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Antithetic effects of agonists and antagonists on the structural fluctuations of TRPV1 channel

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This file includes:

Main Text
Figures 1 to 2

Abstract

Transient receptor potential vanilloid member 1 (TRPV1) is a heat and capsaisin receptor that allows cations to permeate and cause pain. As the molecular basis for temperature sensing, the heat capacity (ΔC_p) model (D. E. Clapham, C. Miller, *Proc. Natl. Acad. Sci. U. S. A.* **108**, 19492–19497 (2011).) has been proposed and experimentally supported. Theoretically, heat capacity is proportional to a variance in enthalpy, presumably related to structural fluctuation; however, the fluctuation of TRPV1 has not been directly visualized. In this study, we directly visualized single-molecule structural fluctuations of the TRPV1 channels in a lipid bilayer with the ligands resiniferatoxin (RTX: agonist, 1000 times hotter than capsaicin) and capsazepine (CPZ: antagonist) by high-speed atomic force microscopy (HS-AFM). We observed the structural fluctuations of TRPV1 in an apo state, and found that RTX binding enhances structural fluctuations, while CPZ suppresses fluctuations. These ligand-dependent differences in structural fluctuation would play a key role in the gating of TRPV1.

Main Text

Introduction

The TRPV1 cation channel in sensory neuron opens by heat (> 43 degree Celsius) or binding of capsaicin, generating pain sensation.(1, 2) As the molecular basis of temperature sensing, the heat capacity (ΔC_p) model has been proposed(3) and experimentally supported.(4) Recently, cryo-electron microscopy (cryo-EM) has revealed the molecular structure of TRPV1 not only with and without ligand, but also in a heat-dependent manner.(5–7) Since C_p is proportional to enthalpy fluctuation that is directly related to conformational fluctuations, the measurement of the latter, which has not yet been performed in TRPV1, is also instrumental to fully elucidate the functioning mechanism of this channel. Furthermore, there are several examples showing that changes in structural fluctuations are closely related to protein activity, including ion channels.(8–10) Thus, it makes sense to analyze structural fluctuations in each gating state. Built upon this lack of knowledge, we use here HS-AFM(11) to directly observe structural fluctuations in TRPV1 under different experimental conditions. Ideally, measurements with temperature-control are the best way to control the activity of this channel; however, there are technical difficulties to obtain high-resolution images by heating the sample, such as thermal drift. As such, in this study, we have directly observed by HS-AFM the structural fluctuations of the TRPV1 channel in a lipid bilayer with and without two ligands, the RTX(12) and the CPZ(13).(11)

Results

HS-AFM of TRPV1 channels in lipid bilayer

Since the TRPV1 channel is homo-tetrameric channel, many clover-like structures were found in the TRPV1-reconstituted membrane (Figure 1, Movie S1-S3). Considering the height of the protrusion from the lipid membrane near the channel (1.4 ± 0.5 nm, n = 24), the observed surface is likely to be the extracellular side of TRPV1 (Figure 1A). The overall structure of the channel was not significantly changed by the presence of ligands (Figure 1 B to D), which is consistent with previous cryo-EM studies that have reported relatively small structural change upon

ligand binding.(6) From these HS-AFM movies, we extracted the trajectories of each of the four domains to analyze fluctuations of the TRPV1, as shown on the bottom right of figures 1 B-C.

Agonist/antagonist changed structural fluctuation of the TRPV1

Using the single-molecule trajectories from a large number of channels, we analyzed structural fluctuations. Distributions of lateral and angular displacement between successive frames in the HS-AFM videos are affected by ligand binding (Figure 2A and B). In both distributions, the RTX binding increases the fluctuations as compared to the Apo state, while, conversely, the CPZ binding suppresses it.

Discussion

Possible contribution of structural fluctuation for gating of TRPV1

In this study, we elucidate that RTX enhances structural fluctuation of TRPV1, while CPZ suppresses it. This is the first experimental evidence showing the correlation between molecular fluctuation and gating state (ligand binging) of the TRPV1 channel. One may thought that the observed fluctuations are too small (angstrom level) to modulate channel function; however, as well described in literature(10), energetics of ion permeation can be altered significantly by small structural fluctuations of the narrow pore structure. Given that even such a small change in the narrow pore alters energetics, small fluctuations at wider locations could alter the energetics more largely.

Previously, Miller and Clapham have proposed that change in heat capacity (ΔC_p) is theoretically a key factor for temperature-gating of TRP channels (3), and Chowdhury et al. supported this concept by mutational experiments.(4) Intriguingly, Kwon et al. have reported that the predicted ΔC_p from SASA of cryo-EM structures in closed and open states is smaller than that predicted by Miller and Clapham. Since RTX act as an agonist at TRPV1, the increase in molecular fluctuation of TRPV1 in the presence of RTX implies an increase in heat capacity during TPRV1 channel activation. It suggests an important role of structural fluctuations in the TRPV1 gating mechanism, which follows the heat capacity model. This study suggests the significance of measuring structural fluctuation, which would be another key factor for heat-sensing of TRPV1, because structural fluctuations are related to a variance in enthalpy, which is directly proportional to heat capacity.

