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Intravenous Anesthetics Inhibit Capacitative Calcium Entry in Pulmonary Venous Smooth Muscle Cells

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Background: **The authors have previously demonstrated that propofol attenuates capacitative calcium entry (CCE) *via* the protein kinase C signaling pathway in pulmonary artery smooth muscle cells (PVSMCs). The current goals were to determine whether CCE exists in PVSMCs; to assess the roles of the protein kinase C, tyrosine kinase (TK), and ρ-kinase signaling pathways in regulating CCE; and to investigate the extent and cellular mechanisms by which intravenous anesthetics (thiopental, midazolam, ketamine, and propofol) alter CCE.**

Methods: **Primary cultures of fura-2–loaded canine PVSMCs were placed in a dish (37°C) on an inverted fluorescence microscope. Intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was measured as the 340/380 fluorescence ratio in individual PVSMCs. Thapsigargin, a sarcoplasmic reticulum Ca^{2+}–adenosine triphosphatase inhibitor, was used to deplete intracellular Ca^{2+} stores after removing extracellular Ca^{2+}. CCE was then activated by restoring extracellular Ca^{2+} (2.2 mM).**

Results: **Thapsigargin caused a transient increase in $[Ca^{2+}]_i$ (160 ± 6%). Restoring extracellular Ca^{2+} caused a rapid peak increase in $[Ca^{2+}]_i$ (155 ± 7% of baseline), followed by a sustained increase in $[Ca^{2+}]_i$ (129 ± 5% of baseline), *i.e.*, CCE was stimulated in PVSMCs. Neither protein kinase C activation nor inhibition had an effect on CCE. ρ-Kinase inhibition also had no effect on CCE, whereas TK inhibition attenuated both peak and sustained CCE. Thiopental, midazolam, ketamine, and propofol each attenuated both peak and sustained CCE. TK inhibition abolished the thiopental-, midazolam-, and ketamine-induced, but not the propofol-induced, decreases in CCE.**

Conclusion: **Capacitative calcium entry is present in canine PVSMCs. Thiopental, midazolam, and ketamine attenuate CCE primarily *via* the TK signaling pathway. Propofol attenuates CCE *via* a TK-independent mechanism.**

CAPACITATIVE calcium entry (CCE) is activated by depletion of intracellular Ca^{2+} stores.[1,2] It is a critical mechanism for refilling intracellular Ca^{2+} stores and maintaining a sustained increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$).[3,4] CCE may also be of importance in the regulation of a number of diverse cellular functions, such as apoptosis, secretion, and gene transcription.[5] Furthermore, it has been suggested that CCE plays an important role in agonist-mediated pulmonary artery contraction.[4] We have previously demonstrated that CCE exits and is involved in $[Ca^{2+}]_i$ oscillations as well as the contractile response induced by α_1-adrenoreceptor activation in pulmonary artery smooth muscle cells (PASMCs).[6]

Pulmonary veins are a primary site for entry of vagal nerves into the left atrium[7] and are likely involved in atrial fibrillation.[8] Pulmonary venous constriction results in pulmonary edema formation in congestive heart failure,[9] as well as in high-altitude pulmonary edema.[10] Pulmonary veins are known to constrict in response to a number of stimuli.[11–13] An increase in $[Ca^{2+}]_i$ is a major trigger for pulmonary venous constriction.[12,13] However, the role of CCE in the regulation of $[Ca^{2+}]_i$ in pulmonary venous smooth muscle cells (PVSMCs) is unknown. Moreover, the effects of intravenous anesthetics on CCE in PVSMCs have not been elucidated.

Our first goal was to determine whether CCE exists in PVSMCs. We have previously demonstrated that tyrosine kinase (TK) positively regulates CCE,[6] whereas protein kinase C (PKC) negatively[14] regulates CCE in PASMCs. Our second goal was to investigate the role of the TK, PKC, and ρ-kinase (ROK) signaling pathways in regulating CCE in PVSMCs. We have previously reported that propofol attenuates CCE *via* the PKC signaling pathway in PASMCs.[14] Therefore, our third goal was to investigate the effects of intravenous anesthetics (ketamine, thiopental, midazolam, and propofol) on CCE in PVSMCs and to identify the signaling pathways involved in anesthesia-induced changes in CCE in PVSMCs.

