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Gs cascade regulates canonical transient receptor potential 5 (TRPC5) through cAMP mediated intracellular Ca^{2+} release and ion channel trafficking

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ABSTRACT

Canonical transient receptor potential (TRPC) channels are Ca^{2+}-permeable, non-selective cation channels those are widely expressed in mammalian cells. Various molecules have been found to regulate TRPC both *in vivo* and *in vitro*, but it is unclear how heterotrimeric G proteins transmit external stimuli to regulate the activity of TRPC5. Here, we demonstrated that TRPC5 was potentiated by the $G\alpha_s$ regulatory pathway. Whole-cell TRPC5 current was significantly increased by β-adrenergic receptor agonist, isoproterenol (ISO, 246 ± 36%, $n = 6$), an activator of the adenylate cyclase, forskolin (FSK, 273 ± 6%, $n = 5$), or a membrane permeable cAMP analogue, 8-Br-cAMP (251 ± 63%, $n = 7$). In addition, robust Ca^{2+} transient induced by isoproterenol was observed utilizing a Ca^{2+} imaging technique. When intracellular $[Ca^{2+}]_i$ was buffered to 50 nM, cAMP-induced potentiation was attenuated. We also found that the Ca^{2+} release is mediated by IP_3 since intracellular IP_3 infusion attenuated the potentiation of TRPC5 by $G\alpha_s$ cascade. Finally, we identified that the membrane localization of TRPC5 was significantly increased by ISO (155 ± 17%, $n = 3$), FSK (172 ± 39%, $n = 3$) or 8-Br-cAMP (216 ± 59%, $n = 3$). In conclusion, these results suggest that the $G\alpha_s$–cAMP pathway potentiates the activity of TRPC5 *via* facilitating intracellular Ca^{2+} dynamics and increasing channel trafficking to the plasma membrane.

1. Introduction

Transient Receptor Potential Canonical 5 (TRPC5) is one of the seven mammalian TRPC proteins [1]. The seven mammalian TRPC proteins are further divided into four subgroups, TRPC1, TRPC2, TRPC3/C6/C7, and TRPC4/C5, based on the sequence similarities. It has been recognized from the earlier studies that TRPC channels, including TRPC5, can be stimulated by G protein-coupled receptor agonists, such as, ATP, acetylcholine, sphingosine-1-phosphate (S1P), glutamate and cholecystokinin [2,3]. G protein is certainly a key downstream effector following receptor activation, since TRPC5 activation by these agonists mimics the effect of a stable GTP analogue, GTPγS (which can be reversed by a stable GDP analogue, GDPβS) [4]. $G\alpha_{i/o}$ has been demonstrated to mediate the effects of S1P or oxidized phospholipids on TRPC5 since pertussis toxin prevented the activation [5,6]. $G\alpha_{q/11}$ has also been implicated in the regulation [4,7] but the requirement may be minor compare to $G\alpha_{i/o}$ [5,6]. Interestingly, although acting *via* different G proteins, endogenous muscarinic receptors and S1P receptors couple apparently similarly to TRPC5 in HEK 293 cells [2]. S1P is known to activate TRPC5 without causing intracellular Ca^{2+}-release in HEK293 cells [2]. Similarly, in both HEK 293 cells and vascular smooth muscle cells, oxidized phospholipids stimulated TRPC5 or TRPC1/5 without evoking Ca^{2+}-release [6]. However, intracellular calcium is involved in the activation of TRPC5 [8,9] since stepwise increase of $[Ca^{2+}]_i$ (by photolysis of caged Ca^{2+}) augmented TRPC5 activity, suggesting a primary mechanism of internal Ca^{2+}[8]. In addition, TRPC4/C5 channel activities are also shown to be facilitated by internal and external Ca^{2+} [3].

It is considered that phospholipase C (PLC), IP_3 and subsequent cytosolic Ca^{2+} increase mediate G-protein activation of TRP channels. Although this pathway has generally been associated with $G\alpha_{q/11}$ activation, recent studies reported that $G\alpha_s$ may also increase cytosolic Ca^{2+} through IP_3-dependent pathway. E.g., β_2-AR, a Class I GPCR, stimulates $G\alpha_s$ and increases cAMP to increase PKA. PKA, in turn, phosphorylates IP_3 receptor (IP_3R) and this phosphorylation alters IP_3 sensitivity of IP_3R [10]. Alternatively, it is reported that cAMP activates Epac and cAMP-triggered Epac activates PLCε to produce IP_3, which binds to IP_3R and increases Ca^{2+} mobilization from endoplasmic reticulum (ER) into cytosol [11]. Since cytosolic Ca^{2+} *per se* is known to activate TRPC5, we hypothesized that $G\alpha_s$ pathway regulates TRPC5 activity by

