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Research Report

Capsaicin differentially modulates voltage-activated calcium channel currents in dorsal root ganglion neurones of rats

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Abstract

It is discussed whether capsaicin, an agonist of the pain mediating TRPV1 receptor, decreases or increases voltage-activated calcium channel (VACC) currents ($I_{Ca(V)}$). $I_{Ca(V)}$ were isolated in cultured dorsal root ganglion (DRG) neurones of rats using the whole cell patch clamp method and Ba^{2+} as charge carrier. In large diameter neurones (>35μm), a concentration of 50μM was needed to reduce $I_{Ca(V)}$ (activated by depolarizations to 0 mV) by 80%, while in small diameter neurones (≤30μm), the IC_{50} was 0.36 μM. This effect was concentration dependent with a threshold below 0.025 μM and maximal blockade (>80%) at 5μM. The current–voltage relation was shifted to the hyperpolarized direction with an increase of the current between −40 and −10mV and a decrease between 0 and +50 mV. Isolation of L-, N-, and T-type calcium channels resulted in differential effects when 0.1 μM capsaicin was applied. While T-type channel currents were equally reduced over the voltage range, L-type channel currents were additionally shifted to the hyperpolarized direction by 10 to 20 mV. N-type channel currents expressed either a shift (3 cells) or a reduction of the current (4 cells) or both (3 cells). Thus, capsaicin increases $I_{Ca(V)}$ at negative and decreases $I_{Ca(V)}$ at positive voltages by differentially affecting L-, N-, and T-type calcium channels. These effects of capsaicin on different VACCs in small DRG neurones, which most likely express the TRPV1 receptor, may represent another mechanism of action of the pungent substance capsaicin in addition to opening of TRPV1.

Theme: Sensory systems
Topic: Pain modulation: pharmacology

Keywords: Voltage-activated calcium channel current; Capsaicin; Pain; TRPV1; Dorsal root ganglion neurones; L-type calcium channel; N-type calcium channel; T-type calcium channel; Small diameter neurones; Electrophysiology; Patch clamp technique

Abbreviations: $[Ca^{2+}]_i$, intracellular calcium concentration; Calciseptine, specific L-type calcium channel blocker; Caps, Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide); DRG, dorsal root ganglion; EGTA, ethylene glycol-bis(2-aminoethylether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; FS-12, specific L-type calcium channel blocker; IC_{50}, inhibitory concentration reducing 50%; $I_{Ca(V)}$, voltage-activated calcium channel currents; $I_{Ca(L)}$, long lasting voltage-activated calcium channel current ("L-type"); $I_{Ca(N)}$, neither–nor voltage-activated calcium channel current ("N-type"); $I_{Ca(T)}$, transient voltage-activated calcium channel current ("T-type"); $I_{Ca(P+Q)}$, purkinje voltage-activated calcium channel current ("P/Q-type"); IV–Curve, current–voltage relation curve; Pimozide, specific T-type calcium channel blocker; TEA, tetraethylammonium; TTX, tetrodotoxin; TRPV1, transient receptor potential channel of the vanilloid receptor subtype, type 1 (formerly VR1); VACCs, voltage-activated calcium channels; ω-Conotoxin GVIA, specific N-type calcium channel blocker; ω-Conotoxin MVIAA, specific N-type calcium channel blocker

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1. Introduction

Capsaicin (8-methyl-*N*-vanillyl-6-nonenamide), the "hot" substance in chilli peppers (*Capsicum frutescens*), is naturally present in the food consumed by humans, being commonly used as a spice. Capsaicin binds to a specific receptor, TRPV1 (formerly VR1-) receptor, a member of the vanilloid receptor subfamily of the transient receptor potential (TRP) channel superfamily [21]. Capsaicin-sensitive neurones that express TRPV1 have a large range of physiological functions (reviewed in [35]). For example, capsaicin acts in human bronchi, where it inhibits the fenoterol induced relaxation of smooth muscles [14]. Capsaicin-sensitive neurones play a major role in the urinary bladder for the maintenance of normal function [3,4] and are involved in the regulation of blood pressure [36]. But most important are the effects in the nociceptive system (reviewed by [35]).

