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

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Abstract

Transient receptor potential vanilloid member 1 (TRPV1) is a heat and capsaicin 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 binding 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 neurons 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 elucidating 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. As a first step in the understanding of structural fluctuations in TRPV1, 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).

Results

HS-AFM of TRPV1 channels in lipid bilayer

Since the TRPV1 channel is a 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 changes 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,

53 the RTX binding increases the fluctuations as compared to the Apo state, while, conversely, the
54 CPZ binding suppresses it.
55
56 **Discussion**
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58 **Possible contribution of structural fluctuation for gating of TRPV1**
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60   In this study, we elucidate that RTX enhances the structural fluctuation of TRPV1, while
61 CPZ suppresses it. This is the first experimental evidence showing the correlation between
62 molecular fluctuation and the gating state (ligand binding) of the TRPV1 channel. One may think
63 that the observed fluctuations are too small (angstrom level) to modulate channel function;
64 however, as well described in the literature(10), the energetics of ion permeation can be altered
65 significantly by small structural fluctuations of the narrow pore structure. Given that even such a
66 small change in the narrow pore alters energetics, small fluctuations at wider locations could alter
67 the energetics more largely.
68   Previously, Miller and Clapham have proposed that change in heat capacity (ΔC_p) is
69 theoretically a key factor for temperature-gating of TRP channels (3), and Chowdhury et al.
70 supported this concept by mutational experiments.(4) Intriguingly, Kwon et al. have reported that
71 the predicted ΔC_p from SASA of cryo-EM structures in closed and open states is smaller than that
72 predicted by Miller and Clapham. Since RTX act as an agonist at TRPV1, the increase in the
73 molecular fluctuation of TRPV1 in the presence of RTX implies an increase in heat capacity during
74 TPRV1 channel activation. It also suggests an important role of structural fluctuations in the TRPV1
75 gating mechanism, which follows the heat capacity model. Notably, the direct observation system
76 of TRPV1 in this study would be an important platform for further direct visualization of TRPV1 in
77 future studies, in particular, to observe heat-dependent changes of structural fluctuations
78 associated with temperature control.
79   Overall, this study suggests the importance of structural fluctuation, which would be a key
80 factor for the heat-sensing of TRPV1, because structural fluctuations are related to a variance in
81 enthalpy, which is directly proportional to heat capacity.
82
83 **Materials and Methods**
84
85 **Expression and purification of TRPV1**
86   See extended method.
87
88 **HS-AFM observation of TRPV1 reconstituted in a lipid bilayer**
89   We reconstituted the TRPV1 channel into a lipid bilayer by a previously reported
90 method.(14) To prevent lateral diffusion of the channels, we modified mica surface by 3-
91 aminopropyltriethoxysilane (APTES) by applying 0.01 % APTES solution onto the mica for 3 min,
92 then rinsed by pure water. Solubilized TRPV1 channels (10 μg/mL in 50 mM HEPES (pH8), 200
93 mM NaCl, 0.025 % DDM, 10 µg/ml soybean lipids, 2 mM TCEP, 10 % glycerol) were applied onto
94 the APTES-modified mica surface for 5 min, then floating channels were washed out by buffer of
95 the solubilized channel. Next, DDM-destabilized DMPC liposomes (100 μg/mL DMPC in 10 mM
96 HEPES [pH 7.5], 150 mM NaCl, 0.006 % DDM) were applied and incubated for 10 min, then rinsed
97 by imaging buffer (10 mM HEPES [pH 7.5], 150 mM NaCl) to remove DDM and excess liposomes.
98 For imaging with ligands, 10 nM RTX (K_i = 43 pM)(12) or 20 μM CPZ (K_i = 0.52 μM) (13) were
99 added to all buffer from dilution of solubilized TRPV1 to imaging buffer. All reconstitution
100 procedures were performed at 25°C. For HS-AFM observations, a laboratory-built HS-AFM was
101 used(11). We used cantilever AC10 (Olympus Co., Tokyo, Japan) with an electron beam deposition
102 (EBD) tip.(11) The typical resonant frequency of AC10 in water was 400 kHz.
103
104 **Analysis of fluctuation**
105   To analyze structural fluctuation, we tracked the position of four subunits by image
106 analysis. Prior to particle tracking, we removed the background, high-frequency noise and lateral

