen-activated protein kinases (MAPKs), are able to phosphorylate L-VDCC at two sites: On Ser^{496} of the β_2 subunit and Ser^{1928} of the α_1 subunit. Phosphorylation on the β_2 subunit or the α_1 subunit decreased or increased the L-VDCC activity, respectively (62). Thus, it may be hypothesized that in ASM, MAPK kinase (MEK)-ERK1/2 signaling may be involved in the continual opening and closing of the channel under basal conditions. This pathway may be associated with receptor tyrosine kinases (RTKs), which are activated by basal cytokines or growth factors. Our group previously demonstrated that ERK1/2 are present in the phosphorylated state in unstimulated bovine ASM (9). Fig. 1D and E show that the addition of U-0126, an inhibitor of ERK1/2, to guinea-pig tracheal myocytes significantly diminished the $_b[Ca^{2+}]_i$ until reaching a plateau. The addition of D-600 did not further modify the $[Ca^{2+}]_i$, confirming that phosphorylation of the L-VDCC through the MEK-ERK1/2 pathway is possibly involved in its constitutive active mode. Therefore, the ERK1/2 signaling pathway may be responsible for phosphorylating the β_2 Ser^{496} and α_1 Ser^{1928} sites, serving to switch the L-VDCC between an open and closed state (Fig. 1F).

Treatment with mibefradil, a T-VDCC blocker, also significantly lowered $_b[Ca^{2+}]_i$ in the guinea-pig tracheal myocytes, implying the participation of this channel in sustaining $_b[Ca^{2+}]_i$ (Fig. 1C and E). The presence of T-VDCC has been reported in this tissue (19), and the expression of $Ca_V3.1$, $Ca_V3.2$ and $Ca_V3.3$ subunits has been detected in ASM by immunohistochemistry (63). In this context, unexpectedly our group found that the addition of mibefradil following U-0126 did not further diminish $_b[Ca^{2+}]_i$ (Fig. 1D). This finding suggested that T-VDCC could also be regulated by the ERK1/2 signaling pathway. Recent studies have shown that T-VDCC may be modified by several serine/threonine protein kinase pathways, suggesting that this channel is susceptible to undergo phosphorylation (64); however, further research is required in this regard to determine the functional impact that ERK1/2 signaling has on the T-VDCC. Notably, in sensitized guinea-pigs that developed an airway inflammatory state, the expression level of L-VDCC was not modified (60). This finding indicated that these channels appear not to participate in the modification of $_b[Ca^{2+}]_i$ that is observed in inflammatory ailments, such as asthma (65).

3. TRPC channels

In smooth muscle, TRPC channel genes code for ROCC and SOCC, which have an important role in intracellular Ca^{2+} homeostasis, while recently transient receptor potential vanilloid 1 (TRPV1) was revealed to be involved in the modulation of

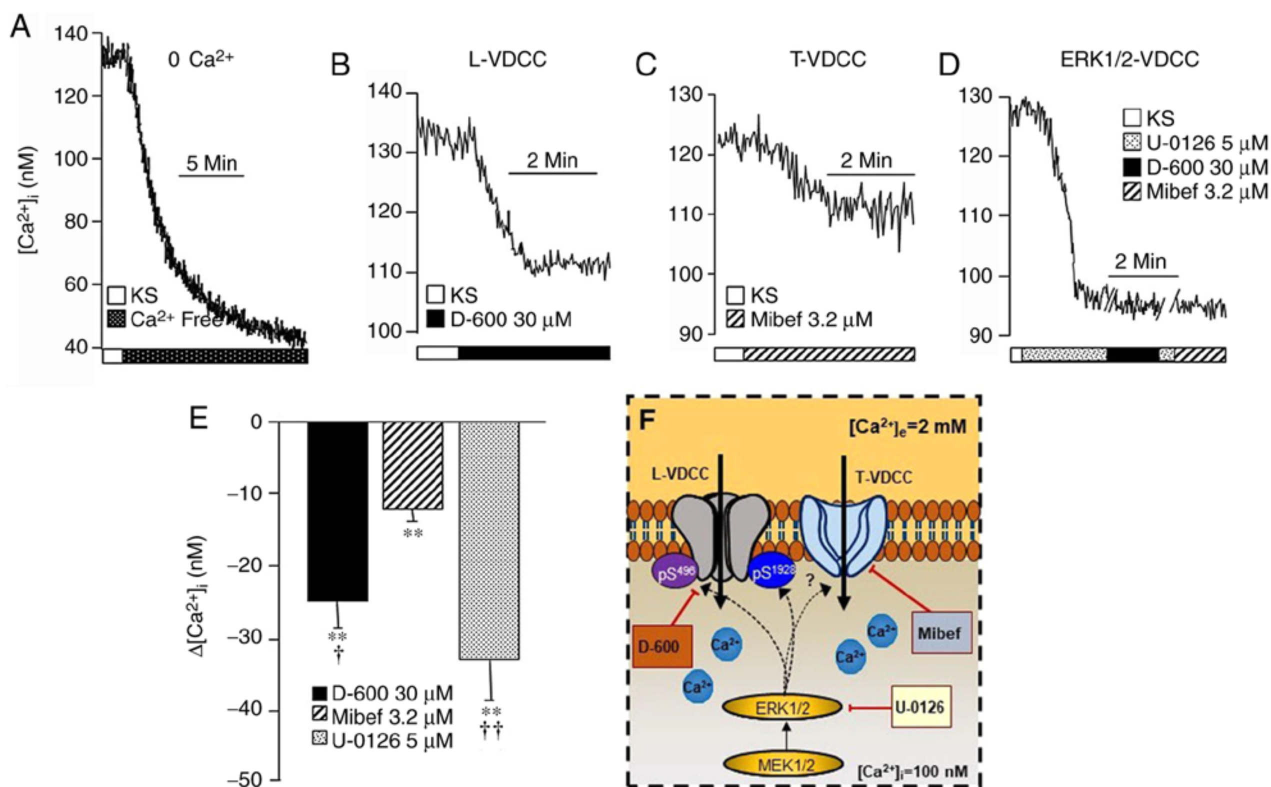

Figure 1. In guinea-pig airway myocytes at rest, L-VDCC and T-VDCC contribute towards maintaining the $_b[Ca^{2+}]_i$, and apparently are phosphorylated through the MEK-ERK1/2 pathway. Upper traces are representative of the intracellular Ca^{2+} measurements through fura-2AM in the different experimental protocols. (A) Representative trace showing the amplitude of the reduction in the $_b[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}. The addition of (B) D-600 (an L-VDCC blocker; n=12) or (C) Mibef (a T-VDCC blocker; n=13) significantly lowered the $_b[Ca^{2+}]_i$ to differing extents. (D) Blockade of MEK-ERK1/2 kinase with U-0126 (n=12) markedly diminished the $_b[Ca^{2+}]_i$ and the administration of D-600 or Mibef did not lead to any further decreases in the altered $[Ca^{2+}]$i (n=6). (E) Bar graph depicting the statistical analysis of the different experimental protocols. Each bar represents the mean ± standard error of the mean. **P<0.01 when compared with their respective $_b[Ca^{2+}]_i$ values; †P<0.05, ††P<0.01 with respect to the Mibef group (according to the Student-Newman-Keuls multiple comparison test). (F) Schematic representation of regulation of the basal activity of the VDCCs. The MEK signaling pathway through ERK1/2 phosphorylates the β_2 Ser^{496} (pS^{496}) and α_1 Ser^{1928} (pS^{1928}) sites, switching the L-VDCC and probably also the T-VDCC between an open and closed state. D-600, Mibef or U-0126 diminished the $_b[Ca^{2+}]_i$, (for further details, see the 'VDCCs' section). These results suggest that, under basal conditions, the two types of VDCC are continuously phosphorylated through the MEK pathway, which is responsible for their constitutive activity. L-VDCC, L-type voltage-dependent channel; T-VDCC, T-type voltage dependent Ca^{2+} channel; $_b[Ca^{2+}]_i$, intracellular basal Ca^{2+} concentration; MEK, mitogen-activated protein kinase kinase; ERK1/2, extracellular-signal-regulated kinase 1/2; Mibef, mibefradil; KS, Krebs' solution.

ASM tone and Ca^{2+} handling during agonist-induced contraction (66). In general, due to their ionic permeability, all TRPC channels are considered to be non-selective cation channels (NSCCs) (67,68). Thus far, all known TRPC channel activity has been shown to be associated with a phospholipase C (PLC) signaling pathway (69,70). In this context, it has been proposed that certain TRPC channels, including TRPC1, -2 and -3, are dependent on SR-Ca^{2+} depletion due to IP_3 production [a process termed store-operated Ca^{2+} entry (SOCE)] (36,71-75). On the other hand, ROCCs also include TRPC channels (TRPC3, -4, -5, -6 and 7), although these are activated by DAG, the other metabolite of PLC activity, and are independent of SR-Ca^{2+} depletion (69,70,76). In this context, only TRPCs 3, 6 and 7 are directly activated by DAG not involving protein kinase C (69,76), whereas TRPCs 4 and 5 are inhibited by protein kinase C, since their activity may be observed when this kinase is blocked (70).

