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Research report

Capsaicin augments synaptic transmission in the rat medial preoptic nucleus

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Abstract

The medial preoptic nucleus (MPN) is the major nucleus of the preoptic area (POA), a hypothalamic area involved in the regulation of body-temperature. Injection of capsaicin into this area causes hypothermia in vivo. Capsaicin also causes glutamate release from hypothalamic slices. However, no data are available on the effect of capsaicin on synaptic transmission within the MPN. Here, we have studied the effect of exogenously applied capsaicin on spontaneous synaptic activity in hypothalamic slices of the rat. Whole-cell patch-clamp recordings were made from visually identified neurons located in the MPN. In a subset of the studied neurons, capsaicin enhanced the frequency of spontaneous glutamatergic EPSCs. Remarkably, capsaicin also increased the frequency of GABAergic IPSCs, an effect that was sensitive to removal of extracellular calcium, but insensitive to tetrodotoxin. This suggests an action of capsaicin at presynaptic GABAergic terminals. In contrast to capsaicin, the TRPV4 agonist 4α-PDD did not affect GABAergic IPSCs. Our results show that capsaicin directly affects synaptic transmission in the MPN, likely through actions at presynaptic terminals as well as on projecting neurons. Our data add to the growing evidence that capsaicin receptors are not only expressed in primary afferent neurons, but also contribute to synaptic processing in some CNS regions.

Theme: Excitable membranes and synaptic transmission
Topic: Presynaptic and postsynaptic mechanisms

Keywords: Vanilloid receptor; Postsynaptic currents; Slice

1. Introduction

The medial preoptic nucleus (MPN) is the major nucleus of the preoptic area (POA). This hypothalamic area is, among other functions, involved in the regulation of body temperature by controlling heat-loss and heat-retention mechanisms. POA neurons can be classified into temperature-sensitive (~30%) and temperature-insensitive neurons. The temperature-sensitive POA neurons can be further grouped into warm-sensitive and cold-sensitive. There is evidence that at least some of the temperature-sensitivity is inherent to these neurons (for review, see [3]). The most direct evidence for this is that warming causes activation of a yet unidentified, non-selective cation channel in warm-sensitive neurons [14]. However, synaptic inputs from other brain regions, the periphery, as well as from local networks within the POA [28] may also contribute to the thermosensitivity of POA neurons. For example, it has been suggested that cold-sensitive neurons lack an inherent temperature sensitivity altogether, but derive their temperature sensitivity through synaptic inhibition by warm-sensitive neurons [3].

The molecular basis for inherent as well as synaptically mediated temperature sensitivity of POA neurons is unknown. However, several lines of arguments support the idea that ion channels of the Transient Receptor Potential

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(TRP) family may be involved in this phenomenon. Morphological studies have shown the presence of such receptors in the POA. Resiniferatoxin, an analogue of the TRPV1 (formerly called VR1) agonist capsaicin, specifically binds in the POA in rat [1] and monkey [30]. In addition, TRPV1 mRNA [26] as well as protein [23] is present in several hypothalamic nuclei of the rat. Recent evidence also indicates the presence of TRPV4 protein, another member of the TRP channel family, in the medial POA of the rat hypothalamus [10]. Further, capsaicin causes hypothermia only when injected into the POA, but not into other regions of the hypothalamus [16]. Likewise, capsaicin applied by microelectrophoresis increases the firing rate of warm-sensitive units and decreases that of cold-sensitive units [14]. In hypothalamic slices, capsaicin also evokes calcium-dependent glutamate release, an effect that can be blocked by capsazepine, a competitive antagonist at TRPV1 [26].

Despite these strong indications that TRP channels may be involved in the thermosensitivity of POA neurons, only little is known about how activation of these receptors affects synaptic transmission within this region. Here, we have studied the effects of exogenously applied capsaicin on spontaneous synaptic activity in MPN neurons in hypothalamic slices of the rat. Our results show that in the MPN capsaicin increases glutamatergic as well as GABAergic synaptic transmission. The effect on both transmitter systems can be observed in the presence of TTX. To our knowledge, the present study is the first to describe in detail a direct enhancement of GABAergic synaptic transmission by the TRPV1 agonist capsaicin. This uncommon effect was not evoked when applying 4α-PDD, an agonist of TRPV4, the only other TRPV channel described in the POA.

