Increased Nociceptive Input Rapidly Modulates Spinal GABAergic Transmission Through Endogenously Released Glutamate

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Stimulation of nociceptive primary afferents elicits pain by promoting glutamatergic transmission in the spinal cord. The lamina II of the spinal cord is a critical site for the relay and processing of nociceptive information from primary afferents to second-order neurons in the spinal dorsal horn. Although stimulation of primary afferents potentiates glutamatergic transmission in second-order and higher-order neurons in the spinal dorsal horn, little is known whether, and how, increased nociceptive input affects spinal GABAergic tone in the spinal dorsal horn. The aim of this study was to determine whether, and how, stimulation of nociceptive primary afferents rapidly modulates GABAergic synaptic transmission in the spinal cord. We found that glutamate spillover after primary afferent stimulation produces a heterosynaptic action to affect GABAergic transmission. Reduction of GABAergic tone can occur rapidly (within minutes) after stimulation of primary afferents, which could facilitate transmission of nociceptive information in the spinal cord. Through group II and group III metabotropic glutamate

tic ionotropic glutamate receptors (iGluRs) in the spinal cord can enhance the excitability of dorsal horn neurons (central sensitization) (Dougherty et al. 1992; Leem et al. 1996). However, the cellular mechanisms for the hypersensitivity of dorsal horn neurons after tissue and nerve injury are not fully known.

The lamina II of the spinal cord is a critical site for the relay and processing of dynamic modulation of sensory information. Yet the detailed circuitry and synaptic control of lamina II neurons involved in the integration and regulation of nociceptive input are largely unclear. Both excitatory and inhibitory interneurons in the lamina II can modulate the input of primary afferents through presynaptic and postsynaptic mechanisms (Lu and Perl 2003; Pan and Pan 2004; Yoshimura and Nishi 1995). Glutamate is an important excitatory neurotransmitter and iGluRs are essential for nociceptive transmission and sensitization of dorsal horn neurons in the spinal cord (Dougherty et al. 1992; Leem et al. 1996; Lu and Perl 2003; Pan and Pan 2004; Yoshimura and Nishi 1995). By contrast, γ-aminobutyric acid (GABA) is the dominant inhibitory neurotransmitter in the spinal cord and spinal nociceptive transmission and dorsal horn neurons in different laminae are under tonic inhibitory control mediated largely by GABA (Light and Kavookjian 1988; Pan and Pan 2004; Yoshimura and Nishi 1995). In this regard, blockade of GABA_\text{A} receptors results in hypersensitivity of dorsal horn neurons and allodynia (Sivilotti and Woolf 1994; Sorkin et al. 1998). Reduced GABAergic input to lamina II neurons is considered an important contributor to chronic neuropathic pain (Moore et al. 2002). The primary afferent terminals and the glutamatergic and GABAergic interneurons are closely intermingled in the superficial dorsal horn. Although stimulation of primary afferents potentiates glutamatergic transmission in second-order and higher-order neurons in the spinal dorsal horn, little is known whether, and how, increased nociceptive input affects local inhibitory GABAergic input to dorsal horn neurons.

The aim of this study was to determine whether, and how, stimulation of nociceptive primary afferents rapidly modulates GABAergic synaptic transmission in the spinal cord. We found that glutamate spillover after primary afferent stimulation produces a heterosynaptic action to affect GABAergic transmission. Reduction of GABAergic tone can occur rapidly (within minutes) after stimulation of primary afferents, which could facilitate transmission of nociceptive information in the spinal cord. Through group II and group III metabotropic glutamate

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receptors (mGluRs) on GABAergic interneurons, modulation of spinal GABAergic tone is directly linked to increased glutamatergic input from nociceptive primary afferents. This new information is important for our understanding of the integrative mechanisms underlying the regulation of nociceptive inflow by spinal dorsal horn neurons.

M E T H O D S

Animals

Male Sprague–Dawley rats (3–4 wk old; Harlan, Indianapolis, IN) were used in this study. All the surgical preparations and experimental protocols were approved by the Animal Care and Use Committee of the University of Texas M. D. Anderson Cancer Center. Some rats were treated with intrathecal pertussis toxin (PTX) to inactivate inhibitory $G_{i/o}$ proteins (Chen and Pan 2004; Zhang et al. 2005). Intrathecal catheters were inserted in rats anesthetized using 2–3% isoflurane. The catheters (polyethylene-10 tubing) were inserted through an incision in the cisternal membrane and advanced 4.5 cm caudal so that the tip of each catheter was positioned at the lumbar spinal level. Rats were injected intrathecally with 1 μg of PTX 5–7 days before the final electrophysiology experiments.

