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Inhibition of the Desensitization of Canonical Transient Receptor Potential Channel 5 by Dimethyl Sulfoxide

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The classic type of transient receptor potential channel (TRPC) is a molecular candidate for Ca^{2+}-permeable cation channel in mammalian cells. TRPC5 is rapidly desensitized after activation by G protein-coupled receptor. Herein we report the effect of dimethyl sulfoxide (DMSO) on the desensitization of TRPC5. TRPC5 was initially activated by muscarinic stimulation with 50 μM carbachol (CCh) and then decayed rapidly even in the presence of CCh (desensitization). DMSO in the pipette solution slowed the rate of this desensitization. Under the control conditions, TRPC5 current spontaneously declined to 6±1% of the initial peak amplitude 60 sec after CCh application and to 1±0.5% after 120 sec. But, in the presence of 0.01%, 0.1% and 1% DMSO, TRPC5 current spontaneously declined to 55±2%, 68±1% and 100±0.2% of the initial peak amplitude 60 sec after CCh application and to 38±2%, 61±1% and 100±1% after 120 sec, respectively. The results suggest that DMSO can internally attenuate the desensitization of TRPC5 current through unknown mechanisms that remain to be elucidated.

Key Words: Transient receptor potential channel, Ca^{2+}-permeable cation channel, Dimethyl sulfoxide

INTRODUCTION

The classic type of transient receptor potential channel (TRPC) is a molecular candidate for Ca^{2+}-permeable cation channel in mammalian cells. It consists of seven types of TRPC. TRPC3, TRPC6, and TRPC7 belong to one group and are activated by diacylglycerol (DAG), whereas TRPC1, TRPC4, and TRPC5 belong to another and are not activated by DAG. Moreover, there are two types of Ca^{2+}-permeable cation channels. Store-operated channels (SOC) are activated by store depletion, whereas receptor-operated channels (ROC) are activated by the stimulation of G protein-coupled receptors (GPCRs).

TRPC5 was initially suggested as a SOC (Philipp et al, 1998). However, after the initial report, TRPC5 was shown to be activated by GPCR stimulation and has been suggested to be an ROC (Okada et al, 1998; Kanki et al, 2001; Lee et al, 2003; Ohta et al, 2004). TRPC4 and TRPC5 are rapidly desensitized after activation by GPCR, and this desensitization does not depend on extracellular monovalent cations such as Na^+ or Cs^+. Under both monovalent cation conditions, TRPC4 and TRPC5 were found to be desensitized after the activation of muscarinic receptors. Even when intracellular GTPγS was used to activate TRPC4 and TRPC5, TRPC4 and TRPC5 currents were rapidly desensitized.

In the case of TRPCs, protein kinase C (PKC) was found to be involved in the desensitization process, whereas DAG activated TRPC3, TRPC6, and TRPC7 but not TRPC1, TRPC4, and TRPC5 (Venkatachalam et al, 2003). When 1-oleoyl-2-acetyl-*sn*-glycerol, a membrane-permeable analog of DAG, was applied before GPCR stimulation by acetylcholine, it inhibited TRPC5 activation (Venkatachalam et al, 2003). Measurements of intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) showed that PKC inhibited all TRPC channels, whereas DAG activated TRPC3, TRPC6, and TRPC7.

We have been studying the desensitization mechanism of TRPC5 using whole cell patch-clamp techniques. In our previous studies (Lee et al, 2003; Zhu et al, 2003), when we increased the extracellular Ca^{2+} concentration ($[Ca^{2+}]_o$) from 0 to 10 mM, it caused initial facilitation and a subsequent faster desensitization; when intracellular GTPγS was used to activate TRPC4 and TRPC5, a similar phenomenon was observed. These results suggest that Ca^{2+}-dependent processes are involved in the desensitization of TRPC5. Also, PKC phosphorylation was involved in the desensitization of TRPC5, and threonine at residue 972 of mouse TRPC5 might be phosphorylated by PKC (Zhu et al, 2005).

