Histamine-mediated increases in cytosolic \([\text{Ca}^{2+}]\) involve different mechanisms in human pulmonary artery smooth muscle and endothelial cells

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Mauban, Joseph R. H., Katherine Wilkinson, Christian Schach, and Jason X.-J. Yuan. Histamine-mediated increases in cytosolic \([\text{Ca}^{2+}]\) involve different mechanisms in human pulmonary artery smooth muscle and endothelial cells. *Am J Physiol Cell Physiol* 290: C325–C336, 2006. First published September 14, 2005; doi:10.1152/ajpcell.00236.2005.—Agonist stimulation of human pulmonary artery smooth muscle cells (PASMC) and endothelial cells (PAEC) with histamine showed similar spatiotemporal patterns of \([\text{Ca}^{2+}]\) release. Both sustained elevation and oscillatory patterns of changes in cytosolic \([\text{Ca}^{2+}]\) concentration (\([\text{Ca}^{2+}]_{\text{cyt}}\)) were observed in the absence of extracellular \([\text{Ca}^{2+}]\). Capacitative \([\text{Ca}^{2+}]\) entry (CCE) was induced in PASMC and PAEC by passive depletion of intracellular \([\text{Ca}^{2+}]_{\text{cyt}}\) stores with 10 \(\mu\)M cyclopiazonic acid (CPA; 15–30 min). The pyrazole derivative BTP2 inhibited CPA-activated \([\text{Ca}^{2+}]_{\text{cyt}}\) influx, suggesting that depletion of CPA-sensitive internal stores is sufficient to induce CCE in both PASMC and PAEC. The recourse of histamine-mediated \([\text{Ca}^{2+}]_{\text{cyt}}\) release was examined after exposure of cells to CPA, thapsigargin, caffeine, ryanodine, FCCP, or bafilomycin. In PASMC bathed in \([\text{Ca}^{2+}]\)-free solution, treatment with CPA almost abolished histamine-induced rises in \([\text{Ca}^{2+}]_{\text{cyt}}\). In PAEC bathed in \([\text{Ca}^{2+}]\)-free solution, however, treatment with CPA eliminated histamine-induced sustained and oscillatory rises in \([\text{Ca}^{2+}]_{\text{cyt}}\) but did not affect initial transient increase in \([\text{Ca}^{2+}]_{\text{cyt}}\). Furthermore, treatment of PAEC with a combination of CPA (or thapsigargin) and caffeine (and ryanodine), FCCP, or bafilomycin did not abolish histamine-induced transient \([\text{Ca}^{2+}]_{\text{cyt}}\) increases. These observations indicate that 1) depletion of CPA-sensitive stores is sufficient to cause CCE in both PASMC and PAEC; 2) induction of CCE in PAEC does not require depletion of all internal \([\text{Ca}^{2+}]_{\text{cyt}}\); stores; 3) the histamine-releasable internal stores in PASMC and PAEC are mainly CPA-sensitive stores; 4) PAEC, in addition to a CPA-sensitive functional pool, contain other stores insensitive to CPA, thapsigargin, caffeine, ryanodine, FCCP, and bafilomycin; and 5) although the CPA-insensitive stores in PAEC may not contribute to CCE, they contribute to histamine-mediated \([\text{Ca}^{2+}]_{\text{cyt}}\) release.

Intracellular calcium stores; oscillations; pulmonary hypertension

The regulation of pulmonary vascular tone determines the regional blood perfusion and optimal ventilation-to-perfusion ratio in the lung, which are required for normal gas exchange. Hypoxia-induced pulmonary vasoconstriction, for example, directs blood flow away from poorly ventilated areas to achieve maximal oxygenation of the venous blood in the pulmonary artery (62, 67). The contractile state of pulmonary arteries is influenced by neural, humoral, and endothelial factors (4). Agonist-mediated changes in cytosolic \([\text{Ca}^{2+}]_{\text{cyt}}\) in pulmonary artery smooth muscle cells (PASMC) (19, 44, 51, 66) and endothelial cells (PAEC) (25, 27) play a pivotal role in the regulation of excitation-contraction coupling in the pulmonary vasculature. The interaction of PAEC with PASMC via paracrine agonists, upon activation of receptors in either type of these cells, determines whether vessels constrict or relax in response to certain agonists (4, 32). Histamine, a molecule synthesized and released mainly from mast cells in the adventitial layer of pulmonary vessels (e.g., during inflammatory response), is a vasoactive substance with dual effects on the pulmonary vasculature. Histamine can elicit vasoconstriction or vasodilation depending on the cell type (e.g., smooth muscle cells or endothelial cells) on which it is acting and on the receptor subtypes (e.g., H1, H2, H3, or H4) activated (1, 11, 28, 30, 31, 50).

Histamine exerts a vasoconstrictive effect, presumably via activation of H1 receptors, but also exerts a vasodilatory effect on pulmonary arterial muscle preparations (9, 39). Whereas the dual effects of histamine on vessel caliber of human pulmonary arteries have been known for some time, the \([\text{Ca}^{2+}]\) signals that underlie stimulation of PASMC and PAEC with histamine have not been extensively examined. Previous studies have shown that histamine evokes oscillatory membrane currents via release of \([\text{Ca}^{2+}]_{\text{cyt}}\) from internal stores in freshly dissociated rabbit PASMC (64) and induces \([\text{Ca}^{2+}]_{\text{cyt}}\) release and influx in human umbilical vein endothelial cells (37) and PAEC (47, 60). PAEC also show an increase in \([\text{Ca}^{2+}]_{\text{cyt}}\) in response to hypercapnia, where \([\text{Ca}^{2+}]_{\text{cyt}}\) is released from thapsigargin (TG)-insensitive stores (38). A relatively higher dose of histamine elicits heparin-inhibitable \([\text{Ca}^{2+}]_{\text{cyt}}\) release (concurrent with significant phosphatidylinositol hydrolysis) in human PAEC (60). The nature (and identity) of the inositol 1,4,5-trisphosphate (IP3)-sensitive stores involved in the response to histamine, however, was not closely examined in these previous reports.

