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·Report·

Pharmacologically inhibiting GluR2 internalization alleviates neuropathic pain

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ABSTRACT

Neuropathic pain is of serious clinical concern and only about half of patients achieve partial relief with currently-available treatments, so it is critical to find new drugs for this condition. Recently, the cell-surface trafficking of pain-related receptors has been suggested as an important mechanism underlying persistent neuropathic pain. Here, we used the short peptide $GluA_{2\text{-}3y}$, which specifically inhibits the GluA2-dependent endocytosis of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors, and tested its anti-nociceptive effect in the periaqueductal grey (PAG) of intact rats and rats with neuropathic pain. Intra-PAG injection of 0.15, 1.5, 7.5, and 15 pmol of $GluA_{2\text{-}3y}$ induced dose-dependent increases in hindpaw withdrawal latencies to noxious thermal and mechanical stimuli in intact rats, suggesting that GluA2 cell-surface trafficking in the PAG is involved in pain modulation. Furthermore, $GluA_{2\text{-}3y}$ had much stronger anti-nociceptive effects in rats with neuropathic pain induced by sciatic nerve ligation. Interestingly, the intra-PAG injection of 15 pmol $GluA_{2\text{-}3y}$ had an analgesic effect similar to 10 μg (35 nmol) morphine in rats with neuropathic pain. Taken together, our results suggested that GluA2 trafficking in the PAG plays a critical role in pain modulation, and inhibiting GluA2 endocytosis with $GluA_{2\text{-}3y}$ has potent analgesic effects in rats with neuropathic pain. These findings strongly support the recent hypothesis that targeting receptor trafficking could be a new strategy for the treatment of neuropathic pain.

Keywords: periaqueductal grey; AMPA receptor; $GluA_{2\text{-}3y}$; internalization; morphine; hindpaw withdrawal latency

INTRODUCTION

Neuropathic pain is of serious clinical concern, ~5% of the European population being severely affected[1, 2]. It can be spontaneous, stimulus-evoked, or a combination of both[3]. It is not only an economic burden, but also disrupts social activities and reduces the quality of life. Although neuropathic pain is generally associated with inflammation and tissue injury, the underlying mechanism is still poorly understood and no effective treatments have been developed. It is estimated that only about half of the patients with neuropathic pain can achieve partial relief with currently-available treatments[4]. Thus, it is imperative to understand its causes and find new therapeutic targets.

Recently, accumulating evidence has suggested that dysregulated cell-surface trafficking of receptors that facilitate pain plays an important role in the development of inflammatory and neuropathic pain[5, 6]. These include

subunits of voltage-gated Ca^{2+} channels, GluA1 and GluA2 of α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs), and NR1 and NR2 of N-methyl D-aspartate receptors[5]. It has been shown that peripheral inflammation evoked by intraplantar injection of complete Freund's adjuvant (CFA) increases GluA1 insertion into the cell surface[7] and increases synaptic GluA2 internalization in spinal dorsal horn neurons[8]. Furthermore, L5 and L6 spinal nerve ligation-induced neuropathic pain was found to increase GluA2 internalization in the spinal cord[9]. However, the cell-surface trafficking of receptors that facilitate pain at the supraspinal level has received little attention.

The periaqueductal grey (PAG) is the grey matter located around the cerebral aqueduct within the midbrain tegmentum[10]. The PAG is an important structure for the transmission and/or modulation of nociceptive information, and is a key station in the analgesia pathway from the brainstem to the spinal cord[11]. $GluA_{2\text{-}3y}$ is a synthetic peptide derived from the rat GluA2 carboxyl tail (^{869}YKEGYNVYG^{877}), and it specifically blocks GluA2-dependent AMPAR internalization[12]. To facilitate intracellular delivery of the peptide, $GluA_{2\text{-}3y}$ was fused with the cell membrane transduction domain of the HIV-1 Tat protein (YGRKKRRQRRR) to generate Tat-$GluA_{2\text{-}3Y}$, which has been shown to be cell-permeable[12]. The present study was performed to explore the antinociceptive effects of intra-PAG delivery of Tat-$GluA_{2\text{-}3y}$ in intact rats and in rats with neuropathic pain.

