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Calcineurin is required for TRPV1-induced long-term depression of hippocampal interneurons

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ABSTRACT

Transient receptor potential vanilloid 1 (TRPV1) mediates a novel form of presynaptic long-term depression (LTD) in hippocampal interneurons. To date, while TRPV1 is currently being heavily studied in the PNS for its anti-nociceptive and anti-inflammatory properties, much less is known regarding TRPV1 signaling and function in the CNS, including the mechanism mediating hippocampal interneuron LTD. Here we performed whole-cell voltage clamp electrophysiology experiments on CA1 hippocampal interneurons from Sprague–Dawley male rats to identify this signaling mechanism. Because calcineurin is linked to multiple synaptic plasticity types, we investigated whether TRPV1 activates presynaptic calcineurin, which in turn induces LTD. To do so we employed calcineurin inhibitors cyclosporin A or FK-506. To determine the location of the calcineurin involved we either bath applied calcineurin antagonists, blocking calcineurin activity ubiquitously in the slice, presynaptically and postsynaptically, or applied antagonists to the internal solution to block calcineurin postsynaptically. Both calcineurin antagonists applied to the bath blocked TRPV1-dependent LTD, indicating calcineurin involvement in LTD. Because calcineurin antagonist applied to the internal solution did not block TRPV1-LTD, it suggests presynaptic calcineurin is required for LTD. Finally, because high frequency stimulus used to induce LTD could potentially activate receptors besides TRPV1, we confirmed that bath, but not intracellularly applied cyclosporin A, also blocked TRPV1 agonist-induced depression of CA1 interneurons. In conclusion, these data illustrate that presynaptic calcineurin activity is required for both TRPV1-induced LTD and TRPV1 agonist-induced depression. This finding is the first to demonstrate the TRPV1-induced signaling mechanism in CA1 hippocampus.

1. Introduction

Declarative learning and memory are thought to depend on hippocampal synaptic plasticity. Synaptic plasticity includes the strengthening and weakening of synapses known as long term potentiation and long term depression (LTD), respectively. LTD is mediated either postsynaptically [1,12,21] or presynaptically [2,28], where presynaptic LTD is caused by decreased neurotransmitter release.

Recently, a novel form of presynaptic LTD was identified for the first time that was induced by transient receptor potential vanilloid 1 (TRPV1) at hippocampal Cornu Ammonis 3 (CA3)–CA1 interneuron synapses [9]. This LTD was mediated by presynaptic TRPV1, activated by the retrograde messenger 12-(S)-HPETE produced in the postsynaptic cell. Both high frequency stimulation (HFS) and TRPV1 agonists induced this LTD. Expression of presynaptic as well as postsynaptic hippocampal TRPV1 was noted previously in the CA3–CA1 region [5] supporting this finding. Interestingly, postsynaptic hippocampal TRPV1 mediates dentate gyrus granule cell LTD [3]. In other brain regions presynaptic TRPV1 can also enhance glutamate release [15,25], in contrast to hippocampal interneuron LTD.

In the PNS, TRPV1 which is activated by lipophilic ligands [26], is being studied for its anti-inflammatory and anti-nociceptive properties [24]. In the CNS, TRPV1 was recently shown to have a behavioral function as TRPV1 knockout mice demonstrate reduced anxiety, fear conditioning and stress sensitization [16,22]. While TRPV1 is expressed in many areas of the brain [5,27] and involved in synaptic plasticity [3,9,10,14,17] its physiological importance in the CNS is still unclear [13].

However, understanding TRPV1 function in the CNS is important such as its modulatory role of hippocampal interneurons [9] because they are critical in hippocampal function. For example,

Abbreviations: TRPV1, transient receptor potential vanilloid 1; LTD, long-term depression; CA3, Cornu Ammonis 3; HFS, high frequency stimulus; EPSC, excitatory postsynaptic potential.

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a single interneuron innervates hundreds of pyramidal cells [7], and synchronizes their firing and oscillatory behavior [4]. In addition, TRPV1 agonist activation enhances CA1 long term potentiation via the GABAergic system [17], illustrating the contribution TRPV1 interneuron LTD has to pyramidal output. Though important the mechanism behind TRPV1-initiated interneuron LTD is still unknown.

Because TRPV1 is a calcium cation channel, one would expect it to increase neurotransmission presynaptically, activating CA1 interneurons like at other synapses, which it does not. Therefore, we investigated second messenger pathways including phosphatases that TRPV1 could activate to decrease neurotransmission. An excellent candidate is calcineurin, also known as protein phosphatase 3/protein phosphatase 2B. Many studies have linked calcineurin activity to synaptic plasticity, including presynaptic plasticity [11,30]. In addition, a link between TRPV1 and calcineurin was identified in dorsal root ganglion cells where TRPV1 mediated Ca^{2+} currents activated calcineurin, causing reduced neurotransmitter release [29].

Our data indicate calcineurin is required for TRPV1-mediated LTD at CA3–CA1 interneuron synapses. We are the first to propose a signal mechanism for how presynaptic TRPV1 functions in the brain at synapses where neurotransmission is decreased. This finding provides us further insight into how TRPV1 may be functioning presynaptically in other areas of the CNS such as the superior colliculus.

