An environmental sensor, TRPV4, is a novel regulator of intracellular Ca\textsuperscript{2+} in human synoviocytes

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Submitted 8 May 2009; accepted in final form 11 September 2009

SYNOVIOCYTES IN THE JOINT SYNOVIAL TISSUE PLAY OBBLIGATORY ROLES IN BOTH JOINT MAINTENANCE AND INTEGRITY AND IN THE INFLAMMATORY RESPONSE OF ARTHRITIS (6, 17). THESE SYNOVIOCYTES EXPRESS MANY BIOLOGICAL SIGNALS INCLUDING RECEPTORS AND ION CHAINS (1, 2, 9, 10). IN THE CHRONIC INFLAMMATION AND PAIN ASSOCIATED WITH RHUMATOID ARTHRITIS AND INJURY, SOME OF THESE SIGNALS ARE ACTIVATED, IMPLYING THAT THEY ARE POTENTIAL TARGETS FOR TREATMENT.

Recent extensive studies have revealed that TRPVs are sensors for a diverse range of stimuli, such as heat, protons, lipids, and/or changes in membrane stretching and extracellular osmolarity (11, 16). In particular, TRPV4 is a sensor of osmotic pressure changes because the channel senses changes in cell volume (12, 20). TRPV4 is also modulated by heat stimulation (24).

In the present study, we examined the effects of 4\textit{o}-PDD on intracellular Ca\textsuperscript{2+} in three types of synoviocytes [human fibroblast-like synoviocytes line MH7A and synoviocytes in patients with (RA) and without (CTR) rheumatoid arthritis]. The application of 4\textit{o}-PDD induced a substantial elevation of Ca\textsuperscript{2+} in the RA and CTR cells but not in the MH7A cells, which was sensitive to the removal of external Ca\textsuperscript{2+} and ruthenium red (RuR). Consistently, the human TRPV4 (hTRPV4) mRNA transcript and the protein were clearly detected in RA and CTR cells, and those in MH7A cells were rather lower. Moreover, the 4\textit{o}-PDD-induced Ca\textsuperscript{2+} response of MH7A cells transfected with hTRPV4 (MH7A-V4) was comparable to those of RA and CTR cells. Hypotonic stimulation also elicited an elevation of Ca\textsuperscript{2+} in the RA cells as well as in the MH7A-V4 cells, but not in the MH7A cells. Activation of TRPV4 in RA cells reduced the production of chemokine, IL-8.

MATERIALS AND METHODS

**Cell culture.** Human fibroblast-like synoviocytes line MH7A was cultured as previously described (8, 13). Human synovial cells derived from patients with (RA) and without (CTR) rheumatoid arthritis were purchased from Cell Applications (San Diego, CA) and cultured in synoviocyte growth medium that contained 10% growth supplement, 100 U/ml of penicillin G, and 100 \(\mu\)g/ml of streptomycin. The cultured cells were maintained at 37°C in a 5% CO2 atmosphere. CTR and RA cells were not used after the sixth passage.

**Recombinant expression of human TRPV4 in MH7A.** Partially confluent MH7A cells (40–60%) were transfected with a pcDNA3.1/hyroro(+) hTRPV4 plasmid DNA, using lipofectamine 2000 (Invitrogen). The expression level of hTRPV4 was substantial; 40–60% cells were transfected 24–48 h after the procedure. All experiments were performed within 96 h of transfection.

**RT-PCR amplification.** RT-PCR amplification for hTRPV4 expression was performed as described previously (14). Total RNAs were

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extracted from CTR, RA, and MH7A cells by the acid guanidium thiocyanate-phenol method following digestion with RNase-free DNase, and RT was performed according to the manufacturer’s instructions. The oligonucleotide sequences of primers specific for hTRPV4 (sense and antisense: 5’ to 3’) were AACTGACCA-GAACCTGCAACCCG and ATGACGCTAGCGCCAGGCTG (10). The thermal cycler program used for PCR amplification included a 0.5-min denaturation step at 94°C, a 0.5-min annealing step at 55°C, and a 0.5-min primer extension step at 72°C for 35 cycles and was carried out using an ABI 2720 thermal cycler (Applied Biosystems, Foster City, CA). The amplified products were separated on 1.5% agarose gels in Tris-acetate-EDTA buffer, visualized with 1 μg/ml ethidium bromide, and assessed on FAS III (TOYOBO, Tokyo, Japan). As a control signal, GAPDH expression was analyzed (sense 5’ to 3’: TGAAGGTGAGGCAGGTAGTTGT; antisense 5’ to 3’: CATGTGGGCACTAGGTCCAC).

