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The effect of temporal adaptation to different temperatures and osmolarities on heat response of TRPV4 in cultured cells

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ABSTRACT

Transient receptor potential vanilloid 4 (TRPV4) channel is a polymodal receptor activated by moderate heat and hypoosmolarity. TRPV4 expressed in the skin area contributes to several skin functions as a barrier to maintain internal body physiology and a transporter of external stimuli. The skin condition such as skin temperature and osmolarity varies with internal and external changes, and may influence the activity of TRPV4 contributing to skin physiology, thermal sensation, and thermoregulation. However, the combination effect of skin conditions such as temperature and osmolarity on the activity of TRPV4 has not been examined. In the current study, we investigated the effect of temporal adaptation (5–10 min) to different temperature (25–35 °C) and osmolarity (250–350 mOsm) conditions on the heat response (until 40 °C) of human TRPV4 in cultured cells using Ca^{2+} imaging. The temperature to activate TRPV4 increased with elevation of the adaptation temperature, and decreased with the adaptation to hypoosmolarity in the range of 25–35 °C. In addition, the heat response was inhibited with the adaptation to hyperosmolarity in the range of 25–35 °C. Thus, we demonstrated that the activation temperature of TRPV4 varied with the temporal sensory adaptation to different temperature and osmolarity conditions. These findings may contribute to gaining better understanding of the variation in several TRPV4-mediated skin functions.

1. Introduction

Skin, which is the outermost layer of the body, works as a barrier to maintain internal body physiology and a transporter of external stimuli. One factor that contributes to these skin functions is the transient receptor potential vanilloid 4 (TRPV4) channels expressed in the skin area. TRPV4 is a polymodal receptor, which is activated by moderate heat (Güler et al., 2002; Watanabe et al., 2002), mechanical or hypoosmotic stimuli (Liedtke et al., 2000; Strotmann et al., 2000; Wissenbach et al., 2000; Güler et al., 2002; Toft- Bertelsen et al., 2017), and chemical stimuli (Watanabe et al., 2002; Thorneloe et al., 2008). Several studies have reported that TRPV4 contributes to form intercellular junction in keratinocytes (Sokabe et al., 2010), sense innocuous warmth (Watanabe et al., 2002; Chung et al., 2004; Lee et al., 2005; Vizin et al., 2015), decrease cell volume after swelling (Hoffmann et al., 2009; Becker et al., 2005), and induce vasodilation (Hartmannsgruber et al., 2007; Mendoza et al., 2009; Sonkusare et al., 2012). Thus, TRPV4 is a common factor that plays various roles in skin physiology, thermal sensation, and thermoregulation.

As characteristics of TRPV4, it was reported that the hypoosmotic response has a sensitivity peak at core body temperature (Liedtke et al., 2000). The heat response under hypoosmolarity is greater than that under isoosmolarity, and inhibited under hyperosmolarity (Güler et al., 2002). These results show that the adaptation to core body temperature range or hypoosmolarity enhances the activity level of TRPV4. In human skin, TRPV4 is adapted to both skin temperature and osmolarity. Human skin temperature is usually lower than core body temperature (Webb, 1992; Taylor et al., 2014), and changes with external environment (Wagner et al., 1974; Martinez-Nicolas et al., 2015) and thermoregulation (Torii et al., 1992; Kräuchi et al., 2000). In addition, skin osmolarity or cell volume changes with internal physiology, such as dehydration and inflammation. Therefore, to understand the characteristics of TRPV4, it is necessary to consider the adaptation to both temperature and osmolarity. Differences in the adaptation conditions may result in variation in the activity of TRPV4, with direct or indirect influences on skin physiology, thermal sensation, and thermoregulation.

Abbreviations: ΔFrate, difference between maximum and minimum fluorescence intensity rate; Frate, fluorescence intensity rate; Tact, activation temperature; Tadp, adaptation temperature; TRPV4, transient receptor potential vanilloid 4.

