



Lack of transient receptor potential melastatin 8 activation by phthalate esters that enhance contact hypersensitivity in mice

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HIGHLIGHTS

- Several phthalate esters enhance skin sensitization in mouse models.
- These phthalate esters activate TRPA1 and TRPV1 but do not activate TRPM8.
- A TRPA1-specific antagonist, HC030031, does not inhibit TRPM8 activation.
- Phthalate esters activate TRP channels with a subtype-specific manner.
- TRPM8 activation is not likely to be involved in the skin sensitization process.

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ABSTRACT

We studied the involvement of sensory neurons in skin sensitization to allergens using a mouse model in which the T-helper type 2 response is essential. Skin sensitization to fluorescein isothiocyanate (FITC) has been shown to be enhanced by several phthalate esters, including dibutyl phthalate (DBP). For different types of phthalate esters, we found a correlation between the ability of transient receptor potential (TRP) A1 activation and that of enhancing skin sensitization. A TRPA1-specific antagonist, HC-030031, was shown to suppress skin sensitization in the presence of DBP. However, since phthalate esters also activate TRPV1, phthalate esters could activate other types of TRP channels non-selectively. Furthermore, sensitization to FITC is also enhanced by menthol, which activates TRPA1 and TRPM8. Here we established an *in vitro* system for measuring TRPM8 activation. The selectivity for TRPM8 was established by the fact that two TRPM8 agonists (menthol and icilin) induced calcium mobilization, whereas agonists of TRPA1 and TRPV1 did not. We demonstrated that phthalate esters do not activate TRPM8. TRPA1-antagonist HC-030031 did not inhibit TRPM8 activation induced by menthol or icilin. These results show that phthalate esters activate TRPA1 and TRPV1 with selectivity. TRPM8 activation is not likely to be involved in the sensitization to FITC.

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1. Introduction

The involvement of sensory neurons in allergic sensitization has been attracting much attention (Palm et al., 2012), especially in connection with the T-helper type 2 (Th2)-driven immune responses.

Abbreviations: CHS, contact hypersensitivity; DBP, dibutyl phthalate; DEHP, di(2-ethylhexyl)phthalate; DEP, diethyl phthalate; DHPP, diheptyl phthalate; DMP, dimethyl phthalate; DPNP, di-*n*-pentyl phthalate; DPP, di-*n*-propyl phthalate; TRPA1, transient receptor potential ankylin 1; TRPM8, transient receptor potential melastatin 8; TRPV1, transient receptor potential vanilloid 1.

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The fluorescein isothiocyanate (FITC)-induced contact hypersensitivity (CHS) mouse model has been recognized as a Th2-type skin sensitization model (Dearman and Kimber, 2000; Maruyama et al., 2007b; Takeshita et al., 2004). Experimentally, dibutyl phthalate (DBP) has been empirically added to solvents for fluorescein isothiocyanate (FITC) (Kripke et al., 1990). In this model, we found some types of phthalate esters including DBP that enhance skin sensitization to FITC (Imai et al., 2006; Sato et al., 1998; Shiba et al., 2009). As to a role of DBP in FITC-induced CHS, there have been reports demonstrating that the production of thymic stromal lymphopoietin (TSLP) is induced in keratinocytes by DBP (Larson et al., 2010; Shigeno et al., 2009). Because TSLP is known as a cytokine associated with Th2-type responses (Ziegler, 2010), such an effect of DBP may contribute to enhanced Th2-driven immune responses.

Another possible role of DBP in skin sensitization could be the delivery of FITC to pilosebaceous units in the skin (Simonsson et al., 2012). However, the molecular targets of DBP leading to enhanced sensitization have not been fully identified.

Phthalate esters are widely used as plasticizers for plastics, synthetic leather, vinyl flooring, wall coverings, paint, adhesive agents and cosmetics. Certain types of phthalate esters in house dust have been demonstrated to be associated with allergic diseases among children (Bornehag et al., 2004; Kolarik et al., 2008).