Quantitative PCR. Real-time quantitative PCR was performed with the use of SYBR Green Chemistry on a Thermal Cycler Dice Real Time System (Takara Bio, Tokyo, Japan). Standard curves were generated for the constitutively expressed GAPDH from regression analysis of the mean values of RT-PCRs for the log10 diluted cDNA. Unknown quantities relative to the standard curve for a particular set of primers were calculated, yielding transcriptional quantification of gene products relative to the endogenous standard (β-actin). Each cDNA sample was tested in triplicate. Oligonucleotide sequences of primers specific for human TRPV4 (sense and antisense, 5’ to 3’) were TCATGATCGGCTACGCTTCA and CCTCATTGCACACCT-H1003 and extension step at 60°C (for 40 cycles), and a dissociation step (15 s) was generated using known concentrations (pg/ml) of IL-8.

Fig. 1. Expression of human (h) vanilloid type 4 transient receptor potential channel (TRPV4) mRNA in cells from patients with (RA) and without (CTR) rheumatoid arthritis and in MH7A cells. A: hTRPV4 mRNA transcript was clearly detected in RA and CTR cells using PCR-amplification. Fullerton, CA). The results were compared with a standard curve that was generated using known concentrations (pg/ml) of IL-8. Unknown quantities relative to the standard curve for a particular set of primers were calculated, yielding transcriptional quantification of gene products relative to the endogenous standard (β-actin). Each cDNA sample was tested in triplicate. Oligonucleotide sequences of primers specific for human TRPV4 (sense and antisense, 5’ to 3’) were TCATGATCGGCTACGCTTCA and CCTCATTGCACACCT-H1003 and extension step at 60°C (for 40 cycles), and a dissociation step (15 s) was generated using known concentrations (pg/ml) of IL-8.

Measurement of Ca2+ fluorescence ratio. Synoviocytes were loaded with 5 μM fura-2-acetoxyethyl ester (fura-2 AM) in standard HEPES solution for 30 min at room temperature. Fura-2 fluorescence signals were measured using the Argus/HisCa imaging system (Hamamatsu Photonics, Hamamatsu) driven by ImageWork Bench v6.0 (Indec). The frequency of image acquisition was selected as 0.2 Hz. The ratios of fluorescence intensity were transformed into intracellular Ca2+ concentration (Ca2+2) using the following equation: Ca2+2 = 224 × B × [(R – Rmin)/(Rmax – R)], where R is the ratio of 340/380 nm; Rmin and Rmax are fluorescence intensity determined by addition of 1 mM EGTA and 2 mM Ca2++, respectively, after the permeabilization of cells with 10 μM monomycin; and B is the ratio of the fluorescence proportionality coefficients obtained at 380 nm under Rmin and Rmax conditions.

Electrophysiology. The resistance of microelectrodes filled with pipette solution was in the range of 3–5 MΩ. Membrane currents and voltage signals were digitized onto a computer using an analog-digital converter (PCI6034E, National Instruments Japan, Tokyo, Japan). Data acquisition and analysis of whole-cell currents were carried out using WinWCP3.7, developed by Dr. J. Dempster (University of Strathclyde, Glasgow, UK). Cell capacitance was measured with capacitance cancellation circuitry in a voltage-clamp amplifier (SEN2400, Nihon Kohden, Tokyo, Japan). The pipette solution for amphotericin B-perforated whole cell recording contained (in mM) 110 Cs-aspartate, 30 CsCl, 2 MgCl2, and 10 HEPES (pH 7.2 with CsOH). All experiments were performed at 25 ± 1°C. Liquid junction potential between pipette and bath solutions (~10 mV) was corrected when aspartate-rich pipette solution was used.

