

Pharmacology of Spinal Glutamatergic Receptors in Post-Thermal Injury-evoked Tactile Allodynia and Thermal Hyperalgesia

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Background: After a focal thermal injury to the heel of a rat, thermal hyperalgesia appears at the injury site (primary thermal hyperalgesia), and tactile allodynia appears at the off-injury site (secondary tactile allodynia). The pharmacology of spinal glutamatergic receptors in the initiation and maintenance of secondary tactile allodynia was examined.

Methods: In rats prepared with chronic intrathecal catheters, the heel of one hind paw was exposed to a 52°C surface for 45 s, resulting in a local erythema without blistering. Intrathecal *N*-methyl-D-aspartate (NMDA) receptor antagonists (MK-801, AP5) and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-kainate (AMPA-KA) receptor antagonists (CNQX, NBQX, NS257, etc.) were administered either before (pretreatment) or after (posttreatment) the induction of the injury. Tactile withdrawal thresholds and thermal paw withdrawal latencies were assessed.

Results: Pretreatment and posttreatment with AMPA-KA antagonists produced a dose-dependent blockade of secondary tactile allodynia. However, NMDA antagonists, in doses that effectively block other models of facilitated states, showed little or no effect. Primary thermal hyperalgesia was blocked only by high-dose AMPA-KA antagonists.

Conclusion: Spinal AMPA-KA receptors play a major role in the initiation of secondary tactile allodynia induced by focal thermal injury. In contrast, spinal NMDA receptors play only a minimal role.

STUDIES of the response properties of dorsal horn neurons show that persistent activation of small afferents initiates a state in which the spinal neuron shows an enhancement of its response to subsequent small and large afferent input and an increased receptive field for natural low- and high-intensity stimuli.¹ These physiologic characteristics parallel the pain behavior profile associated with tissue injury. After a focal burn restricted to the heel of a rat, thermal stimulus to the injury site results in a shortened escape latency (e.g., primary ther-

mal hyperalgesia), and a light touch applied adjacent to the injury site results in a lower threshold (e.g., secondary tactile allodynia).²⁻⁴

Current work on spinal pharmacology of this injury-induced cascade emphasizes the role played by the spinal release of glutamate and the activation of ionotropic (*N*-methyl-D-aspartate [NMDA] and α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid-kainate [AMPA-KA]) receptors.⁵ Electrophysiologic studies with AMPA-KA antagonists show the following: (1) acute depolarization is blocked by AMPA-KA receptor antagonists; (2) such blockade eliminates responses to cutaneous mechanical, chemical, and thermal stimulation⁶; (3) AMPA-KA receptors mediate low-threshold input, presumably from large myelinated fibers⁷; and (4) such a blockade serves to block facilitation induced by persistent small afferent activation. Thus, spinal non-NMDA receptors play an important role in the processing of both noxious and innocuous stimuli and in mediating afferent traffic that leads to a state of central sensitization. In contrast, antagonists of the NMDA glutamate ionophore have little effect on acute excitation but typically diminish the facilitated response that otherwise accompanies repetitive small afferent stimulation.^{7,8} Thus, to the degree that the secondary tactile allodynia represents a state of central sensitization, we hypothesized that the secondary tactile allodynia would be blocked by both ionotropic antagonists. The blockade of allodynia could result either from the direct blockade of noxious stimuli, thus preventing central sensitization, or blockade of the allodynia itself. Accordingly, the effect of drugs on primary thermal hyperalgesia was also addressed to clarify the underlying mechanisms.

The current study assessed spinal glutamatergic pharmacology defining the hyperalgesia and allodynia induced by injury. We examined the effects of agents with relative selectivity for these several classes of receptors given intrathecally before and after the thermal injury.

Materials and Methods

All studies were conducted in accordance with the guidelines of the Institutional Animal Care Committee of the University of California-San Diego.

Animals

Male Holtzman-Sprague-Dawley rats (weight, 275-350 g; Harlan Industries, Indianapolis, IN) were housed in cages

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Received from the Department of Anesthesiology, University of California-San Diego, La Jolla, California. Submitted for publication March 20, 2001. Accepted for publication October 30, 2001. Supported in part by grant No. DA02110 from the National Institutes of Health, National Institute on Drug Abuse, Rockville, New Jersey (to Dr. Yaksh).

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with a 12-12-h light-dark cycle, and were allowed free access to food and water. After surgical interventions, rats were housed individually.

Intrathecal Cannulation

Lumbar intrathecal cannulation was conducted using a modification of the method described by Yaksh and Rudy.⁹ In brief, during halothane anesthesia (1-2%, mixed with O₂-air), a polyethylene catheter (PE-10) was inserted through an incision in the atlantooccipital membrane and threaded caudally to the lumbar enlargement. Rats with discernible neurologic deficits after intrathecal implantation were discarded. Rats were given at least 5 days to recover after surgery before any testing was performed.

Testing

Paw Withdrawal Threshold to Mechanical Stimuli. von Frey filaments with logarithmic incremental stiffness (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10 g; Stoelting, Wood Dale, IL) were used. Rats were placed in plastic cages with a wire-meshed bottom allowing free access to their paws. The 50% probability of paw withdrawal (thresholds) to mechanical stimulus were determined using a previously described method.¹⁰ Briefly, beginning with the 2.0-g probe, von Frey filaments were applied to the plantar surface of a hind paw for 6-8 s, in ascending or descending order after a negative or positive response, respectively. A cutoff threshold of 15 g was determined based on the previous study.¹⁰ Interpolation of 50% threshold was conducted according to the method of Dixon.¹¹ The testing site for mechanical threshold was determined at a site near but off injury (fig. 1) to evaluate the effects of drugs on secondary tactile allodynia.⁴ Animals were allowed at least 30 min to acclimate in the testing cages, after which baseline thresholds were assessed. Rats with a baseline threshold less than 10 g were excluded from the study.

Paw Withdrawal Threshold to Noxious Thermal Stimuli. Paw withdrawal latency (PWL; seconds) to noxious thermal stimulus was determined by a previously reported device modeled after Hargreaves *et al.*¹² Details of the device are given elsewhere.¹³ Rats were placed in individual plastic cages on a glass maintained at 30°C. A radiant heat source was focused on the heel portion of the plantar surface of the hind paw. The time interval between the application of the heat source and the hind paw withdrawal response was defined as PWL. After an acclimation time of at least 30 min, baseline PWL was assessed. Thermal threshold was examined on the heel portion of the hind paw (injury site; fig. 1) to evaluate the effects of drugs on primary thermal hyperalgesia.