We have been studying the mechanism underlying the phthalate ester-induced enhancement of skin sensitization using FITC-induced CHS. The initial study that suggested involvement of the nervous system included experiments in which the initiation of dermal dendritic cell (DC)-trafficking was measured using an organ culture system (Chun et al., 2000). As a DC marker, we used mouse macrophage C-type lectin mMGL (CD301), which was subsequently shown to be expressed on DC as well (Denda-Nagai et al., 2002). When DBP was epicutaneously applied on mouse skin and then skin explants were cultured *in vitro*, mMGL-positive cells disappeared during organ culture, presumably due to movement out of the skin explants. In contrast, when DBP was applied on the skin explants after dissection, the mMGL-positive cell number in the dermis did not change. These results indicated that the integrity of the whole animal is required for the reception of the effects of DBP (Chun et al., 2000). Subsequently, we examined the effect of desensitization of nociceptive receptors. When mice were epicutaneously treated with allyl isothiocyanate (AITC) or capsaicin before sensitization to FITC in the presence of DBP, FITC-induced CHS was attenuated (Maruyama et al., 2007a). Such pre-treatment also decreased the trafficking of FITC-presenting DC to draining lymph nodes. Furthermore, subcutaneous injection of a calcitonin gene-related peptide (CGRP) antagonist at the skin site of sensitization suppressed FITC-induced CHS (Maruyama et al., 2007a). Collectively, these results suggested the involvement of the peripheral nerve system in the enhancing effect of DBP on the sensitization phase of CHS.

As a molecular mechanism underlying the enhancing effect of phthalate esters on FITC-sensitization, we have proposed the involvement of activation of transient receptor potential ankyrin 1 (TRPA1) on sensory neurons in the skin sensitization process. As mentioned above, pre-treatment of skin with AITC, an agonist of TRPA1, attenuated the effect of DBP (Maruyama et al., 2007a). We then directly found that DBP activates TRPA1 in *in vitro* experiments involving nerve cells isolated from mouse dorsal root ganglia as well as cultured cells transfected with TRPA1 cDNA (Imai et al., 2006; Shiba et al., 2009).

Transient receptor potential (TRP) channels are calcium-permeable cation channels. They constitute a family of channel molecules on sensory neurons (Clapham, 2003). We have provided several lines of evidence showing a connection between TRPA1 activation and enhanced skin sensitization. First, by comparing phthalate esters with different alkyl chain lengths, we found that phthalate esters that enhance sensitization to FITC activate TRPA1 *in vitro* (Shiba et al., 2009). Second, known agonists of TRPA1 (cinnamaldehyde, carvacrol and menthol) enhanced the skin sensitization to FITC (Shiba et al., 2012). Third, pre-treatment with a TRPA1-selective antagonist, HC-030031 (Eid et al., 2008), suppressed the skin sensitization to FITC in the presence of DBP (Shiba et al., 2012).

However, we found that phthalate esters also activate transient receptor potential vanilloid 1 (TRPV1) channels, another type of molecule in the TRP family, *in vitro* (Shiba et al., 2009). One may wonder whether phthalate esters selectively activate TRPA1 and TRPV1 or not. In other words, phthalate esters may activate TRP channels non-selectively regardless of their subtype. Although menthol was shown to enhance skin sensitization to FITC, menthol is known not only as a TRPA1 agonist (Karashima et al., 2007),

but also as an agonist of transient receptor potential melastatin 8 (TRPM8) (Bautista et al., 2007). It is possible that the effect of menthol on skin sensitization is mediated by TRPM8 activation. If so, there is a possibility that TRPM8 may also be involved in the enhancing effect on skin sensitization.

To examine the selectivity of phthalate esters as to TRP channel subtypes, we determined whether or not phthalate esters activate TRPM8 *in vitro*. We also compared several types of phthalate esters with or without enhancing activity toward FITC-induced CHS as to the TRPM8-agonistic activity. Finally, we examined the TRPA1-specificity of HC-030031 as an antagonist by examining its effect on TRPM8 activation.

2. Materials and methods

2.1. Chemicals and reagents

L-(−)-Menthol, allyl isothiocyanate (AITC), dimethyl phthalate (DMP), diethyl phthalate (DEP), DBP, diheptyl phthalate (DHPP), di(2-ethylhexyl)phthalate (DEHP), and kanamycin sulfate were purchased from Wako Pure Chemicals (Osaka, Japan); di-*n*-propyl phthalate (DPP) and di-*n*-pentyl phthalate (DPNP) from Kanto Chemicals (Tokyo, Japan); and capsaicin (CAP), ionomycin, bovine serum albumin (BSA; fraction V), Ham's F-12 medium, icilin and probenecid from Sigma-Aldrich (St. Louis, MO). Dulbecco's modified Eagle's medium (DMEM) was purchased from Nissui Pharmaceuticals (Tokyo, Japan); Fluo-4-AM and *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) from Dojindo Laboratories (Kumamoto, Japan); dimethylsulfoxide (DMSO) and Opti-MEM 1 from Nacalai Tesque (Kyoto, Japan); blasticidin, tetracycline, zeocin and Hanks' balanced salt solution (HBSS) from Life Technologies (Carlsbad, CA); fetal bovine serum (FBS) from Hyclone (South Logan, UT); 2-(1,3-dimethyl-2,6-dioxo-1,2,3,6-tetrahydro-7H-purin-7-yl)-*N*-(4-isopropylphenyl)acetamide (HC-030031) from ENZO Life Sciences (Farmingdale, NY); and FuGENE 6 Transfection Reagent from Roche Diagnostics (Indianapolis, IN).