Solutions. HEPES-buffered solution of following composition was used (in mM): 137 NaCl, 5.9 KCl, 2.2 CaCl2, 1.2 MgCl2, 14 glucose,
and 10 HEPES (pH 7.4 with NaOH). When the cells were superfused with Ca²⁺/H¹¹⁰⁰¹⁻*-free (0Ca²⁺/H¹¹⁰⁰¹⁻*) bathing solution, Ca²⁺ was omitted from the standard HEPES solution. To induce cell swelling using hypotonic stimulation, the cells were first superfused with solution containing (in mM) 91.3 NaCl, 5.9 KCl, 2.2 CaCl₂, 1.2 MgCl₂, 14 glucose, 10 HEPES, and 91 mannitol, pH 7.4 (mannitol solution, 310 mosM), and then with the solution without mannitol [227 mosM (14)]. All experiments were performed at 25 ± 1°C.

**Chemicals.** The following drugs were used: 4αPDD (Sigma, St. Louis, MO), RuR (Wako, Tokyo, Japan), amphotericin B (Sigma), and sphingosine-1-phosphate (S1P, Sigma). RuR, 4αPDD and amphotericin B, and S1P were dissolved in distilled water, DMSO, and methanol, respectively. All drug concentrations are expressed as their final concentration in the solutions, and the pH of the solution was readjusted after the addition of drugs. A change in the solution was achieved within 30 s.

**Statistical analysis.** Data are expressed as means ± SE or SD. Statistical significance between two and among multiple groups was examined using paired and unpaired Student’s t-test and Tukey’s multiple-comparison test, respectively. P < 0.05 was considered statistically significant.

**RESULTS**

Expression of hTRPV4 in human synoviocytes at mRNA and protein level. First, we evaluated the expression of hTRPV4 mRNA transcripts in synoviocytes in patients with (RA) and
without (CTR) rheumatoid arthritis, and human fibroblast-like synoviocytes line MH7A (MH7A). The total RNA isolated from these cells was subjected to RT-PCR. Among these cells, hTRPV4 mRNA transcripts were clearly detected in RA and CTR cells (Fig. 1A, two separate experiments). The mRNA expression level of hTRPV4 in human synoviocytes was further examined based on real-time PCR (Fig. 1B). The expression of hTRPV4 mRNA transcript between RA and CTR cells was comparable, while that in MH7A cells was substantially lower. As shown in Fig. 2, immunocytochemical analysis revealed that the expression of TRPV4 protein was significant in RA (Fig. 2A) as well as CTR cells (Fig. 2B), whereas it was faint in MH7A cells (Fig. 2C). As a control, MH7A cells transfected with hTRPV4 (MH7A-V4) were positively detected by the anti-TRPV4 antibody (Fig. 2D). Each positive signal disappeared when cells were pretreated with an IP against TRPV4 antibody (Fig. 2, bottom).

Effects of 4αPDD on Ca^{2+}i in human synoviocytes. To examine whether human synoviocytes are sensitive to 4αPDD, a potent TRPV4 agonist, we applied 0.3 μM 4αPDD to the RA, CTR, and MH7A cells while measuring Ca^{2+}i (Fig. 3). In more than 90% of RA (24 of 26 cells) and CTR cells (23 of 24 cells), 0.3 μM 4αPDD induced a significant elevation of Ca^{2+}i (change in Ca^{2+}i: 260.3 ± 36.4 nM in RA, n = 26, and 258.7 ± 37.9 nM in CTR, n = 24, Fig. 3D). In contrast, MH7A cells had no response to 0.3 μM 4αPDD (58 of 58 cells; Fig. 3, C and D), whereas they were affected by 0.3 μM S1P [change in Ca^{2+}i: 531.6 ± 54.2 nM, n = 58 (9, 26)].