Induction of Thermal Injury. To induce a well-defined focal thermal injury, the rat was briefly anesthetized with halothane (2-3%, mixed with O₂-air) delivered through a nose cone. The plantar surface of one

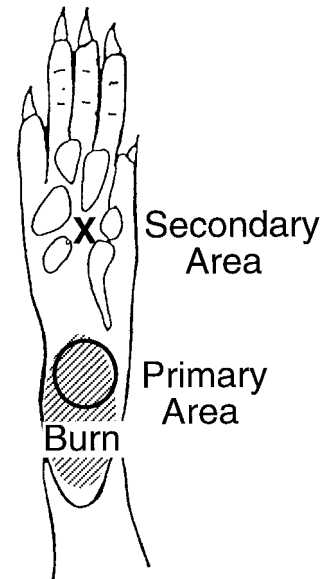


Fig. 1. Figure of a rat hind paw with thermal injury (dashed area) at the heel portion. The "X" indicates the testing point for secondary tactile allodynia, and the circle indicates the testing area for primary thermal hyperalgesia.

hind paw was placed on a $52 \pm 1^\circ\text{C}$ surface for 45 s, with constant pressure applied to the heel portion of the hind paw with a 10-g sand pouch. Our previous observation showed that this exposure results in mild local erythema on the heel portion without excessive tissue damage such as blistering. No erythema is observed on the toe portion. The rat subsequently displays significant primary thermal hyperalgesia and secondary tactile allodynia²⁻⁴

Experimental Paradigms

After baseline paw withdrawal threshold (PWT) or PWL measurement, thermal injury was induced as described. Intrathecal drugs were given either 5 min before (pretreatment) or 30 min after (posttreatment) the induction of injury. Mechanical threshold was assessed every 30 min after the injury for 3 h. Thermal withdrawal latency was assessed at 30, 45, 60, 90, and 120 min after the injury. Retesting of the same rat occurred with intervals of not less than 3 days for the contralateral paw and 7 days for the ipsilateral. Rats were used no more than three times. Our preliminary testing supports that this interval resulted in no effect from the previous injury on the hyperalgesia. The vehicle-tested rats were interspersed throughout the drug testing, and one set of control data was used for each vehicle (saline or H₂O).

Motor dysfunction was evaluated by testing the righting and stepping reflexes, as well as the ability to ambulate in a normal posture. Abnormalities observed within 30 min of drug administration were noted.

Drugs

Drugs used in the study are summarized in table 1. All drugs were dissolved in either 0.9% sterile preservative-

Table 1. List of Drugs

Drugs	Molecular Weight	Source	Solvent
NMDA antagonists			
MK-801	337	RBI, Natick, Massachusetts	Saline
AP5	197	RBI	Saline
AMPA-kainate antagonist			
CNQX	276	RBI	Saline
NBQX	380	RBI	H ₂ O
NS257	360	RBI	Saline
PD1 60725 (NA)	325	Warner Lambert-Park Davis, Pharmaceutical Research, Ann Arbor, Michigan	H ₂ O
PD1 61989-0054 (NA)	356	Warner Lambert-Park Davis, Pharmaceutical Research	H ₂ O
Subunit-specific antagonists			
LY293558	350	Lilly Research Laboratories, Indianapolis, Indiana	Saline
LY377770	350	Lilly Research Laboratories	Saline
metGluR antagonists/agonists			
L-AP3	169	RBI	Saline
L-AP4	183	RBI	Saline
S-4CPG	195	RBI	NaOH-Saline

RBI = Research Biochemicals; MK-801 = (+)-5-methyl-10,11-dihydro-5H-dibenzo[a,d]cycloheptan-5,10-imine hydrogen maleate; AP5 = 2-amino-5-phosphonovaleric acid; CNQX = 6-cyano-7-nitroquinoxaline-2,3-dione disodium; NBQX = 1,2,3,4-tetrahydro-6-nitro-2,3-dioxo-benzo[f]quinoxaline-7-sulfonamide disodium; NS257 = 1,2,3,6,7,8-hexahydro-(hydroxyimino)-N,N,7-trimethyl-2-oxo-benzo[2,1-b:3,4-c'] dipyrrole-5-sulfonamide hydrochloride; LY293558 = (3S,4aR,6R,8aR)-6-[2-(1 (2H)-tetrazole-5-yl)ethyl]decahydroisoquinoline-3-carboxylic acid monohydrate; LY377770 = (3S,4aR,6R,8aR)-6-[2-(1(2H)-tetrazole-5-yl)decahydroisoquinoline-3-carboxylic acid; L-AP3 = *R*(+)-2-amino-3-phosphonopropanoic acid; L-AP4 = 2-aminoA-phosphono-S-butanoic acid; S-4CPG = *S*(+)- α -amino-4-carboxy-benzeneacetic acid; NA = not available.

free saline or distilled water (H₂O), as indicated in table 1. The pH of the solution was adjusted within the range of 5–9 when required. Unless restricted by motor dysfunction or drug solubility, the highest dose of intrathecal drug tested was 100 μ g. Drug doses were injected in a volume of 10 μ l, followed by 10 μ l of saline flush, using calibrated PE-90 tubing connected to a microinjector.

Statistics

Results are presented as either the raw PWT (grams) or PWL (seconds), or as an efficacy, represented as a percent decrease from baseline measurements (algesic index [AI]; percent). The following formula was used to compute AI:

$$AI = \frac{[(\text{baseline value} - \text{postinjury value}) / \text{baseline value}] \times 100.}{}$$

In all graphs indicating AI, the y-axis was converted for easier understanding, showing a decrease in the threshold to be graphed in a negative direction (e.g., worsening of allodynia or hyperalgesia). To evaluate the effects of the drugs on the secondary tactile allodynia, AI values 30 min after drug injection (30 min after injury for pretreatment, 60 min after injury for posttreatment) were evaluated. Effects of the drugs on primary thermal hyperalgesia were evaluated with AI values 30 min after injury for pretreatment and AI values at 45 min after injury for posttreatment.

Raw PWTs are presented as median values and were compared using nonparametric analyses. Multiple comparisons were performed using the Friedman test for nonparametric analysis.¹⁴ All other data are presented as

mean \pm standard error of the mean, and the comparisons were performed using analysis of variance followed by Tukey test for multiple comparisons. Significance was set at $P < 0.05$.

