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ION CHANNELS, RECEPTORS AND TRANSPORTERS

Functional transient receptor potential canonical type 1 channels in human atrial myocytes

Yan-Hui Zhang · Hui-Jun Wu · Hui Che · Hai-Ying Sun · Lik-Cheung Cheng · Xin Li · Wing-Kuk Au · Hung-Fat Tse · Gui-Rong Li

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Abstract Transient receptor potential (TRP) channels are not well understood in human atrium, and the present study was therefore designed to investigate whether TRPC channels would mediate the nonselective cation current reported previously and are involved in the formation of store-operated Ca^{2+} entry (SOCE) channels in human atrial myocytes using approaches of whole-cell patch voltage-clamp, RT-PCR, Western blotting, co-immunoprecipitation, and confocal scanning approaches, etc. We found that a nonselective cation current was recorded under K^+-free conditions in human atrial myocytes, and the current was inhibited by the TRP channel blocker La^{3+}. Thapsigargin enhanced the current, and its effect was suppressed by La^{3+} and prevented by pipette inclusion of anti-TRPC1 antibody. Endothlin-1 and angiotensin II enhanced the current that could be inhibited by La^{3+}. Gene and protein expression of TRPC1 channels were abundant in human atria. In addition, mRNA and protein of STIM1 and Orai1, components of SOCE channels, were abundantly expressed in human atria. Co-immunoprecipitation analysis demonstrated an interaction of TRPC1 with STIM1 and/or Orai1. Ca^{2+} signaling mediated by SOCE channels was detected by a confocal microscopy technique. These results demonstrate the novel evidence that TRPC1 channels not only mediate the nonselective cation current, but also form SOCE channels in human atria as a component. TRPC1 channels can be activated by endothelin-1 or angiotensin II, which may be involved in the atrial electrical remodeling in patients with atrial fibrillation.

Keywords Human atrial myocytes · Nonselective cation current · TRPC1 · SOCE channels

Introduction

Recent studies have reported a large family of nonselective cation ion channels, i.e., transient receptor potential (TRP) family of ion channels. The superfamily of TRP channels comprises at least 28 cation-permeable channels that are functionally expressed in different tissues/cells of mammals including humans. They include TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPP (polycystin), TRPML (mucolipin), TRPA (ankyrin), and TRPN (no mechanopotential) [2, 7]. Several types of TRP channel genes are reported in the heart, including TRPC3/6, TRPV2/4, TRPM3/7, and TRPP2 in mouse cardiac tissue and/or myocytes, and TRPC1/3, TRPC6, and TRPM4 in rat cardiac myocytes [21], and TRPC5 [4], TRPC6 [24], and TRPM4 [13] in human cardiac tissue and/or myocytes. A recent report showed that functional TRPC3 channels regulate proliferation in rat cardiac fibroblasts [16]. TRPC

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Y.-H. Zhang · H.-J. Wu · H. Che · H.-Y. Sun · H.-F. Tse · G.-R. Li
Department of Medicine, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China

L.-C. Cheng · X. Li · W.-K. Au
Department of Surgery, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China

G.-R. Li
Department of Physiology, Li Ka Shing Faculty of Medicine, The University of Hong Kong, Pokfulam, Hong Kong, China

G.-R. Li (✉)
L4-59, Laboratory Block, FMB, The University of Hong Kong, 21 Sassoon Road, Pokfulam, Hong Kong, China
e-mail: grli@hkucc.hku.hk

channels are believed to form store-operated Ca^{2+} entry (SOCE) channels as a component [22, 35]. Nonetheless, most studies on TRP channels in cardiac tissues from these species only demonstrate the presence of genes and/or protein expression of these channels. The information regarding the current properties of the TRP channels in native human cardiac myocytes is scarce in published literature.

In human cardiac myocytes, earlier reports demonstrated a nonselective cation current in human atrial myocytes [8, 26], and this cation current may play a role in human atrial electrophysiology [8]. However, molecular identity of this cation channel current is unknown. Our recent study described the functional TRPM7 channels in human atrial myocytes [46]. TRPM7 current is a Mg^{2+}-sensitive current, which is activated upon removing intracellular Mg^{2+} ions [9, 27, 46]. The property of the nonselective cation current [8, 26] in human atrial myocytes is clearly different from that of TRPM7 current [9, 27, 46] or Ca^{2+}-activated TRPM4 current [46]. In the present study, we are interested in investigating whether TRPC channels are involved in the mediation of the nonselective cation current and participate in the formation of the SOCE channels [22, 35] in human atrial myocytes.

Materials and methods

Human atrial myocyte preparation

Atrial myocytes were enzymatically isolated from specimens of human atrial appendage obtained from patients undergoing coronary artery bypass grafting. The experimental procedure for obtaining the human atrial tissue was approved by the Ethics Committee of the University of Hong Kong (UW-10-174) based on the patients' consent. The atria for myocyte dissociation were free from supraventricular tachyarrythmias and were grossly normal at the time of surgery. The human cardiac cell isolation procedure was adopted as described previously [29, 30], and in Supplementary Methods. The isolated human atrial myocytes were used for whole-cell patch voltage-clamp recording of membrane current and identifying gene expression of TRP channels in human atrial myocytes.

