Role of peripheral glutamate in mechanical hyperalgesia in a traumatic neuropathy model of the rat

Jun Ho Jang

Department of Medical Science
The Graduate School, Yonsei University

Role of peripheral glutamate in mechanical hyperalgesia in a traumatic neuropathy model of the rat

Jun Ho Jang

Department of Medical Science

The Graduate School, Yonsei University

Role of peripheral glutamate in mechanical hyperalgesia in a traumatic neuropathy model of the rat

Directed by Professor Joong Woo Leem

The Doctoral Dissertation
submitted to the Department of Medical Science,
the Graduate School of Yonsei University
in partial fulfillment of the requirements for the
degree of Doctor of Philosophy

Jun Ho Jang

June 2005

This certifies that the Doctoral Dissertation of Jun Ho Jang is approved.

Joong Woo Leem _____

Kwang Se Paik _____

Taick Sang Nam _____

Bae Hwan Lee _____

Jin Woo Chang _____

The Graduate School

Yonsei University

June 2005

ACKNOWLEDGEMENTS

I would especially like to thank my advisor, professor Joong Woo Leem. He first sparked my interest in neuroscience, and continually stimulated me to develop scientific thinking and research skills. His encouragement has been of great help in overcoming many of the difficulties which arose throughout my doctoral program. In fact, it would not have been possible to complete this work without his enthusiastic guidance.

I am also very grateful for having an exceptional doctoral committee and wish to thank professors Kwang Se Paik, Taick Sang Nam, Jin Woo Chang and Bae Hwan Lee for their advice. I extend many thanks to all the people in the Department of Physiology, who showed me how to study science and reminded me that there is more to life than experiment.

There is no way to thank my family enough. They deserve a special acknowledgement for their love and care. My mother has been a constant source of support and encouragement throughout my life. Though I often lost my course and crossed her whishes, she was always my side. A special thank goes also to my brother for his generous care and always keeping his door open.

Finally, let me say 'sorry' to the sacrificed rats without any guilt for my work.

TABLE OF CONTENTS

LIST OF FIGURES

Abstract

Role of peripheral glutamate in mechanical hyperalgesia in a traumatic neuropathy model of the rat

Jun Ho Jang

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Joong Woo Leem)

Peripheral nerve injury leads to neuropathic pain, including mechanical hyperalgesia (MH). When a peripheral nerve is injured, damaged primary sensory afferents emit a barrage of brief impulses, or an injury discharge, which are conducted both orthodromically to the spinal cord and antidromically to the periphery. There is evidence indicating that centrally conducting injury discharge plays a role in triggering neuropathic pain. Although peripherally conducting impulses are observed to lead to peripheral nociception through glutamate (Glu) receptor activation, their role in neuropathic pain is not clearly understood. The present study was conducted to provide evidence for a hypothesis that nerve injury-induced peripheral effects contribute to neuropathic pain. To this, using rats with lumbar 5 (L5) dorsal rhizotomy (DR), it was investigated 1) whether nerve lesion or electrical stimulation (ES) of L5 spinal nerve induced neuropathic pain and, if so, 2) whether or not peripheral Glu receptors were involved in mediating this pain.

Rats that received left L5 DR were subjected to either spinal nerve lesion (SNL) or ES, each of which was done at left L5 spinal nerve. For SNL, spinal nerve was tightly ligated and transected. For ES, a

train of electrical pulses (0.5-ms, 2-5 mA, 4 Hz) were applied for 5 min to the spinal nerve with a pair of flexible silver wires. Mechanical sensitivity of hind paw was assessed by measuring paw withdrawal threshold (PWT) to von Frey filament application. The effects of an intraplantar (i.pl.) injection of Glu receptor antagonists or agonist on mechanical sensitivity into the affected hind paw were investigated.

SNL reduced PWT of the affected hind paw with a peak reduction on day 3 post-SNL. This reduced PWT, or MH, was maintained for at least 42 days of the test period. ES also reduced PWT in a similar way to SNL, lasting for 7 days. When an i.pl. injection was given immediately before SNL, NMDA receptor antagonist MK-801 (20 nmol), group-I metabotropic Glu (mGlu) receptor antagonist DL-AP3 (70 nmol), and selective group-II mGlu receptor agonist APDC (20 nmol) delayed the onset of MH. For ES-induced MH, the same application of above drugs completely blocked it's onset as long as for 42-test period. Pre-treatment of AMPA/kainate receptor antagonist NBQX (100 nmol) was without an effect on either SNL- or ES-induced onset of MH. When drugs were given after SNL induced-MH had been established, MK-801 reversed MH for about 45 minutes, whereas NBQX, DL-AP3, or APDC had no effect. The same was true for ES-induced MH.

These results suggest that 1) peripherally conducting impulses play a crucial role in the generation of neuropathic pain, regardless of whether these impulses are directly from injury discharge or secondarily from nerve injury and 2) that peripheral Glu receptors are involved in mediating this pain.

Key words: neuropathic pain, nerve injury, injury discharge, glutamate, electrical stimulation

Role of peripheral glutamate in mechanical hyperalgesia in a traumatic neuropathy model of the rat

Jun Ho Jang

Department of Medical Science
The Graduate School, Yonsei University

(Directed by Professor Joong Woo Leem)

I. INTRODUCTION

Peripheral nerve injury leads to neuropathic pain, which is characterized by spontaneous burning pain, pain evoked by normally innocuous stimuli (allodynia), and exaggerated pain in response to painful stimuli (hyperalgesia). When a peripheral nerve is injured, damaged primary sensory afferents emit a barrage of brief impulses, or an injury discharge, lasting several minutes.[1] Injury discharges are conducted both orthodromically to the spinal cord and antidromically to the periphery. There is evidence indicating that centrally conducting injury discharge plays a role in the triggering neuropathic pain. The abnormal pain behavior following peripheral nerve transection is suppressed by local anesthetic blockade of centrally conducting injury discharge[2,3] or by intrathecal injection of glutamate (Glu) receptor blockers at the lumbar level just prior to transection.[4] Brief electrical stimulation (ES) of C-fibers, which mimics injury discharge, produces prolonged hyperalgesia,[5] although in which the effects exerted by stimulation-produced discharge that is peripherally conducted could be not excluded.

Several lines of evidence suggest that peripherally conducting impulses play a role in peripheral nociception through Glu receptor

activation. The antidromic stimulation of primary sensory afferents results in increment of Glu levels in the extracellular space of the rat hind paw.[6] Both ionotropic Glu (iGlu)[7,8] and metabotropic Glu (mGlu)[9-11] receptors have been localized in the peripheral processes of primary unmyelinated afferents. The activation of peripheral Glu receptors by an intraplantar (i.pl.) injection of Glu[7,12] or of Glu receptor agonists[13,14] produces nociceptive behaviors that are blocked by appropriate antagonists.[9,11,15] However, a role of peripherally conducting impulses in neuropathic pain has not received much attention.

It has been demonstrated that in a neuropathy model in which centrally conducting discharges had been blocked by lumbar 5 (L5) dorsal rhizotomy (DR), L5 spinal nerve lesion (SNL) still produces long-lasting mechanical hyperalgesia.[16] Thus, this model is useful for studying the nerve injury-induced peripheral effects on neuropathic pain. Nerve injury-induced peripheral effects are presumably exerted by peripherally conducting injury discharge stemming from injured afferents and by spontaneous discharge from intact afferents nearby degenerating axon during Wallerian degeneration following the lesion. In fact, the development of spontaneous discharges in intact afferent neighbors following peripheral nerve lesion has been demonstrated.[17-19] Therefore, it is impossible to assess contribution of peripherally conducting injury discharge only, as being separated from Wallerian degeneration-related discharge, to long-lasting mechanical hyperalgesia shown in this neuropathic pain model. An alternate approach to studying the role of injury discharge is to use ES mimicking injury discharge, or an artificial injury discharge. The present study was conducted using rats with L5 DR to investigate 1) whether nerve lesion or ES of L5 spinal nerve induced neuropathic pain and, if so, 2) whether or not peripheral Glu receptors were involved in mediating this pain.

II. MATERIALS AND METHODS

1. Subjects

Male Sprague-Dawley rats (200-250g) that were housed in groups of 3-4, with food and water available ad libitum under a light-dark cycle, were used in this study. Rats were allowed to acclimate at least for a week before surgery and behavioral testing. Experiments were carried out in accordance with NIH regulations for animal care and with the approval of the Institutional Animal Care and Use Committee of Yonsei University, Seoul, Korea.

2. Preparation of a neuropathy model

The neuropathy model was prepared with rats that were first subjected to left L5 DR, followed 6 days later by left L5 SNL. Both operations were performed under enflurane anesthesia (induction 3% and maintenance 2%). For left L5 DR, the surgical field was shaved, a longitudinal incision made exposing the L4 to L6 vertebral segments, and a laminectomy performed at the left L5 segment. After making a small incision of the dura matter, the exposed L5 dorsal root was transected. For left L5 SNL, an incision was made above the lumbar spine and the left transverse process of L6 vertebra was exposed. After carefully removing this process, left L5 spinal nerve was exposed, tightly ligated with 6-0 silk thread, and cut about 1 mm distal to the ligation. The wound was sutured and maintained with postoperative care. In the sham operation group, all operations were performed in the same manner, but without DR and SNL.

3. Electrical stimulation

Rats that received left L5 DR 6 days earlier were subjected to ES. Left L5 spinal nerve was exposed under pentobarbital anesthesia (50 mg/kg, i.p.) in a same manner as the animal model preparation. Exposed spinal nerve was freed from surrounding connective tissue, and a piece of parafilm was placed underneath the spinal nerve, isolating it from the surrounding tissue and nearby other spinal nerve. To prevent the spinal nerve from damage or stretching due to a muscle twitch during ES, nerve was surrounded with a pair of silver wires that were very thin and flexible (0.005 inches in diameter, 2-4 mm apart). Square-wave pulses of 0.5 ms duration at 4 Hz were applied for 5 minutes. The lowest level of electrical current needed to elicit a muscle twitch was defined as threshold current. Electrical stimuli were applied at strengths of 200 times a threshold current, which ranged from 2 to 5 mA and were reported to be strong enough to activate primary afferent C-fibers. The wound was sutured and maintained with postoperative care. In the sham operation group, all operations were performed in the same manner, but without applying electrical stimuli.