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However, the combination effect of the adaptation of TRPV4 to these skin conditions (temperature and osmolarity) have not been examined. Moreover, the effect on the activation temperature has also not been examined. To understand the activation temperature of TRPV4, assuming skin condition, may help to understand the variation in several skin functions influenced by skin temperature changes. Thus, in the current study, we examined the effect of temporal sensory adaptation to different temperature and osmolarity conditions on the heat response of TRPV4 in cultured cells.

2. Material and methods

2.1. Cell culture and solutions

HeLa cells (JCRB9004, JCRB Cell Bank), which do not express TRPV4 (Thul et al., 2017) were maintained in DMEM (FUJIFILM Wako Pure Chemical Corporation) supplemented with 10% fetal bovine serum (FBS; Biosera) and 1% penicillin-streptomycin (Sigma-Aldrich) at 37 °C in 5% CO_2. The cells were seeded in ϕ35-mm dish for Ca^{2+} imaging and a 384-well plate for screening test, and incubated for a day. The cells were transfected with pcDNA3.1 (+) plasmid DNA (Thermo Fisher Scientific, 2.5 μg per ϕ35-mm dish, 0.03 μg per a well of 384-well plate) containing human TRPV4 (Promega) using FuGENE HD Transfection Reagent (Promega) and OPTI-MEM medium (Thermo Fisher Scientific). The cells transfected with pcDNA3.1 (+) plasmid DNA without TRPV4 were also prepared as a control. For Ca^{2+} imaging, after incubating for 4–5 h, the cells were reseeded into ϕ35-mm dish with ϕ8-mm coverslips. After incubating for a day, the cells were loaded with 10 μM fluorescent calcium indicator (Cal-520 AM, AAT Bioquest) in standard solution containing 0.02% pluronic F-127 (Sigma-Aldrich). After incubating for 1 h at 33 °C in 5% CO_2 to avoid heat activation, the solution was replaced with fresh solution. The standard solution (300 mOsm) contained 130 mM NaCl, 3 mM KCl, 2.5 mM $CaCl_2$, 0.6 mM $MgCl_2$, 10 mM HEPES, 1.2 mM $NaHCO_3$, and 10 mM glucose with NaOH to adjust the pH to 7.45 (Güler et al., 2002). Hypoosmotic and hyperosmotic solutions were prepared by reducing NaCl or adding mannitol, respectively. The osmolarity of each solution was measured using an osmometer (Fiske Micro-Osmometer Model210, Advanced Instruments).

2.2. Screening test

The responses of TRPV4 to osmolarity and 1 μM GSK1016790A, a TRPV4 agonist, were confirmed using 384-well plate and Ca^{2+} imaging with functional drug screening system (FDSS/μCELL, Hamamatsu Photonics, sampling frequency 1 Hz) at room temperature (25.38 ± 0.74 °C). Stimuli solutions were added into each well with standard 300 mOsm solution in 1:1 vol rate in 30 s after the start of recording. The osmolarity conditions were hypoosmotic (250 and 275 mOsm), standard (300 mOsm), and hyperosmotic (325 and 400 mOsm), which are added stimuli solutions of 200, 250, 300, 350, and 500 mOsm to standard solution. Fluorescence intensity rate (F_{rate}) at every second was calculated by the obtained fluorescence intensity divided by the baseline fluorescence intensity which was the mean value for 30 s before stimulation. Then, differences between maximum and minimum fluorescence intensity rates were calculated (ΔF_{rate}).

2.3. Ca^{2+} imaging with fluorescence microscope

The effects of the adaptation to different temperatures (T_{adp}) and osmolarities were measured using a fluorescence microscope (ECLIPSE TE200-U, NIKON), a CCD digital camera (ImagEM C9100-13, Hamamatsu Photonics), a heating system, and a thermocouple. The sampling frequencies of all devices were 1 Hz. The heating system (Fig. 1) consisted of a stage heater and a Peltier element with ϕ10-mm well (TES1-4903T125 with 3-V batteries). The coverslip with cultivated cells was put in the well with 200 μl solution and adapted to the temperature and the solution's osmolarity for 5–10 min before recording. After recording the baseline condition for 30 s, heating was started until 40 °C (0.1–0.3 °C /s). T_{adp} conditions were 25, 30, and 35 °C and the osmolarity conditions were 250, 300, and 350 mOsm. The fluorescence intensity of each cell was measured using ImageJ (National Institutes of Health). F_{rate} at every second was calculated by the obtained fluorescence intensity divided by the baseline fluorescence intensity which was the mean value for 30 s before heating.

