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Research Paper

Inflammation-induced GluA1 trafficking and membrane insertion of Ca^{2+} permeable AMPA receptors in dorsal horn neurons is dependent on spinal tumor necrosis factor, PI3 kinase and protein kinase A☆

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ABSTRACT

Peripheral inflammation induces sensitization of nociceptive spinal cord neurons. Both spinal tumor necrosis factor (TNF) and neuronal membrane insertion of Ca^{2+} permeable AMPA receptor (AMPAr) contribute to spinal sensitization and resultant pain behavior, molecular mechanisms connecting these two events have not been studied in detail. Intrathecal (i.t.) injection of TNF-blockers attenuated paw carrageenan-induced mechanical and thermal hypersensitivity. Levels of GluA1 and GluA4 from dorsal spinal membrane fractions increased in carrageenan-injected rats compared to controls. In the same tissue, GluA2 levels were not altered. Inflammation-induced increases in membrane GluA1 were prevented by i.t. pre-treatment with antagonists to TNF, PI3K, PKA and NMDA. Interestingly, administration of TNF or PI3K inhibitors followed by carrageenan caused a marked reduction in plasma membrane GluA2 levels, despite the fact that membrane GluA2 levels were stable following inhibitor administration in the absence of carrageenan. TNF pre-incubation induced increased numbers of Co^{2+} labeled dorsal horn neurons, indicating more neurons with Ca^{2+} permeable AMPAr. In parallel to Western blot results, this increase was blocked by antagonism of PI3K and PKA. In addition, spinal slices from GluA1 transgenic mice, which had a single alanine replacement at GluA1 ser 845 or ser 831 that prevented phosphorylation, were resistant to TNF-induced increases in Co^{2+} labeling. However, behavioral responses following intraplantar carrageenan and formalin in the mutant mice were no different from littermate controls, suggesting a more complex regulation of nociception. Co-localization of GluA1, GluA2 and GluA4 with synaptophysin on identified spinoparabrachial neurons and their relative ratios were used to assess inflammation-induced trafficking of AMPAr to synapses. Inflammation induced an increase in synaptic GluA1, but not GluA2. Although total GluA4 also increased with inflammation, co-localization of GluA4 with synaptophysin, fell short of significance. Taken together these data suggest that peripheral inflammation induces a PI3K and PKA dependent TNFR1 activated pathway that culminates with trafficking of calcium permeable AMPAr into synapses of nociceptive dorsal horn projection neurons.

1. Introduction

Peripheral inflammation elicits a state of central or spinal sensitization that, in part, underlies development of the resultant pain behavior (Latremoliere and Woolf, 2009). This spinal plasticity encompasses a plethora of mechanisms and transduction pathways, many working in parallel. We have focused our efforts on delineating one such pathway initiated by spinal tumor necrosis factor (TNF), which is presumably released from activated glia, and culminates with trafficking of GluA1 and GluA4 subunit enriched, GluA2 lacking, α-amino-3-hydroxy-5-methyl-4-isoxazol proprionic acid (AMPA) receptors into plasma membranes

Abbreviations: AMPA, α-amino-3-hydroy-5-methyl-4-isoxazolepropionic acid; AMPAr, AMPA receptor; EPSC, excitatory post-synaptic current; ETA, Etanercept; i.t., intrathecal; LTD, long term depression; LTP, long term potentiation; NMDA, *N*-methyl-D-aspartic acid; NMDAr, NMDA receptor; PI3K, phosphatidylinositol 3 kinase; PKA, protein kinase A; PKC, protein kinase C; TNF, tumor necrosis factor; TNFR1, TNF receptor 1.

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(Beattie et al., 2002; Choi et al., 2010). These newly-inserted receptors are Ca^{2+} permeable AMPA receptors (Katano et al., 2008; Park et al., 2009; Vikman et al., 2008).