2.2. Stable expression of TRP channels on CHO cells

Chinese hamster ovary (CHO) cells stably expressing TRPM8 were prepared according to the method described previously for TRPA1 expression (Shiba et al., 2009). Mouse TRPM8 (mTRPM8) cDNA was prepared by the RT-PCR method. Total RNA from mouse dorsal root ganglion cells was isolated using ISOGEN (Nippon Gene, Tokyo, Japan). The first strand cDNAs were synthesized from mRNA with reverse-transcriptase SuperScript III (Life Technologies) using oligo dT primers. The mTRPM8 cDNA was amplified with Phusion DNA Polymerase (New England Biolabs; Ipswich, MA) using a forward and reverse primer set with a restriction enzyme recognition site: mTRPM8 forward-*Xho*I (5'-CCGCTCGAGATGCTCTTCGAGGGAGCCAG-3') and mTRPM8 reverse-*Xba*I (5'-GCTCTAGACGCCAGCCTTACTTGATGTT-3'). The underline nucleotides refer to restriction sites. The PCR products were ligated into the *Xho*I-*Xba*I sites of pcDNA4/TO (Life Technologies), and the DNA sequence was verified. The resulting construct was transfected into T-REx CHO cells (Life Technologies), which stably expressed the tetracyclin repressor protein transcribed from pcDNA6TR in the cells, by means of FuGENE 6. The cells were cultured in Opti-MEM 1 for 24 h at 37 °C under an atmosphere of 5% CO₂/95% air. After selection and cloning in the presence of 200 µg/ml zeocin (for pcDNA4/TO) and 5 µg/ml blasticidin (for pcDNA6/TR), we obtained CHO cell clones stably expressing TRPM8. TRPM8-expressing CHO cells were maintained in Ham's F-12 medium/DMEM (1:1) supplemented with 10% FBS, 10 mM HEPES (pH 7.4), 60 µg/ml kanamycin sulfate, 200 µg/ml zeocin and 5 µg/ml blasticidin.

2.3. Calcium mobilization

TRPM8-expressing CHO cells were cultured at a density of 4×10^4 cells/well of a 96-well plate (#3340; Corning Inc., Corning, NY) for 24 h at 37 °C in the presence of 1 µg/ml tetracycline to induce TRPM8 expression. The cells were then loaded with 3 µM Fluo 4-AM in HBSS supplemented with 20 mM HEPES (pH 7.4), 0.1% BSA and 2.5 mM probenecid for 60 min at 37 °C. Measurements were performed with a fluorometric imaging plate reader, FLEXstation II (Molecular Devices, Sunnyvale, CA), as described (Shiba et al., 2009). A sample was added at 30 s and ionomycin (5 µM) was added at 150 s after the start of measurement. Ionomycin was used to determine the maximum level of calcium. Samples were dissolved in DMSO to make stock solutions. The stock solutions were diluted on the day of the experiment. The final concentration of DMSO did not exceed 0.1%.

2.4. Statistics

Data are summarized as means ± standard errors of means (SEMs). Dose-response relationships were investigated with the use of William's test. Differences in the responses between conditions at each dose were analyzed with the use of the unpaired Student's *t*-test with Bonferroni's correction for multiplicity.