Spinal cord slice preparation

Under isoflurane anesthesia, the lumbar segment of the spinal cord was removed through laminectomy at the L2–L5 level. The segment of the spinal cord was immediately placed in an ice-cold sucrose artificial cerebrospinal fluid (aCSF) presaturated with 95% O$_2$-5% CO$_2$. The sucrose aCSF contained (in mM) 234 sucrose, 3.6 KCl, 1.2 MgCl$_2$, 2.5 CaCl$_2$, 12 NaH$_2$PO$_4$, 12.0 glucose, and 25.0 NaHCO$_3$. The tissue was then placed in a shallow groove formed in a gelatin block and glued onto the stage of a vibratome (Technical Product International, St. Louis, MO). Transverse spinal cord slices (400 μm) were cut in the ice-cold sucrose aCSF and incubated in Krebs’ solution oxygenated with 95% O$_2$-5% CO$_2$ at 34°C for 1–2 h before they were transferred to the recording chamber. The Krebs’ solution contained (in mM) 117.0 NaCl, 3.6 KCl, 1.2 MgCl$_2$, 2.5 CaCl$_2$, 1.2 NaH$_2$PO$_4$, 11.0 glucose, and 25.0 NaHCO$_3$.

Electrophysiological recordings

Recordings of postsynaptic currents were performed using the whole cell voltage-clamp method, as previously described (Li et al. 2002; Pan and Pan 2004; Zhang et al. 2005). The slice was placed in a glass-bottom chamber (Warner Instrument, Hamden, CT) and fixed with parallel nylon threads supported by a U-shape stainless steel weight. The slice was continuously perfused with Krebs’ solution at 5.0 ml/min at 34°C maintained by an inline solution heater and a temperature controller (TC-324; Warner Instrument). The lamina II was identified as a distinct translucent band across the superficial lamina II neurons. TRPV1 is located predominantly on primary afferents, we tested the effect of capsaicin, a known agonist for TRPV1 channels, on GABAergic sIPSCs of lamina II neurons. TRPV1 is located predominantly on primary afferents (Pan and Pan 2004; Yang et al. 1998). Bath application of 2 μM capsaicin, a nonselective TRPV1 agonist for TRPV1 channels, on GABAergic sIPSCs of lamina II neurons. TRPV1 is located predominantly on primary afferents and terminals in the superficial laminae of the spinal cord (Chen and Pan 2006; Guo et al. 2001; Pan et al. 2003). We used capsaicin to stimulate primary afferents (Pan and Pan 2004; Yang et al. 1998). Bath application of 2 μM capsaicin, a nonselective TRPV1 agonist for TRPV1 channels, on GABAergic sIPSCs of lamina II neurons. TRPV1 is located predominantly on primary afferents and terminals in the superficial laminae of the spinal cord (Chen and Pan 2006; Guo et al. 2001; Pan et al. 2003). We used capsaicin to stimulate primary afferents (Pan and Pan 2004; Yang et al. 1998). Bath application of 2 μM capsaicin, a nonselective TRPV1 agonist for TRPV1 channels, on GABAergic sIPSCs of lamina II neurons. TRPV1 is located predominantly on primary afferents and terminals in the superficial laminae of the spinal cord (Chen and Pan 2006; Guo et al. 2001; Pan et al. 2003). We used capsaicin to stimulate primary afferents (Pan and Pan 2004; Yang et al. 1998). Bath application of 2 μM capsaicin, a nonselective TRPV1 agonist for TRPV1 channels, on GABAergic sIPSCs of lamina II neurons. TRPV1 is located predominantly on primary afferents and terminals in the superficial laminae of the spinal cord (Chen and Pan 2006; Guo et al. 2001; Pan et al. 2003). We used capsaicin to stimulate primary afferents (Pan and Pan 2004; Yang et al. 1998). Bath application of 2 μM capsai
capsaicin for 3 min significantly decreased both the frequency and amplitude of GABAergic sIPSCs in 13 of 26 (50%) neurons recorded (Fig. 1). These 13 neurons consisted of nine inner and four outer lamina II neurons. The cumulative probability analysis of sIPSCs revealed that the distribution pattern of the interevent interval but not the amplitude of sIPSCs was shifted toward the right in response to capsaicin (Fig. 1A). The mean sIPSC frequency and amplitude were reduced 53.0 ± 5.2% (n = 13) and 16.4 ± 3.7% (n = 13), respectively, by capsaicin. In contrast, in nine of the 26 neurons (34.6%), capsaicin significantly increased the frequency but not the amplitude of sIPSCs (Fig. 1, B and C). The latency of both the inhibitory and stimulatory effects of capsaicin was about 1.5 min and these effects lasted for ≥6 min after capsaicin application was discontinued (Fig. 1). In the remaining four (15.4%) neurons, capsaicin had no significant effect on the frequency and amplitude of sIPSCs (Fig. 1). We did not observe the biphasic effect of capsaicin on sIPSCs. Bath application of 10 μM bicuculline, a GABA_A-receptor blocker, abolished sIPSCs of all lamina II neurons tested (Fig. 1). These results suggest that activation of nociceptive primary afferents inhibits GABAergic input to a population of dorsal horn neurons.