In ASM, previous studies have reported the presence of almost all TRPC channel subtypes (TRPC1, -2, -3, -4, -5 and -6), with the exception of TRPC7 (67,68). Several TRPC channels have been shown to be constitutively active in different types of tissue. For example, TRPC1 and -4 were proposed to be continuously active in C57 mice skeletal myocytes (77); likewise, TRPC7 in human embryonic kidney cells (76), while TRPC3 was also observed to be constitutively active in rabbit ear artery and mouse airway myocytes (78,79). In this regard, our recent study demonstrated that, in guinea-pig ASM, this channel was also involved in maintaining the $_b[Ca^{2+}]_i$ and preserving smooth muscle basal tone (18). The role of this channel in $_b[Ca^{2+}]_i$ is illustrated in Fig. 2, where the addition of 2-aminoethoxydiphenyl borate (2-APB), a blocker of the TRPC3 channel (80), markedly diminished the $_b[Ca^{2+}]_i$ (Fig. 2A and E). Furthermore, Pyr3, another specific TRPC3 channel blocker (81), also lowered $_b[Ca^{2+}]_i$ by a similar extent (Fig. 2B and E). These results suggested that TRPC3 is constitutively active in guinea-pig ASM, even though the mechanism underlying this phenomenon has yet to be fully elucidated.

Since almost all TRPC channel subtypes are expressed in ASM, in this review the DAG analog, 1-oleoyl-2-acetyl-sn-glicerol (OAG), was used to investigate the possible functional role of the channels present in this tissue. Fig. 2C shows that the addition of OAG to tracheal myocytes induced a transient

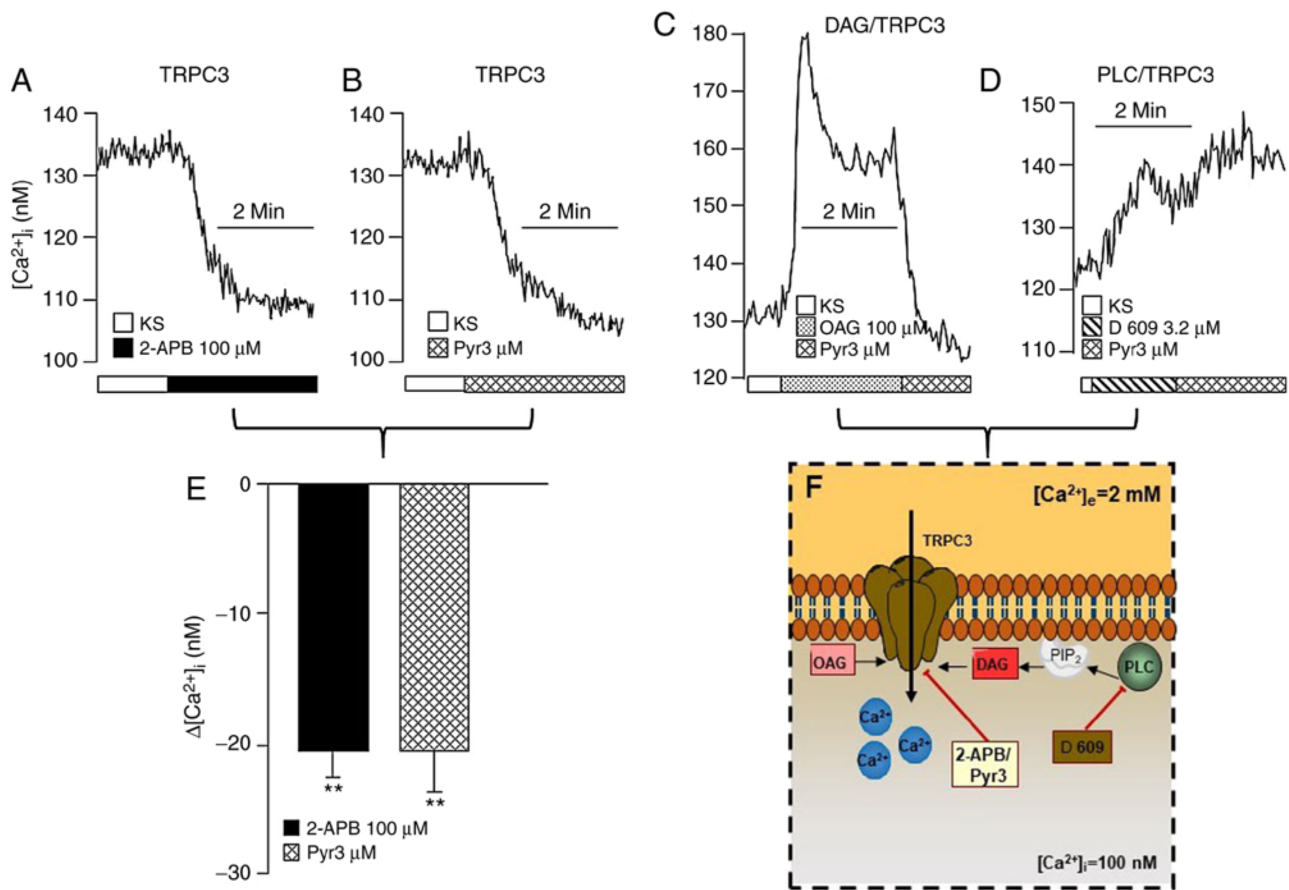

Figure 2. Membrane TRPC3 channel also contributes to $_b[Ca^{2+}]_i$ in guinea-pig airway smooth muscle. The upper traces shown are representative of the different experimental protocols. The addition of (A) 2-APB (a blocker of TRPC3; n=5) or (B) Pyr3 (a specific TRPC3 blocker; n=5) lowered the $_b[Ca^{2+}]_i$. (C) The addition of OAG, a DAG analog, induced a transient peak of the $[Ca^{2+}]_i$, followed by a plateau. The application of Pyr3 to the Ca^{2+} plateau returned Ca^{2+} to its basal level, indicating that the main TRPC channel functionally active in airway smooth muscle at rest is TRPC3. (D) Incubation with D-609, an inhibitor of PLC, produced a small incremental increase in the $[Ca^{2+}]_i$, and the addition of Pyr3 no longer diminished the $_b[Ca^{2+}]_i$. (E) Bar graph illustrating that the effects elicited by 2-APB and Pyr3 on $_b[Ca^{2+}]_i$ are similar. Each bar represents the mean ± standard error of the mean. $^{**}P<0.01$ compared with the respective $_b[Ca^{2+}]_i$ value. (F) Schematic representation of the basal activity regulation of the TRPC3 channel. The results suggest that, under basal conditions, TRPC3 may oscillate between an open and closed state in the plasma membrane, i.e., these channels are constitutively active in this tissue, and are regulated by PLC through DAG. See the 'Transient receptor potential canonical channels' section for further details. PIP_2, phosphatidylinositol 4,5-bisphosphate; TRPC3, transient receptor potential canonical-3; 2-APB, 2-aminoethoxydiphenyl borate; OAG, 1-oleoyl-2-acetyl-sn-glycerol; DAG, diacylglycerol; PLC, phospholipase C.

peak in the $[Ca^{2+}]_i$ followed by a plateau. This response could have been developed through TRPC3 and/or TRPC6 channels, since these are both directly activated by DAG (69). However, after having reached the Ca^{2+} plateau induced by OAG, the addition of Pyr3 led to a return of $[Ca^{2+}]_i$ to its basal level. This finding indicated that the predominant TRPC channel that is functionally active in guinea-pig ASM, is TRPC3. Our group has postulated that TRPC3 is one of the channels involved in the maintenance of $_b[Ca^{2+}]_i$ (18), probably in a DAG-dependent manner. This lipid molecule is produced via the PLC or phospholipase D (PLD) pathways. It has been reported in rabbit ear artery myocytes that the PLD pathway produces DAG to sustain the constitutive activity of TRPC3 that contributes to the resting membrane potential (78,82). In ASM, protein kinase A was reported to regulate PLD activity, and it has been postulated that this phospholipase may be involved in the molecular mechanism underlying cyclic adenosine 5'-phosphate (c-AMP)-mediated relaxation in this tissue (83). By contrast, PLC has been shown to be predominantly involved in the IP_3-Ca^{2+} signaling pathway and in contraction (35). Therefore, in this review, we investigated if PLC may participate in DAG production in ASM at rest by using tricyclodecan-9-yl xanthogenate (D-609, a relatively specific inhibitor of PLC) (84) to inhibit this enzyme activity. It was observed that the addition of Pyr3 following D-609 to tracheal myocytes did not result in any further notable perturbations of the $_b[Ca^{2+}]_i$ (Fig. 2D). Thus, these results suggested that PLC generates DAG, which subsequently leads to the activation of TRPC3 under basal conditions in order to maintain $_b[Ca^{2+}]_i$ in ASM (Fig. 2F). Conceivably, the activity of PLC may be regulated by endogenous ligands of RTKs, or by G-protein-coupled receptors.

It has been demonstrated that the expression levels and activity of the TRPC3 channel are greatly augmented in ASM cells obtained from sensitized mice (79). This may lead to an increase in the $_b[Ca^{2+}]_i$, which could contribute to airway hyperresponsiveness in asthma.