To elucidate the mechanisms involved in the 4αPDD-induced elevation of Ca^{2+}i, we examined the effects of removing Ca^{2+} from the bathing solution on the 4αPDD-induced Ca^{2+} response in RA cells. As shown in Fig. 4A, application of 0.3 μM 4αPDD in the absence of external Ca^{2+} did not produce
any Ca\(^{2+}\) response, but it did induce a clear elevation of Ca\(^{2+}\); in the presence of 2.2 mM Ca\(^{2+}\) (Fig. 4, A and C). Moreover, in Fig. 4B, the effects of RuR, an effective blocker of TRPVs, were examined. Pretreatment of RA cells with 10 \(\mu\)M RuR effectively inhibited the Ca\(^{2+}\) response to 0.3 \(\mu\)M 4aPDD, and after withdrawal of RuR, reintroduction of 4aPDD caused the elevation of Ca\(^{2+}\); which was relatively smaller than that by the first application of 4aPDD (Fig. 4, B and C; see also Fig. 3). In 10–20% cells, 4aPDD-independent spontaneous elevation of Ca\(^{2+}\); was observed after removal of the first application of 4aPDD with RuR (Fig. 4B) or 0Ca\(^{2+}\) (not shown).

Comparison of Ca\(^{2+}\) response to 4aPDD among RA, CTR, MH7A-V4, and MH7A cells. In Fig. 5, the concentration and response relationships of the 4aPDD-induced Ca\(^{2+}\) responses in RA and CTR cells are summarized. As a control, 4aPDD was applied to MH7A cells transfected with hTRPV4 (MH7A-V4). Although 4aPDD-induced elevation of Ca\(^{2+}\) in RA and CTR cells was substantially smaller than that in MH7A-V4 cells (Fig. 5A), the sensitivity to 4aPDD was comparable among RA, CTR, and MH7A-V4 cells (Fig. 5B). In contrast, MH7A cells were rather resistant to 4aPDD and had a small increase in Ca\(^{2+}\) in the presence of 3 \(\mu\)M 4aPDD (38.9 ± 12.5 nM, \(n = 24\); Fig. 5A).

Activation of nonselective cation currents by 4aPDD in human synoviocytes. To confirm that nonselective cationic currents (NSCCs) are involved in 4aPDD-induced Ca\(^{2+}\) response, 3 \(\mu\)M 4aPDD was applied to RA and MH7A cells under voltage-clamp conditions with amphotericin B-perfused whole cell recording technique (Fig. 6). Cells were voltage-clamped at a holding potential of −40 mV, and ramp voltage-clamp waveforms for 400 ms from −120 to +40 mV were applied every 5 s. As shown in Fig. 6A, application of 3 \(\mu\)M 4aPDD caused inward currents in RA cells, while it did not affect the membrane currents in MH7A cells (Fig. 6, B and C; \(n = 10\)). The reversal potential of the inward currents in the current-voltage (I-V) relationship was −5.2 ± 2.1 mV (Fig. 6Ab; \(n = 6\)), indicating that NSCCs could be involved in the currents. In Fig. 6C, the amplitude of 4aPDD-induced currents at −40 mV, where contamination of the Cl\(^−\) current was negligible due to being close to the equilibrium potential of Cl\(^−\), was summarized in RA and MH7A cells. In RA cells, the current density was significantly increased in the presence of 4aPDD, demonstrating that 4aPDD effectively activates NSCCs in RA cells.

Hypotonic stimulation to RA, MH7A and MH7A-V4 cells. To demonstrate that TRPV4 in synoviocytes is also activated by hypotonic stimulation, we applied a hypotonic osmotic solution to RA, MH7A, and MH7A-V4 cells while measuring Ca\(^{2+}\) (Fig. 7). The application of the hypotonic solution [reduction in osmolarity from 310 to 227 mosM (14)] caused an elevation of Ca\(^{2+}\) in RA and MH7A-V4 cells but not in MH7A cells (Fig. 7, A and B). Moreover, the hypotonic

![Fig. 6](image-url)
solution-induced Ca\(^{2+}\) response in the RA cells was significantly inhibited by the removal of external Ca\(^{2+}\) and the application of 10 \(\mu\)M RuR (Fig. 7C), indicating that hypotonic stimulation activates TRPV4 and elevates Ca\(^{2+}\) in human synoviocytes. After withdrawal of RuR, the hypotonic solution caused the elevation of Ca\(^{2+}\) which was relatively smaller than that by the first exposure (Fig. 5, B and C).

