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Molecules and Cells

Functional Characteristics of TRPC4 Channels Expressed in HEK 293 Cells

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The classical type of transient receptor potential (TRPC) channel is a molecular candidate for Ca^{2+}-permeable cation channels in mammalian cells. Because TRPC4 and TRPC5 belong to the same subfamily of TRPC, they have been assumed to have the same physiological properties. However, we found that TRPC4 had its own functional characteristics different from those of TRPC5. TRPC4 channels had no constitutive activity and were activated by muscarinic stimulation only when a muscarinic receptor was co-expressed with TRPC4 in human embryonic kidney (HEK) cells. Endogenous muscarinic receptor appeared not to interact with TRPC4. TPRC4 activation by GTPγS was not desensitized. TPRC4 activation by GTPγS was not inhibited by either Rho kinase inhibitor or MLCK inhibitor. TRPC4 was sensitive to external pH with pK_a of 7.3. Finally, TPRC4 activation by GTPγS was inhibited by the calmodulin inhibitor W-7. We conclude that TRPC4 and TRPC5 have different properties and their own physiological roles.

INTRODUCTION

Transient receptor potential (TRP) channels constitute a superfamily of cation permeable channels. The *trp* genes in *Drosophila melanogaster* as a founding member of this superfamily are required for visual transduction, which is a phospholipase C-dependent process (Hardie and Minke, 1993). After the *trp* gene was reported in the fruit fly, TRP channels encoded by mammalian homologues of the *trp* gene were viewed as molecular candidates for cation permeable channels or nonselective cation channels (NSCCs) activated by G protein coupled receptors. Based on their amino acid sequences similarities, the TRP-related proteins fall into seven subfamilies: classical TRPs (TRPC), which display the greatest similarity to Drosophila TRP; vanilloid receptor TRPs (TRPVs); melastatin TRPs (TRPMs); mucolipins TRPs (TRPMLs); polycystins TRPs (TRPPs); NOMPC (TRPN); ankyrin 1 TRPs (TRPA1).

Of all the TRP cation channel family members, TRPCs are distinguished from other TRP cation channel families by containing both 3-4 ankyrin repeats in the N-terminus and a TRP box (EWKFAR) in the C-terminus. To date, seven TRPC subtypes have been identified: TRPC1 to TRPC7 (Lee et al., 2006). TRPC channels are classified into four subgroups base on their amino acid sequences: TRPC1, TRPC2, TRPC3/6/7, and TRPC4/5. The primary difference between TRPC3/6/7 and TRPC4/5 is the response to the extracellular diacylglycerol analog, OAG. OAG activates TRPC3/6/7 channels but it has no effect on TRPC4/5. TRPC6 has been suggested as a molecular candidate for α1-adrenoceptor-activated nonselective cation channel (NSCC) in portal vein smooth muscle cells (Inoue et al., 2001) and for vasopressin-activated NSCC in cultured aortic smooth muscle cells (Jung et al., 2002).

TRPC6 was suggested to be involved with the regulation of blood pressure. However, in TRPC6 knockout mice, a blood pressure increased contrary to the expectation that there is a hypotension due to lack of TRPC6 responding to adrenaline, hypertension inducer (Dietrich et al., 2005). In the absence of TRPC6, there was a compensatory up-regulation of TRPC3 and consequent hypertension due to the constitutive activity of increased TRPC3. While TRPC6 was suggested to be a receptor-operated calcium channels (ROC channels), like α1-adrenoceptor-activated nonselective cation channel, TRPC4 was initially suggested to be a molecular candidate for store-operated calcium channels (SOC channels) that are activated by the depletion of calcium store in aortic endothelial cells (Freichel et al., 2003), even though it belongs to the same TRPC subfamily. Recently, stromal interaction molecule (STIM) (Zhang et al., 2005) and ORAI protein (Feske et al., 2006) were shown to be the molecular candidates for store-operated calcium channels, especially calcium selective SOC channels, (i.e. calcium release activated calcium channel, CRAC channel). In contrast, we previously showed that TRPC4 is a candidate for NSCC activated by muscarinic stimulation in gastric smooth muscle cells (Lee et al., 2005) rather than a candidate for SOC channels. Thus, we investigated the functional characteristics of TRPC4 and compared its electrophysiological properties to those of TRPC5, a similar TRPC channel belonging to the same TRPC subfamily.

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MATERIALS AND METHODS

Cell culture and transient transfection

Human embryonic kidney (HEK293) cells (ATCC, USA) were maintained according to the supplier's recommendations. For transient transfection, cells were seeded in 12-well plates. The following day, 0.5 μg/well of pcDNA vectors containing the cDNAs for mouse TRPC4β, M_1 muscarinic receptor and M_3 muscarinic receptor were mixed with 50-100 ng/well of pEGFP-C1 (Clontech), and transfected into the cells using the transfection reagent FuGENE 6 (Roche Molecular Biochemicals) according to the manufacturer's protocol. cDNAs for mouse TRPC4α and human TRPC4α-EGFP were also transfected in the same way. After 30-40 h, the cells were trypsinized and used for whole-cell recording.