**Effect of capsaicin on postsynaptic GABA_A currents of lamina II neurons**

To determine whether changes in the postsynaptic GABA_A receptors account for the inhibitory effect of capsaicin on sIPSCs, we recorded the GABA_A current elicited by puff application of GABA in lamina II neurons before and after bath application of 2 μM capsaicin for 3–10 min. In all 10 neurons tested, the current elicited by puff application of 1 mM GABA was not significantly altered by capsaicin. The mean amplitude of the current elicited by GABA was 1,169.0 ± 289.7 and 1,142.2 ± 323.5 pA (P > 0.05) before and after capsaicin, respectively (Fig. 2). These data suggest that stimulation of nociceptive primary afferents acutely modulates GABAergic transmission through a presynaptic mechanism.

**Role of G_{i/o} proteins in the inhibitory effect of capsaicin on synaptic GABA release**

To determine whether the G_{i/o}-protein–coupled receptors are involved in the inhibitory effect of capsaicin on synaptic GABA release, a group of rats was pretreated with intrathecal PTX to inactivate inhibitory G_{i/o} proteins (Chen and Pan 2004;...
In none of the lamina II neurons from PTX-treated rats did capsaicin cause an inhibition of sIPSCs. In most (23 of 25, 92%) neurons tested, 2 mM capsaicin significantly increased the frequency but not the amplitude of GABAergic sIPSCs and shifted the distribution pattern of the interevent interval of sIPSCs to the left (Fig. 3). In the remaining two (8%) neurons, capsaicin had no evident effect on the frequency and amplitude of GABAergic sIPSCs. These data suggest that the G_\text{i/o}-coupled receptors on GABAergic interneurons are involved in the inhibitory effect of capsaicin on GABAergic transmission in the spinal cord.

Role of ionotropic glutamate receptors in the effect of capsaicin on synaptic GABA release

Stimulation of primary afferents evokes glutamate release, which activates ionotropic glutamate receptors (iGluRs) on the postsynaptic dorsal horn neurons in the spinal cord (Pan and Pan 2004; Wang et al. 2005; Yoshimura and Nishi 1995). We next examined whether endogenous glutamate release plays a role in the effect of capsaicin on GABAergic transmission in the spinal cord. In PTX-treated rats, in the presence of both 20 mM CNQX [a non-N-methyl-D-aspartate (NMDA) receptor antagonist] and 20 mM MK-801 (an NMDA receptor antagonist), bath application of 2 mM capsaicin failed to inhibit GABAergic sIPSCs in all cells recorded (n = 21, Fig. 4A). Capsaicin did not significantly alter the frequency of sIPSCs in 17 of 21 (81%) cells in the presence of CNQX and MK-801. In the remaining four cells (19%, Fig. 4A), capsaicin still signifi-

![FIG. 2. Lack of effect of capsaicin on the postsynaptic \( \gamma \)-aminobutyric acid type A (GABA\text sub A) current of lamina II neurons. A: original tracings of the current elicited by puff application of 1 mM GABA in a lamina II neuron before and after bath application of 2 \( \mu \)M capsaicin. B: summary data showing that capsaicin had no significant effect on the current elicited by GABA in 10 lamina II neurons.](http://jn.physiology.org/)

![FIG. 3. Effect of capsaicin on GABAergic sIPSCs of spinal lamina II neurons in pertussis toxin (PTX)-treated rats. A: raw tracings showing sIPSCs of a lamina II neuron during control and application of 2 \( \mu \)M capsaicin. Cumulative plot analysis of sIPSCs of the same neuron showing distribution of the interevent interval and amplitude during control and application of 2 \( \mu \)M capsaicin. B: summary data showing the effect of capsaicin on the frequency and amplitude of GABAergic sIPSCs in 23 lamina II neurons. *\( P < 0.05 \) compared with control.](http://jn.physiology.org/)
significantly increased the frequency of sIPSCs. These data suggest that the potentiating effect of capsaicin on synaptic GABA release in the spinal cord of PTX-treated rats is primarily caused by increased glutamate release and iGluRs.