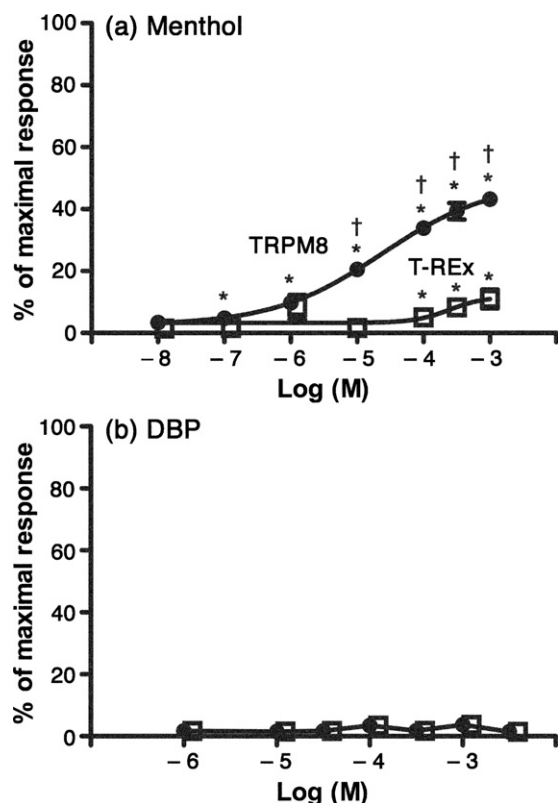


Fig. 1. Elevation of the intracellular calcium level in TRPM8-CHO cells. The concentrations (abscissa) of menthol (a) or DBP (b) are plotted against the percentages of the maximal calcium responses (ordinate) in TRPM8-CHO (closed circles) or control T-Rex CHO (open squares). Each datum is the mean $\pm$ SEM ($n=4$) (* $p<0.05$ for dose–response relationships with William's test; † $p<0.05$ for comparison of the response at each dose between TRPM8-CHO and T-Rex CHO with the t -test with Bonferroni's correction for multiplicity).

All probability values were based on the two-sided test, and values of $p<0.05$ were considered statistically significant. Statistical analyses were performed using R statistical software (Version 2.12.2; The R Foundation for Statistical Computing, Boston, MA) and Graphpad Prism 5 (Version 5.02; Graphpad Software, San Diego, CA).

3. Results

3.1. Menthol but not DBP induces calcium influx into cells expressing TRPM8

We selected CHO cell clones expressing functional TRPM8 by means of calcium mobilization induced by menthol. Using one such clone, menthol was shown to induce calcium mobilization in a dose-dependent manner (Fig. 1a). The dose–response relationships were statistically significant between 10⁻⁷ and 10⁻³ M menthol according to William's test. In contrast, only high concentrations of menthol induced calcium mobilization in CHO cells that lacked TRPM8 expression (T-Rex CHO), the dose–response relationships being statistically significant between 10⁻⁴ and 10⁻³ M. Menthol induced a higher calcium response in TRPM8-expressing CHO cells (TRPM8-CHO) than in T-Rex CHO. The differences were statistically significant between 10⁻⁵ and 10⁻³ M menthol. These results indicate that the menthol-induced calcium response is dependent on TRPM8 expression. EC₅₀ for menthol was calculated to be 23.9 μ M. Under the same conditions, DBP did not induce calcium mobilization into CHO cells regardless of TRPM8 expression (Fig. 1b). Neither TRPM8-CHO nor T-Rex CHO exhibited a statistically significant dose–response relationship as high as with 3 $\times$ 10⁻³ M DBP.

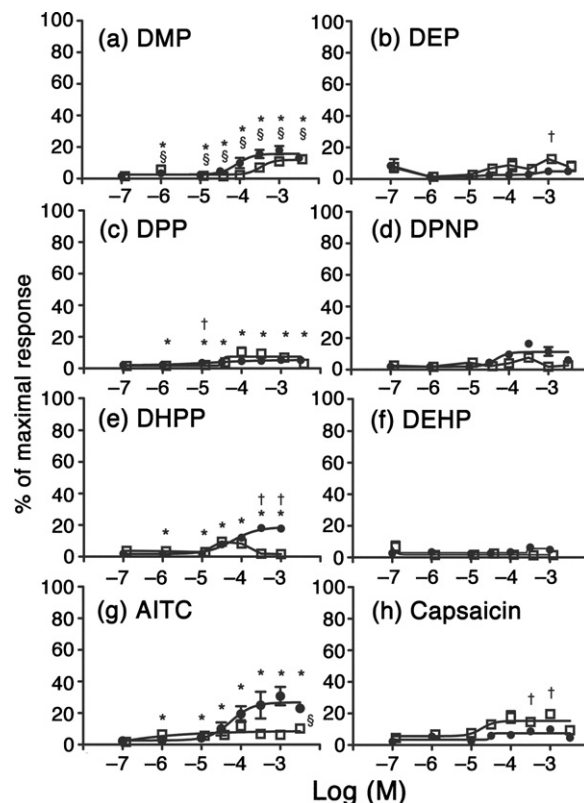


Fig. 2. Intracellular calcium levels in TRPM8-CHO cells upon treatment with various phthalate esters. The concentrations (abscissa) of samples are plotted against the percentages of the maximal calcium responses (ordinate) in TRPM8-CHO (closed circles) or control T-Rex CHO (open squares). The samples were DMP (a), DEP (b), DPP (c), DPNP (d), DHPP (e), and DEHP (f). A TRPA1 agonist, AITC (g), and a TRPV1 agonist, capsaicin (h), were also tested. Each datum is the mean $\pm$ SEM ($n=4$) (* $p<0.05$ for dose–response relationships in TRPM8-CHO; † $p<0.05$ for comparison of the response at each dose between TRPM8-CHO and T-Rex CHO with the t -test with Bonferroni's correction for multiplicity).