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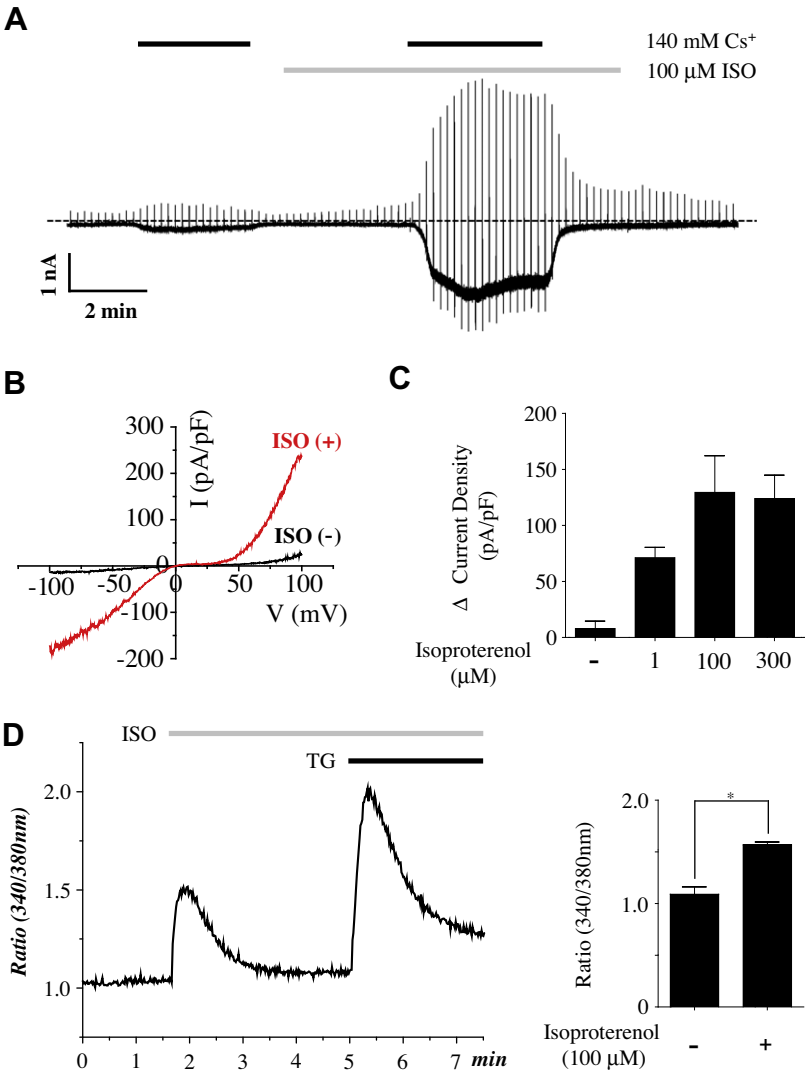

Fig. 1. The effect of isoproterenol on TRPC5 current A. A representative current trace of TRPC5 stimulated by the β–adrenergic receptor agonist isoproterenol (ISO), in hTRPC5-expressing HEK293 cells using the whole-cell patch-clamp technique. Slow depolarizations from +100 to −100 mV were applied from a holding potential of −60 mV. Whole-cell currents were recorded under the condition of 140 mM $[Cs^+]_i$. When the external solution was changed to a 140 mM $[Cs^+]_o$ solution containing ISO, the TRPC5 current increased. B. The Current (*I*)–Voltage (*V*) curve measured in the same cell shown above, demonstrating a typical doubly rectifying TRPC5 induced by ISO. Two colored lines indicate *I–V* relationship at 1st Cs^+ application (black) and 2nd Cs^+ application with ISO (red), as shown in the representative current trace in A. C. A summarized current amplitude of TRPC5 induced by various concentrations of ISO at −60 mV. D. Left, a representative trace of intracellular Ca^{2+} response induced by 100 μM ISO in HEK293 cells, measured through a fluorescent $[Ca^{2+}]_i$ assay using Fura-2. After ISO evoked a Ca^{2+} transient, the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor thapsigargin (TG) was applied at 1 μM. Right, summarized fluorescence intensity evoked by ISO at 340/380 nm. $^*p < 0.05$ ($n = 3$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

inducing the mobilization of intracellular Ca^{2+} from ER to cytosol. Therefore, we examined the signaling cascade mediates β-adrenergic stimulation and TRPC5 activation in HEK293 cells heterologously expressed with human TRPC5 genes.