**Activation of TRPV4 on production of IL-8 in human synoviocytes.** Finally, to confirm the function of TRPV4 in synoviocytes, we examined the effects of 4\(\alpha\)PDD on production of IL-8 in human synoviocytes with and without treatment with 1 U/ml interleukin-1\(\alpha\) (IL-1\(\alpha\)). As a control groups, vehicle solution without 4\(\alpha\)PDD was applied to these cells. In both RA and MH7A cells, exposure to IL-1\(\alpha\) for 24 h substantially increased production of IL-8 (28.8 \pm 0.57 and 0.084 \pm 0.001 ng/ml in RA cells, and 5.91 \pm 0.68 and 0.148 \pm 0.001 ng/ml in MH7A cells with and without IL-1\(\alpha\), respectively). As shown in Fig. 8A, the IL-1\(\alpha\)-induced increase in production of IL-8 in RA cells was significantly reduced to 80% of the control by treatment with 1 \(\mu\)M 4\(\alpha\)PDD, whereas the basal release of IL-8 in RA cells was not changed by 4\(\alpha\)PDD (0.095 \pm 0.0004 and 0.084 \pm 0.001 ng/ml with and without 4\(\alpha\)PDD). In contrast, the IL-1\(\alpha\)-induced increase in production of IL-8 in CTR cells was not significantly changed by treatment with 1 \(\mu\)M 4\(\alpha\)PDD (34.35 \pm 0.25 and 33.87 \pm 0.26 ng/ml with and without 4\(\alpha\)PDD). The production of IL-8 in MH7A cells was not also changed by 4\(\alpha\)PDD under the same experimental conditions with (4.8 \pm 0.14 and 5.9 \pm 0.68 ng/ml with and without 4\(\alpha\)PDD) and without (0.14 \pm 0.001 and 0.15 \pm 0.002 ng/ml with and without 4\(\alpha\)PDD) treatment with IL-1\(\alpha\) (Fig. 8B).

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*Fig. 7. Ca\(^{2+}\) response induced by the application of hypotonic solution to RA, MH7A, and MH7A-V4 cells. Changes in Ca\(^{2+}\), induced by the application of hypotonic solution to RA, MH7A, and MH7A-V4 cells are shown in two representative cells (**Aa**, **Ab**, and **Ac**), and pooled data are summarized as a bar graph (**B**). Each cell was superfused with mannitol compensated isotonic (310 mosM) and then with 227 mosM hypotonic solution to swell the cell. The numbers in parentheses in **B** indicate the numbers of cells employed. **C**: the sensitivity of the 227 mosM-induced Ca\(^{2+}\) response of RA cells to the removal Ca\(^{2+}\) and RuR is summarized and shown as a bar graph. Numbers in parentheses indicate the number of cells studied. **Statistically significant difference: \(P < 0.01\) vs. wash-out control. Bars = SE.*
expressed in SW982, the information concerning its role is not entirely clear. In the present study, real-time PCR analysis revealed that the expression level of TRPV4 mRNA in RA and CTR cells was six to eight times higher than that in MH7A cells. Although the expression of TRPV4 protein was not quantitatively examined, immunocytochemical analysis showed that TRPV4 protein was clearly detected in RA and CTR cells. MH7A cells also had slight expression of TRPV4 at protein level as well as mRNA level. Functional analyses with 4αPDD support that the TRPV4 protein was present in RA and CTR cells. In particular, application of 4αPDD to RA cells under voltage-clamp conditions activated NSCCs, demonstrating that Ca\(^{2+}\) entry through TRPV4 is involved in 4αPDD-induced Ca\(^{2+}\) response (21).

