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Pain Models Display Differential Sensitivity to Ca^{2+}-Permeable Non-NMDA Glutamate Receptor Antagonists

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Background: **Ca^{2+}-permeable non–*N*-methyl-D-aspartate receptors are found in the spinal dorsal horn and represent a presumptive target for glutamatergic transmission in nociceptive processing. This study characterized the analgesic profile associated with the blockade of these spinal receptors by intrathecally delivered agents known to act at these receptors, the spider venom Joro toxin (JST) and philanthotoxin.**

Methods: **Philanthotoxin (0.5, 2.5, or 5 μg) or JST (5 μg) was given spinally before thermal injury to the paw. JST (5 μg) was also given 10 min before subcutaneous formalin injection, after intraplantar administration of carrageenan, and to rats that were allodynic due to tight ligation of spinal nerves. Lower doses of JST (0.25 and 1.0 μg) were given before formalin injection and testing of thermal latencies. Thermal latencies were measured using a Hargreaves box, mechanical thresholds using von Frey hairs, and formalin response by means of counting flinches.**

Results: **Both agents blocked thermal injury–induced mechanical allodynia. JST (5 μg) given 1 h after carrageenan blocked induction of thermal hyperalgesia and mechanical allodynia. JST (5 μg) had no effect in the formalin test, on allodynia after spinal nerve ligation, or when given 3 h after carrageenan. The lowest dose (0.25 μg JST) at pretreatment intervals of 60–120 min resulted in modest hypoalgesia during phase 1 formalin and thermal testing.**

Conclusions: **The behavioral effect of intrathecal Ca^{2+}-permeable non–*N*-methyl-D-aspartate antagonists indicates an important role for this spinal receptor in regulating hyperalgesic states induced by tissue injury and inflammation and reveals an action that is distinct from those observed with other glutamate receptor antagonists.**

α-AMINO-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are composed of GluR1-GluR4 subunits.[1] Most are impermeable to Ca^{2+}; however, a subset of receptors lacking GluR2 subunits are Ca^{2+}-permeable when activated.[2,3] Ca^{2+} entry through this receptor subtype strengthens synaptic transmission.[4] In superficial spinal cord, the majority of Ca^{2+}-permeable non–*N*-methyl-D-aspartate (non-NMDA) receptors are thought to be AMPA rather than kainate receptors.[5] They frequently are found on γ-aminobutyric acid (GABA)–mediated neurons in the superficial dorsal horn[6,7] and are colocalized with neurokinin 1 receptors.[7,8] This distribution suggests that Ca^{2+}-permeable AMPA receptors are so placed to mediate glutamatergic input onto nociceptive projection cells, as well as onto inhibitory interneurons. Ca^{2+} permeability is also seen in homomeric unedited GluR5 or GluR6 kainate receptors.[9] The dorsal horn lacks GluR6 subunits but has a small number of GluR5-containing receptors.[10] Adult dorsal root ganglion has both subunits, allowing for the possibility of presynaptic as well as postsynaptic Ca^{2+}-permeable kainate receptors.[11]

In our laboratory, we have been using a model of secondary hyperalgesia generated by a first-degree burn on the heel, which results in a spinally mediated allodynia on the plantar surface of the distal paw.[12] Intrathecal treatment with non-NMDA receptor antagonists but not NMDA receptor antagonists before the burn blocks this mechanical allodynia.[13] The allodynia is also blocked by intrathecal Joro spider toxin (JST, 3 μg), a Ca^{2+}-permeable non-NMDA receptor antagonist.[14] These data imply that the net effect of intrathecal JST at this dose is the blockade of an excitatory linkage rather than or in addition to an inhibitory one. There were three aims of the current study. We wanted to determine if intrathecal pretreatment with another Ca^{2+}-permeable non-NMDA receptor antagonist, philanthotoxin-433 (PhTX), produced a similar anti-allodynia, thus strengthening the hypothesis that this is a specific receptor-mediated event. We wished to examine the effects of spinal JST in other mechanistically distinct models of nociception to see if animal models of pain that are sensitive to NMDA receptor antagonists might also be blocked by Ca^{2+}-permeable non-NMDA receptor antagonists. We also gave lower doses of JST in an attempt to observe hyperalgesia, as seen in deep dorsal horn neurons, perhaps mediated by a selective blockade at inhibitory interneurons.[15] Some of these results have been published in an abstract.[16]

Materials and Methods

Experiments were approved by the Animal Care Committee of the University of California–San Diego. Male Sprague Dawley rats (Harlan Industries, Indianapolis, IN) weighing 300–350 g were given free access to food and water. Rats were implanted with intrathecal cannulae ending over the lumbar enlargement.[17] Animals were given 5 ml lactated Ringer's solution intraperitoneally immediately after surgery and for each of the following 2 days. After surgery, rats were housed individually. Experiments were performed at least 7 days after intrathecal implantation. Only animals that displayed no post-surgical motor or sensory deficits were used. The person conducting the behavioral tests had no knowledge of the drug or dose administered.