All AMPA receptors are comprised of four subunits (GluA1-4) that form a tetramer when assembled (Keinänen et al., 1990). The majority of AMPA receptors in spinal cord of adult rats contain GluA2 paired with either GluA1 or GluA3 and are Ca^{2+} impermeable. Receptors lacking GluA2 subunits are Ca^{2+} permeant (Burnashev et al., 1992; Hollmann et al., 1991) and, in naïve animals, are found in low levels throughout laminae I–IV (Engelman et al., 1999; Kopach et al., 2011). Significantly, there is a small population of nociceptive lamina I projection neurons that lack GluA1, but instead contain GluA4, (Polgár et al., 2008, 2010). Other nociceptive neurons that develop Ca^{2+} permeable AMPA receptors with GluA4 have more recently been identified in deep dorsal horn (Cabañero et al., 2013). Plasmalemmal AMPA receptors are normally in dynamic equilibrium with those in the cytosolic compartment (Scannevin and Huganir, 2000). Equilibrium is altered by changes in afferent drive. Acutely, increased nociceptive drive favors membrane insertion of GluA1 enriched and GluA2 lacking AMPA receptors and removal of GluA2 containing receptors from the membrane of some cell types (Choi et al., 2010, 2012; Galan et al., 2004; Park et al., 2008; Pezet et al., 2008). Analogous membrane trafficking of GluA4 subunit enriched, GluA2 lacking AMPA receptors occurs in deep dorsal horn during morphine-induced hypersensitivity (Cabañero et al., 2013). Following endocytosis, AMPA receptors are either recycled or degraded depending, among other things, on the proportion of AMPA and NMDA receptor activation and the phosphorylation state of GluA1 (Ehlers, 2000; Fernández-Monreal et al., 2012).

Levels of spinal cord TNF increase during the acute phase of inflammation (Christianson et al., 2012; Raghavendra et al., 2004) and pharmacological blockade of spinal TNF attenuates inflammation-induced pain behavior (Choi et al., 2012; Christianson et al., 2012). In cortical neurons, acute application of TNF increases the magnitude of AMPA evoked miniature excitatory post-synaptic currants (EPSCs) (Steinmetz and Turrigiano, 2010), while in hippocampal neurons (Leonoudakis et al., 2008; Ogoshi et al., 2005; Stellwagen et al., 2005) and spinal motor neurons (Ferguson et al., 2008; Yin et al., 2012), TNF is known to elicit membrane insertion of GluA2 lacking AMPA receptors.

A small body of evidence implicates phosphatidylinositol 3 kinase (PI3K) as an intermediary between acute peripheral inflammation (intraplantar formalin and intracolonic capsaicin) and activity driven increases in GluA1 in membrane fractions obtained from dorsal horn (Galan et al., 2004; Pezet et al., 2008). Other data exist linking protein kinase A (PKA) with spinal AMPA receptor trafficking down stream of NMDA receptor activation (Ehlers, 2000) and many studies have shown that peripheral injury elicits phosphorylation of GluA1 ser 845 by PKA (Choi et al., 2010; Fang et al., 2003; Peng et al., 2011) and of GluA1 ser 831 by PKC and/or CaMKII. Spinal blockade of either PKA or PKC activity prevents peripheral inflammation induced hyperalgesia (Jones and Sorkin, 2005; Peng et al., 2011; Sluka, 2002; Willis, 2001).

In this study, using an animal model of intraplantar carrageenan, combined with subcellular fractionation techniques, we demonstrate peripheral inflammation induced increases in dorsal spinal cord plasma membrane GluA1 and GluA4 as well as a small, simultaneous loss of GluA2. Furthermore, we show that this trafficking is dependent on spinal TNF, NMDA, PI3K and PKA. Immunohistochemistry and confocal microscopy indicate that a significant portion of the increased membrane GluA1 on identified spinal projection neurons is synaptic. In vitro studies conducted in parallel, which used kainate induced cobalt labeling of Ca^{2+} permeable AMPA receptors showed that TNF induces a PI3K and PKA dependent insertion of Ca^{2+} permeable AMPA receptors in dorsal horn neurons. In a follow up study, using knock-in mice with point mutations on GluA1 that do not permit phosphorylation at ser 831 (CaMKII/PKC site) or ser 845 (PKA site), we demonstrated that TNF induced trafficking of Ca^{2+} permeable AMPA receptors is both PKC and PKA dependent.

