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Short communication

A novel method for evaluating activity of transient receptor potential channels using a cellular dielectric spectroscopy

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ABSTRACT

Cellular dielectric spectroscopy (CDS) is a novel technology enabling pharmacological evaluation of multiple receptor types with a label-free cell-based assay. We evaluated activities of a family of ligand-gated channels, transient receptor potential vanilloid 1 (TRPV1) and transient receptor potential ankyrin 1 (TRPA1) channels by an electrical impedance-based biosensor (CellKey™ system) using CDS. Measures of both potency (EC_{50}) and efficacy (E_{max}) of these agonists with CellKey™ were almost identical to those made using the traditional Ca^{2+} influx assay in TRPV1- or TRPA1-expressing cells, suggesting that CellKey™ is a simpler and easier means of evaluating TRP activities.

Among the transient receptor potential (TRP) family, the TRP vanilloid 1 (TRPV1) and TRP ankyrin 1 (TRPA1) are important detectors of pain sensation in sensory afferent neurons.[1–3] These non-selective cation channels are activated by numerous stimuli. TRPV1 is stimulated by capsaicin, heat (>45 °C), and protons.[2,4,5] TRPA1 is activated by allyl isothiocyanate (AITC), formaldehyde, and reactive oxygen species.[2,6,7] We have previously shown that these agonists induced neuropeptide substance P (SP) release from sensory afferent neurons, indicating that some of the pain information activated by these channels is transmitted to the spinal dorsal horn via SP.[8] Many studies using pain models, including knock-out mice lacking the channels and their selective inhibitors, have demonstrated that both TRPV1 and TRPA1 contribute to severe pain, including allodynia or hyperalgesia.[3,6,8,9] This indicates that these channels are involved in pain transmission in primary sensory neurons and could serve as targets for analgesic development.

Cellular dielectric spectroscopy (CDS) is a novel technology enabling comprehensive pharmacological evaluation of cell exogenous/endogenous receptors using a label-free, real-time, kinetic cell-based assay.[10–12] This technology is universal, allowing measurement of multiple types of receptors using the same platform without a need for any modification of the cells.[10–12] Among the biosensors utilizing CDS technology, the CellKey™ system is superior in detecting activation of G-protein-coupled receptors (GPCRs) as changes of intracellular impedance (ΔZ).[10] We have previously reported a novel evaluation method for activity of GPCRs using this system, and found that CellKey™ distinguishes signals activating different subtypes of the Gα protein (Gs, Gi/o, and Gq).[10] However, few reports have evaluated ligand-gated ion channels,