DMSO is an ampiphatic compound that possesses both a hydrophilic sulfoxide moiety and two hydrophobic methyl groups. As a result, DMSO is frequently used as a solvent for drugs and reagents in biological studies and clinical practice. However, DMSO is not inert and many effects on biological systems have been suspected. For example, DMSO has been identified as a cell differentiation inducer (Morley and Whitfield, 1993), an ion channel expression inducer (Jiang et al, 2004; Smith-Maxwell et al, 2004) as well as a neuroprotective agent (Karaca et al, 2001; Lu &

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ABBREVIATIONS: DAG, diacylglycerol; DMSO, dimethyl sulfoxide; GPCR, G protein-coupled receptor; ROC, receptor-operated channel; TRPC, classic type of transient receptor potential.

Mattson, 2001; Ali & Mousa, 2002; Farkas et al, 2004). DMSO was also reported to block Na^+, K^+ and Ca^{2+} currents in neuroblastomaglioma hybrid cells (Jourdon et al, 1986), block Na^+ current in frog muscle cells (Larsen et al, 1996), and water channels (Gradilone et al, 2003; Marinelli et al, 2003; Laforenza et al, 2005) and induce shifts of the voltage-dependence of hERG channel gating (Du et al, 2006). Since DMSO has broad pharmacological actions (Santos et al, 2003), possible interference of drug effects under investigation is usually considered when DMSO is applied as a solvent.

We have undertaken this work to investigate the effect of DMSO on the desensitization of these TRPC5 currents internally.

METHODS

Cell culture and transient transfection

Human embryonic kidney (HEK-293) cells (ATCC, Manassas, VA) were maintained according to the supplier's recommendations. For transient transfection, cells were seeded in 12-well plates. The following day, 1.5~2 μg/well of pcDNA vector containing the cDNA for TRPC5 was transfected into the cells using the transfection reagent FuGENE 6 (Roche Molecular Biochemicals) according to the manufacturer's protocol. After 30~40 h, the cells were trypsinized and used for whole cell recording.

Whole cell patch-clamp experiment

Isolated cells were transferred to a small chamber on the stage of an inverted microscope (model TE2000, Nikon) and were constantly perfused with normal Tyrode solution at a rate of 2~3 ml/min. A glass microelectrode with a resistance of 2~5 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 1-D patch-clamp amplifier (Axon Instruments). For data acquisition and the application of command pulses, pCLAMP software version 6.0 (Axon Instruments) was used. Data were filtered at 5 kHz and displayed on a digital oscilloscope (PM 3350, Phillips), a pen recorder (model 220, Gould), and a computer monitor. Data were analyzed with the use of pCLAMP and Microcal Origin software, version 6.0.

Solutions

Normal Tyrode contained (in mM) 135 NaCl, 5 KCl, 1.8 $CaCl_2$, 1 $MgCl_2$, 5 glucose, and 10 HEPES, and pH was adjusted to 7.4 with the use of NaOH. Cs^+-rich external solution was made by replacing NaCl and KCl with equimolar CsCl. The pipette solution contained (in mM) 140 CsCl, 10 HEPES, 0.5 Tris-GTP, 0.5 EGTA, and 3 Mg-ATP, and its pH was adjusted to 7.3 with CsOH.

Statistics

All data are expressed as means±SE. Statistical significance was determined using the Student's paired or unpaired *t*-tests. P values of less than 0.05 were considered statistically significant, and *n* refers to the number of cell recordings.

