Electrophysiology of human cardiac atrial and ventricular telocytes

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Abstract

Telocytes (TCs) with exceptionally long cellular processes of telopodes have been described in human epicardium to act as structural supporting cells in the heart. We examined myocardial chamber-specific TCs identified in atrial and ventricular fibroblast culture using immunocytochemistry and studied their electrophysiological property by whole-cell patch clamp. Atrial and ventricular TCs with extended telopodes and alternating podoms and podomers that expressed CD34, c-Kit and PDGFR-b were identified. These cells expressed large conductance Ca2+-activated K+ current (BKCa) and inwardly rectifying K+ current (IKir), but not transient outward K+ current (Ito) and ATP-sensitive potassium current (KATP). The active channels were functionally competent with demonstrated modulatory response to H2S and transforming growth factor (TGF)-β1 whereby H2S significantly inhibited the stimulatory effect of TGF-β1 on current density of both BKCa and IKir. Furthermore, H2S attenuated TGF-β1-stimulated KCa1.1/Kv1.1 (encode BKCa) and Kir2.1 (encode IKir) expression in TCs. Our results show that functionally competent K+ channels are present in human atrial and ventricular TCs and their modulation may have significant implications in myocardial physiopathology.

Keywords: Telocyte ● potassium channel ● hydrogen sulphide ● fibrosis

Introduction

Telocytes (TC) are newly identified cells that are found in the interstitial space of many organs, including heart [1], bladder [2], lungs [3] and skeletal muscle [4] and intimately contacting the parenchymal tissues. They have previously been described as interstitial Cajal-like cells in atrial and ventricular myocardium [5, 6] and very little was known of their function. Recently, TCs have been increasingly recognized to play key supporting physiological roles in the heart because of the seminal work of Popescu and colleagues [7–9]. Telocytes have exceptionally long (10–1000 μm), moniliform cellular processes named telopodes (Tps) with intervening podoms and podomers that form an interstitial network connecting different segments of the heart [10] including those of the epicardium [11, 12], myocardium [8, 13] and endocardium [14]. Their close physical association with cardiac progenitors and cardiomyocytes in the stem cell niche has been suggested to be important in the repair and regeneration of infarcted myocardium [11].

Recently, human myometrial TCs have been reported to express calcium-dependent hyperpolarization-activated chloride inward current [15]. However, very limited is known about the electrophysiological activity of cardiac TCs although they have been suggested to act as intercellular signalling intermediary in the myocardium with potential role in cardiac rhythm [11] and atrial fibrillation [16]. We have recently showed that hydrogen sulphide (H2S) modulated the activity of ion channels in human atrial fibroblasts and suppressed their transformation into myofibroblasts in response to transforming growth factor (TGF)-β1. This may have important implications in TGF-β1-mediated cardiac fibrosis and its associated atrial fibrillation [17]. In this study, we present first evidence of electrophysiological properties of human TCs derived from atrial and ventricular myocardium and demonstrate the modulatory role of H2S on the ion channel activity.

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Materials and methods

Telocyte identification

Informed consent was gathered from male patients (n = 10) with mean age of 63 ± 8.7 years old who were scheduled for coronary bypass surgery and were hyperlipidemic, managed with anti-hypertensive and diabetic medications. The protocol was approved by institutional review board of Singapore General Hospital that conformed to the Declaration of Helsinki. Atrial appendages were collected as surgical by-product. Human atrial interstitial cells were isolated by mincing the appendages to less than 1 mm³ and followed by 0.1% trypsin digestion for 20 min. before plating onto tissue culture-treated 60-mm dishes to produce fibroblastic outgrowth from minced tissue pieces. Outgrown fibroblasts were harvested and re-plated onto a fresh tissue culture dish as passage 1 to isolate homogeneous fibroblast culture that expressed collagen I and fibroblast marker as described previously by our group [18]. Atrial fibroblasts were passaged as monolayer in 10% foetal bovine serum supplemented DMEM and antibiotics (100 U/ml penicillin G and 100 μg/ml streptomycin) at 37°C in a humidified atmosphere of 95% air and 5% CO₂. Human cardiac ventricular fibroblasts (cat no. CC-2904) were acquired from Lonza Inc (Singapore). These cells were isolated from normal adult heart and certified to express greater than 90% collagen I and negative for von Willebrand factor VIII-related antigen. After 3 passages of the atrial and ventricular culture, TCs were distinguishable from other interstitial cells and were typically identified by their characteristic ultra-long moniliform cellular processes of Tps under light microscopy and the cultures from passages 3 to 5 were used for subsequent experiments. These TCs containing cultures were seeded on glass coverslips for immunostaining or in 35 mm dish for whole-cell patch-clamp recordings.