Materials and Methods

Animals

Pulmonary veins were isolated from adult mongrel dogs. The technique of euthanasia was approved by the Cleveland Clinic Institutional Animal Care and Use Committee (Cleveland, Ohio). All steps were performed aseptically during general anesthesia with intravenous pentobarbital sodium (30 mg/kg) and intravenous fentanyl citrate (20 μg/kg). The dogs were intubated and ventilated. After administration of heparin (6,000 U), the dogs were exsanguinated by controlled hemorrhage *via* a femoral artery catheter and killed with electrically induced ventricular fibrillation. A left lateral thoracotomy was performed, and the heart and lungs were removed *en bloc*. The pulmonary veins ware isolated and dissected in a laminar flow hood using sterile procedures.

Cell Culture of PVSMCs

Primary cultures of PVSMCs were obtained from segmental and subsegmental branches of pulmonary vein

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(the third and fourth generations having diameters < 4 mm). The intralobar veins were carefully dissected and prepared for tissue culture. Explant cultures were prepared according to the method of Campbell and Campbell,[15] with minor modifications. Briefly, the endothelium was removed by gently rubbing with a sterile cotton swab. The tunica adventitia was carefully removed, together with the most superficial part of the tunica media. The remaining part of the media was cut into 2-mm^2 pieces that were explanted in 25-cm^2 culture flasks. The explants were nourished by Dulbecco's modified Eagle medium/F-12 containing 10% fetal bovine serum and 1% antibiotic mixture solution (10,000 U/ml penicillin and 10,000 μg/ml streptomycin) and kept in a humidified atmosphere of 5% CO_2–95% air at 37°C. PVSMCs began to proliferate from explants after 7 days in culture. Cells were allowed to grow for an additional 10–14 days until subconfluence was achieved. Cells were then subcultured to 35-mm glass dishes specially designed for fluorescence microscopy (Bioptechs ΔT system; Butler, PA). Cells from the first passage were used for experiments. The cells exhibited morphologic characteristics of vascular smooth muscle and expressed α-actin as assessed by Western blot analysis.

Fura-2 Loading Procedure

Twenty-four hours before experimentation, the culture medium containing 10% fetal bovine serum was replaced with serum-free medium to arrest cell growth, to allow for establishment of steady state cellular events independent of cell division, and to prevent a false estimate of $[Ca^{2+}]_i$ resulting from binding of available dye to serum protein in the medium. PVSMCs were loaded with the acetoxymethyl ester form of fura-2 (fura-2 AM: 2 μM) at ambient temperature. After the 30-min loading period, the cells were washed twice in Krebs-Ringer's buffer, which contained 125 mM NaCl, 5 mM KCl, 1.2 mM $MgSO_4$, 11 mM glucose, 2.5 mM $CaCl_2$, and 25 mM HEPES at pH 7.40 adjusted with NaOH at ambient temperature for an additional 20 min before initiating the study. This provided enough time to wash away any extracellular fura-2 AM and for intracellular esterases to cleave fura-2 AM into the active fura-2.

Measurement of Intracellular Ca^{2+} Concentration

Intracellular Ca^{2+} concentration was measured as previously described.[6] Culture dishes containing fura-2–loaded PVSMCs were placed in a temperature-regulated (37°C) chamber (Bioptechs, Inc., Butler, PA) mounted on the stage of an Olympus IX-70 inverted fluorescence microscope (Olympus America Inc., Lake Success, NY). Fluorescence measurements were obtained from individual PVSMCs in a culture monolayer using a dual-wavelength spectrofluorometer (Deltascan RFK6002; Photon Technology International, Lawrenceville, NJ) at excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. The volume of the chamber was 1.5 ml. The temperature of all solutions was maintained at 37°C in a water bath. Solution changes were accomplished rapidly by aspirating the buffer in the dish and superfusing it with a pipet. Just before data acquisition, background fluorescence (*i.e.*, fluorescence between cells) was measured and subtracted automatically from the subsequent experimental measurements. Fura-2 fluorescence signals (340, 380, and 340/380 ratio) originating from PVSMCs were continuously monitored at a sampling frequency of 25 Hz and were collected using a software package from Photon Technology International.