The TRPV receptors, which are other members of the TRP family, have been implicated in mechanical stretch-induced Ca^{2+} influx in human ASM (85). In this context, TRPV1 is expressed in these cells, and was shown to be involved in Ca^{2+} oscillations and the maintenance of contraction by cholinergic

agonists (66). However, any role in terms of maintaining the $_b[Ca^{2+}]_i$ has not yet been elucidated, and this requires further research.

4. Capacitative Ca^{2+} entry

SR-Ca^{2+} depletion mediated by IP_3 induces the established mechanism of capacitative Ca^{2+} entry. The first studies on this were performed by Putney (31) in non-excitable cells. Capacitative Ca^{2+} entry also occurs in smooth muscle via Ca^{2+} influx through diverse membrane channels (32,86). One of these Ca^{2+} influx mechanisms involves two types of protein associated with the SOCE pathway: Stromal interaction molecules (STIMs) and Orai proteins (87,88), both of which have been characterized in vascular smooth muscle and ASM (89,90). Orai are plasma membrane proteins, and three isoforms from different genes have been characterized: Orai1, -2 and -3 (91). On the other hand, two homologs of STIM have been identified: STIM1 and STIM2, both of which are located in the SR membrane (88,92,93). Regarding the two protein groups, Orai1 and STIM1 are the proteins that are chiefly expressed in ASM, and are responsible for the capacitative Ca^{2+} entry (89,94). Briefly, STIM1 on the SR functions as a Ca^{2+} sensor, monitoring the organelle's Ca^{2+} content (95). When the SR-Ca^{2+} store is depleted, STIM1 forms an aggregate with other STIM1 molecules, thereby forming structures designated as 'puncta', which interact with Orai1 plasma membrane proteins to promote capacitative Ca^{2+} entry (89). Additionally, in several cell types it has been postulated that STIM/Orai may interact with TRPC channels, thereby establishing an alternative mechanism for capacitative Ca^{2+} entry (89,96). It is noteworthy that, in ASM, IP_3 has been demonstrated to directly open membranal TRPC3 channels. This recent finding implies that IP_3 mediates SR-Ca^{2+} depletion (i.e., capacitative Ca^{2+} entry) and also a direct, independent Ca^{2+} influx by TRPC channels (36). In this context, in one of our previous studies, we demonstrated that, in unstimulated airway myocytes, capacitative Ca^{2+} entry was not activated unless the SR Ca^{2+} content fell below 50% (8). However, it is well known that capacitative Ca^{2+} entry is activated by contractile agonists that act through the PLC_β-IP_3 signaling cascade (32), therefore providing no certainty that it does contribute to the maintenance of $_b[Ca^{2+}]_i$.

5. Na^+/Ca^{2+} exchanger

The Na^+/Ca^{2+} exchanger (NCX) is a membrane Ca^{2+}-handling protein that introduces three Na^+ ions to the cytoplasm, while extruding one Ca^{2+} when in its forward mode. By contrast, in its reverse mode, it introduces Ca^{2+} and extrudes Na^+ (42). To activate the reverse mode (NCX_{REV}), the entry of Na^+ through an NSCC, and probably L-VDCC in proximity to the NCX, is required (21,41,48,97). The NCX is encoded by three gene isoforms, which generate NCX1, -2 and -3 (98-100). NCX1, extensively distributed in mammalian cells, has 17 different splicing variants that are tissue-specific and define the exchanger's ionic sensitivity and regulation (101). NCX2 has no splicing variants and is located predominantly in the brain, spinal cord, gastrointestinal and kidney tissues, whereas NCX3 has five splice variants expressed in brain and skeletal muscle (101). In ASM, the NCX1.3 splicing variant is the main isoform present (102).

In airway myocytes, it has been proposed that NCX participates in the physiology of $[Ca^{2+}]_i$, including SR-Ca^{2+} refilling (10,57), although it has been given a minor role in Ca^{2+} homeostasis (43). In this context, we have observed that NCX blockade with amiloride, a blocker of both the forward and reverse NCX modes, or KB-R7943, a blocker of NCX_{REV}, had no noticeable effect on $_b[Ca^{2+}]_i$, indicating a minor role of this protein in terms of $_b[Ca^{2+}]_i$ regulation (unpublished data). Nevertheless, its participation in Ca^{2+} regulation, accomplished mainly through NCX_{REV}, becomes evident when $_b[Ca^{2+}]_i$ is increased and acquires a new steady-state (Fig. 3A). In this context, in a murine chronic model of allergen-induced airway hyperresponsiveness, it was shown that the levels of NCX1 were significantly augmented, and that NCX_{REV} activity was increased (103). Furthermore, in human myocytes, the addition of pro-inflammatory cytokines, including tumor necrosis factor-α (TNFα) and interleukin (IL)-13, also increased the expression of NCX1 and favored NCX_{REV} activity (104). These findings suggested that, during inflammation, NCX_{REV} could significantly contribute to an increase in the $_b[Ca^{2+}]_i$, which would predispose airway smooth muscle to hyperresponsiveness.

6. Ca^{2+}-ATPases in ASM

Ca^{2+}-ATPases form part of a large family of membrane proteins defined as P-type ATPases, including the plasmalemmal Ca^{2+}-ATPase (PMCA) and the SR Ca^{2+}-ATPase (SERCA, or sarco/endoplasmic reticulum Ca^{2+}-ATPase) (105).

The PMCA extrudes Ca^{2+} against a high concentration gradient to contribute to $_b[Ca^{2+}]_i$. It exists in a 1:1 relationship with ATP, is electroneutral via H^+/Ca^{2+} exchange, and its affinity for Ca^{2+} and transport efficiency is increased by calmodulin. PMCA1-4 are the products of four different genes with several splice variants (105). PMCA1 and -4 are ubiquitous, and have lower affinity for calmodulin, whereas PMCA2 and PMCA3 have high calmodulin affinity (105,106).

In ASM, the primordial function of PMCA in Ca^{2+} homeostasis was demonstrated late in the 20th century (43). Shortly afterwards, the expression of this pump in canine ASM was reported (107). More recently, in rat bronchial myocytes, the presence of PMCA1 and PMCA4 was confirmed, and the participation of these two isoforms in Ca^{2+} homeostasis was demonstrated (108).

On the other hand, SERCA is, in part, electrogenic, since it introduces two Ca^{2+} ions to the SR, at the same time releasing at least four H^+ ions to the cytoplasm (105). Additionally, it has been demonstrated that SERCA transports two Ca^{2+} ions for each hydrolyzed ATP molecule, and it appears to be the main system for controlling $[Ca^{2+}]_i$ in muscular cells (105).

SERCA pumps are produced by three genes: SERCA1, -2 and -3. They are subjected to alternative splicing, resulting in the isoforms, SERCA1a-b, SERCA2a-c and SERCA3a-f (105,109). In smooth muscle cells, the SERCA isoforms predominantly present are 2a and 2b (109), whereas in ASM, SERCA2b is the predominant isoform (110).

By measuring $[Ca^{2+}]_i$ in the absence of extracellular Ca^{2+}, the addition of thapsigargin, a SERCA blocker, to rat bronchial