Immunocytochemistry/Immunohistochemistry

Telocytes were cultured on coverslips, were fixed with 2% paraformaldehyde/PBS for 15 min., permeabilized by 20 min. incubation in PBS containing 0.1% Triton X-100, and blocked in PBS containing 2.5% bovine serum albumin (BSA). Samples were then incubated overnight at 4°C with primary antibodies in PBS containing 0.1% BSA, after which they were incubated for 1 hr at room temperature with secondary antibody. Coverslips were rinsed and mounted on glass slides and examined by using confocal microscopy. Primary antibodies against CD34 (1:10; BD Biosciences, San Jose, CA, USA), c-Kit (1:100; Novus Biologicals, Littleton, CO, USA) and PDGF receptor (PDGFR)-β (1:1000; Cell Signaling, Danvers, MA, USA) were used to identify TCs. Primary antibodies used were anti-Kv1.1 (1/1000; Abcam, Cambridge, MA, USA) to label BKCa channels, anti-Kv4.3 (1/500; Abcam) to label Ito channels and anti-Kir2.1 (1/1000; Abcam) to label IKᵦ channels. Secondary antibodies used were Alexa Fluor555 (1/1000) and Alexa Fluor488 (1/500)-labelled antibodies (Life Technologies, Carlsbad, CA, USA). Counter staining was performed using DAPI (4', 6-diamidino-2-phenylindole) to visualize nuclei.

Electrophysiological recordings

Cell were placed on the stage of a Nikon Diaphot inverted microscope and superfused continuously at 36 ± 1°C with Tyrode solution containing (in mM) 140 NaCl, 5.4 KCl, 1.8 CaCl₂, 1 MgCl₂, 10 HEPES and 10 Glucose (pH adjusted to 7.4 with NaOH). The patch-clamped cell was superfused by means of a temperature-controlled micro-superfuser (TC-324B; Warner Instruments, Hamden, CT, USA). Patch pipettes were made from borosilicate glass shanks (Sutter Instrument, Novato, CA, USA) and pulled with a Brown–Flaming puller (Model P-97; Sutter Instrument Co), and had tip resistances of 2–3 MΩ when filled with pipette solution. Pipette tips were polished (Microforge MF830; Narishige, Tokyo, Japan). The patch pipettes were filled with a standard solution containing (in mM) 140 KCl, 1.2 MgCl₂, 0.05 EGTA, 10 HEPES, 0.1 GDP and 5.0 Mg ATP (pH adjusted to 7.2 with KOH). After a gigahm seal was obtained by negative pressure suction, the cell membrane was ruptured by a gentle suction to establish whole-cell configuration with a seal resistance >800 MΩ. The cell membrane capacitance (40.3 ± 8.2 pF) was electrically compensated with the pulse software. The series resistance (Rs, 3–5 MΩ) was compensated by 50–70% to minimize voltage errors. Currents were elicited with voltage protocols as described in the following results section for different individual current recordings. Whole-cell voltage-clamp experiments were performed with an Axopatch 200B amplifier (Axon Instruments, Foster City, CA, USA) interfaced to a Digidata 1322A data acquisition system controlled by Clampex version 8.1 software (Axon Instruments). Data were analysed with pCLAMP software (Version 10.0; Axon Instrument) and Origin 8.0 (OriginLab, Northampton, MA, USA).

Statistical analysis

All data were presented as mean ± SE. Statistical significance of the difference between groups was determined using Student’s t-test as appropriate. A value of P < 0.05 was considered statistically significant.