Evidence in this study indicates that 4αPDD induces elevation of Ca\(^{2+}\), in synoviocytes through activation of TRPV4. Elevation of Ca\(^{2+}\) by 4αPDD in RA cells was abolished in the presence of RuR and after the removal of external Ca\(^{2+}\), implying that a RuR-sensitive Ca\(^{2+}\) entry pathway is involved in the response. It is noted that some phorbol ester analogs modulate the activity of cation channels including TRPC6 and TRPM4 (5, 15). In the present study, a small but substantial elevation of Ca\(^{2+}\) was detected by treatment of MH7A with 3 μM 4αPDD (Fig. 5). It is not clear that this response was caused by activation of TRPV4 in MH7A cells. Nevertheless, MH7A cells with lower expression of TRPV4 were relatively resistant to 4αPDD with 0.3 and 1 μM (Fig. 5A). In addition, Ca\(^{2+}\) responses of RA and CTR cells to 4αPDD were comparable to that of MH7A-V4 cells, demonstrating that 4αPDD effectively activates the TRPV4 expressed in RA and CTR as well as MH7A-V4 cells. Since 4αPDD is highly hydrophobic, elevation of Ca\(^{2+}\); often remained sustained even after removal of 4αPDD and spontaneous Ca\(^{2+}\) response was elicited in some cells (Figs. 3 and 4). It is, therefore, suggested that 0.3 μM 4αPDD is an effective probe to activate TRPV4 in native organs where TRPV4 is functionally expressed (21, 24). On the other hand, the second application of hypotonic stimulation and 4αPDD after removal of RuR elicited smaller Ca\(^{2+}\) response than the first application. Although TRPV4 tends to be desensitized by successive stimulations (23, 24), an involvement of the desensitization will be ruled out, because the second application of hypotonic stimulation and 4αPDD after removal of 0Ca\(^{2+}\) elicited comparable Ca\(^{2+}\) response to the first application. One possible reason for the decrease in Ca\(^{2+}\) response by the second stimulation is that RuR is highly effective and therefore difficult to be washed away.

Hypotonic stimulation induced a Ca\(^{2+}\) response in human synoviocytes via the activation of TRPV4. Cell-swelling induced by hypotonic solution activates TRPV4 by means of the phospholipase A\(_2\) (PLA\(_2\))-dependent formation of arachidonic acid, whereas phorbol esters and heat do so by a distinct, PLA\(_2\)-independent pathway (22). On the other hand, hypotonic stimulation can also activate TRPV2 and TRPM4 (3, 14). In the present study, 227 mosM hypotonic stimulation induced a substantial elevation of Ca\(^{2+}\) in MH7A-V4 cells, but not in MH7A cells, providing strong evidence that human synoviocytes containing TRPV4 are sensitive to hypotonic stimulation. Moreover, the hypotonic solution-induced Ca\(^{2+}\) response in RA cells was significantly inhibited by the removal of external Ca\(^{2+}\) and the application of RuR. Taken together, cell-swelling activates TRPV4 in human synoviocytes, suggesting that

**Fig. 8.** Change in production of IL-8 by 4αPDD in human synoviocytes. Production of IL-8 from RA (A) and MH7A (B) cells treated for 24 h with and without 1 μM 4αPDD was measured with an ELISA assay (see MATERIALS AND METHODS). In some experiments, cells were pretreated with 1 U/ml IL-1α to potentiate the production of IL-8. **Statistically significant difference: P < 0.01. Bars = SD.

DISCUSSION

It was clearly shown in the present study that TRPV4 plays an obligatory role as a molecular component in the nonselective cation channel activation induced by 4αPDD and hypotonic stimulation in human synoviocytes and also regulates production of IL-8. This conclusion is based on the following lines of evidence: 1) expression of the TRPV4 mRNA transcript and protein was clearly detected in RA and CTR cells, both of which were sensitive to 4αPDD; 2) the elevation of Ca\(^{2+}\), induced by 4αPDD was inhibited by the removal of external Ca\(^{2+}\); and application of RuR; 3) the 4αPDD-induced Ca\(^{2+}\) response of MH7A cells transfected with hTRPV4 was comparable to those of the RA and CTR cells; 4) 4αPDD induced nonselective cation currents in RA cells; 5) hypotonic stimulation induced a Ca\(^{2+}\) response in RA and MH7A-V4 cells but not in MH7A cells; and 6) the activation of TRPV4 in RA cells reduced production of IL-8.