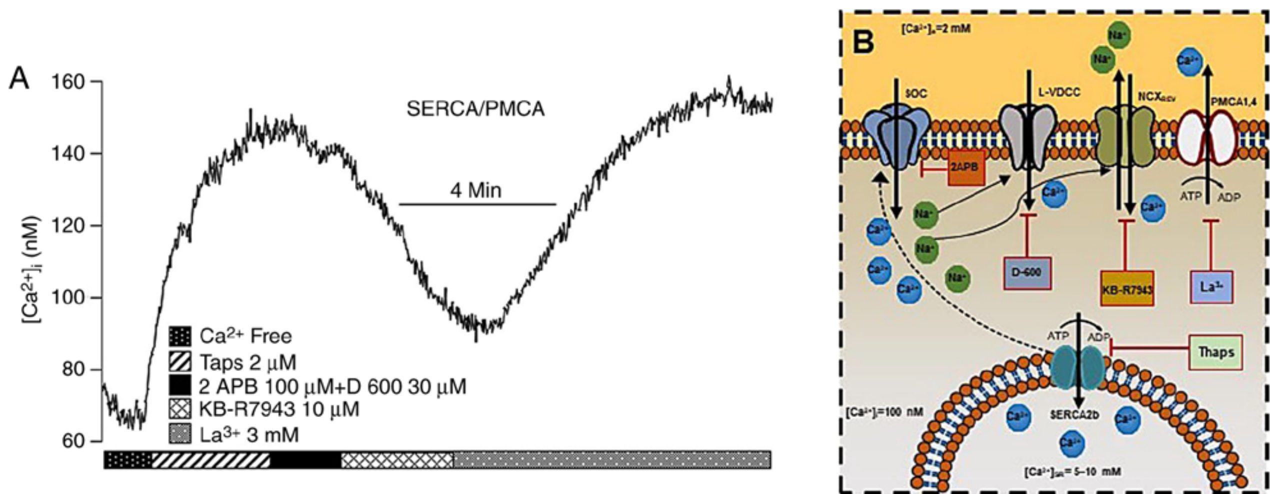

Figure 3. In guinea-pig airway smooth muscle, SERCA and PMCA actively participate in maintaining the $_b[Ca^{2+}]_i$ (A) The blockade of SERCA with Thaps (n=6) increased the $[Ca^{2+}]_i$ until a new basal steady state was reached due to capacitative Ca^{2+} entry involving L-VDCC and SOCC. At this point, the NCX changes to its reverse mode, probably due to the entry of Na^+ through SOCC and L-VDCC, and thereby becomes the main contributor to sustaining the Ca^{2+} plateau since KB-R7943 brought $[Ca^{2+}]i$ to a new basal steady state. The addition of lanthanum (La^{3+}), a non-specific PMCA blocker, led to large increase in $[Ca^{2+}]i$, thus indicating that the former new Ca^{2+} basal state was maintained by PMCA activity. Note that all experimental protocols were performed in Ca^{2+}-containing Krebs solution, with the exception of the first 1.5 min at the beginning of the experiment. (B) Schematic representation of the roles of SERCA, PMCA, NCX and NCX_{REV} in maintaining $_b[Ca^{2+}]i$. For further details, see the 'Na^+/Ca^{2+} exchanger' and the 'Ca^{2+}-ATPases in ASM' sections. NCX, Na^+/Ca^{2+} exchanger; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase; PMCA, plasmalemmal Ca^{2+}-ATPase; Thaps, thapsigargin; L-VDCC, L-type voltage-dependent channel; SOCC, store-operated Ca^{2+} channel.

myocytes produced a transient Ca^{2+} peak that returned to its basal value. At this point, lanthanum, a PMCA blocker, induced a sustained $[Ca^{2+}]_i$ increment that promoted apoptosis (108), demonstrating the central functional role of the two pumps in Ca^{2+} handling in ASM. In this regard, it has been proposed that there is a functional coupling between PMCA and SERCA to maintain Ca^{2+} homeostasis (49). Under physiological conditions (i.e., in the presence of extracellular Ca^{2+}), we found in guinea-pig tracheal myocytes that thapsigargin increased $[Ca^{2+}]_i$ until a plateau was reached (Fig. 3A). It is well known that, in ASM, this Ca^{2+} increment is due to capacitative Ca^{2+} entry (i.e., SOCE) predominantly via the TRPC3 channel, a process that also produces membrane depolarization due to the entry of Na^+ (79,111), consequently leading to L-VDCC opening and further Ca^{2+} and Na^+ entry (10,18,21,36,79,112). At this stage, the NCX may change to its reverse mode (i.e., NCX_{REV}) due to the Na^+ entry, thereby becoming the main contributor towards sustaining the Ca^{2+} plateau due to SERCA blockade. This proposition was corroborated using an NCX_{REV}-mode blocker, KB-R7943, which brought $[Ca^{2+}]_i$ to a new basal Ca^{2+} steady state (Fig. 3A) that was maintained by the PMCA activity. At this point, the addition of lanthanum, a non-specific PMCA blocker, led to a marked increase in $[Ca^{2+}]_i$, probably inducing cellular apoptosis, as was suggested by a previous study (108). Taken together, these results corroborated that, under physiological conditions, SERCA and PMCA exert a primordial role in regulating $[Ca^{2+}]_i$ homeostasis, whereas NCX_{REV} only participates when $_b[Ca^{2+}]_i$ is modified and acquires a new steady state (Fig. 3A and B).

Studies associated with the effects of pro-inflammatory cytokines on the ASM SERCA have demonstrated that overnight exposure of human airway myocytes to TNFα or IL-13 decreases the expression of SERCA that, in turn, diminishes the reuptake of SR-Ca^{2+} (113). Notably, these authors also revealed that, unlike other species, e.g., in porcine airways (114), human ASM SERCA does not express phospholamban, but is directly phosphorylated by Ca^{2+}/calmodulin-dependent protein kinase II (113). Thus, it is possible that in an inflammatory process such as asthma, SR-ATPase activity is decreased, which may lead to an increase in the $_b[Ca^{2+}]_i$ to a new steady state, favoring an augmented response to bronchoconstrictor agonists. The same phenomenon may also be occurring as far as the PMCA is concerned; however, further research is required in this field.

7. Ryanodine and IP_3 receptors

RyR is a non-selective cation channel that releases Ca^{2+} from the SR and, in mammals, its three isoforms, RyR1, -2 and -3, are the products of different genes (115). All three isoforms are expressed in smooth muscle, including ASM (115,116). Cyclic ADP-ribose (cADPR) is considered to be their endogenous ligand in airway myocytes, which is regulated by the membrane-bound protein, CD38 (117). This protein has ADP-ribosyl cyclase and hydrolase activity, and is involved in the synthesis or degradation of cADPR, respectively (118,119).

The IP_3 receptor (ITPR) is another non-selective cation channel that releases Ca^{2+} from the SR via IP_3 generated by the $G_q\alpha$ signaling pathway (35). It has three isoforms (ITPR1, -2 and -3) derived from different genes, which share ~60-80% amino acid homology (120,121). These receptors have also been identified in different smooth muscles types, including ASM (36,122-124).

In 1993, Ca^{2+} 'sparks' were described in heart muscle (125), and these were associated with the Ca^{2+}-induced Ca^{2+} release from RyRs (126). In guinea-pig tracheal myocytes, the presence of spontaneous Ca^{2+} sparks was observed for the first time in