4. Drug preparation

Non-competitive NMDA (N-methyl-D-aspartate) receptor antagonist MK-801 (dizocilpine maleate) and competitive AMPA (α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid)/kainate receptor antagonist NBQX (2,3-dioxo-6-nitro-1,2,3,4,-tetrahydrobenzo[f] quinoxaline-7-sulfonamide) were dissolved in phosphate-buffered saline, pH 7.4 (PBS). Competitive group-I mGlu receptor antagonist DL-AP3 (DL-amino-3-phosphonopropionic acid) and selective Group-II mGlu receptor agonist APDC ((2R,4R)-4-aminopyrrolidine-2,4-dicarboxylate) were first prepared as a stock solution in 100 mM NaOH and then

diluted to final concentrations with PBS. All drugs were purchased from Tocris Cookson (Bristol, UK). Vehicles were prepared as described for the corresponding drug solution without adding the drug.

5. Drug administration

Drugs or vehicles were administered under enflurane anesthesia (3%, 2-3 min) in a volume of 30 ㎕, subcutaneously, into the plantar surface of the hind paw using a 50-㎕ Hamilton syringe with a 28-gauge needle. The needle was inserted into the plantar skin proximal to the midpoint of the hind paw and advanced about 10 mm so that it reached the midpoint of the hind paw, where the solution was injected forming a bleb that disappeared within 10 minutes. The doses used were 20 nmol MK-801, 100 nmol NBQX, 70 nmol DL-AP3, and 20 nmol APDC. A pilot study showed that all drugs at these doses, except NBQX, produced a maximal response in terms of a delay in the onset of SNL-induced mechanical hyperalgesia when injected into the affected hind paw 15 minutes before SNL. No drug at these doses produced stereotypical alterations in motor activity, such as hyper-locomotion or balance loss. As a vehicle control group, rats were treated with an i.pl. injection of PBS- or NaOH-containing vehicle.

6. Experimental protocol

The first set of experiments was aimed at testing whether the neuropathy model, in which the potential central effects induced by a direct input of injury-evoked impulses to the spinal cord were prevented without affecting peripherally conducting injury-evoked impulses, show a long-lasting pain behavior. Rats were randomly assigned to two groups: one that received L5 SNL preceded by L5 DR in the same side and the other that received a sham operation.

The second set of experiments was performed to test whether artificial injury discharge produced by ES of L5 spinal nerve induce a long-lasting pain behavior. Rats were randomly assigned to two groups: one that received L5 ES following L5 DR in the same side and the other that received a sham operation.

In the third set of experiments, the effects of drugs and vehicle (PBS- or NaOH-containing) on L5 SNL-induced mechanical hyperalgesia, administered before SNL, were examined. Rats that received L5 DR 6 days earlier were assigned to one of the following ten groups: animals injected with MK-801, NBQX, DL-AP3, or ADPC into the hind paw ipsilateral to DR, animals injected with each of the same drugs into the contralateral hind paw, animals injected with PBS- or NaOH-vehicle into the ipsilateral hind paw. In each rat, an injection of drugs or vehicle was followed 15 min later by L5 SNL.

The fourth set of experiments examined the effects of drugs or vehicle on L5 SNL-induced mechanical hyperalgesia, when administered after SNL-induced mechanical hyperalgesia had been already established. Rats that received L5 SNL preceded by L5 DR on the same side with established mechanical hyperalgesia were assigned to one of ten groups that were the same as those described above in the third set of experiments. In each rat, an injection of drugs or vehicle was performed on day 10 post-SNL. In both the pre- and post-treatment paradigms, we included groups of rats that received an i.pl. injection of the same drugs into the contralateral hind paw to rule out the possibility that an i.pl. drug injection had some systemic effect.

In the fifth set of experiments, the effects of drugs and vehicle on L5 ES-induced mechanical hyperalgesia, administered before ES, were examined. Rats that received L5 DR 6 days earlier were assigned to one of the following six groups: animals injected with MK-801, NBQX,

DL-AP3, or ADPC into the hind paw ipsilateral to DR, animals injected with PBS- or NaOH-vehicle into the ipsilateral hind paw. In each rat, an injection of drugs or vehicle was followed 15 min later by L5 ES.

The sixth set of experiments examined the effects of drugs or vehicle on L5 ES-induced mechanical hyperalgesia, when administered after ES-induced mechanical hyperalgesia had been already established. Rats that received L5 ES preceded by L5 DR on the same side with established mechanical hyperalgesia were assigned to one of six groups that were the same as those described above in the fifth set of experiments. In each rat, an injection of drugs or vehicle was performed on day 3 post-ES.

7. Behavioral testing

Behavioral testing began with a habituation period, in which rats were placed in plexiglass cubicles for 30 min. Mechanical hyperalgesia was evaluated by measuring the paw withdrawal threshold (PWT) to the application of a von Frey filament using the up-down testing paradigm. An ascending series of von Frey filaments of incremental force (0.35, 0.53, 0.78, 2.5, 3.7, 5.2, 6.0, and 12.5 g) were applied for 2-3 seconds perpendicularly to the middle of the plantar surface of the hind paw until each filament just bent, starting with the 2.5-g stimulus. The testing procedure and the calculations of the mechanical thresholds were performed as described by Chaplan et al..[20] All behavioral testing was done by experimenters blinded to preceding surgeries and drug treatments.

8. Statistical analysis

Data are presented as means ± SEM for all values. Statistical analysis was performed using Friedman one-way ANOVA for repeated

measurements, followed by Wilcoxon signed-rank test when appropriate, to analyze the variance in PWT between testing days. Statistical analysis on the variance in PWT between groups on a given testing day was performed using Kruskal-Wallis ANOVA, followed by the Mann-Whitney rank-sum test when appropriate. A P value of less than 0.05 was considered statistically significant and Bonferroni corrections were made.

III. RESULTS

1. Mechanical hyperalgesia in rats that received L5 SNL following L5 DR

The time courses of changes in the PWTs of rats that received L5 SNL following L5 DR and of those that received sham-operation are explored and shown in Fig. 1. After unilateral L5 DR, the PWT of the affected hind paw to von Frey filament stimuli reduced transiently on day 1 post-DR and returned to the pre-DR baseline level on day 6 post-DR. Animals that showed this transient change in PWTs were all included in this study. L5 SNL performed following L5 DR produced a significant drop in the PWTs of the affected hind paw compared with the pre-SNL baseline ($P<0.05$, Friedman ANOVA). This decrease was also statistically significant versus the PWTs of the contralateral hind paw or the hind paw of sham-operated animals ($P<0.05$, Kruskal-Wallis ANOVA). PWTs reached a minimum (83.6 % change from pre-SNL baseline level of 17.7 ± 0.3 g, n=8) on day 3 post-SNL and were maintained at low levels up to day 42 post-SNL, which is an indication of the long-lasting nature of mechanical hyperalgesia. No significant changes in PWT were observed on hind paws contralateral to SNL and in the sham-operated group.

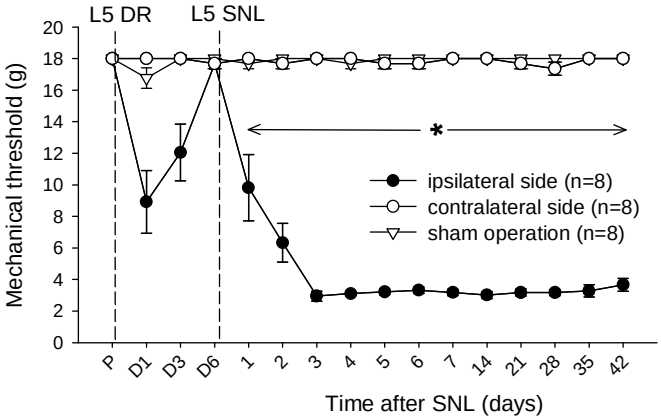

Figure 1. Time course of changes in paw withdrawal threshold (PWT) in rats that received a spinal nerve lesion (SNL) following dorsal rhizotomy (DR). Unilateral lumbar 5 (L5) DR caused PWT of the affected hind paw to decrease transiently and return to pre-DR baseline on day 6 post-DR. After ipsilateral L5 SNL, performed by L5 spinal nerve ligation-and-cut, PWT dropped rapidly and was maintained at low levels up to day 42 post-SNL (closed circles). P, pre-DR values; D, days after DR. Asterisks indicate significantly different from the pre-SNL baseline ($P<0.05$, Friedman ANOVA, followed by Wilcoxon signed-rank test) and from contralateral hind paws (open circles) or sham-operated animals (open triangles) ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test).

2. Mechanical hyperalgesia in rats that received L5 ES following L5 DR

The time courses of changes in the PWTs of rats that received L5 ES following L5 DR and of those that received sham-operation are explored and shown in Fig. 2. After unilateral L5 DR, the PWT of the affected hind paw to von Frey filament stimuli reduced transiently on day 1 post-DR and returned to the pre-DR baseline level on day 6 post-DR. Animals that showed this transient change in PWTs were all included in this study. L5 ES performed following L5 DR produced a significant drop in the PWTs of the affected hind paw on days 1, 2, 3, 4, 5, 6 and 7 post-ES compared with the pre-ES baseline ($P<0.05$, Friedman ANOVA). This decrease was also statistically significant when compared with the PWTs of the contralateral hind paw or the hind paw of sham-operated animals ($P<0.05$, Kruskal-Wallis ANOVA). No significant changes in PWT were observed on hind paws contralateral to ES and in the sham-operated group.