Results

Of 400 rats prepared with intrathecal catheters and examined in this series of experiments, 28 rats were initially excluded from the study for having baseline mechanical thresholds less than 10 g (< 7%). Of the remainder, after thermal exposure as described, only one rat was observed to have blister formation 24 h after the injury, and those data were excluded.

Secondary Tactile Allodynia

Vehicle and Secondary Tactile Allodynia. After the thermal injury, median PWT to von Frey filaments at off-injury site decreased from preinjury values of 15 g to 3.2 g ($n = 20$) and 10.3 g to 2.8 g ($n = 7$) 30 min after the injury with intrathecal saline and H₂O pretreatment, respectively. A significant decrease was observed at 90 min in both groups (figs. 2A and B; $P < 0.05$ vs. preinjury). These results were similar to our previous data for naïve rats,^{4,15} and no effect of vehicle pretreatment was seen on the development of secondary tactile allodynia. Also in the posttreatment group, intrathecal treatment with both saline and H₂O showed no effect on the allodynia observed after the thermal injury. A significant reduction in the PWT was continuously observed at 90 and 120 min after injury in vehicle (saline and H₂O) treatment groups ($P < 0.05$ vs. preinjury; figs. 2C and D).

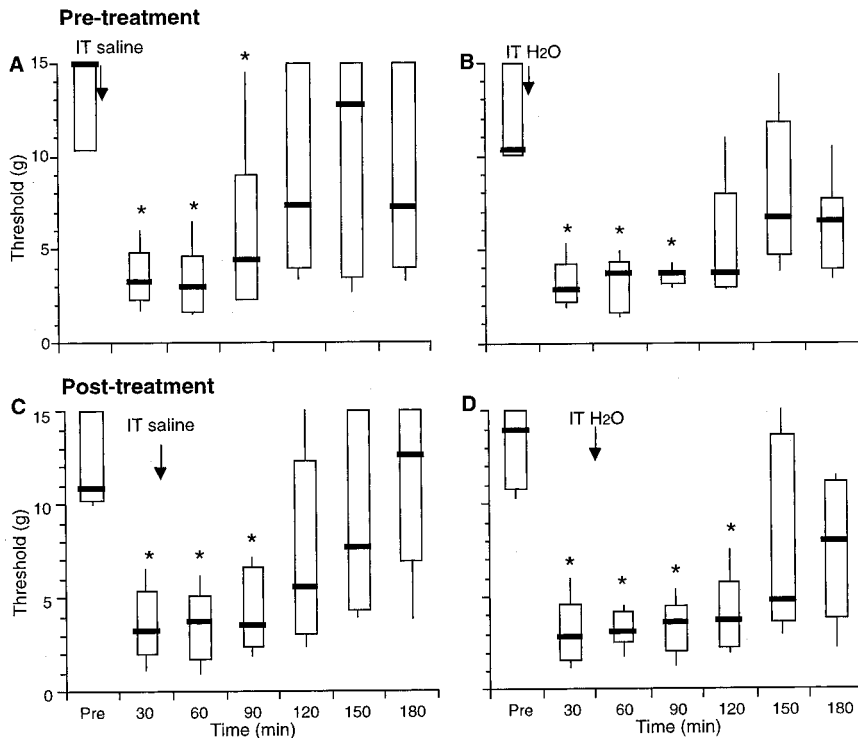


Fig. 2. Effect of vehicle (saline, H₂O) pretreatment and posttreatment on secondary tactile allodynia observed after mild thermal injury. Results are expressed as median (horizontal line) with 25th and 75th percentiles (boxes) and 10th and 90th percentiles (vertical lines). (A and B) Time courses of paw withdrawal threshold of injured paws with saline (A: n = 20) and H₂O (B: n = 7) pretreatment. Intrathecal (IT) drug (arrow) was given 5 min before the injury (time = 0). (C and D) Time courses of paw withdrawal threshold of injured paws with saline (C: n = 10) and H₂O (D: n = 7) posttreatment. Intrathecal (IT) drug (arrow) was given 30 min after the injury (time = 0). **P* < 0.05 versus preinjury threshold by Friedman and modified Dunnett tests.

NMDA Receptor Antagonists and Secondary Tactile Allodynia. Pretreatment and posttreatment with intrathecal MK-801 (30 nmol) or AP5 (50 nmol) had only modest effects on the secondary tactile allodynia after thermal injury (figs. 3A-D). AI at 30 min showed no effect with MK-801 pretreatment and a slight but not significant threshold increase in rats pretreated with AP5. In the posttreatment group, a decrease in the threshold at 30 min after injury in both groups was observed. Intrathecal MK-801 (30 nmol) at this point had no effect. Intrathecal AP5 (50 nmol) produced a small but transient reduction of allodynia. However, 50% of these animals treated with AP5 (50 nmol) displayed modest motor dysfunction at this dose that corresponded to the rats with increased thresholds, and higher doses could not be used (table 2).

AMPA-KA Receptor Antagonists and Secondary Tactile Allodynia. Pretreatment with intrathecal AMPA-KA receptor antagonists reliably reduced the secondary tactile allodynia. As shown in the time courses, the intrathecal NBQX (34 nmol) pretreatment prevented the thermal injury-evoked secondary tactile allodynia (fig. 4A). Results of all AMPA-KA antagonist pretreatments examined are summarized in figure 4B. AI at 30 min after injury showed a dose-dependent blockade of secondary tactile allodynia with all drugs tested. Significant blockade was observed with NBQX (11, 34 nmol), CNQX (36 nmol), PD160725 (68, 225 nmol), PD161989 (70 nmol), and NS257 (28 nmol). The maximum achievable antiallodynic effects were limited in most cases by the onset of motor impairment at the higher

doses and, in the case of CNQX, by a limited vehicle solubility. Similarly, posttreatment with intrathecal NBQX (34 nmol) significantly reversed the secondary tactile allodynia produced by thermal injury (fig. 4C). Comparison of AI at 60 min after injury showed a dose-dependent reversal of secondary tactile allodynia in all AMPA-KA receptor antagonists tested. Significant reversal was observed with NBQX (11, 34 nmol), CNQX (11, 36 nmol), PD160725 (225 nmol), PD161989 (7, 70 nmol), and NS257 (28 nmol) (fig. 4D).

