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GROUP III METABOTROPIC GLUTAMATE RECEPTORS AND TRANSIENT RECEPTOR POTENTIAL VANILLOID 1 CO-LOCALIZE AND INTERACT ON NOCICEPTORS

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Abstract—Several lines of evidence indicate group III metabotropic glutamate receptors (mGluRs) have systemic anti-hyperalgesic effects. We hypothesized this could occur through modulation of transient receptor potential vanilloid 1 (TRPV1) receptors on nociceptors. To address this question we performed anatomical studies to determine if group III mGluRs were expressed on cutaneous axons and if they co-localized with TRPV1. Immunostaining at the electron microscopic level demonstrated that 22% of unmyelinated axons labeled for mGluR8. Immunostaining at the light microscopic level in lumbar dorsal root ganglia (DRG) demonstrated that 80% and 28% of neurons labeled for mGluR8 or TRPV1, respectively. Of those neurons labeled for mGluR8, 25% labeled for TRPV1; of those labeled for TRPV1, 71% labeled for mGluR8. In behavior studies intraplantar injection of the group III mGluR agonist, L-(+)-2-amino-4-phosphonobutyric acid (L-AP-4: 0.1, 1.0, and 10.0 μM) had no effect on paw withdrawal latency (PWL) to heat in naïve rats but administration of 10 μM L-AP-4 prior to 0.05% capsaicin (CAP), significantly attenuated CAP-induced lifting/licking and reduced flinching behavior. The L-AP-4 effect was specific since administration of a group III antagonist α-methyl-3-methyl-4-phosphonophenylglycine (UBP1112) (100 μM) blocked the L-AP-4 effect on CAP, resulting in behaviors similar to CAP alone. Intraplantar injection of UBP1112 alone did not result in nociceptive behaviors, indicating group III mGluRs are not tonically active. Finally, the anti-hyperalgesic effect of group III in this paradigm was local and not systemic since intraplantar administration of L-AP-4 in one hind paw did not attenuate nociceptive behaviors following CAP injection in the contralateral hind paw. Adenyl cyclase/cyclic AMP/PKA may be the second messenger pathway linking these two receptor families because intraplantar injection of forskolin (FSK, 10 μM) reduced PWL to heat and L-AP-4 reversed this FSK effect. Taken together, these results suggest group III mGluRs can negatively modulate TRPV1 through inhibition of adenyl cyclase and downstream intracellular activity, blocking TRPV1-induced activation of nociceptors.

Key words: L-AP-4, mGluR8, capsaicin, anti-hyperalgesia, primary afferents, sensory neurons.

INTRODUCTION

Glutamate, a major excitatory neurotransmitter in the nervous system, is important in peripheral pain transmission and acts on both ionotropic and metabotropic receptors (Carlton, 2001; Goudet et al., 2009). The eight cloned metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors and are divided into three groups depending on homology and pharmacological properties. Group I (mGluRs 1 and 5) are excitatory and coupled to G_q, thus associated with the stimulation of phospholipase C (PLC) and intracellular calcium signals. Groups II (mGluRs 2 and 3) and III (mGluRs 4, 6, 7, and 8) are inhibitory and coupled to $G_{i/o}$, thus negatively coupled to adenyl cyclase (AC) (Goudet et al., 2009). The inhibitory functions of groups II and III make these receptors attractive targets for peripheral pain modulation. Several lines of evidence have implicated group II receptor agonists in the attenuation of peripheral pain (Anjaneyulu et al., 2008; Du et al., 2008). Data indicating that systemic administration of group III mGluR agonists is also effective in treatment of pain are accumulating. For example, administration of the group III agonist, L-(+)-2-amino-4-phosphonobutyric acid (L-AP-4), in the primate dorsal horn, inhibits capsaicin (CAP)-induced central sensitization (Neugebauer et al., 2000; Goudet et al., 2009). Additionally, application of L-AP-4 to spinal lamina II in nerve injured rats results in greater inhibition of evoked excitatory postsynaptic currents (EPSCs) compared to control rats (Zhang et al., 2009). Finally, systemic administration of (*S*)-3,4-dicarboxyphenylglycine (DCPG), a selective mGluR8 agonist, attenuates formalin and carrageenan-induced hyperalgesia (Marabese et al., 2007). Currently, studies investigating the effects of group III mGluR activation in the periphery are limited (Walker