Whole-cell patch-clamp experiments

Isolated cells were transferred to a small chamber on the stage of an inverted microscope (IX50, Olympus, Japan), and were continuously perfused with physiological salt solution (PSS) at a rate of 1-2 ml/min. A glass microelectrode with a resistance of 2-4 MΩ was used to make a gigaohm seal. The conventional whole-cell patch-clamp technique was adopted to hold the membrane potential at -60 mV using an Axopatch 200B patch-clamp amplifier (Axon Instrument, USA). For data acquisition and the application of command pulses, pCLAMP software v.9.2 and Digidata 1322A (Axon Instruments) were used. Data were filtered at 5 kHz and displayed on a computer monitor. Data were analyzed using pCLAMP and Origin software (Microcal origin v.6.0, USA).

Solutions and drugs

Physiological salt solution (PSS, in mM) contained NaCl 135, KCl 5, $CaCl_2$ 2, $MgCl_2$ 1, glucose 10, and HEPES (*N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulphonic acid]) 10, and pH was adjusted to 7.4 using NaOH. Cs-rich external solution was made by replacing NaCl and KCl with equimolar CsCl. The pipette solution (in mM) contained CsCl 140, HEPES 10, Tris-GTP (Tris-Guanosine 5′-triphospate) 0.2, EGTA 0.5, Mg-ATP (Adenosine 5′-triphosphate) 3. Its pH was adjusted to 7.3 with CsOH.

Calmidazolium, W-7, Y-27632 and ML-7 were purchased from Calbiochem (Canada), and carbachol, HEPES, and GTPγS were from Sigma (USA).

Statistics

All data are expressed as means ± SEM. Comparisons between two groups used Student's paired *t*-tests. Comparisons among four groups used ANOVA followed by post-hoc test. $p < 0.05$ was considered statistically significant. The number of cell recordings is given by *n*.

RESULTS

The effect of carbachol on TRPC4β expressed in HEK cells

Whole cell currents were recorded using patch clamp techniques to study the electrophysiological properties of TRPC4 channels. In the beginning, whole cell currents were recorded under the condition of PSS and 140 mM $[Cs^+]_i$. In order to obtain current-voltage (I-V) relationships, we applied a ramp pulse from +100 mV to -100 mV for 500 ms. To activate TRPC4 channels, we applied carbachol (CCh), an analog of acetylcholine, to stimulate muscarinic receptors in HEK cells. In non-transfected HEK cells, TRPC4-like inward current was not activated by 50 μM CCh (Fig. 1A). In HEK cells that expressed mouse TRPC4β only, basal currents did not change when the external solution was changed from PSS to 140 mM $[Cs^+]_o$ solution. In contrast to TRPC5 (Kim et al., 2006), TRPC4β did not show any constitutive activity. Whole cell current was also recorded under the condition of 140 mM $[Cs^+]_o$ and $[Cs^+]_i$ as a control current for subtraction in order to abtain a current-voltage relationship for TRPC4β activated by CCh. When 50 μM CCh was applied at a holding potential of -60 mV, a slight inward current was activated. The I-V relationship, obtained by subtracting the current in the absence of CCh from that in the presence of CCh, showed a typical doubly rectifying shape (Fig. 1B). Endogenous muscarinic receptor was not sufficient for stimulation of TRPC4β by CCh.

After M_1 muscarinic receptor was co-expressed with TRPC4β, 50 μM CCh was applied. The TRPC4β current was activated by stimulation of muscarinic receptors and was quickly desensitized during the application. The I-V relationship showed a typical doubly rectifying shape (Fig. 1C).

GTPγS-induced current of TRPC4 expressed in HEK cells

In order to directly activate G proteins rather than G protein coupled receptors like muscarinic receptors, we added GTPγS to the intracellular pipette solution and recorded whole cell current. Intracellular GTPγS (0.4 mM) induced an inward current at a holding potential of ~60 mV in HEK cells that expressed mouse TRPC4β. The I-V relationship showed a typical doubly rectifying shape (Fig. 2A). The current amplitude for mouse TRPC4β was 2527 ± 242 p*A* ($n = 6$). In contrast, intracellular GTPγS (0.4 mM) did not induce an inward current at a holding potential of -60 mV in HEK cells that expressed mouse TRPC4α (Fig. 2B). The I-V relationship did not show a typical doubly rectifying shape (Fig. 2B, right panel).