Electrophysiology

Whole-cell currents were recorded as described previously [29, 30] under K^+-free conditions. Briefly, the cells were superfused with a modified Tyrode's solution which contained (in millimolar) NaCl 140.0, CsCl 5.0, $MgCl_2$ 1.0, $CaCl_2$ 1.8, 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) 10.0, and glucose 10.0 (pH adjusted to 7.3 with NaOH). Nifedipine (3 μM) was included in bath solutions to block L-type Ca^{2+} channels. The pipette solution contained (in millimolar) Cs-Aspartate 110.0, CsCl 20.0, $MgCl_2$ 1.0, Na-phosphocreatine 5.0, HEPES 10.0, Cs-EGTA 5.0, GTP 0.1, and Mg-ATP 5.0, with pH adjusted to 7.2 with CsOH. Membrane currents were recorded using borosilicate glass electrodes with tip resistances of 1.5–3 MΩ when filled with the pipette solution and measured using an EPC-10 amplifier and Pulse software (Heka Elektronik, Lambrecht, Germany). Cell membrane capacitance (n=61, 91.3±3.5 pF) was compensated electrically. Command pulses were generated by a 12-bit digital-to-analog converter controlled by Pulse software. The data were stored on a PC computer for offline data analysis. All experiments were conducted at room temperature (22–23 °C). Reagents used in the present study are described in Supplementary Methods.

Reverse transcript polymerase chain reaction

The reverse transcript polymerase chain reaction (RT-PCR) for detecting the gene expression of TRPC channels was performed with a procedure as described previously [28]. Briefly, total RNA isolated using the TRIzol method (Invitrogen) from human atrial myocytes, was treated with DNase I (Promega, Madison, WI). Reverse transcription (RT) was performed with RT system (Promega). The RT reaction mixture (cDNA) was used for polymerase chain reaction (PCR). The specific forward and reverse PCR oligonucleotide primers (Table 1) designed with human genes were chosen to amplify the cDNA using the procedure and protocol described previously [28, 46]. The PCR products were electrophoresed through a 1.5 % agarose gel, and the amplified cDNA bands were visualized by ethidium bromide staining. The bands were imaged by Chemi-Genius Bio Imaging System (Syngene, Cambridge, UK).

Western blot analysis and co-immunoprecipitation

The Western blot analysis was performed using protein from human right atrial appendage obtained from patients undergoing coronary artery bypass grafting as described previously [46, 47] and in Supplementary Methods.

The co-immunoprecipitation was performed to identify the possible interaction among TRPC1, STIM1, and Orai1. The protein was immunoprecipitated overnight at 4 °C using 2 μg of anti-TRPC1, anti-STIM1, and anti-Orai1 antibodies with 20 μl protein A/G agarose beads (Santa Cruz Biotech). Immunoprecipitated proteins bound to the pelleted protein A/G beads were washed thoroughly in PBS, denatured in Laemmli sample buffer, electrophoresed, and blotted. Proteins were detected by using anti-TRPC1, anti-STIM1, or anti-Orai1 primary antibody, respectively. As a negative control, protein samples were mock-immunoprecipitated

Table 1 Human gene-specific primers for RT-PCR

Gene (Accession No.)	Primer sequences (5′-3′)	Fragment size (bp)
GAPDH (J_02642)	Forward AACAGCGACACCCACTCCTC Reverse GAGGGGAGATTCAGTGTGGT	258
TRPC1 (NM_003304)	Forward CTGGTATGAAGGGTTGGAAGA Reverse AAAGCAGGTGCCAATGAAC	451
TRPC3 (NM_003305)	Forward ATGACAGTGATGCGGGAGA Reverse CCTCGTCGTAAGCGTAGAAGT	430
TRPC4 (NM_016179)	Forward TGGATGATATTACCGTGGGT Reverse CTTCAAAATGTCCAGGAGC	345
TRPC5 (NM_012471)	Forward CTCCCTCTACCTGGCAACTAT Reverse GCTCCTACAAACTCGGTGAAT	477
TRPC6 (NM_004621)	Forward TTTACTGGTTTGCTCCATGC Reverse AGAGGGGTCCCACTTTATCC	500
TRPC7 (NM_020389)	Forward CGACGACGACTTCTATGCCT Reverse CGCCCACTACAAAATCCTT	389
STIM1 (NM_003156)	Forward GCAGAGTTTTGCCGAATTG Reverse TGAGGTGATTATGGCGAGTC	499
Orai1 (NM_032790)	Forward GAGTTACTCCGAGGTGATGA Reverse GACCGAGTTGAGATTGTGC	307
Orai2 (NM_032832)	Forward CATGGATTACCGGGACTG Reverse CACGGGGAGGAACTTGAT	460
Orai3 (NM_032790)	Forward TTGCTGAAGTTGTCCTGG Reverse TCCTCTAGTTCCTGCTTGTAG	364

GAPDH glyceraldehyde 3-phosphate dehydrogenase, *TRPC* transient receptor potential canonical, *STIM1* stromal interaction molecule 1, *Orai1* calcium release-activated calcium modulator 1

with preimmuno-IgG and treated in the same way as described above.

Immunocytochemistry

The immunocytochemical staining was conducted with the procedure as described previously [46] and in Supplementary Methods.

Ca^{2+} signaling measurement

Ca^{2+} activity was measured using a laser scanning confocal microscopy technique as described previously [17, 42] and in Supplementary Methods. Briefly, the cells were loaded with 5 μM fluo-3 acetoxymethyl ester (AM) (Biotium, Hayward, CA, USA) in Tyrode's solution for 60 min at 37 °C, and then washed with Tyrode's solution without extracellular fluo-3 AM and incubated in Tyrode's solution at room temperature for 30 min before recording Ca^{2+} signals. The cells were placed into a cell chamber (0.3 ml) mounted on the microscope, allowed to adhere to the bottom for 20–30 min, and then superfued with Tyrode's solution (10–15 min). Rod-shaped quiescent myocytes with clear striations were chosen for Ca^{2+} signal recording. When Ca^{2+}-free Tyrode was used for superfusion (15 min), Ca^{2+} in Tyrode's solution was omitted and 1 mM EDTA was added. $Ca^{2+}{}_i$ level was monitored every 2 s in human atrial myocytes using a confocal microscopy (Olympus FV300, Tokyo, Japan) during superfusion using Tyrode's solution with different interventions at room temperature (22–23 °C). Fluo-3 was excited at 488 nm, and emission was detected at 505 nm.