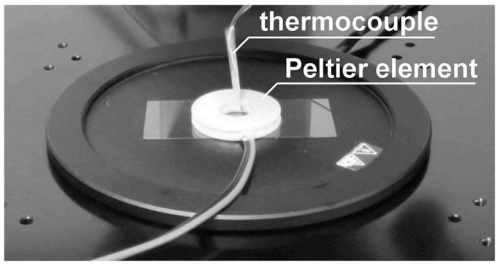

Fig. 1. Heating system. A slide glass, a Peltier element, and a thermocouple on a stage heater. A coverslip with cultivated cells are in the ϕ10-mm well of the Peltier element.

2.4. Statistical analysis

In all analyses, unpaired *t*-test was conducted for the comparison of two conditions. To confirm the response of osmolarity in the screening test, one-way analysis of variance (ANOVA) and comparisons between the standard solution and hypoosmotic or hyperosmotic solutions were performed. To validate the effects of the temporal adaptation to T_{adp} and osmolarity on the heat response, activation temperature (T_{act}) of each cell was found automatically as a point which F_{rate} started to increase against the baseline, then two-way ANOVA was performed. The method to find T_{act} is shown in Fig. 2. To verify the combination effect of T_{adp} and osmolarity, stepwise regression analysis was applied. The significance level was set at 0.05. All analyses were performed using MATLAB R2018b (Mathworks).

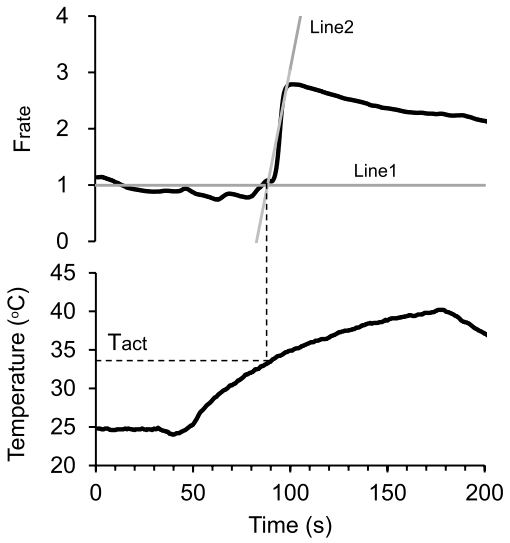

Fig. 2. The method to find activation temperature. Line1: baseline which is the mean value of F_{rate} for 30 s before the stimulation, Line2: fitted line. T_{act} is the temperature on the time which is the intersection point between Line1 and Line2. F_{rate}: fluorescence intensity rate, T_{act}: activation temperature.

3. Results

3.1. Screening test of osmolarity response

Upon stimulation with 250 mOsm solution, the ΔF_{rate} of TRPV4 was found to be significantly higher than that of pcDNA transfected without TRPV4 ($p < 0.001$, Fig. 3a), and same as that after stimulation with 1 μM GSK1016790A ($p < 0.001$, Fig. 3b). The activation by 250 mOsm was inhibited with ruthenium red, which is an antagonist of TRPV4 (Fig. 3a). In addition, the activation varied with osmolarity ($F_{4,75} = 13.87$, $p < 0.001$, Fig. 4). Hypoosmotic solutions (250 and 275 mOsm) activated more than standard solution ($p < 0.001$, $p < 0.01$, respectively, Fig. 4). On the other hand, there were no significant differences between effects of hyperosmotic (325 and 400 mOsm) and standard solutions. Therefore, the activation of TRPV4 by lower osmolarities than standard solution was confirmed.

