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Research Report

Group II metabotropic glutamate receptor activation on peripheral nociceptors modulates TRPV1 function

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ARTICLE INFO

Article history:
Accepted 23 October 2008
Available online 7 November 2008

Keywords:
mGluR
Pain
APDC
LY341495
Capsaicin
Primary afferent
Sensitization

ABSTRACT

Transient receptor potential vanilloid 1 (TRPV1) receptors are critical to nociceptive processing. Understanding how these receptors are modulated gives insight to potential therapies for pain. We demonstrate using double labeling immunohistochemistry that Group II metabotropic glutamate receptors (mGluRs) are co-expressed with TRPV1 on rat dorsal root ganglion (DRG) cells. In behavioral studies, intraplantar 0.1 μM APDC, a group II agonist, significantly attenuates capsaicin-induced nociceptive behaviors through a local effect. The APDC-induced inhibition of capsaicin responses is blocked by 1 μM LY341495, a group II antagonist. At the single fiber level, nociceptor responses to capsaicin are significantly decreased following exposure to APDC and this effect is blocked by LY341495. Finally, activation of peripheral group II mGluRs inhibits forskolin-induced thermal hyperalgesia and nociceptor heat sensitization, suggesting group II receptors are negatively coupled to the cAMP/PKA pathway. The data indicate that group II mGluRs and TRPV1 receptors are co-expressed on peripheral nociceptors and activation of mGluRs can inhibit painful sensory transmission following TRPV1 activation. The data are consistent with group II and TRPV1 receptors being linked intracellularly by the cAMP/PKA pathway. Peripheral group II mGluRs are important targets for drug discovery in controlling TRPV1-induced nociception.

1. Introduction

The transient receptor potential vanilloid 1 (TRPV1) receptor is considered a molecular integrator of chemical and physical stimuli (Caterina et al., 1997; Tominaga et al., 1998). These receptors are localized almost exclusively on nociceptors and when activated, they can result in intense painful sensations (Caterina and Julius, 2001). TRPV1 knock-out (KO) mice have relatively normal responses to acute noxious thermal and mechanical stimulation. In pain models, the TRPV1 KO's develop formalin-induced pain behaviors, carrageenan-evoked mechanical hyperalgesia and nerve-injury-induced mechanical hyperalgesia, (Davis et al., 2000; Caterina et al., 2000; Bolcskei et al., 2005). However, they show an obvious lack of heat hyperalgesia following carrageenan-induced inflammation (Davis et al., 2000; Caterina et al., 2000; Bolcskei et al., 2005) and a significant reduction in the thermal and mechanical hyperalgesia following a mild burn injury (Bolcskei et al., 2005). Thus, modulation of the TRPV1 receptor could be key to controlling pathophysiological pain.

Activation of group II metabotropic glutamate receptors (mGluRs) has been shown to play a role in reducing spinal cord injury pain (Mills et al., 2002), neuropathic pain (Simmons et al., 2002; Chiechio et al., 2002) and various types of inflammatory pain (Sharpe et al., 2002; Simmons et al., 2002; Yang and Gereau, 2003). Importantly, behavioral studies demonstrate

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0006-8993/$ – see front matter © 2008 Published by Elsevier B.V.
doi:10.1016/j.brainres.2008.10.066

group II activation can block prostaglandin E_2 (PGE_2)- and carrageenan-induced mechanical allodynia (Yang and Gereau, 2003) as well as intradermal capsaicin (CAP)-induced central sensitization of dorsal horn cells (Neugebauer et al., 2000).

The demonstration that primary sensory neurons express group II mGluRs (Carlton et al., 2001b) and their activation results in a significant reduction in PGE_2-induced potentiation of CAP responses (Yang and Gereau, 2002) offered the first suggestion that *peripheral* group II mGluRs might be an important target for the development of novel peripheral analgesics. In the present study, we further investigate peripheral group II mGluRs expressed on cutaneous nociceptors and their role in modulating TRPV1 function. We demonstrate using double labeling immunohistochemistry that group II mGluRs co-localize with TRPV1 receptors on small to medium diameter dorsal root ganglion (DRG) cells, providing a morphological basis for interaction of these receptors. We show that intraplantar injection of group II agonists inhibits CAP- and forskolin (FK)-induced nociceptive behaviors. *In vitro* electrophysiological recordings in the glabrous skin show that group II agonists attenuate CAP-induced excitation of nociceptors and FK-induced heat sensitization. Some of these data have been previously presented in abstract form (Zhou and Carlton, 2005; Du and Carlton, 2005).