Statistical analysis

The data are expressed as mean±SEM. Paired and/or unpaired Student's *t* test were used as appropriate to evaluate the statistical significance of differences between two group means, and ANOVA was used for multiple groups. Values of $P<0.05$ were considered to be statistically significant.

Results

Inhibition of nonselective cation current by the TRP channel blocker La^{3+}

Crumb and colleagues reported a nonselective cation current in human atrial myocytes early in 1995 [8]. We also recorded a similar cation current in human atrial myocytes and found that the current was not affected by activation of β-adrenoceptor activation or cAMP/PKA [26]. Here, we determined which TRP channels mediate this cation current in human atrial myocytes in the following experiments.

Figure 1a shows the voltage-dependent current recorded in a representative human atrial myocyte with 300-ms voltage steps between −100 and +60 mV from a holding potential of −40 mV (to inactivate Na^+ current) under K^+-free

conditions. With 3 to 5 min dialysis, the nonselective cation current with variable amplitudes of 100 to 500 pA (at + 80 mV) in individual cells reached a steady-state level. The current was significantly inhibited by the TRP channel blocker La^{3+} (100 and 300 μM). Current–voltage (*I–V*) relationships of the nonselective cation current in the absence and presence of 10 to 300 μM La^{3+} were illustrated in Fig. 1b. The inward and outward currents were inhibited by La^{3+} in a concentration-dependent manner, and the concentration-response curve (Fig. 1c) was fitted to a Hill equation (IC_{50}=45.6 μM). The results suggest that the nonselective cation current may be carried by La^{3+}-sensitive TRP channels in human atrial myocytes.

Molecular identities of non-selective cation channels in human atrial myocytes

To determine the potential molecular identities of the nonselective cation current, we determined the gene and protein expression of TRPC channels in human atrial myocytes. TRPC1 gene is abundant, while mRNA expression of TRPC3, TRPC4, and TRPC6 channels was weak (Fig. 2a). TRPC1 protein level was high in atrial tissues; however, TRPC3 protein level was very low (Fig. 2b). The results from RT-PCR and Western blot analysis suggest that TRPC1 channels are dominantly expressed in human atrium. This is further confirmed in human atrial myocytes immuostained with anti-TRPC1 antibody (Fig. 2c), suggesting that nonselective cation current is likely mediated by TRPC1 channels in human atrial myocytes.

Effect of thapsigargin on membrane current in human atrial myocytes

We then tested whether thapsigargin [3] stimulate the nonselective current in human atrial myocytes. Figure 3a shows the time course of membrane conductance recorded in a representative human atrial myocytes with a 3-s ramp voltage protocol from −100 to +80 mV from a holding potential of −40 mV. Thapsigargin (2 μM) rapidly enhanced the membrane conductance, and basal current and the current activated by thapsigargin was inhibited by 300 μM La^{3+}. Figure 3b displays the voltage-dependent current activated by step-voltage protocol as shown in the *inset* in a typical experiment, in which La^{3+} (100 and 300 μM) significantly inhibits thapsigargin-induced voltage-dependent step current and basal current. Mean values of *I–V* relationships of the voltage-dependent step current are illustrated in

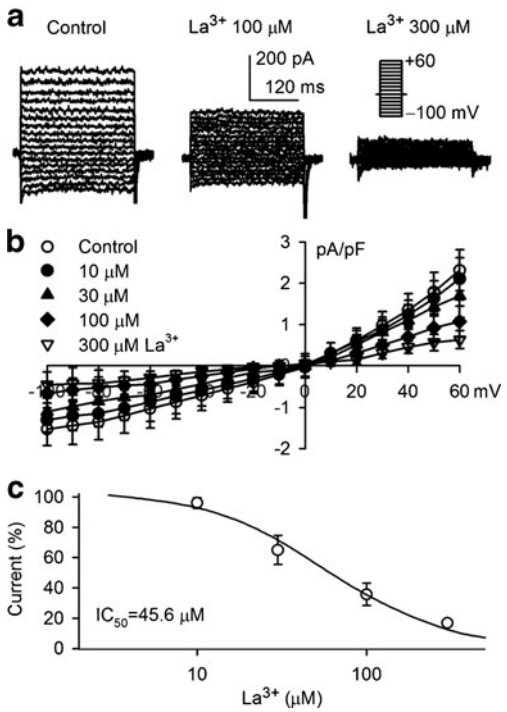

Fig. 1 TRC channels in human atrial myocytes. **a** Nonselective cation current recorded in a representative human atrial myocyte with the voltage steps as shown in the *inset* before (control) and after application of 100 and 300 μM La^{3+}. **b** *I–V* relationships of membrane currents in the absence and presence of different concentrations (n=7 for each concentration) of La^{3+}. **c** Mean value of concentration–response curve of La^{3+} for inhibiting nonselective cation current (at +60 mV, n=7–10 for each concentration) fitted to a Hill equation

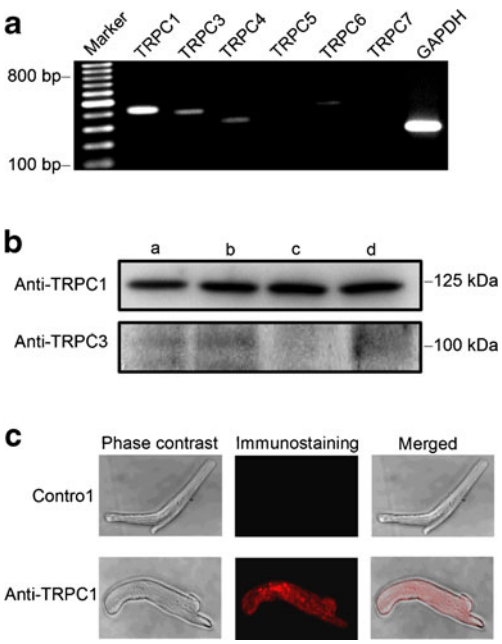

Fig. 2 Molecular identities of nonselective cation current in human atrial mtocytes. **a** Image of RT-PCR in human atrial myocytes for detecting the TRPC channels: TRPC1, C3, C4, C5, C6, and C7 (n=3). **b** Western immunoblots showing abundant protein expression for TRPC1 channels in four human atrial specimens, but not for TRPC3 channels (n=3). The label *a*, *b*, *c*, or *d* indicates the sample from individual subject. **c** Immunostaining showing the expression of TRPC1 channels in human atrial myocytes with anti-TRPC1 antibody (n=3)

