Differences in calcium signalling in rat peripheral sensory neurons

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Received 4 July 2003; received in revised form 29 September 2003; accepted 2 October 2003

Abstract

Calcium influx and the resulting increase in intracellular calcium concentration \([Ca^{2+}]\) can induce enhanced sensitivity to temperature increases in nociceptive neurons. Using the patch-clamp technique and simultaneous calcium microfluorimetry we show that experimental elevation of \([Ca^{2+}]\), using the calcium ionophore ionomycin resulted in a significant potentiation of heat-activated currents. This was not the case when rises in \([Ca^{2+}]\) were elicited by depolarization of the cell membrane by current injection via the patch pipette. Our data provide first, however, indirect evidence that in sensory neurons calcium ions may be guided into different intracellular microdomains depending on the type of ion channel or pore through which they enter the cell. We conclude that the compartmentalization of sensory neurons for calcium ions may be decisive on further signalling cascades accounting, for example, for neuronal plasticity.

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Keywords: TRPV1; Microdomain; Calcium signalling; Nociceptor; Heat hyperalgesia

Excitatory inflammatory mediators like ATP, acetylcholine or extracellular acidosis can not only cause burning pain but also increase nociceptor responsiveness to cause heat hyperalgesia [1,2,6,14,15]. Similarly, the pungent ingredient of red hot chilli peppers, capsaicin, sensitizes nociceptive afferents to heat and leaves the application site in a hypersensitive state [17,18]. One common feature of these compounds is that they activate ionic currents that cause calcium influx and consecutive rises in intracellular calcium concentration \([Ca^{2+}]\) [3,5,21,26]. Such influx of \(Ca^{2+}\) accounted for the heat sensitization induced by the algogens ATP, acidic pH, capsaicin and a number of experimental compounds [10,14,15]. Very recently, a number of different stimuli including electrical stimulation have been shown to induce calcium signals in single peripheral sensory nerve terminals of the rat cornea [13]. In rat skin, depolarization-induced calcium-dependent release of calcitonin gene-related peptide from nociceptive terminals was mediated by N- and L-type voltage-gated calcium channels [16].

In the present study we therefore investigate if calcium influx induced by depolarizing voltage pulses causes a similar potentiation of heat-activated ionic currents (I_\text{heat}) in primary afferent neurons as previously observed, for example, following application of capsaicin or ionomycin.

A detailed description of the dissociation procedure has been published elsewhere [14]. Briefly, lumbar dorsal root ganglia (DRG, L1–L5) were harvested from adult female Wistar rats weighing 110–160 g from an inbred colony. The connective tissue was removed and the ganglia were treated with collagenase (0.28 units/ml, Roche Biochemicals, Mannheim, Germany) for 75 min and trypsin (25,000 units/ml in PBS, PAA Laboratories, Coelbe, Germany) for 12 min. The cells were dissociated with a fire-polished Pasteur pipette, plated on poly-L-lysine coated (200 \(\mu\)g/ml, Sigma) cover slips and cultivated in serum-free TNB 100° medium (Biochrom, Berlin, Germany) supplemented with penicillin–streptomycin (each 20,000 IU/100 ml), 2 mM L-glutamine (both from Gibco), and 100 ng/ml NGF (mouse NGF 7S, 100 ng/ml; Alomone Labs, Tel Aviv, Israel) in a humid atmosphere containing 5% CO2 at 37 °C.

Whole cell current measurements in the voltage-clamp configuration of the patch-clamp technique were performed up to 36 h after dissociation at −80 mV holding potential and 3 kHz sampling rate filtered at 3 kHz with an Axopatch 200A amplifier and pClamp6.0 software package on a PC-type computer (Axon Instruments, Forster City, CA). Borosilicate glass electrodes (Science Products, Hofheim, Germany) pulled on a horizontal puller (Sutter Instrument...
Company, Novato, USA) had resistances of 2–5 MΩ after filling with (in mM) KCl 148, MgCl₂ 4, Na-ATP 2, HEPES 10, Li-GTP 0.2 (all from Sigma) and 100 μM FURA-2 (penta-potassium salt, Molecular Probes, Leiden Netherlands) with the pH adjusted to 7.3 with KOH. The external solution consisted of (in mM) NaCl 145, KCl 5, CaCl₂ 2, MgCl₂ 1, Glucose 10 and HEPES 10 at pH 7.3 adjusted with NaOH. Depolarizing voltage pulses (−80 to 0 mV, 5 s duration) were applied via the patch pipette.

