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Vanilloids selectively sensitize thermal glutamate release from TRPV1 expressing solitary tract afferents

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ABSTRACT

Vanilloids, high temperature, and low pH activate the transient receptor potential vanilloid type 1 (TRPV1) receptor. In spinal dorsal root ganglia, co-activation of one of these gating sites on TRPV1 sensitized receptor gating by other modes. Here in rat brainstem slices, we examined glutamate synaptic transmission in nucleus of the solitary tract (NTS) neurons where most cranial primary afferents express TRPV1, but TRPV1 sensitization is unknown. Electrical shocks to the solitary tract (ST) evoked EPSCs (ST-EPSCs). Activation of TRPV1 with capsaicin (100 nM) increased spontaneous EPSCs (sEPSCs) but inhibited ST-EPSCs. High concentrations of the ultra-potent vanilloid resiniferatoxin (RTX, 1 nM) similarly increased sEPSC rates but blocked ST-EPSCs. Lowering the RTX concentration to 150 pM modestly increased the frequency of the sEPSCs without causing failures in the evoked ST-EPSCs. The sEPSC rate increased with raising bath temperature to 36 °C. Such thermal responses were larger in 150 pM RTX, while the ST-EPSCs remained unaffected. Vanilloid sensitization of thermal responses persisted in TTX but was blocked by the TRPV1 antagonist capsazepine. Our results demonstrate that multimodal activation of TRPV1 facilitates sEPSC responses in more than the arithmetic sum of the two activators, i.e. co-activation sensitizes TRPV1 control of spontaneous glutamate release. Since action potential evoked glutamate release is unaltered, the work provides evidence for cooperativity in gating TRPV1 plus a remarkable separation of calcium mechanisms governing the independent vesicle pools responsible for spontaneous and evoked release at primary afferents in the NTS.

1. Introduction

The transient receptor protein vanilloid type 1 (TRPV1) is commonly associated with transduction of potentially damaging noxious stimuli at nociceptive primary afferents (Tominaga et al., 1998). TRPV1 possesses two particularly interesting features: tripartite gating and high calcium permeability. Three specific attributes associated with damaged tissue separately gate TRPV1 to open: low pH, vanilloid ligands, and/or heat (>42 °C), and this triple-gating property of TRPV1 makes it a multimodal integrator (Kaszas et al., 2012). As a cation permeable ion channel, TRPV1 activation raises intracellular calcium and causes depolarization (Caterina et al., 1997). Thus, the dense expression of TRPV1 within the terminal fields of cranial visceral afferents (Tominaga et al., 1998) in the nucleus of the solitary tract (NTS) raises the prospect of a unique trigger of afferent neurotransmission.

Early neurotransmission work discovered that presynaptic action potentials triggered a rise in the calcium levels responsible for evoked release of neurotransmitter and that neurotransmission consisted of the release of quantal vesicles of transmitter (Fatt and Katz, 1952; del Castillo and Katz, 1954). Even in the absence of action potentials, however, quantal events occur spontaneously suggesting low rates of spontaneous vesicle fusion in most neurons. Mounting evidence now suggests that spontaneous events may arise from a separate pool of vesicles triggered by mechanisms distinct from action potential evoked neurotransmitter release (Kavalali, 2014). Examples of independent spontaneous release exist throughout the brain with widespread variations in spontaneous synaptic activity and regional characteristics in areas such as the cortex (Sara et al., 2005; Hofmann et al., 2011), cerebellum

Abbreviations: CAP, capsaicin; CPZ, capsazepine; NTS, nucleus of the solitary tract; RTX, resiniferatoxin; sEPSC, spontaneous EPSC; ST, solitary tract; ST-EPSC, solitary tract evoked EPSC; TRPV1, transient receptor potential vanilloid type 1; VACC, voltage activated calcium channel.

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(Glitsch, 2006), and brainstem (Peters et al., 2010; Largent-Milnes et al., 2014).

Solitary tract (ST) afferent transmission at NTS neurons is sensitive to vanilloid activation of TRPV1 at a majority of neurons (Doyle et al., 2002). TRPV1 located at unmyelinated, C-fiber ST afferent synaptic terminals acts as a unique calcium source to trigger glutamate vesicle release registered as spontaneous EPSCs (sEPSCs). Surprisingly, physiological temperatures vigorously activate TRPV1 to trigger spontaneous glutamate release only from TRPV1-expressing ST afferents, whereas cooling steeply suppresses spontaneous but not evoked synaptic responses (Peters et al., 2010; Shoudai et al., 2010; Fawley et al., 2015). This ongoing drive of TRPV1 at 37 °C contrasts with the canonical noxious heat thresholds of other primary sensory afferents. Here, we tested whether vanilloid stimulation cooperatively sensitizes thermal activation of TRPV1 in ST afferent transmission as it does in the spinal cord (Matta and Ahern, 2011). Low levels of TRPV1 activation with the ultra-potent vanilloid agonist resiniferatoxin (RTX) (Szallasi and Blumberg, 1999) augmented sEPSC rates without reducing evoked ST-EPSC amplitudes and substantially sensitized responses to thermal changes near 37 °C. TRPV1 activation primarily slowed the conduction of evoked transmission and was opposite to the enhanced spontaneous release. Thus, temperature and vanilloid binding cooperatively gate TRPV1 to augment spontaneous release of glutamate without affecting action potential triggered EPSCs. Our findings highlight the independence of the two release mechanisms controlling the spontaneous pool and the evoked pool of vesicles, which rely on separate and independent calcium sources for release. Together, the multimodal activation of TRPV1 plus its coupling to release from the spontaneous glutamate pool imbues C-fiber afferent central terminals with a capacity to respond to a wide dynamic range of inputs as a unique afferent signaling mechanism.

