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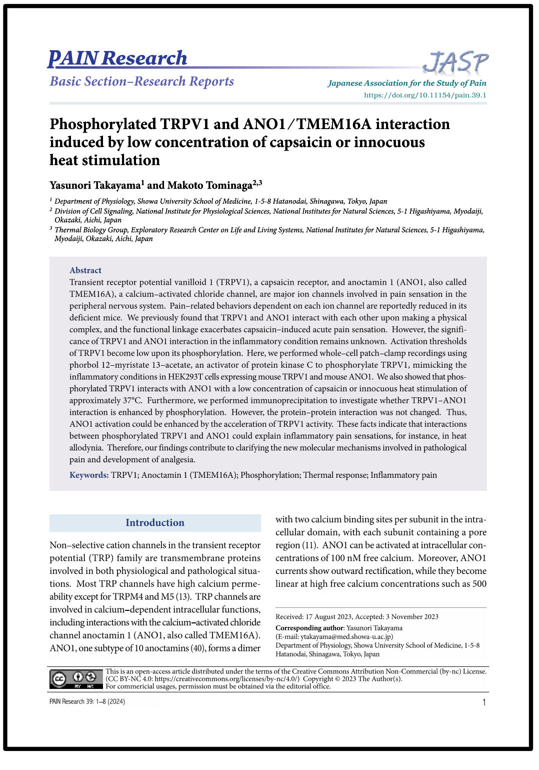
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Basic Section–Research Reports
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https://doi.org/10.11154/pain.39.1

Phosphorylated TRPV1 and ANO1/TMEM16A interaction induced by low concentration of capsaicin or innocuous heat stimulation

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Abstract
Transient receptor potential vanilloid 1 (TRPV1), a capsaicin receptor, and anoctamin 1 (ANO1, also called TMEM16A), a calcium–activated chloride channel, are major ion channels involved in pain sensation in the peripheral nervous system. Pain–related behaviors dependent on each ion channel are reportedly reduced in its deficient mice. We previously found that TRPV1 and ANO1 interact with each other upon making a physical complex, and the functional linkage exacerbates capsaicin–induced acute pain sensation. However, the significance of TRPV1 and ANO1 interaction in the inflammatory condition remains unknown. Activation thresholds of TRPV1 become low upon its phosphorylation. Here, we performed whole–cell patch–clamp recordings using phorbol 12–myristate 13–acetate, an activator of protein kinase C to phosphorylate TRPV1, mimicking the inflammatory conditions in HEK293T cells expressing mouse TRPV1 and mouse ANO1. We also showed that phosphorylated TRPV1 interacts with ANO1 with a low concentration of capsaicin or innocuous heat stimulation of approximately 37°C. Furthermore, we performed immunoprecipitation to investigate whether TRPV1–ANO1 interaction is enhanced by phosphorylation. However, the protein–protein interaction was not changed. Thus, ANO1 activation could be enhanced by the acceleration of TRPV1 activity. These facts indicate that interactions between phosphorylated TRPV1 and ANO1 could explain inflammatory pain sensations, for instance, in heat allodynia. Therefore, our findings contribute to clarifying the new molecular mechanisms involved in pathological pain and development of analgesia.

Keywords: TRPV1; Anoctamin 1 (TMEM16A); Phosphorylation; Thermal response; Inflammatory pain

Introduction

Non–selective cation channels in the transient receptor potential (TRP) family are transmembrane proteins involved in both physiological and pathological situations. Most TRP channels have high calcium permeability except for TRPM4 and M5 (13). TRP channels are involved in calcium–dependent intracellular functions, including interactions with the calcium–activated chloride channel anoctamin 1 (ANO1, also called TMEM16A). ANO1, one subtype of 10 anoctamins (40), forms a dimer with two calcium binding sites per subunit in the intracellular domain, with each subunit containing a pore region (11). ANO1 can be activated at intracellular concentrations of 100 nM free calcium. Moreover, ANO1 currents show outward rectification, while they become linear at high free calcium concentrations such as 500

Received: 17 August 2023, Accepted: 3 November 2023
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hyperthermia and abnormal temperature sensation (3, 25). Research has identified several side effects of ANO1 inhibitors. First, ANO1 is involved in secretions of cerebrospinal fluid (34), salivary (1, 5, 12, 27, 33), tear (12), and mucin (14). Second, ANO1 activation increases blood pressure via smooth muscle contraction (23, 38) and small intestinal peristalsis depending on interstitial cells of Cajal (15). Third, ANO1 expressed in intestinal epithelial cells inhibits dextran sodium sulfate–induced colitis (21). Finally, ANO1 activation in keratinocytes could accelerate wound healing (39). Therefore, it is hard to imagine the systemic application of ANO1 inhibitors. However, local or topical application could work to reduce pain, as has been reported in mice (35). Instead of selective ANO1 inhibitors, development of substances targeting the protein–protein interactions between TRPV1 and ANO1 could also be fruitful.

Because no antagonists have become available to target TRPV1 since its initial cloning in 1997 (6), our study is an intriguing approach for developing pain killers targeting the complex of TRPV1 and ANO1, and ANO1 itself.

Acknowledgements
Mouse TRPV1 cDNA was a generous gift from Dr. David Julius at the University of California San Francisco, and mouse ANO1 cDNA was a generous gift from Dr. Uhtaek Oh at the Korea Institute of Science and Technology. We thank Dr. rer. nat. Alexander S. Chockley from Edanz (https://jp.edanz.com/ac) for editing a draft of this manuscript.

Declarations
Funding: This study was partly supported by Grant–in–Aid for Young Scientists B (17K15793 to YT), Grant–in–Aid for Scientific Research C (21K06632 to YT), Takeda Science Foundation (to YT), the Uehara Memorial Foundation (to YT), and Grant–in–Aid for Scientific Research on Innovative Areas "Thermal Biology" (15H05927 to MT).
Competing interests: The authors declare that there is no conflict of interest.
Author contributions: YT and MT designed the study. YT performed and analyzed all experiments. YT and MT wrote the manuscript.
Ethics approval: Not applicable
Consent for participation: Not applicable
Consent for publication: Not applicable

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