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BRAIN RESEARCH

Research Report

Sustained increase of Ca^{+2} oscillations after chronic TRPV1 receptor activation with capsaicin in cultured spinal neurons

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ABSTRACT

Hyperalgesia and allodynia occur as a consequence of peripheral and central sensitization that follows sustained nociceptive activation. The cellular alterations associated to this state of nociceptive network hyperexcitability represent a form of neuronal plasticity, but they are not well understood because of its complexity *in situ*. In this study, after treating primary spinal neuron cultures with capsaicin (0.5–1 μM) for 48 h fluorimetric recordings were performed. The activation of TRPV1 receptors with capsaicin (0.5–1.0 μM) increased the frequency of calcium transients (0.03±0.002 Hz vs. 0.05±0.006 Hz, $P<0.05$), mediated by AMPAergic transmission, as well as the percent of neurons with activity (37±3% vs. 65±4%, $P<0.05$). The effect of capsaicin was long lasting and the neurons were found to be hyperfunctional and with increased levels of phosphorylated CREB (cAMP responsive element binding) even after 72 h of treatment with capsaicin (32±5% vs. 52±5%). The effect of capsaicin was blocked by capsazepine (1 μM), TTX (100 nM) and KN-62 (1 μM), but not by K252a (200 nM) or PD98059 (50 μM) indicating the involvement of TRPV1. The results suggest the participation of Ca^{2+}, CaMKII and CREB on the prolonged enhancement of excitability following chronic exposure to capsaicin. Thus, it is likely that chronic TRPV1 activation is capable of inducing prolonged increases in neurotransmission mediated by glutamatergic receptors.

1. Introduction

Hyperalgesia and allodynia occur as a consequence of peripheral and central sensitization induced by several forms of sustained nociceptive activation (Andersen et al., 1996; Cervero and Laird, 1999; Julius and Basbaum, 2001). Experimentally, it is known that applications of either capsaicin or strychnine to sensory pathways can induce a syndrome of neuropathic pain activating separate pathways (Sherman and Loomis, 1996; Ressot et al., 2001). However, the cellular and network mechanisms associated to this form of neuronal plasticity are poorly understood.

Capsaicin, a vanilloid derivative, is well known to produce a combination of nociceptive actions (Caterina, 2003). The acute effects of capsaicin are mediated by the activation of TRPV1 receptors that have been cloned and characterized (Caterina, 2003; Dedov et al., 2001; Ferrer-Montiel et al., 2004; Cortright and Szallasi, 2004). The effects of capsaicin are inhibited by ruthenium red and capsazepine (Ellis and Undem, 1994; Winter et al., 1993; Grant et al., 2001). TRPV1 receptors are widely

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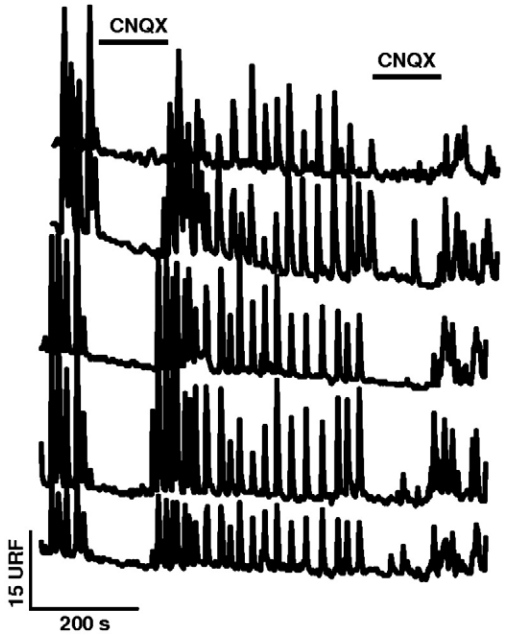

Fig. 1 – Cultured spinal neurons display AMPAergic-driven spontaneous calcium transients. Each trace corresponds to spontaneous calcium transients recorded from a single spinal neuron cultured over a glial monolayer and loaded with Calcium Green. The excitability was blocked with localized application of CNQX (1 μM). URF = Unit of relative fluorescence.