MATERIALS AND METHODS

Male Sprague-Dawley rats weighing 200–300 g were from the Experimental Animal Center of the Academy of Military Medical Science, Beijing, China. Experiments were conducted according to the Guidelines of the International Association for the Study of Pain. All the animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) of Peking University. Every effort was made to minimize both the suffering and the number of animals used. The rats were housed in cages with free access to food and water, and maintained in a climate-controlled room with a 12:12 h day/night cycle.

Solutions for intra-PAG injection were prepared with 0.9% sterile saline, each in a volume of 1 μL containing 15, 7.5, 1.5, or 0.15 pmol of Tat-$GluA_{2\text{-}3y}$ (GL Biochem, Shanghai, China). The drug was always injected into the PAG on one side, as descending PAG projection is bilateral. Left side stands for the ipsilateral side, right side stands for the contralateral side for all the experiments.

The nerve injury model was produced as previously reported[13]. Rats were anesthetized by intraperitoneal injection of sodium pentobarbital (50 mg/kg) and 8–10 mm of the left sciatic nerve was exposed at the level of the mid-high. Four loose ligatures (4-0 chromic gut sutures) were placed around the dissected nerve at 1.0–1.5 mm intervals. The ligations were carefully manipulated so that the nerve was barely constricted, and the skin incision was closed with 4-0 silk sutures. To avoid repeated anesthesia, nerve injury and cannulation surgeries were performed at the same time. Each anesthetized rat was mounted on a stereotaxic instrument. A stainless-steel guide cannula of 0.8 mm outer diameter was directed into the PAG (AP, –5.5 mm; ML, 0.5 mm; DV, 6.0 mm) according to the atlas of Paxinos and Watson, and then fixed to the skull with dental acrylic. The rats were given >3 days to recover from the operation. On the day of experiment, a stainless-steel needle 0.4 mm in diameter was inserted into the guide cannula, to extend 2 mm beyond its tip. One microliter of solution was then infused into the PAG over 1 min.

The Randall Selitto and hot-plate tests were performed after sciatic nerve ligation as described previously[14]. In the hot-plate test, the entire ventral surface of the left or right hindpaw was placed on a hot-plate maintained at 52 ± 2°C, and the time to hindpaw withdrawal was measured in seconds and referred to as the hindpaw withdrawal latency (HWL) to thermal stimulation. The Randall Selitto test (Type 7200, Ugo Basile, Varese, Italy) was used to assess the HWL to mechanical stimulation. A wedge-shaped pusher with a loading rate of 30 g/s was applied to the dorsal surface of a hindpaw and the time required to initiate a struggle response was recorded. The HWL to thermal stimulation was usually between 3 and 6 s, and the HWL to mechanical stimulation between 4 and 7 s.

Data from the nociception tests are presented as mean ± SEM. Statistical differences between groups were determined by two-way analysis of variance (ANOVA) for repeated measurements or two-tailed Student's *t*-test where applicable. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

In intact rats, the HWLs to thermal and mechanical stimulation increased in a dose-dependent manner after intra-PAG injection (Fig. 1)(see supplementary data for statistics). These results demonstrated that intra-PAG injection of $GluA_{2\text{-}3y}$ has antinociceptive effects in intact rats, and suggested that GluA2 internalization modulates the pain response in the PAG.

To assess the anti-nociceptive effect of $GluA_{2\text{-}3y}$ on neuropathic pain, we performed sciatic nerve injury in rats. A group of eight rats received left common sciatic nerve injury. Ten days after injury, thermal hyperalgesia and mechanical allodynia were detected on the left but not the right side (Fig. 2), suggesting that the mononeuropathic pain model had been created successfully.

The HWLs to thermal and mechanical stimulation increased in a dose-dependent manner after intra-PAG injection (Fig. 3) (see supplementary data for statistics). Interestingly, intra-PAG injection of $GluA_{2\text{-}3y}$ had a much stronger anti-nociceptive effect on the left side where rats had received sciatic nerve injury, suggesting that GluA2 internalization in the PAG might play a critical role in neuropathic pain.