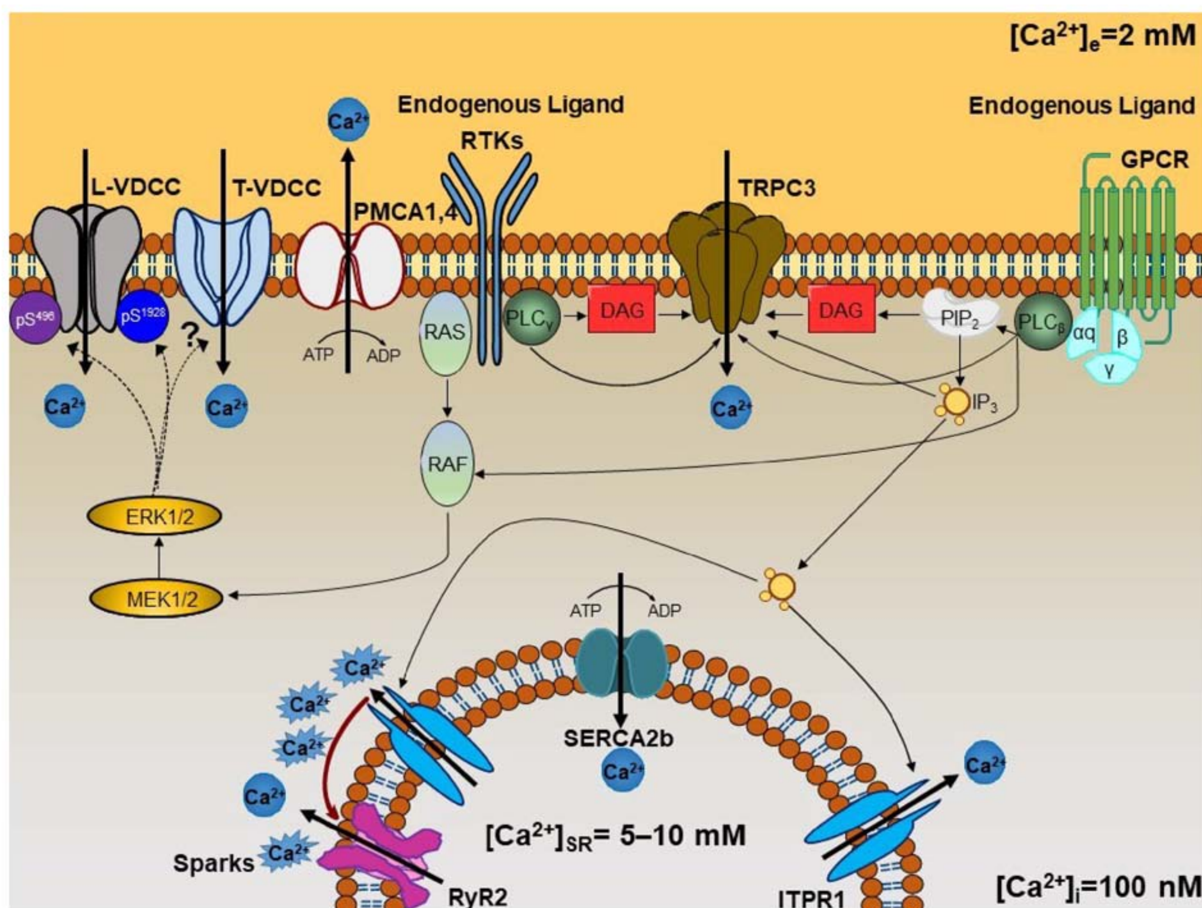

Figure 4. Schematic representation of the mechanisms involved in the maintenance of $_b[Ca^{2+}]$i. Membranal Ca^{2+} channels, such as L-VDCC, T-VDCC and TRPC3, appear to be constitutively active under basal conditions through different signaling pathways. The two types of voltage-dependent Ca^{2+} channel may be modulated by phosphorylation processes mediated by mitogen-activated protein kinase ERK1/2 signaling. This signaling pathway can be activated by GPCRs through the α_q subunit when the endogenous ligand is present under basal conditions (i.e., acetylcholine, histamine, leukotrienes, etc.). It may also be stimulated when RTKs are occupied by the appropriate ligand (cytokines, growth factors, etc.). ERK1/2 phosphorylates L-VDCC on Ser^{496} of the β_2 subunit and Ser^{1928} of the α_1 subunit, decreasing or increasing the channel activity, respectively, enabling it to switch between an open and closed state. T-VDCC is probably also phosphorylated by ERK1/2, but further research is needed to identify the phosphorylation sites (see Fig. 1D). TRPC3 is directly activated by DAG and IP_3 arising from PLC_β or PLC_γ, the first coupled to the α_q subunit of GPCR, and the second to RTKs. Constitutive IP_3 production induces SR-Ca^{2+} release through ITPR1. This Ca^{2+} induces Ca^{2+}-induced Ca^{2+} release through the RyR2 (designated as Ca^{2+} 'sparks'). Finally, $[Ca^{2+}]_i$ is efficiently regulated by the SERCA2b and PMCA1 or PMCA4. L-VDCC, L-type voltage-dependent channel; T-VDCC, T-type voltage dependent Ca^{2+} channel; TRPC3, transient receptor potential canonical-3; ERK1/2, extracellular-signal-regulated kinase 1/2; GPCR, G-protein-coupled receptor; RTK, receptor tyrosine kinase; DAG, diacylglycerol; IP_3, inositol 1,4,5-trisphosphate; PLC, phospholipase C; SR, sarcoplasmic reticulum; ITPR, IP_3 receptor; RyR, ryanodine receptor; SERCA, sarcoplasmic reticulum Ca^{2+}-ATPase; PMCA, plasmalemmal Ca^{2+}-ATPase.

1998 (127). Subsequently, in urinary bladder smooth muscle, these Ca^{2+} sparks were characterized as the elementary release of Ca^{2+} from RyRs (128), and this finding was later corroborated in mouse ASM, occurring predominantly through RyR2 (116,129). In this context, studies on the pulmonary artery revealed that Ca^{2+} sparks are activated by Ca^{2+} released via ITPR (130), as well as in ASM (129). The physiological role of these Ca^{2+} sparks in guinea-pig tracheal myocytes was well established. Essentially, they produce spontaneous transient outward currents caused by large-conductance Ca^{2+}-activated K^+ channels; they also induce spontaneous transient inward currents accomplished through Ca^{2+}-activated Cl-channels (127). Therefore, all these components may serve an important role in the basal state regulation of the ASM by stabilizing the membrane potential, the $_b[Ca^{2+}]_i$ and the basal contractile tone.

Interestingly, further lines of research have demonstrated that pro-inflammatory cytokines (predominantly TNFα), promote the augmentation of CD38-cADPR signaling and increase Ca^{2+} responses to agonists (117,131), a phenomenon that is probably mediated by an augmentation of $_b[Ca^{2+}]_i$. Furthermore, TNFα also enhances $G_q\alpha$ protein expression, thereby increasing the ASM response to carbachol (132). However, upregulation of the IP_3-Ca^{2+} signaling pathway and any consequent modification of the $_b[Ca^{2+}]_i$ in an inflammatory context, such as in asthma, has not readily been identified, and this requires further research.

8. Conclusion

The current review has discussed how several Ca^{2+} handling mechanisms are finely tuned to regulate the $_b[Ca^{2+}]_i$, summarized in Fig. 4. It is conceivable that alterations in any of these processes could render ASM susceptible to developing the type of hyperresponsiveness that is commonly observed in ailments such as asthma, and this warrants further study.

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Availability of data and materials

The datasets presented in the current review are available from the corresponding author on reasonable request.

Authors' contributions

With particular regard to the previously unpublished work presented herein, the contribution of each author was as follows. JRG and ACG performed the assays of intracellular Ca^{2+} levels. EFS performed enzymatic isolation of tracheal myocytes, participated in the assays of intracellular Ca^{2+} levels and data analysis, and provided critical ideas during the writing of the manuscript. BS contributed to the data analysis and writing of the manuscript. LMM contributed to the design and global supervision of the study, data analysis and writing of the manuscript, and was responsible for submitting the paper for publication. All authors read and approved the final manuscript.

Ethics approval and consent to participate

Not applicable.

Patient consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

References

1. Albert AP, Piper AS and Large WA: Properties of a constitutively active Ca^{2+}-permeable non-selective cation channel in rabbit ear artery myocytes. J Physiol 549: 143-156, 2003.
2. Demirel E, Laskey RE, Purkerson S and van Breemen C: The passive calcium leak in cultured porcine aortic endothelial cells. Biochem Biophys Res Commun 191: 1197-1203, 1993.
3. Fayazi AH, Lapidot SA, Huang BK, Tucker RW and Phair RD: Resolution of the basal plasma membrane calcium flux in vascular smooth muscle cells. Am J Physiol 270: H1972-H1978, 1996.
4. Hodgkin AL and Keynes RD: Movements of labelled calcium in squid giant axons. J Physiol 138: 253-281, 1957.
5. Holland WC and Sekul A: Influence of potassium and calcium ions on the effect of ouabain on Ca^{45} entry and contracture in rabbit atria. J Pharmacol Exp Ther 133: 288-294, 1961.
6. Rutter GA, Hodson DJ, Chabosseau P, Haythorne E, Pullen TJ and Leclerc I: Local and regional control of calcium dynamics in the pancreatic islet. Diabetes Obes Metab 19 (Suppl 1): S30-S41, 2017.
7. Wu X, Weng L, Zhang J, Liu X and Huang J: The plasma membrane calcium ATPases in calcium signaling network. Curr Protein Pept Sci 19: 813-822, 2018.
8. Bazan-Perkins B, Flores-Soto E, Barajas-Lopez C and Montaño LM: Role of sarcoplasmic reticulum Ca^{2+} content in Ca^{2+} entry of bovine airway smooth muscle cells. Naunyn Schmiedebergs Arch Pharmacol 368: 277-283, 2003.
9. Carbajal V, Vargas MH, Flores-Soto E, Martinez-Cordero E, Bazán-Perkins B and Montaño LM: LTD_4 induces hyperresponsiveness to histamine in bovine airway smooth muscle: Role of SR-ATPase Ca^{2+} pump and tyrosine kinase. Am J Physiol Lung Cell Mol Physiol 288: L84-L92, 2005.
10. Flores-Soto E, Reyes-Garcia J, Sommer B and Montaño LM: Sarcoplasmic reticulum Ca^{2+} refilling is determined by L-type Ca^{2+} and store operated Ca^{2+} channels in guinea pig airway smooth muscle. Eur J Pharmacol 721: 21-28, 2013.
11. Montaño LM and Bazán-Perkins B: Resting calcium influx in airway smooth muscle. Can J Physiol Pharmacol 83: 717-723, 2005.
12. Hu Z, Ma R and Gong J: Investigation of testosterone-mediated non-transcriptional inhibition of Ca^{2+} in vascular smooth muscle cells. Biomed Rep 4: 197-202, 2016.
13. Braunstein TH, Inoue R, Cribbs L, Oike M, Ito Y, Holstein-Rathlou NH and Jensen LJ: The role of L- and T-type calcium channels in local and remote calcium responses in rat mesenteric terminal arterioles. J Vasc Res 46: 138-151, 2009.
14. Wakle-Prabagaran M, Lorca RA, Ma X, Stamnes SJ, Amazu C, Hsiao JJ, Karch CM, Hyrc KL, Wright ME and England SK: BKCa channel regulates calcium oscillations induced by alpha-2-macroglobulin in human myometrial smooth muscle cells. Proc Natl Acad Sci USA 113: E2335-E2344, 2016.
15. Aguilar HN and Mitchell BF: Physiological pathways and molecular mechanisms regulating uterine contractility. Hum Reprod Update 16: 725-744, 2010.
16. Asano M, Nomura Y, Hayakawa M, Ito KM, Uyama Y, Imaizumi Y and Watanabe M: Increased Ca^{2+} influx in the resting state maintains the myogenic tone and activates charybdotoxin-sensitive K^+ channels in femoral arteries from young SHR. Clin Exp Pharmacol Physiol Suppl 22 (Suppl): S225-S227, 1995.
17. Bae YM, Park MK, Lee SH, Ho WK and Earm YE: Contribution of Ca^{2+}-activated K^+ channels and non-selective cation channels to membrane potential of pulmonary arterial smooth muscle cells of the rabbit. J Physiol 514: 747-758, 1999.
18. Flores-Soto E, Reyes-García J, Carbajal-García A, Campuzano-González E, Perusquía M, Sommer B and Montaño LM: Sex steroids effects on guinea pig airway smooth muscle tone and intracellular Ca^{2+} basal levels. Mol Cell Endocrinol 439: 444-456, 2017.
19. Janssen LJ: T-type and L-type Ca^{2+} currents in canine bronchial smooth muscle: Characterization and physiological roles. Am J Physiol 272: C1757-C1765, 1997.
20. Montaño LM, Barajas-Lopez C and Daniel EE: Canine bronchial sustained contraction in Ca^{2+}-free medium: Role of intracellular Ca^{2+}. Can J Physiol Pharmacol 74: 1236-1248, 1996.
21. Sommer B, Flores-Soto E, Reyes-García J, Diaz-Hernández V, Carbajal V and Montaño LM: Na^+ permeates through L-type Ca^{2+} channel in bovine airway smooth muscle. Eur J Pharmacol 782: 77-88, 2016.
22. Worley JF III and Kotlikoff MI: Dihydropyridine-sensitive single calcium channels in airway smooth muscle cells. Am J Physiol 259: L468-L480, 1990.
23. Bolton TB: Mechanisms of action of transmitters and other substances on smooth muscle. Physiol Rev 59: 606-718, 1979.
24. Godin N and Rousseau E: TRPC6 silencing in primary airway smooth muscle cells inhibits protein expression without affecting OAG-induced calcium entry. Mol Cell Biochem 296: 193-201, 2007.
25. Hallam TJ and Rink TJ: Receptor-mediated Ca^{2+} entry: Diversity of function and mechanism. Trends Pharmacol Sci 10: 8-10, 1989.
26. Martinsen A, Dessy C and Morel N: Regulation of calcium channels in smooth muscle: New insights into the role of myosin light chain kinase. Channels (Austin) 8: 402-413, 2014.
27. McFadzean I and Gibson A: The developing relationship between receptor-operated and store-operated calcium channels in smooth muscle. Br J Pharmacol 135: 1-13, 2002.
28. Murray RK and Kotlikoff MI: Receptor-activated calcium influx in human airway smooth muscle cells. J Physiol 435: 123-144, 1991.
29. Ay B, Prakash YS, Pabelick CM and Sieck GC: Store-operated Ca^{2+} entry in porcine airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 286: L909-L917, 2004.