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Experiment 1: Mechanical Allodynia after First-degree Burn

Rats were acclimated for 30 min to individual compartments (26 × 11 × 20 cm) with mesh bottoms for mechanical testing. Calibrated von Frey filaments (Stoelting, Wood Dale, IL) with buckling forces between 4 and 148 mN were applied perpendicular to the mid-paw plantar surface to determine 50% probability withdrawal thresholds.[18] After two baseline measurements, 10 μl JST (5 μg), PhTX (0.5, 2.5, or 5 μg), or sterile saline (groups of 7–11 rats) was administered to the rats, followed by a 10-μl saline flush into the intrathecal space. Five minutes after injection, the heel of the left hind paw was held on a 52.5°C surface for 45 s with use of a 10-g sand pouch to maintain constant pressure. Rats were briefly anesthetized with halothane during this procedure. Animals were returned to the test compartments; recovery from anesthesia took approximately 2 or 3 min. Mechanical thresholds were then retested at 30-min intervals for 3 h.

At all time points, rats were evaluated for behavioral reactivity after measurement of withdrawal threshold by assessment of four parameters: pinna reflex, corneal reflex, startle reflex, and righting reflex. A rat was assigned a score of 3 for brisk reflexes (PE-10 tubing was used to touch the ear and eye), orientation of movement to a cotton swab inserted through the wire mesh, and prompt righting after being turned on its back. A score of 2 was given for slightly blunted reflexes, and 1 for a delayed or minimal response. A score of 0 was given for no response.

Experiment 2: Response to Subcutaneous Formalin

Small metal bands were placed around the base of one hind paw; these encompassed about 270° of the circumference, leaving a large window on the dorsal surface. Bands weighed 0.5 g and were fixed in place with cyanoacrylate. Rats were placed in a cylindrical test chamber to become acclimated and then intrathecally injected with either 0.25 or 5 μg JST or saline in a volume of 10 μl, followed by a 10-μl saline flush. Formalin (0.5, 1.5, or 5%; 50 μl) was then injected subcutaneously into the paw dorsum. The low dose of JST (0.25 μg) is reported to be optimal for enhancing C-fiber–evoked discharge and C-fiber response after discharge, as recorded in deep dorsal horn neurons[15]; 0.25 μg JST or saline was given 60 min before the 0.5% formalin and 10 min before the 1.5% formalin. The longer pretreatment time was tried because the maximal electrophysiologic response occurs 90 min after spinal drug administration. Lower concentrations of formalin were used to avoid a potential plateau effect, so that if the C-fiber response were enhanced,[15] hyperalgesia could be observed. The higher dose of JST was given 10 min before 5% formalin to see if it reduced the formalin-induced hyperalgesia, similar to its effect on burn-induced allodynia.[14] After formalin injection, rats were returned to the chamber and flinches were counted for 60 min with an automated device.[19] Briefly, the test chamber was placed above a loop antenna that generated a low-wattage electromagnetic wave. Movement of the metal band on the rat's paw (*e.g.*, flinching) alters the electromagnetic field. The resulting signal is fed to a computer that uses the response amplitude and duration to separate flinches from normal locomotor activity. Correlation between the results obtained by this system and those of an experienced observer was $r^2 = 0.94$.[19]

Experiment 3: Mechanical Allodynia in Rats with Tight Spinal Nerve Ligation

Rats were surgically prepared with tight ligation of the L4 and L5 spinal nerves.[20] After 14 days of recovery they were acclimated to the testing device, and baseline 50% probability withdrawal thresholds were determined as in experiment 1. Rats were then intrathecally injected with either 5 μg JST or saline in 10 μl, followed by a 10-μl saline flush. Animals were returned to their test compartments and mechanical thresholds were tested at 15-min intervals for 1 h and at 30-min intervals for a second hour.

Experiment 4: Thermal Hyperalgesia and Mechanical Allodynia after Intraplantar Carrageenan

Rats were acclimated to the mechanical testing apparatus, and measurements of the withdrawal threshold were taken. They were then transferred to a modified Hargreaves apparatus with a heated glass plate maintained at 25°C.[21] A focused projection bulb below the plate was aimed at the mid-plantar surface of the paw. A photodiode-activated timer measured the withdrawal latency, and a cutoff time of 20 s was used to prevent tissue damage. After a 30-min acclimation period, basal thermal withdrawal latency was measured twice on each hind paw; tests were a minimum of 5 min apart and the results were averaged. Rats were then lightly anesthetized with halothane and injected with lambda carrageenan (2 mg in 100 μl sterile saline Sigma, St Louis, MO) in the left hind paw and returned to their home cages, with food and water available. One hour after intraplantar injection, 5 μg JST in 10 μl of sterile saline was administered intrathecally, followed by a 10-μl saline flush (N = 7) or 20 μl of sterile saline (N = 10) to see if the JST would block induction of hyperalgesia in this model. Three hours after administration of carrageenan, animals were returned to the thermal testing apparatus for a 30-min acclimation period, and withdrawal latencies were measured twice on each hind paw. Rats were moved to the mechanical testing chambers and left for 30 min to acclimate, and withdrawal thresholds were measured. Rats were then killed with an anesthetic overdose, and paw thickness was measured with spring-loaded calipers. In a variation on this experiment, the

intrathecal injection was 3 h after administration of carrageenan. Thermal latencies and mechanical withdrawal thresholds were then measured at 3.5 and 4.0 h after administration of carrageenan.

