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Calcium Release-Activated Calcium (CRAC) Channels Mediate the β_2-Adrenergic Regulation of Na,K-ATPase

Michael J. Keller[1], **Emilia Lecuona**[1], **Murali Prakriya**[2], **Yuan Cheng**[1], **Saul Soberanes**[1], **G.R. Scott Budinger**[1], and **Jacob I. Sznajder**[1]

[1]Division of Pulmonary and Critical Care Medicine, Department of Medicine, Northwestern University, Feinberg School of Medicine, Chicago IL, 60611

[2]Department of Molecular Pharmacology and Biological Chemistry, Northwestern University, Feinberg School of Medicine, Chicago IL, 60611

Abstract

β_2-adrenergic agonists have been shown to regulate Na,K-ATPase in the alveolar epithelium by recruiting Na,K-ATPase-containing vesicles to the plasma membrane of alveolar epithelial cells (AEC). Here, we provide evidence that β_2-agonists induce store-operated calcium entry (SOCE) in AECs. This calcium entry is necessary for β_2-agonist-induced recruitment of Na,K-ATPase to the plasma membrane of AECs. Specifically, we show that β_2-agonists induce SOCE via stromal interaction molecule 1 (STIM1)-associated calcium release-activated calcium (CRAC) channels. We also demonstrate that the magnitude of SOCE affects the abundance of Na,K-ATPase at the plasma membrane of AECs.

Keywords

Calcium channels; calcium signaling; store-operated calcium entry; epithelial cell; Na,KATPase

INTRODUCTION

In patients with acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), there is fluid accumulation in the alveoli and impaired gas exchange, in part due to the decreased ability of the lungs to clear edema (1, 2). It is well known that alveolar fluid reabsorption occurs mainly by active transport of sodium ions out of the alveolar spaces with water following the osmotic gradient (3). Sodium transport across the epithelium is regulated in part by the basolateral Na,K-ATPase in addition to apical sodium channels and possibly chloride channels (4, 5). Previous studies in animal models of ARDS demonstrated

To whom correspondence should be addressed: Emilia Lecuona, Division of Pulmonary and Critical Care Medicine, Department of Medicine, 240 E. Huron Ave., McGaw Mezzanine, Chicago, IL 60611, USA, Tel.: (312) 908-8163; Fax: (312) 503-0411; e-lecuona@northwestern.edu.

enhanced alveolar fluid clearance (AFC) following treatment with β-agonists (6–9). The increase in AFC is mediated through the β_2-receptor and is due in large part to recruitment of the Na,K-ATPase to the plasma membrane and consequent increased Na,K-ATPase activity (7, 10–13).

Calcium is a second messenger that is important in regulating vesicle fusion and exocytosis in many cell types (14), and β_2-agonists have been shown to increase intracellular calcium levels in cardiac myocytes (15, 16). One of the primary modes of calcium entry in non-excitable cells is store-operated calcium entry (SOCE). SOCE consists of two phases: release of Ca^{2+} from intracellular stores (mainly the endoplasmic reticulum), which then leads to a second phase of sustained Ca^{2+} entry across the plasma membrane through store-operated channels (17, 18). The most common and well described mechanism of SOCE occurs via calcium release-activated calcium (CRAC) channels (19). CRAC channels have two key components which are the calcium-sensing ER transmembrane protein STIM1 and the plasma membrane pore forming Orai proteins (20).

In this work, we found that β_2-agonists elicit SOCE via STIM1-associated CRAC channels in alveolar epithelial cells (AEC). In addition, we show that β_2-agonist induced calcium entry is necessary for the β_2-agonist-induced recruitment of Na,K-ATPase to the plasma membrane of AECs. Finally, we demonstrate that the magnitude of calcium entry in AECs impacts the abundance of Na,K-ATPase at the plasma membrane.

MATERIALS AND METHODS

Reagents

All cell culture reagents were from Corning Life Sciences. Albuterol sulfate (0.083%) vials from Nephron Pharmaceuticals Corporation were purchased through the Northwestern Memorial Hospital pharmacy store. 1,2-Bis(2-aminophenoxy)ethane-*N*,*N*,*N*’,*N*’-tetraacetic acid-acetoxymethyl ester (BAPTA-AM), thapsigargin (TG), and lanthanum (III) chloride were from Sigma-Aldrich. Forskolin was obtained from Ascent Scientific. ICI-118,551 and SQ-22536 were from EMD-Millipore. Rat STIM1 small interfering RNA (siRNA) modified with 3’-AlexaFluor546 was purchased from Qiagen. Non-silencing siRNA and Lipofectamine RNAiMAX were from Life Technologies. EZ-Link N-hydroxysuccinimide-SS-biotin and streptavidin-agarose beads were purchased from Thermo Scientific Pierce Protein Biology. All other chemicals were from Sigma-Aldrich and were the highest grade available.

Cell Lines and Culture

Alveolar type II (ATII) cells were isolated from the lungs of pathogen-free adult male Sprague-Dawley rats (200 – 225 g), as described previously (21). Cells were used on days 2 and 3 after the isolation. Rat lung epithelial (RLE-6TN) cells (ATCC CRL-2300) were grown in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/ml penicillin, 100 μg/ml streptomycin, and 20 μM HEPES.

Transfections

RLE cells were transfected with rat STIM1 siRNA duplexes (100 pmol) by using Lipofectamine RNAiMAX according to the manufacturer's recommended protocol, and experiments were performed 48–72 h later. A nonsilencing siRNA was used as a control.