We then compared the anti-nociceptive effect of $GluA_{2\text{-}3y}$ with that of morphine. Ten micrograms (35 nmol) of morphine was used, as this dose has been shown to have a potent anti-nociceptive effect when microinjected into the PAG, while concentrations <1 μg (3.5 nmol) have no anti-nociceptive effect[15]. Intra-PAG injection of 15 pmol $GluA_{2\text{-}3y}$ had an anti-nociceptive effect similar to 10 μg morphine on the left side where rats received sciatic nerve injury (Figs. 3 and 4). These results suggested that administration of $GluA_{2\text{-}3y}$ in the PAG has a potent analgesic effect at a low concentration in rats with neuropathic pain.

AMPARs are widely expressed in the central nervous system, and the great majority exist as heteromers containing GluA2. GluA2 is a critical subunit controlling

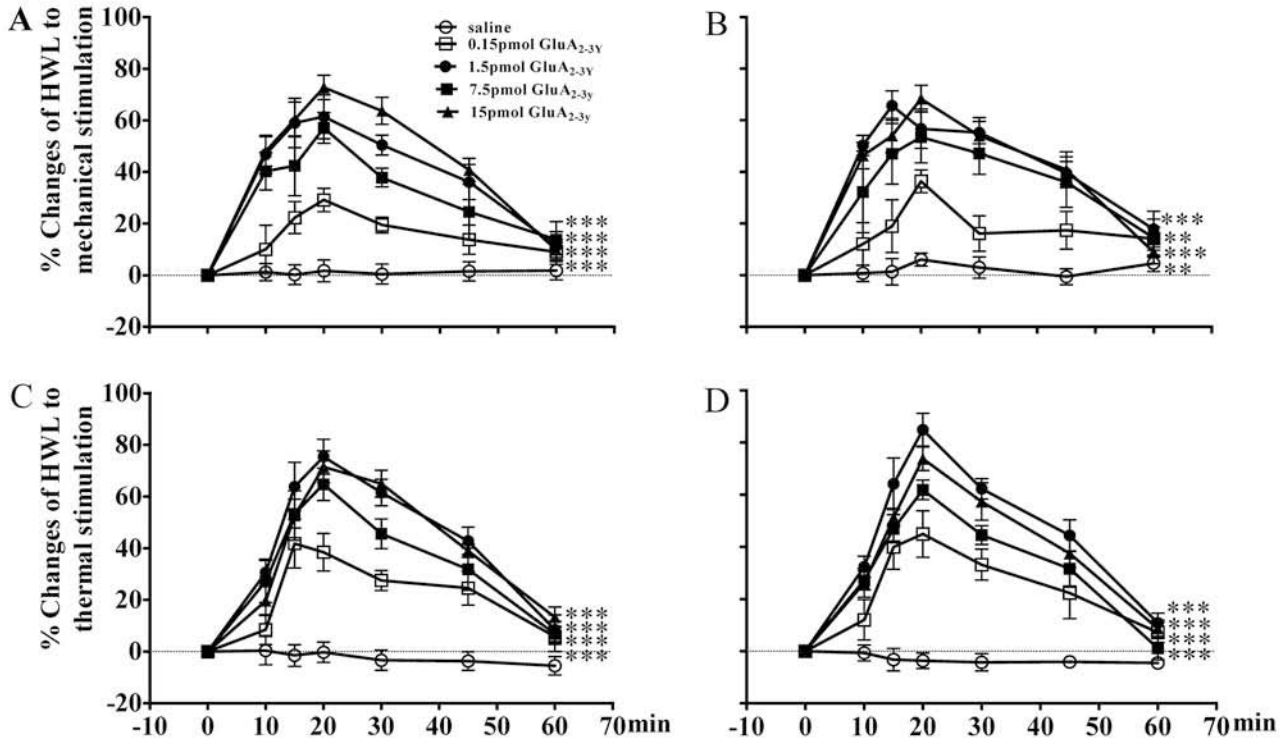

Fig. 1. Effects of intra-PAG injection of $GluA_{2\text{-}3y}$ on the HWLs to mechanical (A and B) and thermal stimulation (C and D) in intact rats. Left HWL: A and C; Right HWL: B and D. Rats received intra-PAG injection of 15 (*n* = 7), 7.5 (*n* = 6), 1.5 (*n* = 7), or 0.15 pmol (*n* = 6) of $GluA_{2\text{-}3y}$, or 1 μL 0.9% saline (*n* = 7) (mean ± SEM; ***P* <0.01, ****P* <0.001 compared with the control group; two-way ANOVA). PAG: periaqueductal grey; HWL: hindpaw withdrawal latency.