2. Results

2.1. Co-localization of group II mGluR and TRPV1 in DRG

Single- and double-labeled profiles were counted in DRG sections from two L5 ganglia from two rats. Immunohistochemical staining for either receptor resulted in a homogenous reaction product that filled the cytoplasm but did not stain the nucleus (Fig. 1). The counts demonstrated that 39±7% of neuronal profiles were labeled positively for mGluR2/3 and 42±5% were labeled positively for TRPV1. Of neuronal profiles expressing mGluR2/3, all (100%) were double labeled for TRPV1. In contrast 93±5% of the TRPV1 cells also expressed mGluR2/3. The mean diameter of single mGluR2/3- and TRPV1-labeled cells was 21.8±3.7 and 21.7±3.6 μm, respectively; for double-labeled profiles it was 21.8±3.7 μm.

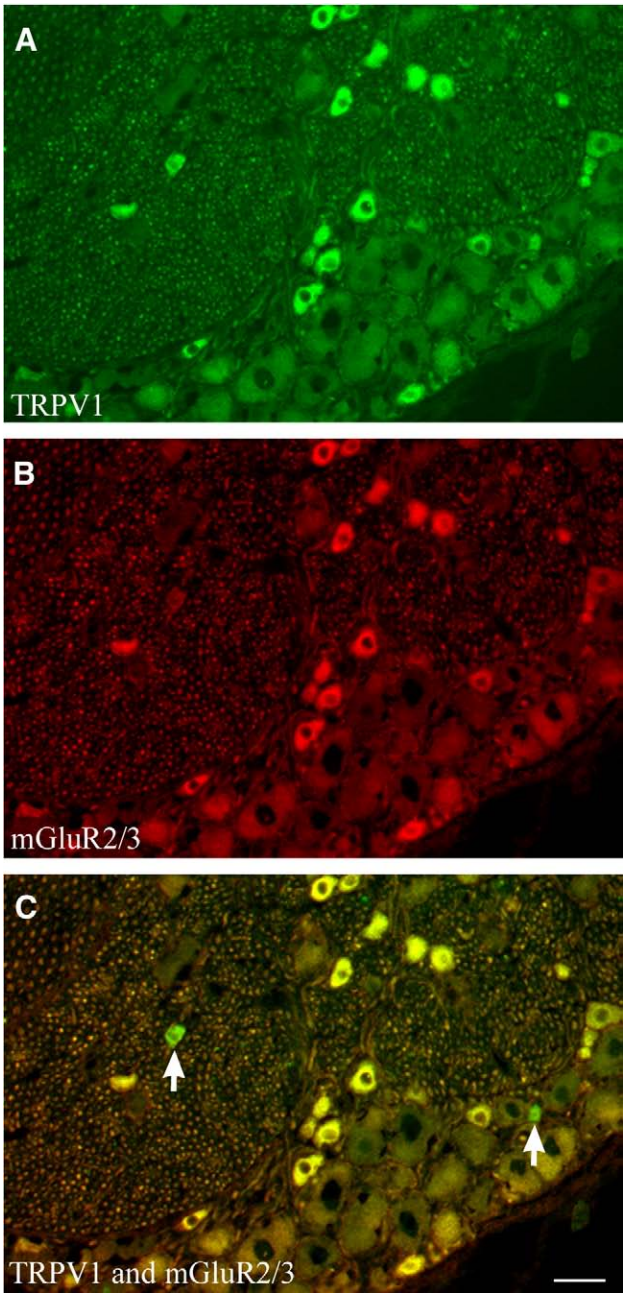

Fig. 1 – Double labeling with immunohistochemistry. The same DRG sections were immunostained with antibodies directed against TRPV1 (A) and mGluR2/3 (B) and the merged images are shown in panel C. Notice that all mGluR2/3-labeled cells also label for TRPV1. However, there is a small population of TRPV1 cells (arrows) which do not label for mGluR2/3. Bar=50 μm.

2.2. Group II mGluR modulation of CAP-induced pain behaviors

Intraplantar injection 0.1% CAP ($n=6$) evoked pain behaviors including flinching and L/L and co-injection of 0.1 μM APDC (a group II agonist) with CAP ($n=6$) significantly reduced both of these behaviors (Figs. 2A and B, $p<0.05$). Injection of the hind paw with the group II antagonist LY+APDC, followed by CAP ($n=6$) blocked the APDC effect. These rats showed nociceptive behaviors that were no different from rats injected with CAP alone. The APDC reduced CAP-evoked behaviors through local activation of group II receptors since injection of APDC in one hind paw did not affect the behavior evoked by CAP injected in the other hind paw (Figs. 2A and B, APDC contra). The time courses are illustrated in Figs. 2C and D. Intraplantar injection of APDC alone (Du et al., 2008) or 1 μM LY alone produced little or no behavioral response (not significantly different from PBS injection).

2.3. Group II mGluR modulation of CAP-induced activity in nociceptors

Application of 0.05% CAP to the receptive fields of C-mechanoheat (CMH) units ($n=10$) induced a robust excitation