Measurement of intracellular calcium

ATII or RLE cells plated on 40-mm coverslips were loaded with fura-2-acetoxymethyl ester (Fura2-AM) (Life Technologies) for 30 min at room temperature in standard buffer solution (150 mM NaCl, 5 mM KCl, 1mM $MgCl_2$, 10 mM glucose, 25 mM sodium bicarbonate, and either 2.5 mM $CaCl_2$ or 0.25 mM EGTA pH 7.4) in the dark, washed with PBS, and further incubated for 30 min at room temperature to complete deesterification of the dye. Fura2 dye was excited through 340-nm and 380-nm interference filters housed in a computer-controlled wheel. The fluorescence emitted was collected at 510 nm. The data acquisition of Fura2 video imaging was obtained using a Nikon TE2000 (Nikon Instruments Inc.) equipped with an environmental control system chamber (FCS2 system; Bioptechs Inc.) and a Plan Super Fluor 40X oil objective (Nikon Instruments Inc.). Images were collected with a Cascade electron-multiplying charge-coupled device (EMCCD) camera TC285 with on-chip multiplication gain (Photometrics) drien by MetaFluor software (Molecular Devices Corp.). Changes in calcium concentration were obtained from the F_{340}/F_{380} ratio and expressed as nM concentrations. To convert Fura2 fluorescence measurements, a calcium imaging calibration kit (Life Technologies) was used to generate a titration standard curve. Drugs were perfused to the cells using a pumping system with tubes equipped with stopcocks.

Biotinylation of cell surface proteins

Cells were labeled for 20 min at 4°C using 1 mg/ml EZ-Link *N*-hydroxysuccinimide-SS-biotin and lysed in cell lysis buffer from Cell Signaling as previously described (22, 23). Surface proteins were pulled down with streptavidin-agarose beads and analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blotting.

Cell lysis and Western blot analysis

After treatment, ATII and RLE cells were washed in ice-cold phosphate-buffered saline (PBS) and solubilized in cell lysis buffer (Cell Signaling). The lysates were cleared by centrifugation for 10 min at 14,000 × g. Protein concentrations were determined by the Bradford assay using a commercial dye reagent (Bio-Rad), and samples containing equal amounts of protein were separated by SDS-PAGE and transferred onto nitrocellulose membranes (GE Healthcare Life Sciences) by using a Trans-Blot Turbo (Bio-Rad) transfer system. The following commercially available antibodies and dilutions were used for Western blotting: mouse anti-Na,K-ATPase subunit α1 (clone 464.6; 1:10,000) was from EMD Millipore; rat anti-STIM1 (1:1000) was from Cell Signaling Technology. Primary antibodies were detected with horseradish peroxidase-conjugated secondary goat anti-mouse antibodies (1:10,000; Bio-Rad) or goat anti-rabbit antibodies (1:2,000; Cell Signaling Technology) by using a chemiluminescence detection kit (Perkin-Elmer Life Sciences). Quantification of protein levels was performed by densitometric scanning with ImageJ 1.29X (NIH).

Statistics

Data are presented as means ± standard errors of the means (SEM) and were statistically analyzed using unpaired t-test or one-way analysis of variance (ANOVA) followed by a multiple comparison (Dunnett) test. P values of less than 0.05 were considered statistically significant.

RESULTS

β_2-adrenergic stimulation elicits a rapid increase in intracellular calcium in alveolar epithelial cells

To determine if β_2-adrenergic receptor activation results in calcium entry in alveolar epithelial cells, we stimulated ATII epithelial cells and RLE cells with albuterol, a selective β_2-receptor agonist. Measurements of calcium concentrations in both cell types revealed a rapid increase in intracellular calcium immediately following the application of albuterol to the media (Figure 1, A and B). The peak $[Ca^{2+}]i$ (Figure 1C) as well as the Ca^{2+} influx (represented as $\Delta [Ca^{2+}]/ \Delta t$, (24, 25)) was very similar in both cell types (Figure 1D).

Calcium is necessary for the β_2-agonist-induced recruitment of Na,K-ATPase to the plasma membrane of alveolar epithelial cells

Our previous work demonstrated that β_2-adrenergic receptor activation leads to increased abundance of Na,K-ATPase at the plasma membrane of AECs (7, 10). To determine if calcium was necessary for the β_2-agonist-induced recruitment of Na,K-ATPase to the plasma membrane of AECs, we pretreated both ATII epithelial cells and RLE cells with BAPTA, a chelator of intracellular and extracellular calcium, prior to stimulation with albuterol. As shown in Figure 1, E and F, BAPTA prevented the β_2-agonist-induced recruitment of Na,K-ATPase to the plasma membrane of both cell types.

Alveolar epithelial cells exhibit store-operated calcium entry (SOCE)

In non-excitable cells, the most common route of calcium signal generation results from activation of cell surface receptors which leads to emptying of intracellular calcium stores, followed by calcium entry into the cell via SOCE channels (17, 18). Other common mode of calcium influx involves voltage-gated calcium channels that respond to depolarization and play a significant role in calcium signaling in excitable cells (16). We observed that ATII epithelial cells lack significant voltage-gated calcium channel activity when stimulated with a depolarizing concentration of potassium chloride, but elicit a robust response to thapsigargin, a non-competitive inhibitor of sarco/endoplasmic reticulum Ca^{2+} ATPase and a powerful stimulus of SOCE (Figure 2A). In a similar fashion, we found that albuterol triggered the release of intracellular calcium stores when calcium levels were measured in media lacking calcium, and that SOCE occurred when calcium was returned to the perfusing media (Figure 2B). Noteworthy, albuterol caused a milder influx of Ca^{2+} in alveolar epithelial cells than thapsigargin reflected in the peak $[Ca^{2+}]_i$ (Figure 2C) and the rate of Ca^{2+} influx (Figure 2D) (26).

STIM1-associated CRAC cha nnels mediate albuterol-induced SOCE and recruitment of Na,K-ATPase to the plasma membrane

STIM1 is a transmembrane protein that functions as a calcium sensor in the ER that is responsible for communicating depletion of ER calcium stores to Orai channels in the plasma membrane (18, 19). Calcium entry via CRAC channels can be inhibited various ways, including binding of the channel with lanthanum chloride ($LaCl_3$). We showed that pre-treating ATII epithelial cells with $LaCl_3$ blunted the degree of calcium entry seen after albuterol is applied to the cells (Figure 3A), as well as the peak $[Ca^{2+}]_i$ (Figure 3C) and the rate of Ca^{2+} influx (Figure 3D). We also demonstrated that CRAC channel inhibition with $LaCl_3$ abrogated the increase in Na,K-ATPase protein abundance at the plasma membrane following stimulation with albuterol (Figure 3E). Transfecting RLE cells with siRNA targeting STIM1 tagged with a fluorescent dye (AlexaFluor546) allowed for selection of transfected cells during calcium measurements. We showed that calcium entry in response to albuterol was blocked or blunted in cells transfected with siRNA targeting STIM1 compared to those cells that were not transfected (Figure 3B; ~80% transfection efficiency), as well as the peak $[Ca^{2+}]_i$ (Figure 3C) and the rate of Ca^{2+} influx (Figure 3D). In addition, silencing STIM1 via siRNA prevented the albuterol-induced recruitment of Na,K-ATPase to the plasma membrane (Figure 3F).