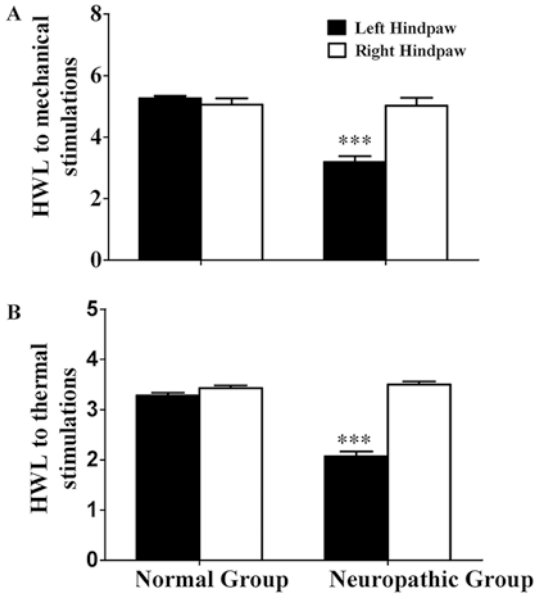

Fig. 2. Effects of left sciatic nerve ligation on the HWLs to mechanical (A) and thermal (B) stimulation (mean ± SEM; ***P <0.001 compared with the normal group; Student's t-test).

AMPAR functions such as Ca^{2+} permeability and synaptic plasticity[16]. Over the last five years, increasing evidence has suggested that cell-surface trafficking of GluA2 plays an important role in pain modulation at the spinal level[5]. In the current study, we showed that blocking GluA2 internalization with $GluA_{2\text{-}3y}$ in the PAG had anti-nociceptive effects in normal rats and in rats with neuropathic pain induced by sciatic ligation, suggesting that GluA2 trafficking is involved in pain responses at the supraspinal level. Furthermore, $GluA_{2\text{-}3y}$ had much stronger anti-nociceptive effects in rats with neuropathic pain than in normal rats. Although it is interesting that $GluA_{2\text{-}3y}$ had less effect on the normal side after mononeuropathy, it should be noted that the normal side was not as hypersensitive as the neuropathic side. The response to pain of the normal side after mononeuropathy was similar to normal rats as suggested by our results (Fig. 2), thus it is reasonable that the normal side is less sensitive to drug treatments, consistent with our previous study[17]. Based on these results, we assume that sciatic nerve