2. Materials and methods

2.1. Cell culture and transient transfection

Human embryonic kidney (HEK)-293 cells (American Type Culture Collection, Manassas, VA) were maintained according to the supplier's recommendations. Plasmids containing human TRPC5 (hTRPC5) were kindly donated by Dr. S. Kaneko. For transient transfection, cells were seeded in 12-well plates. The next day, 0.5–2 μg/well of pcDNA vector containing the GFP tagged cDNA of human TRPC5 transfected into cells using FuGENE 6 transfection reagent (Roche Molecular Biochemicals) according to the manufacturer's protocol. After 18–24 h, cells were trypsinized and used for whole cell recordings.

2.2. Electrophysiology

Whole cell currents were recorded using an Axopatch 200B amplifier (Axon Instruments). Currents were filtered at 5 kHz (−3 dB, 4-pole Bessel), digitized using a Digidata 1440A Interface (Axon Instruments), and analyzed using a personal computer equipped with pClamp 10.2 software (Axon Instruments) and Origin software (Microcal origin v.8.0, USA). Patch pipettes were made from borosilicate glass and had resistances of 3–5 MΩ when filled with standard intracellular solutions. For whole cell experiments, we used an external bath medium (normal Tyrode solution) of the following composition (in mM): 135 NaCl, 5 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 glucose, and 10 *N*-[2-hydroxyethyl]piperazine-*N*′-[2-ethanesulfonic acid] (HEPES) with pH adjusted to 7.4 using NaOH. Cs^+-rich external solution was made by replacing NaCl and KCl