Experiment 5: Low-dose JST on Acute Pain Threshold

Rats were acclimated to individual compartments in the thermal testing device, as in experiment 4. After a 30-min acclimation period, basal thermal withdrawal latency was measured 4 times on each foot, at 15-min intervals. The average of the last three measurements was called baseline. Rats were then given JST (0.25 or 1.0 μg) or saline through the intrathecal catheter, and thermal thresholds were tested at 30-min intervals for the next 2 h.

Drugs

Philanthotoxin 433 (molecular weight, 777.69; RBI, Natick, MA) and JST (molecular weight, 565.71; RBI or Calbiochem, La Jolla, CA) were aliquoted and kept at −70°C. Shortly before use, drugs were diluted in sterile saline to the desired concentration. PhTX is a synthetic analog of a toxin isolated from the venom of the digger wasp.

Statistical Analysis

Since the 50% probability mechanical-threshold data from the burn and carrageenan models is not normally distributed because of the frequent use of the cutoff, these data were examined with use of nonparametric statistics. Data are presented as box plots, with medians and quartiles indicated. The Friedman test for repeated measures was used to assess significance in the burn model. Before-and-after comparisons between data for the same animals in the carrageenan model were made with the Wilcoxon test. Comparisons between mechanical thresholds for different groups were made with the Mann–Whitney test. Mechanical threshold (50% probability) of animals with spinal nerve ligations are presented as mean ± SEM for convenience; however, because of the noncontinuous nature of the stimuli, the Friedman test for repeated measures was used to assess change from the basal allodynia. Thermal latency data and formalin-induced flinching are presented as mean ± SEM. Significant differences from baseline for thermal latency data were calculated by means of analysis of variance (ANOVA) for repeated measures, followed by *post hoc* testing with use of a Bonferroni correction. Before-and-after comparisons of thermal latencies for the same group were made with a paired *t* test. Postinflammation differences between different treatment groups were determined with a non-paired *t* test. In all cases, $P \leq 0.05$ was considered to be significant.

Results

Experiment 1

Rats treated with saline before a first-degree burn (N = 11) displayed mechanical allodynia to von Frey hairs

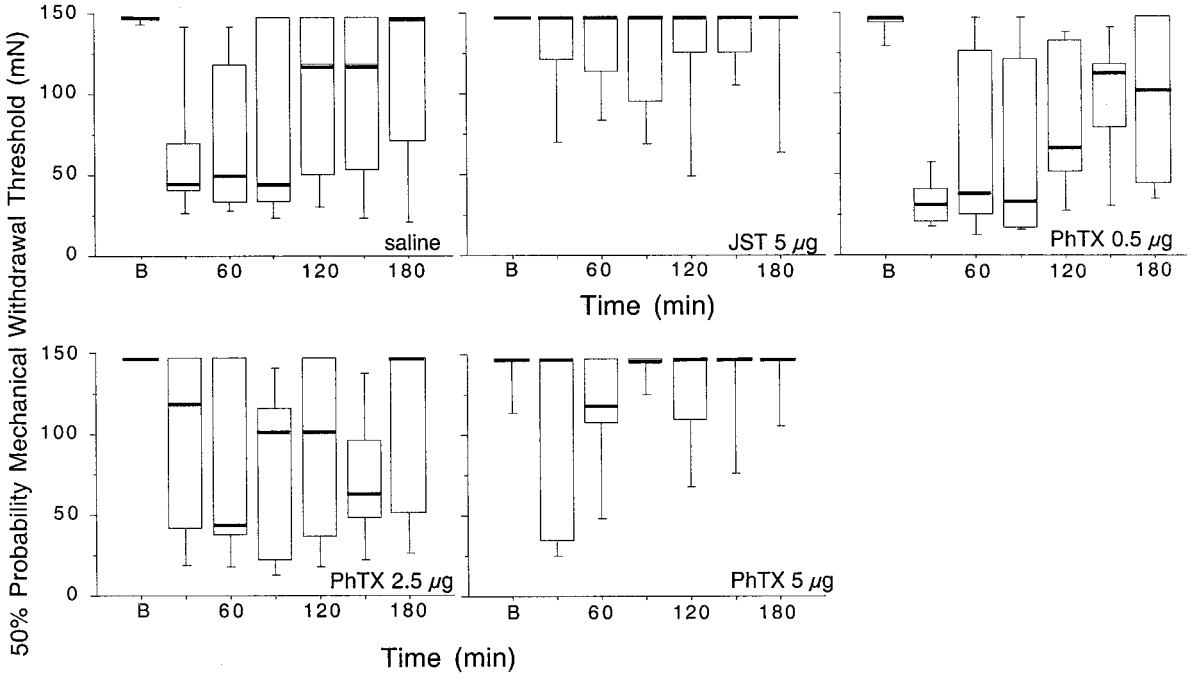

Fig. 1. Plots show mean (heavy line) and quartiles of 50% probability withdrawal threshold to mechanical stimulation. Error bars indicate 10th and 90th percentiles. After determination of baseline response, B, rats were given intrathecal injections of the indicated substance (JST = Joro spider toxin; PhTX = philanthotoxin-433) 5 min before thermal injury. The x axis refers to time after the thermal injury.