3.2. Lack of effects of various phthalate esters on calcium influx into TRPM8-expressing cells

We then examined whether or not some phthalate esters might induce calcium mobilization into TRPM8-CHO. As to DMP, statistically significant dose–response relationships were observed for TRPM8-CHO and T-Rex CHO. However, no statistically significant difference in the calcium response was found between these two cell lines (Fig. 2a). DEP did not show dose–response relationships in either TRPM8-CHO or T-Rex CHO. With 10⁻³ M DEP, there was a statistically significant difference between TRPM8-CHO and T-Rex CHO. However, the response was higher in T-Rex CHO, and the calcium mobilization was at a background level (Fig. 2b). In the case of DPP, statistically significant dose–response relationships were observed for TRPM8-CHO but not for T-Rex CHO. With 10⁻⁵ M DPP, there was a statistically significant difference between TRPM8-CHO and T-Rex CHO, while the actual value was at a background level (Fig. 2c). For DPNP and DEHP, neither statistically significant dose–response relationships nor a difference between TRPM8-CHO and T-Rex CHO was observed (Fig. 2d and f). In the case of DHPP, statistically significant dose–response relationships were observed for TRPM8-CHO. There were also statistically significant differences between TRPM8-CHO and T-Rex CHO with 3 $\times$ 10⁻⁴ and 10⁻³ M (Fig. 2e). At such high concentrations, DHPP may exhibit some activity toward TRPM8. Overall, DMP, DEP, DPP, DPNP, DHPP and DEHP did not induce significant responses (Fig. 2a–f), with some exceptions at high concentrations of DHPP.

Known agonists of TRPA1 (AITC) (Jordt et al., 2004) and TRPV1 (capsaicin) (Caterina et al., 1997) were examined in the present system. Dose–response relationships were observed in TRPM8-CHO between 10^{-6} and 3×10^{-3} M, and in T-Rex CHO with 3×10^{-3} M AITC (Fig. 2g). However, there was no statistically significant difference in the response between TRPM8-CHO and T-Rex CHO at any dose. Therefore, AITC did not exhibit a TRPM8-specific response in the present study. There was no dose–response relationship in the case of capsaicin (Fig. 2h). There were statistically significant differences between TRPM8-CHO and T-Rex CHO with 3×10^{-4} and 10^{-3} M capsaicin. However, the responses in T-Rex CHO were higher with these doses. Therefore, capsaicin did not exhibit a TRPM8-specific response. These results also validated the present experimental systems.

3.3. Calcium mobilization into TRPM8-expressing cells is not inhibited by a TRPA1 antagonist

To confirm the specificity of the experimental systems, we examined the effects of a TRPA1 antagonist, HC-030031, which was shown to inhibit TRPA1 activation in our previous study (Shiba et al., 2012). The calcium mobilization induced by 30 μ M menthol was compared in the presence of various concentrations of HC-030031. There were no dose–response relationships in the responses to HC-030031 (10^{-8} to 10^{-4} M). There was a significant difference in the calcium mobilization between TRPM8-CHO cells and T-Rex CHO ones at each concentration of HC-030031 (Fig. 3a). The results indicated that HC-030031 did not inhibit TRPM8-mediated calcium mobilization. We further examined the effect of HC-030031 on TRPM8 activation induced by another agonist, icilin. The dose–response relationships for various icilin concentrations were observed in the absence or presence of 3 μ M or 30 μ M HC-030031. There was no difference in the calcium mobilization regardless of the presence or absence of HC-030031 except in the responses between 30 μ M and 0 μ M HC-030031 at icilin concentrations of 3×10^{-6} and 10^{-5} M (Fig. 3b). However, the responses in the presence of 30 μ M HC-030031 were higher than those in its absence. Therefore the results indicated that HC-030031 did not inhibit calcium mobilization into TRPM8-CHO cells induced by icilin.

4. Discussion

We produced CHO cells stably expressing TRPM8 to examine whether or not DBP could activate TRP channels other than TRPA1 and TRPV1. We selected clones that responded to menthol. The specificity of TRPM8-CHO cells was confirmed in experiments showing that neither AITC nor capsaicin induced significant calcium mobilization (Fig. 2).

Menthol and icilin (Chuang et al., 2004) induced calcium mobilization in TRPM8-CHO cells in a dose-dependent manner (Figs. 1 and 3). On the other hand, DBP at 1 μ M to 3 mM did not induce calcium mobilization (Fig. 1). We have already shown that both menthol and DBP induce calcium mobilization into TRPA1-expressing CHO cells (Shiba et al., 2012). The lack of TRPM8 activation by DBP *in vitro* suggests that TRPM8 activation is not necessary for the enhancement of skin sensitization.