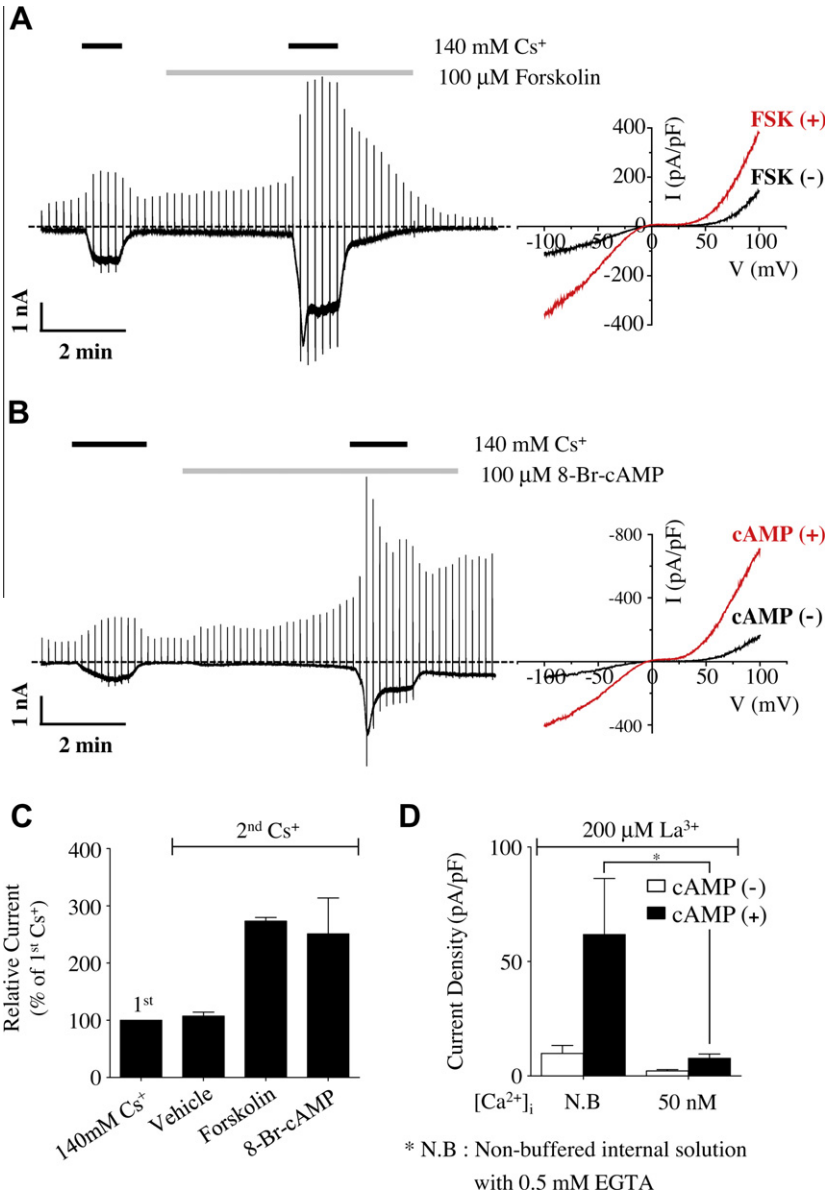

Fig. 2. The effect of forskolin and 8-Br-cAMP on TRPC5 current A. Left, a representative current trace of TRPC5 increased by forskolin (FSK). Right, The *I-V* curve of TRPC5 induced by FSK. The two colored lines indicate the *I–V* relationships at the respective intervals shown in the trace on the left. B. Left, a representative current trace of TRPC5 increased by a cell-permeable analogue of cAMP, 8-Br-cAMP. 8-Br-cAMP the TRPC5 current. Right, The *I–V* curve of TRPC5 activated by 8-Br-cAMP. The two colored lines indicate the *I–V* relationships at the respective intervals shown on the left. C. A summarized current amplitude of TRPC5 by FSK and 8-Br-cAMP at −60 mV. When FSK or 8-Br-cAMP was applied, the TRPC5 current increased relative to the no-treatment condition. D. A summarized current amplitude of TRPC5 induced by 8-Br-cAMP at −60 mV under low Ca^{2+} concentration $[Ca^{2+}]_i$ in the internal 140 mM $[Cs^+]_i$ pipette solution (50nM calculated free Ca^{2+}). Bath solution was Normal Tyrode solution containing 200 μM lanthanide ion (La^{3+}). The effect of 8-Br-cAMP was significantly attenuated when internal Ca^{2+} concentration was buffered to 50 nM. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

with equimolar CsCl. The standard pipette solution contained (in mM) 140 CsCl, 10 HEPES, 0.2 Tris-GTP, 0.5 EGTA, and 3 Mg-ATP with pH adjusted to 7.3 using CsOH. Intracellular 5 μM Ca^{2+} pipette solution was 140 CsCl, 10 HEPES, 0.2 Tris-GTP, 10 EGTA, 3 Mg-ATP, and 9.8 $CaCl_2$ with pH adjusted to 7.3 using CsOH (5 μM calculated free Ca^{2+}). Voltage ramp pulse was applied from +100 to −100 mV for 500 ms at −60 mV holding potential. The calculated junction potential between the pipette and bath solutions used for all cells during sealing was 5 mV (pipette negative) using pClamp 10.2 software. No junction potential correction was applied. Experiments were performed at room temperature (18–22 °C). Cells were continuously perfused at a rate of 0.5 ml/min.

2.3. Confocal imaging

Transfected cells were fixed by incubation with 4% formaldehyde in PBS for 15 min. After being washed in PBS, cells were incubated with 5 μg/ml Alexa Fluor 594 wheat germ agglutinin (WGA) and 1 μg/ml Hoechst 33342 dye (Invitrogen) for 10 min at room temperature. Subsequently, Confocal images were visualized on an Olympus FV-1000 confocal microscope with a 60× 1.35 NA oil lens. TRPC5 expression was detected based on excitation of signal with 488 nm wavelength light (from an argon laser). The cell membrane was visualized by WGA, which has an excitation wavelength of 594 nm light. Hoechst dye excited with 350 nm light emits blue