Table 1. Sedation/Motor Dysfunction Index

Drug	Test	Time Points (min)			
		Baseline	30	90	150
5 μg JST	Pinna	3	3	3	3
	Cornea	3	3	3	3
	Startle	3	2.5 ± 0.3	2.8 ± 0.3	2.0 ± 0.5
	Righting	3	3	2.8 ± 0.3	2.8 ± 0.3
5 μg PhTX	Pinna	3	3	3	3
	Cornea	3	3	3	3
	Startle	3	2.7 ± 0.2	2.9 ± 1.4	3
	Righting	2.8 ± 0.3	2.6 ± 0.3	2.3 ± 0.4	2.3 ± 0.4
2.5 μg PhTX	Pinna	3	3	3	3
	Cornea	3	3	3	3
	Startle	3	3	3	3
	Righting	3	3	3	3
Saline	Pinna	3	3	3	3
	Cornea	3	3	3	3
	Startle	3	3	2.6 ± 0.4	2.3 ± 0.5
	Righting	3	3	2.8 ± 0.2	2.6 ± 0.3

Four subjective measures of sedation and motor dysfunction were taken just after mechanical threshold testing under the various drug conditions. Means ± SEM are given. In many cases, all animals achieved the maximum score of 3.

JST = Joro spider toxin; PhTX = philanthoxin-433.

applied to the distal paw (fig. 1; $P \leq 0.02$). The median threshold response was 148 mN during the baseline testing. During the first hour after burn, median threshold fell to less than 45 mN. Rats given 0.5 μg PhTX (N = 7) also displayed a marked allodynia compared with baseline, with a median response of less than 40 mN for the first 90 min ($P \leq 0.003$), and their responses were no different than those of saline-treated animals. Increasing the dose of PhTX to 2.5 μg (N = 7) resulted in a blunting of the burn-induced allodynia ($P \leq 0.08$, in comparison with baseline), and the median response fell to less than 50 mN for the 60-min time point. When the intrathecal dose of PhTX was 5 μg (N = 9), there was no allodynia ($P \leq 0.3$) and the median response was never less than 100 mN, indicating a complete blockade of the burn-induced allodynia.

After administration of 5 μg PhTX, the pinna and corneal reflexes were totally intact, and scores for both the saline- and PhTX-treated animals were uniformly 3 at each time point. Saline-treated animals all received scores of 3 for these reflexes as well as for orienting toward the cotton swab (startle) and righting. The high dose of PhTX was followed by modest decreases in the orienting (30 min only) and righting responses, which did not reach statistical significance (table 1).

Rats pretreated with 5 μg JST (N = 7) displayed no sensitization to application of von Frey hairs (fig. 1; $P \leq 0.43$). *Post hoc* testing indicated a significant difference from rats given saline at all time points between 60 and 180 min, inclusive. Like the PhTX, 5 μg JST had no effect on the pinna or corneal reflexes. The righting reflex was scored as 2 for one animal at the 90-min time point, but it was unaffected for the rest of the animals. Orienting to the cotton swab (startle) was also no different after the highest dose of JST than it was after saline dosing (table 1).

Experiment 2

Rats pretreated with 5 μg JST in the intrathecal space before subcutaneous 5% formalin injection displayed behavior identical to that of rats given saline before the formalin injection (fig. 2A). Both groups had a prominent phase 1 response, evident through the first 5 min. There was then a quiescent period lasting for 15 min, followed by phase 2 behavior. The timing and magnitude of phase 1 and phase 2 were equivalent. Rats given 0.25 μg JST (N = 8) 10 min before 1.5% formalin were also no different than saline-treated animals (N = 8), with an equally prominent phase 1 and phase 2 (data not shown). However, when low-dose (0.25 μg) JST was given 60 min before 0.5% formalin, there was a clear difference between the groups. Saline-treated animals (N = 8) had a reduced phase 1 response in comparison with the response of those injected with 1.5 or 5% formalin. Pretreatment with 0.25 μg JST (N = 8) resulted in a further reduced phase 1 (fig. 2B), which was significantly smaller than in the saline-treated animals ($P \leq 0.02$). Neither group of animals had an appreciable phase 2 response.

Experiment 3

Rats with tight ligations of spinal nerves L5 and L6 were allodynic. Baseline 50% probability withdrawal thresholds were between 5 and 25 mN. Intrathecal administration of saline (N = 5) or 5 μg of JST (N = 5) produced no change in withdrawal behavior (fig. 3). Variability in the mean response of JST-treated rats was due to the transient behavior of one animal.