Experimental Protocols

In the absence of extracellular Ca^{2+}, thapsigargin was used to deplete intracellular Ca^{2+} stores. Thapsigargin is an irreversible sarcoplasmic reticulum Ca^{2+}-adenosine triphosphatase inhibitor and can induce CCE.[16] After depletion of sarcoplasmic reticulum Ca^{2+} stores, CCE was induced when extracellular Ca^{2+} ($[Ca^{2+}]_o$, 2.2 mM) was restored. The effects of L-type voltage dependent Ca^{2+} channel inhibition (verapamil, 10 μM), nonselective Ca^{2+} channel inhibition (SKF 96365, 50 μM), TK inhibition (tyrphostin 23, 100 μM), PKC activation (phorbol 12-myristate 13-acetate, 1 μM), PKC inhibition (bisindolylmaleimide I, 1 μM), and ROK inhibition (Y27632, 10 μM) on CCE were investigated. The concentration of the inhibitors was chosen based on previous experience in PASMCs[6,14] and PVSMCs.[12,13] The effects of intravenous anesthetics (thiopental, 10–100 μM; midazolam, 10–100 μM; ketamine, 10–100 μM; and propofol, 10–100 μM), alone or in combination with a signaling pathway inhibitor, on CCE were assessed.

Drug Preparation

Verapamil, SKF 96365, tyrphostin 23, phorbol 12-myristate 13-acetate, bisindolylmaleimide (Sigma, St. Louis, MO), and propofol (Aldrich Chemical Co., Milwaukee, WI) were dissolved in dimethyl sulfoxide. The final chamber concentration of dimethyl sulfoxide was less than 0.1% (vol/vol). This diluent had no effect on CCE at the concentration used in these studies. Y27632 (Calbiochem, La Jolla, CA), ketamine (Fort Dodge Animal Health, Fort Dodge, IA), midazolam (American Pharmaceutical Partners Inc., Schaumburg, IL), and thiopental (Sigma) were dissolved in distilled water.

Data Analysis

Data analysis was performed as previously described.[14] Peak and sustained increases in $[Ca^{2+}]_i$ were measured in PVSMCs when the superfusion solution was switched from a Ca^{2+}-free solution to a solution containing 2.2 mM Ca^{2+}. Peak and sustained fluorescence ratio values were averaged before and after each intervention and are expressed as percent of control. The control response to

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which all interventions were compared was the first CCE response after thapsigargin pretreatment. This value was set at 100%. Therefore, each cell served as it own control. The peak response was calculated as the fluorescence change from baseline to peak fluorescence. The sustained response represents the fluorescence values measured when the 340/380 ratio was stable after reintroduction of Ca^{2+} to the buffer. Results are presented as mean ± SEM. Statistical analysis was performed with analysis of variance and the Student *t* test. Differences were considered statistically significant at $P < 0.05$.

Results

CCE in PVSMCs

To identify the presence of CCE in PVSMCs, thapsigargin was used to deplete sarcoplasmic reticulum Ca^{2+} stores in the absence of extracellular Ca^{2+}. Thapsigargin transiently increased $[Ca^{2+}]_i$ by 160 ± 6%, which gradually returned to baseline. Restoring extracellular Ca^{2+} ($[Ca^{2+}]_o$, 2.2 mM) then caused a rapid peak increase in $[Ca^{2+}]_i$ (155 ± 7% of baseline; $P < 0.05$), followed by a sustained increase in $[Ca^{2+}]_i$ (129 ± 5% of baseline; $P < 0.05$), *i.e.*, CCE was stimulated in pulmonary venous smooth muscle cells (fig. 1A). The sustained increase in $[Ca^{2+}]_i$ returned to baseline when $[Ca^{2+}]_o$ was removed. To identify the reproducibility of inducing CCE in the same PVSMC, $[Ca^{2+}]_o$ was sequentially restored and removed three consecutive times. There were no significant differences in the peak or sustained increases in $[Ca^{2+}]_i$ between the first and the second CCE, but the third CCE was slightly smaller in magnitude in the peak and sustained increases in $[Ca^{2+}]_i$ compared with the first CCE (fig. 1B).