Functional TRPV1 receptors are associated with a calcium permeable channel pore and are located in the plasma membrane of neurones as well as in the membrane of the endoplasmatic reticulum of these cells. These TRPV1 receptors are mainly expressed in a functionally distinguishable subpopulation of dorsal root ganglia (DRG) neurones. These neurones are small and presumably have also nociceptive receptors [1,6–8,18]. TRPV1 is activated by a variety of chemicals and by thermal stimuli and was therefore suggested to represent a transducer molecule for noxious heat [6,21]. Accordingly, small capsaicin-sensitive DRG neurones were shown to specifically respond to noxious heat pulses [19,22,23,27,32], whereas large DRG neurones, that do not express TRPV1, are not heat-sensitive [22,23,27]. Thus, activation of TRPV1 seems to play an essential role in nociception, e.g., in the transduction process for noxious heat stimuli and in capsaicin-induced hyperalgesia [9,24,35].

As a main second messenger intracellular calcium ($[Ca^{2+}]_i$) is tightly regulated and plays a major role, in the mediation of nociception. Nonetheless, in a defined range, changes of $[Ca^{2+}]_i$ are allowed and were shown to have functional relevance for the transduction of painful stimuli [19]. The activation of the TRPV1 receptor results in a rise of the intracellular calcium [6,17,29]. This calcium could either have been released from internal stores or could enter through calcium permeable pores from the extracellular space [13,17]. While the amount of calcium entering through the TRPV1 associated pore was relatively low in voltage-clamp experiments at −80 mV [40], larger amounts of extracellular calcium may enter the cell when voltage-activated calcium channels (VACCs) are activated, e.g., by action potential discharges [17].

It has been shown that capsaicin directly blocks calcium entry through voltage-activated calcium channels in a variety of different cell types [25,33] with effective concentrations between 5.8 μM and 20 μM (IC_{50} values [25]), while it seems to be highly effective in sensory neurones at a concentration of 1 μM [38]. It has also been shown that capsaicin (30 μM) shifts the current–voltage relation of voltage-activated calcium channels to the hyperpolarizing range [30], indicating a facilitated activation of these channels with smaller depolarizations. This results in an intracellular rise of calcium within this voltage range. Furthermore, Cheng and colleagues [10] demonstrated that myocytes might react differently, since they found an increase of the voltage-activated calcium channel current after the application of nanomolar concentrations of capsaicin.

To shed light on the possible different effects of capsaicin on $I_{Ca(V)}$, we describe a reduction in small DRG neurones (IC_{50} = 0.36 μM), while large neurones were six times less sensitive. To analyze the effect of capsaicin in small DRG neurones in more detail, we isolated currents through the L-, N- and T-type calcium channels and found differential effects on these calcium channel subtypes. These results might give hints to understand pain modulating processes following the initial transduction process.

A preliminary report has been published in abstract form [20].

2. Methods

2.1. Preparation of dorsal root ganglion neurones

Dorsal root ganglion neurones were isolated from 21- to 28-day-old "Wistar" or "Lewis" rats. Animals were deeply anaesthetized with Isofluran (Curamed), until pinching the rat tail and feet revealed complete analgesia. Thereafter, the rats were decapitated, and the vertebral column was opened by a dorsal approach, starting at the cranial end. Spinal cord was removed, and dorsal root ganglia (DRGs) were collected by fine forceps from both sides of the spinal column and transferred into ice cooled F-12 Medium (Biowest and Sigma, Taufkirchen, Germany). Spinal nerves were cut off under optical control using fine ophthalmological scissors. Thereafter, the ganglia were transferred into a mixture of 0.9 ml F12 medium and 0.1 ml collagenase medium (2612.5 U/ml, Sigma Type II) and digested in a humidified atmosphere (5% CO_2) at 37 °C for 40–55 min. In the next step, the collagenase was removed by washing the ganglia with 1.5 ml of F12 medium for three times. Then the ganglia were transferred into trypsin containing saline (2525 U trypsin per ml in F12 medium, Sigma Type IX) and incubated for 2–3 min under the same condition. Trypsinated ganglia were washed two times. After adding F12 medium (final volume 0.7 ml), the DRG neurones were triturated with a fire polished Pasteur pipette (tip diameter 150 μm) until the ganglion capsules were opened and the neurones were released from the ganglia. A portion of 50 μl of the resulting suspension was placed in the middle of small petri dishes (3 cm; Falcon "easy Grip") and incubated for 4–6 h, allowing cells to attach to the petri dish. Thereafter, 1 ml F12 (with 10% horse serum, Sigma) and 100 μl nerve growth factor were added to each petri dish. Cultures were used for electrophysiological experiments within the next 24 h.