3.2. Heat activation under adaptation to each condition

Fig. 5 shows the maximum F_{rate} during heating until 40 °C, which are averaged values of 5–14 trials per condition. To validate the heat activation of TRPV4, we compared the F_{rate} of TRPV4 and that of pcDNA under adaptation to each condition. F_{rate} of TRPV4 under the adaptation to all T_{adp} conditions with hypoosmotic (250 mOsm) and standard (300 mOsm) solutions were significantly higher than those of pcDNA, and the statistical results are shown in Fig. 5. Heat activation of pcDNA was not observed under all conditions. On the other hand, there were no significant differences between TRPV4 and pcDNA under the adaptation to all T_{adp} conditions with hyperosmotic solution (350 mOsm). Therefore, the heat activation of TRPV4 under adaptation to hypoosmotic and standard solutions at all T_{adp} was observed.

3.3. Activation temperature under adaptation to each condition

To evaluate T_{act}, activated cells of TRPV4 under all T_{adp} conditions with 250 and 300 mOsm, which were found by the method shown in Fig. 2, were extracted. Firstly, the cumulative probabilities of T_{act} were verified. Under both 250 and 300 mOsm conditions, 50%ile cumulative probabilities of T_{act} were shifted to higher temperatures according to the increase of T_{adp} (Fig. 6). Compared with 300 mOsm condition, 50%ile cumulative probabilities of T_{act} were shifted to lower temperatures

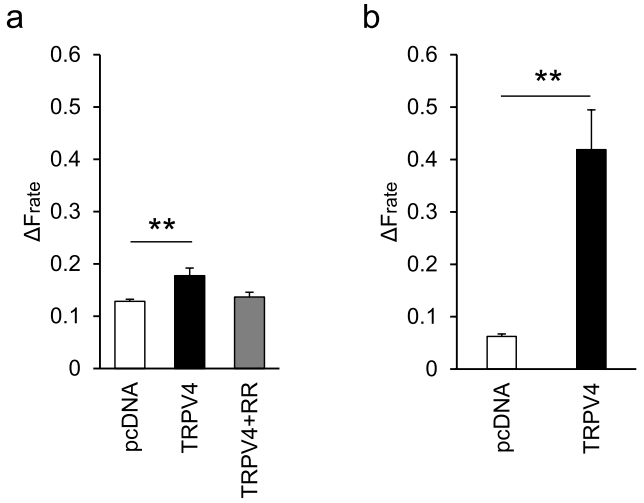

Fig. 3. Activation of TRPV4 by agonist. n = 16 in two trials per condition, mean ± SEM. Comparisons with pcDNA. a: stimulation by 250 mOsm solution. b: stimulation by GSK1016790A. TRPV4+RR: TRPV4 with ruthenium red. **p < 0.01.

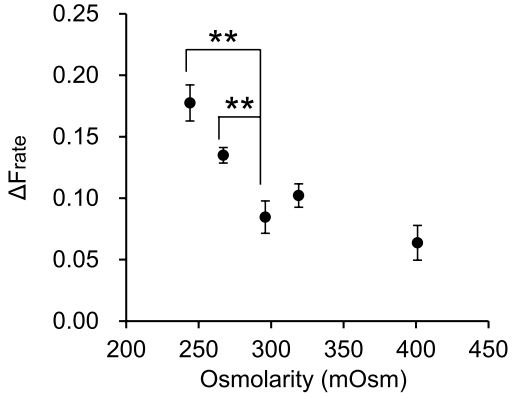

Fig. 4. Dose response relationship between osmolarity and fluorescent rate of TRPV4. n = 16 in two trials per condition, mean ± SEM. The measured osmolarities of 250, 275, 300, 325, and 400 mOsm conditions were 244, 267, 296, 319, and 401 mOsm, respectively. **p < 0.01.