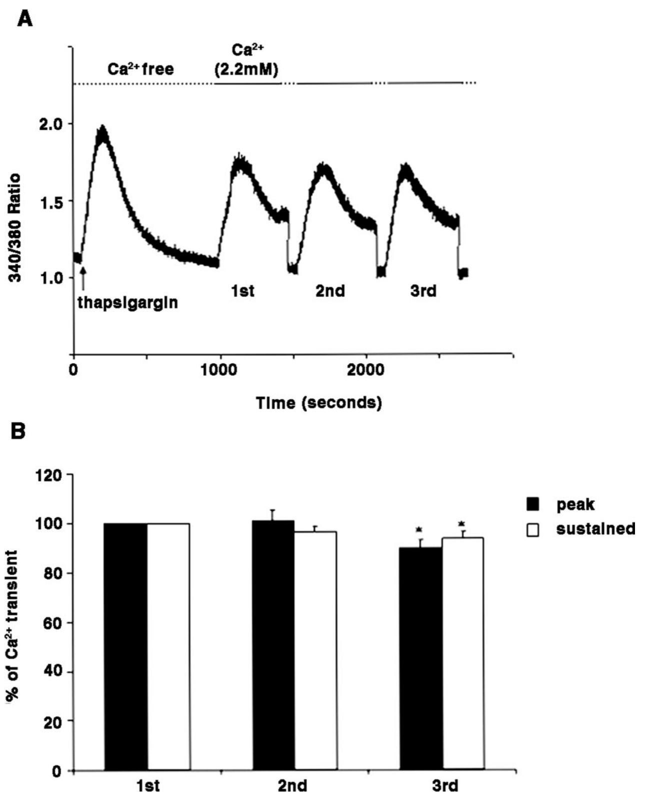

Fig. 1. (*A*) Representative trace depicting capacitative Ca^{2+} entry after depletion of sarcoplasmic reticulum Ca^{2+} stores with thapsigargin in pulmonary venous smooth muscle cells. Extracellular Ca^{2+} was sequentially added and removed three times. (*B*) Summarized data showing the reproducibility of capacitative Ca^{2+} entry. The third capacitative Ca^{2+} entry response was decreased (* $P < 0.05$) slightly compared with the first capacitative Ca^{2+} entry response. n = 13.

Effect of Receptor-operated Ca^{2+} Channel Inhibition on CCE

SKF 96365 is a nonselective Ca^{2+} channel inhibitor that has been used by many investigators to inhibit CCE.[17] SKF 96365 (50 μM) was applied 5 min before $[Ca^{2+}]_o$ was restored the second time (fig. 2A). SKF 96365 attenuated both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 2B).

Effect of Voltage-operated Ca^{2+} Channel Inhibition on CCE

Verapamil was used to inhibit voltage-dependent Ca^{2+} channels. Verapamil (10 μM) was applied 5 min before $[Ca^{2+}]_o$ was restored the second time. Verapamil had no effect on the peak or sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 2B).

Effect of TK Inhibition on CCE

We previously demonstrated that TK plays a role in regulating CCE in PASMCs.[6,14] Tyrphostin 23 was used to inhibit TK. Tyrphostin 23 (100 μM) was applied 5 min before $[Ca^{2+}]_o$ was restored the second time (fig. 3A). Tyrphostin 23 attenuated both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 3B).

Effects of PKC Activation and Inhibition on CCE

We previously demonstrated that PKC plays a role in regulating CCE in PASMCs.[14] Phorbol 12-myristate 13-acetate (1 μM) and bisindolylmaleimide I (1 μM) were used to activate and inhibit PKC, respectively. They were applied 5 min before $[Ca^{2+}]_o$ was restored the second time. Neither phorbol 12-myristate 13-acetate nor bisindolylmaleimide I had an effect on the peak or sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 3B).

Effect of ROK Inhibition on CCE

Y27632 was used to inhibit ROK. Y27632 (10 μM) was applied 5 min before $[Ca^{2+}]_o$ was restored the second time. Y27632 had no effect on the peak or sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 3B).