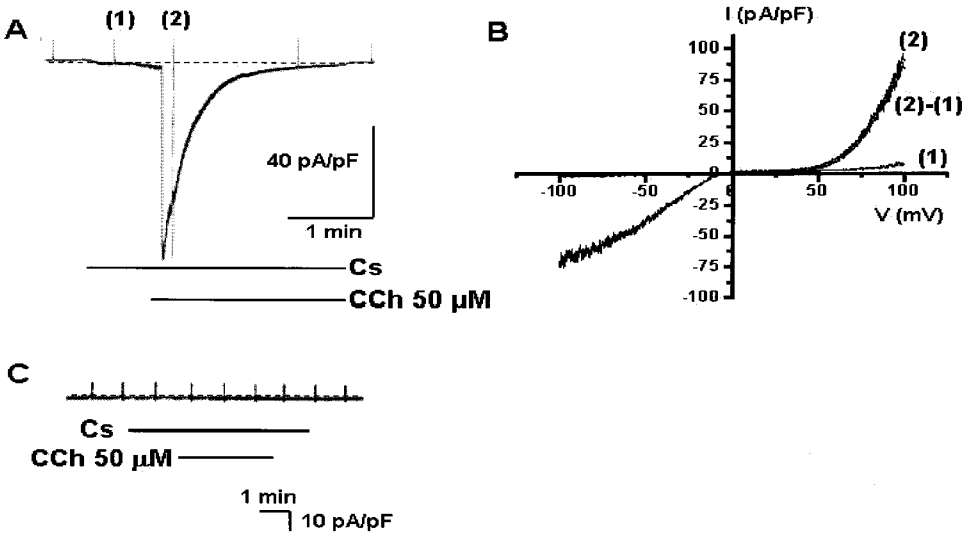

Fig. 1. Carbachol (CCh)-activated inward current and its current-voltage (*I-V*) relationship recorded from human transient receptor potential channel (hTRPC5)-expressing human embryonic kidney (HEK) cells using the whole cell patch-clamp technique. (A) Whole cell currents were recorded under the condition of 140 mM intracellular $[Cs^+]$ ($[Cs^+]_i$). CCh (50 μM) induced an inward current, which decayed spontaneously during CCh treatment. Slow ramp depolarizations from −100 to +100 mV were applied from a holding potential of −60 mV before (1) and during (2) 50 μM CCh treatment. (B) *I-V* relationships were obtained by subtracting (1) from (2). *I-V* relationships showed a typical doubly rectifying shape. (C) Whole cell currents were recorded in mock transfected cells.

RESULTS

Effect of Carbahol on TRPC5 expressed in HEK cells

Whole cell currents were recorded using patch-clamp techniques. In the beginning, whole cell currents were recorded under the condition of normal Tyrode solution (140 mM $[Na^+]_o$) and $[Cs^+]_i$. To determine the current-voltage (*I-V*) relationship, we applied a ramp pulse from −100 mV to +100 mV for 1 s. After the external solution was changed from normal Tyrode to 140 mM $[Cs^+]_o$ solution, basal currents increased slightly due to a constitutive activity of TRPC5. Sometimes, the currents activated up to 1 nA without any stimulation of TRPC5 with CCh. Thus, we usually waited for at least 2 min before the application of CCh. Whole cell current was also recorded under the condition of 140 mM $[Cs^+]_o$ and $[Cs^+]_i$ as a control current for the subtraction to get the *I-V* relationship of hTRPC5 activated by CCh. When 50 μM CCh was applied at a holding potential of −60 mV, an inward current was activated (Fig. 1A). The *I-V* relationship (Fig. 1B, 2-1), obtained by subtracting current in the absence of CCh (Fig. 1A, 1) from that in the presence of CCh (Fig. 1A, 2), showed a typical doubly rectifying shape (Fig. 1B). We used only results obtained from cells showing the typical *I-V* relationship of TRPC5. hTRPC5 currents were not activated by CCh in mock transfected cells (Fig. 1C).

Effects of intracellular DMSO on hTRPC5 desensitization

The TRPC5 current activated by stimulation of muscarinic receptors decayed spontaneously to the basal level even during the first application (Fig. 1A). This phenomenon is called desensitization. The degree of desensitization was variable among cells. When we increased the concentration of DMSO in the pipette solution, the rate of desensitization slowed (Fig. 2). We defined the desensitization score as the ratio of the current at 100 s to peak current. Desensitization scores were increased from 0.03±0.01 (n=7) under the control condition to 0.5±0.05 (n=5) at internal 0.01% DMSO, 0.7±0.05 (n=4) at internal 0.1% DMSO and 1±0.02 (n=3) at internal 1% DMSO (Fig. 3A), suggesting that the desensitization process depends on intracellular DMSO concentration. Under the control conditions, TRPC5 current spontaneously declined to 6±1% of the initial peak amplitude 60 sec after CCh application and to 1±0.5% after 120 sec (n=7). In the case of internal 0.01%, 0.1% and 1% DMSO, TRPC5 current spontaneously declined to 55±2%, 68±1% and 100±0.2% of the initial peak amplitude 60 sec after CCh application and to 38±2%, 61±1% and 100±1% after 120 sec, respectively (Fig. 3B; n=7). However, when