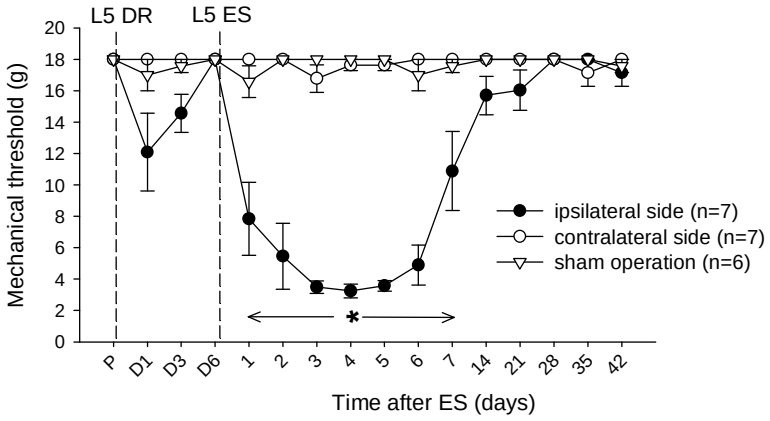

Figure 2. Time course of changes in paw withdrawal threshold (PWT) in rats that received a electrical stimulation (ES) of spinal nerve following dorsal rhizotomy (DR). Unilateral lumbar 5 (L5) DR caused PWT of the affected hind paw to decrease transiently and return to pre-DR baseline on day 6 post-DR. After ipsilateral L5 ES, PWT dropped rapidly and was maintained at low levels up to day 7 post-ES (closed circles). P, pre-DR values; D, days after DR. Asterisks indicate significantly different from the pre-ES baseline ($P<0.05$, Friedman ANOVA, followed by Wilcoxon signed-rank test) and from contralateral hind paws (open circles) or sham-operated animals (open triangles) ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test).

3. Effects of pre-treatment in the periphery with MK-801, NBQX, DL-AP3, or APDC on SNL-induced mechanical hyperalgesia

To test whether peripheral NMDA and AMPA/kainate receptors are involved in the induction phase of SNL-induced mechanical hyperalgesia, the effect of MK-801 or NBQX injected into the hind paw immediately before SNL on PWT scores was investigated. As seen in Fig. 3A, an i.pl. injection of 20 nmol of MK-801, performed 15 min before SNL, into the hind paw ipsilateral to SNL demonstrated a significant increase in PWTs on days 1, 2, 3, and 4 post-treatment compared with PBS-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). These animals that received MK-801 in the ipsilateral hind paw also showed increased PWTs on days 1, 2, 3 and 4 post-treatment compared with animals treated with MK-801 in the contralateral hind paw ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). No significant difference in PWTs was observed between animals treated with MK-801 (20 nmol) in the contralateral hind paw and those treated with PBS-vehicle. In Fig. 3B, the effect of pre-treatment in the periphery with NBQX is shown. Animals that received SNL preceded by an i.pl. injection of 100 nmol of NBQX into the hind paw ipsilateral to SNL displayed similar PWTs to animals treated with PBS-vehicle or with the same dose of NBQX in the contralateral hind paw.

To determine if peripheral mGlu receptors are involved in the induction phase of SNL-induced mechanical hyperalgesia, the effect of non-selective mGlu receptor antagonist MCPG (α-methyl-4-carboxyphenylglycine, 50 nmol) injected into the hind paw before SNL on PWT scores was first analyzed. However, we observed no effect due to the local injection of MCPG into the hind paw on SNL-induced PWT reduction (data not shown). We thought that this might be due to the

non-selective antagonism of MCPG at both group-I and group-II mGlu receptors, which produce opposite effects when activated, e.g., increased or decreased neuronal excitability, respectively. Next, we examined the effects of pre-treatment with competitive Group-I mGlu receptor antagonist DL-AP3 or selective Group-II mGlu receptor agonist APDC in the hind paw on PWT scores. As seen in Fig. 4A, an i.pl. injection of 70 nmol of DL-AP3, performed 15 min before SNL, into the hind paw ipsilateral to SNL resulted in a significant increase in PWTs on days 1, 2, 3, and 4 post-treatment compared with NaOH-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). These animals that received DL-AP3 in the ipsilateral hind paw also showed higher PWTs on days 1, 2, 3 and 4 post-treatment than animals treated with DL-AP3 (70 nmol) in the contralateral hind paw ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). No significant difference in PWTs was observed between animals treated with DL-AP3 in the contralateral hind paw and those treated with NaOH-vehicle. In Fig. 4B, the effect of pre-treatment in the periphery with APDC is shown. An i.pl. injection of 20 nmol of APDC, performed 15 min before SNL, into the hind paw ipsilateral to SNL produced a significant increase in PWTs on day 1 post-treatment compared with NaOH-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). A similar increase was observed when these animals that received APDC in the ipsilateral hind paw were compared with animals treated with APDC (20nmol) in the contralateral hind paw ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). No significant difference in PWTs was observed between animals treated with APDC in the contralateral hind paw and those treated with NaOH-vehicle.

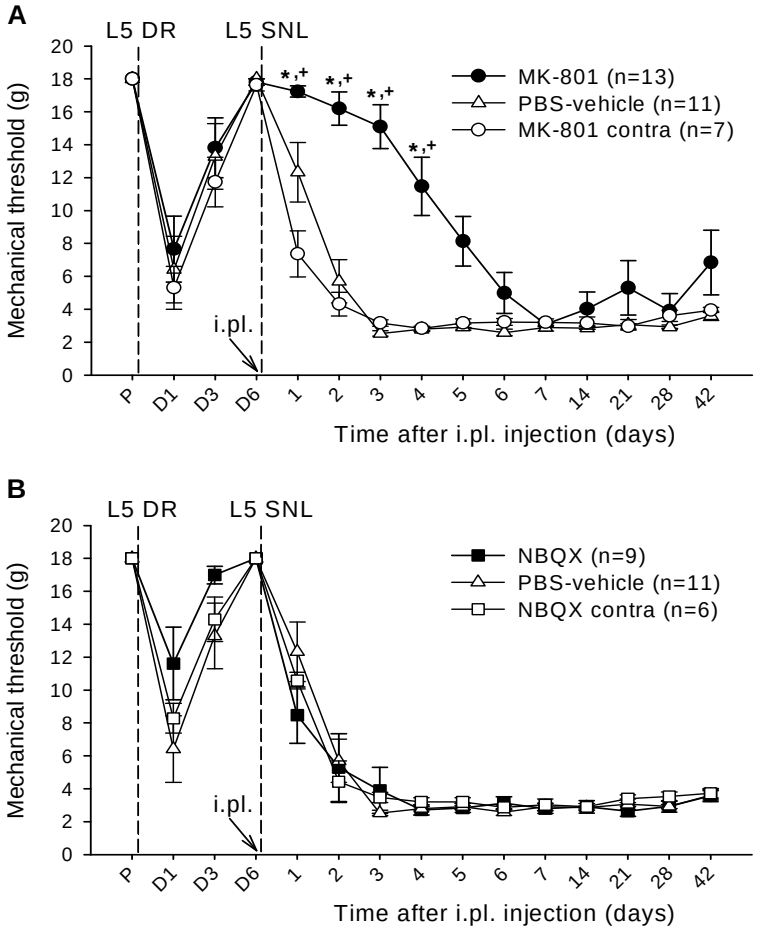

Figure 3. Effects of MK-801 or NBQX pre-treatment in the periphery on lumbar 5 (L5) spinal nerve lesion (SNL)-induced paw withdrawal threshold (PWT) reduction. (A) An intraplantar (i.pl.) injection of MK-801 given before L5 SNL into the affected hind paw (closed circles) resulted in increased PWTs compared with animals treated with PBS-vehicle (open triangles) or with MK-801 in the contralateral hind paw (open circles), as indicated by * or +, respectively ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). (B) The PWTs of animals treated with NBQX before L5 SNL in the affected hind paw (closed rectangles) were similar to those of animals treated with PBS-vehicle (open triangles) or with NBQX in the contralateral hind paw (open rectangles). DR, dorsal rhizotomy; P, pre-DR values; D, days after DR.

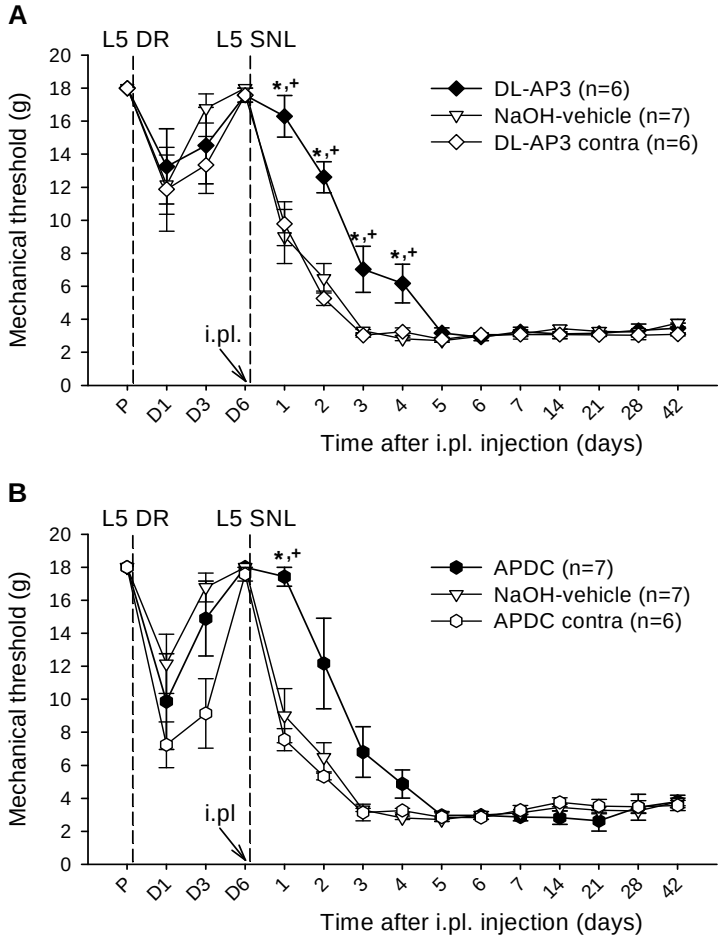

Figure 4. Effects of DL-AP3 or APDC pre-treatment in the periphery on lumbar 5 (L5) spinal nerve lesion (SNL)-induced paw withdrawal threshold (PWT) reduction. (A) Animals that received an intraplantar (i.pl.) injection of DL-AP3 before L5 SNL into the affected hind paw (closed diamonds) displayed higher PWTs than those treated with NaOH-vehicle (open triangles) or with DL-AP3 in the contralateral hind paw (open diamonds), as indicated by * or +, respectively. (B) Animals treated with APDC before L5 SNL in the affected hind paw (closed hexagons) produced higher PWTs than animals treated with NaOH-vehicle (open triangles) or with APDC in the contralateral hind paw (open hexagons), as indicated by * or +, respectively. * and +; $P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test. DR, dorsal rhizotomy; P, pre-DR values; D, days after DR.