Effects of Intravenous Anesthetics on CCE

The intravenous anesthetics were applied 15 min before $[Ca^{2+}]_o$ was restored the second time. Ketamine

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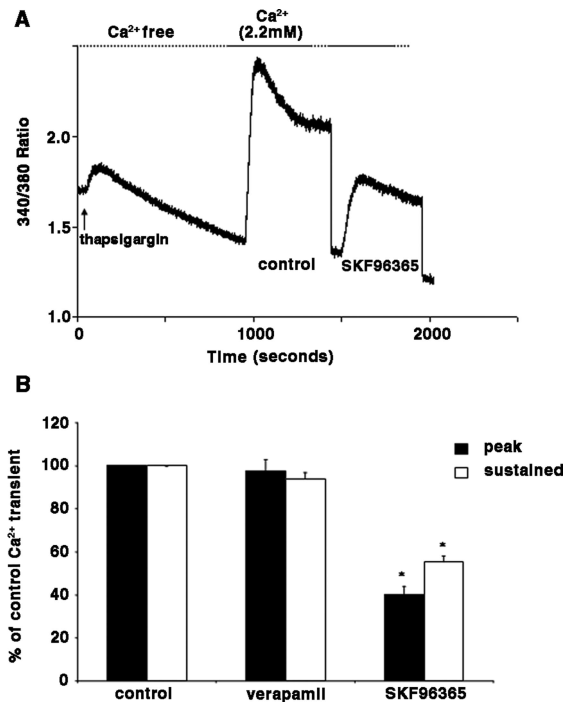

Fig. 2. (*A*) Representative trace depicting the effect of the nonselective Ca^{2+} channel blocker SKF 96365 (50 μM) on capacitative Ca^{2+} entry. After depletion of sarcoplasmic reticulum Ca^{2+} stores with thapsigargin, capacitative Ca^{2+} entry was compared in the absence and presence of SKF 96365, which was added to the buffer 5 min before restoring extracellular Ca^{2+} concentration. (*B*) Summarized data showing the effects of the voltage-dependent Ca^{2+} channel blocker verapamil (10 μM, n = 7) and SKF 96365 (50 μM, n = 9) on capacitative Ca^{2+} entry, respectively. * $P < 0.05$ compared with control.

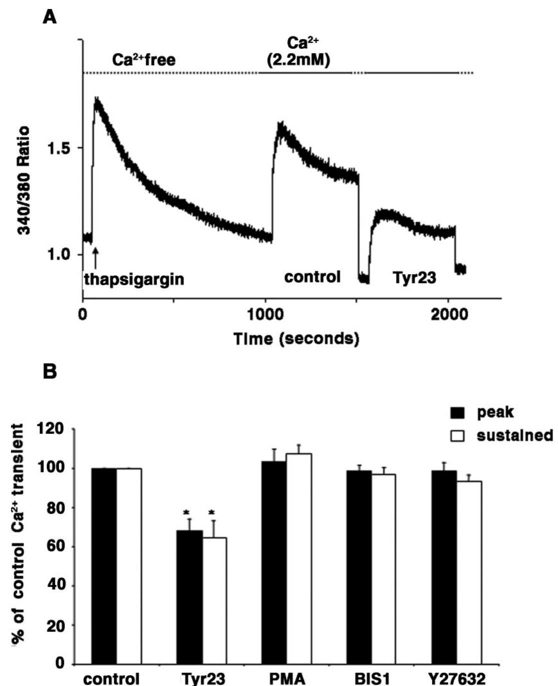

Fig. 3. (*A*) Representative trace depicting the effect of the tyrosine kinase inhibitor tyrphostin 23 (Tyr 23: 100 μM) on capacitative Ca^{2+} entry. After depletion of sarcoplasmic reticulum Ca^{2+} stores with thapsigargin, capacitative Ca^{2+} entry was compared in the absence and presence of tyrphostin 23, which was added to the buffer 5 min before restoring extracellular Ca^{2+} concentration. (*B*) Summarized data showing the effects of tyrphostin 23 (Tyr 23, 100 μM, n = 9), the protein kinase C activator phorbol 12-myristate 13-acetate (PMA, 1 μM, n = 16), the protein kinase C inhibitor bisindolylmaleimide I (BIS 1, 1 μM, n = 6), and the ρ-kinase inhibitor Y27632 (10 μM, n = 6) on capacitative Ca^{2+} entry. * $P < 0.05$ compared with control.