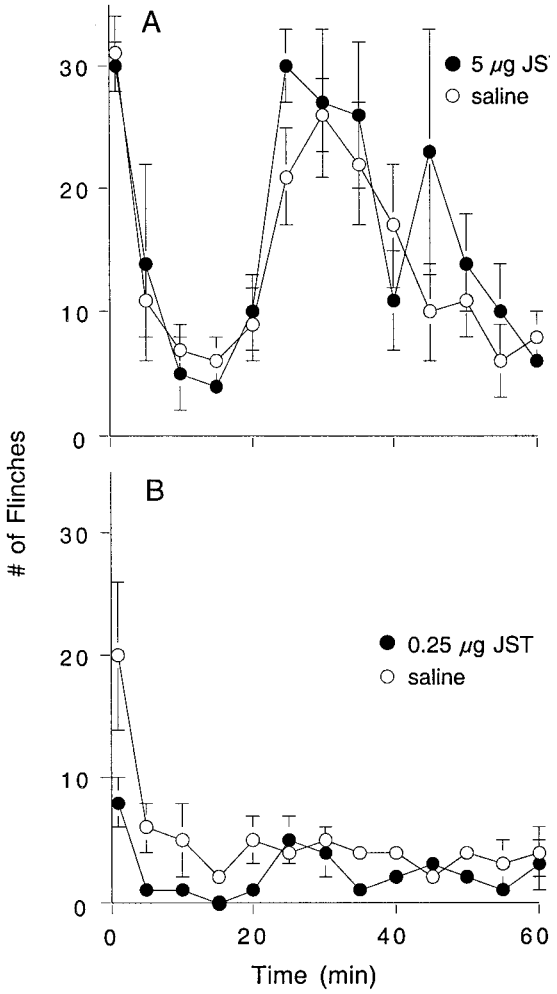

Fig. 2. These graphs show the number of formalin-induced flinches (mean ± SEM) over 60 min. (*A*) The response to 5% formalin injected 10 min after intrathecal saline (N = 5) or 5 μg JST (Joro spider toxin) (N = 5); responses are not different. (*B*) Responses to 0.5% formalin injected 60 min after saline (N = 8) or 0.25 μg JST (N = 8). Phase 1 was smaller in the JST-treated group.

Experiment 4

Before carrageenan injection, thermal latency of the left hind paw was 11.2 s (N = 17) for the first set of animals. There was no basal difference between rats given saline and those given JST. Rats administered intrathecal saline 1 h after carrageenan exhibited increased sensitivity to the stimulus, and their latencies fell to 5.9 ± 1.3 s ($P \leq 0.005$; paired *t* test) 3 h after intraplantar injection. In marked contrast, animals given 5 μg JST 1 h after carrageenan did not develop thermal hyperalgesia (fig. 4A; $P \leq 0.84$). Two and one half hours after the intrathecal injection (3.5 h after administration of carrageenan), thermal latency of the JST-treated group was 11.1 ± 2.0 s ($P \leq 0.84$; paired *t* test). The post-carrageenan difference between saline- and JST-treated groups was also significant ($P \leq 0.04$; unpaired *t* test). Contralateral paw latencies did not change over the course of the experiment for either group (data not shown).

Basal 50% probability mechanical withdrawal thresholds for the left paw also did not differ between the two groups; the median threshold was 147 mN for each. The median threshold of the saline-treated animals fell to 83 mN ($P \leq 0.02$; Wilcoxon signed rank test) 3.5 h after carrageenan injection (fig. 4B). This decrease was blocked by the intrathecal JST ($P \leq 0.18$). Withdrawal thresholds of the noninjected paw were unchanged after carrageenan injection (data not shown).

Paw thickness measured at the end of the experiment did not differ between the two groups. Injected paws were uniformly swollen. Those of the saline-treated animals were 8.7 ± 0.6 mm, and those of the JST-treated animals were 8.1 ± 0.3 mm ($P \leq 0.39$; unpaired *t* test).

When JST was administered 3 h after carrageenan to an already-inflamed paw, it affected neither thermal nor mechanical sensitization (fig. 5). Mean basal thermal latency was 11.6 ± 0.8 s (N = 17), with no difference between the treatment groups. Thirty minutes after late administration or saline (N = 9) or JST (N = 8), carrageenan inflammation elicited a drop in latency to 5.0 ± 0.8 s and 3.8 ± 0.7 s, respectively ($P \leq 0.0001$ in comparison with basal). There was no post-carrageenan difference between the groups ($P \leq 0.27$). Latencies for the contralateral paw did not change over the course of the experiment for either group (data not shown). The 50% probability mechanical-withdrawal threshold also fell for both groups post-carrageenan. Median mechani-

