Galangal Pungent Component, I’-Acetoxychavicol Acetate, Activates TRPA1

Masataka NARUKAWA,1,2 Kanako KOIZUMI,1,2 Yusaku Iwasaki,1,2 Kikue KUBOTA,3 and Tatsuo WATANABE1,2,

1Graduate School of Nutritional and Environmental Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
2Global COE Program, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan
3Department of Nutrition and Food Science, Ochanomizu University, 2-1-1 Otsuka, Bunkyo-ku, Tokyo 112-8610, Japan

Received February 24, 2010; Accepted April 27, 2010; Online Publication, August 7, 2010
[doi:10.1271/bbb.100133]

We investigated the activation of transient receptor potential cation channel (TRP) subfamily V, member 1 (TRPV1) and TRP subfamily A, member 1 (TRPA1) by I’-acetoxychavicol acetate (ACA), the main pungent component in galangal. ACA did not activate TRPV1-expressing human embryonic kidney (HEK) cells, but strongly activated TRPA1-expressing HEK cells. ACA was more potent than allyl isothiocyanate, the typical TRPA1 agonist.

Key words: I’-acetoxychavicol acetate (ACA); transient receptor potential cation channel, subfamily A, member 1 (TRPA1); transient receptor potential cation channel, subfamily V, member 1 (TRPV1); intracellular Ca²⁺ concentration

Galangal, Alpinia galanga L. Swartz, is a perennial plant belonging to the Zingiberaceae family. The rhizomes and seeds of galangal are used as a spice and herbal medicine. Galangal has various pharmacological functions of antioxidative,1) antigastric ulcer,2) antibacterial,3) and antitumor effects.3) I’-Acetoxychavicol acetate (ACA) is known as the pungent component of galangal.4)

Many pungent substances like the hot pepper pungent component, capsaicin (CAP), and black pepper pungent component, piperine (PIP), act on the transient receptor potential cation channel, subfamily V, member 1 (TRPV1); intracellular Ca²⁺ concentration

The racemate of ACA was synthesized from 4-hydroxybenzaldehyde and vinyl magnesium bromide according to the method of Mitsui et al.2) Capsaicin (CAP) was purchased from Sigma (St. Louis, MO, USA), allyl isothiocyanate (AITC) was obtained from Wako Pure Chem. Ind. (Osaka, Japan), and HC030031 was purchased from ChemBridge (San Diego, CA, USA). All other chemicals were of guaranteed reagent grade.

Human TRPV1 and TRPA1 cDNA were amplified by a reverse transcriptase-polymerase chain reaction (RT-PCR), respectively using mRNA obtained from human brain first-strand cDNA (Agilent Technologies, Santa Clara, CA, USA) and human WI38 cells. Human TRPV1 cDNA was subcloned into pcDNA3 (Invitrogen, Carlsbad, CA, USA) and then transfected into human embryonic kidney (HEK)293T cells with the SuperFect transfection reagent (Qiagen, Hilden, Germany). After culturing in the presence of 750 µg/ml of G418, we obtained a stable HEK293T cell line expressing human TRPV1.

The expression of full-length human TRPA1 in stable HEK cells was induced with a tetracycline-inducible T-REX™ expression system from Invitrogen. hTRPA1 cDNA was subcloned into pcDNA4/TO (Invitrogen) and then transfected into HEK T-REX™ cells by using the Lipofectamine 2000 reagent (Invitrogen). HEK T-REX™ cells that stably maintained the hTRPA1 gene were selected by using 500 µg/ml of zeocin and 10 µg/ml of blasticidin, and grown according to the manufacturer’s instructions.