2. Methods

2.1. Ethical approval

All animal procedures were approved by the Institutional Animal Care and Use Committee at Oregon Health and Science University and conformed to animal welfare guidelines issued by the National Institutes of Health publication *Guide for the Care and Use of Laboratory Animals*.

2.2. Slice preparation

Brain slices containing NTS were prepared from adult (>130 g, n = 51) male Sprague–Dawley rats (Charles River Laboratories, Wilmington, MA), fed *ad libitum*, as previously described (Doyle and Andresen, 2001). Briefly, rats were deeply anesthetized with 3% isoflurane and the brainstem removed and placed into ice-cold ACSF. The brainstem was tilted in order to cut a horizontal slice (250 μm) containing 1–3 mm of the ST in the same plane as the NTS. While in the ice-cold ACSF, slices were cut using a sapphire blade (Delaware Diamond Knives, Wilmington, DE) mounted on a vibrating microtome (VT1000 S; Leica Microsystems, Bannockburn, IL), and immediately submerged in a recording chamber containing ACSF. The ACSF consisted of, in mM: 125 NaCl, 3 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 25 $NaHCO_3$, 10 glucose, and 2 $CaCl_2$, bubbled with 95% O_2–5% CO_2. The slice was perfused continuously (1.6–2.0 ml/min) at a bath temperature of 32 °C controlled by an in-line heating system (TC2BIP with HPRE2HF and TH-10Km bath probe; Cell MicroControls, Norfolk, VA). This cooler than physiological base temperature maximized recording time periods compared to 37 °C.

2.3. Patch-clamp recording

Under infrared illumination, neurons were visualized using 40 nM bandpass limited, differential interference contrast microscopy (Doyle et al., 2004) on a fixed-stage Axioskop 2 FS Plus (Zeiss, Thornwood, NJ) with a digital camera (SPOT Pursuit USB Camera; SPOT Imaging Solutions, Sterling Heights, MI). Patch pipettes (2.5–4.0 MΩ) were filled with an internal solution composed of, in mM: 6 NaCl, 4 NaOH, 130 K-gluconate, 11 EGTA, 1 $CaCl_2$, 10 HEPES, 1 $MgCl_2$, 2 Na_2ATP, and 0.2 Na_2GTP (pH 7.3). Using a MultiClamp 700B amplifier (Molecular Devices, Sunnyvale, CA), neurons were voltage clamped at −60 mV without liquid junction potential correction. Data were sampled at 20 kHz and filtered at 10 kHz (Digidata 1321A analog-to-digital converter using Clampex 9; Molecular Devices). Repeated −5 mV (200 ms) hyperpolarizing steps were applied throughout each experiment to monitor access resistance and input resistance. The ACSF included the $GABA_A$ antagonist gabazine (SR-95531; 3 μM) to isolate EPSCs in all experiments. Drugs were bath-applied and purchased from either Tocris (R&D Systems, Minneapolis, MN) or Sigma–Aldrich (St. Louis, MO).

2.4. Afferent activation and identification

After submerging and securing the slice in the recording chamber, a concentric bipolar stimulating electrode was placed onto the ST at least 1 mm rostral from medial NTS. Following acquisition of a stable patch recording, bursts of five shocks (100 μs duration at 50 Hz) tested for ST evoked synaptic responses. Variations in stimulation intensity allowed for the determination of the threshold for obtaining a solitary tract evoked EPSC (ST-EPSC). Gradual increases in intensity created an ST-EPSC recruitment profile and generally, a minimal intensity above threshold was chosen for experiments. To verify the neuron as second-order, the ST burst of shocks was applied once every 6 s and the latency of the ST-EPSC was measured as the time from the stimulus artifact to the onset of the ST-EPSC. Using at least 50 iterations, the synaptic jitter was measured as the standard deviation of the latency. Evoked ST-EPSCs with a jitter <200 μs were classified as monosynaptic (Doyle and Andresen, 2001; Andresen and Peters, 2008). After collecting data for the jitter measurements, the protocol switched to stimulating the ST once every 10 s for the thermal and pharmacological tests. Classifying second-order neurons as receiving TRPV1+ ST afferents generally relied on the following criteria: high control (resting) frequency of sEPSCs in the unstimulated state, increased sEPSC frequencies in the 1 s following suprathreshold ST stimulation compared to the 1 s prior to stimulation (asynchronous sEPSCs) (Peters et al., 2010), and increases in sEPSC rate to increases in temperature. Neurons were verified as TRPV1+ using either capsaicin (CAP, 100 nM) or RTX (150 pM or 1 nM).

2.5. Data analysis and statistics

Analysis was performed using O-phys (see Hofmann et al., 2011; Gainesville, FL) and Origin 8.6 (OriginLab; Northampton, MA) except for latency measurements (Clampfit 9; Molecular Devices). For ST evoked synaptic responses, average values for ST-EPSC amplitude and latency were calculated using the final 3 min before drug application (i.e. 18 trials) and again for a 3 min period beginning 7 min after drug application. A two-sample t-test was performed within each individual neuron and a paired t-test was used for summary of group data for both ST-evoked amplitude and latency (OriginLab). From these same records, corresponding information of spontaneous transmission was assessed for sEPSC event frequencies and amplitudes collected from each test sweep for the 5 s prior to ST stimulation. Given that sEPSCs represent