(10–100 μM) caused dose-dependent decreases in both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 4A). Thiopental (30–100 μM) caused dose-dependent decreases in both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 4B), although the lowest concentration of thiopental had no effect (fig. 4B). Midazolam (30–100 μM) caused dose-dependent decreases in both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 5A), whereas the lowest concentration of midazolam (10 μM) had no effect (fig. 5A). Propofol (100 μM) attenuated both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE (fig. 5B), but lower concentrations of propofol (30 μM, 10 μM) had no effect (fig. 5B).

Effect of TK Inhibition on Anesthesia-induced Attenuation of CCE

To determine whether the TK signaling pathway is involved in the anesthesia-induced attenuation of CCE, we investigated the effects of the anesthetics on CCE in the presence of TK inhibition. The intravenous anesthetics were applied before $[Ca^{2+}]_o$ was restored the second time. In the presence of tyrphostin 23, propofol further attenuated both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE compared with TK inhibition alone (fig. 6). However, ketamine, thiopental, and midazolam had no effect on the peak or sustained increases in $[Ca^{2+}]_i$ due to CCE in the presence of TK inhibition compared with TK inhibition alone (fig. 6).

Discussion

Our results demonstrate that CCE exists in canine PVSMCs. The TK signaling pathway, but not the PKC and ROK pathways, is involved in CCE in canine PVSMCs. Clinically relevant concentrations of ketamine and thiopental attenuate CCE, whereas only supraclinical concentrations of midazolam and propofol have this effect. The TK signaling pathway is involved in the ketamine-, thiopental-, and midazolam-induced attenuation of CCE, whereas it is not involved in the propofol-induced attenuation of CCE.

CCE in PVSMCs

Capacitative Ca^{2+} entry has been demonstrated in a variety of cell types, including vascular smooth muscle cells.[18,19] In the current study, we used thapsigargin to deplete the sarcoplasmic reticulum pool of Ca^{2+} in the absence of extracellular Ca^{2+} and thereby activated CCE.[20] Restoring $[Ca^{2+}]_o$ caused a rapid peak increase

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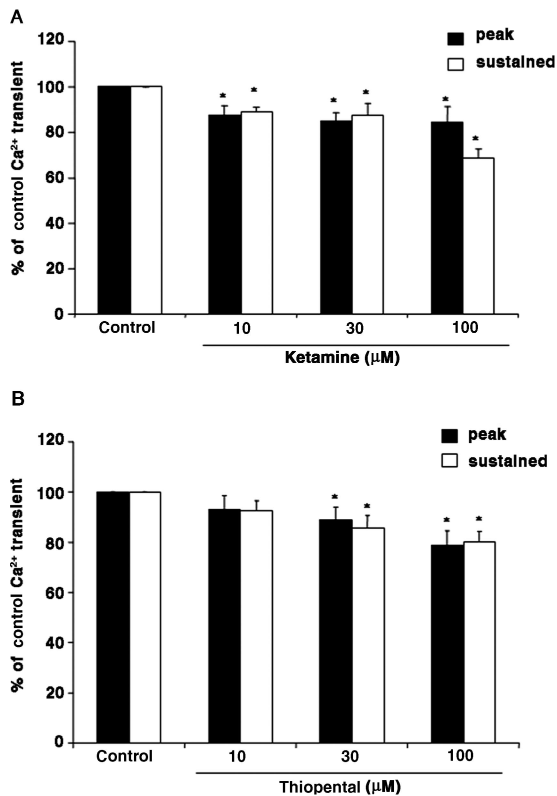

Fig. 4. (*A*) Summarized data showing the dose-dependent inhibitory effects of ketamine (10 μM, n = 9; 30 μM, n = 9; 100 μM, n = 8) on capacitative Ca^{2+} entry. (*B*) Summarized data showing the dose-dependent inhibitory effects of thiopental (30 μM, n = 9; 100 μM, n = 10) on capacitative Ca^{2+} entry. However, 10 μM thiopental had no effect on capacitative Ca^{2+} entry (n = 9). * *P* < 0.05 compared with control.