Fig. 3c, showing a linear *I–V* relationship with a reversal potential close to zero. Thapsigargin (2 μM) activated the membrane current at all test potentials. Basal current and the current activated by thapsigargin was inhibited by 300 μM La^{3+}. Interestingly, thapsigargin no longer enhanced the membrane conductance, when anti-TRPC1 antibody (1:100), which targets the intracellular and trans-membrane epitope of TRPC1 channels, was included in pipette solution (Fig. 3d). These results suggest that the current activated by thapsigargin is likely mediated by TRPC1 channels.

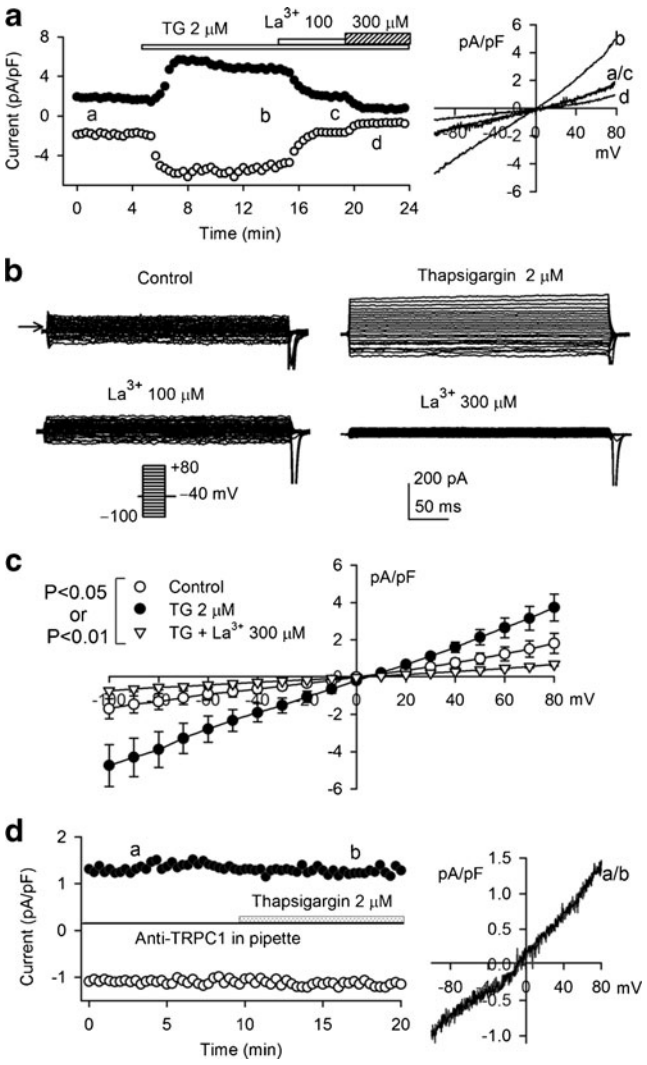

Fig. 3 Activation of TRPC1 channels by thapsigargin in human atrial myocytes. **a** Time course of membrane current recorded in a representative cell with a 3-s ramp from −100 to +80 from a holding potential of −40 mV in control, 2 μM thapsigargin (TG), and thapsigargin plus 100 or 300 μM La^{3+}. Original ramp *I–V* current traces at corresponding time points are shown in right side of the panel. **b** Voltage-dependent current recorded in a typical experiment with the voltage steps as shown in the *inset* in control, 2 μM thapsigargin, and thapsigargin plus 100 or 300 La^{3+}. **c** *I–V* relationship curves of membrane current in control, 2 μM thapsigargin, and thapsigargin plus 300 La^{3+} (n=6). **d** Time course of membrane current recorded in a typical cell using a pipette solution which contained anti-TRPC1 antibody (1:100) with a ramp protocol before and application of 2 μM thapsigargin in bath solution. Original ramp *I–V* current traces at corresponding time points are shown in right side of the panel (n=5)

Effects of endothelin-1 and angiotensin II on membrane current in human atrial myocytes

A recent study reported that knockdown of TRPC1 channels was found to prevent cardiac hypertrophy induced by entothelin-1 or angiotensin II in rats [34], suggesting a close relation of endothelin-1 or angiotensin II to TRPC1 channel activation. We therefore tested whether endothelin-1 or angiotensin II would activate TRPC1 channels in human atrial myocytes.

Figure 4a displays the time course of membrane current recorded in a representative human atrial myocyte with a ramp protocol as in Fig. 3a in control and 10 nM endothelin-1 superfusion. The membrane conductance was gradually increased after endothelin-1 exposure for 2 min, and then reached a steady-state level. The current activated by endothelin-1 was fully inhibited by 300 μM La^{3+}. Endothelin-1 also increased the step current elicited by voltage steps protocol, and the current activated by endothelin-1 was fully decreased by La^{3+} (Fig. 4b). Mean values of voltage step-activated current at −100 and + 80 mV are illustrated in Fig. 4c. Endothelin-1 remarkably stimulated the current (n=6, P<0.01 vs. control), and the current activated by endothelin-1 was fully inhibited by 300 μM La^{3+} (Fig. 4c). In addition, the inclusion of anti-TRPC1 antibody in pipette solution prevented the stimulation by endothelin-1 (n=4). These results suggest that the current activated by endothelin-1, as that induced by thapsigargin, may be mediated by TRPC1 channels. However, the current activation by endothelin-1 is clearly slower than that by thapsigargin (Fig. 3a), suggesting that endothelin-1 receptors may be involved.