2. Methods

2.1. Brain slice preparation

All experiments were performed in accordance with Institutional Animal Care and Use Committee (IACUC) protocols and follow the NIH guidelines for the care and use of laboratory animals. These guidelines include minimizing animal suffering and the number of animals used to perform the required experiments. Sprague–Dawley male rats (Charles River; 15–27 days old) were deeply anesthetized with isoflurane using a vapomatic chamber and decapitated. The brain was rapidly removed and placed in ice-cold ringers (in mM): NaCl, 119; $NaHCO_3$, 26; KCl, 2.5; NaH_2PO_4, 1.0; $CaCl_2$, 2.5; $MgSO_4$, 0.6; glucose, 11; saturated with 95% O_2, 5% CO_2 (pH 7.4). Coronal slices (350–400 μm) were prepared and placed in a submersion chamber containing oxygenated artificial cerebrospinal fluid (ACSF) at room temperature. A surgical cut was made between the CA3 and CA1 regions to avoid bursting activity. Slices were either kept in this ACSF or transferred to oxygenated ACSF containing calcineurin antagonists for at least a 1 h recovery period. Slices remained in these solutions for 1–6 h.

2.2. Electrophysiology

Slices were then transferred to a submerged recording chamber bathed in oxygenated ACSF (28–32 °C) containing elevated divalent cations to reduce epileptiform activity (4 mM $CaCl_2$ and 4 mM $MgCl_2$, replacing 2.5 mM $CaCl_2$ and 1.3 mM $MgSO_4$) as described previously [9]. Slices were continuously perfused with ACSF at a flow rate of 2–3 ml/min. Whole-cell patch clamp recordings were made from interneurons identified visually in the CA1 stratum radiatum of the hippocampus with infrared optics, CCD camera and monitor, using an Olympus BX51WI microscope with 40× water immersion objective. A bipolar stainless steel stimulating electrode was placed in stratum radiatum, located approximately 200–500 μm from the recorded cell to stimulate CA3 glutamatergic afferents of the Schaffer Collateral pathway at 0.1 Hz and evoke excitatory postsynaptic currents (EPSCs; stimulus intensities were typically 50–300 μA, 100 μs). Recordings were performed in voltage clamp at −65 mV using a multiclamp 700B amplifier (Molecular Devices, Sunnyvale, CA). Borosilicate glass patch pipettes (2–4 MΩ) were filled with Cs^+-gluconate (117 mM) based internal solution containing (in mM): NaCl, 2; $MgCl_2$, 5; HEPES, 20; ATP, 2; GTP, 0.3; QX-314, 1 and EGTA, 0.6. AMPAR-mediated currents were measured while blocking $GABA_A$ receptors with picrotoxin (100 μM). Once a stable baseline recording of AMPAR-mediated currents was obtained by stimulating at 0.1 Hz, either the Schaffer collateral pathway was stimulated using a conditioning HFS (100 Hz for 1 s repeated again 20 s later) or TRPV1 agonists were added directly to the ACSF for 10 min. EPSCs were evoked and monitored for at least 15 min post-tetanus or post-TRPV1 agonist application at 0.1 Hz.

2.3. Analysis

EPSCs were filtered at 4 kHz, digitized with an axon 1440A digitizer (Molecular Devices, Sunnyvale, CA) interfaced with a Dell personal computer (Optiplex 745) and recorded with pClamp10.2 clampex software (Molecular Devices). Data was analyzed using pClamp10.2 clampfit software, EPSC amplitudes were determined by measuring against a 10 ms baseline prior to evoking EPSCs. Microsoft Excel and Origin (Natwick, MA) were used to organize, graph, perform statistical analysis and average EPSC amplitude values in 1 min intervals. To positively identify interneuron LTD or TRPV1-induced depression, average EPSCs taken from a 5 min period immediately before tetanus or drug application were compared to a 5 min period 10–15 min post-tetanus or post-drug application to determine statistical significance, using a two way, unpaired *t* test ($p < 0.05$). To determine significance between intracellularly and extracellularly applied drug we compared a 5 min period 10–15 min post-tetanus or post drug application to examine the difference between the two. Average reported EPSC amplitudes are means and standard error of the mean.

The cell input resistance was monitored throughout the experiment and if changed by more than 10%, the cell was discarded. Only one experiment was performed per slice and the reported *n* is the number of slices. One to three slices were used per animal. Interneurons were distinguished from pyramidal cells visually, electrophysiologically (higher input resistance and faster EPSCs as compared to pyramidal cells) and based on their location in the radiatum.

2.4. Materials

All salts were purchased from Sigma–Aldrich, Fisher Scientific, JT Baker or Mallinckrodt. Capsaicin, cyclosporin A, picrotoxin, QX-314 bromide and resiniferatoxin were purchased from Tocris Bioscience (Ellisville, MO). FK-506 was purchased from Ascent Scientific (Princeton, NJ). Stock solutions of capsaicin (25 mM) and resiniferatoxin (2 mM) were dissolved in DMSO and then stored at −90 °C for no more than one month. These stock solutions were then diluted to their final concentration in ACSF. As a note, the concentration of 50 μM for cyclosporin A and FK-506 was chosen as this amount has been demonstrated to effectively permeate cell membranes, blocking calcineurin throughout brain slices [11,20]. We also determine that 10 μM cyclosporin A applied extracellularly was sufficient to only partially block LTD in our experiments.

3. Results

3.1. Calcineurin is required for HFS-induced LTD of CA1 hippocampal interneurons

To determine whether calcineurin is required for HFS-induced LTD of CA1 stratum radiatum interneurons we first used the