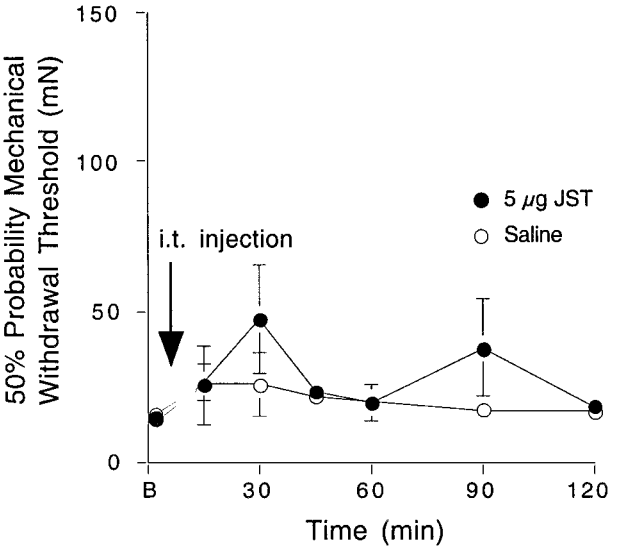

Fig. 3. This graph shows the mean ± SEM 50% probability withdrawal threshold to mechanical stimulation. Rats had tight ligations of spinal nerves L5 and L6 7 days before the experiment and were severely allodynic before any further treatment. Saline or 5 μg Joro spider toxin (JST) was given at the time indicated by the arrow. i.t. = intrathecal.

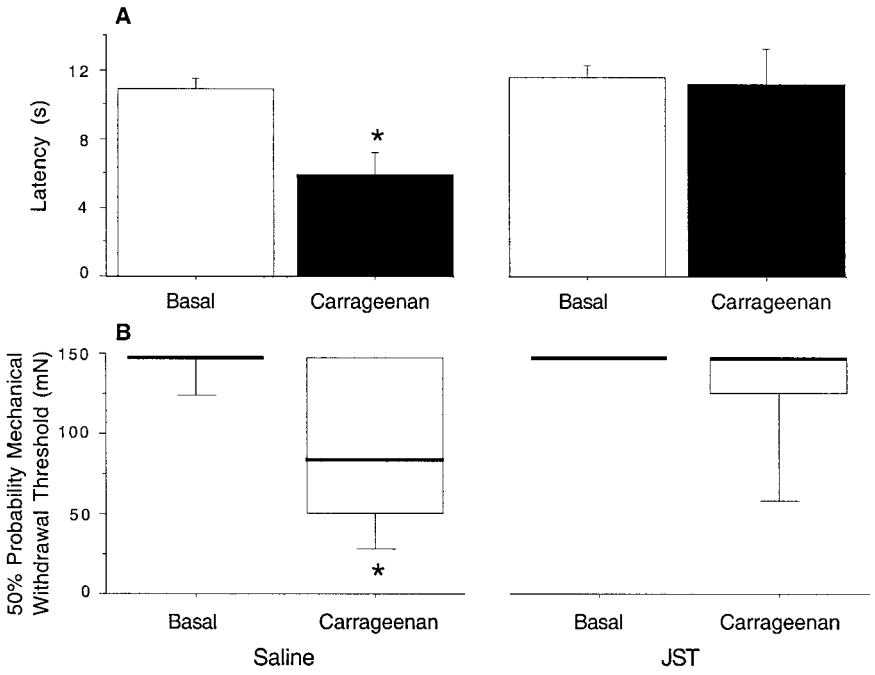

Fig. 4. Responses to (*A*) thermal and (*B*) mechanical stimulation. Latencies are shown as mean ± SEM and mechanical thresholds as medians, quartiles, and 10th and 90th percentiles. Basal responses were measured before (open bars) and carrageenan responses 3.5 and 4.0 h after (dark bars) intraplantar injection of the irritant. The intrathecal injection (saline or 5 μg Joro spider toxin [JST]) was given 30 min after carrageenan (*statistical difference [$P \leq 0.05$] between the two treatment groups).

cal threshold of saline-treated animals fell from 147 mN to 43.8 mN ($P \leq 0.03$). JST-treated animals were also allodynic post-carrageenan; median thresholds fell from 147 mN to 70.2 mN ($P \leq 0.02$). Withdrawal thresholds of the noninjected paw were unchanged (data not shown). Again, there was no difference in the size of the inflamed paw between the two groups.

Experiment 5

Basal thermal withdrawal latencies were 11.3 ± 0.5, 9.6 ± 0.5, and 11.8 ± 0.3 s for the animals treated with saline, 0.25 μg JST, and 1.0 μg JST, respectively. The 0.25-μg JST group (N = 6) had slightly faster latencies than the other two groups ($P \leq 0.04$). After intrathecal administration of saline (N = 9) and the 1.0 μg JST pretreatments (N = 6), latencies were unchanged (fig. 6). An ANOVA for repeated measures revealed a change in latency for the 0.25 μg JST–treated animals ($P \leq 0.001$). *Post hoc* testing showed a significant increase in latency (hypoalgesia) in comparison with saline-treated animals at 90 min ($P \leq 0.02$) and 120 min ($P \leq 0.02$) after drug administration.