Materials and Methods

Expression and purification of TRPV1

See extended method.

HS-AFM observation of TRPV1 reconstituted in a lipid bilayer

We reconstituted the TRPV1 channel into a lipid bilayer by a previously reported method.(14) To prevent lateral diffusion of the channels, we modified mica surface by 3-aminopropyltriethoxysilane (APTES) by applying 0.01 % APTES solution onto the mica for 3 min, then rinsed by pure water. Solubilized TRPV1 channels (10 μg/mL in 50 mM HEPES (pH8), 200 mM NaCl, 0.025 % DDM, 10 μg/ml soybean lipids, 2 mM TCEP, 10 % glycerol) were applied onto the APTES-modified mica surface for 5 min, then floating channels were washed out by buffer of solubilized channel. Next, DDM-destabilized DMPC liposomes (100 μg/mL DMPC in 10 mM HEPES [pH 7.5], 150 mM NaCl, 0.006 % DDM) were applied and incubated for 10 min, then rinsed by imaging buffer (10 mM HEPES [pH 7.5], 150 mM NaCl) to remove DDM and excess liposomes. For imaging with ligands, 10 nM RTX (K_i = 43 pM)(12) or 20 μM CPZ (K_i = 0.52 μM) (13) were added to all buffer from dilution of solubilized TRPV1 to imaging buffer. All reconstitution procedures were performed at 25°C. For HS-AFM observations, a laboratory-built HS-AFM was

used(11). We used cantilever AC10 (Olympus Co., Tokyo, Japan) with an electron beam deposition (EBD) tip.(11) The typical resonant frequency of AC10 in water was 400 kHz.

Analysis of fluctuation

To analyze structural fluctuation, we tracked position of four subunits by image analysis. Prior to particle tracking, we removed background, high-frequency noise and lateral drift of HS-AFM movies using subtract background, FFT filter and template matching plugin on ImageJ, respectively. Trajectories of particles were analyzed using TrackMate plugin on ImageJ.(15) Estimated blob diameter was set to 4.0 nm. Obvious particle misrecognitions and trajectory swaps were corrected manually. We analyzed 91 channels and 95,347 frames in total.

Data Availability.

The datasets generated and/or analyzed during the current study are available from the corresponding author (sumino@staff.kanazawa-u.ac.jp) on reasonable request.

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168 **Figures**
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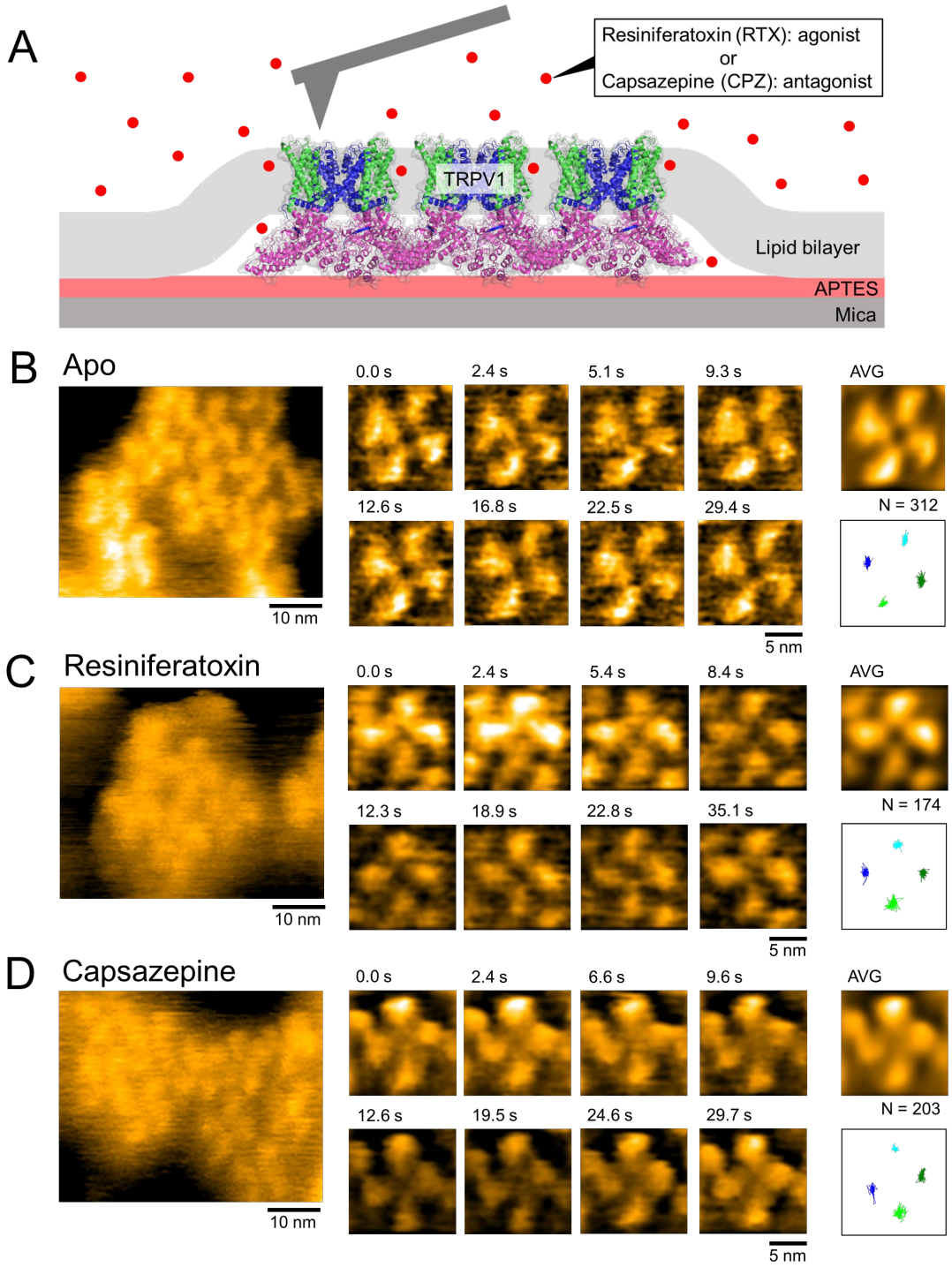