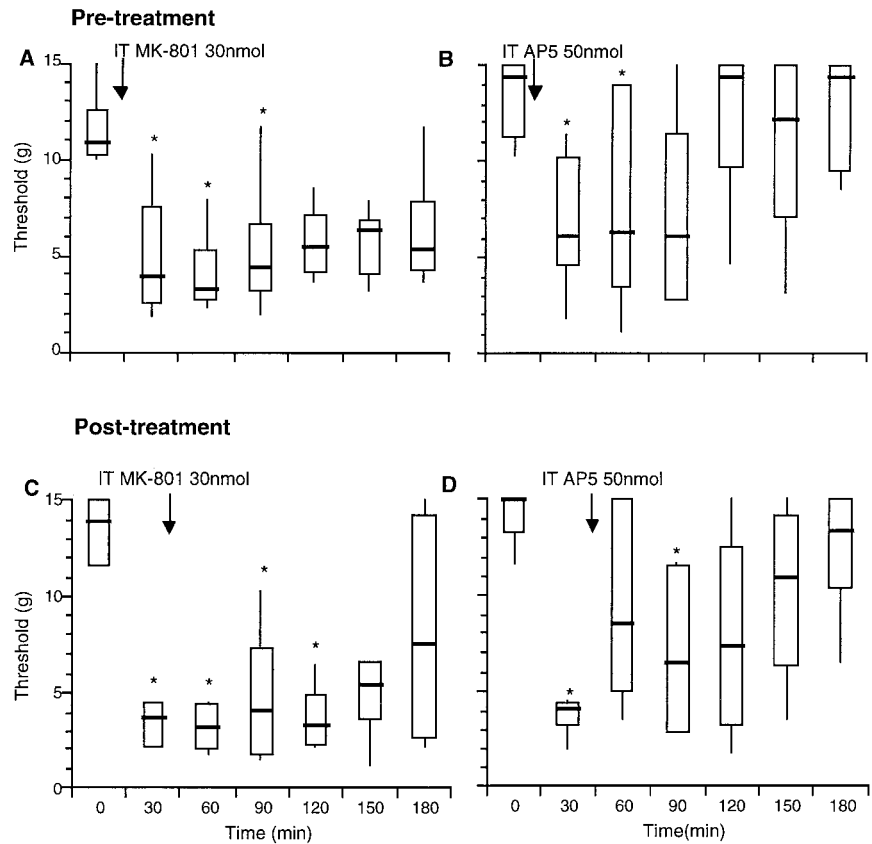
AMPA-KA Receptor Subunit-Specific Antagonists and Secondary Tactile Allodynia. Pretreatment with intrathecal LY293558, but not by LY377770, antagonists of Glu-r 2/5 and Glu-r 5, respectively, reduced the secondary tactile allodynia (fig. 5A; *P* < 0.05 and *P* > 0.08, respectively).

Posttreatment with either intrathecal LY293558 or LY377770 had no significant effect on the secondary tactile allodynia (fig. 5B; *P* > 0.08) at the maximum usable dose.

Primary Thermal Hyperalgesia

Vehicle and Primary Thermal Hyperalgesia. Average PWL showed a decrease from 10.9 ± 0.3 s to 5.2 ± 0.3 s (AI = $52 \pm 3\%$) and 11.2 ± 0.8 s to 5.0 ± 0.5 s (AI = $54 \pm 5\%$) with saline (n = 13) and H₂O (n = 6) pretreatment, respectively, showing a maximum reduction at 30 min after injury (fig. 6A). In the posttreatment group, similar reduction of the PWL was observed, and no effect of intrathecal saline (n = 12) or H₂O (n = 6) posttreatment was observed (fig. 6B).

Fig. 3. Effects of intrathecal NMDA receptor antagonists pretreatment and post-treatment on secondary tactile allodynia observed after mild thermal injury. Graph plots are described in figure 2. Time courses of injured paw withdrawal threshold with intrathecal (IT) MK-801 (A) and AP5 (B) pretreatment (n = 6 in each group) and MK-801 (C) and AP5 (D) post-treatment (n = 6 in each group). *P < 0.05 versus preinjury threshold by Friedman and modified Dunnett tests.



NMDA Receptor Antagonists and Primary Thermal Hyperalgesia. Intrathecal NMDA receptor antagonists MK801 (20 nmol) and AP5 (50 nmol) had been shown with no effect on the primary thermal hyperalgesia ($P > 0.08$; Jun and Yaksh, unpublished observation, 1997).

AMPA-KA Receptor Antagonists and Primary Thermal Hyperalgesia. Pretreatment with AMPA-KA receptor antagonists, doses that effectively antagonized secondary tactile allodynia, were tested on the primary thermal hyperalgesia. Comparison of AI at 30 min after

Table 2. Incidence of Motor Dysfunction

Drugs	Dose: nmol/Incidence of Motor Deficit % (n/N)			Preliminary Observation
NMDA antagonists				
MK-801			30*	
			8% (1/12)†	
AP5			50*	
			50% (6/12)†	
AMPA-KA antagonists				
CNQX	3.6*	11*	36*	
	0% (0/12)	0% (0/12)	0% (0/12)	
NBQX	1*	11*	34*	
	0% (0/12)	6% (1/18)†	48% (11/23)†	
NS257	2.8*	8.4*	28*	
	0% (0/12)	0% (0/18)	0% (0/18)	
PD160725	23*	68*	225*	
	0% (0/12)	0% (0/12)	21% (5/24)	
PD161989	0.7*	7*	23*	70*
	0% (0/6)	0% (0/12)	0% (0/6)	4% (1/24)†
LY293558	0.03*	0.09*	0.3*	230*
	0% (0/12)	0% (0/12)	0% (0/24)	100% (2/2)†
LY377770			0.9*	0.9*
			0% (0/24)	60% (3/5)†
				3*
				75% (3/4)†

* Dose shown in nanomoles for drug. † Observed with motor deficits.