Discussion

These results confirm that secondary mechanical allodynia occurring in the area around a first-degree burn is sensitive to spinal administration of JST, an antagonist for Ca^{2+}-permeable AMPA and kainate receptors. Earlier findings[14] have been extended to include a dose-dependent sensitivity of the allodynia to a second receptor-specific antagonist, PhTX, consistent with the presumed receptor-specificity of the effect. Both toxins block mechanical allodynia in the burn model at doses that produce minimal side effects. In spinal cord, JST and PhTX block the responses of individual neurons to AMPA, quisqualate, and kainate while producing small to negligible antagonism of NMDA-evoked responses.[22] Although some studies attribute these arthropod toxins with the ability to inhibit NMDA-evoked responses,[23,24] this does not seem to be the case in either the dorsal or ventral horn of the spinal cord.[22] The lack of effect of JST pretreatment on phase 2 of the formalin test supports this, because pretreatment with NMDA receptor antagonists is known to block this response.[25–27] In addition, the burn-induced allodynia is reported to be resistant to spinal NMDA receptor antagonists.[13] Not all non-NMDA receptors are uniformly inhibited by these toxins; JST and PhTX appear to be specific inhibitors of Ca^{2+}-permeable but not Ca^{2+}-impermeant non-NMDA receptors in a number of systems.[4,28–30]

One problem with the hypothesis that selective spinal blockade of Ca^{2+}-permeable non-NMDA receptors inhibits some forms of spinal sensitization and allodynia–hyperalgesia is the fact that the largest percentage of these receptors in the superficial dorsal horn are found on GABA and glycine-containing neurons. It is counterintuitive to imagine that blockade of inhibitory interneurons (disinhibition) leads to a reduction or loss of sensitization. Stanfa *et al.*[15] have recently shown that at low doses, spinal administration of JST (0.25–1.0 μg) results in an increase in dorsal horn responses to C-fiber input as well as to the late C-fiber-induced response associated with wind-up. The maximum hyperexcitability was ob-

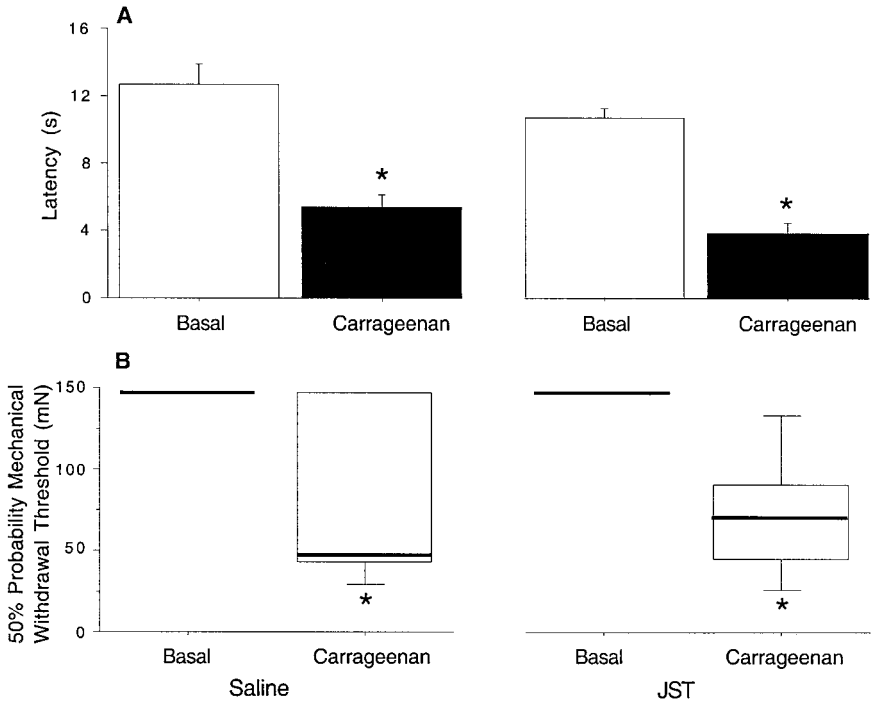

Fig. 5. Responses to (*A*) thermal and (*B*) mechanical stimulation, as in figure 4. Rats were given intrathecal saline or 5 μg Joro spider toxin (JST) 3.0 h after carrageenan injection (*statistical difference [$P \leq 0.05$] between the two treatment groups).

served 90 min after JST was applied. No change was observed for either Aδ-fiber–evoked or Aβ-fiber–evoked responses. As activity initiated by both Aδ or Aβ fibers is enhanced by nonselective blockade of inhibitory amino acid receptors, Stanfa and colleagues hypothesize that blockade of Ca^{2+}-permeable non-NMDA receptors must effect a selective subset of the inhibitory interneurons, resulting in a complex alteration in dorsal horn circuitry. Within this specified low-dose range at times from 15–120 min after JST administration, we have observed no hyperalgesic effect on acute thermal latency, on magnitude of phase 1 or phase 2 of the formalin test, or on allodynia in the burn model. There was, however, an association between 0.25 μg JST and a modest hypoalgesic effect on thermal latency and phase 1 of the formalin test. This was apparent only at 60–120 min after JST treatment. Given that both acute thermal stimulation and the phase 1 formalin response are believed to be driven to a great extent by C-fiber activation, the hypoalgesia was unexpected.