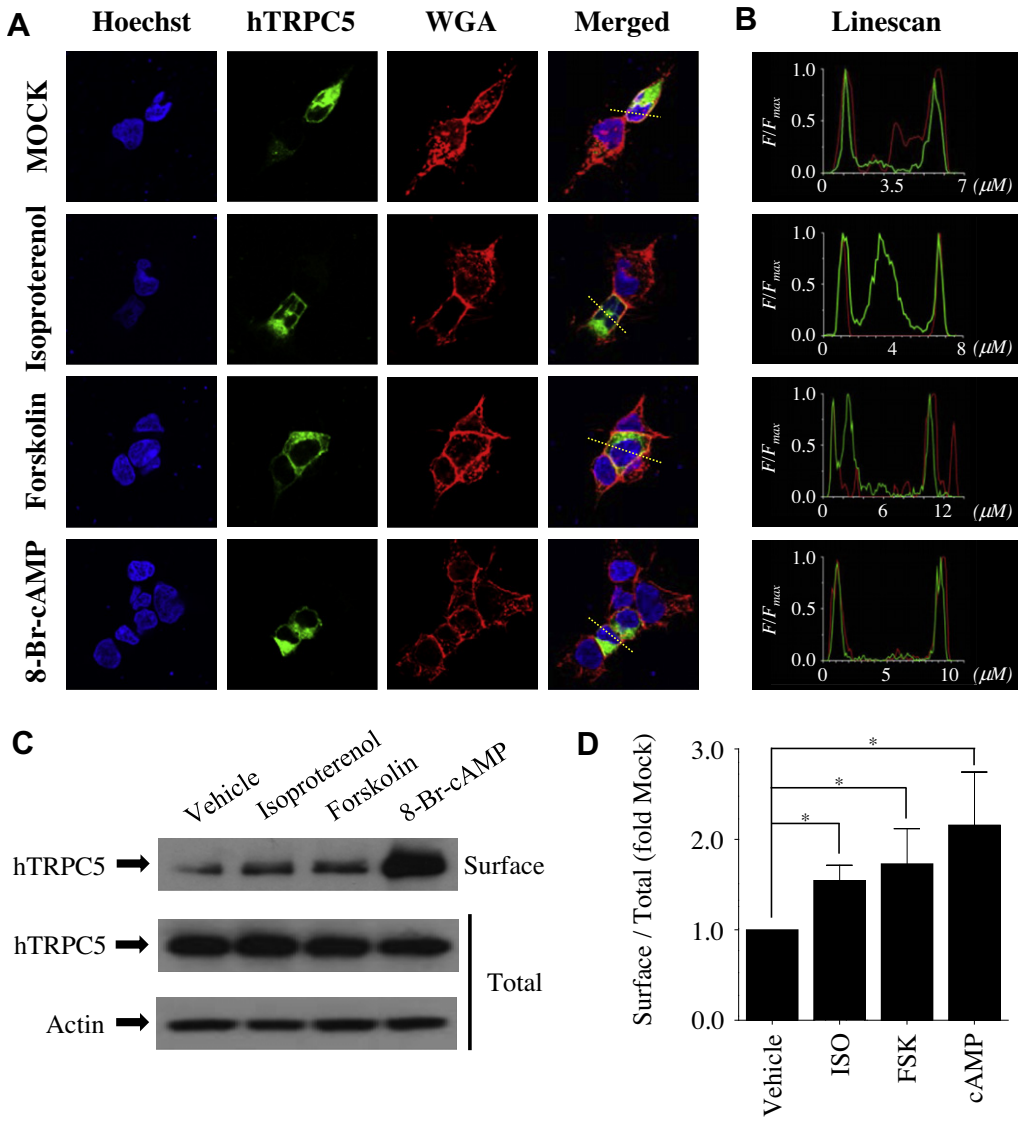

Fig. 3. The effect of $G\alpha_s$ signaling cascade on the plasma membrane expression of TRPC5 determined through treatment with agonists and effectors A. Left, the expression of hTRPC5-Enhanced Green Fluorescent Protein (EGFP, green) in HEK293 cells after pretreatment with ISO, FSK and 8-Br-cAMP, respectively. To assess the expression of hTRPC5, Alexa Fluor 594-conjugated wheat germ agglutinin (WGA) (red) and Hoechst (blue) were used to stain the plasma membranes and nuclei, respectively, in the same cells. B. The fluorescence intensity profiles were measured along lines drawn across the cell membrane near the nucleus, as shown in A. C. Representative hTRPC5-EGFP surface expression level changes caused by ISO, FSK and 8-Br-cAMP in HEK293 cells. The surface expression of hTRPC5-EGFP was determined by surface biotinylation. D. The summarized surface expression of TRPC5 was determined by densitometry. The surface expression was normalized relative to the total expression and then calculated as fold change relative to cells pretreated with vehicle solvent (MOCK). $^*p < 0.05$. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

fluorescence when bound to DNA. Colocalization analysis was performed using Metamorph software (Molecular Devices).

2.4. Surface biotinylation

Cells were washed in PBS and incubated in PBS with 100 μM isoproterenol, 100 μM forskolin, or 100 μM 8-Br-cAMP (Sigma Aldrich) for 4 min on ice. After washed in PBS, cells were incubated in 0.5 mg/ml sulfo-NHS-LC-biotin (Pierce) in PBS for 30 min on ice. Free biotin was quenched by the addition of 100 mM glycine in PBS. Lysates were prepared in lysis buffer by passing 7–10 times through a 26-gauge needle after sonication. Lysates were centrifuged at 13,300*g* for 10 min at 4 °C, and protein concentration in the supernatants was determined. Forty microliters of a 50% slurry of avidin beads (Pierce) were added to cell lysates (400 μg protein). After incubation for 1 h at RT, beads were washed three times with 0.5% Triton-X-100 in PBS, and proteins were extracted in sample buffer. Collected proteins were then analyzed by Western blot.

2.5. Statistics

Results are expressed as means ± SEM. Results were compared using Student's *t*-test between two groups, or using ANOVA followed by post hoc test among three groups or more. $p < 0.05$ was considered statistically significant. The number of cell recordings is given by *n*.