Sustained pulmonary vasoconstriction and excessive pulmonary arterial medial hypertrophy significantly contribute to the elevated pulmonary vascular resistance and pulmonary arterial pressure in patients with idiopathic pulmonary arterial hypertension (46). Agonist-mediated PASMC contraction and mitogen-mediated cell proliferation play important roles in triggering pulmonary vasoconstriction and stimulating pulmonary vascular remodeling. The agonist- or mitogen-mediated rises in \([\text{Ca}^{2+}]_{\text{cyt}}\) serve as important stimuli for cell contraction and motility, gene expression, cell cycle progression, and cell growth (6, 17, 20, 21, 52). Increased resting \([\text{Ca}^{2+}]_{\text{cyt}}\) and enhanced \([\text{Ca}^{2+}]_{\text{cyt}}\) influx have been demonstrated to cause contraction and stimulate proliferation in PASMC and to increase AP-1 DNA binding activity and stimulate gene expression of...
growth factors in PAEC (13). Interactions between PASMC and PAEC have been implicated in the development of pulmonary arterial hypertension (24).

In this study, we examined and compared the histamine-mediated regulation of [Ca\(^{2+}\)]\(_{cyt}\) in human PASMC and PAEC. Our results indicate that similar pathways of Ca\(^{2+}\) influx, such as capacitative Ca\(^{2+}\) entry (CCE), can be activated by Ca\(^{2+}\) release from internal stores or by depletion of Ca\(^{2+}\) from intracellular stores in both cell types. In PASMC, the intracellular stores involved in regulating CCE are also the major stores responsible for histamine-mediated Ca\(^{2+}\) mobilization. In PAEC, however, the internal stores involved in triggering CCE contribute only partially to histamine-mediated initial transient Ca\(^{2+}\) mobilization but play a critical role in histamine-mediated oscillatory Ca\(^{2+}\) changes in the absence of extracellular Ca\(^{2+}\). More important, our results indicate that human PAEC may contain a hitherto undescribed histamine releasable store that is insensitive to cyclopiazonic acid (CPA), TG, caffeine, ryanodine, FCCP, and bafilomycin and is minimally present in human PASMC.

MATERIALS AND METHODS

Cell preparation and culture. Human PASMC and PAEC from normal subjects (Cambrex, Walkersville, MD) were used in the experiments. PASMC were derived from intrapulmonary arteries of three individuals who were 8 (male Caucasian), 15 (male Caucasian), and 26 (female) years old, respectively, whereas PAEC were derived mainly from extrapulmonary arteries (including main pulmonary artery and left and right branches of main pulmonary artery) from two individuals ages 18 (male Caucasian) and 24 (female) years. Both PASMC and PAEC were cryopreserved at passage 3, replated onto coverslips to amplify cell number for two or three passages, and then used for the proposed experiments for two or three passages. In other words, PASMC and PAEC were used for the experiments at the fifth to ninth passages.

The cells were plated onto coverslips or petri dishes and incubated in a humidified atmosphere of 5% CO\(_2\) in air at 37°C in smooth muscle growth medium (SmGM; Cambrex) for PASMC and in endothelium growth medium (EGM; Cambrex) for PAEC. SmGM was composed of smooth muscle basal medium (SmBM) supplemented with 5% FBS, 0.5 ng/ml human epidermal growth factor, 2 ng/ml human fibroblast growth factor, and 5 μg/ml insulin. EGM was composed of endothelium basal medium (EBM) supplemented with 2% FBS, 0.5 ng/ml human epidermal growth factor, 2 ng/ml human fibroblast growth factor, and 5 μg/ml insulin. Cells were subcultured or plated onto 25-mm coverslips or petri dishes and incubated in a 5% CO\(_2\) atmosphere at 37°C in smooth muscle growth medium (SmGM; Cambrex) for PASMC and in endothelium growth medium (EGM; Cambrex) for PAEC. SmGM was composed of smooth muscle basal medium (SmBM) supplemented with 5% FBS, 0.5 ng/ml human epidermal growth factor, 2 ng/ml human fibroblast growth factor, and 5 μg/ml insulin. EGM was composed of endothelium basal medium (EBM) supplemented with 2% FBS, 0.5 ng/ml human epidermal growth factor, 2 ng/ml human fibroblast growth factor, and 5 μg/ml insulin. Cells were subcultured or plated onto 25-mm coverslips with trypsin-EDTA buffer when 70–90% confluency was achieved. In some experiments, PAEC were grown by incubation in EBM for 24 h. There was no significant morphological difference between proliferating (cultured in EGM) and growth-arrested (cultured in EBM for 24 h) cells. Our previous studies (unpublished observations) showed that most cells (>90%) cultured in the basal medium (without serum and growth factors) are in G0/G1 phases of the cell cycle, whereas >66% of cells cultured in the growth medium (with serum and growth factors) are in S/G2/M phases.

Measurement of [Ca\(^{2+}\)]\(_{cyt}\). Human PASMC and PAEC on coverslips were placed in a recording cell chamber on the stage of an inverted microscope (Nikon). [Ca\(^{2+}\)]\(_{cyt}\) was measured in single PASMC and PAEC was measured with the Ca\(^{2+}\)-sensitive fluorescent indicator fluo-4 AM. Cells were loaded with fluo-4 at room temperature for 15 min using a physiological salt solution (PSS) containing 10 μM fluo-4 AM, 1.5% DMSO (vol/vol), and 0.03% cremophor EL (vol/vol). The fluo-4-loaded cells were then washed with PSS for 15 min to remove excess extracellular dye and to allow intracellular esterases to cleave cytosolic fluo-4 AM into active fluo-4. The PSS contained (in mM) 140 NaCl, 5 KCl, 1.8 CaCl\(_2\), 1 MgCl\(_2\), 5 HEPES, and 5.6 glucose (pH 7.4 at 22°C). In Ca\(^{2+}\)-free PSS, 1–2 mM EGTA was added to chelate residual Ca\(^{2+}\). In nominally Ca\(^{2+}\)-free PSS, CaCl\(_2\) was omitted and no EGTA was added.

Excitation (488 nm) was provided by a xenon arc lamp. Fluorescence emission (>510 nm) was collected with a ×40 Nikon Plan Fluor objective (0.75 numerical aperture) and a charge-coupled device (CCD) camera (Stanford Photonics). “Ca\(^{2+}\) images” or fluorescence images based on the fluorescence signals emitted from the cells were acquired at 1 Hz and stored on a Macintosh computer for later analysis. Although we may refer to the figures as Ca\(^{2+}\) images, all figures are simply Ca\(^{2+}\)-dependent fluo-4 fluorescence images.