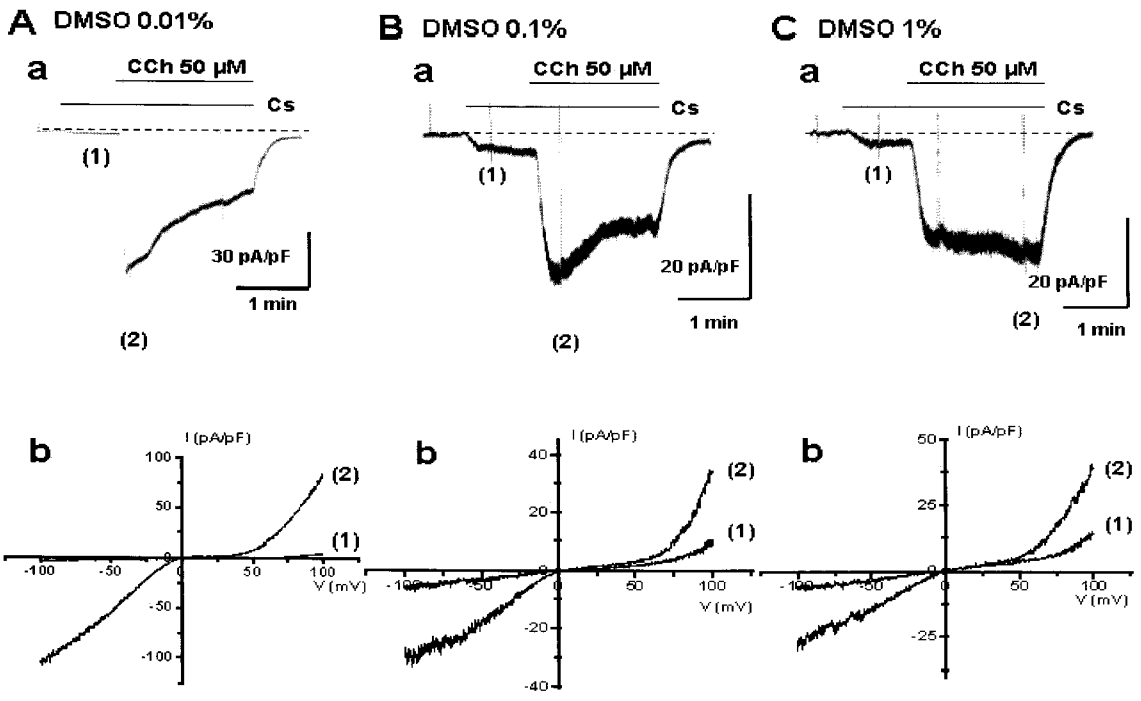

Fig. 2. Effect of intracellular DMSO concentration ($[DMSO]_i$) on human transient receptor potential channel 5 (hTRPC5) desensitization. Whole cell currents were recorded under conditions of 0.01% $[DMSO]_i$ (A), 0.1% $[DMSO]_i$ (B), or 1% $[DMSO]_i$ (C) using the patch-clamp technique. When 50 μM carbachol (CCh) was applied at a holding potential of −60 mV, an inward current was activated in TRPC5-expressing human embryonic kidney (HEK)-293 cells (a). The degree of TRPC5 desensitization depended on $[DMSO]_i$. Dotted lines indicate zero currents. (1) and (2) indicate ramp pulses before and during the application of CCh, respectively. To obtain current-voltage (*I-V*) relationships, we applied a ramp pulse from −100 to +100 mV for 1 s. The *I-V* relationship of TRPC5 had a doubly rectifying shape (b).