3. Results

3.1. β-adrenergic stimulation potentiates TRPC5 via intracellular Ca^{2+} release

It has been reported that TRPC5 expressed in HEK293 cells have a basal channel activity and measurements of TRPC5 activity can

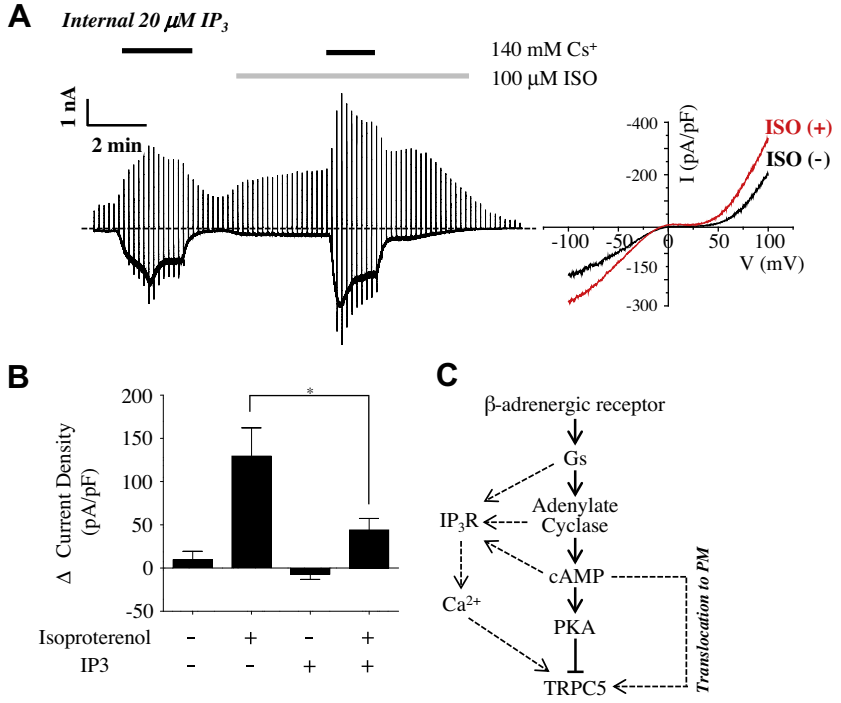

Fig. 4. The attenuated effect of ISO-induced TRPC5 current by IP_3 and GTPS A. Left, the representative current trace of ISO-induced TRPC5 following inositol 1,4,5-triphosphate (IP_3) infusion in the pipette solution. When 20 M IP_3 was included in the internal 140 mM $[Cs^+]_i$ solution, the increase in TRPC5 current induced by ISO was reduced. Right, the *I–V* curve TRPC5 induced by ISO. The two colored lines indicate respective conditions. B. Summarized current amplitude of ISO-induced TRPC5 in the presence of IP_3 at −60 mV. The ISO-induced TRPC5 activity was attenuated by the infusion of IP_3. $^*p < 0.05$ C. Schematic model depicting the positive feedback mechanism by which Ca^{2+} transients and increased membrane translocation due to $G\alpha_s$ signaling induced TRPC5 channel activity in HEK293 cells.

be manipulated by altering the extracellular ion composition [7,12,13]. In order to efficiently measure TRPC5 activity, we used symmetrical Cs^+-rich solution (140 mM Cs^+) both intracellularly and extracellularly. To investigate the effect of β-adrenergic stimulation on TRPC5, we designed an electrophysiological recording scheme that compares two consecutive TRPC5 currents. β-adrenergic receptor was stimulated by a well-known agonist isoproterenol (ISO, 100 μM). Treatment with bath solution containing 100 μM ISO increased the TRPC5 current to 129 ± 32 pA/pF ($n = 5$) (Fig. 1A and B). Likewise, 1 μM ISO increased the TRPC5 current to 71 ± 9 pA/pF ($n = 3$, Fig. 1C).

Lanthanides have been reported to stabilize the open state of the TRPC5 channels by directly interacting with glutamate residue near the pore region of the channels [13]. Because La^{3+} and Gd^{3+} are known to activate TRPC5 in the absence of any exogenous stimulators, we examined whether β-adrenergic stimulation was able to potentiate La^{3+}-activated TRPC5 current. As expected, La^{3+} increased TRPC5 current under basal conditions; application of ISO induced a further increase in the amplitude of TRPC5 (Supplementary Fig. S1A).

In addition to our electrophysiologic approach, we measured intracellular Ca^{2+} level *via* a fluorescent $[Ca^{2+}]_i$ measurement technique using Fura-2 to determine whether β-adrenergic stimulation increases cytosolic $[Ca^{2+}]_i$. In HEK293 cells, ISO induced a robust and transient release of intracellular Ca^{2+}. Subsequent application of thapsigargin (TG), a sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) inhibitor, induced an even larger Ca^{2+} transient (Fig. 1D).

These results imply that β-adrenergic stimulation induces partial Ca^{2+} release but not full depletion, which in turn may potentiate TRPC5.