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107 drift of HS-AFM movies using subtract background, FFT filter and template matching plugin on
108 ImageJ, respectively. Pixels were interpolated to 0.1 nm/pixel using the bilinear method.
109 Trajectories of particles were analyzed using TrackMate plugin on ImageJ.(15) Estimated blob
110 diameter was set to 4.0 nm. Obvious particle misrecognitions and trajectory swaps were corrected
111 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.

Acknowledgments

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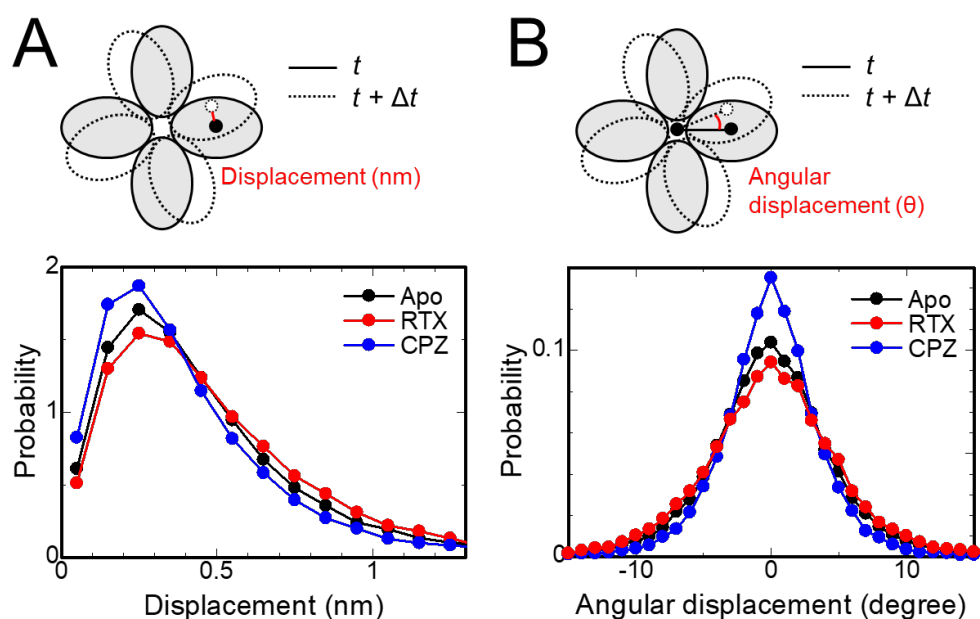167   tracking. *Methods* **115**, 80–90 (2017).