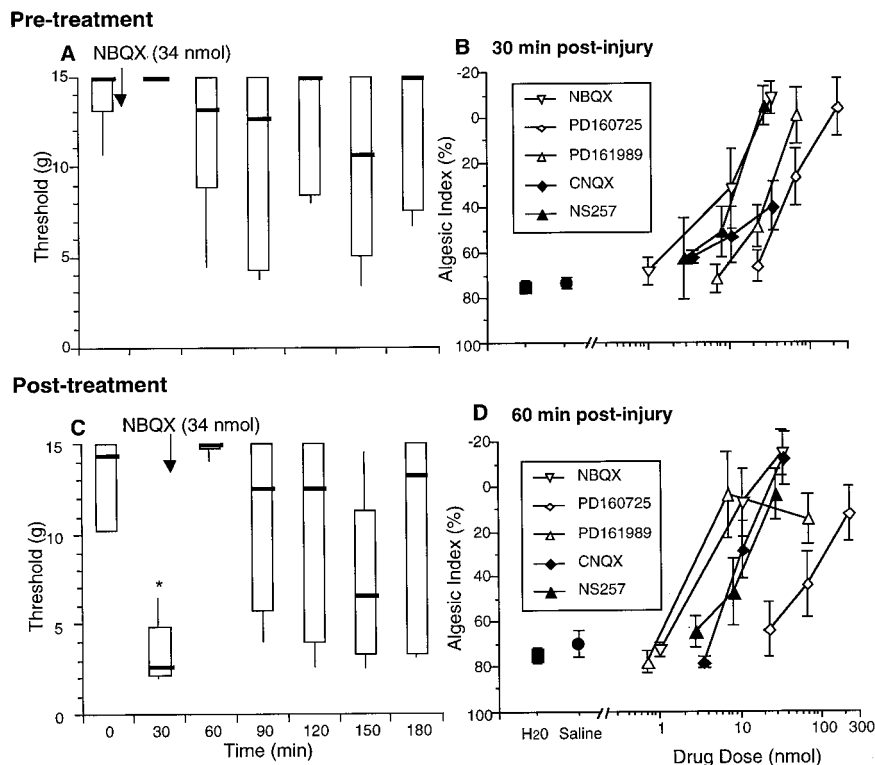


Fig. 4. Effects of intrathecal AMPA-KA receptor antagonists pretreatment and posttreatment on the secondary tactile allodynia observed after mild thermal injury. Graph plots are described in figure 2. (A) Time course of injured paw withdrawal threshold with intrathecal (IT) NBQX pretreatment ($n = 6$). (B) Comparison of the algescic index (mean \pm standard error of the mean) at 30 min after injury with AMPA-KA receptor antagonist pretreatment ($n = 6-7$ in each group). (C) Time course of injured paw withdrawal threshold with intrathecal (IT) NBQX posttreatment ($n = 6$). (D) Comparison of the algescic index (mean \pm standard error of the mean) at 60 min after injury with AMPA-KA receptor antagonists posttreatment.

injury showed a significant blockade of the primary thermal hyperalgesia only with CNQX (36 nmol) and NBQX (34 nmol) (analysis of variance, $P < 0.003$; fig. 7A).

In the case of primary thermal hyperalgesia, unlike the secondary tactile allodynia, the peak decrease of PWL is usually observed by 30 min after injury with a gradual recovery to baseline latency by 60 min after injury, which may obscure the effect of the drug. Accordingly, in the posttreatment group, effects of the drugs were assessed at 45 min after the injury (15 min after intrathecal drug administration). A significant dose-dependent reversal of the thermal hyperalgesia was observed with NBQX (34 nmol) and NS257 (28 nmol) treatment (fig. 7B).

Excitatory Amino Acid Antagonists on Motor Function and Other Behavioral Side Effects

As noted previously, for most drugs, aside from issues of solubility, the dose-limiting factor was motor dysfunction. Typically, these motor effects were a bilateral paralysis of the hind limb and a loss of the hind-limb placing and stepping reflexes. All of the observed instances of motor dysfunction caused by these agents were transient and completely reversible. For data analysis, any level of motor deficiency within 30 min of intrathecal drug was noted as "positive," and this analysis is summarized in table 2.

For NBQX, a high incidence of motor dysfunction was observed with 36 nmol. However, the duration of the motor effects was short (< 5 min), and full recovery was observed by the time of the first testing. NMDA antagonists also showed a high incidence of motor deficits in

the doses used. These deficits, although completely reversible, often lasted more than 30 min in the highest doses.

All AMPA-KA receptor antagonists displayed a dose-dependent incident of an atypical behavior, which was characterized by exaggerated glooming, scratching of the trunk, and "wet dog" shakes. These behaviors were not observed with intrathecal NMDA receptor antagonists (data not shown).

Discussion

The focal thermal injury model used in the current study revealed a well-defined thermal hyperalgesia at the injury site, but not elsewhere, and a concurrent off-site tactile allodynia. There are two principal findings regarding the pharmacology of spinal glutamate receptors in the primary thermal hyperalgesia and secondary tactile allodynia induced by focal thermal injury. First, blockade of the NMDA receptor, with competitive or noncompetitive antagonists, had only a minor effect or none at all on the subsequent development of primary thermal hyperalgesia and secondary tactile allodynia after thermal injury. Second, AMPA-KA receptor-selective antagonists at doses that did not cause motor dysfunction showed a dose-dependent blockade of both the primary and secondary hyperalgesia.

Spinal Glutamatergic Receptors after Tissue Injury

Activation of sensory afferents releases excitatory amino acids from the rat spinal cord *in vitro* and *in*

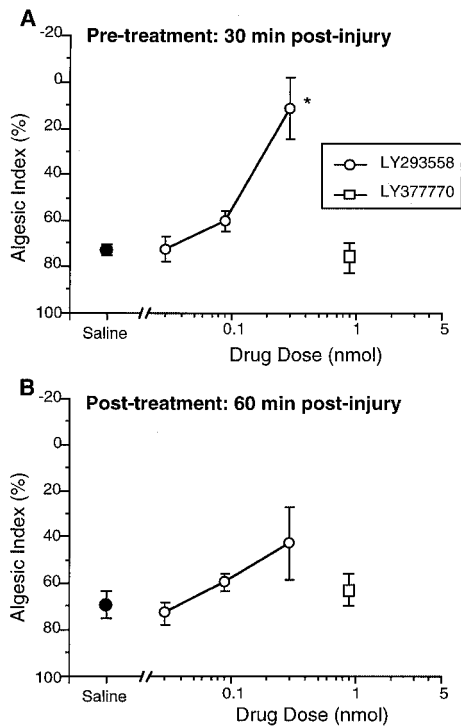


Fig. 5. Effects of intrathecal AMPA-KA receptor subunit-specific antagonists on the secondary tactile allodynia after mild thermal injury. (A) Comparison of the algesic index (mean \pm standard error of the mean) at 30 min after injury (n = 6 in each group). *P < 0.05 versus saline values. (B) Comparison of the algesic index at 60 min after injury with intrathecal posttreatment (n = 6 in each group).