2. Materials and methods

2.1. Slice preparation

All animal experiments were approved by the regional ethics committee for animal research (S123/01). Fourteen days pregnant female Sprague–Dawley rats were purchased from Möllegård (Skensved, Denmark), and housed at 20 ± 0.5 °C with a 12-h light/dark cycle and free access to food and water. Offspring of both sexes were used at ages from 26 to 34 days postnatal.

For recording, a rat was lightly anaesthetized with enflurane and decapitated. The brain was rapidly removed and placed throughout the entire slicing procedure in pre-oxygenated ice-cold (4 °C) slice preparation solution containing (in mM): Sucrose 225, KCl 5, $CaCl_2$ 2.5, $MgCl_2$ 1.5, NaH_2PO_4 1.2, $NaHCO_3$ 25, glucose 10, pH 7.4 (95% O_2, 5% CO_2). A block of tissue containing the anterior hypothalamus was used to cut 200–250 μm thick coronal slices using a vibratome (Vibratome 100 plus, Ted Pella, Redding, CA, USA). After cutting, slices were allowed to recover for at least 1 h in artificial cerebrospinal fluid (aCSF, see below) at room temperature (21–23 °C).

2.2. Acute dissociation of MPN neurons

The procedure for dissociation of MPN neurons followed the details previously described [19].

2.3. Recording solutions

The aCSF used for slice recordings contained (in mM): NaCl 124, KCl 3.0, $CaCl_2$ 2.4, $MgSO_4$ 1.3, NaH_2PO_4 1.4, NaOH 18, HEPES 5.0, glucose 11, pH 7.4 (95% O_2, 5% CO_2). Tetrodotoxin (TTX), 6-nitro-7-sulphomoylbenzo[f]-quinoxaline-2,3-dione (NBQX), or bicuculline methiodide were added to the aCSF without further adjustments, as indicated in the text. In some experiments, a Ca^{2+}-free extracellular solution of the following composition was used (in mM): NaCl 124, KCl 3.0, $MgSO_4$ 10, NaH_2PO_4 1.4, NaOH 18, HEPES 5.0, glucose 11, pH 7.4 (95% O_2, 5% CO_2).

IPSCs were recorded with an intracellular solution containing (in mM): KCl 140, NaCl 3.0, $MgCl_2$ 1.2, HEPES 10, EGTA 1.0, Mg-ATP 4, pH 7.2 (KOH). The intracellular solution used to record EPSCs contained (in mM): Cs-gluconate 140, NaCl 3.0, $MgCl_2$ 1.2, HEPES 10, EGTA 10, Mg-ATP 4.0, pH 7.2 (CsOH).

Recordings from acutely dissociated neurons were made with an extracellular solution of composition (in mM): NaCl 137, KCl 5.0, $CaCl_2$ 1.0, $MgCl_2$ 1.2, HEPES 10, glucose 10, pH 7.4 (NaOH). The intracellular solution used for these experiments was the same as the one used for IPSC recordings in slices (see above). All ATP-containing intracellular solutions were stored on ice until used in the experiments.

2.4. Electrophysiological recordings

The recording chamber used for slice recordings (Warner Instrument, Hamden, CT, USA) had a volume of 180 μl. It was perfused at a rate of ~1.5 ml/min with continuously bubbled (95% O_2, 5% CO_2) aCSF, that was preheated before entering the recording chamber using an automatic temperature controller TC-324B with inline heater SH-27B (Warner Instrument Corp., Hamden, CT, USA). The temperature was measured in the recording chamber, between the solution inlet and the slice, and was adjusted to 36 ± 2 °C. For recording, a slice was fixed with a slice anchor grid with parallel threads (Warner Instrument, Hamden, CT, USA). Slices were observed using a Zeiss Axioskop (Carl Zeiss, Göttingen, Germany) mounted with an infrared CCD camera C7500 with camera controller C2741 (Hamamatsu, Japan). Whole-cell recordings were made from visually identified MPN neurons, using the