To determine whether the activation of TRPC1 channels by endothelin-1 is mediated by endothlin-1 receptors, the endothelin-1 receptor-A antagonist BQ-123 [20] was used in another set of experiments. Figure 4d shows the time course of membrane current recorded in a representative human atrial myocyte with a ramp protocol in control, 1 μM BQ-123, and BQ-123 plus 10 nM endothelin-1 superfusion. The membrane conductance was not increased by endothelin-1 in the presence of 1 μM BQ-123. The basal current was inhibited by 300 μM La^{3+}. Figure 4e displays the step current recorded in a representative human atrial myocyte with the protocol as shown in inset in Fig. 4b. The mean values of the basal current at +80 and −100 mV are illustrated in Fig. 4f. TRPC1 current was not enhanced by endothelin-1 in cells pretreated with BQ-123. These results suggest that the increase of the current by endothelin-1 is likely mediated by endothelin-1 receptor-A.

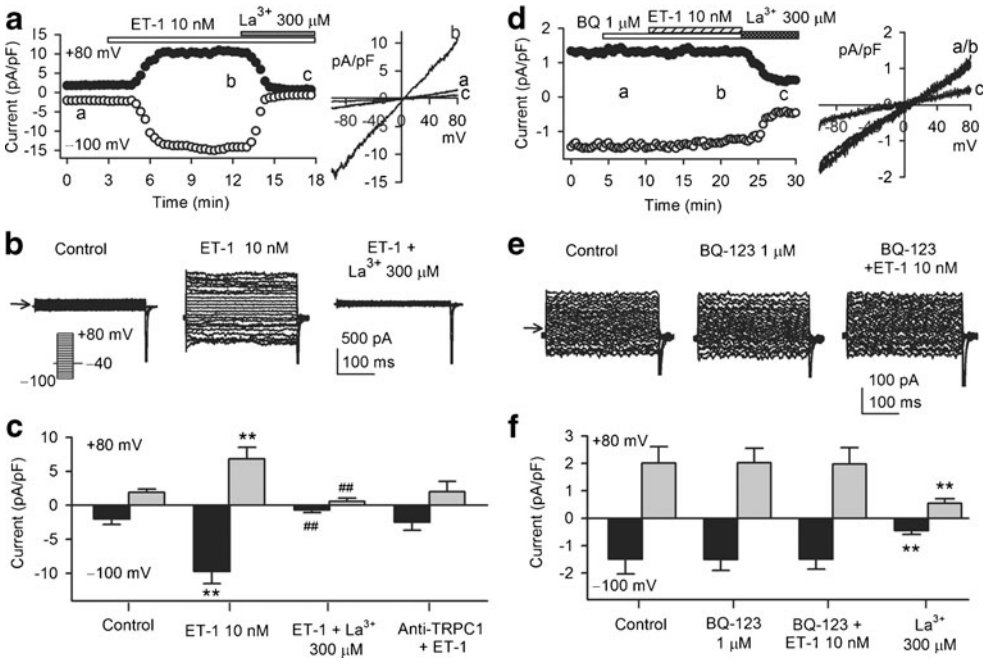

Fig. 4 Activation of TRPC1 channels by endotyhelin-1 in human atrial myocytes. **a** Time course of membrane current recorded in a representative atrial myocyte with a ramp protocol as in Fig. 2a in control, 10 nM endothelin-1 (ET-1), and endothelin-1 plus 300 μM La^{3+}. Original ramp *I–V* current traces at corresponding time points are shown in right side of the panel. **b** Voltage-dependent current recorded in a typical cell with the voltage steps as shown in the *inset* in control, 10 nM endothelin-1 and endothelin-1 plus 300 μM La^{3+}. **c** Mean values of membrane current at −100 and +80 mV in control, 10 nM endothelin-1, endothelin-1 plus 300 μM La^{3+} (n=6, **P<0.01 vs. control; ##P<0.01 vs. endothelin-1 alone), and anti-TRPC1 antibody inclusion in pipette solution plus endothelin-1 (n=4). **d** Time course of membrane current recorded in a representative human atrial myocyte with a ramp protocol as in Fig. 2a in control, 1 μM BQ-123 (BQ), and BQ-123 plus 10 nM endothelin-1. Original ramp *I–V* current traces at corresponding time points are shown in *right side* of the panel. **e** Voltage-dependent current recorded in a typical cell with the voltage steps (as in **b**) in control, 1 μM BQ-123, and BQ-123 plus 10 nM endothlin-1. **f** Mean values of membrane current at −100 and +80 mV in control, 1 μM BQ-123, BQ-123 plus 10 nM endothelin-1, or 300 μM La^{3+} (n=6, **P<0.01 vs. control)

It has been reported that angiotensin II activates TRPC1 and TRPC6 channels [40]. We therefore tested whether angiotensin II activates TRPC1 current in human atrial myocytes. Figure 5a displays the time course of the membrane current elicited in a typical experiment using a ramp protocol with application of 1 μM angiotensin II in bath solution. Angiotensin II activated the membrane conductance in human atrial myocytes with a relatively long period of exposure (>5 min), and the activated current was remarkably inhibited by 300 μM La^{3+}.

Figure 5b shows the voltage-dependent current recorded in a typical experiment with voltage steps as shown in the *inset* in control, 1 μM angiotensin II (for 10 min), and angiotensin II plus 300 μM La^{3+}. Mean values of membrane current at −100 and +80 mV are illustrated in Fig. 5c. As the current activated by thapsigargin or endothelin-1, angiotensin II-induced current was fully inhibited by 300 μM La^{3+} (n=6, P<0.01 vs. angiotensin II alone), and inclusion of anti-TRPC1 antibody prevented the current increase by angiotensin II (n=4).