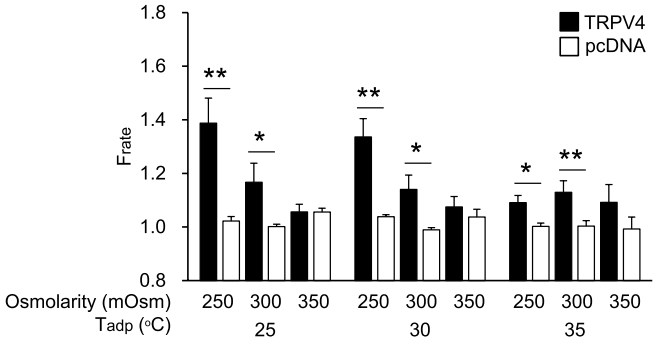

Fig. 5. Maximum fluorescence intensity rate during heating under all temperature and osmolarity conditions. n = 5–14. Mean ± SEM. *p < 0.05, **p < 0.01.

under all T_{adp} conditions with 250 mOsm.

Secondly, the effects of T_{adp} and osmolarity were validated by two-way ANOVA. The main effect of T_{adp} and osmolarity on T_{act} were significant ($F_{2,233} = 10.29$, $p < 0.001$, $F_{1,238} = 14.91$, $p < 0.001$, respectively. Fig. 7a). Significant interaction between T_{adp} and osmolarity was not observed. T_{act} at 30 and 35 °C conditions were significantly higher than that at 25 °C condition ($p < 0.01$, $p < 0.001$, respectively. Fig. 7b). T_{act} at 250 mOsm condition was significantly lower than that at 300 mOsm condition ($p < 0.001$, Fig. 7c).

3.4. Combination effect of adaptation temperature and osmolarity

We conducted stepwise regression analysis to examine the combination effect of T_{adp} and osmolarity on T_{act} using the data shown in Fig. 7. T_{adp} and osmolarity were used as independent variables, and T_{act} was used as the dependent variable. Results of the analysis revealed significant coefficients both of T_{adp} and osmolarity for T_{act} (Table 1). T_{act} increased with increase in T_{adp} and osmolarity under the present experimental conditions (25–35 °C with 250 and 300 mOsm).

4. Discussion

We evaluated the heat response of TRPV4 in cultured cells under the temporal sensory adaptation to different temperature and osmolarity conditions. The current results revealed that T_{act} by heat increased with the elevation of T_{adp}, and decreased with the adaptation to hypo-osmolarity in the range of 25–35 °C. There was no interaction between

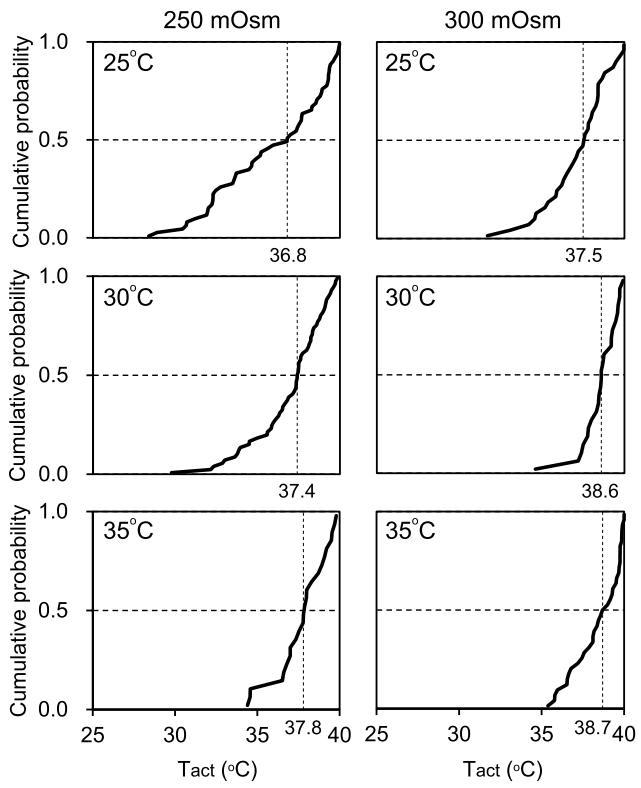

Fig. 6. Cumulative probabilities of activation temperature under adaptation to all temperature conditions with 250 and 300 mOsm. n = 24–63 in 10–14 trials. Left panels: 250 mOsm conditions, right panels: 300 mOsm conditions, upper panels: 25 °C conditions, middle panels: 30 °C conditions, lower panels: 35 °C conditions. The measured adaptation temperature was 25.11 ± 0.51 °C at 25 °C condition, 29.99 ± 0.48 °C at 30 °C condition, and 33.79 ± 0.54 °C at 35 °C condition. 50%ile = 0.5 of vertical axis.