However, when the C-terminus for human TRPC4α was linked to a GFP protein, human TRPC4α was activated by intracellular 0.4 mM GTPγS and 50 μM CCh (Fig. 3). When we applied Cs repeatedly to determine whether or not there was a desensitization phenomenon, human TRPC4α currents were repeatedly activated by intracellular GTPγS. The inward currents for human TRPC4α, however, were desensitized (Fig. 3A). In the absence of exogenous muscarinic receptor, 50 μM CCh activated the human TRPC4α current. The human TRPC4α current activated by 50 μM CCh decayed spontaneously to the basal level even during the application. The I-V relationship showed a typical doubly rectifying shape (Fig. 3B).

The TRPC4β current activated by intracellular GTPγS did not decay spontaneously to the basal level even during the application. When we activated TRPC4β repeatedly by applying Cs after the first application, the current was activated like that of control (Fig. 4). A third application of Cs could still induce an inward current at a holding potential of ~60 mV under the condition of intracellular GTPγS (0.4 mM).

The effect of DAG analog, OAG, on TRPC4β expressed in HEK cells

The activation mechanism for TRPC4 remains still unknown. We first applied OAG to determine if TRPC4 responded to lipid metabolites of PIP_2 by phospholipase C. After M_3 muscarinic receptor was co-expressed with TRPC4β, 50 μM CCh was applied. CCh induced an inward current at a holding potential of -60 mV, but 100 μM OAG did not (Fig. 5A). The I-V relationship did not show a typical doubly rectifying shape (Fig. 5B).

The effects of calmodulin inhibitors or MLCK inhibitor on TRPC4β expressed in HEK cells

In a previous study we examined the activation mechanism for

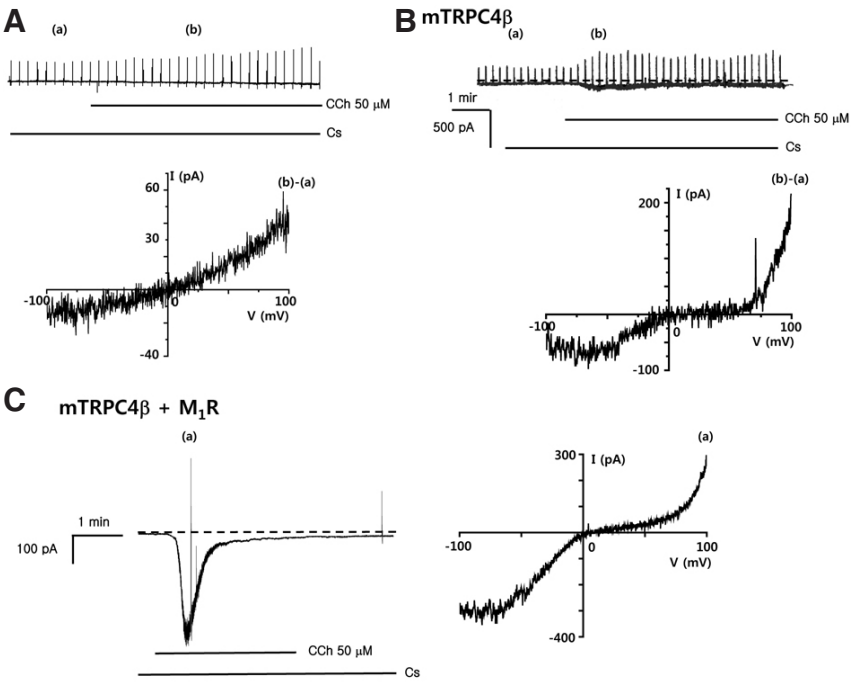

Fig. 1. Carbachol (CCh)-activated inward currents and current-voltage (*I-V*) relationships in human embryonic kidney (HEK) cells. Non-transfected and mouse transient receptor potential channel 4β (mTRPC4β) expressed in HEK cells. Whole cell currents were recorded under the condition of 140 mM intracellular $[Cs^+]$ ($[Cs^+]_i$) and extracellular $[Cs^+]$ ($[Cs^+]_o$) using whole cell patch-clamp technique. Typical *I-V* relationship was obtained using a ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. The 50 μM CCh was added in the bath. (A) Whole cell currents and *I-V* relationships for non-transfected HEK cells. An inward current for TRPC4 was not activated by 50 μM CCh application. The *I-V* relationship was obtained by subtracting (a) from (b). (B) Whole cell currents and *I-V* relationships for mTRPC4β expressed in HEK cells. A slight inward current was activated by 50 μM CCh. The *I-V* relationship was obtained by subtracting (a) from (b). (C) M_1 muscarinic receptor was co-expressed with mTRPC4β in HEK cells. 50 μM CCh induced an inward current, which decayed spontaneously during CCh treatment. *I-V* relationship showed a typical doubly rectifying shape.