Mn\(^{2+}\) quench experiments. Human PASMC and PAEC were incubated in control PSS containing 10 μM fura-2 AM and 1.5% DMSO for 30 min. Cells were then washed for 15 min with PSS to allow cleavage of the loaded fura-2 AM to active fura-2. Excitation of fura-2 to yield Ca\(^{2+}\)-insensitive signals was selected with a narrow-bandwidth excitation filter (360 nm, bandwidth 10 nm). To maximize signal-to-noise ratio, the excitation was left on for 920 ms and the CCD camera was configured to collect photons throughout this period. Fluorescence emission (510 nm) was collected at 1 Hz. To evaluate Mn\(^{2+}\) entry triggered by store depletion, cells were first incubated in Ca\(^{2+}\)-free medium for 5 min and then bathed in Ca\(^{2+}\)-free medium (with 1–2 mM EGTA) containing 10 μM CPA for 15 min. After incubation with CPA, the solution was then switched to nominally Ca\(^{2+}\)-free solution containing 1 mM Mn\(^{2+}\). The narrow-bandwidth excitation filter ensured that the signals collected were not affected by calcium ions. The Mn\(^{2+}\) quench experiments were designed to be Ca\(^{2+}\) insensitive. The excitation wavelength for this protocol was 360 nm, which is a Ca\(^{2+}\)-insensitive wavelength for fura-2. Therefore, fura-2 fluorescence should not change (or should change very little) with increases in [Ca\(^{2+}\)]\(_{cyt}\). Indeed, we did not observe an increase in fluorescence signals (at 360-nm wavelength) in the absence of extracellular Mn\(^{2+}\) during application of histamine to cells (both PASMC and PAEC), thus validating that the signal we detected with 360-nm excitation was not artificially altered by changes in intracellular Ca\(^{2+}\) concentration.

Measurement of area of cells. The area of PASMC and PAEC was determined using ImageJ software (version 1.32j; National Institutes of Health, Bethesda, MD). Briefly, the outlines of the cells were traced manually, and the corresponding areas of cells were calculated by the software and recorded in the computer for further analysis. Values of the area were then reported as a normalized value, A/\(A_0\), where \(A_0\) is the cell area after exposure to histamine and \(A\) is the area of the same cell before exposure to histamine. The percent change of cell area ([\(A/\A_0\) × 100]) was used to indicate histamine-mediated changes in cell area and size.

Chemicals. All chemicals were purchased from Sigma (St. Louis, MO) and prepared as stock solutions in the appropriate solvent. Histamine was prepared as an aqueous stock solution and then diluted to the final concentrations with the appropriate saline solution. CPA and TG were dissolved in DMSO to make a stock solution of 10 mM. Aliquots of the stock solution were then diluted into the appropriate solutions to their final concentration on the day of use. Solutions’ pH values were measured after addition of the drugs and readjusted to 7.4 when necessary.

A cell-permeant analog of 3,5-bis(trifluoromethyl)pyrazole (BTP), or \(N\)-(4-(3,5-bis(trifluoromethyl)-1H-pyrazol-1-yl)phenyl)-4-methyl-1,2,3-thiadiazole-5-carboxamide (Calbiochem), that specifically inhibits store-operated Ca\(^{2+}\) channels with an IC\(_50\) of ~10 nM (23, 26, 69) was dissolved in DMSO to make a stock solution of 10 mM, which was then diluted to the final concentration in bath solutions on the day of use. A specific inhibitor of PLC, 1-6-[[17β]-3-methoxyestra-1,3,5[10]-trien-17-yl]amino][hexyl]-1H-pyrole-2,5-dione (U-73122; Sigma), was used in some experiments to examine the involvement of PLC in histamine-mediated increases in [Ca\(^{2+}\)]\(_{cyt}\).
U-73122 was dissolved in DMSO (50%) and ethanol (50%) to make a stock solution of 25 mM; aliquots were then diluted to the final concentration in bath solutions on the day of use (48, 68).

Statistics and data analysis. Regions of interest (ROI) were chosen in each cell to extract fluorescence values. The fluorescence (F) data were then normalized relative to the basal fluorescence value (Fo) of each individual ROI to yield the normalized value F/Fo. Results are summarized as means ± SE of n cells obtained from multiple coverslips. Each experiment was performed at least three times. Statistical significance was tested with paired and unpaired Student’s t-tests as well as one-way ANOVA, with a P value <0.05 taken as significant.

RESULTS

Histamine-mediated increases in [Ca^{2+}]_cyt are comparable in human PASMC and PAEC in absence of extracellular Ca^{2+}. Extracellular application of 12.5 μM histamine did not change the cell shape or size (Fig. 1, A and B) but significantly increased [Ca^{2+}]_cyt in a similar fashion in PASMC and PAEC bathed in Ca^{2+}-free solution (Fig. 1C), although these two cell types are morphologically different (Fig. 1A). The histamine-mediated increases in [Ca^{2+}]_cyt were composed of an initial rapid transient rise followed by either a sustained or an oscillatory increase due to Ca^{2+} release and Ca^{2+} release-resquestration cycling (Fig. 1C). The oscillatory patterns of histamine-induced [Ca^{2+}]_cyt responses were composed of traveling waves that were not always evident at a 1-Hz sampling rate.

The amplitude of histamine-induced initial [Ca^{2+}]_cyt transients, although varying individually among cells, was comparable between PASMC and PAEC (Fig. 1, C and D). However, in cells that showed [Ca^{2+}]_cyt oscillations (or oscillatory responses to histamine), the frequency of histamine-induced oscillations was quite different between PASMC and PAEC; the oscillation with higher frequency was more common in PAEC. As shown in Fig. 1E, oscillations with frequency >0.08 Hz were observed only in PAEC; mean frequency was 0.04 ± 0.003 Hz in PASMC (17 of 35 cells) and 0.06 ± 0.004 Hz in PAEC (44 of 111 cells). These observations indicate that histamine-mediated Ca^{2+} release may share the same mechanisms (or Ca^{2+} release channels) in PASMC and PAEC, whereas the mechanisms involved in histamine-mediated [Ca^{2+}]_cyt oscillation, which is often closely related to the balance of Ca^{2+} release from and sequestration into various intracellular Ca^{2+} stores [e.g., the sarcoplasmic reticulum (SR)], may be different.