Activation of adenylyl cyclase results in SOCE

Binding of an agonist to the β_2-adrenergic receptor results in dissociation of the $G_{s\alpha}$ subunit of the G protein and activation of adenylyl cyclase with subsequent increase in cAMP levels and downstream signaling (27). We first confirmed the specificity of albuterol as a β2-agonist by inhibiting the effect of albuterol in calcium entry by pre-incubation with 1 μM of the β2-antagonist ICI-118,551 (Figure 4A, 4D and 4E). The role of cAMP on SOCE was determined by preventing the albuterol-induced calcium entry by pre-incubation with 10 μM of the adenylyl cyclase inhibitor SQ-22536 (Figure 4B, 4D and 4E). We also found that direct activation of adenylyl cyclase by forskolin resulted in SOCE with similar magnitude of peak calcium but with an increased rate of calcium entry as that seen with albuterol (Figure 4C, 4D and 4E). In addition, we showed that forskolin-induced calcium entry is inhibited in RLE cells transfected with siRNA targeting STIM1 and tagged with a fluorescent dye (AlexaFluor546) (Figure 4F).

The magnitude of SOCE affects the abundance of Na,K-ATPase at the plasma membrane of AECs

Previous work in our lab has demonstrated that treatment of A549 cells with 1 μM thapsigargin results in significant endocytosis of Na,K-ATPase (28). In attempt to explain why a different stimulus, albuterol, that also elicits SOCE results in recruitment of Na,K-ATPase to the plasma membrane, we compared the effect of treatment with different concentrations of thapsigargin on plasma membrane Na,K-ATPase abundance. We showed that a concentration of 1 nM thapsigargin results in SOCE of similar magnitude as albuterol (Figure 5A) as well as similar peak $[Ca^{2+}]_i$ (Figure 5B) and rate of Ca^{2+} influx (Figure 5C). We also found that only high concentrations of thapsigargin result in endocytosis of the Na,K-ATPase, were as lower concentrations have no significant effect on the abundance of

Na,K-ATPase at the plasma membrane (Figure 5D). These results suggest that both calcium and cAMP are necessary for the β_2-agonist induced recruitment of Na,K-ATPase to the plasma membrane in AECs.

DISCUSSION

Previous *in vitro* and animal studies have shown that β-agonists enhance alveolar fluid clearance, and this effect is mediated in part by increased abundance of Na,K-ATPase at the plasma membrane of AECs (6–11, 28). In this work, we demonstrated that β_2-agonists elicit store-operated calcium entry (SOCE) in AECs, and this calcium entry is necessary for the β_2-agonist induced upregulation of Na,K-ATPase at the plasma membrane. Specifically, we showed that SOCE via STIM1-associated CRAC channels is involved in the response. We demonstrated that calcium entry is necessary, but not sufficient for the β_2-agonist-induced recruitment of Na,K-ATPase to the plasma membrane of alveolar epithelial cells. Finally, we showed that the magnitude of SOCE independently affected the abundance of Na,K-ATPase at the plasma membrane of AECs.

SOCE has been reported in AECs (29–31), but this is the first report of β_2-agonists eliciting SOCE in AECs. Calcium is an important component of vesicle fusion and exocytosis in neurons as well as endocrine cells and T-cells (14). Both cAMP and calcium are recognized to play important independent as well as cooperative roles in exocytosis in non-neuronal cells (32). Within alveolar cells, cAMP promotes the recruitment of Na,K-ATPase from intracellular vesicle pools into the plasma membrane (33, 34). By chelating calcium with BAPTA, we showed that calcium was also necessary for the β_2-agonist-induced recruitment of Na,K-ATPase to the plasma membrane.

STIM1 is the primary calcium sensing protein in the ER and a major component of CRAC channels along with the Orai family of proteins (20). Through pharmacologic and siRNA inhibition of CRAC channels and STIM1, we showed that the β_2-agonist induced SOCE is mediated by STIM1-containing CRAC channels. In addition to its role in CRAC channels, STIM1 has been shown to activate adenylyl cyclase and increase cAMP following calcium store depletion in a process which is independent of cytosolic calcium (35). This process of store-operated cAMP signaling may also be contributing in a regulatory mechanism to the β_2-agonist induced SOCE and recruitment of Na,K-ATPase to the plasma membrane of alveolar epithelial cells. STIM1 has also been shown to bind to Na,K-ATPases through a STIM1-POST (partner of STIM1) complex that is triggered by intracellular store depletion (36). This raises the possibility that STIM1 may have a direct stabilization effect on Na,K-ATPase abundance at the plasma membrane and contribute directly to the β_2-agonist induced response.

Activation of β_2-agonist receptors leads to dissociation of the $G_{s\alpha}$ subunit of the G protein and activation of adenylyl cyclase with subsequent increase in cAMP levels. In this work, we show that forskolin elicits SOCE in AECs of similar magnitude as albuterol, indicating that the $G_{s\alpha}$ subunit/adenylyl cyclase and increased cAMP mediate the β_2-agonist induced SOCE; and we confirmed this observation by preventing SOCE in cells treated with albuterol in the presence of the adenylyl cyclase inhibitor SQ22536. We hypothesize that

cAMP by a still not determined mechanism stimulate the increase in intracellular calcium, a phenomenon that might be cell specific as cAMP does not have a direct effect on increasing intracellular calcium in kidney cells (37). A similar interaction between cAMP and calcium entry has been shown to regulate prostaglandin E2-mediated chloride secretion in mouse inner medullary collecting duct cells (38). Inhibiting STIM1 with a fluorescently labeled siRNA confirms the role of STIM1-associated CRAC channels in the cAMP-mediated SOCE.

