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SURGICAL INCISION INDUCES PHOSPHORYLATION OF AMPA RECEPTOR GLUR1 SUBUNITS AT SERINE-831 SITES AND GLUR1 TRAFFICKING IN SPINAL CORD DORSAL HORN VIA A PROTEIN KINASE Cγ-DEPENDENT MECHANISM

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Abstract—Spinal α-amino-3-hydroxy-5-methy-4-isoxazole propionate (AMPA) receptor plays an important role in acute pain induced by surgical tissue injuries. Our previous study has shown that the enhanced phosphorylation of AMPA receptor GluR1 subunits at Serine-831 sites by protein kinase C (PKC) in the spinal cord dorsal horn is involved in post-surgical pain hypersensitivity. However, which isoforms of PKC are responsible for the phosphorylation of AMPA receptor GluR1 subunits at Serine-831 sites remains to be established. In the present study, using an animal model of postoperative pain, we found that surgical tissue injuries enhanced the membrane translocation level of PKCγ, but not PKCα, βI, and βII, and induced the trafficking of GluR1, but not GluR2 into neuronal plasma membrane. Intrathecal (i.t.) pretreatment of small interfering RNA targeting PKCγ to reduce the PKCγ expression in the spinal cord significantly attenuated the pain hypersensitivity and inhibited the phosphorylation of AMPA receptor GluR1 subunits at Serine-831 sites as well as GluR1 membrane trafficking. Our study indicates that the surgical incision-induced phosphorylation of AMPA receptor GluR1 subunits at Serine-831 sites and GluR1 trafficking are regulated by a PKCγ-dependent mechanism.

Key words: surgery, pain, AMPA receptor, PKC, trafficking.

INTRODUCTION

Postoperative pain is a common form of acute pain associated with daily surgical procedures. Efficacious postoperative pain analgesia may improve patient satisfaction, and decrease morbidity and mortality following surgery (Liu and Wu, 2007). Despite multiple therapeutic approaches, the medical community still faces a great challenge to relieve acute postoperative pain. Opioids are still used as the mainstay in clinical treatment of postoperative pain (Wu and Raja, 2011). A major concern in the current management of postoperative pain is the risk of serious side effects with opioid use, such as nausea, vomiting, urinary retention, pruritus, respiratory depression, tolerance, addiction and hyperalgesia, etc. There is hence an overwhelming need for the development of novel, safer, non-opioid analgesics in alleviating postoperative pain.

Brennan's group developed a rat model of incisional pain (Brennan et al., 1996). The plantar incision in rats produces a variety of nociceptive responses that parallel the time course of postoperative pain in humans. Using this model, it has been shown that the mechanisms underlying postoperative pain are very different from those of inflammatory or neuropathic pain. For example, spinal *N*-methyl-D-aspartate (NMDA) receptor antagonists might inhibit exaggerated pain behaviors in most models of persistent pain, but they have no significant effects on pain behaviors after a plantar incision (Zahn and Brennan, 1998; Zahn et al., 2005). Interestingly, several lines of evidence have shown that epidural or intrathecal (i.t.) administration of α-amino-3-hydroxy-5-methy-4-isoxazole propionate (AMPA)/kainate receptor antagonist surprisingly produced postoperative analgesia (Zahn et al., 2005; Lee et al., 2006; Jin et al., 2007). These studies indicate a critical role of AMPA receptors in signaling postoperative pain. AMPA receptor presents some unique characteristics in the mechanisms of its subunits regulation during nociceptive stimulation, which is involved in the trafficking and phosphorylation of AMPA receptor subunits (Fang et al., 2002, 2003; Hartmann et al., 2004; Wang et al., 2010b). Our recent study has shown that AMPA receptors are implicated in the mechanisms underlying postoperative pain through the phosphorylation regulation of GluR1 subunits at the Serine-831 site and a protein kinase C (PKC) inhibitor that selectively targets several PKC isoforms, Gö6983 significantly inhibits the

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Abbreviations: AMPA, α-amino-3-hydroxy-5-methy-4-isoxazole propionate; aPKC, atypical PKC; BCA, bicinchoninic acid; CFA, complete Freund's adjuvant; cPKC, conventional PKC; DAG, diacylglycerol; EDTA, ethylenediaminetetraacetic acid diacylglycerol; GRIP, glutamate receptor-interacting protein; i.t., intrathecal; NMDA, *N*-methyl-D-aspartate; nPKC, novel PKC; PICK1, protein interacting with C kinase 1; PKC, protein kinase C; PVDF, polyvinylidene difluoride; siRNA, small interfering RNA.

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phosphorylation of AMPA receptor GluR1 subunit at Serine-831 site and attenuates pain hypersensitivity (Wang et al., 2011b). Additionally, the activation of PKC was found to be the upstream event of the phosphorylation of AMPA receptor GluR1 subunit at Serine-831 site (Lee et al., 2000, 2010). The trafficking of GluR1 subunits into neuronal membrane may be triggered by the phosphorylation of GluR1 subunits and is known to be a critical step in the functional regulation of AMPA receptors (Thomas et al., 2008; Anggono and Huganir, 2012). Previous studies have demonstrated that the trafficking of GluR1 subunits plays an important role in pain hypersensitivity in different pain models (Galan et al., 2004; Choi et al., 2010; Tao, 2012). However, which isoforms of PKC are responsible for the phosphorylation of GluR1 subunit at Serine-831 site as well as its subsequent trafficking into neuronal membrane remains unclear.