In vascular smooth muscle cells, there are at least two functionally distinct receptor-mediated Ca^{2+} pools in the SR: an IP3-releasable store that is sensitive to CPA and TG and a ryanodine-sensitive store that is sensitive to caffeine (8, 14, 58). The next set of experiments was designed to define 1) whether the Ca^{2+}-storing capacity, or the concentration of releasable Ca^{2+}, in the SR or endoplasmic reticulum (ER) ([Ca^{2+}]_SR/ER) is different between PASMC and PAEC; 2) which intracellular stores, i.e., the CPA- or caffeine-sensitive stores, are responsible for histamine-mediated [Ca^{2+}]_cyt increases in PASMC and PAEC; and 3) whether histamine-

Fig. 1. Characteristics of histamine-mediated Ca^{2+} release in human pulmonary artery smooth muscle cells (PASMC) and endothelial cells (PAEC). A: contrasting morphology typical of human PASMC and PAEC in which cytosolic Ca^{2+} concentration ([Ca^{2+}]_cyt) was measured before (control, left) and during (Hist, right) application of histamine (12.5 μM). B: summary data (means ± SE) showing % changes in area of PASMC (n = 27 cells) and PAEC (n = 42 cells) after application of histamine. Cell area was determined from images like those in A. Values are reported as the normalized value A/Ao, where A is area during exposure to histamine and Ao is cell area in basal condition. C: representative records of [Ca^{2+}]_cyt changes (denoted by fluorescence value normalized to basal fluorescence value (F/F0)) in PASMC (left) and PAEC (right) before, during, and after application of 12.5 μM histamine in Ca^{2+}-free (0Ca, with 2 mM EGTA) solution. Each trace in C represents F/F0 changes in an area of interest located in the cytosol of a single cell. D: amplitude distributions of the histamine-mediated initial peak [Ca^{2+}]_cyt transients in PASMC (top) and PAEC (bottom). Mean frequency ratios (F/F0) are comparable between PASMC (2.48 ± 0.08, n = 55 cells) and PAEC (2.48 ± 0.04, n = 111 cells). E: frequency distribution of the histamine-induced oscillatory changes in [Ca^{2+}]_cyt in PASMC (top) and PAEC (bottom); 48% of PASMC (17 of 35) and 40% of PAEC (44 of 111) exhibited oscillatory changes in [Ca^{2+}]_cyt upon stimulation with histamine.
mediated Ca\(^{2+}\) release is derived from different internal stores in PASMC and PAEC.

Passive depletion of intracellularly stored Ca\(^{2+}\) with CPA causes CCE in PASMC and PAEC. Treatment of human PASMC and PAEC with 10 \(\mu\)M CPA, a sarco(endo)plasmic reticulum Ca\(^{2+}\)-ATPase (SERCA) inhibitor (49) that passively depletes intracellularly stored Ca\(^{2+}\) (potentially in the CPA-sensitive or IP\(_3\)-sensitive SR/ER), induced a relatively slow increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) in the absence of extracellular Ca\(^{2+}\) (Fig. 2A). Prolonged or long-term blockade of SERCA with 1 \(\mu\)M CPA in the absence of extracellular Ca\(^{2+}\) (Fig. 2B) further indicate that CPA-mediated CCE can be induced successfully in both cell types by a 15-min incubation with CPA.

A set of Mn\(^{2+}\) quench experiments (measuring fura-2 fluorescence at 360-nm wavelength) was conducted to measure the rates of Mn\(^{2+}\) influx induced by treatment of the two cell types with CPA. As shown in Fig. 2B, no significant differences were observed in CPA-triggered quenching rates between PASMC and PAEC. Blockade of SOC with 1 \(\mu\)M BTP2 for 15 min abolished the CPA-mediated CCE (Fig. 2C).

The initial rise in [Ca\(^{2+}\)]\(_{\text{cyt}}\) induced by CPA (due to Ca\(^{2+}\) leakage from the SR/ER to the cytosol) in the absence of extracellular Ca\(^{2+}\) was kinetically different (\(P < 0.05\)) between PASMC and PAEC (Fig. 2Ab, left), whereas the [Ca\(^{2+}\)]\(_{\text{cyt}}\) increase due to store depletion-mediated CCE was virtually similar in PASMC and PAEC (Fig. 2Ab, right, B, and C). The quenching experiments (Fig. 2B) further indicate that CPA-mediated CCE is not different between PASMC and PAEC.

As shown in a separate set of Mn\(^{2+}\) quench experiments, the CPA-mediated Ca\(^{2+}\) influx was partially inhibited by nifedipine (1 \(\mu\)M) in PASMC (by 39%; Fig. 3A) but not in PAEC (Fig. 3B). In PASMC, CCE may shift the resting membrane potential to a less negative level and activate nifedipine-sensitive, voltage-dependent Ca\(^{2+}\) channels, contributing to the sustained Ca\(^{2+}\) influx. However, in PAEC, nifedipine had no effect on the CPA-mediated CCE because these cells do not express voltage-dependent Ca\(^{2+}\) channels.

These data indicate that 1) PASMC and PAEC possess similar mechanisms for triggering CCE when the SR/ER is passively depleted by CPA; 2) passive “depletion” of Ca\(^{2+}\) from the CPA-sensitive SR/ER causes CCE with similar spatiotemporal patterns (e.g., in terms of amplitude and kinetics) in PASMC and PAEC; and 3) the intracellular pools that are

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**Fig. 2.** Cyclopiazonic acid (CPA)-mediated passive store depletion induces capacitative Ca\(^{2+}\) entry (CCE) in human PASMC and PAEC. A: representative records (a) of [Ca\(^{2+}\)]\(_{\text{cyt}}\) changes (denoted by F/F\(_0\)) in PASMC (left) and PAEC (right) before, during, and after extracellular application of 10 \(\mu\)M CPA in the absence (0Ca) or presence of extracellular Ca\(^{2+}\). Summarized data (b; means ± SE) show the time course of CPA-mediated changes in [Ca\(^{2+}\)]\(_{\text{cyt}}\) in PASMC (\(n = 36\) cells) and PAEC (\(n = 23\) cells). Reintroduction of extracellular Ca\(^{2+}\) after CPA treatment increased [Ca\(^{2+}\)]\(_{\text{cyt}}\), apparently as a result of CCE (right). The records of CCE-mediated [Ca\(^{2+}\)]\(_{\text{cyt}}\) changes in the absence of extracellular Ca\(^{2+}\) (left) and the records of CCE-mediated [Ca\(^{2+}\)]\(_{\text{cyt}}\) changes after restoration of extracellular Ca\(^{2+}\) (right) are from different cells. B: representative records (a) and summarized data (b; means ± SE) showing Mn\(^{2+}\) quench of fura-2 fluorescence signals at 360 nm in PASMC (\(n = 24\) cells) and PAEC (\(n = 16\)). The cells were pretreated with CPA (10 \(\mu\)M) for 15 min before addition of Mn\(^{2+}\). C: summarized data (means ± SE) showing the time course of CCE-induced increases in [Ca\(^{2+}\)]\(_{\text{cyt}}\) (denoted by F/F\(_0\)) in PASMC (a; \(n = 43\)) and PAEC (b; \(n = 59\)) in the absence (Cont) or presence (BTP2) of 1 \(\mu\)M BTP2. The control curves were constructed from the data presented in Ab (right).