vivo.¹⁶⁻¹⁸ With peripheral inflammation, this release increases, reflecting, in part, the role of facilitatory products, including prostanooids and nitric oxide.¹⁹ This extracellular increase in glutamate activity serves to activate local populations of glutamatergic receptors. Immunohistochemistry indicates that NMDA, non-NMDA, and metabotropic sites are found in the substantia gelatinosa and marginal layers.²⁰ Functionally, non-NMDA receptors are postsynaptic to the primary afferents and mediate the postsynaptic excitation evoked by sensory afferents excited by thermal, mechanical, and chemical stimuli. In contrast, most studies on NMDA antagonist have been shown not to block acute primary afferent-evoked excitation^{8,21} and thus are not considered to be a major mediator of acute monosynaptic-evoked excitation by primary afferent input. In the face of ongoing small afferent traffic, there is a persistent depolarization that leads to an alleviation of the NMDA channel magnesium block.²² The ability of spinally delivered NMDA antagonists to attenuate such ongoing processing in electrophysiologic models such as “wind-up”⁸ and in behavioral models such as the carrageenan-evoked thermal hyperalgesia²³ and the formalin test^{24,25} is taken to support the importance of this spinal NMDA mechanism in post-tissue injury-induced hyperalgesic states.¹⁹

NMDA Receptor

Despite their role in the electrophysiologic phenomena of “wind-up” and several models of central sensitization, spinal NMDA receptor antagonists, at doses equal to those previously reported, did not alter the burn injury-induced primary thermal hyperalgesia and secondary tactile allodynia. These results are in accord with the failure of intrathecal NMDA antagonists to reverse the mechanical allodynia in the paw incision model.^{26,27} Higher doses of NMDA antagonists could not be used because of evolving motor dysfunction. In AP5-treated rats, there was a tendency for the reversal; however, the animals that showed an increase in the threshold exactly corresponded to the animals with motor impairment, suggesting that, without motor impairment, no rats showed any effect from AP5 treatment. Accordingly, we believe that the absence of effect in these tissue injury models distinguishes them mechanistically from models of spinal sensitization in which the NMDA receptors do play a significant role in nonmotor impairing doses. We suggest two possible components to this difference. First, the mild thermal erythema generated in the focal burn model is limited to a local site and accordingly to a limited population of sensory afferents. In contrast, the formalin injection initially evokes activity in afferents projecting from the injection site. However, this activation spreads over the next hour to include afferents that project to the skin surface distant from the injection site, reflecting a diffusion of the injected irritant.²⁸ Second, the acute thermal injury is thought to activate a population of A δ afferents and C-polymodal nociceptors. In

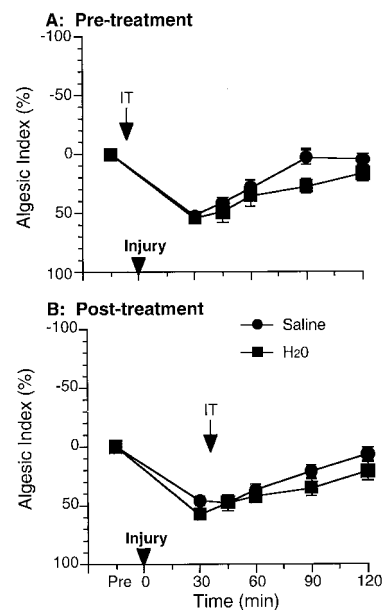


Fig. 6. Effect of intrathecal (IT) vehicle treatment on the primary thermal hyperalgesia observed after mild thermal injury. (A) Time courses for thermally injured paw after pretreatment with saline (n = 12) and H₂O (n = 6) are presented as algesic index. (B) Time courses after posttreatment with saline (n = 12) and H₂O (n = 6) as algesic index.

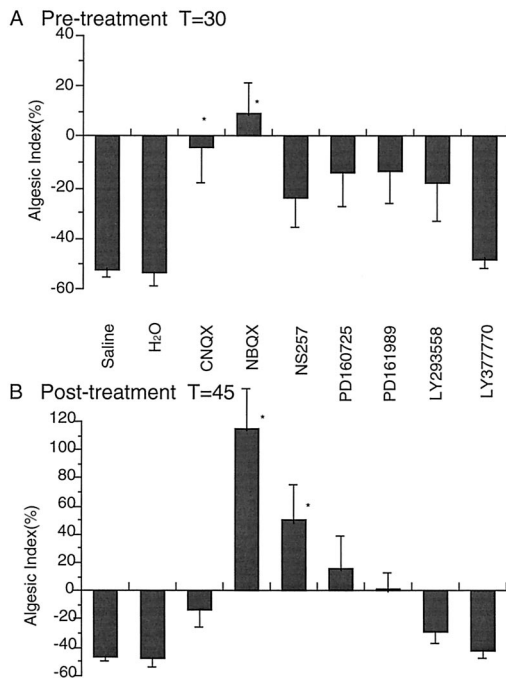


Fig. 7. Effects of intrathecal AMPA-KA receptor antagonists pretreatment and posttreatment on the primary thermal hyperalgesia observed after mild thermal injury. (A) Comparison of the algesic index of the injured paw 30 min after injury with drug pretreatment ($n = 6$ in each group except for saline [$n = 12$]). (B) Comparison of the algesic index of the injured paw 45 min after the injury with drug posttreatment at 30 min ($n = 6$ in each group except for saline [$n = 12$]). * $P < 0.05$ versus vehicle treatment by analysis of variance and Tukey test.

contrast, in the work of Puig and Sorokin,²⁸ formalin-evoked activity was observed in slow C, as well as fast conducting $A\beta$ afferents. We hypothesize that the exaggerated response evolving from the formalin injection represents a change in the anatomic distribution of the peripheral stimulus, leading to the concurrent activation of larger pools of spinal neurons that are linked by glutamatergic interneurons acting through NMDA receptors and by the activation of other pools of afferents not activated by a mild local injury. This speculation is consistent with previous work reported on the incision model and the change in the response patterns of dorsal horn neurons.²⁹

AMPA-KA Receptors

Consistent with the finding that spinal AMPA-KA antagonists abolish monosynaptic excitation evoked by all modalities,⁶ AMPA-KA antagonists also decrease the behavioral response to acute thermal and mechanical noxious stimulus. With regard to the role of the AMPA receptor in spinal sensitization or facilitated pain states, conflicting results have been reported with intrathecal AMPA antagonists. In the rat formalin model, Hunter and Singh³⁰ reported that intrathecal NBQX blocked the first but not the second phase of the formalin test. In contrast, Simmons *et al.*,³¹ using a more subunit selective