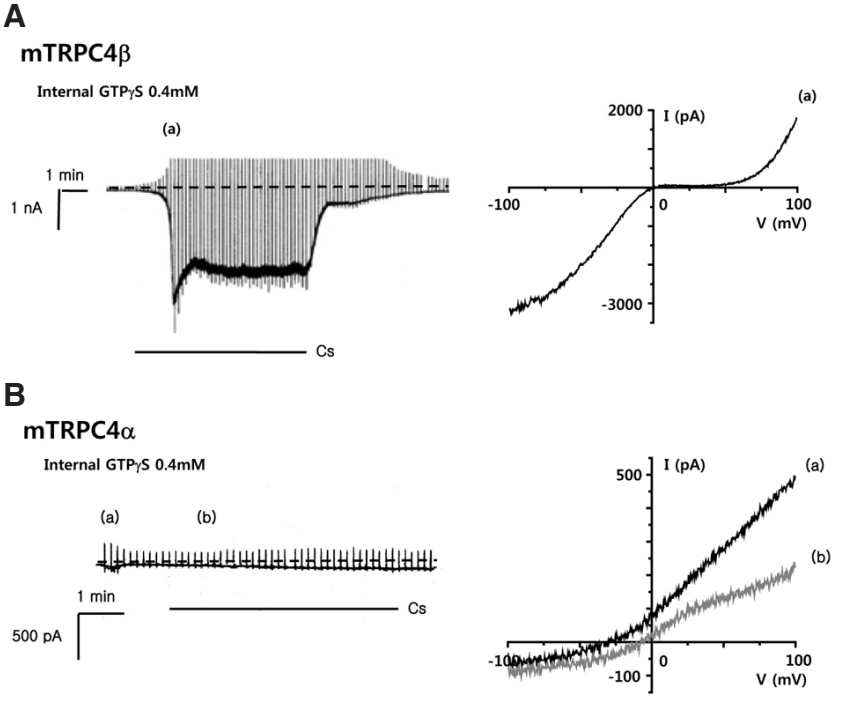

Fig. 2. GTPγS-activated inward currents and current-voltage (*I-V*) relationships for TRPC4 expressed in HEK cells. Whole cell currents were recorded under the condition of 140 mM intracellular $[Cs^+]$ ($[Cs^+]_i$) and extracellular $[Cs^+]$ ($[Cs^+]_o$) using whole cell patch-clamp technique. Typical *I-V* relationship was obtained using a ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. (A) When external solution was changed to 140 mM extracellular $[Cs^+]$ ($[Cs^+]_o$) solution, an inward current was induced in the presence of internal 0.4 mM GTPγS for mTRPC4β. *I-V* relationship showed a typical doubly rectifying shape. (B) Whole cell currents and *I-V* relationships for mTRPC4α expressed in HEK cells. Unlike mTRPC4β, an inward current was not induced in the presence of internal 0.4 mM GTPγS for mTRPC4α.

TRPC5, a member of the same subfamily as TRPC4, and showed that TRPC5 was activated by either myosin light chain kinase (MLCK) or Rho kinase (Kim et al., 2006). We first investigated the involvement of calmodulin (CaM) in the activation of TRPC4. W-7, an inhibitor of CaM, inhibited the TRPC4β current induced by intracellular GTPγS from 1327 ± 82 p*A* (*n* = 7) to 116 ± 37 p*A* (*n* = 9, *p* < 0.01) (Fig. 6). However, another CaM antagonist, calmidazolium (CMZ), did not inhibit TRPC4β current activated by intracellular GTPγS (1882 ± 141 p*A*, *n* = 3) (Fig. 6B). We next investigated the involvement of MLCK, a target of calmodulin, in the activation of TRPC4. ML-7, an inhibitor of MLCK, also did not inhibit TRPC4β current induced by intracellular GTPγS, either (1948 ± 442 p*A*, *n* = 3) (Fig. 6B).