We also demonstrated that various phthalate esters did not induce calcium mobilization in TRPM8-CHO cells. This is true for phthalate esters regardless of whether or not they exhibit enhancing activity toward FITC-induced CHS. Thus, neither phthalate esters with enhancing activity (DEP, DPP, DBP and DPNP) nor ones without such activity (DMP and DEHP) exhibited TRPM8 activation. A lack of TRPM8 activation has been observed for phthalate esters that have been shown to activate TRPA1 (DEP, DPP, DBP and

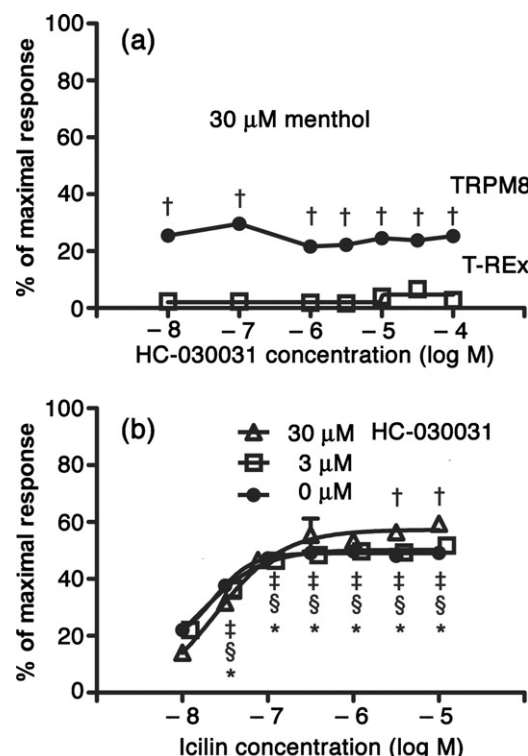


Fig. 3. Lack of inhibition of the calcium response in TRPM8-CHO cells by a selective TRPA1 antagonist, HC-030031. (a) Effects of varying concentrations of HC-030031 (abscissa) on the calcium response (ordinate) induced by 30 μ M menthol. The calcium mobilization into TRPM8-CHO (closed circles) or control T-Rex CHO (open squares) is shown. Each datum is the mean $\pm$ SEM ($n = 4$) (* $p < 0.05$ for dose–response relationships with William's test; $^\dagger p < 0.05$ for comparison of the response at each dose between TRPM8 and T-Rex with the t -test with Bonferroni's correction for multiplicity). (b) Effects of varying concentrations of icilin (abscissa) on the calcium response (ordinate) of TRPM8-CHO cells in the presence of 3 μ M (open squares) or 30 μ M (open triangles), or the absence (close circles) of HC-030031. Each datum is the mean $\pm$ SEM ($n = 4$). The symbols † , § and * denote $p < 0.05$ for the dose–response relationships as to icilin concentration in the presence of 30, 3 and 0 μ M HC-030031, respectively, with William's test; † denotes $p < 0.05$ between 0 and 30 μ M HC-030031 with the t -test with Bonferroni's correction for multiplicity.

DPNP) or TRPV1 (DPP, DBP and DPNP) (Shiba et al., 2009). Only DHPP appeared to show significantly higher calcium mobilization in TRPM8-CHO cells than in T-Rex CHO cells. However, high concentrations of DHPP were required, and the levels of the responses were low.

Calcium mobilization induced by menthol or icilin in TRPM8-expressing CHO cells is not inhibited by HC-030031, a TRPA1-specific antagonist (Eid et al., 2008). Similar dose–response curves as to icilin concentration were obtained in the presence of 0 μ M, 3 μ M or 30 μ M HC-030031. This dose range of HC-030031 has been shown to affect calcium mobilization into TRPA1-expressing CHO cells induced by AITC (Shiba et al., 2012). Thus, the higher the concentration of HC-030031 is, the more AITC is required to obtain the same level of calcium mobilization. The lack of an inhibitory effect of HC-030031 on TRPM8 activation indicated that the inhibitory effect of HC-030031 on skin sensitization is not mediated by TRPM8 inhibition.