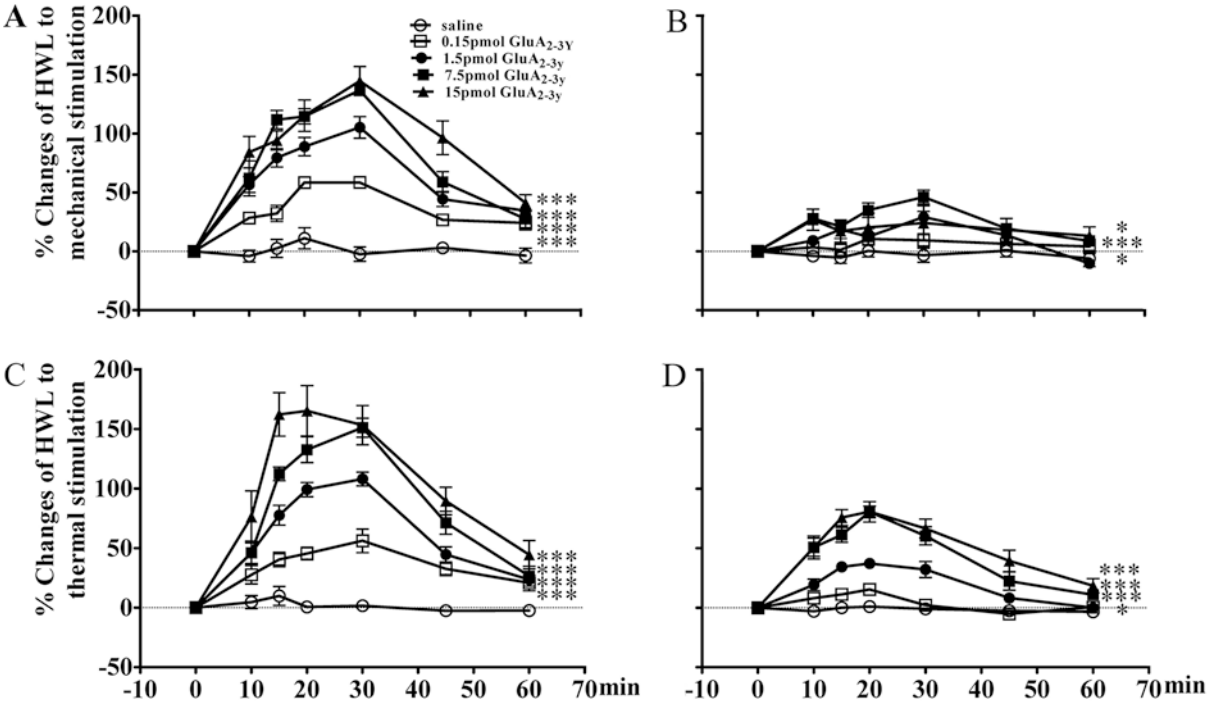

Fig. 3. Effects of intra-PAG injection of $GluA_{2\text{-}3y}$ on the HWLs to mechanical (A and B) and thermal stimulation (C and D) in rats with sciatic nerve injury. Left HWL: A and C; Right HWL: B and D. Rats with mononeuropathy received intra-PAG administration of 0.15 (n = 7), 1.5 (n = 7), 7.5 (n = 8), or 15 pmol (n = 8) $GluA_{2\text{-}3y}$ or 1 μL 0.9% saline (n = 8) (mean ± SEM; *P <0.05, ***P <0.001 compared with the control group; two-way ANOVA). PAG: periaqueductal grey; HWL: hindpaw withdrawal latency.