Results

Identification of cardiac telocytes

Telocytes were clearly identifiable in our human atrial and ventricular cardiac fibroblast culture by phase contrast after passage 3. Telocytes displayed characteristic long Tps (100–500 μm) [9] with occasional podomers and podoms that were often in contact with fibroblasts and other TCs [19]. In accordance with previous reports [1, 8, 20], these human TCs stained positive for CD34, c-Kit (CD34/c-Kit double staining in Fig. S1) and PDGFR-β markers (Fig. 1).

Ion currents in human atrial and ventricular telocytes

To record and characterize ion currents in single TCs, cells with long Tps were selected under light microscopy with patch pipettes (Fig. 2) and conventional whole-cell voltage-clamp experiment was performed at 36 ± 0.5°C. Outward currents were activated at depolarization voltages between −30 mV and −90 mV in 10 mV increments from a holding potential of −40 mV. Compared to baseline current density (8.3 ± 2.8 pA/pF, n = 6 atrial TCs; 8.9 ± 2.1 pA/pF, n = 6 ventricular TCs), the activated currents were sensitive to inhibition.
Identification of cardiac atrial and ventricular telocytes (TCs). Immunofluorescent staining for anti-CD34 (red), c-kit (red) and vimentin (green) demonstrated cells with very small cell bodies (~1:1 ratio cytoplasm to nucleus) and extremely long and thin cellular processes of telopode (Tp) scattered among atrial and ventricular fibroblasts (Fb). TC: telocyte; Fb: cardiac fibroblast; Tp: telopode. Scale bar corresponds to 10 μm.

(1.8 ± 0.4 pA/pF, P < 0.01, n = 6 atrial TCs; 2.3 ± 0.5 pA/pF, P < 0.01, n = 6 ventricular TCs) at +90 mV by 1 mM Paxilline (a specific BKCa inhibitor) and were responsive (32.4 ± 3.1 pA/pF, P < 0.01, n = 6 atrial TCs; 20.9 ± 4.2 pA/pF, P < 0.01, n = 6 ventricular TCs) at +90 mV to stimulation by 10 μM (+/-) Naringenin (a specific BKCa opener), confirming the presence of BKCa currents in
atrial and ventricular TCs (Fig. 3A). Furthermore, under identical voltage-clamp condition, 1 ng/ml TGF-β1 significantly increased the peak BKCa current density (17.1 ± 2.7 pA/pF, P < 0.01, n = 6 atrial TCs; 17.8 ± 2.1 pA/pF, P < 0.05, n = 6 ventricular TCs) at +90 mV. However, additional presence of H2S (100 μM) reduced the effect of TGF-β1 (12.6 ± 0.8 pA/pF, P < 0.05, n = 6 atrial TCs; 12.2 ± 1.6 pA/pF, P < 0.05, n = 6 ventricular TCs), thereby demonstrating functionality of the channels and confirming the inhibitory effect of H2S on BKCa currents in TCs (Fig. 3B). Moreover, the rising phase of BKCa currents at +100 mV with activation τ (τact) at baseline (29.3 ± 1.7 ms, atrial TCs; 41.6 ± 1.6 ms, ventricular TCs) was lowered significantly by TGF-β1 (7.3 ± 0.7 ms, P < 0.05, n = 6 atrial TCs; 11.1 ± 0.1 ms, P < 0.05, n = 6 ventricular TCs), but was significantly reversed (11.9 ± 0.3 ms, P < 0.05, n = 6 atrial TCs; 29.1 ± 0.3 ms, P < 0.05, n = 6 ventricular TCs) by H2S, confirming its modulation of BKCa channel kinetics in TCs (Fig. 3C).

Similarly, under conditions that elicited total outward K+ currents, it was found that the activated currents were insensitive to 4-aminopyridine (4-AP; 0.5 mM) inhibition, indicating the absence of transient outward currents (Ito) in atrial and ventricular TCs (Fig. 4A). Furthermore, currents elicited from the holding potential of −40 mV that ramped every 9 sec. from −120 mV to +60 mV at 20 mV/sec. and subsequently ramped to −40 mV at −100 mV/sec. were not responsive to 30 μM pinacidil (KATP specific channel enhancer), suggesting ATP-sensitive K current (KATP) was not present in atrial and ventricular TCs (Fig. 4B). Such differential ion channel profile of TCs further differentiated them from cardiac fibroblasts that showed higher BKCa current density and presence of Ito and KATP currents that were previously reported by our group [18].