We have previously demonstrated that robust SOCE elicited by 1μM thapsigargin results in endocytosis of the Na,K-ATPase in alveolar epithelial cells (28). However, albuterol causes SOCE of significantly lower magnitude and similar to that of a lower dose (1 nM) of thapsigargin. We showed that low magnitude SOCE does not have significant effect on the abundance of Na,K-ATPase at the plasma membrane and supports the statement that calcium is necessary but not sufficient for the β_2-agonist induced recruitment of Na,K-ATPase. We have previously demonstrated that PKA is also necessary for the β2-adrenergic-induced recruitment of the Na,K-ATPase to the plasma membrane (12, 13); therefore, we hypothesize that cAMP by increasing intracellular Ca^{2+} and activating PKA leads to the recruitment of the Na,K-ATPase in alveolar epithelial cells. There are numerous potential sites of crosstalk between cAMP and calcium signaling within non-excitable cells that may impact the spatio-temporal pattern of calcium signaling (39). We hypothesize that compartmentalized cAMP signaling in concert with mild increases in calcium levels, likely in spatially related microdomains, work together to augment the recruitment of Na,K-ATPase from intracellular vesicles to the plasma membrane of alveolar epithelial cells.

In summary, as shown schematically in Figure 6, we have shown that calcium entry via STIM1-associated CRAC channels is necessary for the recruitment of Na,K-ATPase to the plasma membrane of ATII epithelial cells following treatment with albuterol and the subsequent increase in cAMP. These findings may allow for further investigations into therapeutic options to enhance alveolar fluid clearance in patients with ARDS.

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HIGHLIGHTS

- β_2-agonists elicit store-operated Ca^{2+} entry (SOCE) in alveolar epithelial cells.
- Ca^{2+} entry is necessary for the β_2-agonist-induced upregulation of Na,K-ATPase.
- STIM1-associated CRAC channels mediate the β_2-agonist-induced SOCE.
- The magnitude of Ca^{2+} entry affects the abundance of plasma membrane Na,K-ATPase.

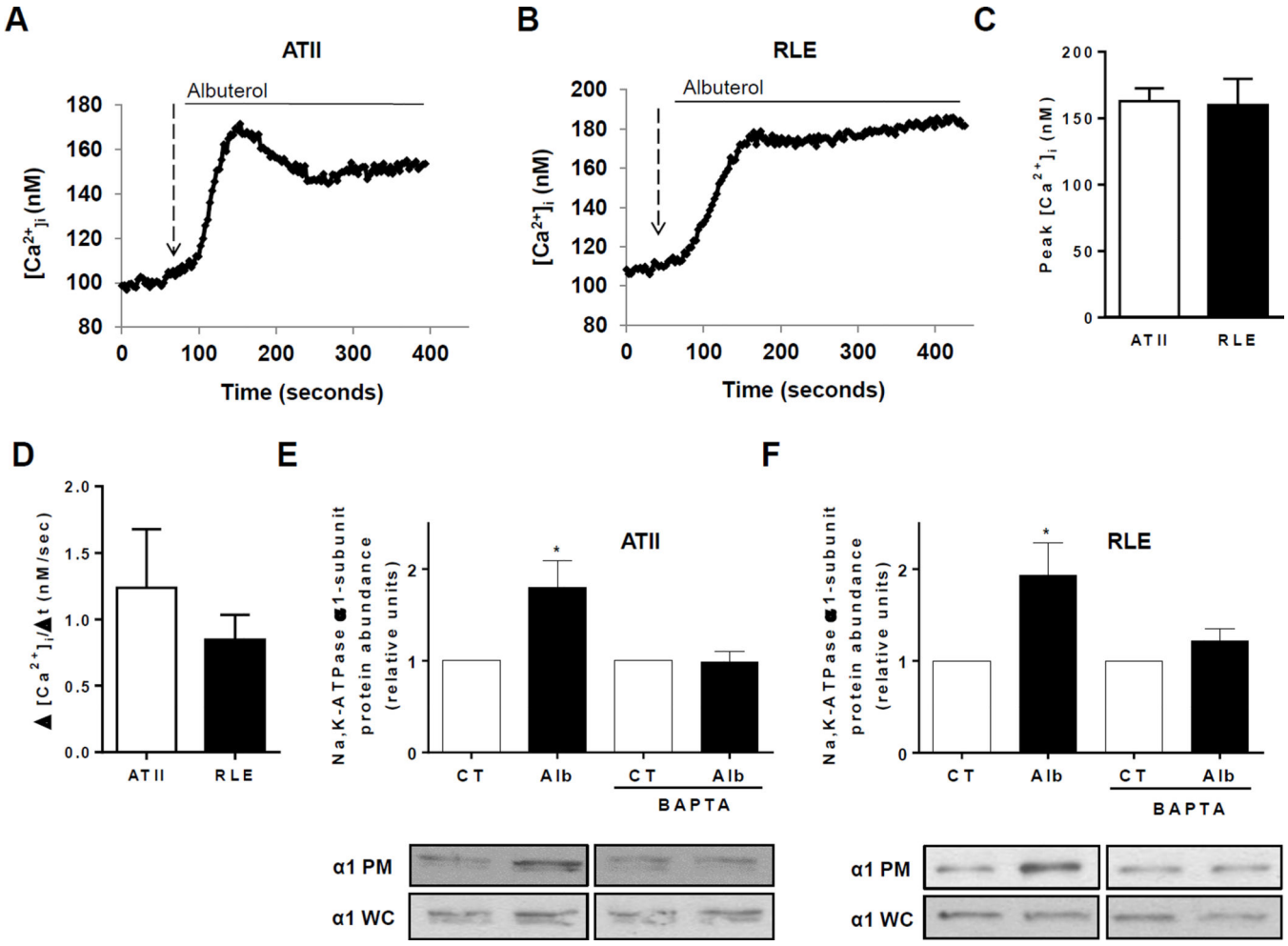

FIGURE 1. β_2-adrenergic stimulation elicits a rapid increase in intracellular calcium that is required for the recruitment of the Na,K-ATPase to the plasma membrane in alveolar epithelial cells

Measurement of intracellular calcium concentration in ATII (A) and RLE (B) cells at baseline and following the addition of albuterol (5 μM) to the media. Peak $[Ca^{2+}]_i$ (C) and rate of Ca^{2+} entry (D) were measured in ATII and RLE cells after addition of 5μM albuterol. Plasma membrane abundance of Na,K-ATPase was measured via biotinylation technique in ATII (E) and RLE (F) cells after treatment with albuterol (5 μM, 15 min) in control conditions and in cells pretreated with BAPTA (50 μM, 5 min pretreatment). Whole-cell lysate Na,K-ATPase was used as a loading control. Results are for 5 experiments with 10–20 cells each.* $p < 0.05$.