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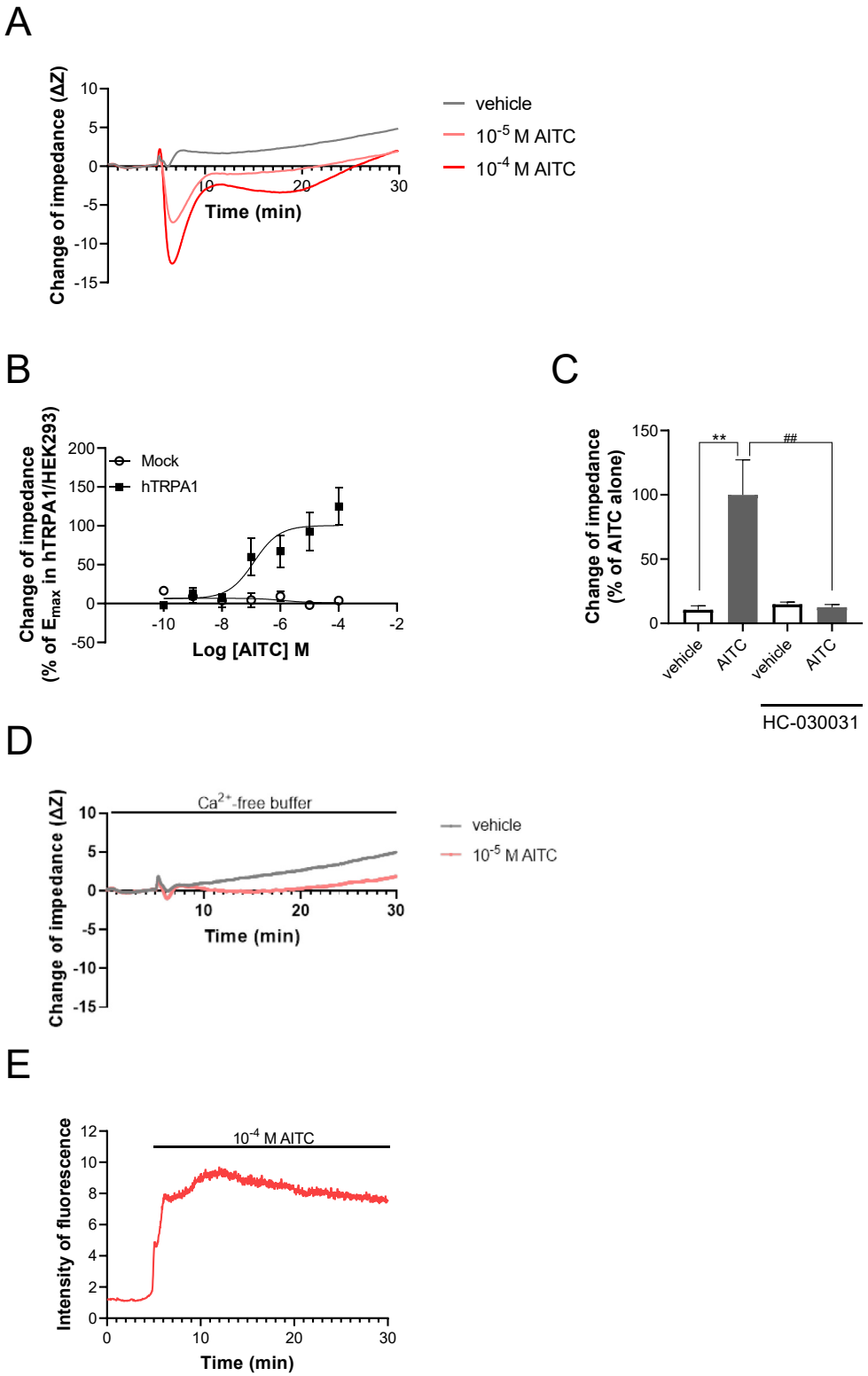

Fig. 1. Changes in impedance (ΔZ) or intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in human transient receptor potential ankyrin 1 (hTRPA1)-expressing cells treated with the TRPA1 agonist allyl isothiocyanate (AITC). (A) hTRPA1-expressing cells were treated with vehicle or AITC (10^{-5} or 10^{-4} M) for 25 min (n = 5–7). (B) Concentration-response relationship for ΔZ induced by AITC. AITC (10^{-10}-10^{-4} M) treated with cells expressing hTRPA1 or empty-vector (Mock), respectively (n = 5–26). (C) Effects of the TRPA1 antagonist HC-030031 on the AITC-induced ΔZ in hTRPA1-expressing cells pretreated with vehicle or HC-030031 (2×10^{-5} M) for 30 min, followed by treating with vehicle or AITC (10^{-4} M) for 25 min, respectively (n = 3). $^{**}p < 0.01$, compared with vehicle alone, $^{\#\#}p < 0.01$, compared with AITC alone. (D) hTRPA1-expressing cells were treated with vehicle or AITC (10^{-5} M) for 25 min in Ca^{2+}-free buffer (n = 2). Similar results were obtained in at least three independent experiments. (E) hTRPA1-expressing cells were treated with AITC (10^{-4} M) for 25 min and measured $[Ca^{2+}]_i$ (n = 5) using a fluorescence microscope IX71 (Olympus Corporation, Tokyo, Japan).