Next, we elicited inwardly rectifying K+ currents by depolarizing the TCs with a 2 sec. ramp protocol of −120 to 0 mV from holding potential of −40 mV. Similar to our previous report of IKir current in
Human atrial fibroblasts that was attenuated by Ba2+ ion [18], addition of Ba2+ attenuated the inwardly rectifying currents in both atrial and ventricular TCs, and a 20 mM K+ in the bath solution induced a strong Kir2.1 expression in cardiac telocytes. Voltage protocol showing no appreciable enhancement in amplitude. Current density of IKir at baseline (P<0.01, n=6) in 5.4 mmol/l [K]o respectively. The baseline inward current in TCs (Fig. 5A). Furthermore, slope conductance [21] of IKir in atrial and ventricular TCs were 9.5 ± 4.1 pS/pF (n = 6) and 3.1 ± 1.2 pS/pF (n = 6) in 5.4 mmol/l [K]o, respectively. The baseline current density of IKir at −120 mV (−4.3 ± 0.4 pA/pF, atrial TCs; −4.1 ± 0.3 pA/pF, ventricular TCs) was increased by 1 ng/ml TGF-β1 (−6.4 ± 0.1 pA/pF, P<0.01, n=6 atrial TCs; −5.2 ± 0.1 pA/pF, P<0.01, n=6 ventricular TCs), confirming the functionality of IKir channel. However, the current density at −120 mV reverted towards baseline (−5.8 ± 0.3 pA/pF, P<0.05, n=6 atrial TCs; −4.3 ± 0.5 pA/pF, P<0.05, n=6 ventricular TCs) in the presence of H2S (Fig. 5B).

H2S attenuated TGF-β1-induced KCa1.1 and Kir2.1 expression in cardiac telocytes

Transforming growth factor-β1 is a major mediator of myocardial fibrosis. Our previous study showed that exogenously applied H2S significantly attenuated TGF-β1-stimulated KCa1.1/Kv1.1 (responsible for BKCa) and Kir2.1 (responsible for IKir) expression and reduced proliferation of human atrial fibroblasts [18]. Consistent with our electrophysiological findings, immunofluorescent staining with anti-Kv1.1 and anti-Kir2.1 antibodies confirmed their presence in cardiac TCs (Fig. 6). Furthermore, stimulation with TGF-β1 enhanced the channel expression in cardiac fibroblasts, but also in the associated TCs. Similarly, pretreatment with H2S for 24 hrs reduced the expression levels and organizational distribution of Kv1.1 and Kir2.1 induced by TGF-β1, supporting its inhibitory role on channel expression in cardiac TCs. Contrary to atrial fibroblasts [22] and cardiac fibroblasts in this study, Kv4.3 (responsible for Ito) expression in TCs was barely above background noise levels (data not shown), which supported the undetectable Ito current in our electrophysiological findings.

Discussion

Telocytes have previously been reported in human epicardium [1]. Our results confirmed that ionic active cardiac TCs are present in the human atrial and ventricular myocardium. Concordance with previous report [23], they expressed c-Kit, CD34 and PDGFR-β [20] and exhibited typical TC phenotype such as Tps, podoms and podomers [19]. Because of these unique structural characteristics, TCs with monolayer processes were relatively straightforward to distinguish from cultured fibroblasts and other interstitial cells that maintained disparate spindle-like and stellate-shaped cells in culture. On the basis of these criteria, we were able to visually isolate single TCs from human atrial and ventricular fibroblasts and other interstitial cells for their electrophysiological studies. The examined cardiac TCs expressed a subset of ionic currents that was previously reported to be present in human atrial fibroblasts [18]. To our knowledge, this is the first study that provides evidence of BKCa and IKir channels and functionally active currents in human atrial and ventricular TCs.