For ratiometric Ca²⁺ measurements background corrected fluorescent images were taken with a slow scan CCD camera system with a fast monochromator (PTI, New Jersey, USA) coupled to an Axiovert with ×40 fluar oil immersion objective (Zeiss, Oberkochen, Germany). FURA-2 was excited at 340 and 380 nm wavelength (λ) with equal exposure time of 200 ms and fluorescence was collected at λ > 500 nm. [Ca²⁺]ᵢ was calculated as previously published [15].

For drug application and heat stimulation of single neurons a fast 7-channel system with common outlet was used and voltage commands for automated heat stimulation were obtained from the pulse generator of the pClamp6.0 software. Ramp-shaped temperature increases from room temperature up to 50 °C within 5 s were applied at 1 min intervals. Solutions were flowing at constant speed which resulted in good reproducibility of the heat stimuli [11].

For detailed statistical analysis the Statistica software package for Windows 6.0 (StatSoft Inc., Tulsa, OK) was used. Differences were considered significant at P < 0.05; see Fig. 1. Differences were considered significant at P < 0.05, n = 9; Fig. 1). In two neurons the recording conditions were stable enough to apply both conditioning stimuli in the same cell and the same difference in outcome for ionomycin as compared to depolarization was obtained even when the second neuron was conditioned in reverse order (Fig. 2).

In previous studies of our group, substances inducing rises in [Ca²⁺]ᵢ, for example capsaicin or ionomycin, caused heat sensitization of nociceptors that was characterized by an increase in discharge activity in response to controlled heat stimuli and by a drop in heat thresholds. In a cellular model, the same substances induced potentiation of heat-activated ionic currents that fully depended on the preceding rises in [Ca²⁺], [14,15]. Since these changes were almost immediate a contribution of calcium-activated enzymatic signalling, for example activation of adenylyl cyclase and protein kinase A, was proposed [10]. As a potential target of PKA phosphorylation the vanilloid receptor TRPV1 was suggested by us and others [4,7,9,19,22].

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Fig. 1. Facilitation of Iheat following application of ionomycin but not depolarization voltage pulses. (A) Example of a neuron in which ramp-shaped heat stimuli evoke reproducible inward currents in control solution. Ionomycin (1 μM) results in a transient rise in [Ca²⁺]ᵢ (lowest panel) which is accompanied by an increase in peak Iheat (middle). A similar increase in [Ca²⁺]ᵢ but no potentiation of Iheat is induced by depolarization in the neuron depicted in (B). Peak Iheat is significantly increased after ionomycin-induced but not following depolarization-induced calcium influx (see insets C,D).
In contrast to reports of calcium-induced heat sensitization following exposure to capsaicin, acidic solutions or ionomycin, the present study demonstrates that calcium influx through voltage-operated calcium channels does not sensitize nociceptive neurons to heat although similar rises in $[\text{Ca}^{2+}]_i$ can be obtained by electrical stimulation via the patch pipette. This finding could have been anticipated from older studies where a change in heat sensitivity has never been observed upon antidromic electrical stimulation of nociceptive primary afferents [20,23]. Furthermore, even desensitizing effects of calcium entering the nociceptive neuron have been reported [8,12]. An explanation for these obviously contradictory findings had so far not been offered.

Nociceptive peripheral nerve terminals express a variety of functional ion channels and receptors, for example for capsaicin or acidic solutions as well as voltage-operated calcium channels, which upon activation let calcium ions enter the cell [13]. Spatio-temporal aspects of $\text{Ca}^{2+}$ signal transduction known from other cell types including neurons may also account for the differences in nociceptive calcium signalling [24,25].

Our data provide first, however, indirect evidence that in sensory neurons calcium ions may be guided into different intracellular microdomains depending on the type of ion channel or pore through which they enter the cell. We conclude that the compartmentalization of sensory neurons for calcium ions may be decisive on further signalling cascades accounting, for example, for neuronal plasticity.

Acknowledgements

The authors thank I. Izydorczyk and A. Wirth-Huecking for expert technical assistance, O. Obreja for carefully reading the manuscript, and H.O. Handwerker for continuous support. The work was supported by the DFG (SFB 353, A10) and the Wilhelm-Sander-Stiftung (1996.058.2).

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