170
171 **Figure 1.** HS-AFM of TRPV1 channels in a lipid bilayer with or without ligands. (A) Schematic
172 illustration of the experiment. (B-D) HS-AFM images and trajectories of TRPV1 without ligand (B),
173 with 10 nM RTX (C) and 20 μM CPZ (D) added to the imaging buffer. Frame rate: 0.3 sec/frame.
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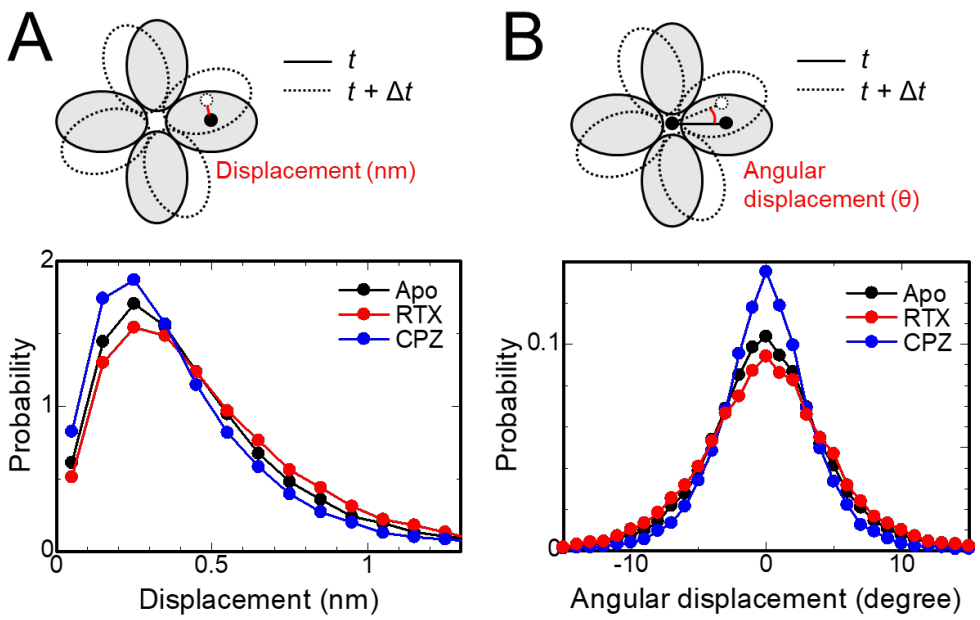

Figure 2. Structural fluctuation of TRPV1 depending on binding of ligands. Distribution of lateral (A) and angular (B) displacement of each subunit between successive frames of HS-AFM video. Frame interval (Δt) is 0.3 sec for A and B. Analyzed number of channels for Apo, RTX and CPZ are, (A): 43, 22 and 26, (B): 33, 13 and 14, respectively. Total frames for analysis of Apo, RTX and CPZ are (A): 46702, 23574 and 25071, (B): 37526, 13802 and 13677, respectively.