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responsible for storing Ca\(^{2+}\) and releasing Ca\(^{2+}\) in response to agonist treatment may be different between PASMC and PAEC.

Differential contribution of CPA-sensitive stores to histamine-mediated Ca\(^{2+}\) release in PASMC and PAEC. Treatment of PASMC and PAEC with CPA (10 \(\mu\)M for 15 min) depletes intracellularly stored Ca\(^{2+}\) in the CPA-sensitive stores (e.g., IP\(_3\)-sensitive SR/ER). In PASMC bathed in Ca\(^{2+}\)-free solution, passive depletion of Ca\(^{2+}\) from the CPA-sensitive stores, in addition to causing CCE (see Figs. 2 and 3), almost abolished the histamine-induced transient increase in \([\text{Ca}^{2+}]_\text{cyt}\) (Fig. 4A, left, and B). Only a small proportion of PASMC showed brief, low-amplitude, transient responses to application of histamine after depletion of intracellular stores by CPA, indicating that internal stores in PASMC were largely depleted by CPA. However, all PAEC (51 of 51 cells examined) treated with CPA still showed a robust transient Ca\(^{2+}\) release in response to application of histamine. Maximal mean fluorescence observed was 1.68 ± 0.03 \((n = 51)\) in PAEC treated with CPA (Fig. 4B). These observations indicate that 15-min CPA treatment could largely deplete intracellular stores that are responsible for histamine-induced Ca\(^{2+}\) release in PASMC but could not deplete the histamine-releasable intracellular stores in PAEC. These data further suggest that, in addition to inducing Ca\(^{2+}\)
influx through Ca²⁺-permeable channels in the plasma membrane, histamine-mediated increases in \([\text{Ca}^{2+}]_\text{cyt}\) occur by promoting Ca²⁺ release from different intracellular stores in PASMC and PAEC.

**CAFE treatment altered kinetics of histamine-mediated Ca²⁺ release in PAEC.** As described above, incubation of PAEC with 10 μM CPA, although capable of inducing CCE, failed to eliminate histamine-induced rise in \([\text{Ca}^{2+}]_\text{cyt}\). The histamine-induced transient \([\text{Ca}^{2+}]_\text{cyt}\) increase in PAEC pretreated with CPA was lower in amplitude, however, and did not have a sustained component relative to the response observed under control conditions (Fig. 4D). Oscillatory signals in response to histamine could no longer be observed after 15-min incubation of PAEC with CPA. These results suggest that 1) activation of SOC or induction of CCE in PAEC does not require depletion of all internal Ca²⁺ stores; 2) CPA-insensitive stores in PAEC do not significantly contribute to store depletion-mediated CCE; 3) depletion of CPA-sensitive stores in PAEC, although unable to eliminate histamine-induced transient Ca²⁺ release, is sufficient to induce CCE; and 4) the agonist-releasable pools responsible for storing Ca²⁺ may be different between PASMC and PAEC.

Caffeine-sensitive stores are not histamine-releasable stores in PASMC and PAEC. In human PASMC, short-term (<5 min) treatment with 1 mM caffeine did not induce an obvious increase in \([\text{Ca}^{2+}]_\text{cyt}\) and had no effect on histamine-mediated increase in \([\text{Ca}^{2+}]_\text{cyt}\) in the absence of extracellular Ca²⁺. Long-term (15 min) pretreatment with caffeine slightly (but insignificantly) increased the histamine-induced \([\text{Ca}^{2+}]_\text{cyt}\) release (Fig. 5A). The amplitude of histamine-induced initial peak \([\text{Ca}^{2+}]_\text{cyt}\) transient in PASMC treated with 1 mM caffeine for 15 min (F/F₀, 3.22 ± 0.22; n = 14 cells from 6 experiments) was slightly but statistically significantly (P < 0.05) higher than in control PASMC (F/F₀, 2.48 ± 0.08; n = 35 cells from 4 experiments) and PASMC treated with 1 mM caffeine for 5 min (F/F₀, 2.51 ± 0.16; n = 23 cells from 5 experiments) (Fig. 5B). The percentage of cells showing oscillatory responses to histamine was fairly similar in control (29%) and caffeine-treated (25% at 5 min and 50% at 15 min) cells. The percentage of cells showing oscillations from previous experiments in Ca²⁺-free solution was 49% (see Fig. 1C), which was similar to that in cells treated with caffeine for 15 min (50%). These results indicate that most of the Ca²⁺ in human PASMC is stored in the CPA-sensitive stores but that very little is stored in "caffeine-sensitive stores." The Ca²⁺ released from caffeine-sensitive stores can actually be sequestered by CPA-sensitive SERCA to the CPA-sensitive stores in vascular smooth muscle cells (14–16, 58); this is probably why histamine-mediated rise in \([\text{Ca}^{2+}]_\text{cyt}\) due to Ca²⁺ release from intracellular stores is slightly enhanced in PASMC treated with caffeine (Fig. 5A, right, and B).

Pretreatment of PAEC with 1 mM caffeine in a Ca²⁺-free solution (with 2 mM EGTA) neither induced a measurable change in \([\text{Ca}^{2+}]_\text{cyt}\) nor affected histamine-mediated increase in \([\text{Ca}^{2+}]_\text{cyt}\) (Fig. 6). In addition, pretreatment of PAEC with 1 μM TG (for 15 min), similar to treatment with CPA, also failed to abolish histamine-mediated increases in \([\text{Ca}^{2+}]_\text{cyt}\) when
cells were bathed in Ca\(^{2+}\)-free solutions (Fig. 7). Actually, the TG-mediated increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) (compare Fig. 7B, left, with Fig. 2Ab, left) and histamine-induced increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) after TG treatment (compare Fig. 7B, right, with Fig. 4B) were both kinetically comparable to the CPA-mediated increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) and histamine-induced increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) after CPA treatment. These results indicate that CPA and TG, both inhibitors of SERCA, deplete the same intracellular stores in human PAEC.