2. Materials and methods

2.1. Animals and surgery

Male Holtzman rats (Harlan Industries, Indianapolis, IN), weighing 250–350 g were used for behavioral experiments and for in vivo inflammation followed by tissue harvest and either Western blots or immunohistochemistry. Female Sprague Dawley rats (175–250 g; Charles River Laboratories, Wilmington, MA) provided spinal cord slices for in vitro cobalt labeling experiments. Transgenic mice, which lacked the ability to phosphorylate GluA1 at ser 845 or at ser 831 due to single alanine substitutions were generated (Johns Hopkins University, (H.-K. Lee et al., 2003)) and bred to C57BL/6 mice (Harlan Industries) to produce heterozygous mating pairs at University of California, San Diego. This mutation prevents insertion of GluA1-containing Ca^{2+} permeable AMPAr into the plasma membrane. These mice were bred and homozygous knock-ins and wild type littermates used for behavioral (both sexes) and in vitro cobalt labeling (female) experiments. Animals were maintained on a 12-h light/dark cycle with free access to food and water; all experiments were performed during the light cycle. Experiments were carried out according to protocols approved by the Institutional Animal Care and Use Committees of the University of California, San Diego and the University of California, Irvine (under the Guide for Care and Use of Laboratory Animals, National Institutes of Health publication 85-23, Bethesda, MD, USA).

To permit bolus intrathecal (i.t.) drug delivery in the in vivo rat studies, lumbar i.t. catheters (PE-5, 8.5 cm in length, Spectranetics, Colorado Springs, CO, USA) were implanted via cisternal exposure under isoflurane anesthesia (2–4%) and externalized as described elsewhere (Yaksh and Rudy, 1976). Following catheter implantation rats were housed singly. Experiments were performed 5–10 days following catheter implantation.

2.2. Drugs

In Vivo: Etanercept (Enbrel, 30 and 100 μg, TNF antagonist, Amgen, Thousand Oaks, CA, USA) and a receptor-neutralizing antibody specific for TNFR1 (125 μg, R&D Systems, Minneapolis, MN, USA) were used for behavioral experiments. The following agents were used prior to tissue harvest and subsequent immunoblotting. Etanercept (ETA), 100 μg; LY294002 (2-morpholin-4-yl-8-phenylchromen-4-one), 50 μg, a non-selective PI3K inhibitor; H-89 (dihydrochloride hydrate, 26 μg, a PKA inhibitor), Calbiochem, La Jolla, CA); AKAP st-Ht31(2.8 μg, Promega, Madison, WI, USA, inhibitor protein for PKA anchoring onto AKAPs); and MK-801 hydrogen maleate, (MK-801, 30 μg, a non-competitive NMDA receptor antagonist) were delivered i.t. in 10 μl vehicle followed by a 10 μl saline flush. LY294002 was obtained from Sigma Aldrich, St. Louis, MO. All agents were dissolved in physiological saline, except for LY294002, which was dissolved in 5% DMSO, 2.5% EtOH and 92.5% saline.

In vitro: Wortmannin (100 μM, a non-selective PI3K antagonist; Tocris Bioscience, Ellisville, MI) and H89 (10 μM) were used as pretreatments prior to adding TNF (20 nM Rats; 12 nM Mice), to slices in the bath.

2.3. Induction of inflammation

2.3.1. Rats

To induce a state of local inflammation 100 μl of a 2% λ carrageenan solution (Sigma, St Louis, MO, USA; v/w in physiological saline) was injected subcutaneously into the ventral aspect of the left hind paw under brief isoflurane anesthesia.

2.3.2. Mice

The injection volume of carrageenan was reduced to 20 μl. After injection, animals were immediately placed in their test compartments