Similarly, in untreated rats, in the presence of both 20 μM CNQX and 20 μM MK-801, 2 μM capsaicin failed to inhibit the GABAergic sIPSCs in all 22 lamina II neurons recorded. In the majority (17 of 22, 77.3%) of lamina II neurons tested, capsaicin had no significant effect on the frequency and amplitude of sIPSCs (Fig. 4B). In the remaining five (22.7%) neurons, capsaicin significantly increased the frequency of GABAergic sIPSCs (Fig. 4B). These results strongly suggest that the inhibitory effect of capsaicin on synaptic GABA release is disynaptic or multisynaptic, which is mediated by endogenous glutamate release and activation of iGluRs in the spinal cord.

**Effect of capsaicin on GABAergic mIPSCs of lamina II neurons**

To determine whether the dorsal horn interneurons are involved in the stimulating effect of capsaicin on synaptic GABA release, we tested the effect of capsaicin on GABAergic mIPSCs in 21 separate lamina II neurons. In most neurons (17/21, 81%) tested, bath application of 2 μM capsaicin did not significantly alter the frequency and the amplitude of mIPSCs in the presence of 1 μM of TTX (Fig. 4C). In the remaining four (19%) neurons, 2 μM capsaicin significantly increased the frequency but not the amplitude of mIPSCs (Fig. 4C). Thus increased nociceptive inflow can inhibit synaptic GABA release to most dorsal horn neurons through interneurons.

**Role of group II and group III metabotropic glutamate receptors in the inhibitory effect of capsaicin on GABA release**

Group II and group III metabotropic glutamate receptors (mGluRs) are G<sub>protein</sub>-coupled receptors (Prezeau et al. 1992; Tanabe et al. 1992). It was previously shown that activation of presynaptic group II and group III mGluRs depresses GABA release in spinal dorsal horn neurons (Gerber et al. 2000). The results described in the previous section suggest that inhibition of synaptic GABA release by capsaicin is mediated by endogenous glutamate release. Also, because the inhibitory effect of capsaicin on synaptic GABA release was abolished by PTX treatment, we hypothesized that the group II and group III mGluRs play a role in the attenuation of synaptic GABA release during stimulation of primary afferents with capsaicin. In the presence of 200 μM CPPG and 100 nM LY341495, which block group II and group III mGluRs, respectively (Conn and Pin 1997; Gerber et al. 2000; Schoepp et al. 1999), 2 μM capsaicin had no inhibitory effect on sIPSCs. In nine of 17 (52.9%) neurons studied, capsaicin significantly increased the frequency of sIPSCs (Fig. 5). These nine neurons consisted of six inner and three outer lamina II neurons. In the remaining eight neurons, capsaicin had no significant effect on the frequency and amplitude of sIPSCs (Fig. 5B).

We then examined the relative contribution of group II mGluRs to the inhibitory effect of capsaicin on synaptic GABA release in the spinal cord. In the presence of 100 nM LY341495, 2 μM capsaicin significantly increased the frequency of sIPSCs in 13 of 21 (62%) neurons recorded (Fig. 6A). However, in four of 21 (19%) neurons, capsaicin still significantly decreased the frequency of sIPSCs (Fig. 6A).
These four neurons consisted of three inner and one outer lamina II neurons. In the remaining four (19%) neurons, capsaicin had no significant effect on the frequency and amplitude of GABAergic sIPSCs.

We also determined the relative role of group III mGluRs in the inhibitory effect of capsaicin on synaptic GABA release in the spinal cord. In the presence of 200 μM CPPG, 2 μM capsaicin significantly increased the frequency of sIPSCs in 12 of 21 (57%) neurons (Fig. 6, B and C). However, in four of 21 (19%) neurons, capsaicin significantly decreased the frequency of sIPSCs (Fig. 6, B and C). These four neurons consisted of one inner and three outer lamina II neurons. In the remaining five (24%) neurons, capsaicin had no significant effect on the frequency or amplitude of sIPSCs. Collectively, these results suggest that acute stimulation of primary afferents inhibits synaptic GABA release by activating both group II and group III mGluRs, located presynaptically on GABAergic interneurons, in the spinal cord.