The receptor mediation of the slow current activation by angiotensin II was determined with the angiotensin II receptor-1 antagonist losartan [15]. Figure 5d illustrates the time course of membrane current recorded in a representative human atrial myocyte with a ramp protocol during control, 1 μM losartan, and losartan plus 1 μM angiotensin II in bath solution. The membrane conductance was not enhanced by angiotensin II in the presence of 1 μM losartan. The basal current was inhibited by 300 μM La^{3+}. Figure 5e displays the step current recorded in a representative human atrial myocyte with the protocol as shown in inset in Fig. 5b. The mean values of the basal current at +80 and −100 mV are illustrated in Fig. 5f. The nonselective cation current was not increased by angiotensin II in cells treated with losartan (n=6, P=NS vs. control) while inhibited by La^{3+} (P<0.01). These results indicate that the activation of TRPC1 current by angiotensin II is mediated by angiotensin II receptor-1.

SOCE channels in human atrial myocytes

The previous studies in neonatal rabbit cardiac ventricular myocytes demonstrated that SOCE channels play an important role in modulating sarcoplasmic reticulum (SR) Ca^{2+} loading [18]. However, functional SOCE channels and the

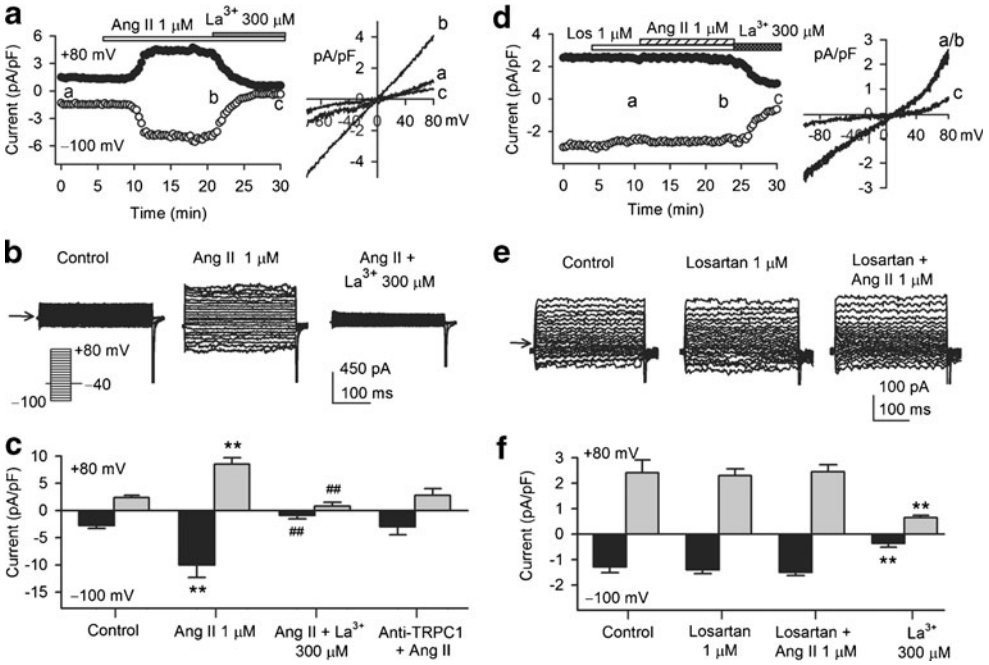

Fig. 5 Activation of TRPC1 channels by angiotensin II in human atrial myocytes. **a** Time course of membrane current recorded in a typical experiment with a ramp protocol as in Fig. 2a in control, 1 μM angiotensin II (Ang II), and angiotensin II plus 300 μM La^{3+}. Original ramp *I–V* current traces at corresponding time points are shown in *right side* of the panel. **b** Voltage-dependent current recorded in a typical cell with the voltage steps as shown in the *inset* in control, 1 μM angiotensin II, and angiotensin II plus 300 μM La^{3+}. **c** Mean values of membrane current at −100 and +80 mV in control, 1 μM angiotensin II, angiotensin II plus 300 μM La^{3+} (n=6, **P<0.01 vs. control; ##P< 0.01 vs. angiotensin II alone), and anti-TRPC1 anti-body inclusion in pipette solution plus angiotensin-II (n=4). **d** Time course of membrane current recorded in a representative human atrial myocyte with a ramp protocol as in Fig. 3a in control, 1 μM losartan (Los), and losartan plus 1 μM angiotensin II or 300 μM La^{3+}. Original ramp *I–V* current traces at corresponding time points are shown in right side of the panel. **e** Voltage-dependent current recorded in a typical cell with the voltage steps (as shown in the *inset* of **b**) in control, 1 μM losartan, and losartan plus 1 μM angiotensin II. **f** Mean values of membrane current at −100 and +80 mV in control, 1 μM losartan, losartan plus 1 μM angiotensin II or 300 μM La^{3+} (n=6, **P<0.01 vs. control)

molecular identities are not understood in human atria. It is believed that TRPC1 channels are part of SOCE channels [22, 35]. To determine whether SOCE channels are present in human atrium, we examined the gene expression of SOCE channel-related components: STIM1 (stromal interaction molecule 1), Orai1 (Ca^{2+} release-activated Ca^{2+} modulator 1), Orai2, and Orai3. Figure 6a shows the result of RT-PCR using the primers designed with human genes as described previously [28]. The mRNA levels for STIM1 and Orai1 were evident, while Orai2 and Orai3 were relatively weak, suggesting that SOCE channels are also present in human atrium, and are likely formed by TRPC1, STIM1, and Orai1. We therefore determined the protein expression of STIM1 and Orai1 in human atrium. Figure 6b displays the Western immunoblots with anti-STIM1 and anti-Orai1 antibodies in human atrial tissues from four individual patients. The protein expression of STIM1 and Orai1, as TRPC1 (Fig. 2b), was abundant in human atrial tissues. These results indicate that the three subunits of SOCE channels are evident in human atria.