4. Effects of post-treatment in the periphery with MK-801, NBQX, DL-AP3, or APDC on SNL-induced mechanical hyperalgesia

To test whether peripheral NMDA and AMPA/kainate receptors were involved in the maintenance phase of SNL-induced mechanical hyperalgesia, the effect of MK-801 or NBQX injected into the hind paw after SNL on PWT scores was examined. As seen in Fig. 5A, all three groups of animals that received SNL following DR demonstrated a significant decrease in PWTs after SNL compared with the pre-SNL baseline ($P<0.05$, Friedman ANOVA). An i.pl. injection of 20 nmol of MK-801, performed on day 10 post-SNL, into the hind paw ipsilateral to SNL showed a significant increase in PWTs at 45, 60, and 75 min post-treatment compared with PBS-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). These animals that received MK-801 in the ipsilateral hind paw also showed increased PWTs at 45, 60, and 75 min post-treatment compared with animals treated with MK-801 (20 nmol) in the contralateral hind paw ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). No significant difference in PWTs was observed between animals treated with MK-801 in the contralateral hind paw and those treated with PBS-vehicle. In Fig. 5B, the effect of post-treatment in the periphery with NBQX is shown. An i.pl. injection of 100 nmol of NBQX, performed on day 10 post-SNL, into the hind paw ipsilateral to SNL produced no significant difference in PWTs compared with animals treated with the same dose of NBQX in the contralateral hind paw or those treated with PBS-vehicle.

The possible involvement of peripheral group-I and group-II mGlu receptors in the maintenance phase of SNL-induced mechanical hyperalgesia was tested by analyzing the effect of DL-AP3 or APDC injected into the hind paw after SNL on PWT scores. As seen in Fig.

6A, an i.pl. injection of 70 nmol of DL-AP3, performed on day 10 post-SNL into the hind paw ipsilateral to SNL, produced no significant difference in PWTs compared with those animals treated with NaOH-vehicle. The same held true when animals were treated with DL-AP3 (70 nmol) in the contralateral hind paw on day 10 post-SNL. In Fig. 6B, the effect of post-treatment in the periphery with APDC is shown. Animals that received an i.pl. injection of 20 nmol of APDC, on day 10 post-SNL, into the hind paw ipsilateral to SNL showed no significant difference in PWTs compared with animals treated with NaOH-vehicle, and this was also the case for animals treated with APDC (20 nmol) in the contralateral hind paw on day 10 post-SNL.

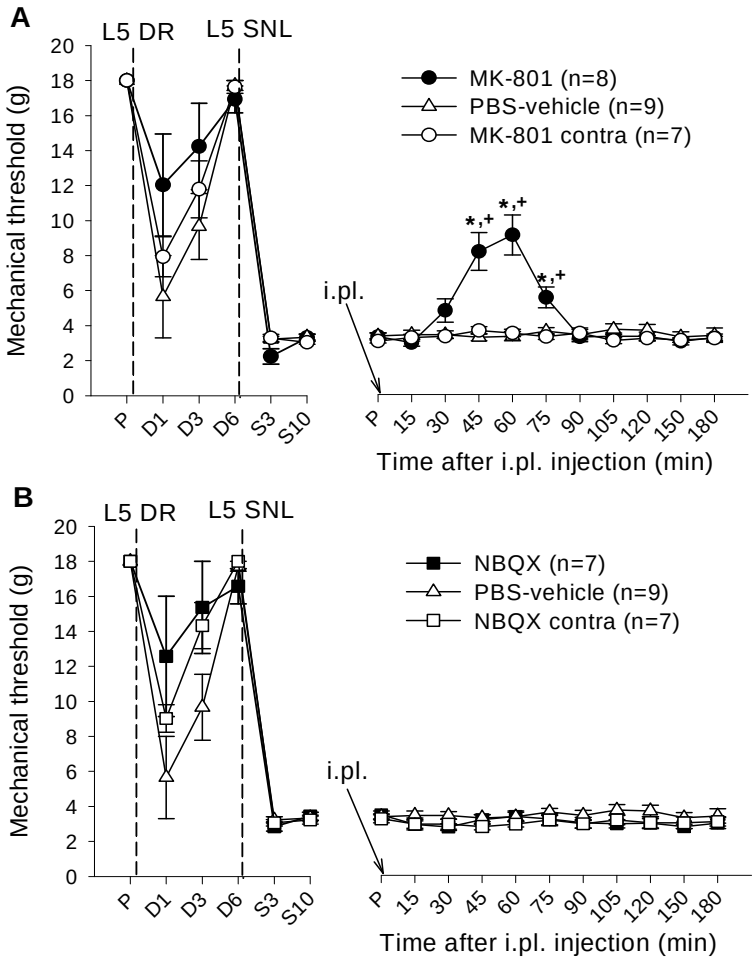

Figure 5. Effects of MK-801 or NBQX post-treatment in the periphery on lumbar 5 (L5) spinal nerve lesion (SNL)-induced paw withdrawal threshold (PWT) reduction. The left hand side of each graph shows changes in the PWTs of the three groups of animals that received L5 SNL following L5 dorsal rhizotomy (DR). (A) An intraplantar (i.pl.) injection of MK-801 given on day 10 post-SNL into the affected hind paw produced a higher PWT than a PBS-vehicle injection into the affected hind paw or an MK-801 injection into the contralateral hind paw, as indicated by * or +, respectively ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). (B) No PWT difference was observed between animals treated with NBQX on day 10 post-SNL in the affected hind paw and animals treated with PBS-vehicle or with NBQX in the contralateral hind paw. Symbols are as described in Fig. 3. P, pre-DR values; D, days after DR; S, days after SNL.

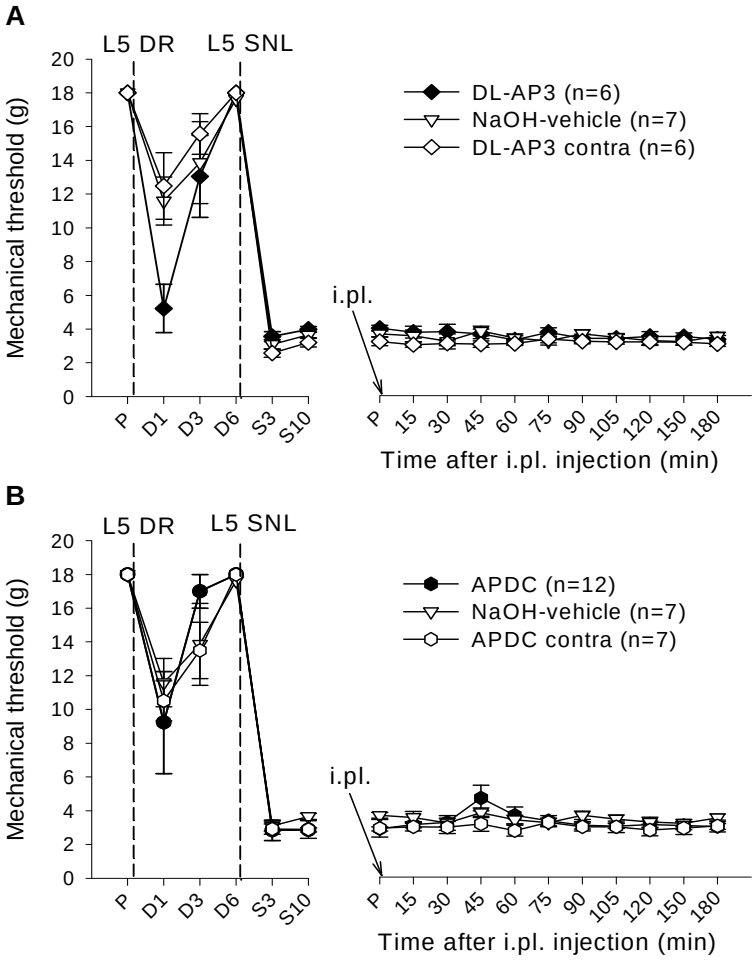

Figure 6. Effects of DL-AP3 or APDC post-treatment in the periphery on lumbar 5 (L5) spinal nerve lesion (SNL)-induced paw withdrawal threshold (PWT) reduction. Changes in the PWTs of the three groups of animals that received L5 SNL following L5 dorsal rhizotomy (DR) are shown on the left hand side of each graph. (A) An intraplantar (i.pl.) injection of DL-AP3 on day 10 post-SNL into the affected hind paw resulted in no PWT differences compared with animals treated with NaOH-vehicle or with DL-AP3 in the contralateral hind paw. (B) No PWT differences were observed between animals treated with APDC on day 10 post-SNL in the affected hind paw and those treated with NaOH-vehicle or with APDC in the contralateral hind paw. Symbols are as described in Fig. 4. P, pre-DR values; D, days after DR; S, days after SNL.