The intracellular Ca²⁺ concentration ([Ca²⁺]i) was measured with FlexStation™ II (Molecular Devices, Sunnyvale, CA, USA). The cells were seeded in 96-well plates 24 h before the assay. To obtain TRPA1-expressing HEK cells, 1 µg/ml of tetracycline was added to induce the expression of the TRPA1 protein. The cells were subcultured every week, and the highest passage number used was 30. The cells were loaded with 3 µM Fluo-4-AM (Molecular Probes, Eugene, OR, USA) for 1 h at 37°C in a loading buffer (5.57 mM KCl, 0.44 mM KH₂PO₄, 137 mM NaCl, 0.34 mM Na₂HPO₄·7H₂O, 5.56 mM d-glucose, 20 mM HEPES, 1 mM CaCl₂, 0.1% BSA, and 250 mM probenecid at pH 7.4). Dose-response

Abbreviations: ACA, I’-acetoxychavicol acetate; AITC, allyl isothiocyanate; [Ca²⁺]i, intracellular Ca²⁺ concentration; CAP, capsaicin; CPZ, capsazepine; HEK, human embryonic kidney; PIP, piperine; TRP, transient receptor potential; TRPA1, transient receptor potential cation channel, subfamily A, member 1; TRPV1, transient receptor potential cation channel, subfamily V, member 1

1 To whom correspondence should be addressed. Fax: 48-51-264-5550; E-mail: watanabe@u-shizuoka.ken.ac.jp
Curves for TRPV1 were obtained by using 1–300 μM ACA and 0.1 nM–10 μM CAP. We used 1 nM–30 μM ACA and 0.01–100 μM AITC for TRPA1. The antagonistic inhibition of TRPA1 activity was studied by adding 30 μM HC030031 to 1 μM ACA. The test compounds were prepared in dimethyl sulfoxide (DMSO) and added to the loading solution to a final DMSO concentration of 0.1% or 0.2%. Five μM ionomycin was added to each well to elicit the maximum fluorescence intensity. The data values for the test compounds are expressed as a percentage of the response to 5 μM ionomycin. Curves were fitted and parameters estimated with Prism 4.0a software (GraphPad Software, San Diego, CA, USA).

TRPV1-expressing HEK293T cells did not respond to 1–300 μM ACA (Fig. 2). However, ACA strongly increased [Ca²⁺]ᵢ in the TRPA1-expressing HEK T-REx™ cells (Fig. 3). The dose-response curve for ACA is shown in Fig. 3A. The EC₅₀ value for ACA (0.16 μM) was 3.8 times lower than that of the representative TRPA1 agonist, AITC (0.60 μM). The maximum activities of ACA and AITC were equal (ACA, 0.82; AITC, 0.86). The addition of TRPA1 antagonist HC030031 (30 μM) significantly decreased the Ca²⁺ response induced by ACA (Fig. 3B). In addition, these compounds did not increase [Ca²⁺]ᵢ in HEK T-REx™ cells that did not express TRPA1 (data not shown). These results indicate that ACA activated TRPA1, but not TRPV1. We have demonstrated in this study that ACA activated TRPA1 more strongly than the typical TRPA1 agonist, AITC.

Spices are known to exhibit energy metabolism-enhancing effects, TRPV1 being involved in this enhancing effect.¹⁴ TRPV1 agonists like CAP are known to increase the energy metabolism by stimulating the sympathetic nervous system. The agonists induce adrenaline secretion by this mechanism, and this secreted adrenaline acts on the β-adrenergic receptors in the liver and white adipose tissues (WAT), resulting in the decomposition of glycogen in the liver and of triglyceride in WAT, the energy metabolism being subsequently enhanced. It has been similarly reported that the activation of TRPA1 elevated the temperature of brown adipose tissue¹⁵ and induced adrenaline secretion in anesthetized rats.¹⁶ TRPA1 activation may therefore play a role in thermogenesis. Energy metabolism is possibly enhanced by the intake of the potent TRPA1 agonist, ACA. However, since this result was obtained from a cultured cell system, we will investigate the effect in the future of ACA in vivo, and clarify the mechanism underlying this effect.

Acknowledgments

This work was supported in part by grant-in-aid for scientific research (C) (19580146) in the priority area of food science from JSPS, Japan and by the Global Center of Excellence (COE) program from the Ministry of Education, Culture, Sports, Science and Technology, Japan.
References