30. Bazan-Perkins B, Carbajal V, Sommer B, Macías-Silva M, González-Martínez M, Valenzuela F, Daniel EE and Montaño LM: Involvement of different Ca^{2+} pools during the canine bronchial sustained contraction in Ca^{2+}-free medium: Lack of effect of PKC inhibition. Naunyn Schmiedebergs Arch Pharmacol 358: 567-573, 1998.
31. Putney JW Jr: A model for receptor-regulated calcium entry. Cell Calcium 7: 1-12, 1986.
32. Sweeney M, McDaniel SS, Platoshyn O, Zhang S, Yu Y, Lapp BR, Zhao Y, Thistlethwaite PA and Yuan JX: Role of capacitative Ca^{2+} entry in bronchial contraction and remodeling. J Appl Physiol (1985) 92: 1594-1602, 2002.
33. Avila-Medina J, Mayoral-González I, Domínguez-Rodriguez A, Gallardo-Castillo I, Ribas J, Ordoñez A, Rosado JA and Smani T: The complex role of store operated calcium entry pathways and related proteins in the function of cardiac, skeletal and vascular smooth muscle cells. Front Physiol 9: 257, 2018.
34. Baron CB, Cunningham M, Strauss JF III and Coburn RF: Pharmacomechanical coupling in smooth muscle may involve phosphatidylinositol metabolism. Proc Natl Acad Sci USA 81: 6899-6903, 1984.
35. Berridge MJ: Inositol trisphosphate and calcium signalling. Nature 361: 315-325, 1993.
36. Song T, Hao Q, Zheng YM, Liu QH and Wang YX: Inositol 1,4,5-trisphosphate activates TRPC3 channels to cause extracellular Ca^{2+} influx in airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 309: L1455-L1466, 2015.
37. Bazan-Perkins B, Sánchez-Guerrero E, Carbajal V, Barajas-López C and Montaño LM: Sarcoplasmic reticulum Ca^{2+} depletion by caffeine and changes of $[Ca^{2+}]_i$ during refilling in bovine airway smooth muscle cells. Arch Med Res 31: 558-563, 2000.
38. Sieck GC, Kannan MS and Prakash YS: Heterogeneity in dynamic regulation of intracellular calcium in airway smooth muscle cells. Can J Physiol Pharmacol 75: 878-888, 1997.
39. Matsuki K, Kato D, Takemoto M, Suzuki Y, Yamamura H, Ohya S, Takeshima H and Imaizumi Y: Negative regulation of cellular Ca^{2+} mobilization by ryanodine receptor type 3 in mouse mesenteric artery smooth muscle. Am J Physiol Cell Physiol 315: C1-C9, 2018.
40. Zhao C, Wu AY, Yu X, Gu Y, Lu Y, Song X, An N and Shang Y: Microdomain elements of airway smooth muscle in calcium regulation and cell proliferation. J Physiol Pharmacol: 69, 2018.
41. Blaustein MP and Lederer WJ: Sodium/calcium exchange: Its physiological implications. Physiol Rev 79: 763-854, 1999.
42. Eisner DA and Lederer WJ: Na-Ca exchange: Stoichiometry and electrogenicity. Am J Physiol 248: C189-C202, 1985.
43. Janssen LJ, Walters DK and Wattie J: Regulation of $[Ca^{2+}]i$ in canine airway smooth muscle by Ca^{2+}-ATPase and Na^+/Ca^{2+} exchange mechanisms. Am J Physiol 273: L322-L330, 1997.
44. Lipskaia L, Bobe R, Chen J, Turnbull IC, Lopez JJ, Merlet E, Jeong D, Karakikes I, Ross AS, Liang L, *et al*: Synergistic role of protein phosphatase inhibitor 1 and sarco/endoplasmic reticulum Ca^{2+}-ATPase in the acquisition of the contractile phenotype of arterial smooth muscle cells. Circulation 129: 773-785, 2014.
45. Liu B, Zhang B, Huang S, Yang L, Roos CM, Thompson MA, Prakash YS, Zang J, Miller JD and Guo R: Ca^{2+} Entry through reverse mode Na^+/Ca^{2+} Exchanger contributes to store operated channel-mediated neointima formation after arterial injury. Can J Cardiol 34: 791-799, 2018.
46. Mazur II, Veklich TO, Shkrabak OA, Mohart NA, Demchenko AM, Gerashchenko IV, Rodik RV, Kalchenko VI and Kosterin SO: Selective inhibition of smooth muscle plasma membrane transport Ca^{2+}, Mg^{2+}-ATPase by calixarene C-90 and its activation by IPT-35 compound. Gen Physiol Biophys 37: 223-231, 2018.
47. Nishiyama K, Azuma YT, Morioka A, Yoshida N, Teramoto M, Tanioka K, Kita S, Hayashi S, Nakajima H, Iwamoto T and Takeuchi T: Roles of Na^+/Ca^{2+} exchanger isoforms NCX1 and NCX2 in motility in mouse ileum. Naunyn Schmiedebergs Arch Pharmacol 389: 1081-1090, 2016.
48. Sommer B, Flores-Soto E and González-Avila G: Cellular Na^+ handling mechanisms involved in airway smooth muscle contraction (Review). Int J Mol Med 40: 3-9, 2017.
49. Zhang WB and Kwan CY: Pharmacological evidence that potentiation of plasmalemmal Ca^{2+}-extrusion is functionally coupled to inhibition of SR Ca^{2+}-ATPases in vascular smooth muscle cells. Naunyn Schmiedebergs Arch Pharmacol 389: 447-455, 2016.
50. Poburko D, Lhote P, Szado T, Behra T, Rahimina R, McManus B, Van Breemen C and Ruegg UT: Basal calcium entry in vascular smooth muscle. Eur J Pharmacol 505: 19-29, 2004.
51. Bean BP: Classes of calcium channels in vertebrate cells. Annu Rev Physiol 51: 367-384, 1989.
52. Yu J and Bose R: Calcium channels in smooth muscle. Gastroenterology 100: 1448-1460, 1991.
53. Green KA, Small RC and Foster RW: The properties of voltage-operated Ca^{2+}-channels in bovine isolated trachealis cells. Pulm Pharmacol 6: 49-62, 1993.
54. Hisada T, Kurachi Y and Sugimoto T: Properties of membrane currents in isolated smooth muscle cells from guinea-pig trachea. Pflugers Arch 416: 151-161, 1990.
55. Kotlikoff MI: Calcium currents in isolated canine airway smooth muscle cells. Am J Physiol 254: C793-C801, 1988.
56. Marthan R, Martin C, Amedee T and Mironneau J: Calcium channel currents in isolated smooth muscle cells from human bronchus. J Appl Physiol (1985) 66: 1706-1714, 1989.
57. Hirota S and Janssen LJ: Store-refilling involves both L-type calcium channels and reverse-mode sodium-calcium exchange in airway smooth muscle. Eur Respir J 30: 269-278, 2007.
58. Catterall WA, Perez-Reyes E, Snutch TP and Striessnig J: International union of pharmacology. XLVIII. Nomenclature and structure-function relationships of voltage-gated calcium channels. Pharmacol Rev 57: 411-425, 2005.
59. Du W, McMahon TJ, Zhang ZS, Stiber JA, Meissner G and Eu JP: Excitation-contraction coupling in airway smooth muscle. J Biol Chem 281: 30143-30151, 2006.
60. Reyes-Garcia J, Flores-Soto E, Solis-Chagoyan H, Sommer B, Diaz-Hernandez V, Garcia-Hernandez LM and Montaño LM: Tumor necrosis factor alpha inhibits L-type Ca^{2+} channels in sensitized guinea pig airway smooth muscle through ERK 1/2 pathway. Mediators Inflamm 2016: 5972302, 2016.
61. Janssen LJ and Daniel EE: Depolarizing agents induce oscillations in canine bronchial smooth muscle membrane potential: Possible mechanisms. J Pharmacol Exp Ther 259: 110-117, 1991.
62. Xu KY, Zhu W and Xiao RP: $Serine^{496}$ of β_2 subunit of L-type Ca^{2+} channel participates in molecular crosstalk between activation of $(Na^+ + K^+)$-ATPase and the channel. Biochem Biophys Res Commun 402: 319-323, 2010.
63. Wang Y, Sun J, Jin R, Liang Y, Liu YY and Xu YD: Influence of acupuncture on expression of T-type calcium channel protein in airway smooth muscle cell in airway remodeling rats with asthma. Zhongguo Zhen Jiu 32: 534-540, 2012 (In Chinese).
64. Blesneac I, Chemin J, Bidaud I, Huc-Brandt S, Vandermoere F and Lory P: Phosphorylation of the Cav3.2 T-type calcium channel directly regulates its gating properties. Proc Natl Acad Sci USA 112: 13705-13710, 2015.
65. Wylam ME, Gungor N, Mitchell RW and Umans JG: Eosinophils, major basic protein, and polycationic peptides augment bovine airway myocyte Ca^{2+} mobilization. Am J Physiol 274: L997-L1005, 1998.
66. Yocum GT, Chen J, Choi CH, Townsend EA, Zhang Y, Xu D, Fu XW, Sanderson MJ and Emala CW: Role of transient receptor potential vanilloid 1 in the modulation of airway smooth muscle tone and calcium handling. Am J Physiol Lung Cell Mol Physiol 312: L812-L821, 2017.
67. Dietrich A, Chubanov V, Kalwa H, Rost BR and Gudermann T: Cation channels of the transient receptor potential superfamily: Their role in physiological and pathophysiological processes of smooth muscle cells. Pharmacol Ther 112: 744-760, 2006.
68. Ong HL, Brereton HM, Harland ML and Barritt GJ: Evidence for the expression of transient receptor potential proteins in guinea pig airway smooth muscle cells. Respirology 8: 23-32, 2003.
69. Hofmann T, Obukhov AG, Schaefer M, Harteneck C, Gudermann T and Schultz G: Direct activation of human TRPC6 and TRPC3 channels by diacylglycerol. Nature 397: 259-263, 1999.
70. Storch U, Forst AL, Pardatscher F, Erdogmus S, Philipp M, Gregoritza M, Mederos Y Schnitzler M and Gudermann T: Dynamic NHERF interaction with TRPC4/5 proteins is required for channel gating by diacylglycerol. Proc Natl Acad Sci USA 114: E37-E46, 2017.
71. Li SW, Westwick J and Poll CT: Receptor-operated Ca^{2+} influx channels in leukocytes: A therapeutic target? Trends Pharmacol Sci 23: 63-70, 2002.
72. Zitt C, Zobel A, Obukhov AG, Harteneck C, Kalkbrenner F, Luckhpoff A and Schultz G: Cloning and functional expression of a human Ca^{2+}-permeable cation channel activated by calcium store depletion. Neuron 16: 1189-1196, 1996.