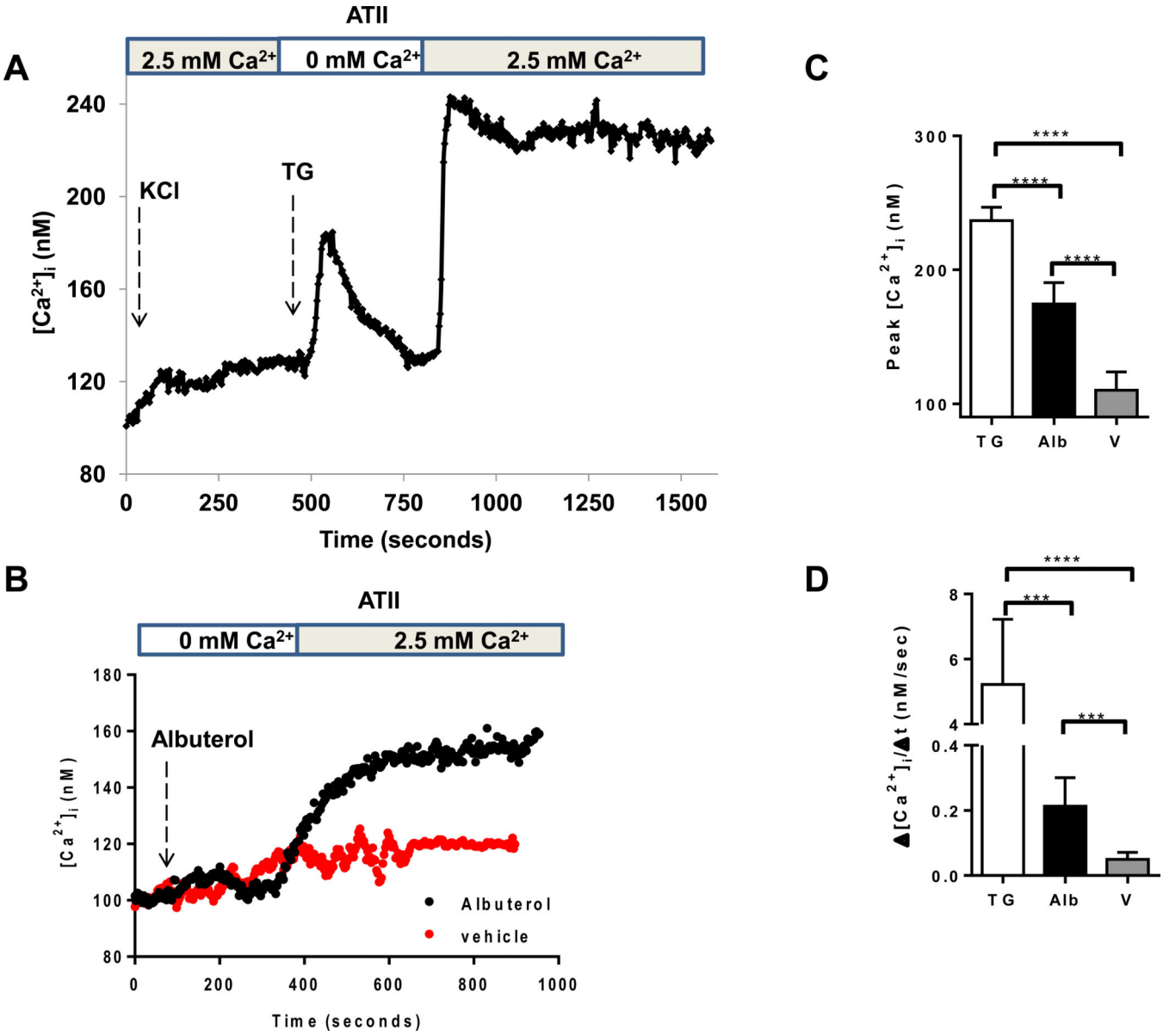

FIGURE 2. Alveolar epithelial cells lack measurable voltage-gated calcium channel activity, but demonstrate store-operated calcium entry in response to thapsigargin and β_2-agonists

(A) Measurement of intracellular calcium concentration in ATII cells during the addition of a depolarizing concentration of KCl (65 mM), followed by the addition of thapsigargin (1 μM) in media lacking calcium. 2.5 mM calcium was then re-instituted into the media as indicated. (B) Levels of intracellular calcium in ATII cells during the addition of albuterol (5 μM) to calcium-free media, followed by the return of calcium to the perfusing media. Results are for 5 experiments with 10–20 cells each. (C) Peak $[Ca^{2+}]_i$ and (D) rate of Ca^{2+} entry were measured in the cells after re-institution of 2.5 mM calcium to the media. Results are for 3 experiments with 10–20 cells each. ****p<0.0001; *** p < 0.001. TG: Thapsigargin. Alb: Albuterol. V: vehicle.