5. Effects of pre-treatment in the periphery with MK-801, NBQX, DL-AP3, or APDC on ES-induced mechanical hyperalgesia

To test whether peripheral NMDA and AMPA/kainate receptors are involved in the induction phase of ES-induced mechanical hyperalgesia, the effect of MK-801 or NBQX injected into the hind paw immediately before ES on PWT scores was investigated. As seen in Fig. 7A, an i.pl. injection of 20 nmol of MK-801, performed 15 min before ES, into the hind paw ipsilateral to ES demonstrated a significant increase in PWTs on days 1, 2, 3, 4, 5, 6 and 7 post-treatment compared with PBS-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). In Fig. 7B, the effect of pre-treatment in the periphery with NBQX is shown. Animals that received ES preceded by an i.pl. injection of 100 nmol of NBQX into the hind paw ipsilateral to ES displayed similar PWTs to animals treated with PBS-vehicle.

To determine if peripheral mGlu receptors are involved in the induction phase of ES-induced mechanical hyperalgesia, we examined the effects of pre-treatment with competitive group-I mGlu receptor antagonist DL-AP3 or selective group-II mGlu receptor agonist APDC in the hind paw on PWT scores. As seen in Fig. 8A, an i.pl. injection of 70 nmol of DL-AP3, performed 15 min before ES, into the hind paw ipsilateral to ES resulted in a significant increase in PWTs on days 1, 2, 3, 4, 5, 6 and 7 post-treatment compared with NaOH-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). In Fig. 8B, the effect of pre-treatment in the periphery with APDC is shown. An i.pl. injection of 20 nmol of APDC, performed 15 min before ES, into the hind paw ipsilateral to ES produced a significant increase in PWTs on day 1, 2, 3, 4, 5, 6 and 7 post-treatment compared with NaOH-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test).

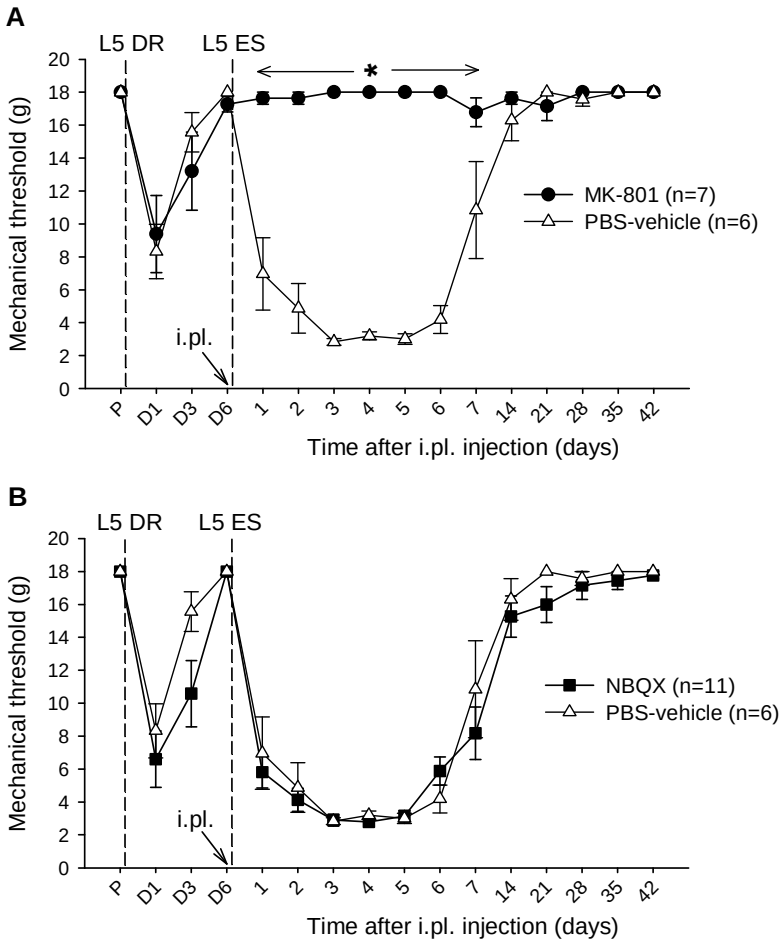

Figure 7. Effects of MK-801 or NBQX pre-treatment in the periphery on lumbar 5 (L5) electrical stimulation (ES)-induced paw withdrawal threshold (PWT) reduction. (A) An intraplantar (i.pl.) injection of MK-801 given before L5 ES into the affected hind paw (closed circles) resulted in increased PWTs compared with animals treated with PBS-vehicle (open triangles), as indicated by asterisks ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). (B) The PWTs of animals treated with NBQX before L5 ES in the affected hind paw (closed rectangles) were similar to those of animals treated with PBS-vehicle (open triangles). DR, dorsal rhizotomy; P, pre-DR values; D, days after DR.

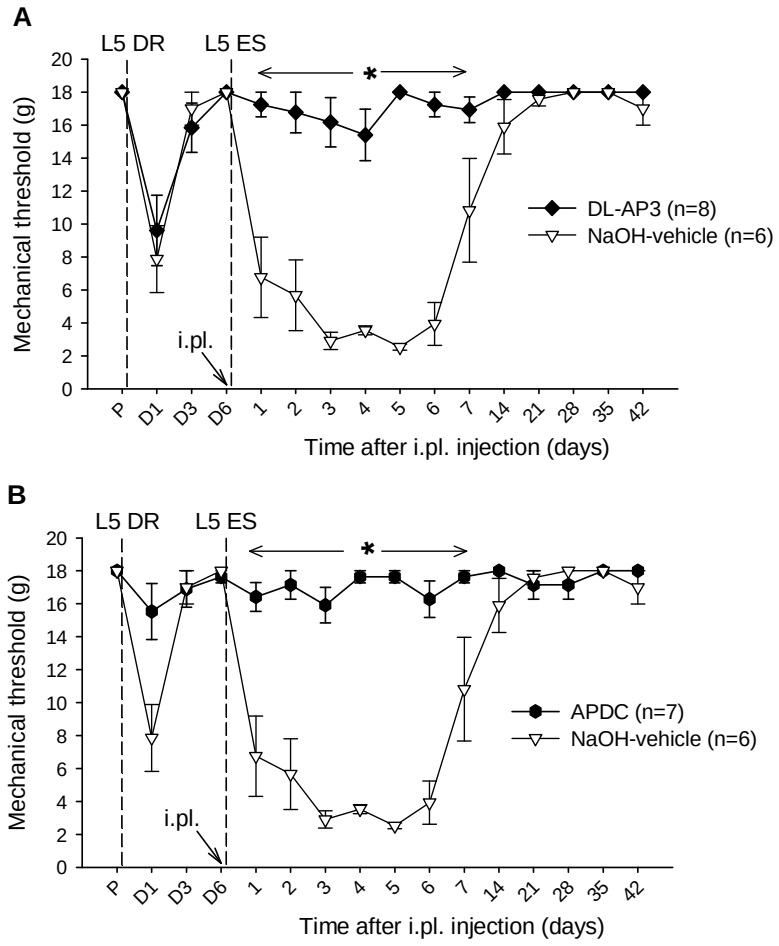

Figure 8. Effects of DL-AP3 or APDC pre-treatment in the periphery on lumbar 5 (L5) electrical stimulation (ES)-induced paw withdrawal threshold (PWT) reduction. (A) Animals that received an intraplantar (i.pl.) injection of DL-AP3 before L5 ES into the affected hind paw (closed diamonds) displayed higher PWTs than those treated with PBS-vehicle (open triangles), as indicated by asterisks. (B) Animals treated with APDC before L5 ES in the affected hind paw (closed hexagons) produced higher PWTs than animals treated with NaOH-vehicle (open triangles), as indicated by asterisks ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). DR, dorsal rhizotomy; P, pre-DR values; D, days after DR.

6. Effects of post-treatment in the periphery with MK-801, NBQX, DL-AP3, or APDC on ES-induced mechanical hyperalgesia

To test whether peripheral NMDA and AMPA/kainate receptors were involved in the maintenance phase of ES-induced mechanical hyperalgesia, the effect of MK-801 or NBQX injected into the hind paw after ES on PWT scores was examined. As seen in Fig. 9A, all two groups of animals that received ES following DR demonstrated a significant decrease in PWTs after ES compared with the pre-ES baseline ($P<0.05$, Friedman ANOVA). An i.pl. injection of 20 nmol of MK-801, performed on day 3 post-ES, into the hind paw ipsilateral to ES showed a significant increase in PWTs at 15, 30, and 45 min post-treatment compared with PBS-vehicle-treated animals ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). In Fig. 9B, the effect of post-treatment in the periphery with NBQX is shown. An i.pl. injection of 100 nmol of NBQX, performed on day 3 post-ES, into the hind paw ipsilateral to ES produced no significant difference in PWTs compared with animals treated with PBS-vehicle.

The possible involvement of peripheral group-I and group-II mGlu receptors in the maintenance phase of ES-induced mechanical hyperalgesia was tested by analyzing the effect of DL-AP3 or APDC injected into the hind paw after ES on PWT scores. As seen in Fig. 10A, an i.pl. injection of 70 nmol of DL-AP3, performed on day 3 post-ES into the hind paw ipsilateral to ES, produced no significant difference in PWTs compared with those animals treated with NaOH-vehicle. In Fig. 10B, the effect of post-treatment in the periphery with APDC is shown. Animals that received an i.pl. injection of 20 nmol of APDC, on day 3 post-ES, into the hind paw ipsilateral to ES showed no significant difference in PWTs compared with animals treated with NaOH-vehicle.