73. Xu SZ and Beech DJ: TrpC1 is a membrane-spanning subunit of store-operated Ca^{2+} channels in native vascular smooth muscle cells. Circ Res 88: 84-87, 2001.
74. Wu X, Babnigg G and Villereal ML: Functional significance of human trp1 and trp3 in store-operated Ca^{2+} entry in HEK-293 cells. Am J Physiol Cell Physiol 278: C526-C536, 2000.
75. Gailly P and Colson-Van Schoor M: Involvement of trp-2 protein in store-operated influx of calcium in fibroblasts. Cell Calcium 30: 157-165, 2001.
76. Okada T, Inoue R, Yamazaki K, Maeda A, Kurosaki T, Yamakuni T, Tanaka I, Shimizu S, Ikenaka K, Imoto K, *et al*: Molecular and functional characterization of a novel mouse transient receptor potential protein homologue TRP7. Ca^{2+}-permeable cation channel that is constitutively activated and enhanced by stimulation of G protein-coupled receptor. J Biol Chem 274: 27359-27370, 1999.
77. Vandebrouck C, Martin D, Colson-Van Schoor M, Debaix H and Gailly P: Involvement of TRPC in the abnormal calcium influx observed in dystrophic (mdx) mouse skeletal muscle fibers. J Cell Biol 158: 1089-1096, 2002.
78. Albert AP, Pucovsky V, Prestwich SA and Large WA: TRPC3 properties of a native constitutively active Ca^{2+}-permeable cation channel in rabbit ear artery myocytes. J Physiol 571: 361-369, 2006.
79. Xiao JH, Zheng YM, Liao B and Wang YX: Functional role of canonical transient receptor potential 1 and canonical transient receptor potential 3 in normal and asthmatic airway smooth muscle cells. Am J Respir Cell Mol Biol 43: 17-25, 2010.
80. Trebak M, Bird GS, McKay RR and Putney JW Jr: Comparison of human TRPC3 channels in receptor-activated and store-operated modes. Differential sensitivity to channel blockers suggests fundamental differences in channel composition. J Biol Chem 277: 21617-21623, 2002.
81. Kiyonaka S, Kato K, Nishida M, Mio K, Numaga T, Sawaguchi Y, Yoshida T, Wakamori M, Mori E, Numata T, *et al*: Selective and direct inhibition of TRPC3 channels underlies biological activities of a pyrazole compound. Proc Natl Acad Sci USA 106: 5400-5405, 2009.
82. Albert AP, Piper AS and Large WA: Role of phospholipase D and diacylglycerol in activating constitutive TRPC-like cation channels in rabbit ear artery myocytes. J Physiol 566: 769-780, 2005.
83. Mamoon AM, Smith J, Baker RC and Farley JM: Activation of protein kinase A increases phospholipase D activity and inhibits phospholipase D activation by acetylcholine in tracheal smooth muscle. J Pharmacol Exp Ther 291: 1188-1195, 1999.
84. Monick MM, Carter AB, Gudmundsson G, Mallampalli R, Powers LS and Hunninghake GW: A phosphatidylcholine-specific phospholipase C regulates activation of p42/44 mitogen-activated protein kinases in lipopolysaccharide-stimulated human alveolar macrophages. J Immunol 162: 3005-3012, 1999.
85. Ito S, Kume H, Naruse K, Kondo M, Takeda N, Iwata S, Hasegawa Y and Sokabe M: A novel Ca^{2+} influx pathway activated by mechanical stretch in human airway smooth muscle cells. Am J Respir Cell Mol Biol 38: 407-413, 2008.
86. Leung FP, Yung LM, Yao X, Laher I and Huang Y: Store-operated calcium entry in vascular smooth muscle. Br J Pharmacol 153: 846-857, 2008.
87. Prakriya M, Feske S, Gwack Y, Srikanth S, Rao A and Hogan PG: Orai1 is an essential pore subunit of the CRAC channel. Nature 443: 230-233, 2006.
88. Roos J, DiGregorio PJ, Yeromin AV, Ohlsen K, Lioudyno M, Zhang S, Safrina O, Kozak JA, Wagner SL, Cahalan MD, *et al*: STIM1, an essential and conserved component of store-operated Ca^{2+} channel function. J Cell Biol 169: 435-445, 2005.
89. Peel SE, Liu B and Hall IP: ORAI and store-operated calcium influx in human airway smooth muscle cells. Am J Respir Cell Mol Biol 38: 744-749, 2008.
90. Potier M, Gonzalez JC, Motiani RK, Abdullaev IF, Bisaillon JM, Singer HA and Treback M: Evidence for STIM1- and Orai1-dependent store-operated calcium influx through ICRAC in vascular smooth muscle cells: Role in proliferation and migration. FASEB J 23: 2425-2437, 2009.
91. Shuttleworth TJ: Orai3-the 'exceptional' Orai? J Physiol 590: 241-257, 2012.
92. Liou J, Kim ML, Heo WD, Jones JT, Myers JW, Ferrel JE Jr and Meyer T: STIM is a Ca^{2+} sensor essential for Ca^{2+}-store-depletion-triggered Ca^{2+} influx. Curr Biol 15: 1235-1241, 2005.
93. Prakriya M and Lewis RS: Store-operated calcium channels. Physiol Rev 95: 1383-1436, 2015.
94. Peel SE, Liu B and Hall IP: A key role for STIM1 in store operated calcium channel activation in airway smooth muscle. Respir Res 7: 119, 2006.
95. Zhang SL, Yu Y, Roos J, Kozak JA, Deerinck TJ, Ellisman MH, Stauderman KA and Cahalan MD: STIM1 is a Ca^{2+} sensor that activates CRAC channels and migrates from the Ca^{2+} store to the plasma membrane. Nature 437: 902-905, 2005.
96. Liao Y, Erxleben C, Yildirim E, Abramowitz J, Armstrong DL and Birnbaumer L: Orai proteins interact with TRPC channels and confer responsiveness to store depletion. Proc Natl Acad Sci USA 104: 4682-4687, 2007.
97. Dai JM, Kuo KH, Leo JM, van Breemen C and Lee CH: Mechanism of ACh-induced asynchronous calcium waves and tonic contraction in porcine tracheal muscle bundle. Am J Physiol Lung Cell Mol Physiol 290: L459-L469, 2006.
98. DiPolo R and Beaugé L: Sodium/calcium exchanger: Influence of metabolic regulation on ion carrier interactions. Physiol Rev 86: 155-203, 2006.
99. Philipson KD and Nicoll DA: Sodium-calcium exchange: A molecular perspective. Annu Rev Physiol 62: 111-133, 2000.
100. Lytton J: Na^+/Ca^{2+} exchangers: Three mammalian gene families control Ca^{2+} transport. Biochem J 406: 365-382, 2007.
101. Khananshvili D: The SLC8 gene family of sodium-calcium exchangers (NCX)-structure, function, and regulation in health and disease. Mol Aspects Med 34: 220-235, 2013.
102. Algara-Suarez P, Mejia-Elizondo R, Sims SM, Saavedra-Alanis VM and Espinosa-Tanguma R: The 1.3 isoform of Na^+-Ca^{2+} exchanger expressed in guinea pig tracheal smooth muscle is less sensitive to KB-R7943. J Physiol Biochem 66: 117-125, 2010.
103. Rahman M, Inman M, Kiss L and Janssen LJ: Reverse-mode NCX current in mouse airway smooth muscle: Na^+ and voltage dependence, contributions to Ca^{2+} influx and contraction, and altered expression in a model of allergen-induced hyperresponsiveness. Acta Physiol (Oxf) 205: 279-291, 2012.
104. Sathish V, Delmotte PF, Thompson MA, Pabelick CM, Sieck GC and Prakash YS: Sodium-calcium exchange in intracellular calcium handling of human airway smooth muscle. PLoS One 6: e23662, 2011.
105. Brini M and Carafoli E: Calcium pumps in health and disease. Physiol Rev 89: 1341-1378, 2009.
106. Carafoli E: Calcium pump of the plasma membrane. Physiol Rev 71: 129-153, 1991.
107. Darby PJ, Kwan CY and Daniel EE: Caveolae from canine airway smooth muscle contain the necessary components for a role in Ca^{2+} handling. Am J Physiol Lung Cell Mol Physiol 279: L1226-L1235, 2000.
108. Chen YF, Cao J, Zhong JN, Chen X, Cheng M, Yang J and Gao YD: Plasma membrane Ca^{2+}-ATPase regulates Ca^{2+} signaling and the proliferation of airway smooth muscle cells. Eur J Pharmacol 740: 733-741, 2014.
109. Bobe R, Bredoux R, Corvazier E, Andersen JP, Clausen JD, Dode L, Kovács T and Enouf J: Identification, expression, function, and localization of a novel (sixth) isoform of the human sarco/endoplasmic reticulum Ca^{2+}ATPase 3 gene. J Biol Chem 279: 24297-24306, 2004.
110. Mahn K, Hirst SJ, Ying S, Holt MR, Lavender P, Ojo OO, Siew L, Simcock DE, McVicker CG, Kanabar V, *et al*: Diminished sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) expression contributes to airway remodelling in bronchial asthma. Proc Natl Acad Sci USA 106: 10775-10780, 2009.
111. Helli PB and Janssen LJ: Properties of a store-operated nonselective cation channel in airway smooth muscle. Eur Respir J 32: 1529-1539, 2008.
112. Perusquia M, Flores-Soto E, Sommer B, Campuzano-González E, Martinez-Villa I, Martinez-Banderas AI and Montaño LM: Testosterone-induced relaxation involves L-type and store-operated Ca^{2+} channels blockade, and PGE_2 in guinea pig airway smooth muscle. Pflugers Arch 467: 767-777, 2015.
113. Sathish V, Thompson MA, Bailey JP, Pabelick CM, Prakash YS and Sieck GC: Effect of proinflammatory cytokines on regulation of sarcoplasmic reticulum Ca^{2+} reuptake in human airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 297: L26-L34, 2009.
114. Sathish V, Leblebici F, Kip SN, Thompson A, Pabelick CM, Prakash YS and Sieck GC: Regulation of sarcoplasmic reticulum Ca^{2+} reuptake in porcine airway smooth muscle. Am J Physiol Lung Cell Mol Physiol 294: L787-L796, 2008.