3.2. Downstream mechanisms mediating the β-adrenergic receptor stimulation of TRPC5

The β-adrenergic receptor is one of the well established G protein-coupled receptors. It couples with different types of heterotrimeric G proteins based on its subtype. HEK293 cells predominantly express the β_2-adrenregic receptor [14], which couples primarily with $G\alpha_s$. In order to determine whether cAMP is downstream molecule of $G\alpha_s$ to elicit TRPC5, the role of an adenylate cyclase (AC)-specific activator, forskolin (FSK, 100 μM), or a cell permeable analogue of cAMP, 8-Br-cAMP (100 μM), was tested. As shown in Fig. 2A–C, FSK and 8-Br-cAMP increased the TRPC5 current to 273 ± 6% ($n = 5$) and to 251 ± 63% ($n = 7$), respectively. Similar to ISO, 8-Br-cAMP further increased the TRPC5 current after the stimulation by La^{3+} (Fig. S2A–C) or by Gd^{3+} (Fig. S2D–F).

These results clearly showed that β-adrenergic stimulation and $G\alpha_s$–cAMP signaling pathway elicited similar increases in TRPC5 channel activity. However, it remains unclear whether signaling pathway downstream of $G\alpha_s$ activates intracellular Ca^{2+} to exert their effects on TRPC5. In order to answer this question, we investigated whether reducing intracellular Ca^{2+} alters the effects of 8-Br-cAMP on TRPC5. When intracellular Ca^{2+} was buffered to 50 nM, the effect of 8-Br-cAMP was significantly attenuated (Fig. 2D).

These results suggest that the activation of β-adrenergic receptor or $G\alpha_s$ pathway potentiates TRPC5 activity in an intracellular Ca^{2+}-dependent pathway.

3.3. $G\alpha_s$ downstream cascade increases the translocation of TRPC5 to the plasma membrane

It is important to establish whether increase in the activity of TRPC5 is associated with its trafficking to plasma membrane. Our previous report showed that TRPC5 [15] expression in the plasma membrane was significantly increased when co-expressed with a constitutive active form of $G\alpha_s$, $G\alpha_s^{Q227L}$. In this chronic system, prolonged activation of $G\alpha_s$ signaling increased the overall expression of the TRPC5 channel.

In the present study, we detected the membrane expression of TRPC5 following acute (i.e., short term) stimulation of $G\alpha_s$

downstream signaling in a confocal microscope. After transient transfection of human TRPC5-EGFP in HEK293 cells, we incubated the cells with ISO, FSK or 8-Br-cAMP for 4 min prior to fixation. Plasma membrane and the nucleus were stained for efficient visualization. In all conditions, TRPC5 was distributed at the cell periphery and in the cytoplasm (Fig. 3A). The fluorescence intensity profiles measured along a line drawn across the cell membrane near the nucleus also confirmed that the areas of highest TRPC5 intensity were at the cell periphery (Fig. 3B). For quantitative analysis, we assayed the surface expression of TRPC5 by performing surface biotinylation. ISO treatment increased the translocation of TRPC5 to the plasma membrane by 155 ± 17% ($n = 3$) compared to the control. Similarly, FSK and 8-Br-cAMP promoted TRPC5 translocation to plasma membrane by 172 ± 39% ($n = 3$) and 216 ± 59% ($n = 3$), respectively (Fig. 3C and D).

These results imply that β-adrenergic stimulation and $G\alpha_s$ signaling not only elicit intracellular Ca^{2+} transients but also increase the translocation of TRPC5 to plasma membrane. The increase in the proportion of membrane-associated channels may contribute to the increase in the whole-cell TRPC5 current.

3.4. IP_3 attenuates $G\alpha_s$ cascade stimulation of TRPC5; $G\alpha_s$ cascade-IP_3R-mediated Ca^{2+} release potentiates TRPC5

The results above clearly demonstrate that the activation of the $G\alpha_s$ cascade caused by β-adrenergic stimulation induces Ca^{2+} transients, which in turn, potentiates TRPC5. Although there are several lines of evidence regarding possible roles for the $G\alpha_s$ cascade in regulating Ca^{2+} transients [10,11,16,17], we were still unable to identify a single molecular candidate that actually mediates the cascade and potentiation of TRPC5 through Ca^{2+} release. Therefore, in order to investigate whether IP_3R-mediated Ca^{2+} release following the stimulation of $G\alpha_s$ signaling potentiates TRPC5, we infused 20 μM of IP_3 into the pipette solution.

In the presence of IP_3, the basal TRPC5 activity was enhanced (Figs. 1A and 4A) but the potentiation of TRPC5 by ISO was greatly attenuated. As shown in Fig. 4A and B, ISO increased TRPC5 current by 129 ± 32 pA/pF without IP_3 ($n = 5$). However, ISO-increased current was only 44 ± 13 pA/pF ($n = 11$) when IP_3 was included (Fig. 4A and B).