T_{adp} and osmolarity on T_{act} by heat. In addition, we demonstrated that the adaptation to hyperosmolarity inhibited the heat response in the range of 25–35 °C.

The temperature to activate TRPV4 increased with the elevation of T_{adp} from 25 to 35 °C under 250 and 300 mOsm, but the variation of T_{act} was small, less than 2 °C. It is reported that T_{act} of TRPM8 (Fujita et al., 2013), which is activated by innocuous and noxious cold (McKemy et al., 2002; Knowlton et al., 2010), also changes with T_{adp}. Therefore, it is considered that T_{act} by moderate heat was more or less maintained at body temperature range under the adaptation to 25–35 °C, but varied with the range of less than 2 °C. Human warmth sensation also varies depending on the skin temperature (Kenshalo, 1970). Some studies have reported that TRPV4 contributed to sense innocuous warmth (Watanabe et al., 2002; Chung et al., 2004; Lee et al., 2005; Vizin et al., 2015), but another study denied the major contribution by behavioral experiments with mice (Huang et al., 2011). Although the role of TRPV4 in warmth sensation remains controversial, it is possible that TRPV4 may have a minor contribution to human warmth sensation or an indirect contribution such as regulating the local blood flow (Dhaka et al., 2006). Humans can detect temperature increase or decrease of less than 1 °C (Kenshalo, 1970; Jamal et al., 1985; Fowler et al., 1987; Stevens and Choo, 1998), even when the skin temperature is adapted to different temperatures (Kenshalo, 1970). The current findings of the sensory adaptation of TRPV4 in cultured cells to different temperatures may help to explain the changes in human warmth sensation depending on the skin temperature.

Previous study showed that the heat response under hypoosmolarity was greater than that under isoosmolarity (Güler et al., 2002). Related to

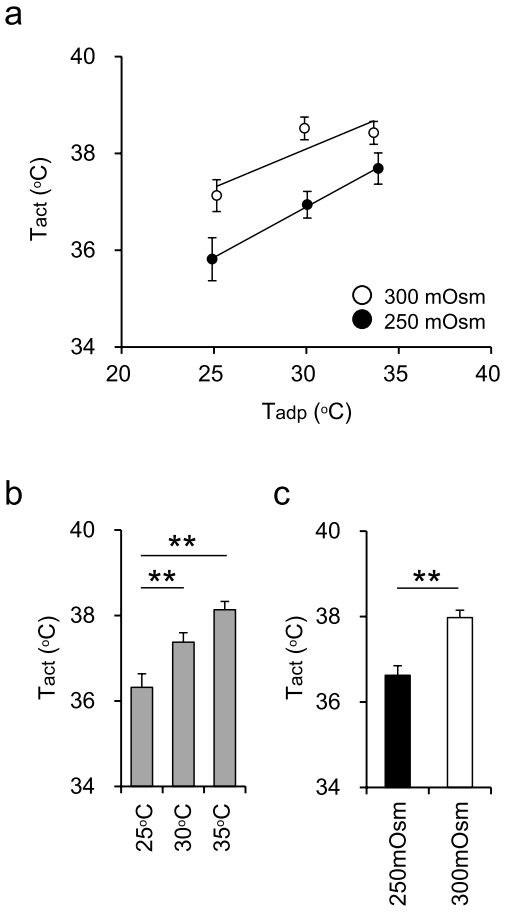

Fig. 7. Activation temperature under adaptation to each condition. a: activation temperatures under all temperature conditions with 250 and 300 mOsm. n = 24–63 in 10–14 trials. mean ± SEM with linear regression line. b: activation temperatures under each temperature condition which includes data under 250 and 300 mOsm conditions. mean + SEM. c: activation temperatures under 250 and 300 mOsm conditions which include data under all temperature conditions. mean + SEM. **p < 0.01.