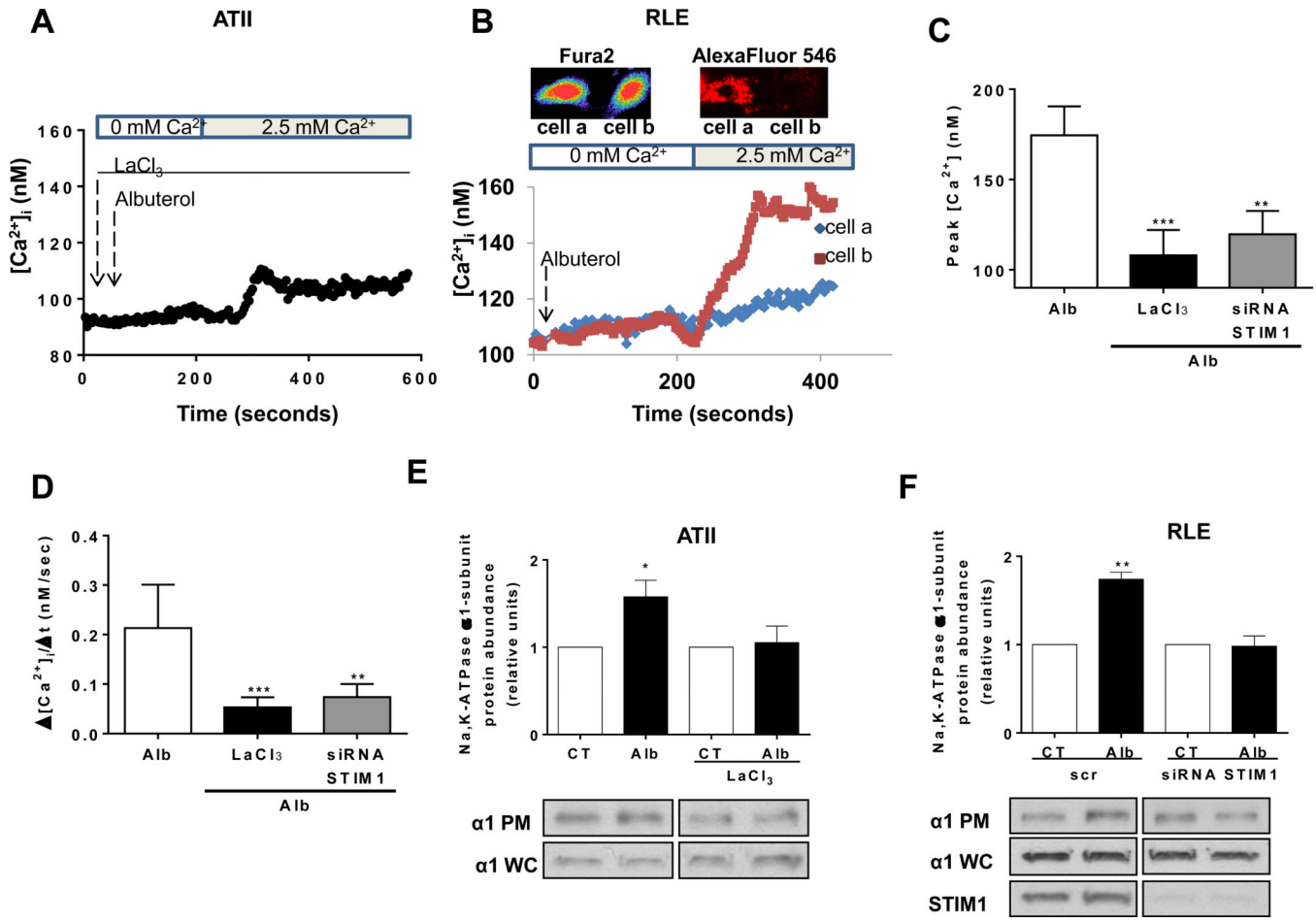

FIGURE 3. STIM1-associated CRAC channels mediate the β_2 agonist-induced increase in intracellular calcium and recruitment of Na,K-ATPase to the plasma membrane of alveolar epithelial cells

(A) Measurement of calcium levels following albuterol administration in ATII cells pretreated with $LaCl_3$ (5 μM, 5 min preincubation). (B) Measurement of calcium in RLE cells transfected with fluorescently labeled siRNA against STIM1 during exposure to albuterol (5 μM), with representative images of Fura-2 and AlexaFluor-546 fluorescence. (C) Peak $[Ca^{2+}]_i$ and (D) rate of Ca^{2+} entry were measured in the cells after re-institution of 2.5 mM calcium to the media in cells pre-incubated with $LaCl_3$ (5 μM, 5 min preincubation) or transfected with siRNA against STIM1 previous addition of albuterol (5 μM). (E) Na,K-ATPase abundance in ATII cells in response to albuterol (5 μM, 15 min treatment) in control conditions and following a 5 min pretreatment with LaCl3 (5 μM). (F) Plasma membrane abundance of Na,K-ATPase in response to albuterol (5 μM, 15min) in RLE cells transfected with scramble siRNA or siRNA targeting STIM1. Whole-cell lysate Na,K-ATPase was used as a loading control. Results are for 3 experiments with 10–20 cells each. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