At higher doses of JST (2.5 and 10.0 μg), Stanfa *et al.*[15] reported that facilitation of C-fiber responses was reduced and that half of the nociceptive neurons sampled were inhibited. These data are consistent with the JST-induced blockade of punctate and thermal hyperalgesia seen in the burn and early treatment carrageenan models. Together these data indicate that at higher doses of JST, a second set of Ca^{2+}-permeable non-NMDA receptors that transmit nociceptive information become antagonized. These could be neurokinin-1 receptor–bearing cells in lamina I[8] or as yet unidentified cells in the deep dorsal horn. Because at least some of these cells are downstream of the inhibitory interneurons, the net effect of combined inhibitory and excitatory neuronal inhibition is a blockade of nociception. If the electrophysiologic experiments were conducted primarily on deep dorsal horn neurons, as is likely, it is possible that at lower doses the JST penetrated to the inhibitory interneurons to cause disinhibition but not to the recorded cells themselves. This would result in the observed cellular excitation. However, antinociceptive behavior could result after a reduction in firing of the neurokinin 1 receptor–bearing cells in lamina 1,[31] a hypothesis consistent with our observations.

The total lack of effect produced by JST treatment in the formalin, tight-spinal-ligation, and carrageenan-main-

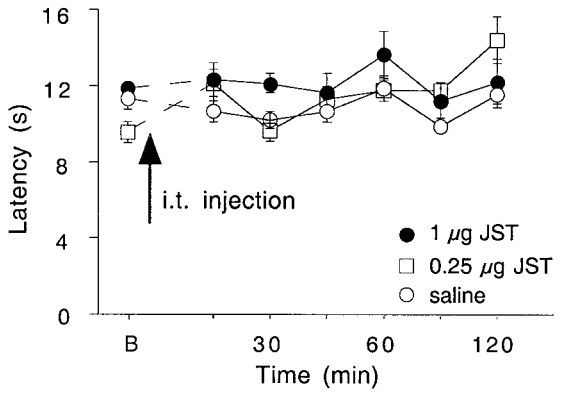

Fig. 6. Saline, 0.25 μg Joro spider toxin (JST), or 1.0 μg JST were injected intrathecally (i.t.) and acute thermal thresholds were measured for the rats for the next 2 h. Animals given only the low-dose JST displayed a slight hypoalgesia at the 90- and 120-min test points.

tenance models supports the idea that Ca^{2+}-permeable non-NMDA receptors play a complex role in dorsal horn circuitry. Either they are not involved in nociception in these models or their inhibitory and excitatory effects roughly balance out and cannot be seen with our behavioral techniques. The clear effect of JST treatment in the first-degree-burn model, after carrageenan administration during induction of the inflammation-related hyperalgesia, and in the post-incisional pain model[32] demonstrates that these receptors are relevant in a variety of hyperalgesic states.

There are reports of NMDA-independent components of long-term potentiation blocked by JST,[33] indicating that in some long-term potentiation models, NMDA and non-NMDA receptors work in parallel. Some sequelae to nociceptive stimulation, including c-Fos expression and phosphorylation of extracellular signal-related kinases, are only partially reversed by NMDA receptor antagonists.[34] It has been postulated that other systems, including perhaps a Ca^{2+}-permeable non-NMDA receptor–mediated pathway, work in parallel to NMDA receptor activation in the generation of spinal sensitization.[34]

Joro spider toxin might work at receptors other than Ca^{2+}-permeable non-NMDA receptors; two potential alternative sites of action are NMDA receptors and voltage-gated calcium channels. It is possible that at some doses or in different tissue types this is the case. However, as mentioned above, 5 μg JST is highly effective in blocking the punctate allodynia resulting from the burn model and has no effect on phase 2 of the formalin test. Thus, because phase 2 formalin responses are reduced by pretreatment with either NMDA receptor antagonists or N-type calcium channel blockers[35] and because allodynia resulting from tight ligation of the spinal nerves is also acutely sensitive to voltage-gated calcium channel blockade,[36] these data strongly imply that 5 μg JST did not appreciably block NMDA receptors or voltage-gated Ca^{2+} channels in spinal tissue. If it did block calcium entry through either of these routes, it would likely reduce the phase 2 formalin response and reverse allodynia in the spinal nerve ligation model, and it clearly does not.

In summary, two Ca^{2+}-permeable AMPA receptor antagonists, JST and PhTX, at spinal doses of 3–5 μg, block the secondary mechanical allodynia surrounding a first-degree burn. JST at the same dose also blocks induction of carrageenan-induced hyperalgesia but has no effect on its maintenance or on phase 1 or 2 of the formalin test or allodynia caused by spinal ligation. At lower doses, we saw no behavioral manifestation of the excitation that has been recorded in wide-dynamic-range cells of the spinal cord. In contrast, we observed hypoalgesia at low doses with long pretreatment times. This points to a role of the Ca^{2+}-permeable AMPA receptor in mediating spinal sensitization in selected pain models.

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