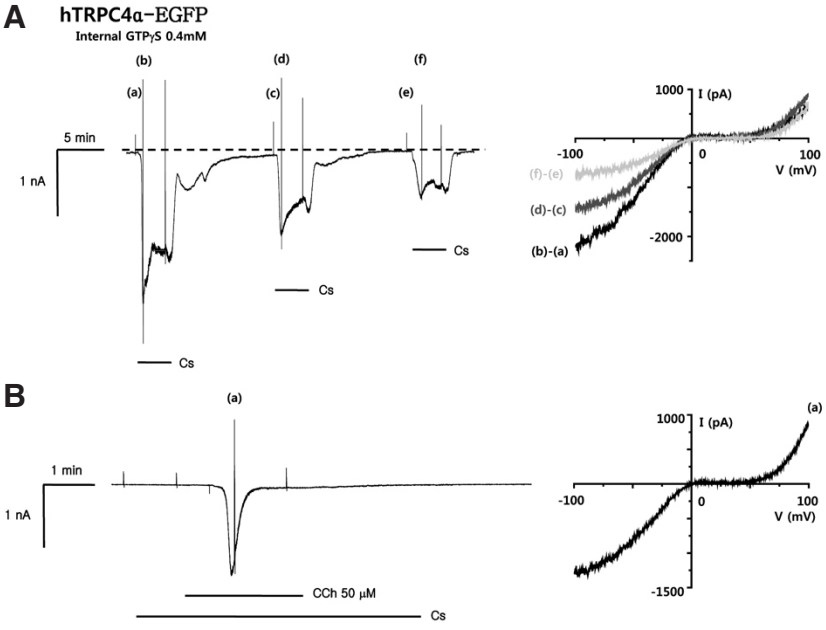

Fig. 3. GTPγS-activated and carbachol (CCh)-activated inward currents and their current-voltage (*I-V*) relationships for human TRPC4α-EGFP (hTRPCα) expressed in HEK cells. Whole cell currents were recorded under the condition of 140 mM $[Cs^+]_i$ and $[Cs^+]_o$ using whole cell patch-clamp technique. Typical *I-V* relationship was obtained by using ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. (A) When external solution was changed to 140 mM extracellular $[Cs^+]_o$ solution, an inward current was induced in the presence of internal 0.4 mM GTPγS for hTRPC4α. The *I-V* relationships in the right panel were obtained by subtracting (a) from (b), (c) from (d), and (e) from (f). *I-V* relationship showed a typical doubly rectifying shape. (B) Whole cell currents and *I-V* relationships for CCh-activated inward currents. Muscarinic receptor was not co-expressed with hTRPC4α in HEK cells. 50 μM CCh induced an inward current, which decayed spontaneously during CCh treatment. *I-V* relationship showed a typical doubly rectifying shape.

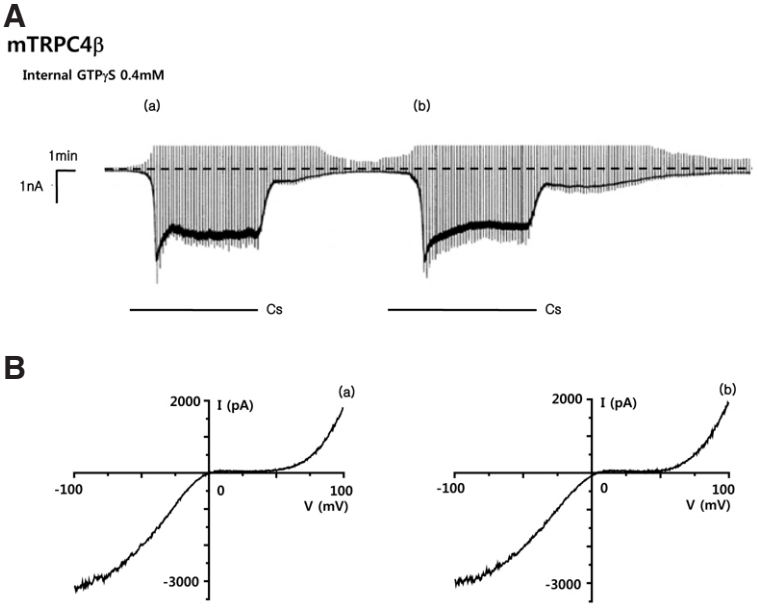

Fig. 4. The effect of GTPγS on mTRPC4β expressed in HEK cells. Whole cell currents were recorded using whole cell patch-clamp technique and typical *I-V* relationship was obtained using a ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. (A) GTPγS-activated inward current did not decay spontaneously in the presence of internal 0.4 mM GTPγS. The currents were repeatedly activated with similar current amplitude when the external solution was changed from physiological salt solution to 140 mM extracellular $[Cs^+]$ ($[Cs^+]_o$) solution. (B) *I-V* relationships showed a typical doubly rectifying shape.

The effects of MLCK inhibitor and Rho kinase inhibitor on TRPC4β expressed in HEK cells

When TRPC4 is activated by intracellular GTPγS, it is possible that GTPγS may activate several G proteins, such as Rho protein or Gq protein, which are endogenously expressed in HEK cells. We investigated the effects of both Rho kinase inhibitor and MLCK kinase inhibitor on GTPγS-induced currents for TRPC4β (Fig. 7). The GTPγS-induced current (1527 ± 170 p*A*, $n = 4$) was not inhibited by Y-27632, Rho kinase inhibitor, alone (2097 ± 460 p*A*, $n = 4$) (Fig. 7A). GTPγS-induced current (1150 ± 141 pA, $n = 4$) was also not inhibited by simultaneous application of Y-27632 and ML-7 (1228 ± 212 pA, $n = 3$) (Fig. 7B).