PKC is a family of phospholipids-dependent serine/threonine kinase that participates in a serial of cellular functions during nociceptive transmission. According to their activation requirements, PKCs are divided into conventional (cPKC), novel (nPKC) and atypical (aPKC) isoforms. cPKCs require Ca^{2+} and diacylglycerol (DAG) for their activation, whereas nPKCs and aPKCs are only responsive to DAG and lipid mediators for activation, respectively (Velazquez et al., 2007). Within the superficial laminae of the dorsal spinal cord, an area containing sensory neurons that has been implicated in major pain processing, cPKCs including PKCα, βI, βII, and γ isoforms have been identified and localized (Igwe and Chronwall, 2001). With the evidence that cPKC isoforms are in the anatomical regions that possibly regulate nociception processing, our aim was to determine whether these cPKC isoforms are involved in the mechanism of painful events and could serve as potential therapeutic targets for developing new treatment of postoperative pain. The membrane translocation of PKC was reported to be used as an index of PKC activation, since this event is generally associated with its activation (Wang et al., 2010a). In the current study, we also tried to determine which individual cPKC isoform activation (membrane translocation) might be involved in the phosphorylation of GluR1 subunit at Serine-831 and its trafficking during postoperative painful stimuli.

EXPERIMENTAL PROCEDURES

Animals

Male Sprague–Dawley (SD) rats weighing 250–300 g were used for all studies. Rats were housed on a 12-h light/12-h dark cycle and maintained at 21 ± 2 °C with free access to food and water. All experimental procedures were approved by the Ethics Committee of Beijing Chaoyang Hospital, Capital Medical University and followed guidelines issued by the International Association for the Study of Pain.

Experiment design

Based on our preliminary time course studies, to identify which individual cPKC and AMPA receptor subunit were possibly involved in postoperative pain, we examined the changes of the membrane translocations of cPKCs, and the trafficking of GluR1 and GluR2 into membrane fractions 3 h after plantar incision. Tissues from normal rats were used as control. For sham surgery group, animals only received right hindpaw sterilization under 5-min isoflurane anesthesia, but no incision. For incision group, rats received right hindpaw sterilization and 1-cm plantar incision under isoflurane anesthesia.

The experiments above showed the activation of PKCγ and GluR1 trafficking into membrane fractions in response to surgical incision (see the section of Results). As these substrates were altered by plantar incision, we further examined the effects of pretreatment of PKCγ small interfering RNA (siRNA) on pain behaviors, phosphorylation of GluR1 subunits at Serine-831, and GluR1 trafficking into the plasmic membrane to confirm whether PKCγ was involved in the upstream regulation of the phosphorylation of AMPA receptor GluR1 subunits at Serine-831, and the GluR1 trafficking into plasmic membrane. Eight rats implanted with i.t. catheters were used as a control and they only received i.t. injections of vehicle, but no isoflurane anesthesia, plantar sterilization and incision. Rats in the sham surgery group underwent i.t. pretreatment of vehicle, isoflurane anesthesia and plantar sterilization, but without incision. For the incision groups, rats received i.t. pretreatments of vehicle, scrambled siRNA, siRNA for PKCγ, respectively and then, they were subjected to plantar sterilization and incision under isoflurane anesthesia 24 h following the last i.t. injection. The rats in different groups were intrathecally pretreated with vehicle, PKCγ siRNA (2 μg, i.t.) or scrambled RNAi as a negative control (2 μg, i.t.) twice daily for three consecutive days before surgical incision. The agents for i.t. injections were administered through the i.t. catheter in a volume of 10 μl followed by a 10-μl saline flush to clear the catheter. The cumulative pain scores and withdrawal threshold to mechanical stimulation were measured 3 h after incision in rats. Finally, the animals were sacrificed and the spinal cord tissue samples were obtained for further experiments.

Plantar incision

Rats were anesthetized for plantar incision using the same method as we described previously (Brennan et al., 1996; Wang et al., 2007, 2008, 2011b). In brief, anesthesia was induced by placing the animal in a sealed plastic box filled with 5% isoflurane mixed with air. Then, anesthesia was maintained with 1.2–1.5% isoflurane delivered through a nose cone to the animal. The plantar aspect of the right hindpaw was sterilized with 10% providone–iodine and the paw was placed through a sterile drape. A 1-cm longitudinal incision was made with a number 11 blade, through skin and fascia of the plantar aspect of the foot, starting 0.5 cm from the proximal edge of the heel and extending toward the toes. The underlying flexor muscle was elevated and incised longitudinally. The muscle origin and insertion remained intact. The wound skin was closed with two mattress sutures of 5–0 nylon and covered with antibiotic ointment and appropriate dressing. After surgery, the rats were allowed to recover in individual cages.

Intrathecal catheter implantation

The i.t. catheter implantation was performed as we described previously (Wang et al., 2007, 2008, 2011b). The catheter (PE-10) was passed 8.5 cm caudally to the level of the lumbar