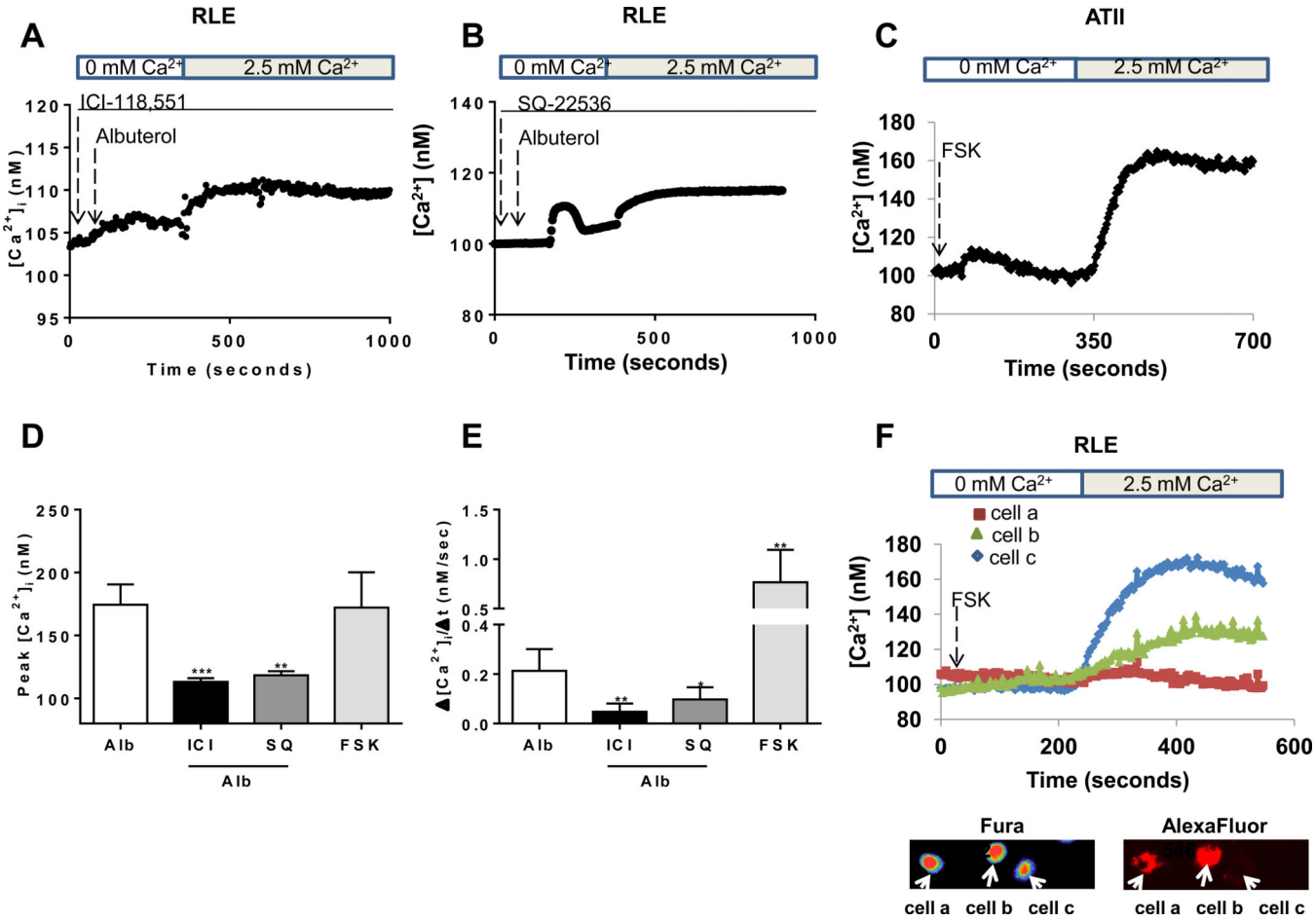

FIGURE 4. Activation of adenylyl cyclase is required for the β_2 agonist-induced store-operated calcium entry in alveolar epithelial cells

(A) Measurement of calcium levels following albuterol administration in RLE cells pretreated with ICI-118,551 (1 μM, 30 min preincubation). (B) Measurement of calcium levels following albuterol administration in RLE cells pretreated with SQ-22536 (10 μM, 30 min preincubation). (C) Measurement of intracellular calcium levels in ATII cells treated with forskolin (50 μM). Perfusion was started in calcium-free media and switched to 2.5 mM calcium as indicated. (D) Peak $[Ca^{2+}]_i$ and (E) rate of Ca^{2+} entry were measured in the cells after re-institution of 2.5 mM calcium to the media in cells incubated with forskolin (50 μM) or pre-incubated with ICI-118,551 (1 μM, 30 min preincubation) or SQ-22536 (10 μM, 30 min preincubation) previous addition of albuterol (5 μm). (F) Calcium concentration in RLE cells transfected with fluorescently labeled siRNA targeting STIM1 during treatment with forskolin (50 μM) along with Fura-2 and AlexaFluor5–46 fluorescence images. Results are for 3 experiments with 10–20 cells each. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