Concurrent treatment of PAEC with 10 \(\mu\)M CPA and 1 mM caffeine also had negligible effect on histamine-mediated increase in [Ca\(^{2+}\)]\(_{\text{cyt}}\) due to Ca\(^{2+}\)-release (Fig. 8). The results suggest that caffeine-sensitive stores in PAEC do not significantly contribute to histamine-stimulated Ca\(^{2+}\) release. In human PAEC, Ca\(^{2+}\) appears to be stored or distributed in various intracellular stores. In addition to the CPA-sensitive store, a CPA- and caffeine-insensitive store exists in human PAEC and plays an important role in mediating histamine-mediated rise in [Ca\(^{2+}\)]\(_{\text{cyt}}\).

Mitochondria and bafilomycin-sensitive stores do not contribute to histamine-mediated Ca\(^{2+}\) release in PAEC. An agonist-releasable internal store that is insensitive to CPA or caffeine has not been described for human PAEC. We therefore sought to define the potential source of the histamine-mediated Ca\(^{2+}\) release in PAEC. As shown in Fig. 8, treatment of PAEC with 10 \(\mu\)M CPA for a longer time (30 min) was unable to abolish histamine-mediated transient [Ca\(^{2+}\)]\(_{\text{cyt}}\) rise (Fig. 8, Aa, Ab, Ba, and Bb), probably because CPA was not able to deplete the histamine-releasable internal stores. CPA has been demonstrated to inhibit SERCA effectively at nanomolar concentrations (49); the inability of prolonged exposure to micromolar levels of CPA to abolish histamine-mediated Ca\(^{2+}\) release indicates that histamine-releasable internal stores may not express high levels of CPA-sensitive SERCA.

Fig. 7. Effects of pretreatment with thapsigargin (TG) on histamine-mediated Ca\(^{2+}\) mobilization in human PAEC. A: representative traces of [Ca\(^{2+}\)]\(_{\text{cyt}}\) changes in PAEC in response to application of 1 \(\mu\)M TG (left) and to application of 12.5 \(\mu\)M histamine (after ~15 min of exposure to TG; right) under Ca\(^{2+}\)-free conditions. B: summarized data (means \pm SE) of [Ca\(^{2+}\)]\(_{\text{cyt}}\) changes in PAEC (denoted by F/F\(_0\)) in response to 1 \(\mu\)M TG in Ca\(^{2+}\)-free (0Ca) solution (left, n = 76) and to 12.5 \(\mu\)M histamine in cells incubated in TG-containing 0Ca solution (right; for 15 min, n = 80).

Fig. 8. Effects of pretreatment with CPA, caffeine, FCCP, and bafilomycin on histamine-mediated Ca\(^{2+}\) mobilization in human PAEC. Representative traces (A) and summarized data (B; means \pm SE) of [Ca\(^{2+}\)]\(_{\text{cyt}}\) changes (denoted by F/F\(_0\)) in response to extracellular application of 12.5 \(\mu\)M histamine in Ca\(^{2+}\)-free (0Ca) solution in PAEC pretreated with 10 \(\mu\)M CPA alone for 30 min (a; n = 107), CPA + 1 mM caffeine (b; n = 114), CPA + 5 \(\mu\)M FCCP (c; n = 71), or CPA + 0.5 \(\mu\)M bafilomycin (d; n = 80). Pretreatment with CPA alone, CPA + caffeine, CPA + FCCP, or CPA + bafilomycin did not significantly affect histamine-mediated increases in [Ca\(^{2+}\)]\(_{\text{cyt}}\) in PAEC superfused with Ca\(^{2+}\)-free solution.
To examine whether mitochondria and lysosomes are potential histamine-releasable Ca\(^{2+}\) stores in PAEC, we investigated the effect of histamine on cells treated with FCCP, a mitochondrial uncoupler that depletes Ca\(^{2+}\) from mitochondria (12), and bafilomycin, a vacuolar proton pump inhibitor (or vacuolar H\(^+-\)ATPase) that depletes Ca\(^{2+}\) from lysosome-related organelles in various cell types (29). Bath application of 5 \(\mu\)M FCCP to PAEC that had previously been incubated in 10 \(\mu\)M CPA (15 min) evoked a small, detectable increase in [Ca\(^{2+}\)]\(_{cyt}\) (data not shown). A robust, brief transient could still be evoked by histamine in PAEC even after treatment (~9 min) with combined CPA and FCCP (Fig. 8, Ac and Bc). The amplitudes of histamine-induced [Ca\(^{2+}\)]\(_{cyt}\) transients in PAEC treated with CPA (30 min), CPA + caffeine, and CPA + FCCP were all comparable (Fig. 8, Ba–Bc). Furthermore, combined treatment with CPA + bafilomycin (0.5 \(\mu\)M for 15 min) also failed to eliminate histamine-induced [Ca\(^{2+}\)]\(_{cyt}\) transients in PAEC (Fig. 8, Ad and Bd). These results suggest that the histamine-induced transient Ca\(^{2+}\) release is neither from CPA- and caffeine-sensitive SR/ER nor from mitochondria or lysosome-like organelles.

To further confirm that a ryanodine-sensitive store is not involved in the residual response to histamine, we examined whether histamine was able to induce Ca\(^{2+}\) release from intracellular stores in PAEC treated with combined CPA, caffeine, and ryanodine. As shown in Fig. 9A, histamine still caused a significant increase in [Ca\(^{2+}\)]\(_{cyt}\) in PAEC treated for 15 min with CPA (10 \(\mu\)M), caffeine (1 mM), and ryanodine (100 \(\mu\)M) in the absence of extracellular Ca\(^{2+}\). Because a high concentration of ryanodine efficiently blocks Ca\(^{2+}\) release through ryanodine receptors, these experiments provide further evidence that there is a unique histamine-releasable store in human PAEC, which is neither the CPA- or TG-sensitive store nor the ryanodine- or caffeine-sensitive store. Although this unique store was insensitive to CPA, TG, caffeine, ryanodine, FCCP, and bafilomycin, our study indicated that the histamine-induced Ca\(^{2+}\) release from this store still depended on activation of PLC. Inhibition of PLC with U-73122 (50 \(\mu\)M) in the presence of CPA (10 \(\mu\)M) abolished the histamine-mediated Ca\(^{2+}\) release in PAEC (Fig. 9B). These data suggest that the histamine-releasable (CPA and TG insensitive) store is functionally coupled to PLC; receptor-mediated activation of PLC is required to trigger Ca\(^{2+}\) release from this store.