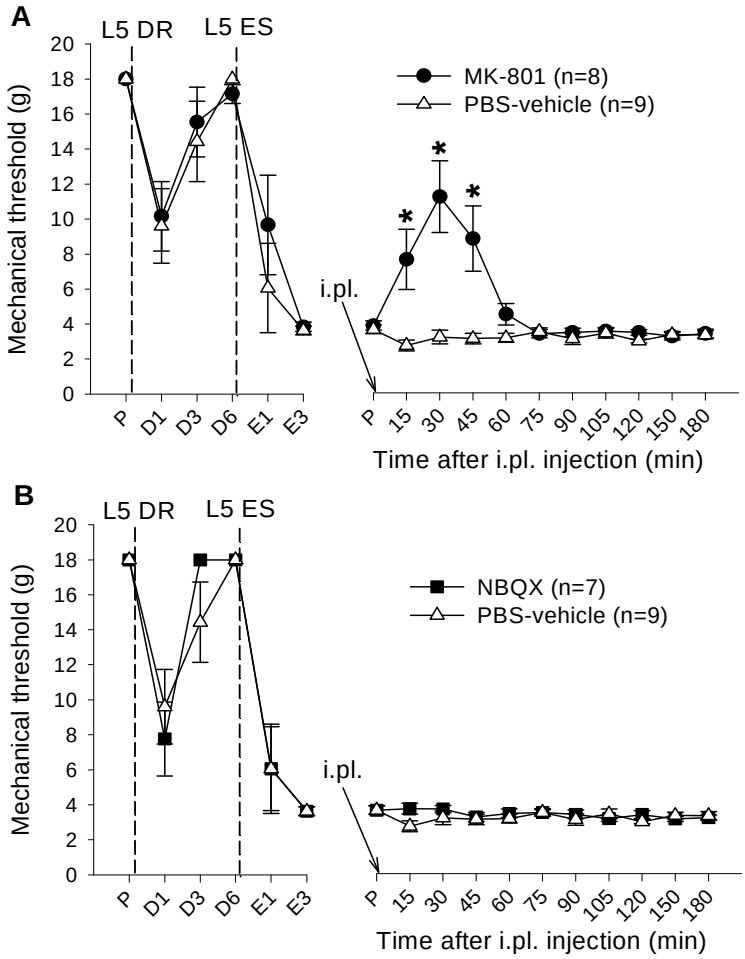

Figure 9. Effects of MK-801 or NBQX post-treatment in the periphery on lumbar 5 (L5) electrical stimulation (ES)-induced paw withdrawal threshold (PWT) reduction. The left hand side of each graph shows changes in the PWTs of the two groups of animals that received L5 ES following L5 dorsal rhizotomy (DR). (A) An intraplantar (i.pl.) injection of MK-801 given on day 3 post-ES into the affected hind paw produced a higher PWT than a PBS-vehicle injection into the affected hind paw, as indicated by asterisks ($P<0.05$, Kruskal-Wallis ANOVA, followed by Mann-Whitney rank-sum test). (B) No PWT difference was observed between animals treated with NBQX on day 3 post-ES in the affected hind paw and animals treated with PBS-vehicle. Symbols are as described in Fig. 7. P, pre-DR values; D, days after DR; E, days after ES.

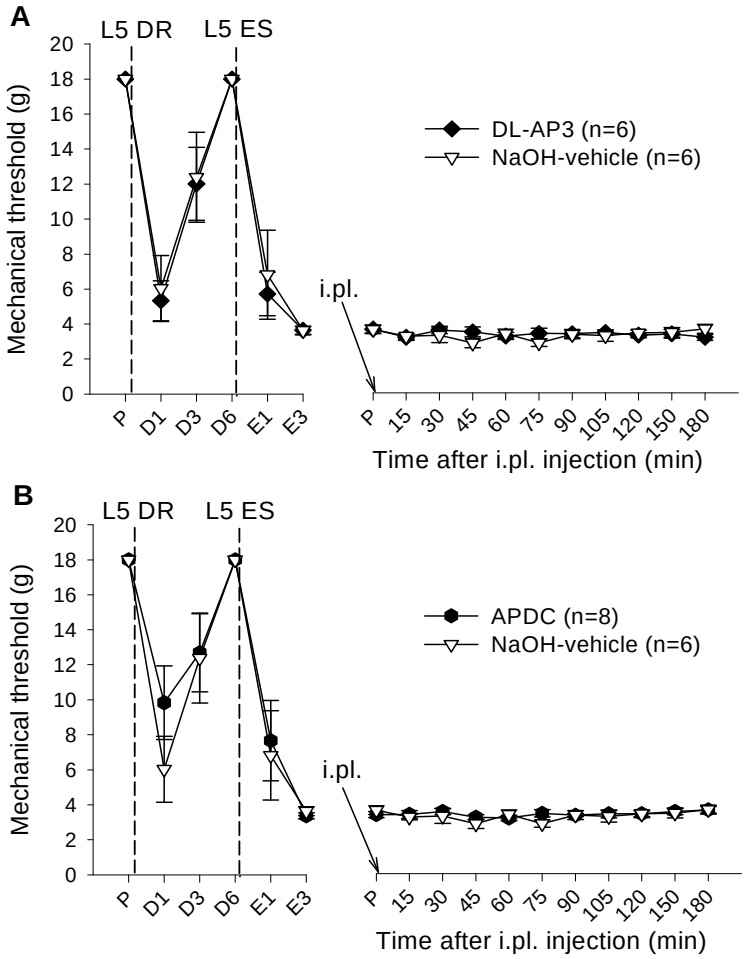

Figure 10. Effects of DL-AP3 or APDC post-treatment in the periphery on lumbar 5 (L5) electrical stimulation (ES)-induced paw withdrawal threshold (PWT) reduction. Changes in the PWTs of the two groups of animals that received L5 ES following L5 dorsal rhizotomy (DR) are shown on the left hand side of each graph. (A) An intraplantar (i.pl.) injection of DL-AP3 on day 3 post-ES into the affected hind paw resulted in no PWT differences compared with animals treated with NaOH-vehicle. (B) No PWT differences were observed between animals treated with APDC on day 3 post-ES in the affected hind paw and those treated with NaOH-vehicle. Symbols are as described in Fig. 8. P, pre-DR values; D, days after DR; E, days after ES.

IV. DISCUSSION

When a peripheral nerve is injured, damaged primary sensory afferents emit a barrage of brief impulses, or injury discharge, which last briefly several minutes.[1] This injury discharge travels orthodromically to central terminals in the spinal cord and antidromically to terminals in peripheral tissues. It has been known that centrally conducting injury discharge is responsible for triggering neuropathic pain by increasing the excitability of spinal dorsal horn neurons which leads to central sensitization. The abnormal pain behavior following peripheral nerve transection is suppressed by local anesthetic blockade of centrally conducting injury discharge[2,3] or by intrathecal injection of glutamate receptor blockers at the lumbar level just prior to transection.[4] Brief ES of C-fibers, which mimics injury discharge, produces prolonged hyperalgesia,[5] although in which the effects exerted by stimulation-produced discharge that is peripherally conducted could be not excluded. In addition to centrally conducting injury discharge, ectopic discharge that is long-lasting[21,22] and generated from neuroma or DRG of injured sensory afferents[23] also accesses to the spinal cord. An advantage of using our rats with previous DR would be that the effect of peripherally conducting injury discharge on neuropathic pain could be examined, without considering the potential effect of centrally conducting impulses.

Our observation that an injury to the L5 spinal nerve, when injury and ectopic discharges that are conducted to the spinal cord are prevented by a previous L5 DR, still induces a mechanical hyperalgesia lasting over 42 days, implies that nerve injury-induced peripheral effects play an important role in the generation of SNL-induced mechanical hyperalgesia. Furthermore, observations that artificial injury discharge

produced by ES of L5 spinal nerve under the condition of a previous L5 DR induces a mechanical hyperalgesia lasting over 7 days and that the onset of such ES-induced mechanical hyperalgesia is completely blocked by MK-801, DL-AP3 and APDC applied into the plantar skin immediately before ES, suggest an involvement of peripherally conducting injury discharge in the induction of SNL-induced mechanical hyperalgesia. Nerve injury-induced peripheral effects are exerted presumably both by peripherally conducting injury discharge stemming from injured afferents and by spontaneous discharge from intact afferents nearby degenerating axon during Wallerian degeneration following the lesion. The development of spontaneous discharges in intact afferent neighbors following peripheral nerve lesion has been demonstrated.[17-19] A possible role of such spontaneous discharges, other than injury discharge, in the generation of SNL-induced mechanical hyperalgesia is supported by our findings that peripherally applied glutamate receptor antagonists block the onset of SNL-induced mechanical hyperalgesia only for several days whereas they block ES-induced mechanical hyperalgesia completely.

Injury-evoked impulses traveling over primary sensory afferent fibers to the periphery would induce Glu release from the peripheral terminals of these afferents. In fact, it has been shown that the ES of primary sensory afferents results in the peripheral release of Glu.[6] Furthermore, it has been observed that various subclasses of Glu receptors are present on the peripheral processes of primary sensory afferents.[7,8,10,14] A question then arises as to whether peripheral Glu plays a role in neuropathic pain. Numerous evidences suggest a critical role for central Glu in neuropathic pain by showing that various subclasses of Glu receptors within the spinal cord contribute to neuropathic pain.[3,24-27] To date, however, little or no evidence has been presented to indicate the

involvement of peripheral Glu in mediating neuropathic pain. Our observation that mechanical hyperalgesia induced by L5 SNL, associated with previous L5 DR, is relieved by blocking the action of particular Glu receptors locally in the hind paw, suggests a contribution of Glu acting at its receptors in the periphery to the generation of neuropathic pain.