Although further studies are needed, there are some evidences as to how activation of sensory neurons through TRPA1 can enhance the sensitization process. As mentioned earlier, we have shown that neuropeptides such as CGRP may be involved in enhanced sensitization to FITC in the presence of DBP (Maruyama et al., 2007a). CGRP is known to be released from peripheral nerve endings following TRPA1 activation (Bautista et al., 2005). Mast cells are reported to produce TNF- α in response to CGRP (Niizeki

et al., 1997). TNF- α on the surface of mast cells was shown to promote DC migration (Suto et al., 2006). Others demonstrated that CGRP enhances FITC-induced CHS while it suppresses 2,4,6-trinitrochlorobenzene (TNCB)-induced CHS (Mikami et al., 2011). There is a possibility that neuropeptides may differently influence the sensitization phase depending on the nature of haptens and immune responses. In this context, it has been shown that FITC-induced CHS is Th2-driven (Dearman and Kimber, 2000; Maruyama et al., 2007b; Takeshita et al., 2004) while TNCB-induced CHS is Th1-driven (Hayashi et al., 2001).

In conclusion, TRPM8 activation is not likely to be involved in the enhancement of skin sensitization to FITC. We also demonstrated that not all TRP channel subtypes are activated by phthalate esters, but that TRPA1 and TRPV1 are selectively activated.

Conflict of interest

None declared.