The effect of extracellular pH on TRPC4α expressed in HEK cells

Extracellular pH modulates nonselective cation channels activated by muscarinic stimulation or intracellular GTPγS (Inoue et al., 1995; Zholos and Bolton, 1997). Thus, we tested whether extracellular pH modulated TRPC4 current in HEK cells that was expressed human TRPC4α. Human TRPC4α tagged with GFP at the C-terminus was expressed. To activate the TRPC4 current, GTPγS was added to the pipette solution. Extracellular pH inhibited the human TRPC4α current as the extracellular pH decreased from pH 8.4 to 4.4, with pK_a of 7.3 (Fig. 8).

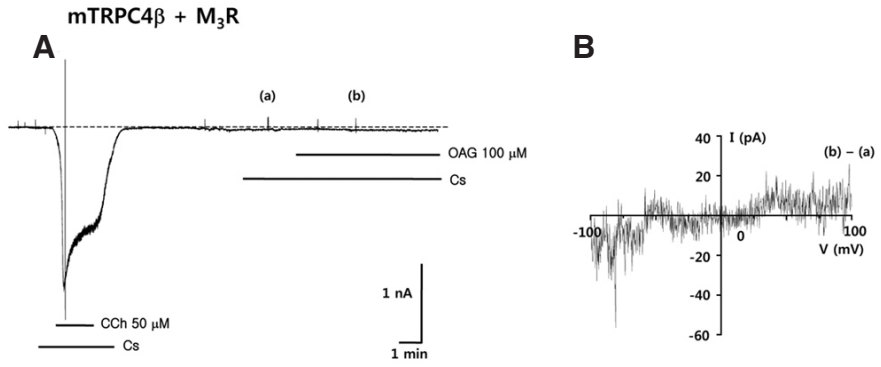

Fig. 5. Diacylglycerol analog (OAG)-activated inward currents and its current-voltage (*I-V*) relationships for mTRPC4β expressed in HEK cells. Whole cell currents were recorded under the condition of 140 mM intracellular $[Cs^+]$ ($[Cs^+]_i$) and extracellular $[Cs^+]$ ($[Cs^+]_o$) using whole cell patch-clamp technique. Typical *I-V* relationship was obtained by using ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. (A) M_3 muscarinic receptor was co-expressed with TRPC4β in HEK cells. 50 μM CCh was applied to confirm whether mTRPC4β was expressed. After CCh was removed, application of OAG (100 μM) did not induce an inward current. (B) The *I-V* relationship was obtained by subtracting (a) from (b).

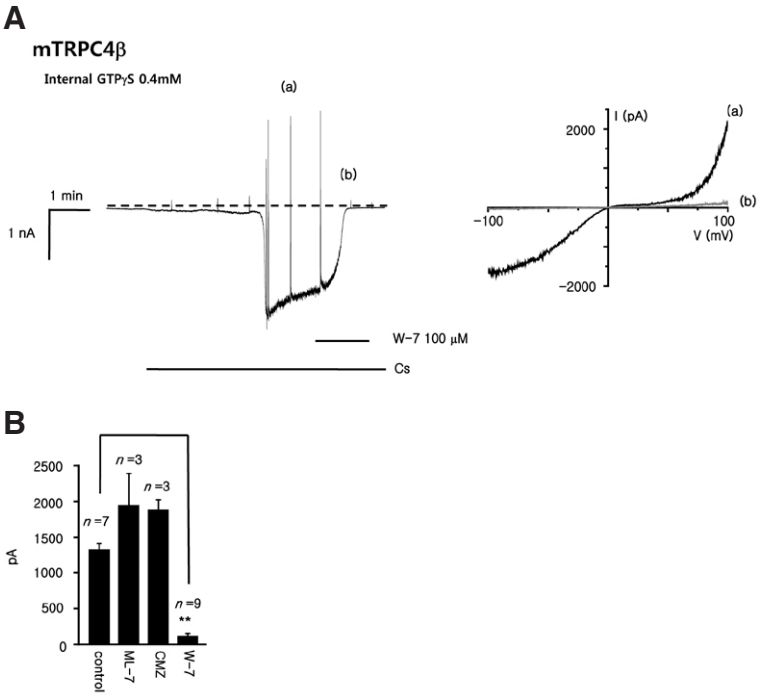

Fig. 6. The effect of calmodulin (CaM) inhibitor on GTPγS-activated inward current of mTRPC4β. (A) Whole cell currents were recorded under the condition of 140 mM intracellular $[Cs^+]$ ($[Cs^+]_i$) and extracellular $[Cs^+]$ ($[Cs^+]_o$) using whole cell patch-clamp technique. CaM inhibitor, W-7, was applied during the activation of GTPγS-induced current. *I-V* relationship was obtained using a ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. *I-V* relationships showed a typical doubly rectifying shape. Unlike TRPC5, W-7 inhibited the TRPC4β current in the presence of intracellular 0.4 mM GTPγS. (B) Amplitudes of inward currents during the application of an MLCK inhibitor, ML-7, and CaM inhibitors, W-7 and calmidazolium (CMZ), are summarized. ML-7 and CMZ did not inhibit GTPγS-induced current for mTRPC4β. ** $p < 0.01$.