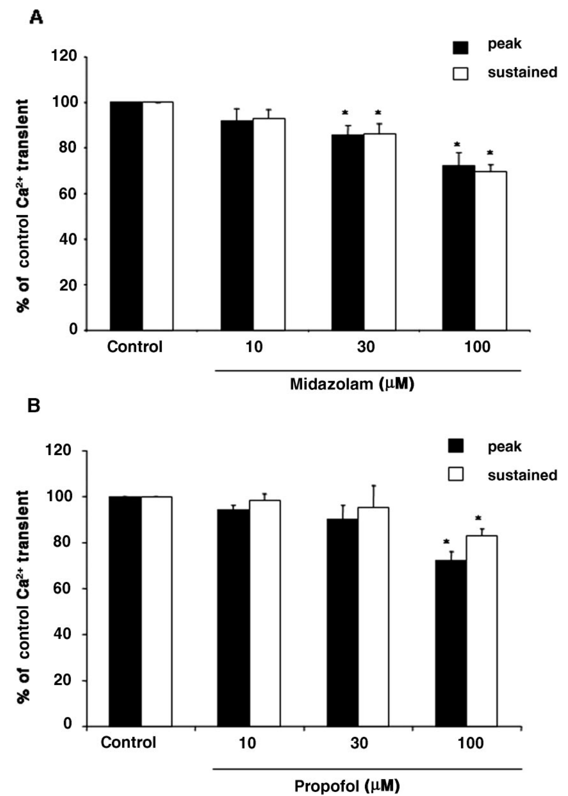

Fig. 5. (*A*) Summarized data showing the dose-dependent inhibitory effects of midazolam (30 μM, n = 6; 100 μM, n = 9) on capacitative Ca^{2+} entry. However, 10 μM midazolam had no effect on capacitative Ca^{2+} entry (n = 6). (*B*) Summarized data showing the inhibitory effects of 100 μM propofol on capacitative Ca^{2+} entry (n = 8). However, lower concentrations of propofol (10 μM, n = 6; 30 μM, n = 7) had no effect on capacitative Ca^{2+} entry. * *P* < 0.05 compared with control.

followed by a sustained increase in $[Ca^{2+}]_i$. This is the first demonstration that CCE exists in PVSMCs. When $[Ca^{2+}]_i$ was restored and removed three times, there were no differences between the first and second CCE responses, but the third CCE was slightly reduced. Therefore, we assessed the effects of interventions by comparing the second CCE response to the first.

Effects of SKF 96365 and Verapamil on CCE

SKF 96365 has been used to block CCE after depletion of sarcoplasmic reticulum Ca^{2+} stores in a variety of cell types.[17] In our study, SKF 96365 markedly attenuated both the peak and sustained increases in $[Ca^{2+}]_i$ due to CCE, whereas verapamil had no effect. These results are consistent with the concept that CCE is insensitive to voltage-gated Ca^{2+} channel inhibitors.

Effect of PKC and ROK Inhibition on CCE

The basis of CCE is that release of Ca^{2+} from intracellular stores increases Ca^{2+} influx. The mechanism linking the decrease in intracellular Ca^{2+} stores to the opening of plasma membrane Ca^{2+} channels remains controversial. One theory postulates the release of a diffusible messenger by the pools, whereas other hypotheses involve a physical interaction between the empty stores and plasma membrane proteins, secretory vesicles, or even cytoskeletal elements.[21] It has been proposed that the sarcoplasmic reticulum might possess protein kinases or phosphatases capable of altering the phosphorylation state of ion channels.[22] Previous studies

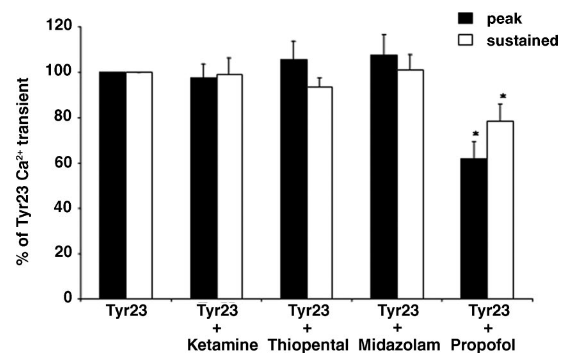

Fig. 6. Summarized data showing the effects of the intravenous anesthetics on capacitative Ca^{2+} entry in the presence of tyrphostin 23 (Tyr 23). In the presence of Tyr 23, ketamine (100 μM, n = 6), thiopental (100 μM, n = 6), and midazolam (100 μM, n = 6) no longer had an inhibitory effect on capacitative Ca^{2+} entry. However, propofol (100 μM, n = 6) continued to decrease capacitative Ca^{2+} entry after pretreatment with Tyr 23. * *P* < 0.05 compared with Tyr23 alone.