**Histamine-mediated Ca\(^{2+}\) release is not different between proliferating and growth-arrested PAEC.** Expression and function of membrane receptors and ion channels responsible for agonist-mediated Ca\(^{2+}\) influx and release may change significantly between proliferating and quiescent (or growth arrested) phenotypes. The experiments described above were all performed in proliferating PAEC cultured in EGM containing serum and growth factors. To examine whether PAEC behave differently in proliferating and growth-arrested phenotypes, we examined the effect of histamine on [Ca\(^{2+}\)]\(_{cyt}\) or Ca\(^{2+}\) mobilization in PAEC preincubated for 24 h in EBM that did not include serum and growth factors. As shown in Fig. 10, histamine-mediated increase in [Ca\(^{2+}\)]\(_{cyt}\) occurred in 96% of growth-arrested PAEC after treatment with CPA (66 of 69 cells; Fig. 10), which was similar to proliferating PAEC (51 of 51 cells; Fig. 4). In addition to the percentage of responsive cells in the total cell population examined, the kinetics of histamine-induced [Ca\(^{2+}\)]\(_{cyt}\) rise or Ca\(^{2+}\) release in CPA-treated cells was also comparable between growth-arrested (Fig. 10B, right) and proliferating PAEC (Fig. 8Ba). These results indicate that the histamine-releasable and CPA-insensitive intracellular store is functionally present in both quiescent and proliferating PAEC phenotypes.
oxide and prostacyclin on activation of H1 receptors on PAEC is involved in histamine-mediated pulmonary vasodilation (30, 31, 39, 55). Furthermore, histamine-mediated Ca2+ mobilization from intracellular stores evokes oscillatory K+ and Cl− currents by opening Ca2+-activated K+ channels and Ca2+-activated Cl− channels (64) that are highly expressed in PASMC and PAEC.

CPA-sensitive 
Ca2+ stores contribute to histamine-mediated 
Ca2+ release. On activation of respective receptors by agonists or ligands (e.g., histamine), Ca2+ release may be derived from one or more types of intracellular Ca2+ stores including the SR/ER, acidic stores, Golgi apparatus, and mitochondria. Ca2+ can be released via action of IP3 on IP3 receptors (IP3R) located on the internal store membrane and/or via Ca2+-induced Ca2+ release from stores that contain ryanodine receptors. The SR/ER Ca2+ stores can be further subdivided into spatially and functionally distinct subcompartments that can unload and refill separately from each other. CPA- and IP3-, as well as caffeine- and ryanodine-sensitive stores have been well demonstrated in vascular smooth muscle cells, neurons, and astrocytes (14–16, 58).

Our results from the present study indicate that the histamine-mediated Ca2+ release in human PASMC is derived mainly from CPA- and IP3-sensitive stores because incubation of cells in 10 μM CPA was able to eliminate the response to stimulation with histamine. In PAEC, a major proportion of Ca2+ is also released from CPA- and IP3-sensitive stores in response to histamine stimulation, because treatment of PAEC with CPA significantly attenuated the histamine-mediated rise in [Ca2+]cyt in the absence of extracellular Ca2+. In addition, preincubation of PAEC with CPA also altered the kinetics of the histamine-mediated rise in [Ca2+]cyt; the CPA treatment eliminated histamine-induced [Ca2+]cyt oscillations. These observations suggest that CPA- and IP3-sensitive stores play a significant role in histamine-stimulated increases in [Ca2+]cyt in both PASMC and PAEC.

Another significant difference between PASMC and PAEC shown in our study was that histamine-mediated oscillation frequency was greater in PAEC than in PASMC (Fig. 1E). Ca2+ oscillations are regulated by 1) the balance between Ca2+ release from intracellular stores through Ca2+ release channels and Ca2+ sequestration back into the stores via Ca2+-Mg2+-ATPase in the SR/ER (SERCA), 2) the balance between Ca2+ influx through Ca2+ channels and Ca2+ extrusion via Ca2+-Mg2+-ATPase in the plasma membrane, and 3) the subcellular organization of the Ca2+ release and sequestration system (18, 65). Because PAEC are morphologically smaller than PASMC and tend to have a much smaller cytosol area or store-to-surface distance than PASMC, the relatively higher frequency of Ca2+ oscillations in PAEC might be due to 1) a shorter distance between the SR/ER (or Ca2+ release channels and SERCA) and the plasma membrane (or Ca2+-permeable channels and Ca2+ pumps), 2) a smaller subpluralmemmal area, and 3) different organization of Ca2+ release and uptake systems. It is unclear whether the activation threshold of Ca2+ channels and Ca2+ pumps in the SR and plasma membrane differs between human PASMC and PAEC.

Depletion of CPA-sensitive 
Ca2+ stores is sufficient to in- 
duce CCE in PASMC and PAEC. Maintaining a high concentration (or capacity) of Ca2+ in the SR/ER ([Ca2+]SR/ER) is not only important in the signal transduction process required for

**DISCUSSION**

Histamine is a physiologically relevant mediator that has been implicated in allergic, inflammatory, and immune responses (2, 53, 59) as well as in neurotransmission and cell differentiation and maturation. In pulmonary arteries from rabbit, guinea pig, and rat, histamine is known to exert vasoconstrictive effects (31, 34, 50). As demonstrated in studies using pulmonary arteries from Wistar-Kyoto and spontaneously hypertensive rats, histamine can also exert endothelium-dependent and -independent relaxation (31, 57). In the lung, histamine is synthesized and released mainly from mast cells that are localized to the adventitia of pulmonary vessels. Histamine can affect multiple cell types that carry one or more specific subtypes of G protein-coupled receptors (H receptors) on the plasma membrane. There are four subtypes of surface receptors that mediate responses to histamine (H1, H2, H3, and H4 receptors). Stimulation with histamine is known to increase hydrolysis of phosphoinositol, enhance accumulation of inositol phosphates, and elevate [Ca2+]cyt; the CPA treatment

![Diagram](http://example.com/diagram.png)