Human synovial sarcoma (SW982) expresses some TRPs at the mRNA level, and it is proposed that TRPV1 is a proton sensor in synovial tissue (1, 4, 10). Although TRPV4 is also
TRPV4 functions as an environmental sensor to sense cell-swelling, mechanical stress, and/or lipid metabolites to which synoviocytes are exposed. Interestingly, the synovial fluid osmolality in patients with rheumatoid arthritis was significantly different from that in the normal group [280 mmol/kg vs. 404 mmol/kg (19)]. The functions of TRPV4 in synoviocytes, particularly under pathophysiological conditions, remain to be determined.

A chemokine, IL-8 is consistently increased in development of rheumatoid arthritis and therefore is a potential target for the treatment of rheumatoid arthritis. In the present study, application of 4αPDD to RA cells effectively inhibited the IL-1α-induced increase in production of IL-8 without affecting the basal release. Moreover, 4αPDD had no effects on production of IL-8 with and without treatment with IL-1α in MH7A cells where TRPV4 does not function. Taken together, these results provide strong evidence of a new function of TRPV4 for regulation of production of cytokine. Of importance is that activation of TRPV4 reduces production of IL-8 in the presence of IL-1α, implying that the production of IL-8 is regulated by TRPV4 under inflammatory conditions. Furthermore, the evidence that IL-1α-induced production of IL-8 in CTR cells was not changed by 4αPDD indicates that the role of TRPV4 regulation of inflammatory responses in synoviocytes is more important under pathophysiological conditions. In TRPC6-deficient mice, production of IL-5 and IL-13 was decreased in the bronchoalveolar lavage (18). Moreover, Ca2+ influx via TRPM2 regulates reactive oxygen species-induced chemokine production, which aggravates inflammation (25). Although the signaling cascades responsible for inhibition of IL-8 production by the activation of TRPV4 remain to be determined, these pooled data strongly point to an important immunological function of these cation channels in immune-related organs including synovial fibroblasts.

The present study revealed that Ca2+ entry through TRPV4 is not involved in a final step to regulate IL-1α-induced production of IL-8 in RA cells. The production of IL-8 was not affected by 4αPDD in CTR cells where functional TRPV4 was expressed, suggesting that disease status including cell conditions has a key role in the regulation by TRPV4. In fact, our preliminary results show that 4αPDD rather increased the production of IL-8 induced by IL-1α in MH7A-V4 cells (14.0 ± 0.39 and 12.3 ± 0.25 ng/ml with and without 4αPDD). However, it is noted that this result includes some experimental limitation, because transfection efficiency of TRPV4 varies from cell to cell, and Ca2+ will be easily overloaded by 4αPDD in high expressing cells (see Fig. 5A; MH7A-V4). Nevertheless, TRPV4 will be a potential target for treatment of inflammation in pathophysiological status of synoviocytes.

In 10–15% RA and CTR cells, spontaneous oscillatory change in Ca2+ was observed (Fig. 3A). The oscillatory change in Ca2+ with and without bioactive substances has been demonstrated in many types of cells, and the frequency tuning is a more effective regulator of Ca2+ than the amplitude tuning (7). However, physiological and pathophysiological significance of change in Ca2+ including these spontaneous Ca2+ oscillations is not clear in synoviocytes. Since Ca2+ plays pivotal roles in many biological events, further extensive studies are required in synoviocytes.

In conclusion, this study demonstrates that human synoviocytes express TRPV4, which underlies the 4αPDD- and hypotonic stimulation-induced Ca2+ response. TRPV4 regulates production of IL-8 in human synoviocytes and may be an environmental sensor activated by cell-swelling in synovial fluid, mechanical stress by joint movement, and lipid metabolites produced under inflammation in joint.

ACKNOWLEDGMENTS

We thank Dr. J. Dempster (University of Strathclyde, Glasgow, United Kingdom) for developing electrophysiology software (WinWCP). IL-1α was kindly provided by Dainippon Pharmaceutical Co. (Osaka, Japan).

GRANTS

This work was supported by Grants-in-Aid for Scientific Research to Y. Itoh and K. Muraki from the Japanese Society of Promotion and Science, Takeda Science Foundation, Smoking Science Foundation, Clinical Pharmacological Foundation, Suzukken Memorial Foundation, and Furukawa Foundation.

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