expressed in the central and peripheral nervous systems (Nagy and Rang, 1999; Dedov et al., 2001; Rashid et al., 2003; Vass et al., 2004; Breese et al., 2005; Cortright and Szallasi, 2004). It is now believed that TRPV1 receptors serve as an integrator of inflammatory pain pathways since they are not only activated by capsaicin, but also by various other stimuli such as low pH, temperature, arachidonic acid and prostaglandins. It has been proposed that a hyperalgesic response to thermal stimuli can result after a few hours of intense activation of TRPV1 (Caterina, 2003; Davis et al., 2000; Pogatzki-Zahna et al., 2005). Therefore, in this study we wanted to learn about the impact of sustained TRPV1 receptor activation on cultured spinal network activity. Additionally, we wanted to study if the capsaicin effects were mediated by increases in intracellular Ca^{2+} following membrane depolarization and leading to CREB activation, a main modulator of neuronal plasticity (Sasamura and Kuraishi, 1999; Ji and Woolf, 2001). Therefore, the present study was undertaken to examine the impact of chronic TRPV1 receptor activation on cultured spinal network excitability.

2. Results

Fig. 1 shows spontaneous calcium transients in spinal neurons cultured over an astrocyte monolayer. These spontaneous variations in intracellular calcium follow synaptic formation in culture and are highly dependent on neuronal excitability and calcium influx (Gu et al., 1994, Tapia el al., 2001, Carrasco et al., 2007a). The rhythmic and somewhat coordinated calcium transients represent bursting neuronal activity that is driven by synaptic inputs, excitatory and inhibitory, occurring in the presence of low extracellular K^+ (Ballerini et al., 1999). Focal applications of CNQX revealed that these spontaneous transients were highly dependent on the activation of AMPA receptors. Furthermore, the applications of TTX (100 nM), a Na^+ channel blocker, also inhibited this spontaneous neuronal network activity showing a dependency on action potential firing (Carrasco et al., 2007a). NMDA receptors at this developmental stage play a small role in neurotransmission (Tapia et al., 2001).

It was previously reported that application of capsaicin to DRG neurons caused a sustained increase in intracellular calcium (Szallasi and Blumberg, 1999). Therefore, in the first series of experiments, we wanted to determine if spinal neurons were sensitive to capsaicin and to examine if TRPV1 receptors were expressed in these cultured spinal cells. Examination of immunoreactivity in spinal neurons with fluorescent microscopy did not reveal the presence of a significant signal (not shown). However, acute application of capsaicin (1 μM) to spinal neurons produced a reversible increase in the frequency of calcium transients (Fig. 2A) showing the presence of functional receptors. In addition, the presence of TRPV1 receptors in these cultured spinal neurons, but not in HEK-293 or hippocampal neurons, was confirmed using immunodetection with Western blot techniques (Fig. 2B).

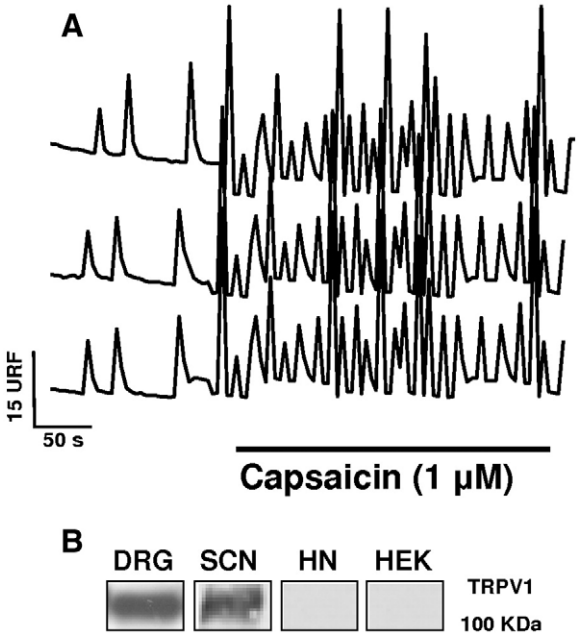

Fig. 2 – Acute capsaicin increased the excitability in cultured spinal neurons. A, The traces show spontaneous calcium transients detected with Calcium Green in three different neurons. Acute application of capsaicin (1 μM) increased the frequency of calcium transients. B, The insert shows the immunodetection of TRPV1 receptors with Western blots in spinal cord neurons (SCN), but not in hippocampal neurons (HN) and HEK 293 cells (HEK). DRG neurons were used as a positive control. Coomassie blue stained gels were used to standardize the protein loaded in each lane.