Our data show that an i.pl. injection of MK-801, but not of NBQX, immediately before L5 SNL delays the onset of L5 SNL-induced mechanical hyperalgesia. They also show that an i.pl. injection of MK-801, but not NBQX, relieves the mechanical hyperalgesia already established following L5 SNL. The application of a higher dose (1000 nmol) of NBQX still failed to either prevent or relieve the mechanical hyperalgesia (data not shown). These results suggest that NMDA, but not AMPA/kainate, receptors contribute to both the induction and maintenance phases of neuropathic pain. Since, as mentioned earlier, NMDA receptors are found to be present on the peripheral processes of primary sensory afferents, it is speculated that Glu released from the terminals of injured primary afferents in hind paw skin acts at NMDA receptors on terminals of nearby intact afferents to sensitize them, particularly nociceptive fibers, and thus participates in the generation of neuropathic pain. This speculation is supported by observations that cutaneous nociceptors are sensitized by exposure either to Glu in the normal state[28] or to NMDA in the inflammatory state.[29]

The underlying mechanisms of an increase in mechanical sensitivity, or mechanical sensitization, that can be induced by Glu in the peripheral terminals of uninjured nociceptive afferents are not clear. It is possible that Ca^{++} dependent protein kinase C (PKC), whose activity increases by NMDA receptor-mediated Ca^{++} influx, is involved in this mechanical sensitization. In support of this possibility, it has been found in cultured of adult rat dorsal root ganglion (DRG) neurons that mechanically

induced response measured as transient increase of intracellular Ca^{++} level, is enhanced by activation of NMDA receptors. This NMDA-mediated potentiation of mechanical response is inhibited by PKC inhibitor or mimicked by PKC activator.[30] Further support of a role of Ca^{++} dependent PKC in nociceptor sensitization is the inhibition of potassium K^{+} currents in cultured capsaicin-sensitive rat DRG neurons by PKC activator in which Ca^{++} entry is necessary.[31]

Our observation of the failure of peripherally injected NBQX to attenuate neuropathic pain behavior conflicts with results showing that acute inflammatory pain behavior induced by formalin, which lasts several hours, is attenuated by treatment locally in the periphery with another AMPA/kainate receptor antagonist CNQX.[32] However, this agrees with the findings that chronic inflammatory pain behaviors induced by either Freunds complete adjuvant[33] or bee venom,[34] which last longer than several days, are relieved by peripheral treatment with MK-801, but not with CNQX treatment. This discrepancy may reflect a difference in pathological conditions, between the acute and chronic pain states, for peripheral Glu to exert its effect on the generation of pathological pain. We speculate that in the periphery Glu acts at both NMDA and AMPA/kainate receptors to generate acute pain, whereas it acts preferentially at NMDA receptors to produce chronic pain state, including SNL-induced mechanical hyperalgesia.

The mGlu receptors are widely expressed in the central nervous system, where they function to modulate neuronal excitability and synaptic transmission. On the basis of studies on the functions and pharmacology of mGlu receptors,[35,36] group-I mGlu receptors are known to associated with the activation of phospholipase C, which results in the release of calcium from intracellular stores, and with the activation of protein kinase C. On the other hand, group-II mGlu receptors are

negatively coupled to the activation of adenylyl cyclase, which results in a decrease in cyclic AMP production. Thus, activation of group-I mGlu receptors leads to increased neuronal excitability, whereas the activation of group-II mGlu receptors results in decreased neuronal excitability. Recent evidence suggests that peripheral mGlu receptors play a substantial role in the modulation of nociceptive processing. Anatomical studies have shown that both group-I and group-II mGlu receptors were localized on peripheral nociceptive afferents.[9-11] It has been observed that the activation of peripheral group-I mGlu receptors by an i.pl. injection of their agonists resulted in nociceptive behaviors that were blocked by appropriate antagonists.[9,11] Consistent with these findings, our results suggest that blocking of the actions of peripheral group-I mGlu receptors prevents the induction of neuropathic pain behavior, because they show that the group-I mGlu receptor antagonist DL-AP3, when administered locally to the hind paw before SNL, delays the onset of SNL-induced mechanical hyperalgesia. Interestingly, we observed that an i.pl. injection of the group-II mGlu receptor agonist APDC also delays the onset of mechanical hyperalgesia, which suggests that the activation of peripheral group-II mGlu receptors prevents the induction of neuropathic pain behavior. The similar findings have been reported, in which the intraperitoneal[26] and the intrathecal[37] administration of group-II mGlu agonist were found to reverse neuropathic pain behavior, although in both reports agonists may have had systemic effects. We speculate that the activation of group-I and group-II mGlu receptors in the periphery, which perhaps occur during the induction phase of neuropathic pan due to peripherally released Glu, may have no effect on the development of neuropathic pain. This is because, based on our results, the induction of neuropathic pain can be facilitated by the activation of peripheral group-I mGlu receptors, but

hindered by the activation of peripheral group-II mGlu receptors. Nevertheless, our findings suggest the potential utility of selective group-I receptor antagonist and group-II mGlu receptor agonist for the treatment of neuropathic pain.

We postulate two events as possible mechanisms that account for the development of mechanical hyperalgesia in rats that have received L5 SNL associated with previous L5 DR. The first is associated with injury discharge that is evoked at an earlier stage by L5 SNL from injured primary afferents, and conducted antidromically to the periphery. The second is associated with spontaneous ectopic discharge induced at a later stage from nearby uninjured nociceptive afferents, due to the degeneration of injured peripheral axons after L5 SNL, which may take several days to occur. This type of ectopic discharge has been demonstrated.[18] An earlier injury discharge would, by causing the peripheral release of Glu, initiate a sensitization of peripheral terminals of nearby uninjured nociceptive afferents, and thus contribute to the induction of mechanical hyperalgesia. A later ectopic discharge would not only produce a sensitization of central neurons by gaining access to the spinal cord through the L4 spinal nerve and dorsal root, but it would also produce a peripheral sensitization of uninjured nociceptor terminals, thus contributing to the maintenance of mechanical hyperalgesia. We observed that a single i.pl. injection of the NMDA receptor antagonist MK-801 prior to SNL exerted anti-hyperalgesic action lasting for 2-4 days. This could be explained if blocking the action of Glu released peripherally at an earlier stage prevents the induction of mechanical hyperalgesia, until a later event occurs to develop mechanical hyperalgesia, which may take several days.

V. CONCLUSION

The present study was conducted to provide evidence for a hypothesis that nerve injury-induced peripheral effects contribute to neuropathic pain. Using rats that received previous L5 DR, in which centrally conducting impulses are prevented, we obtained the following results.

L5 SNL reduced PWT of the affected hind paw with a peak reduction on day 3 post-SNL. This reduced PWT was maintained for at least 42 days of the test period. L5 ES also reduced PWT in a similar way to SNL, lasting for 7 days. When an i.pl. injection was given immediately before SNL, NMDA receptor antagonist MK-801, group-I mGlu receptor antagonist DL-AP3, and selective group-II mGlu receptor agonist APDC delayed the onset of mechanical hyperalgesia. For ES-induced mechanical hyperalgesia, the same application of above drugs completely blocked it's onset as long as for 42-test period. Pre-treatment of AMPA/kainate receptor antagonist NBQX was without an effect on either SNL- or ES-induced onset of mechanical hyperalgesia. When drugs were given after SNL induced-mechanical hyperalgesia had been established, MK-801 reversed mechanical hyperalgesia for about 45 minutes, whereas NBQX, DL-AP3, or APDC had no effect. The same was true for ES-induced mechanical hyperalgesia.

The results suggest that 1) peripherally conducting impulses play a crucial role in the generation of neuropathic pain and 2) that peripheral Glu receptors are involved in mediating this pain. It would be possible that various subclasses of peripheral Glu receptors could be therapeutically targeted to manage neuropathic pain.

REFERENCES

1. Wall PD, Waxman S, Basbaum Al. Ongoing activity in peripheral nerve: injury discharge. Exp Neurol 1974;45:576-589.
2. Gonzales-Darder JM, Barbera J, Abellan MJ. Effect of prior anesthesia on autotomy following sciatic transection in rats. Pain 1986;24:87-91.
3. Seltzer Z, Beilin B, Ginzburg R, Paran Y, Shinko T. The role of injury discharge in the induction of neuropathic pain behavior in rats. Pain 1991;46:327-336.
4. Seltzer Z, Cohn S, Ginzburg R, Beilin B. Modulation of neuropathic pain behavior in rats by spinal disinhibition and NMDA receptor blockade of injury discharge. Pain 1991;45:69-75.
5. Vatine J-J, Argov R, Seltzer Z. Brief electrical stimulation of c-fibers in rats produces thermal hyperalgesia lasting weeks. Neurosci Lett 1998:125-128.
6. deGroot J, Zhou S, Carlton SM. Peripheral glutamate release in the hind paw following low and high intensity sciatic stimulation. Neuroreport 2000;11:497-502.
7. Carlton SM, Hargett GL, Coggeshall RE. Localization and activation of glutamate receptors in unmyelinated axons of rat glabrous skin. Neurosci Lett 1995;197:25-28.
8. Coggeshall RE, Carlton SM. Ultrastructural analysis of NMDA, AMPA, and Kainate receptors on unmyelinated and myelinated axons in the periphery. J Comp neurol 1998;391:78-86.
9. Bhave G, Karim F, Carlton SM, Gereau IV RW. Peripheral group I metabotropic glutamate receptors modulate nociception in mice. Nat Neurosci 2001;4:417-423.
10. Carlton SM, Hargett GL, Coggeshall RE. Localization of metabotropic glutamate receptors 2/3 on primary afferent axons in the rat. Neuroscience 2001;105:957-969.