Role of group II and group III mGluRs in the inhibitory effect of bradykinin on GABA release

Because capsaicin can cause rapid desensitization of TRPV1 (Szallasi and Blumberg 1999), we were unable to examine the capsaicin effect before and after application of group II and group III mGluRs antagonists in the same dorsal horn neuron. Bradykinin is a nonapeptide, which can be used repeatedly to stimulate glutamate release from nociceptive primary afferents in the spinal cord (Jeftinija 1994; Wang et al. 2005). We chose bradykinin to stimulate nociceptive primary afferent terminals not restricted to TRPV1-expressing nerves in the spinal cord. Bath application of 10 μM bradykinin for 3 min significantly decreased the frequency of GABAergic sIPSCs in 18 of 49 (36.7%) neurons studied (Fig. 7). These 18 neurons consisted of 15 inner and three outer lamina II neurons. In separate (14 of 49, 28.6%) neurons, bradykinin significantly increased the frequency of sIPSCs (Fig. 7). The bradykinin effect persisted for ≥6 min during washout of bradykinin. In the remaining 17 (34.7%) neurons, bradykinin had no significant effect on the frequency or amplitude of sIPSCs (Fig. 7). Repeated application of 10 μM bradykinin had a reproducible effect on sIPSCs (data not shown).

In 12 lamina II neurons in which bradykinin reduced the frequency of sIPSCs, we determined the role of group II and group III mGluRs in this action. In nine of 12 cells, 200 μM CPPG alone abolished the inhibitory effect of bradykinin on GABAergic sIPSCs (Fig. 8). These nine neurons consisted of one outer and eight inner lamina II neurons. In the remaining three cells, CPPG alone did not significantly reduce the effect of bradykinin on sIPSCs. However, subsequent application of 100 nM LY341495 completely blocked the reduction of the frequency of sIPSCs by bradykinin (Fig. 8). These three neurons consisted of one outer and two inner lamina II neurons. In no case was a decrease-type response converted to an
increase-type response in the presence of CPPG and LY341495. These results provide further evidence that stimulation of nociceptive primary afferents inhibits GABAergic input to dorsal horn neurons through combined group II and group III mGluRs in the spinal cord.

**DISCUSSION**

The increased pain sensitivity after tissue and nerve injury likely arises from a net result of peripheral and central sensitization and an imbalance between excitatory and inhibitory synaptic inputs to spinal dorsal horn projection neurons. This is the first study to demonstrate that increased nociceptive inflow can rapidly alter GABAergic synaptic transmission in the spinal cord. We found that stimulation of nociceptive primary afferents with either capsaicin or bradykinin increased the frequency of GABAergic sIPSCs in many lamina II neurons. Interestingly, capsaicin or bradykinin also caused a rapid and long-lasting inhibition of sIPSCs in a population of lamina II neurons. Subsequent experiments with iGluR antagonists provided evidence that the reduction in GABAergic input to dorsal horn neurons is through endogenously released glutamate. Using PTX and specific group II and group III mGluR antagonists, we provided further evidence that group II and group III mGluRs function as heteroreceptors to inhibit GABAergic tone after stimulation of primary afferents. Therefore our study provides novel information that group II and group III mGluRs play a critical role in the rapid modulation of GABAergic transmission in the spinal dorsal horn in response to increased nociceptive input. Acute disinhibition of lamina II neurons by endogenous glutamate may contribute to central sensitization and pain hypersensitivity.

The lamina II is critical for the relay of nociceptive information in the spinal cord (Cervero and Iggo 1980; Lu and Perl 2003). In this study, we used capsaicin and bradykinin to stimulate nociceptive primary afferents in the spinal cord slice preparation. The capsaicin receptors (TRPV1 channels) were localized to the central terminals of primary afferent neurons in the superficial dorsal horn (Chen and Pan 2006; Guo et al. 2001; Pan et al. 2003). Previous immunocytochemical and electrophysiological studies documented that TRPV1-expressing primary afferent terminals are glutamatergic (Chen and Pan 2006; Hwang et al. 2004; Pan et al. 2003). Furthermore, bradykinin receptors (kinin B2 receptors) are also expressed on primary sensory neurons (Prado et al. 2002; Wang et al. 2005) and bradykinin can elicit glutamate release from primary afferent terminals (Jeffinna 1994; Wang et al. 2005). We found that although capsaicin and bradykinin increased synaptic GABA release in a population of lamina II neurons, they also...