To date, only very limited is known about the electrophysiological property of isolated TCs. In human myometrium, patch-clamp recordings of TCs revealed a calcium-dependent hyperpolarization-activated chloride inward current, but absence of L-type calcium channels, which was postulated to modulate myometrial smooth muscle contractions [15]. Inwardly rectifying chloride current has been identified in rodent interstitial cells of Cajal (ICC) in regulating gut pacemaker activity [24]. Transient outward potassium current that modulated pacemaker potential has also been described in rodent ICC [25]. Although transient outward Ito and ATP-sensitive KATP currents were not detected in both the atrial and ventricular TCs in this study, the presence of functionally active BKCa and hyperpolarized-activated inwardly rectifying IKir channels and their close proximity to cardiac fibroblasts and cardiomyocytes may suggest a probable role of cardiac TCs within the multicular framework that facilitates mechanoelectrical coupling of the heart.

Myofibroblasts are abundant in fibrosis [26] and have been associated with TGF-β1-mediated atrial fibrillation in rodent [27] and in patients with inflammation-linked atrial remodelling [17]. Preventing aberrant proliferation of cardiac fibroblasts and their transformation to myofibroblasts has been an attractive target in limiting fibrosis. Our previous study showed that H2S effectively suppressed proliferation of atrial fibroblasts by inhibiting BKCa, IKir and Ito channels [18]. Consistently, we found that H2S attenuated TGF-β1-induced BKCa.
A. Effect of Ba\(^{2+}\) on membrane current in human atrial and ventricular telocytes. Representative I–V relationships of membrane currents recorded with a 2-sec. ramp protocol (−120 to 0 mV from a holding potential of −40 mV) in 5 mM K\(^{+}\) or 20 mM K\(^{+}\) and after application of 0.5 mM Ba\(^{2+}\).

B. Effect of H\(_2\)S (100 μM) on IK\(_{ir}\) currents in the presence of transforming growth factor-β1 (1 ng/ml) in human atrial and ventricular telocytes.

Fig. 6 Immunostaining of BK\(_{ca}\) (Kv1.1) and IK\(_{ir}\) (Kir2.1) in human atrial (top) and ventricular (bottom) telocytes. Cells were stained with anti-Kv1.1 (red), anti-Kir2.1 (red) and the nuclei were stained using DAPI (blue). H\(_2\)S attenuates the Kv1.1 and Kir2.1 upregulation induced by transforming growth factor-β1. TC: telocyte; Fb: cardiac fibroblast; Tp: telopode. Scale bar corresponds to 50 μm.
activation and decelerated the transition from closed to open state of the channel, suggesting a role for H₂S in regulating BK₈s channel kinetic and voltage sensitivity in human cardiac TCs. Furthermore, H₂S down-regulated the voltage-dependent relationships of IKᵥ and IKₛ induced by TGF-β1 in the TCs. It is currently unclear about the implications of greater BK₈s (Fig. 3C) and IKᵥ (Fig. 5B) channel responsiveness observed in the atrial TCs as compared to ventricular TCs when stimulated with TGF-β1. This was despite both TCs exhibited similar current density of BK₈s (Fig. 3B) and IKᵥ (Fig. 5A) at baseline. Although atrial fibroblasts are known to participate more actively in cardiac fibrosis as compared to ventricular fibroblasts [28, 29], it remains to be ascertained if such differential sensitivity of atrial TCs to TGF-β1 stimulation has a role in more aggressive fibrotic response of atrial fibroblasts. Furthermore, the differential disease status of the atrial and ventricular tissue donors (from which TCs were derived in our study), in affecting the observed outcome could not be totally discounted.

In summary, our study shows the presence of functionally competent BK₈s and IKᵥ channels, but not Iₖᵥ and KᵥATP channels, in cardiac atrial and ventricular TCs. Their close physical association with cardiac fibroblasts and cardiomyocytes may suggest additional active roles in myocardial physiopathology apart from mere passive structural supporting function in the heart.

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Conflict of interest

The authors confirm that there are no conflicts of interest.

Supporting information

Additional Supporting Information may be found in the online version of this article:

Figure S1 Double immunofluorescent staining of human atrial and ventricular telocytes demonstrating positivity for CD34 and c-Kit. Scale bar corresponds to 10 μm.

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