We then used the co-immunoprecipitation to examine the possible interaction among TRPC1, STIM1, and/or Orai1. Anti-TRPC1, anti-STIM1, and anti-Orai1 antibodies were used respectively as bait in co-immunoprecipitation process, and then used to pull down each corresponding protein, respectively. As Fig. 6c shows, the immunoprecipitation revealed that the protein bands at expected molecular weight was detected, respectively. These results suggest that TRPC1, STIM1, and Orai1 can form the ternary complex of SOCE channels in human atrial myocytes.

Ca^{2+} signaling mediated by SOCE channels in human atrial myocytes

Ca^{2+} signaling mediated by SOCE channels was determined with a confocal microscopy technique [17, 42]. It is well recognized that activation of SOCE channels is relied on the depletion of intracellular Ca^{2+} store (SR/ER) [1]. We therefore used a Ca^{2+}-free Tyrode's solution which contained 1 mM EDTA, 10 μM cyclopiazonic acid (an inhibitor of Ca^{2+}-ATPase in the intracellular Ca^{2+} store), and 10 μM ryanodine to superfuse the human atrial myocytes loaded with 5 μM flou-3 AM (for 15 min) to deplete SR Ca^{2+} store. Figure 6d illustrates the representative Ca^{2+} signal recordings in

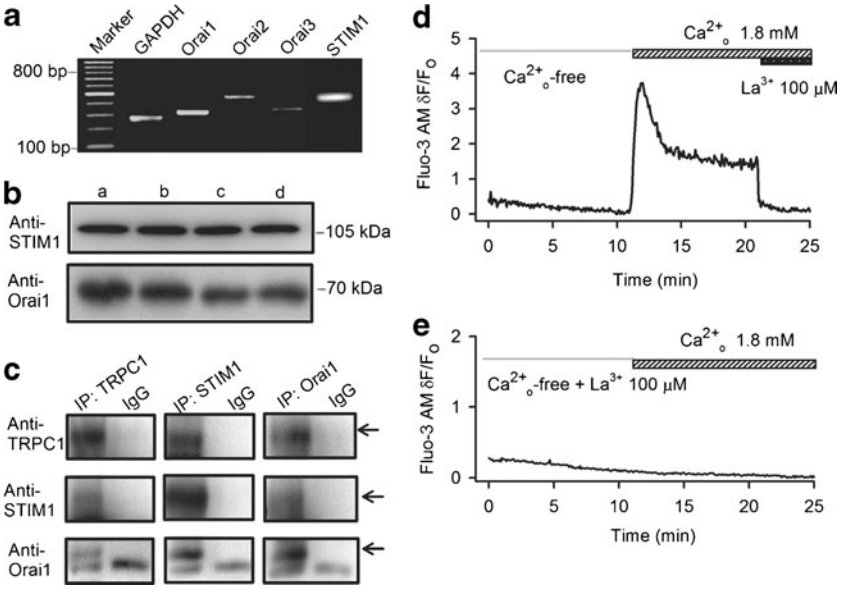

Fig. 6 SOCE components and the mediation of Ca^{2+} signaling in human atrium. **a** RT-PCR image showing abundant gene expression of Orai1 and STIM1 in human atrial myocytes (n=3). **b** Western blots showing abundant protein expression of STIM1 and Orai1 in four human atrial specimens (n=4). **c** Co-immunoprecipitation showing interaction between TRPC1, STIM1, and Orai1. The IP represents the immunoprecipitation using indicated antibody to pull down the interacting proteins: The interaction of proteins (*arrows*) was determined using anti-TRPC1, anti-STIM1, and anti-Orai1 antibody respectively. The other lanes represent the immunoprecipitation with IgG, which are used as negative control (n=3). **d** Ca^{2+} signaling was recorded before and after restoration of bath Ca^{2+} and La^{3+} (100 μM) application in a human atrial myocyte. The pseudo-ratio $\delta F/F_0$: $\delta F/F_0 = (F - F_{base})/F_{base}$ was applied to express intracellular free Ca^{2+} level, where F is the measured fluorescence intensity of Fluo-3 AM, F_{base} is the lowest level of fluorescence intensity in the cell (n=18). **e** Ca^{2+} signaling was recorded before and after restoration of bath Ca^{2+} in a human atrial myocyte treated with 100 μM La^{3+} (n=15)

quiescent myocytes treated with different conditions. The Ca^{2+}-free superfusion (>10 min) induced a decline of basal Ca^{2+} level. Intracellular Ca^{2+} level was immediately increased upon restoration of extracellular Ca^{2+} and then showed a slow decline phase, and the effect was remarkably reduced by application of 100 μM La^{3+} (Fig. 6d, n=18). When La^{3+} (100 μM) was applied in the Ca^{2+}-free bath solution, no change was observed in intracellular Ca^{2+} level upon restoring extracellular Ca^{2+} (Fig. 6e, n=15). As in human cardiac fibroblasts [6], Ca^{2+} signaling mediated by SOCE channels was inhibited by La^{3+} in human atrial myocytes. These results indicate that functional SOCE channels are present in human atrial myocytes.

Discussion

The present study shows that functional TRPC1 channels are present in human atrial myocytes, which likely mediate the non-selective cation current previously described in human atrial myocytes [8, 26]. Thapsigargin, endothlin-1, and angiotensin II enhanced the current, and their effects were inhibited by the TRP channel blockers La^{3+}. In addition, TRPC1 channels interact with STIM1 and Orai1 for the formation of SOCE channel complex.