115. Guerrero-Hernandez A, Ávila G and Rueda A: Ryanodine receptors as leak channels. Eur J Pharmacol 739: 26-38, 2014.
116. Liu QH, Zheng YM, Korde AS, Yadav VR, Rathore R, Wess J and Wang YX: Membrane depolarization causes a direct activation of G protein-coupled receptors leading to local Ca^{2+} release in smooth muscle. Proc Natl Acad Sci USA 106: 11418-11423, 2009.
117. Deshpande DA, Walseth TF, Panettieri RA and Kannan MS: CD38/cyclic ADP-ribose-mediated Ca^{2+} signaling contributes to airway smooth muscle hyper-responsiveness. FASEB J 17: 452-454, 2003.
118. Rusinko N and Lee HC: Widespread occurrence in animal tissues of an enzyme catalyzing the conversion of NAD^+ into a cyclic metabolite with intracellular Ca^{2+}-mobilizing activity. J Biol Chem 264: 11725-11731, 1989.
119. White TA, Johnson S, Walseth TF, Lee HC, Graeff RM, Munshi CB, Prakash YS, Sieck GC and Kannan MS: Subcellular localization of cyclic ADP-ribosyl cyclase and cyclic ADP-ribose hydrolase activities in porcine airway smooth muscle. Biochim Biophys Acta 1498: 64-71, 2000.
120. Ross CA, Danoff SK, Schell MJ, Snyder SH and Ullrich A: Three additional inositol 1,4,5-trisphosphate receptors: Molecular cloning and differential localization in brain and peripheral tissues. Proc Natl Acad Sci USA 89: 4265-4269, 1992.
121. Taylor CW, Genazzani AA and Morris SA: Expression of inositol trisphosphate receptors. Cell Calcium 26: 237-251, 1999.
122. Narayanan D, Adebiyi A and Jaggar JH: Inositol trisphosphate receptors in smooth muscle cells. Am J Physiol Heart Circ Physiol 302: H2190-H2210, 2012.
123. Wang YX, Zheng YM, Mei QB, Wang QS, Collier ML, Fleischer S, Xin HB and Kotlikoff MI: FKBP12.6 and cADPR regulation of Ca^{2+} release in smooth muscle cells. Am J Physiol Cell Physiol 286: C538-C546, 2004.
124. Montaño LM, Flores-Soto E, Reyes-Garcia J, Diaz Hernández V, Carbajal-Garcia A, Campuzáno González E, Ramirez-Salinas GL, Velasco-Velázquez M and Sommer B: Testosterone induces hyporesponsiveness by interfering with IP_3 receptors in guinea pig airway smooth muscle. Mol Cell Endocrinol 473: 17-30, 2018.
125. Cheng H, Lederer WJ and Cannell MB: Calcium sparks: Elementary events underlying excitation-contraction coupling in heart muscle. Science 262: 740-744, 1993.
126. Fabiato A: Calcium-induced release of calcium from the cardiac sarcoplasmic reticulum. Am J Physiol 245: C1-C14, 1983.
127. ZhuGe R, Sims SM, Tuft RA, Fogarty KE and Walsh JV Jr: Ca^{2+} sparks activate K^+ and Cl^- channels, resulting in spontaneous transient currents in guinea-pig tracheal myocytes. J Physiol 513: 711-718, 1998.
128. Collier ML, Ji G, Wang Y and Kotlikoff MI: Calcium-induced calcium release in smooth muscle: Loose coupling between the action potential and calcium release. J Gen Physiol 115: 653-662, 2000.
129. Liu QH, Zheng YM and Wang YX: Two distinct signaling pathways for regulation of spontaneous local Ca^{2+} release by phospholipase C in airway smooth muscle cells. Pflugers Arch 453: 531-541, 2007.
130. Zhang WM, Yip KP, Lin MJ, Shimoda LA, Li WH and Sham JS: ET-1 activates Ca^{2+} sparks in PASMC: Local Ca^{2+} signaling between inositol trisphosphate and ryanodine receptors. Am J Physiol Lung Cell Mol Physiol 285: L680-L690, 2003.
131. Jude JA, Solway J, Panettieri RA Jr, Walseth TF and Kannan MS: Differential induction of CD38 expression by TNF-α in asthmatic airway smooth muscle cells. Am J Physiol Lung Cell Mol Physiol 299: L879-L890, 2010.
132. Hotta K, Emala CW and Hirshman CA: TNF-α upregulates Giα and Gqα protein expression and function in human airway smooth muscle cells. Am J Physiol 276: L405-L411, 1999.

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