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Abbreviations: AMPA, alpha-amino-3-hydroxy-5-methylisoxazolone-4-propionic acid; AC, adenyl cyclase; cAMP, cyclic AMP; CAP, capsaicin; DCPG, (*S*)-3,4-dicarboxyphenylglycine; DRG, dorsal root ganglia; EPSC, excitatory postsynaptic current; FSK, forskolin; iGluR, ionotropic glutamate receptor; L-AP-4, L-(+)-2-amino-4-phosphonobutyric acid; L/L, lifting and/or licking; mGluR, metabotropic glutamate receptor; NGS, normal goat serum; NDS, normal donkey serum; NGF, nerve growth factor; NMDA, N-Methyl-D-aspartate; PB, phosphate buffer; PBS, phosphate buffered saline; PGE2, prostaglandin E2; PGI2, prostaglandin I2; PWT, paw withdrawal threshold; PKA, protein kinase A; PLC, phospholipase C; PWL, paw withdrawal latency; RT, room temperature; SCG, satellite glial cells; TRPV1, transient receptor potential vanilloid 1; TSA, tyramide signal amplification; TrkA, tyrosine kinase A; UBP, α-methyl-3-methyl-4-phosphonophenylglycine (UBP1112).

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et al., 2001; Jin et al., 2009). For example, group III mGluR activation has been shown to be anti-hyperalgesic on bee venom-induced nociception in the rat hind paw (Chen et al., 2010). Furthermore, no studies have examined the relationship between peripheral group III mGluR agonists and the transient receptor potential ion channels.

Transient receptor potential vanilloid 1 (TRPV1) is most widely known as the CAP receptor. CAP is an ingredient found in hot chili peppers and activates the channel (Caterina et al., 1997). In addition to CAP, TRPV1 can be activated by a variety of noxious stimuli: heat (above 40 °C) (Caterina et al., 1997), acidic pH (Tominaga et al., 1998; Jordt et al., 2000), cannabinoid anandamide (Zygmunt et al., 1999; Smart et al., 2000), HETE (Hwang et al., 2000), and spider toxins (Costa et al., 1997; Siemens et al., 2006). Thus, TRPV1 is a polymodal receptor. Several studies have demonstrated that TRPV1 knockout mice maintain responses to acute noxious stimuli while showing attenuated development of hyperalgesia in the inflammatory state (Caterina et al., 2000; Davis et al., 2000; Bölcskei et al., 2005). These studies highlight the role TRPV1 plays in development of hyperalgesia and the importance of targeting TRPV1 in the treatment of pain.

Pain modulation at its source is an attractive strategy, especially in aiming to reduce side-effects common to most widely available analgesics (Patapoutian et al., 2009; McDougall, 2011). Since the TRP family makes up a large group of temperature sensing ion channels (Patapoutian et al., 2009), it is of great interest to examine the modulation of TRP channels, TRPV1 in particular, in the periphery. In the present study, we examined the effects of group III mGluR activation by L-AP-4 on CAP-induced nociception in the periphery.

EXPERIMENTAL PROCEDURES

All experiments were approved by the University Animal Care and Use committee and followed the guidelines for the ethical care and use of laboratory animals (Zimmermann, 1983). Steps were taken to minimize both the number of animals used and their discomfort.