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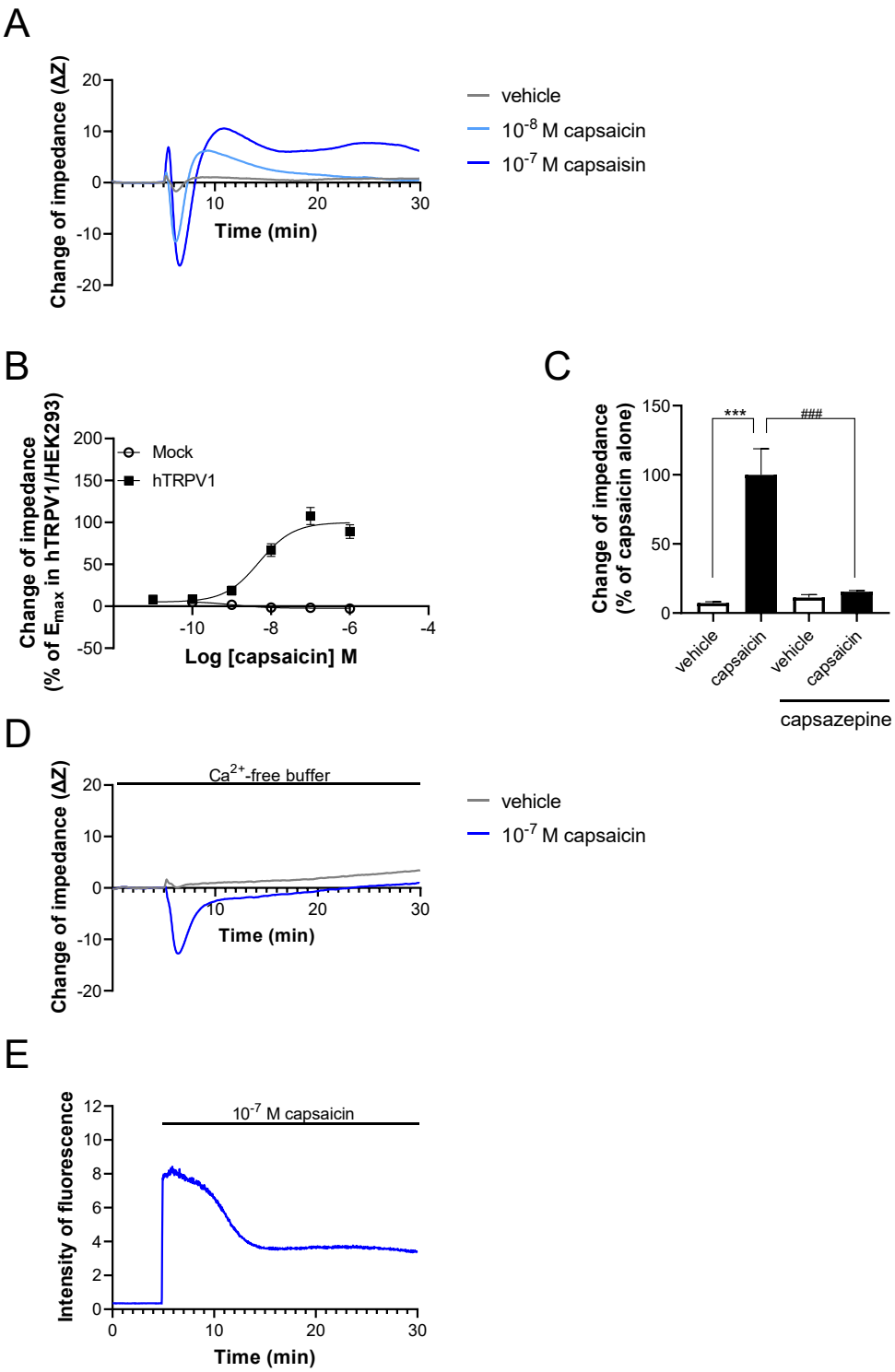

Fig. 2. Changes in impedance (ΔZ) or intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in human transient receptor potential vanilloid 1 (hTRPV1)-expressing cells treated with a TRPV1 agonist capsaicin. (A) hTRPV1-expressing cells were treated with vehicle or capsaicin (10^{-8} or 10^{-7} M) for 25 min (n = 2). Similar results were obtained in at least three independent experiments. (B) Concentration-response relationship for ΔZ induced by capsaicin. Capsaicin (10^{-11}-10^{-6} M) was used to treat cells expressing hTRPV1 or empty-vector (Mock), respectively (n = 4–36). (C) Effects of a TRPV1 antagonist capsazepine on the capsaicin-induced ΔZ in hTRPV1-expressing cells. The cells were pretreated with vehicle or capsazepine (10^{-5} M) for 30 min followed by treating with vehicle or capsaicin (10^{-6} M) for 25 min, respectively (n = 3). ***p < 0.001, compared with vehicle alone; $^{###}$p < 0.001, compared with capsaicin alone. (D) hTRPV1-expressing cells were treated with vehicle or capsaicin (10^{-7} M) for 25 min in Ca^{2+}-free buffer (n = 2). Similar results were obtained in at least three independent experiments. (E) hTRPV1-expressing cells were treated with capsaicin (10^{-7} M) for 25 min and measured $[Ca^{2+}]_i$ using a fluorescence microscope IX71 (n = 18). Ca^{2+} imaging assay was performed using a fluorescence microscope IX71 (Olympus Corporation, Tokyo, Japan).