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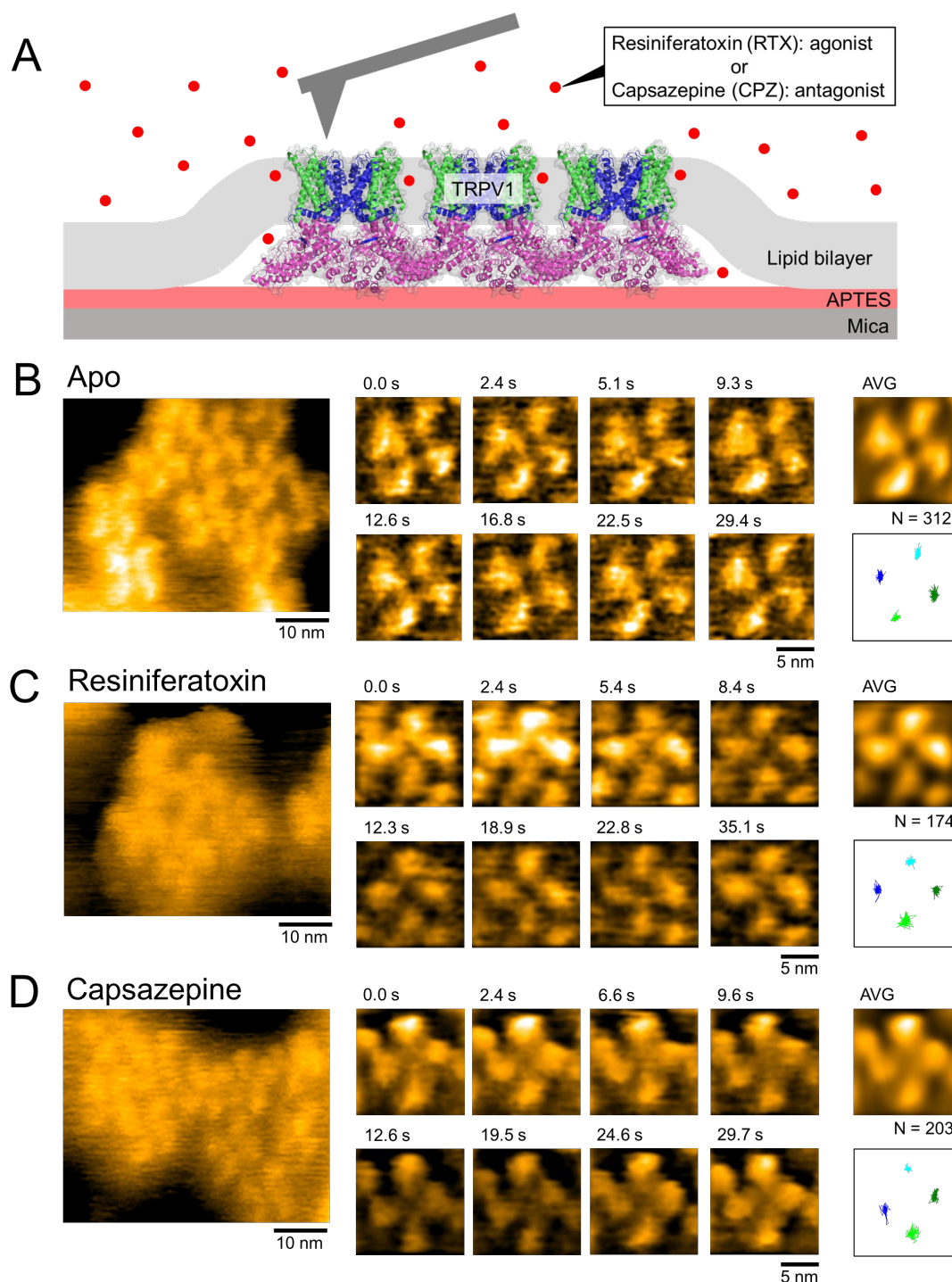
Figure 1. HS-AFM of TRPV1 channels in a lipid bilayer with or without ligands. (A) Schematic illustration of the experiment. (B-D) HS-AFM images and trajectories of TRPV1 without ligand (B), with 10 nM RTX (C) and 20 μM CPZ (D) added to the imaging buffer. Frame rate: 0.3 sec/frame.

177
178 **Figure 2.** Structural fluctuation of TRPV1 depending on binding of ligands. Distribution of lateral
179 (A) and angular (B) displacement of each subunit between successive frames of HS-AFM video.
180 Frame interval (Δt) is 0.3 sec for A and B. Analyzed number of channels for Apo, RTX and CPZ
181 are, (A): 43, 22 and 26, (B): 33, 13 and 14, respectively. Total frames for analysis of Apo, RTX and
182 CPZ are (A): 46702, 23574 and 25071, (B): 37526, 13802 and 13677, respectively.
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