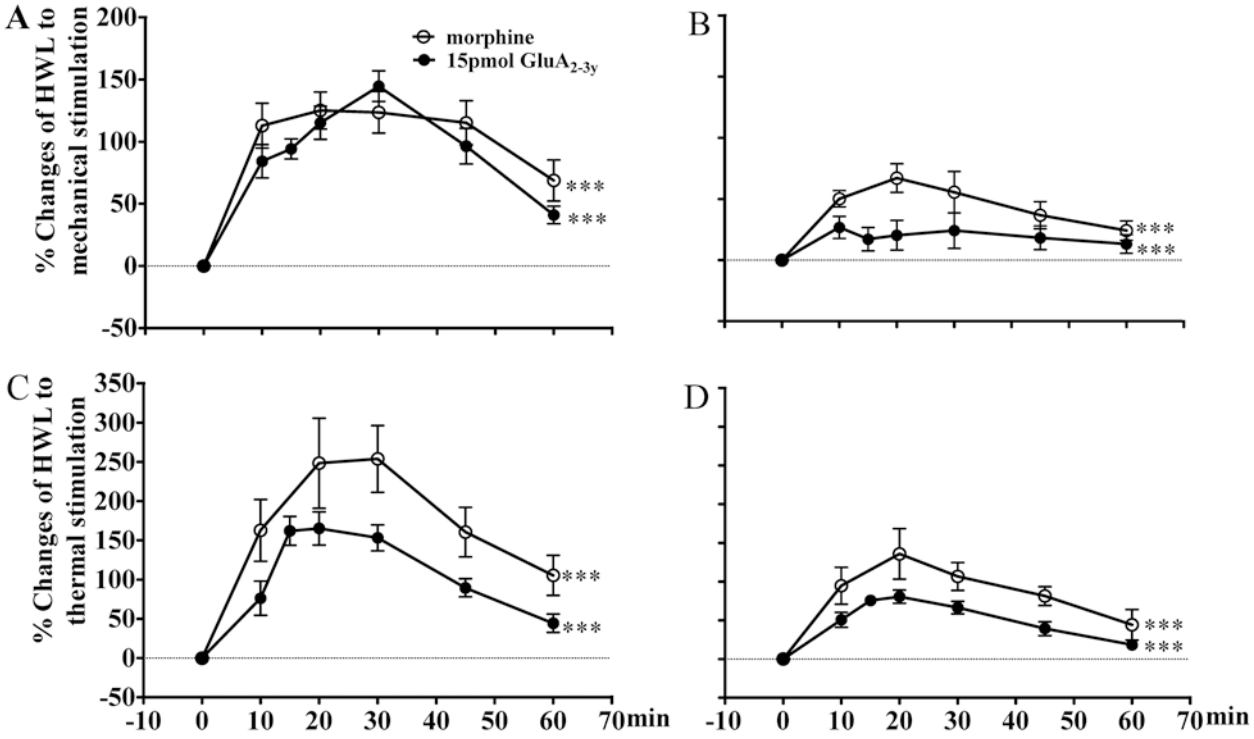

Fig. 4. Comparison of intra-PAG injection of morphine and $GluA_{2\text{-}3y}$ on the HWLs to mechanical (A and B) and thermal (C and D) stimulation in rats with mononeuropathy (mean ± SEM; ***P <0.001 compared with the control group; two-way ANOVA).

ligation-induced neuropathic pain disrupted normal GluA2 trafficking, probably by increasing its internalization. This is supported by a study showing that spinal nerve ligation-induced neuropathic pain significantly decreases the amount of membrane GluA2 and correspondingly increases the cytosolic vesicle fraction in the dorsal spinal cord[9]. Consistently, another group reported that CFA-induced peripheral inflammation induces synaptic GluA2 internalization in dorsal horn neurons during the maintenance of nociceptive hypersensitivity[8]. We also found that sciatic nerve ligation did not change the total protein level of GluA2 in the PAG (data not shown), consistent with a report showing that the expression of the GluA2 subunit of AMPAR in the ventrolateral PAG does not change after spinal nerve ligation-induced neuropathic pain[18]. These results indicated that GluA2 undergoes dynamic trafficking between the cell membrane and the cytosol under various conditions such as inflammation and nerve injury.

Although the PAG is known to be the primary control center for descending pain modulation, it is still not fully understood how it regulates pain responses under physiological and/or pathological conditions. Here, we found that the regulation of GluA2 cell-surface trafficking in the PAG plays an important role in pain modulation; this was demonstrated by the anti-nociceptive effects of intra-PAG injection of $GluA_{2\text{-}3y}$ which specifically blocks GluA2 internalization. We further compared the anti-nociceptive effects of $GluA_{2\text{-}3y}$ with morphine, which is the most prescribed opioid for pain relief, although the side-effects (such as addiction) limit its use. It is well known that the PAG–ventromedial medulla–spinal cord pathway is essential for the analgesic actions of both systemic and intra-PAG morphine[19]. We showed that intra-PAG injection of 15 pmol $GluA_{2\text{-}3y}$ produced an analgesic effect similar to 35 nmol morphine in rats with neuropathic pain induced by sciatic nerve ligation. That $GluA_{2\text{-}3y}$ was able to achieve pain relief at this low concentration suggested that drugs targeting GluA2 trafficking such as $GluA_{2\text{-}3y}$ could be useful in the treatment of persistent pain.

In conclusion, the results of the present study

demonstrated that inhibition of GluA2 internalization by $GluA_{2\text{-}3y}$ in the PAG had anti-nociceptive effects in intact rats and in rats with neuropathic pain. To our knowledge, this is the first evidence showing that GluA2 cell-surface trafficking is important in pain modulation at the supraspinal level, and targeting a glutamate receptor can achieve pain relief. The analgesic effect of $GluA_{2\text{-}3y}$ is potent at a low dose under neuropathic pain conditions, thus further studies are needed to investigate its therapeutic potential for the treatment of neuropathic pain. Moreover, our results strongly support the recent hypothesis that cell-surface receptor trafficking is a new target for the treatment of neuropathic pain.

ELECTRONIC SUPPLEMENTARY MATERIAL

Supplementary material is available in the online version of this article at http://dx.doi.org/10.1007/s12264-015-1556-2.

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