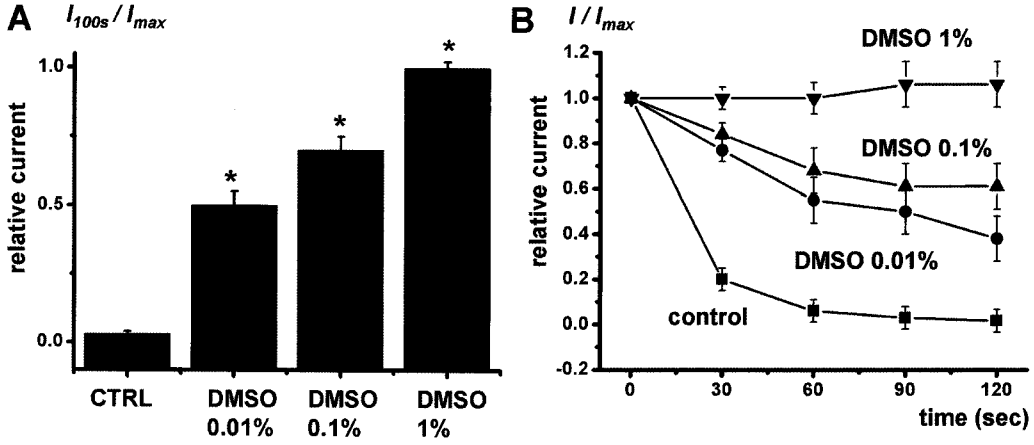

Fig. 3. (A) Degree of desensitization. Desensitization scores (I_{100s}/I_{max}) were found to depend on $[DMSO]_i$. *$p < 0.05$. (B) hTRPC5 currents were normalized against the peak amplitude at 1 sec and average values are plotted against time (■, control; ●, 0.01% DMSO; ▲, 0.1% DMSO; ▼, 1% DMSO).

we applied DMSO externally, there were no changes in currents (data not shown). We are in a process to investigate what makes the differences.

DISCUSSION

The present data showed that DMSO attenuated the desensitization of TRPC5 current internally. Although the mechanisms for the action of DMSO internally on TRPC5 channel are not clear, it might be suggested that the primary mechanism might be PKC phosphorylation (Zhu et al, 2005).

The desensitization of TRPC5 by PKC was found to be dependent on extracellular and intracellular Ca^{2+}, intracellular Mg-ATP or EGTA, and PKC inhibitors. Threonine at residue 972 of mouse TRPC5 might be the site of phosphorylation by PKC (Zhu et al, 2005).

The effect of DMSO on endogenous TRP channel in HEK cells has not been reported. Therefore, the results of this paper were entirely due to the effect of DMSO on heterogeneously expressed hTRPC5 on HEK cells.

In gastric guinea pig myocytes, a nonselective cation channel is activated by muscarinic stimulation. Desensitization of this channel has been found to depend on PKC activity (Kim et al, 1997), and $[Ca^{2+}]_i$, and attenuated by PKC inhibitors. Conventional PKC is involved in the desensitization process. When $[Ca^{2+}]_i$ was increased to 200 nM, PKC-α was activated and induced desensitization. However, at less than 200 nM $[Ca^{2+}]_i$, PKC-α was not activated, therefore the current was not desensitized. We also showed that TRPC5 is a molecular candidate for the nonselective cation channel that is activated by muscarinic stimulation in murine gastric myocytes (Lee et al, 2003; Zhu et al, 2003). The desensitization of TRPC5 might correspond to the desensitization of the nonselective cation channels activated by muscarinic stimulation in native tissues, such as in the gastric myocytes of guinea pigs and murine gastric myocytes.

In the gastric myocytes of guinea pigs and murine gastric myocytes, we did not find the involvement of DMSO in desensitization. More detailed studies of the involvement and the mechanism of DMSO in native tissue are also needed.

In conclusion, DMSO can attenuate the desensitization of TRPC5 current in internally.

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