FEBS Letters 588 (2014) 4686–4693

journal homepage: www.FEBSLetters.org

Calcium release-activated calcium (CRAC) channels mediate the β_2-adrenergic regulation of Na,K-ATPase

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ARTICLE INFO

Article history:
Received 27 June 2014
Revised 23 October 2014
Accepted 29 October 2014
Available online 11 November 2014

Edited by Maurice Montal

Keywords:
Calcium channel
Calcium signaling
Store-operated calcium entry
Epithelial cell
Na,K-ATPase

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.

1. 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 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.

2. Materials and methods

2.1. Reagents

All cell culture reagents were from Corning Life Sciences. Albuterol sulfate (0.083%) vials from Nephron Pharmaceuticals

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http://dx.doi.org/10.1016/j.febslet.2014.10.041
0014-5793/

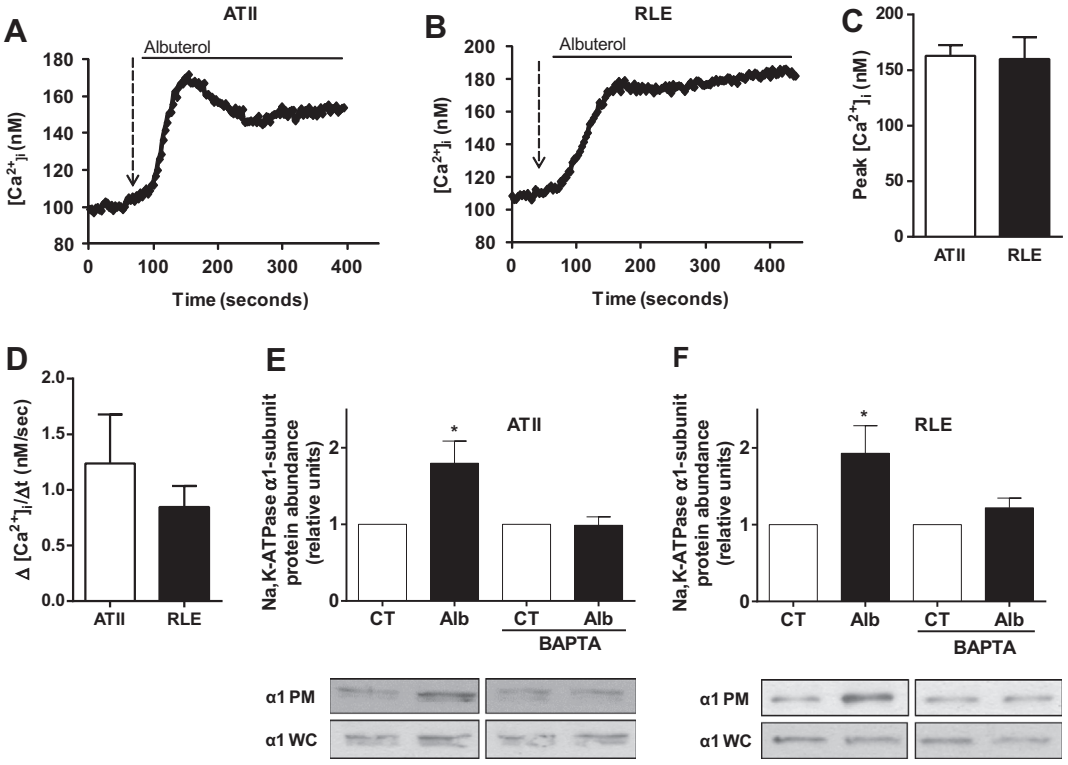

Fig. 1. β_2-Adrenergic stimulation elicits a rapid increase in intracellular calcium that is required for the recruitment of the Na,K-ATPase to the plasma membrane in alveolar epithelial cells. Measurement of intracellular calcium concentration in ATII (A) and RLE (B) cells at baseline and following 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 5 μM 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. $^*P < 0.05$.

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.

2.2. 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.

2.3. 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.

2.4. 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, 1 mM $MgCl_2$, 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 40× 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.

2.5. 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