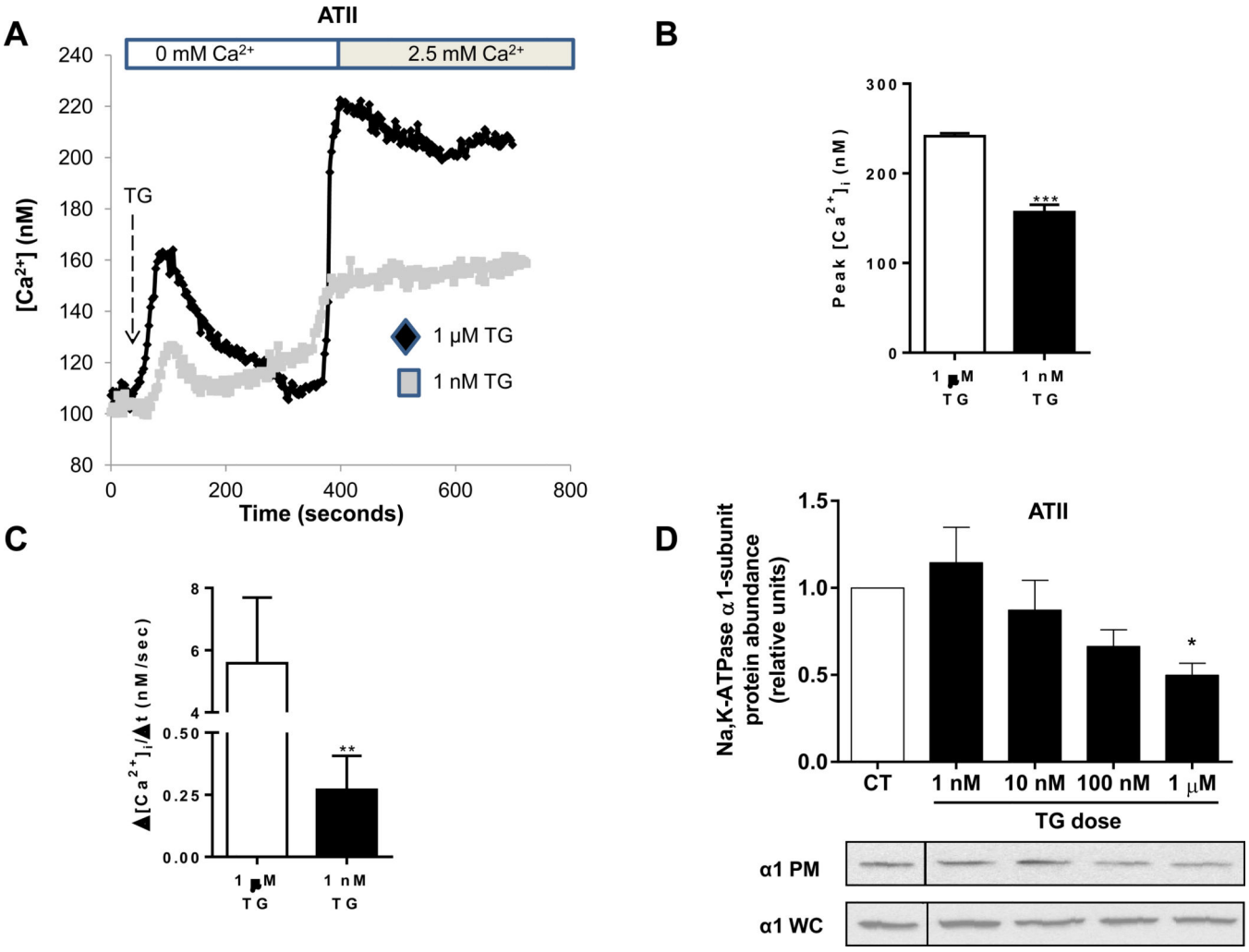

FIGURE 5. The magnitude of activation of store-operated calcium entry affects the abundance of Na,K-ATPase at the plasma membrane

(A) Measurement of intracellular calcium in ATII cells exposed to two doses of thapsigargin (1 nM and 1 μM). Perfusion was initially in calcium-free media and then switched to 2.5 mM calcium as indicated. (B) Peak $[Ca^{2+}]_i$ and (C) rate of Ca^{2+} entry were measured in the cells after re-institution of 2.5 mM calcium to the media in cells incubated with 1 μM or 1 nM Thapsigargin (TG). (D) Amount of plasma membrane Na,K-ATPase in ATII cells measured via biotinylation technique after treatment with different doses of thapsigargin (20 min). Whole-cell lysate Na,K-ATPase was used as a loading control.. Results are for 3 experiments with 10–20 cells each. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

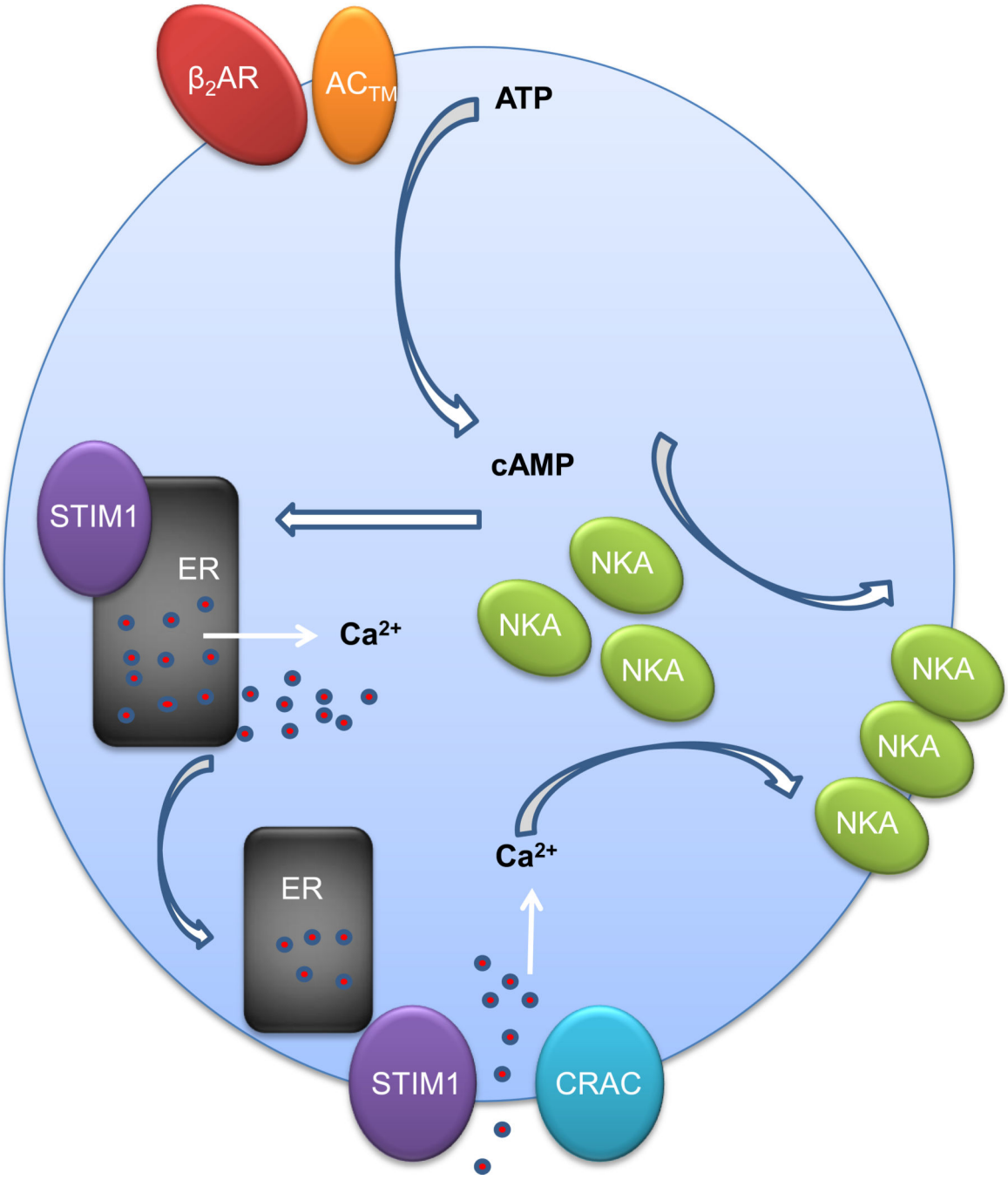

FIGURE 6. Schematic representation of β_2 agonist-induced SOCE and recruitment of Na,K-ATPase vesicles to the plasma membrane of alveolar epithelial cells

β_2AR (β_2-adrenergic receptor), AC™ (transmembrane adenylyl cyclase), NKA (Na,K-ATPase).