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Table 1
EC_{50} and E_{max} of TRPA1 or TRPV1 agonists using CellKey™ system and Ca^{2+} imaging assay.

TRP channels	agonist	CellKey system		Ca^{2+} imaging assay	
		Log EC_{50} (M)	E_{max} (%)	Log EC_{50} (M)	E_{max} (%)
hTRPA1	AITC	−7.20 ± 0.36	100.0 ± 10.5	−6.69 ± 0.06	100.0 ± 1.73
	allicin	−5.91 ± 0.24	185.3 ± 25.5	−6.68 ± 0.05	112.5 ± 2.27
	cinnamaldehyde	−6.77 ± 0.46	134.4 ± 18.4	−5.36 ± 0.04	101.4 ± 1.76
hTRPV1	capsaicin	−8.12 ± 0.29	100.0 ± 10.6	−8.66 ± 0.10	100.0 ± 3.84
	alvanil	−8.84 ± 0.35	90.3 ± 9.51	−8.25 ± 0.04	110.7 ± 1.97
	olvanil	−9.10 ± 0.49	85.4 ± 12.1	−8.95 ± 0.05	107.4 ± 1.90

Ca^{2+} imaging assay was performed using a FlexStation 3 microplate reader (Molecular Devices, Sunnyvale, CA). Log EC_{50} and E_{max} were calculated using GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA).

such as TRPV1 or TRPA1. In this study, we evaluated their activities using the CellKey™ system.

We first used CellKey™ to examine the effects of selective agonists of TRPA1 or TRPV1 on ΔZ in human embryonic kidney 293 (HEK293) cells stably expressing hTRPV1 or hTRPA1. These cells were prepared using the tetracycline-inducible T-Rex™ expression system (Invitrogen, Carlsbad, CA, USA), using methods described previously.[2] Procedures for the CellKey™ system have also been described previously.[13,14] The cells were cultured at a density of 2.0×10^4 cells/well on a standard CellKey™ 96-well microplate containing electrodes at the bottom of the wells. After incubation for 24 h, cells were washed with Hanks' balanced salt solution (Sigma–Aldrich, St Louis, MO, USA) with 20 mM HEPES and 0.1% BSA and allowed to equilibrate in the assay buffer for 30 min before beginning the assay. The CellKey™ instrument applies small voltages to these electrodes every 10 s and measures ΔZ in the cell layer. In this study, we recorded a 5 min baseline, added drugs, and measured changes in ΔZ for 25 min.

The TRPA1 agonist AITC (10^{-5} and 10^{-4} M) induced transient decreases of ΔZ and then returned to baseline, compared to vehicle (Fig. 1A). The extent of changes in ΔZ were therefore expressed as the value subtracted the minimum ΔZ from the maximum ΔZ after drug injection. Results are shown as the mean ± standard error (S.E.M.). Concentration-response curves were fitted using GraphPad Prism 8 software (GraphPad Software, La Jolla, CA, USA). In hTRPA1-expressing HEK293 cells, AITC increased in ΔZ in a concentration-dependent manner (Fig. 1B), without changing ΔZ in HEK293 cells transfected with empty-vector (Mock, Fig. 1B). We further examined the effects of the TRPA1 selective antagonist HC-030031 on the AITC-induced increase in ΔZ in hTRPA1-expressing HEK293 cells. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test (GraphPad Prism 8). A p value of <0.05 was considered statistically significant. We found that HC-030031 (2×10^{-5} M) significantly reduced the AITC-induced increase in ΔZ (Fig. 1C). In addition, we investigated involvements of extracellular Ca^{2+} on ΔZ induced by AITC to clear mechanisms of the AITC-induced ΔZ. In Ca^{2+}-free buffer, the AITC-induced ΔZ was remarkably smaller than that in Ca^{2+}-containing buffer (Fig. 1A and D), suggesting that the AITC-induced ΔZ is mediated mainly through Ca^{2+} influx.