Immunostaining of the digital nerve at the electron microscopic level

Naïve male Sprague–Dawley rats (n = 3, 250–300 g) were deeply anesthetized with pentobarbital (100 mg/kg) and perfused transcardially with heparinized saline followed by a mixture of 2.5% glutaraldehyde, 1.0% paraformaldehyde and 0.1% picric acid in 0.1 M phosphate buffer (PB) at 4 °C. The digital nerves were dissected and cut into 2.0–2.5 mm segments. Prior to immunostaining, all tissue was placed for 1 h in 1% sodium borohydride to remove excess glutaraldehydes and rinsed in graded alcohols to increase antibody penetration. The tissue was placed in 10% normal goat serum (NGS) for 1 h and incubated in antiserum made in guinea pig directed against mGluR8 (1:2000, Chemicon, Temecula, CA, USA) for 72 h at 4 °C. Following incubation, the tissues were rinsed in phosphate buffered saline (PBS), followed by 3% NGS for 30 min, incubated in biotinylated goat anti-guinea-pig IgG in 1% NGS for 1 h, rinsed in 1% NGS followed by 3% NGS for 30 min each and incubated in the ABC reagent (Vector Laboratories, Burlingame, CA, USA) for 2 h. The tissue was incubated in a solution of diaminobenzidine (0.05%) containing 0.01% hydrogen peroxide for approximately 4–6 min. After immunostaining, blocks were placed in 1% phosphate-buffered osmium tetroxide for 2 h, dehydrated and embedded in plastic. For all blocks, ultrathin sections of the digital nerves were cut at right angles to the long axis of the fibers. The sections were mounted on formvar-coated slot grids and viewed on a JEOL 100CX electron microscope (Peabody, MA, USA). A digital nerve cut in cross section from each animal was photographed and montaged, and all labeled and unlabeled axons counted in order to obtain a percentage of mGluR8-labeled axons. For some tissues, the primary antiserum was omitted and the tissue was otherwise treated as described above. Absorption controls were also run with the peptide obtained from Chemicon. Tissue was incubated in a solution containing antibody (1:2000) that was preabsorbed with 100 μg/ml of peptide. Specific immunoreaction product was absent in both of these controls.

Immunostaining the dorsal root ganglia (DRG) at the light microscopic level

Tissue collection. Naïve male Sprague–Dawley rats (n = 3, 250–300 g) were deeply anesthetized with pentobarbital (100 mg/kg) and perfused transcardially with 4% paraformaldehyde and 0.1% picric acid in 0.1 M PB at 4 °C. The L4 DRG were dissected out. The ganglia were fast frozen using liquid nitrogen in a cryoembedding compound. The tissue was sectioned (8 μm thick) on a cryostat (Microm International, GmbH, Walldorf, Germany), along the short axis and placed on gelatin-dipped 3-well, Teflon-printed slides (Electron Microscopy Sciences, Fort Washington, PA, USA). Serial section sets (3–5 sets per ganglia) were collected with a defined section separation so that cell counts could be determined by stereological analysis. Tissue sections were allowed to dry overnight at room temperature (RT).

Immunohistochemistry. Slides containing tissues were first incubated in PB + 0.2% TritonX for 10 min. Sections were blocked in 10% normal donkey serum (NDS) for 1 h. Sections were incubated in guinea-pig polyclonal anti-mGluR8 (1:200,000, Chemicon) for 48 h at RT and rinsed with PBS. Next, sections were incubated in biotinylated goat anti-guinea-pig IgG (1:200, Vector Laboratories) for 1 h. After rinsing in PBS, sections were incubated in Vectastain Elite ABC peroxidase reagent (avidin–biotin complex, Vector Laboratories) for 1 h and rinsed in PBS. Sections were incubated in Cyanine 3-labeled tyramide (1:75, Perkin-Elmer Life Science, Waltham, MA, USA) for 7 min, using the TSA (Tyramide Signal Amplification) protocol, rinsed in PBS, and incubated in 0.03% H_2O_2 for 20 min to inactivate residual peroxidase, followed by a rinse in PBS. Next, sections were incubated in goat anti-TRPV1 (1:500 Santa Cruz, Santa Cruz, CA, USA) for 24 h. Sections were rinsed in PBS and incubated in a Cyanine 2-conjugated mouse anti-goat IgG (1:300 Jackson Laboratories, West Grove, PA, USA) for 1 h and were rinsed in PBS, then dH_2O. Slides were cover slipped using Vecta-Shield mounting media (Vector Laboratories). In separate sets of tissues used for controls, the primary antiserum was omitted and the tissue was otherwise treated as described above. Absorption controls for TRPV1 (Carlton et al., 2009) and mGluR8 (Carlton and Hargett, 2007) have been reported previously from our lab.

Cresyl Violet. To obtain total cell counts, one serial section set representing each DRG was stained with Cresyl Violet for 2 min.

Analysis. Pairs of serial sections (disector pairs) were photographed and analyzed using the physical disector method (Coggeshall, 1992; Coggeshall and Lekan, 1996; West, 1999). Total cell counts were determined for each DRG by multiplying the number of Tops (cells only present in one of the two disector sections) by section separation, and dividing by 2 (the number of disector sections). Tissues labeled with mGluR8 and TRPV1 were used to determine percentages of single- and double-