AMPA-KA receptor antagonist, LY293558, found the second but not the first phase to be significantly reduced. In injury models, the mechanical hyperalgesia observed in the postoperative pain model was observed to decrease with NBQX.^{26,27} These results, as well as the effective blockade of secondary tactile allodynia shown in the current study, demonstrate the important role of spinal AMPA-KA receptors in certain types of hyperalgesia. Because non-NMDA receptors are assumed to mediate monosynaptic transmission from primary afferent fibers to the spinal dorsal horn neurons, blockade of this receptor would be hypothesized to prevent central sensitization. However, only the highest doses of some of the AMPA-KA antagonists tested were observed to have an analgesic effect, suggesting a need for significantly higher doses of antagonists for a complete blockade of nociceptive or C-fiber input. In contrast, the antiallodynic effect was observed dose-dependently with doses that did not affect the stepping and righting reflexes. The preservation of these reflexes reflects not only intact motor function but also shows the presence of normal proprioceptive sensation mediated by low threshold mechanoreceptive ($A\beta$) afferents. In addition, the observed thermal analgesic effect of AMPA-KA antagonists was of a short duration, approximately 15–30 min. If the antiallodynic effect was only caused by its blockade of the monosynaptic transmission, reappearance of allodynia is likely in the posttreatment group. Accordingly, it is hypothesized that either the AMPA-KA receptor plays a specific role in the course of central sensitization leading to secondary tactile allodynia, or the AMPA-KA receptor, by blocking low-threshold input, shows a specific antiallodynic effect.

It has recently been shown that certain subclasses of AMPA sites lacking the GLU-R2 subunit are calcium permeable.³² Ca^{2+} entry through this receptor subtype likely serves to strengthen synaptic transmission.³³ Electrophysiologic studies have indicated that these sites, when activated, may subsequently internalize.³⁴ This raises the possibility that such sites may be responsible for the first step in the cascade initiated by acute afferent activation and dependent on increases in intracellular calcium. The relevance of these spinal sites is suggested in recent work showing that intrathecal jorotoxin, an agent that preferentially blocks the Ca^{2+} -permeable AMPA site, also blocked secondary tactile allodynia.³⁵

Different Role of AMPA and Kainate Receptors Evaluated by the Receptor Subunit-Specific Antagonists

Multiple receptor protein subunits for non-NMDA receptors have been identified. Of the AMPA and kainate receptor subunit proteins that have been cloned, iGluR 1–4 are AMPA-sensitive and iGluR 5–7, KA 1–2 are kainate-preferring.³⁶ The development of receptor subtype-selective antagonists has allowed us to suggest different

roles for spinal AMPA or kainate subunits in the pain transmission. Our study revealed the following: (1) GluR2 and 5 receptor antagonist LY293558^{31,37} pretreatment effectively blocked the secondary tactile allodynia; (2) posttreatment with LY293558 displayed only a limited efficacy; and (3) a GluR5-preferring antagonist, LY377770,³⁸ showed no effect in the development and maintenance of primary thermal hyperalgesia and secondary tactile allodynia. These results suggest that, although the GluR2 subunit may play a major role in the development of secondary tactile allodynia, participation of other subunit(s) is more likely required for its maintenance. Furthermore, in this model, the GluR5 subunit does not play a major role in the development of both primary thermal hyperalgesia and secondary tactile allodynia after acute tissue injury. This result contrasts with the result of Simmons *et al.*,³¹ who reported a role for GluR5 in the sustained hyperalgesia induced by formalin injection in the rat hind paw. This again suggests different pharmacologic mechanisms underlying the development of hyperalgesia-allodynia after acute tissue injury.

In the current study, intrathecal AMPA-KA receptor antagonists were observed to produce an atypical behavior: "wet-dog shaking." The implications of this phenomenon are not clear. As it was always associated with an increased incidence of scratching behavior, the drugs may be associated with a sensation of itching. As the phenomenon was observed principally with AMPA-KA antagonists, it is likely to be a specific effect of these agents. As AMPA-KA antagonists are associated with abolition of responses to a variety of cutaneous stimuli,⁶ the behavior may reflect a dysesthesia secondary to a loss of such afferent input. Alternately, glutamate receptor subunits are collocated on dorsal horn γ -aminobutyric acid-mediated neurons.³⁹ This raises the possibility that certain forms of afferent traffic may lead to the activation of local inhibitory circuits, and blockade of those inhibitory components may lead to a paradoxical change in afferent encoding leading to the observed aberrant behavior. The clinical implications of this phenomenon are yet to be investigated.

In conclusion, a distinct spinal excitatory amino acid pharmacology is responsible for the development of hyperalgesia-allodynia after acute tissue injury as compared with those of chronic nerve injury and persistent inflammation. There may be a clinical importance for AMPA-KA antagonists in the treatment of hyperalgesia-allodynia after acute tissue injury, such as postoperative pain.