TRP channels are extensively studied in cardiovascular system from different species [21, 44]. Several types of TRP channel genes have recently been described in the heart. They include TRPC3/6, TRPV2/4, TRPM3/7, and TRPP2 in mouse cardiac tissue and/or myocytes [21], and TRPC1/3, TRPC6, and TRPM4 in rat cardiac myocytes [21], TRPC1 channels are widely expressed in atrial, ventricular, and Purkinje cells in rat hearts [19]. TRPC5 [4] and TRPC6 [24] channels were expressed in human ventricular tissues. TRPM4 channels were reported in human atrial myocytes [13, 46]. Recent studies demonstrated that TRPM7 channels [9] and TRPC1/4/6 channels [6] are expressed in human cardiac fibroblasts. We have recently found that TRPM7 channels are present in human atrial myocytes [46]. The present study has provided the new information that TRPC1 channels are present in human atria, mediate the nonselective cation current, and participate in forming SOCE channels. TRP channels, as other classical channels, may play important roles in maintaining normal cellular electrophysiology in the heart.

Increasing evidences show that TRP channels play regulatory roles in the progression of cardiac hypertrophy [11, 38, 45]. It has been reported that genes and proteins of several TRP channels are upregulated in the diseased hearts. They include TRPC5 in failing human ventricular myocardium [4], TRPC1 in hypertrophic rat ventricle [34], TRPM4 in ventricular myocytes isolated from spontaneously hypertensive rats [14]. The upregulation of TRP channels is

believed to mediate the progression of electrical remodeling and the arrhythmogenesis of the diseased heart [21].

Intracellular Ca^{2+} is considered as an inducer of different hypertrophic responses [44]. In addition to Ca^{2+} entry through voltage-gated L-type Ca^{2+} channels to trigger Ca^{2+} release from sarcoplasmic reticulum, TRP channels mediate the spatial segregation of increased Ca^{2+}. TRPC-derived accumulation of intracellular Ca^{2+} is believed to contribute to selective activation of calcineurin in diseased heart [4]. In addition, TRPC channels (especially TRPC1) are the candidates for SOCE channels [35]. TRPC3 and TRPC6 channels participate in the hypertrophic growth induced by angiotensin II in rat neonatal cardiomyocytes [36]. It is interesting to note that TRPC1-knockout mice effectively prevent cardiac hypertrophy induction by aortic banding or angiotensin II infusion [41, 44]. A recent study has reported that SOCE channels are present in neonatal rat cardiomyocytes and regulate normal and hypertrophic cell growth [43].

Mechanical stretch-activated nonselective cation channels was described in isolated human atrial myocytes, and the cation channel preferentially passing Na^+ ions was found to play a role in generating cardiac arrhythmias [23]. An earlier report showed that the nonselective cation current is also carried by Na^+ and/or Cs^+ (or K^+) in human atrial myocytes [8], which is likely mediated by TRPC1 channels. It is generally believed that TRPC1 channels are sensitive to cell membrane stretch [21, 33], and therefore mediate the mechanical stretch-activated nonselective cation channels in isolated human atrial myocytes [23].

The non-selective cation current described previously in human atrial myocytes [8, 26] may be carried by TRPC1 channels. Angiotensin II increases single channel open probability of TRPC1 channels by activating phospholipase C (PLC) and nuclear factor of activated T cells (NFAT) in rabbit mesenteric artery myocytes [21, 33], and in TRPC3/6 channels in cultured neonatal rat cardiac myocytes [36]. Interestingly, angiotensin II could induce membrane depolarization and spontaneous activity in cultured neonatal rat cardiac myocytes [36], which suggests that activation of TRPC channels is of arrhythmogenesis. In the present study, we demonstrated that the inward and outward currents of nonselective cation (TRPC1) channels were enhanced by angiotensin II in human atrial myocytes, and the effect was prevented by the angiotensin II receptor-1 antagonist losartan. This suggests that the effective treatment of atrial fibrillation with angiotensin II blockers or angiotensin converter enzyme inhibitor [10, 12, 25] is likely related to the inhibition of angiotensin II effect on TRPC1 channels.

In addition to the permeability to mono-cation ions, TRPC channels are also permeable to Ca^{2+} [37]. Endothelin-1 (by stimulating ET-A and ET-B receptors) was found to increase single channel open probability of TRPC1 channels by activating PLC in rabbit coronary artery myocytes [21, 39]. Activation of TRPC1 channels by endothelin-1 may mediate coronary artery contraction by opening this Ca^{2+}-permeable non-selective cation channel [31]. In the present study, we found that endothelin-1 activated TRPC1 channels in human atrial myocytes, and the effect was prevented in cells pretreated with the endothelin-1 receptor-A antagonist BQ-123, and also with anti-TRPC1 antibody dialysis in pipette solution. Although no report is available to compare our observation that endothelin-1 increases TRPC1 channel current in human atrial myocytes, the increased expression of endothelin-1 receptors which has been demonstrated in patients with atrial fibrillation [5, 32] implicates that the activation of TRPC1 channels by endothelin-1 is likely involved in the genesis of atrial fibrillation.

One of limitations was that the direct inhibition of anti-TRPC1 antibody was not observed due to the dialysis of pipette solution containing Cs^+ to inhibit the predominant K^+ currents, e.g., I_{Kur} and I_{to} in human atrial myocytes, although it effectively prevented the activation of TRPC1 channels by thapsigargin. Another limitation was that no selective inhibitor of Na^+/Ca^{2+} exchanger is available to differentiate how much it is involved in the intracellular Ca^{2+} rising in human atrial myocytes. Nonetheless, all these limitations would not affect the main outcome of the present study for demonstrating the evidence of TRPC1 channels in mediating the nonselective cation current and participating in the formation of SOCE channels in human atrial myocytes.

Collectively, the present study demonstrates for the first time that TRPC1 channels likely mediate the nonselective cation current, and are involved in formation of SOCE channel complex in human atrial myocytes. TRPC1 channels can be activated by endothelin-1 or angiotensin II, which may be involved in the atrial electrical remodeling in patients with atrial fibrillation.

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Conflict of interest None declared.

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