4. Discussion

TRPC5 is a polymodal channel that can be stimulated by many molecules, including extracellular lanthanides (e.g., Gd^{3+} and La^{3+}) and extracellular or intracellular lysophosphatidylcholine (LPC) [3,5,13]. Recently, it was suggested that internal Ca^{2+} is an important second messenger that is involved in facilitating TRPC5 channel activity [8,9]. It was also reported that increase in cAMP levels induce calcium transients in HEK293 cells [16]. Moreover, intracellular Ca^{2+} level plays a part in the sensitization of IP_3R by PKA at low IP_3 concentrations [17]. In a sense, the assumption that local Ca^{2+} release is regulated by PKA relies on two experimental observations. First, some G protein-coupled signaling pathways activate PLC and regulate adenylate cyclase at the same time, leading to IP_3 production and altering PKA activity via changes in the cAMP level. Second, phosphorylation by PKA alters the properties of the IP_3 receptor (IP_3R) itself.

Previously, we reported that $G\alpha_s$ negatively regulated GTPγS-activated TRPC5 via direct phosphorylation by PKA [15]. GTPγS-activated TRPC5 current was inhibited by treatment of ISO, FSK and 8-Br-cAMP. Co-expression of constitutively active form of $G\alpha_s$, $G\alpha_s^{Q227L}$, also inhibited GTPγS-activated TRPC5 current. We identified that cAMP-PKA pathway inhibited TRPC5 current *via* phosphorylation of Ser794 and Ser796 site at C-terminus of TRPC5.

GTPγS is one of the best understood TRPC4/5 activators [18]. When GTPγS was infused in a pipette solution, as we performed in the present study (Fig. S3A) or reported previously, the TRPC5 channel was significantly potentiated prior to ISO application (Fig. 1A and Fig. S3A). Therefore, under the GTPγS activation system, we would have dominantly observed negative regulation of TRPC5 by PKA. In other words, we would not have been able to observe the potentiating effect of $G\alpha_s$ cascade-IP_3R signaling. Although it was easier to observe the negative effect of signaling pathway downstream of $G\alpha_s$ – PKA phosphorylation – on GTPγS activation system, we should not ignore the positive effect – $G\alpha_s$ cascade-IP_3R signaling. The latter pathway may have remained operative throughout the experiment despite our inability to observe its activity.

In this study, we also infused a solution containing both IP_3 and GTPγS into the pipette. Because IP_3 binds to the IP_3 receptor, ER Ca^{2+} was expected to be reduced, thereby reducing further Ca^{2+} mobilization, on which $G\alpha_s$ signaling utilizes. Under these conditions, a decrease in GTPγS-activated TRPC5 current in response to ISO was again observed, but the inhibition was considerably larger (Fig. S3B and D). No run-down was observed in the TRPC5 currents activated by IP_3 and GTPγS (Fig. S3C). We alleviated the effect of the β-adrenergic stimulation-dependent Ca^{2+} response by using a pipette solution representing a high free Ca^{2+} state. As expected, ISO inhibited GTPγS-activated TRPC5 when intracellular Ca^{2+} was buffered to 5 μM (Fig. S4A).

HEK293 cells express endogenous β_2-adrenergic receptors [14]. As discussed above, isoproterenol elicited both positive and negative effects on TRPC5 depending on the constituents of the internal solution, even when only endogenous levels of β_2-adrenergic receptors were present. When the β_2-adrenergic receptor was co-expressed with TRPC5 in HEK293 cells, it facilitated the effect of isoproterenol on TRPC5 channel (Fig. S1B). However, denopamine (10 μM), a β_1-adrenergic receptor agonist, had no effect on TRPC5 current in the presence of Cs^+ (Fig. S1C). These results suggest that the β_2-adrenergic receptor is involved in the action of isoproterenol on TRPC5 channels.

In addition to our results regarding TRPC5, stimulating effects of cAMP on TRPC3 and TRPC6 have been reported [13,19]. This effect is attributable to cAMP-dependent plasma membrane translocation or exocytosis [20,21]. The TRPC3 channel is translocated to the apical membrane by stimulated vasopressin receptor 2 via the AC-cAMP-PKA pathway [20]. Further evidence in the literature suggests that TRPC4 and TRPC5 can be inserted into the surface membrane through a process that requires PI3K [22,23]. Both the AC-cAMP-PKA pathway and cAMP-PI3K seem to be involved in the exocytosis of TRPC5.

In conclusion, the results of this study clearly support bidirectional roles for β-adrenergic stimulation on TRPC5 channels; i.e., it exerts both positive and negative effects on TRPC5 (Fig. 4C). β-adrenergic stimulation followed by the $G\alpha_s$ cascade induces a Ca^{2+} release mediated by IP_3R, and this Ca^{2+} release potentiates TRPC5 channels. The cascade also promotes the plasma membrane translocation of TRPC5 channels. In addition, the furthest downstream effector molecule, PKA, phosphorylates and inhibits the TRPC5 channel, as we reported previously [15].

Acknowledgments

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.03.123.

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