We next investigated the effects of the TRPV1 agonist capsaicin on hTRPV1-expresing HEK293 cells using the CellKey™ system. Capsaicin (10^{-8} and 10^{-7} M) induced transient decrease and subsequent increase in ΔZ, compared to vehicle (Fig. 2A). While capsaicin did not increase ΔZ in HEK293 cells transfected with empty-vector (Mock, Fig. 2B), it increased ΔZ in a concentration-dependent manner in hTRPV1-expressing HEK293 cells (Fig. 2B). A TRPV1 selective antagonist capsazepine (10^{-5} M) inhibited the capsaicin-induced increase in ΔZ in hTRPV1-expressing HEK293 cells (Fig. 2C). In Ca^{2+}-free buffer, capsaicin also induced a transient decrease (Fig. 2D), but its level was smaller than that in Ca^{2+}-containing buffer (Fig. 2A and D). After the capsaicin-induced transient decrease in ΔZ, the response gradually returned toward the basal level within several minutes (Fig. 2D), which was smaller than that in Ca^{2+}-containing buffer (Fig. 2A and D). Both TRPA1 and TRPV1 are non-selective cation channels, but these permeability among cations is different.[15] Although further studies are needed, the TRPV1-activated ΔZ might be related to not only Ca^{2+} influx but also Ca^{2+}-independent signaling, such as ion fluxes of other cations.

To compare the sensitivity between the CellKey™ assay and a traditional Ca^{2+} influx assay, we examined the effects of AITC and capsaicin on hTRPA1-or hTRPV1-expressing cells with a Ca^{2+} influx assay as described previously.[2,16] In TRPA1-expressing cells, AITC induced increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$), which was sustained for at least 25 min (Fig. 1E). On the other hand, capsaicin induced a rapid and transient increase and subsequent sustained $[Ca^{2+}]_i$ for at least 25 min (Fig. 2E). These data suggest that the difference of shapes of $[Ca^{2+}]_i$ between AITC and capsaicin could be involved in those of ΔZ in the present study. In Table 1 regarding the comparison of values of EC_{50} and E_{max} of AITC- or capsaicin-induced cellular responses between the CellKey™ assay and Ca^{2+} influx assay, the extent of changes in $[Ca^{2+}]_i$ were expressed as the value subtracted the baseline value from the maximum value after drug injection. Data are expressed as the means ± S.E.M. (CellKey™ assay: n = 7–27, Ca^{2+} influx assay: n = 3). Statistical analysis was performed using two-way ANOVA followed by Bonferroni's multiple comparison test (GraphPad Prism 8). $p < 0.05$ was considered statistically significant. We found that the values of EC_{50} and E_{max} of AITC or capsaicin were not significantly different between the CellKey™ and the Ca^{2+} influx assays [Table 1: AITC (EC_{50}, E_{max}; $p = 0.999$, $p = 0.999$), capsaicin ($p = 0.999$, $p = 0.999$)]. When testing other agonists of TRPA1 and TRPV1, measured EC_{50} and E_{max} were also not significantly different between the assays [Table 1: Allicin (EC_{50}, E_{max}; $p = 0.999$, $p = 0.3924$), cinnamaldehyde ($p = 0.999$, $p = 0.999$), alvanil ($p = 0.999$, $p = 0.999$), olvanil ($p = 0.999$, $p = 0.999$)]. In evaluating activities of GPCRs, we previously reported that the CellKey™ system has similar or higher sensitivity than a traditional cAMP assay systems using label-based assays.[10,14] Our present findings suggest that sensitivity of the CellKey™ system for TRPA1 and TRPV1 seems to be as same as the traditional Ca^{2+} influx assay.

In conclusion, we established a novel evaluation method for TRPA1 and TRPV1 activities for the first time using the CellKey™ system, which has similar sensitivity to a traditional Ca^{2+} influx assay. As such, we suggest that the CellKey™ system is applicable for screening of TRPV1 and TRPA1 agonists.

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Conflict-of-interest disclosure

Y.U. received a grant support from Tsumura & Co. and Maruho Co. Ltd. K.O. and M.Y. are employees of Tsumura & Co, Japan. Other authors declare no competing interests.

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