11. Walker K, Reeve A, Bowes M, Winter J, Wotherspoon G, Davis A, et al. mGlu5 receptors and nociceptive function II. mGlu5 receptors functionally expressed on peripheral sensory neurons mediate inflammatory hyperalgesia. Neuropharmacology 2001;40:10-19.
12. Carlton SM, Zhou S, Coggeshall RE. Evidence for the interaction of glutamate and NK1 receptors in the periphery. Brain Res 1998;790:160-169.
13. Jackson DL, Graff CB, Richardson JD, Hargreaves KM. Glutamate participates in the peripheral modulation of thermal hyperalgesia in rats. Eur J Pharmacol 1995;284:321-325.
14. Zhou S, Komak S, Du J, Carlton SM. Metabotropic glutamate 1á receptors on peripheral primary afferent fibers: their role in nociception. Brain Res 2001;913:18-26.
15. Zhou S, Bonasera L, Carlton SM. Peripheral administration of NMDA, AMPA or KA results in pain behaviors in rats. Neuroreport 1996;7:895-900.
16. Jang JH, Nam TS, Paik KS, Leem JW. Involvement of peripherally released substance P and calcitonin gene-related peptide in mediating mechanical hyperalgesia in a traumatic neuropathy model of the rat. Neurosci Lett. 2004;360:129-132.
17. Wu G, Ringkamp M, Hartke TV, Murinson BB, Campbell JN, Griffin JW, Meyer RA. Early onset of spontaneous activity in uninjured C-fiber nociceptors after injury to neighboring nerve fibers. J Neurosci 2001;21:RC140.
18. Wu G. Ringkamp M, Murinson BB, Pogatzki EM, Hartke TV, Weerahandi HM, et al. Degeneration of myelinated errerent fibers induces spontaneous activity in uninjured C-fiber afferents. J. Neurosci 2002;22(17):7746-7753.
19. Shim B, Kim D-W, Kim BH, Nam TS, Leem JW and Chung JM. Mechanical and heat sensitization of cutaneuous nociceptors in rats with experimental peripheral neuropathy. Neuroscience 2005;132:193-201.

20. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL. Quantitative assessment of tactile allodynia in the rat paw. J Neurosci Methods 1994;53:55-63.
21. Liu X, Eschenfelder S, Blenk KH, Jänig W, Häbler H. Spontaneous activity of axtomized afferent neurons after L5 spinal nerve injury in rats. Pain 2000;84:309-318.
22. Han HC, Lee DH, Chung JM. Characteristics of ectopic discharges in a rat neuropathic pain model. Pain 2000;84:253-261.
23. Liu CN, Wall PD, Ben Dor E, Michaelis M, Amir R, Devor M. Tactile allodynia in the absence of C-fiber activation: altered firing properties of DRG neurons following spinal nerve injury. Pain 2000;85:503-521.
24. Fisher K, Fundytus ME, Cahill CM, Coderre TJ. Intrathecal administration of mGluR compound, (S)-4CPG, attenuates hyperalgesia and allodynia associated with sciatic nerve constriction injury in rats. Pain 1998;77:59-66.
25. Mao J, Price DD, Hayes RL, Lu J, Mayer DJ, Frenk H. Intrathecal treatment with dextrorphan or ketamine potently reduces pain-related behaviors in a rat model of peripheral mononeuropathy. Brain Res 1993;605:164-168.
26. Simmons RMA, Webster AA, Kalra AB, Iyengar S. Group II mGluR receptor agonists are effective in persistent and neuropathic pain models in rats. Pharmacol Biochem Behav 2002;73:419-427.
27. Tal M, Bennett GJ. Neuropathic pain sensations are differentially sensitive to dextrophan. Neuroreport 1994;5:1438-1440.
28. Du J, Koltzenburg M, Carton S. Glutamate-induced excitation and sensitization of nociceptors in rat glabrous skin. Pain 2001;89:187-198.
29. Du J, Zhou S, Coggeshall RE, Carlton SM. *N*-methyl-D-aspartate-induced excitation and sensitization of normal and inflamed nociceptors. Neuroscience 2003;118:547-562.

30. Chaban VV, Li J, Ennes HS, Nie J, Mayer EA, Mcroberts JA. *N*-methyl-D-aspartate receptors enhance mechanical responses and voltage-dependent Ca^{2+} channels in rat dorsal root ganglia neurons through protein kinase C. Neuroscience 2004;128:347-357.
31. Zhang Y-H, Kenyon JL, Nocol GD. Phorbol easter-induced inhibition of potassium currents in rat sensory neurons requires voltage-dependent entry of calcium. J Neurophysiol 2001;85:362-373.
32. Davidson EM, Coggeshall RE, Carlton SM. Peripheral NMDA and non-NMDA glutamate receptors contribute to nociceptive behaviors in the rat formalin test. Neuroreport 1997;8:941-946.
33. Leem JW, Hwang JH, Hwang SJ, Park H, Kim Mk, Choi Y. The role of *N*-methyl-D-aspartate receptors in Freund's complete adjuvant induced mechanical hyperalgeisa in rats. Neurosci Lett 2001;297:155-158.
34. You H-J, Chen J, Morch CD, Arendt-Nielsen L. Differential effect of peripheral glutamate (NMDA, non-NMDA) receptor antagonists on bee venom-induced spontaneous nociception and sensitization. Brain Res Bull 2002;58:561-567.
35. Conn PJ, Pin J-P. Pharmacology and functions of metabotropic glutamate receptors. Annu Rev Pharmacol Toxicol 1997;37:205-237.
36. Pin J-P, Duvoisin R. Review: Neurotransmitter receptors I, The metabotropic glutamate receptors: structure and functions. Neuropharmacology 1995;34:1-26.
37. Fisher K, Lefebvre C, Coderr TJ. Antinoccieptive effects following intrathecal pretreatment with selective metabotropic glutamate receptor compounds in a rat model of neuropathic pain. Pharmacol Biochem Behav 2002;73:411-418.

Abstract (in Korean)

외상성 신경병증통증 모델 쥐의 기계적 통각과민 현상에서 말초 glutamate의 역할

<지도교수 임 중 우>

연세대학교 대학원 의과학과

장 준 호

말초신경 손상은 기계적 통각과민을 포함하는 신경병증통증을 유발한다. 말초신경이 손상되면 일시적으로 신경방전을 생성하는데 이러한 손상방전은 중추 혹은 말초 양방향으로 전도될 수 있다. 중추 쪽으로 전도되는 손상방전은 중추신경계를 민감화 함으로써 신경병증통증의 생성에 기여하는 것으로 알려져 있다. 한편, 말초 쪽으로 전도되는 신경방전 역시 신경병증통증 생성에 기여할 가능성이 있는데, 실제로 말초로 전도되는 신경활동은 말초 쪽 신경종말에서 glutamate를 분비하고 결과적으로 glutamate 수용체를 활성화함으로써 통증을 일으키는 것으로 보고 되었다. 하지만, 이러한 가능성에도 불구하고 역방향성 신경방전이 신경병증통증에 기여하는지는 아직 밝혀지지 않은 실정이다. 본 연구에서는 신경손상에 의해 말초신경계 수준에서 유발되는 변화(peripheral effect)가 신경병증통증의 형성에 기여한다는 가설을 설정하고 이를 증명하고자 하였다. 이를 위해 제5요수 후근(L5 dorsal root)을 미리 절단한 백서를 이용하여 1) L5 척수신경(spinal nerve)의 손상 또는 전기자극이 신경병증통증을 유발하는지, 2) 이러한 신경병증통증 유발에 있어서 이에 말초 glutamate이 관여하는지 조사하였다.

신경손상은 L5 척수신경을 결찰 후 절단함으로써 시행하였고, 전기자극은 L5 척수신경에 접촉한 은선(silver wire)을 통해 연발성 펄스(train pulses; 0.5-ms, 2-5 mA, 4 Hz)를 5분간 가해줌으로써 시행하였다. 신경병증통증의 특징인 기계적 통각과민에 관한 행동반응은 von Frey 필라멘트를 뒷 발바닥에 가하고 이에 대한 발의 회피반응 정도를 측정함으로써 분석하였다. 말초 glutamate 수용체의 신경병증통증에 관한 기여도는 glutamate 수용체 아형의 길항제나 효현제를 신경손상 측 발바닥 피하에 처치하고 이

러한 처치가 기계적 통각과민에 미치는 영향을 분석함으로서 조사하였다.

제5요수 후근을 미리 절단한 백서를 이용한 이상의 실험을 통해, 다음과 같은 연구결과를 얻었다.

1. L5 척수신경을 손상한 다음날부터 손상 측 발의 회피반응역치는 감소하였고, 이러한 감소는 손상 후 3일째에 최대에 도달하여 약 42일간 유지되었다. 이러한 역치감소를 기계적 통각과민이 생성된 것으로 간주하였다.
2. L5 척수신경을 전기자극한 경우도 신경을 손상한 경우와 비슷하게 기계적 통각과민이 유발되었는데, 이는 7일 정도 유지되었다.
3. 척수신경 손상에 의해 유도되는 기계적 통각과민은, 손상 직전에 발바닥 피하에 처치한, NMDA 수용기 길항제인 MK-801 (20 nmol), group-I mGlu 수용기 길항제인 DL-AP3 (70 nmol), 그리고 group-II mGlu 수용기 효현제인 APDC (20 nmol)에 의해 1~4일 그 유도가 지연되었다. 하지만, AMPA/kainate 수용기 길항제인 NBQX (100 nmol)에 의해서는 영향을 받지 않았다.
4. 척수신경 손상에 의해 이미 통각과민이 형성된 상황에서는, MK-801만 통각과민을 45분 동안 완화하였고, NBQX, DL-AP3 그리고 APDC에 의해서는 영향을 받지 않았다.
5. 전기자극에 의해 유도되는 통각과민은, 자극 직전에 처치한 MK-801, DL-AP3 그리고 APDC에 의해 완전히 차단되었다.
6. 전기자극에 의해 이미 기계적 통각과민이 형성된 상황에서는, MK-801만 통각과민을 45분 동안 완화하였고, NBQX, DL-AP3 그리고 APDC에 의해서는 영향을 받지 않았다.

이상의 실험 결과는 1) 신경 손상에 의해 말초로 전도되는 신경활동(직접적인 손상방전, 혹은 신경손상에 의해 이차적으로 유발되는 신경활동)이 신경병증통증의 형성에 중요한 역할을 하며, 2) 각 경우 모두에 있어서 말초 glutamate가 공헌함을 보여준다.

핵심되는 말 : 신경병증통증, 신경손상, 손상방전, glutamate, 전기자극