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reported that PKC activation could inhibit[23] or facilitate[24] CCE. We have demonstrated that PKC negatively regulates CCE in PASMCs.[14] In the current study, neither the PKC activator, phorbol 12-myristate 13-acetate, nor the PKC inhibitor, bisindolylmaleimide I, had an effect on CCE in PVSMCs. We also assessed the role of another kinase, ROK, in CCE. We have recently reported that ROK is involved in agonist-induced pulmonary venous contraction[12,13] However, Y27632, a ROK inhibitor, had no effect on CCE in PVSMCs. This suggests that the ROK signaling pathway is not involved in CCE in PVSMCs.

Role of TK in CCE in PVSMCs

It has been reported that depletion of intracellular Ca^{2+} stores triggers tyrosine phosphorylation,[25] and inhibition of TK attenuates CCE in a number of cell types,[26,27] including smooth muscle.[28] We have demonstrated that inhibition of TK attenuates CCE in PASMCs.[6,14] In the current study, the TK inhibitor, tyrphostin 23, attenuated CCE in PVSMCs. This suggests that the TK signaling pathway is involved in CCE in PVSMCs.

Effects of Intravenous Anesthetics on CCE in PVSMCs

It is well known that $[Ca^{2+}]_i$ plays an important role in the contraction of smooth muscle. The intravenous anesthetics ketamine,[29] propofol,[30] midazolam,[31] and thiopental[30] have been reported to inhibit smooth muscle contractile responses by reducing $[Ca^{2+}]_i$. Recently, we reported that ketamine attenuated acetylcholine-induced contraction in pulmonary veins.[32] Because CCE is involved in the regulation of $[Ca^{2+}]_i$ in PVSMCs, CCE may serve as a cellular target for intravenous anesthetics in PVSMCs. Therefore, we investigated the effects of intravenous anesthetics on CCE in PVSMCs. Our results indicate that clinical concentrations of ketamine[33] and thiopental[34] caused dose-dependent decreases in CCE in PVSMCs. Midazolam and propofol attenuated CCE only in supraclinical concentrations (midazolam: 100 μM, clinical concentration is 0.3–10 μM[35]; propofol: 100 μM, clinical concentration is 5–50 μM[36]). It has been reported that intravenous anesthetics differentially inhibit phenylephrine-induced $[Ca^{2+}]_i$ oscillations by inhibiting CCE in PASMCs.[6,37] Our results suggest that intravenous anesthetics may alter pulmonary venous tone by inhibiting CCE. To investigate the role of TK as a mechanism by which the anesthetics attenuated CCE, we performed experiments in PVSMCs after pretreatment with the TK inhibitor tyrphostin 23. Compared with TK inhibition alone, ketamine, thiopental, and midazolam no longer attenuated CCE after pretreatment with tyrphostin 23, suggesting that the TK signaling pathway is involved in the reductions in CCE caused by these anesthetics. In contrast, propofol continued to decrease CCE in the presence of tyrphostin 23, suggesting that inhibition of TK is not the primary mechanism for the propofol-induced inhibition of CCE. This result is consistent with a previous report from our laboratory that inhibition of TK is not the primary mechanism for propofol-induced inhibition of CCE in PASMCs.[14] We also reported that propofol attenuated CCE *via* a PKC-dependent mechanism in PASMCs.[14] However, our current study demonstrated that PKC inhibition did not attenuate CCE in PVSMCs. Therefore, the propofol-induced attenuation of CCE in PVSMCs is not likely to involve PKC.

We acknowledge that results obtained from this *in vitro* study can only be cautiously extrapolated to clinical practice. However, because pulmonary venous resistance is an important component of total pulmonary vascular resistance, our results provide new insight concerning the effect of intravenous anesthetics on pulmonary venous contraction.

In summary, CCE exists in PVSMCs. The TK signaling pathway positively regulates CCE, whereas the PKC and ROK signaling pathways are not involved. Ketamine, thiopental, and midazolam attenuate CCE *via* a TK-dependent mechanism.

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