DISCUSSION

In the present study, when expressed in HEK cells, TRPC4β showed its own unique electrophysiological properties that were different from TRPC5. 1) TRPC4β had no basal activity. 2) TRPC4β was activated by muscarinic stimulation only when a muscarinic receptor was co-expressed. 3) Both a Rho kinase inhibitor and an MLCK kinase inhibitor did not decrease GTPγS-induced currents. 4) TRPC4α was more sensitive to decreased extracellular pH than TRPC5. 5) W-7 inhibited the TRPC4β current activated by intracellular GTPγS.

The two most abundant TRPC4 variants are a "full-length" TRPC4α and a slightly shorter TRPC4β, which lacks a stretch of 84 amino acids residues (781-864 in the case of mouse TRPC4) in the cytosolic C terminus (Δ84AA) (Otsuguro et al., 2008). There are several different functional features not only between TRPC4α and TRPC4β but also between TRPC4 and TRPC5 (Figs. 1, 3 and 4). These results suggest that members of the TRPC subfamily have their own characteristics features as NSCC, although their sequences are very similar.

Although seven TRPC channels belong to the same family, each TRPC channel has its own function for regulating intracellular calcium. TRPC1 was suggested to be involved with mechanosensation. In TRPC1 knockout mice, however, mechanosensation was normal. TRPC2 is involved with pheromone responses, and TRPC2 knockout mice respond abnormally to urine-based olfactory cues (Liman et al., 1999; Lucas et al., 2003; Stowers et al., 2002; Vannier et al., 1999). TRPC6 is a molecular candidate for nonselective cation channel activated by adrenergic stimulation in vascular smooth muscle (Inoue et al., 2001). TRPC7 is a receptor-operated DAG-activated channel in human keratinocytes and acts as a G protein-activated Ca^{2+} channel mediating angiotensin II-induced myocardial apoptosis (Beck et al., 2006; Satoh et al., 2007). TRPC4 and TRPC5 are molecular candidates for nonselective cation channel activated by muscarinic stimulation in visceral smooth muscle (Kim et al., 2006; 2007; 2008; Lee et al., 2003; 2005; Zhu et al., 2003; 2005). In fact, there was no activation of nonselective

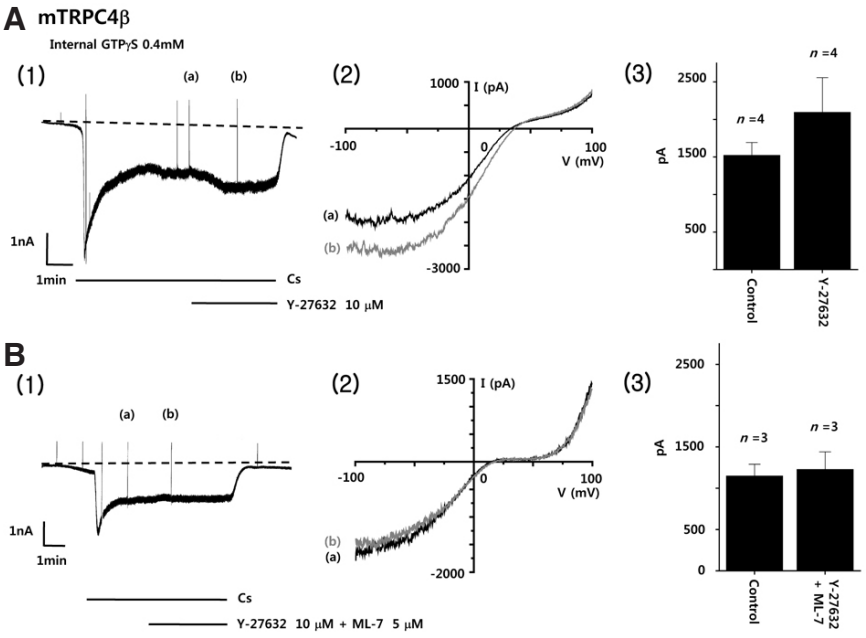

Fig. 7. The effect of Rho kinase inhibitor and myosin light chain kinase (MLCK) inhibitor on GTPγS-activated inward current of mTRPC4β. Whole cell currents were recorded under the condition of 140 mM intracellular $[Cs^+]$ ($[Cs^+]_i$) and extracellular $[Cs^+]$ ($[Cs^+]_o$) using whole cell patch-clamp technique. Rho kinase inhibitor, Y-27632, or both Y-27632 and ML-7 (MLCK inhibitor) were applied during the activation of GTPγS-induced current (A(1) and B(1)). These did not inhibit GTPγS-induced current. *I-V* relationship was obtained using a ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. *I-V* relationships showed a typical doubly rectifying shape (A(2) and B(2)). Amplitudes of inward currents during the application of ML-7 and Y -27632 are summarized (A(3) and B(3)).