**Fig. 10.** Effect of pretreatment with CPA on histamine-mediated Ca2+ mobilization in growth-arrested human PAEC. Representative traces (A) and summarized data (B; means ± SE) of [Ca2+]cyt changes in PAEC (precultured in endothelium basal medium for 24 h) in response to application of 10 μM CPA (left; n = 74) and to application of 12.5 μM histamine (after ~15 min of exposure to CPA; right; n = 69), under Ca2+-free conditions. Almost all (66 of 69) growth-arrested cells treated with CPA showed a response to bath application of histamine similar to that of proliferating PAEC cultured in endothelium growth medium.
cell contraction, migration, and gene expression but also necessary for appropriate lipid and protein synthesis in the SR/ER. Depletion of Ca^{2+} from intracellular stores (e.g., the SR/ER) as a result of activation of IP$_3$ receptors or inhibition of SERCA has been demonstrated to mediate CCE by opening SOC in many cell types. CCE plays a significant role in maintaining the filling state of SR/ER (41). The intracellular Ca^{2+} store that contributes to regulating SOC activity or controlling CCE can be depleted actively by opening Ca^{2+} release channels or passively by blocking Ca^{2+}-Mg^{2+}-ATPase in the SR/ER. Our results from this study indicate that emptying or reduction of IP$_3$-sensitive stores by CPA is sufficient to induce CCE in both human PASMC and PAEC. The CPA-mediated CCE in PASMC and PAEC is sensitive to the SOC blocker BTP2 (23, 26, 69). At present, the exact mechanisms involved in the induction of CCE are still unclear. Several models have been described: 1) a chemical coupling mechanism in which a soluble messenger released from intracellular stores serves as a trigger to open plasmalemmal SOC and induce CCE, 2) a conformational coupling mechanism in which the direct or indirect (via cytoskeleton) interaction between SOC in the plasma membrane and IP$_3$R in the SR mediates opening of SOC and induction of CCE, and 3) a physical coupling mechanism in which the SR is physically, yet indirectly, tethered to the sarcolemmal SOC by one or more scaffolding proteins (40, 42, 45, 61).

Histamine-mediated Ca^{2+} release derives from multiple internal stores in PAEC. A significant difference exists between PASMC and PAEC with regard to the stores involved in the response to stimulation with histamine. In PASMC, pretreatment with CPA, which depletes CPA- or IP$_3$-sensitive stores, almost abolished histamine-mediated increase in fCa$^{2+}$_cyt in the absence of extracellular Ca^{2+}. In PASMC bathed in Ca^{2+}-free solutions, however, pretreatment with effective doses of CPA (5–10 μM) alone almost abolished histamine-mediated increase in fCa$^{2+}$_cyt. These results suggest that PAEC contain an alternate source of histamine-releasable Ca^{2+} that is minimally present in PASMC. This internal Ca^{2+} store is insensitive to CPA (or TG) and caffeine (or ryanodine), so it is not the CPA- and IP$_3$-sensitive SR/ER or caffeine- and ryanodine-sensitive SR/ER.

An intimate relationship exists between the SR/ER and mitochondria. Ca^{2+} released from the SR/ER can be taken up by neighboring mitochondria and result in a transient increase in mitochondrial [Ca$^{2+}$]. The continuous flux of Ca^{2+} into and out of mitochondria, which occurs during stimulation with histamine of some cell types, is then linked to the refilling of nearby SR/ER stores (3, 33, 35). The inability of CPA and FCCP (a proton ionophore that dissipates the mitochondrial membrane potential and depletes Ca^{2+} from mitochondria) to eliminate histamine-mediated Ca^{2+} release, however, suggests that mitochondria do not directly contribute to the histamine-induced elevation of fCa$^{2+}$_cyt in PAEC.

The Ca^{2+} pools (or the SR/ER) that are resistant to SERCA inhibitors (e.g., CPA, TG) have been shown in some mammalian cells (56, 63). The bafilomycin-sensitive V-type ATPases (10) that are associated with acidic Ca^{2+} storage compartments may also exist in the SR/ER and other unknown internal stores. However, the inability of CPA and bafilomycin to eliminate histamine-mediated Ca^{2+} mobilization indicates that the internal stores utilizing bafilomycin-sensitive V-type ATPases do not directly contribute to histamine-mediated Ca^{2+} release in human PAEC.

Together, the results from this study indicate that human PAEC possess a unique internal Ca^{2+} store that is minimally present in PASMC. The identity of this PAEC-specific internal Ca^{2+} store is presently unknown; whether it is the Golgi apparatus requires further investigation. Identification of this novel Ca^{2+} pool in human PAEC and its possible contribution to normal or pathological cellular processes represents a new dimension in our understanding of Ca^{2+} homeostasis and its effects on pulmonary vascular endothelial function. Although it seems not to be coupled to CCE, this unique internal Ca^{2+} store (insensitive to CPA, TG, caffeine, ryanodine, FCCP, and bafilomycin) has sufficient capacity to store and release Ca^{2+} to mediate histamine-induced functional effect on PAEC. This unique store may serve as a "reserved" resource for agonist- and receptor-mediated increases in fCa$^{2+}$_cyt, especially when PAEC are exposed to multiple agonists and growth factors. Amplitude and frequency modulation of intracellular Ca^{2+} signals play important roles in regulating cell contraction, mobility, proliferation, differentiation, and apoptosis; this unique Ca^{2+} store in human PAEC may greatly contribute to the agonist (or histamine)-mediated activation of cytoplasmic enzymes, such as nitric oxide synthase, adenyl cyclases, and calmodulin kinases, and of transcription factors, such as c-Jun, c-Fos, and cAMP response element binding protein (5, 7, 54).

In summary, a rise in fCa$^{2+}$_cyt in PASMC serves as a major trigger for pulmonary vasoconstriction, whereas an increase in fCa$^{2+}$_cyt in PAEC, in addition to inducing cell mobility and migration, may also be an important stimulus for activating enzymes that produce endothelium-derived relaxing factors (e.g., nitric oxide). Our data from this study demonstrate the importance of defining other potential stores that are responsible for histamine-mediated Ca^{2+} release.
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following. 1) PASMC and PAEC share the same intracellular stores and mechanisms for triggering CCE but use different intracellular stores and mechanisms for agonist (e.g., histamine)-mediated Ca2+ mobilization. 2) Both PASMC and PAEC are able to generate oscillations in the absence of extracellular Ca2+; the Ca2+ oscillation in PASMC seems to be due mainly to release-uptake cycling through the same store (i.e., CPA-sensitive store), whereas the Ca2+ oscillation in PAEC is caused predominantly by release-uptake cycling among different stores. 3) Multiple intracellular Ca2+ stores in PAEC ensure agonist-mediated Ca2+ release or signaling; a unique internal store that is not required for triggering CCE but partially involved in agonist-induced Ca2+ release exists in PAEC. 4) The major intracellular stores in PASMC are CPA-sensitive stores that not only are responsible for agonist-mediated Ca2+ mobilization but also contribute to controlling the activity of SOC and regulation of CCE.

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