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References

- Bautista, D.M., Movahed, P., Hinman, A., Axelsson, H.E., Sterner, O., Högestätt, E.D., Julius, D., Jordt, S.E., Zygmunt, P.M., 2005. Pungent products from garlic activate the sensory ion channel TRPA1. *Proceedings of the National Academy of Sciences of the United States of America* 102, 12248–12252.
- Bautista, D.M., Siemens, J., Glazer, J.M., Tsuruda, P.R., Basbaum, A.I., Stucky, C.L., Jordt, S.E., Julius, D., 2007. The menthol receptor TRPM8 is the principal detector of environmental cold. *Nature* 448, 204–208.
- Bornehag, C.G., Sundell, J., Weschler, C.J., Sigsgaard, T., Lundgren, B., Hasselgren, M., Hagerhed-Engman, L., 2004. The association between asthma and allergic symptoms in children and phthalates in house dust: a nested case–control study. *Environmental Health Perspectives* 112, 1393–1397.
- Caterina, M.J., Schumacher, M.A., Tominaga, M., Rosen, T.A., Levine, J.D., Julius, D., 1997. The capsaicin receptor: a heat-activated ion channel in the pain pathway. *Nature* 389, 816–824.
- Chuang, H.H., Neuhauser, W.M., Julius, D., 2004. The super-cooling agent icilin reveals a mechanism of coincidence detection by a temperature-sensitive TRP channel. *Neuron* 43, 859–869.
- Chun, K.H., Imai, Y., Higashi, N., Irimura, T., 2000. Migration of dermal cells expressing a macrophage C-type lectin during the sensitization phase of delayed-type hypersensitivity. *Journal of Leukocyte Biology* 68, 471–478.
- Clapham, D.E., 2003. TRP channels as cellular sensors. *Nature* 426, 517–524.
- Dearman, R.J., Kimber, I., 2000. Role of CD4⁺ T helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. *Immunology* 101, 442–451.
- Denda-Nagai, K., Kubota, N., Tsuiji, M., Kamata, M., Irimura, T., 2002. Macrophage C-type lectin on bone marrow-derived immature dendritic cells is involved in the internalization of glycosylated antigens. *Glycobiology* 12, 443–450.
- Eid, S.R., Crown, E.D., Moore, E.L., Liang, H.A., Choong, K.C., Dima, S., Henze, D.A., Kane, S.A., Urban, M.O., 2008. HC-030031, a TRPA1 selective antagonist, attenuates inflammatory- and neuropathy-induced mechanical hypersensitivity. *Molecular Pain* 4, 48.
- Hayashi, M., Higashi, K., Kato, H., Kaneko, H., 2001. Assessment of preferential Th1 or Th2 induction by low-molecular-weight compounds using a reverse transcription-polymerase chain reaction method: comparison of two mouse strains, C57BL/6 and BALB/c. *Toxicology and Applied Pharmacology* 177, 38–45.
- Imai, Y., Kondo, A., Iizuka, H., Maruyama, T., Kurohane, K., 2006. Effects of phthalate esters on the sensitization phase of contact hypersensitivity induced by fluorescein isothiocyanate. *Clinical and Experimental Allergy* 36, 1462–1468.
- Jordt, S.E., Bautista, D.M., Chuang, H.H., McKemy, D.D., Zygmunt, P.M., Högestätt, E.D., Meng, I.D., Julius, D., 2004. Mustard oils and cannabinoids excite sensory nerve fibres through the TRP channel ANKTM1. *Nature* 427, 260–265.
- Karashima, Y., Damann, N., Prenen, J., Talavera, K., Segal, A., Voets, T., Nilius, B., 2007. Bimodal action of menthol on the transient receptor potential channel TRPA1. *Journal of Neuroscience* 27, 9874–9884.
- Kolarik, B., Naydenov, K., Larsson, M., Bornehag, C.G., Sundell, J., 2008. The association between phthalates in dust and allergic diseases among Bulgarian children. *Environmental Health Perspectives* 116, 98–103.
- Kripke, M.L., Munn, C.G., Jeevan, A., Tang, J.M., Bucana, C., 1990. Evidence that cutaneous antigen-presenting cells migrate to regional lymph nodes during contact sensitization. *Journal of Immunology* 145, 2833–2838.
- Larson, R.P., Zimmerli, S.C., Comeau, M.R., Itano, A., Omori, M., Iseki, M., Hauser, C., Ziegler, S.F., 2010. Dibutyl phthalate-induced thymic stromal lymphopoietin is required for Th2 contact hypersensitivity responses. *Journal of Immunology* 184, 2974–2984.
- Maruyama, T., Iizuka, H., Tobisawa, Y., Shiba, T., Matsuda, T., Kurohane, K., Imai, Y., 2007a. Influence of local treatments with capsaicin or allyl isothiocyanate in the sensitization phase of a fluorescein-isothiocyanate-induced contact sensitivity model. *International Archives of Allergy and Immunology* 143, 144–154.
- Maruyama, T., Shiba, T., Iizuka, H., Matsuda, T., Kurohane, K., Imai, Y., 2007b. Effects of phthalate esters on dendritic cell subsets and interleukin-4 production in fluorescein isothiocyanate-induced contact hypersensitivity. *Microbiology and Immunology* 51, 321–326.
- Mikami, N., Matsushita, H., Kato, T., Kawasaki, R., Sawazaki, T., Kishimoto, T., Ogita, Y., Watanabe, K., Miyagi, Y., Sueda, K., Fukada, S.I., Yamamoto, H., Tsujikawa, K., 2011. Calcitonin gene-related peptide is an important regulator of cutaneous immunity: effect on dendritic cell and T cell functions. *Journal of Immunology* 186, 6886–6893.
- Niizeki, H., Alard, P., Streilein, J.W., 1997. Calcitonin gene-related peptide is necessary for ultraviolet B-impaired induction of contact hypersensitivity. *Journal of Immunology* 159, 5183–5186.
- Palm, N.W., Rosenstein, R.K., Medzhitov, R., 2012. Allergic host defences. *Nature* 484, 465–472.
- Sato, K., Imai, Y., Irimura, T., 1998. Contribution of dermal macrophage trafficking in the sensitization phase of contact hypersensitivity. *Journal of Immunology* 161, 6835–6844.
- Shiba, T., Maruyama, T., Kurohane, K., Iwasaki, Y., Watanabe, T., Imai, Y., 2009. TRPA1 and TRPV1 activation is a novel adjuvant effect mechanism in contact hypersensitivity. *Journal of Neuroimmunology* 207, 66–74.
- Shiba, T., Tamai, T., Sahara, Y., Kurohane, K., Watanabe, T., Imai, Y., 2012. Transient receptor potential ankyrin 1 activation enhances hapten sensitization in a T-helper type 2-driven fluorescein isothiocyanate-induced contact hypersensitivity mouse model. *Toxicology and Applied Pharmacology* 264, 370–376.
- Shigeno, T., Katakuse, M., Fujita, T., Mukoyama, Y., Watanabe, H., 2009. Phthalate ester-induced thymic stromal lymphopoietin mediates allergic dermatitis in mice. *Immunology* 128, e849–e857.
- Simonsson, C., Stenfeldt, A.L., Karlberg, A.T., Ericson, M.B., Jonsson, C.A., 2012. The pilosebaceous unit—a phthalate-induced pathway to skin sensitization. *Toxicology and Applied Pharmacology* 264, 114–120.
- Suto, H., Nakae, S., Kakurai, M., Sedgwick, J.D., Tsai, M., Galli, S.J., 2006. Mast cell-associated TNF promotes dendritic cell migration. *Journal of Immunology* 176, 4102–4112.
- Takeshita, K., Yamasaki, T., Akira, S., Gantner, F., Bacon, K.B., 2004. Essential role of MHC II-independent CD4⁺ T cells, IL-4 and STAT6 in contact hypersensitivity induced by fluorescein isothiocyanate in the mouse. *International Immunology* 16, 685–695.
- Ziegler, S.F., 2010. The role of thymic stromal lymphopoietin (TSLP) in allergic disorders. *Current Opinion in Immunology* 22, 795–799.