Table 1
Stepwise regression analysis explaining activation temperature with adaptation temperature and osmolarity. The result was calculated with the data under 25–35 °C with 250 and 300 mOsm conditions. B: unstandardized regression coefficient, SE: standard error, β: standardized regression coefficient, T_{adp}: adaptation temperature.

	B	SE	β	p	R^2
T_{adp}	0.20	0.04	0.29	< 0.001	0.15
Osmolarity	0.02	0.01	0.24	< 0.001	

this report, we showed that T_{act} by moderate heat decreased with the adaptation to hypoosmolarity in the range of 25–35 °C. The result of previous study may have a relation to the decrease of T_{act}. In addition, we confirmed that heat responses were inhibited with the adaptation to hyperosmolarity in the range of 25–35 °C. Hyperosmolarity can occur due to dehydration after sweating, and it inhibits thermoregulation such as sweating and vasodilation in the skin (Takamata et al., 2001). The inhibition of the heat response of TRPV4 in the skin may be involved in this inhibition mechanism of thermoregulation under dehydration.

Under the present experimental conditions, T_{act} of TRPV4 under the combination of reduced temperature and hypoosmolarity was the lowest, however, there was no significant interaction of temperature and osmolarity on T_{act}. Distinct pathways have been reported to activate

TRPV4 under stimulation of heat and cell swelling (Vriens et al., 2004). Therefore, it is suggested that each adaptation condition influenced TRPV4 independently. TRPV4 is expressed in the skin area and activated by a combination of several stimuli. Thus, the findings may contribute to understand the roles of TRPV4 in the variation of several skin functions such as skin barrier formation in skin physiology and temperature sensitivity in thermal sensation and thermoregulation. However, to better understand the specific contribution of TRPV4 to these skin functions, it is necessary to elucidate the contributions of other candidates in the skin, such as TRPV6 (Bianco et al., 2007) and TRP melastatin 4 (TRPM4) (Wang et al., 2019) in skin formation, and TRPV3 (Peier et al., 2002; Smith et al., 2002; Xu et al., 2002) and TRPM2 (Tan and McNaughton, 2016) in innocuous warmth sensation.

We demonstrated the decrease in T_{act} under the adaptation to hypoosmolarity for 5–10 min. TRPV4 is activated by hypoosmolarity (Liedtke et al., 2000; Strotmann et al., 2000; Wissenbach et al., 2000; Güler et al., 2002) or cell swelling (Toft-Bertelsen et al., 2017). After cell swelling, a system to recover the cell volume involves TRPV4 (Hoffmann et al., 2009; Becker et al., 2005). However, in an experiment, cell volume was not fully recovered upon transfection with TRPV4 (Becker et al., 2005), suggesting a requirement for other factors for cell volume regulation. In addition, we examined the heat response of TRPV4 in cultured cells using Ca^{2+} imaging and hypoosmotic or hyperosmotic solutions adjusted by NaCl or mannitol. Osmotic changes in the extracellular fluid in the skin can occur with changes in ion concentrations as well as changes in water volume. Therefore, to gain further insight into the contribution of TRPV4 to several skin functions, in vivo studies including neurological or behavioral studies performed under real-life conditions are required.

5. Conclusions

In summary, we found that the temperature to activate TRPV4 in cultured cells increased with the elevation of adaptation temperature, and decreased with the adaptation to hypoosmolarity in the range of 25–35 °C. In addition, the heat response was inhibited with hyperosmolarity in the range of 25–35 °C. Thus, the activation temperature of TRPV4 varies with the temporal sensory adaptation to different temperature and osmolarity conditions. These findings may contribute to understand the variation of several TRPV4-mediated skin functions.

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Declaration of competing interest

The authors are employees of Toyota Central R&D Labs, Inc. (who also provided the funding for this study), which is funded by its stockholder companies (https://www.tytlabs.com/company/profile.html). There are no patents, products in development, or marketed products to declare.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jtherbio.2019.102424.

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