References

1. Simone DA, Sorkin LS, Oh U, Chung JM, Owens C, LaMotte RH, Willis WD: Neurogenic hyperalgesia: Central neural correlates in responses of spinothalamic tract neurons. *J Neurophysiol* 1991; 66:228-46
2. Jun JH, Yaksh TL: The effect of intrathecal gabapentin and 3-isobutyl gamma-aminobutyric acid on the hyperalgesia observed after thermal injury in the rat. *Anesth Analg* 1998; 86:348-54
3. Jones D, Sorkin L: Systemic gabapentin and S(+)-3-isobutyl-gamma-aminobutyric acid block secondary hyperalgesia. *Brain Res* 1998; 1-2:93-9
4. Nozaki-Taguchi N, Yaksh TL: A novel model of primary and secondary hyperalgesia after mild thermal injury in the rat. *Neurosci Lett* 1998; 254:25-8
5. Bettler B, Mülle C: Review: Neurotransmitter receptors: II. AMPA and kainate receptors. *Neuropharmacology* 1995; 34:123-39
6. Dougherty PM, Palecek J, Paleckova V, Sorkin LS, Willis WD: The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. *J Neurosci* 1992; 12:3025-41
7. Dickenson AH, Sullivan AF: Differential effects of excitatory amino acid antagonists on dorsal horn nociceptive neurones in the rat. *Brain Res* 1990; 506:31-9
8. Dickenson AH, Sullivan AF: Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. *Neuropharmacology* 1987; 26:1235-8
9. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1976; 17:1031-6
10. 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
11. Dixon WJ: Efficient analysis of experimental observations. *Ann Rev Pharmacol Toxicol* 1980; 20:441-62
12. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. *Pain* 1988; 32:77-88
13. Dirig DM, Salami A, Rathbun ML, Ozaki GT, Yaksh TL: Characterization of variables defining hindpaw withdrawal latency evoked by radiant thermal stimuli. *J Neurosci Methods* 1997; 76:183-91
14. Siegel S, Castellan NJ Jr: *Nonparametric Statistics for the Behavioral Sciences*. New York, McGraw-Hill, 1988, pp 174-83
15. Nozaki-Taguchi N, Yaksh TL: Characterization of the antihyperalgesic action of a novel peripheral mu-opioid receptor agonist-loperamide. *ANESTHESIOLOGY* 1999; 90:225-34
16. Kangrga I, Randic M: Outflow of endogenous aspartate and glutamate from the rat spinal dorsal horn in vitro by activation of low- and high-threshold primary afferent fibers: Modulation by mu-opioids. *Brain Res* 1991; 553:347-52
17. Malmberg AB, Yaksh TL: The effect of morphine on formalin-evoked behaviour and spinal release of excitatory amino acids and prostaglandin E2 using microdialysis in conscious rats. *Br J Pharmacol* 1995; 114:1069-75
18. Malmberg AB, Yaksh TL: Cyclooxygenase inhibition and the spinal release of prostaglandin E2 and amino acids evoked by paw formalin injection: A microdialysis study in unanesthetized rats. *J Neurosci* 1995; 15:2768-76
19. Yaksh TL, Hua XY, Kalcheva I, Nozaki-Taguchi N, Marsala M: The spinal biology in humans and animals of pain states generated by persistent small afferent input. *Proc Natl Acad Sci U S A* 1999; 96:7680-6
20. Furuyama T, Kiyama H, Sato K, Park HT, Maeno H, Takagi H, Tohyama M: Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with special reference to nociception. *Brain Res Molecular Brain Res* 1993; 18:141-51
21. Davies J, Watkins JC: Role of excitatory amino acid receptors in mono- and polysynaptic excitation in the cat spinal cord. *Exp Brain Res* 1983; 49:280-90
22. Chen L, Huang LY: Protein kinase C reduces Mg²⁺ block of NMDA-receptor channels as a mechanism of modulation. *Nature* 1992; 356:521-3
23. Ren K, Williams GM, Hylden JL, Ruda MA, Dubner R: The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan-induced behavioral hyperalgesia in rats. *Eur J Pharmacol* 1992; 219:235-43
24. Yamamoto T, Shimoyama N, Mizuguchi T: The effects of morphine, MK-801, an NMDA antagonist, and CP-96,345, an NK1 antagonist, on the hyperesthesia evoked by carrageenan injection in the rat paw. *ANESTHESIOLOGY* 1993; 78:124-33
25. Chaplan SR, Malmberg AB, Yaksh TL: Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. *J Pharmacol Exp Ther* 1997; 280:829-38
26. Zahn PK, Umali E, Brennan TJ: Intrathecal non-NMDA excitatory amino acid receptor antagonists inhibit pain behaviors in a rat model of postoperative pain. *Pain* 1998; 74:213-23
27. Pogatzki EM, Zahn PK, Brennan TJ: Effect of pretreatment with intrathecal excitatory amino acid receptor antagonists on the development of pain behavior caused by plantar incision. *ANESTHESIOLOGY* 2000; 93:489-96
28. Puig S, Sorkin LS: Formalin-evoked activity in identified primary afferent fibers: Systemic lidocaine suppresses phase-2 activity. *Pain* 1996; 64:345-55
29. Zahn PK, Brennan TJ: Incision-induced changes in receptive field properties of rat dorsal horn neurons. *ANESTHESIOLOGY* 1999; 91:772-85
30. Hunter JC, Singh L: Role of excitatory amino acid receptors in the mediation of the nociceptive response to formalin in the rat. *Neurosci Lett* 1994; 174:217-21
31. Simmons RM, Li DL, Hoo KH, Deverill M, Ornstein PL, Iyengar S: Kainate GluR5 receptor subtype mediates the nociceptive response to formalin in the rat. *Neuropharmacology* 1998; 37:25-36

32. Brorson JR, Zhang Z, Vandenberghe W: Ca(2+) permeation of AMPA receptors in cerebellar neurons expressing glu receptor 2. *J Neurosci* 1999; 19:9149-59
33. Gu JG, Albuquerque C, Lee CJ, MacDermott AB: Synaptic strengthening through activation of Ca2+-permeable AMPA receptors. *Nature* 1996; 381:793-6
34. Liu SQ, Cull-Candy SG: Synaptic activity at calcium-permeable AMPA receptors induces a switch in receptor subtype. *Nature* 2000; 405:454-8
35. Sorkin LS, Yaksh TL, Doom CM: Mechanical allodynia in rats is blocked by a Ca2+ permeable AMPA receptor antagonist. *Neuroreport* 1999; 10: 3523-6
36. Nakanishi S, Masu M: Molecular diversity and functions of glutamate receptors. *Annu Rev Biophys Biomol Struct* 1994; 23:319-48
37. Bleakman R, Schoepp DD, Ballyk B, Bufton H, Sharpe EF, Thomas K, Ornstein PL, Kamboj RK: Pharmacological discrimination of GluR5 and GluR6 kainate receptor subtypes by (3S,4aR,6R,8aR)-6-[2-(1(2)H-tetrazole-5-yl)ethyl]-decahydroisoquinoline-3-carboxylic acid. *Mol Pharmacol* 1996; 49:581-5
38. O'Neill MJ, Bond A, Ornstein PL, Ward MA, Hicks CA, Hoo K, Bleakman D, Lodge D: Decahydroisoquinolines: Novel competitive AMPA/kainate antagonists with neuroprotective effects in global cerebral ischaemia. *Neuropharmacology* 1998; 37:1211-22
39. Kerr RC, Maxwell DJ, Todd AJ: GluR1 and GluR2/3 subunits of the AMPA-type glutamate receptor are associated with particular types of neurone in laminae I-III of the spinal dorsal horn of the rat. *Eur J Neurosci* 1998; 10:324-33