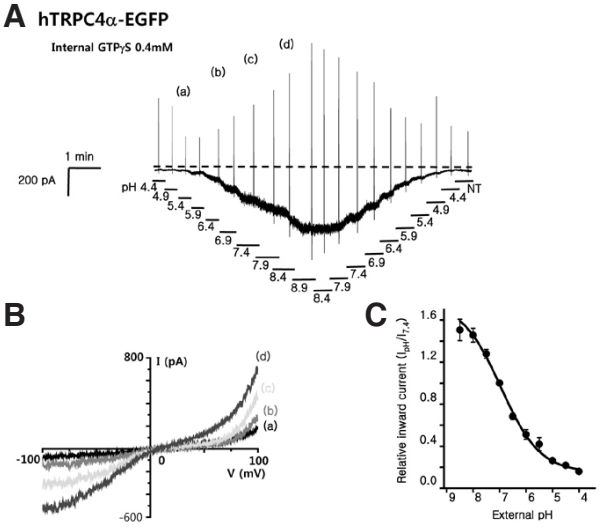

Fig. 8. The effect of extracellular pH (pH_e) on humanTRPC4α-EGFP expressed in HEK cells. (A) Current trace at a holding potential of -60 mV at pH_e from 8.9 to 4.4 (shown by horizontal bars in both acidifying and alkalinizing order). The dotted line indicates the zero current level. The vertical lines indicate the ramp pulses applied for obtaining *I-V* relationships. (B) The *I-V* relationships obtained at the time indicated at (A). Typical *I-V* relationship was obtained by using ramp pulse from +100 mV to -100 mV for 500 ms from a holding potential of -60 mV. Extracellular acidosis decreased the TRPC4α current. (C) Dose-response curve for TRPC4α currents against extracellular pH. The normalized current amplitudes at -60 mV ($I_{pH}/I_{7.4}$) were plotted against the extracellular pH. The dose-response curves for TRPC4α were fit to a Hill function with pKa of 7.3.

cation channel activated by muscarinic stimulation in gastric smooth muscle of TRPC4 knockout mice (Lee et al., 2005).

The primary difference between TRPC3/6/7 and TRPC4/5 is the response to extracellularly applied diacylglycerol analog, OAG. OAG can activate TRPC3/6/7 channels but has no effect on TRPC4/5 except one study (Lee et al., 2003). In our study, OAG could induced an inward current for TRPC5, which was very small compared to that activated by carbachol or intracellular GTPγS (Lee et al., 2003). However, OAG could not activate TRPC4β (Fig. 4). Recently Shi et al. (2004) showed that CaM kinase, rather than MLCK, is involved in the activation of TRPC6 in contrast to TRPC5 (Kim et al., 2006; Shimizu et al., 2006). In our previous study, we showed that MLCK and Rho kinase were involved in the activation of TRPC5. However, both MLCK and Rho kinase were not involved in the activation of TRPC4. Another protein, G_{i2} protein, was involved in the activation of TRPC4 (Jeon et al., 2008).

Although TRPC4 and TRPC5 belong to the same subgroup, their amino acid sequences are different at the three regions: the pore region, N-terminus and C-terminus of the TRPC channel. In particular the pore region is so different that it was used for selective inhibition of TRPC4/5 channel with selective antibody for TRPC5 (Xu et al., 2005). TRPC3 has constitutive activity like that of TRPC5, while TRPC6 dose not similar to TRPC4. There was even a difference between TRPC4α and TRPC4β that lacks 84 amino acids in the C-terminus. Intracellular PIP_2 inhibited TRPC4α but not TRPC4β (Otsuguro et al., 2008). Even the nitrosylation of TRPC channel by nitric oxide (NO) was different between TRPC5 and TRPC4 (Yoshida et al., 2006). Also, the G proteins involved with activation were different between TRPC4 and TRPC5 (Jeon et al., 2008). In the present study, TRPC4α was more sensitive to decreased extracellular pH than TRPC5 (see Kim et al., 2008). W-7 might be used to distinguish TRPC4 from TRPC5, although it is known as an inhibitor of calmodulin (Fig. 6B).

In conclusion, each TRPC channel has its own physiological function and is involved with a specific response to G protein coupled receptors (acetylcholine, adrenaline, pheromone, or angiotension II) or to intracellular store depletion.

Note: Supplementary information is available on the Molecules and Cells website (www.molcells.org).

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