![FIG. 6. Effect of capsaicin on GABAergic sIPSCs of spinal lamina II neurons in the presence of LY341495 or CPPG. A: summary data show the effect of 2 μM capsaicin on the frequency and amplitude of sIPSCs in the presence of 100 nM LY341495 in 21 lamina II neurons. B: summary data show the effect of 2 μM capsaicin on the frequency and amplitude of sIPSCs in the presence of 200 μM CPPG in another 21 lamina II neurons. *P < 0.05 compared with control. C: summary data show the percentage of increase- and decrease-type neurons in which capsaicin significantly altered the frequency of sIPSCs in different protocols. *P < 0.05 compared with the percentage of decrease-type neurons in the control group. #P < 0.05 compared with the percentage of increase-type neurons in the control group (Fisher’s exact test).](http://jn.physiology.org/abstracts/877)
induced a long-lasting inhibition of GABAergic input to other lamina II neurons. Because we measured GABAergic sIPSCs [not miniature (m)IPSCs], the decrease in the sIPSC amplitude by capsaicin or bradykinin can be explained by the reduced presynaptic GABA release. This presynaptic action is further supported by our finding that the postsynaptic GABA_A current was not altered by capsaicin. Notably, capsaicin was reported to have no effect on the GABAergic and glycinergic inhibitory synaptic transmission (Yang et al. 1998). The lack of capsaicin effect on synaptic GABA release in the previous studies might have been attributable to the short duration (30 s) of capsaicin application. We found in the present study that the effect of capsaicin on GABAergic sIPSCs took about 2 min, reflecting a disynaptic or multisynaptic effect. Also, a longer onset latency of capsaicin effect on sIPSCs compared with that on excitatory postsynaptic currents (<30 s) (Pan and Pan 2004; Yang et al. 1998) suggests that the effect of capsaicin on GABAergic transmission is probably an indirect effect secondary to increased glutamatergic input. The use of iGluR antagonists to isolate IPSCs in the previous study also may account for the failure to observe the inhibitory effect of capsaicin (Pan and Pan 2004; Yang et al. 1998). As shown in this study, the iGluR antagonists abolished the inhibitory effect of capsaicin on GABAergic input, suggesting that this capsaicin effect is critically dependent on endogenous glutamate and activation of iGluRs. This finding suggests that stimulation of primary afferents can rapidly (within minutes) reduce GABAergic tone in the spinal cord, which possibly can increase glutamatergic synaptic efficacy in the dorsal horn. Thus “disinhibition” of lamina II neurons could contribute to the sensitization of dorsal horn neurons, leading to long-lasting pain hypersensitivity after tissue and nerve injury.

We found that in rats treated with PTX to inactivate G_{i/o} proteins, capsaicin failed to inhibit synaptic GABA release in all lamina II neurons tested. Therefore the receptors coupled to G_{i/o} proteins are probably involved in the inhibitory effect of capsaicin. Glutamate released from primary afferents and interneurons acts through two broad classes of glutamate receptors: ionotropic (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, NMDA, and kainate) receptors and G-protein-coupled metabotropic receptors (mGluRs). Eight mGluRs have been cloned and are classified into three groups. Group I receptors (mGluRs 1 and 5) couple to phospholipase C by G_{q/11} proteins (Houamed et al. 1991; Masu et al. 1991) and increase
neuronal firing and synaptic transmission. Stimulation of group II (mGluRs 2 and 3) and group III (mGluRs 4, 6, 7, and 8) receptors coupled to G\textsubscript{i/o} proteins (Prezeau et al. 1992; Tanabe et al. 1992, 1993), on the other hand, generally reduces neuronal excitability and synaptic transmission (Conn and Pin 1997; Macek et al. 1996; Schoepp et al. 1999). Both group II and group III mGluR agonists can reduce chronic neuropathic pain (Chen and Pan 2005; Fisher et al. 2002). Bath application of exogenous group II and group III mGluR agonists reduces both glutamatergic and GABAergic transmission in the rat spinal cord (Gerber et al. 2000). We hypothesized that when glutamate release is enhanced from primary afferents and glutamatergic interneurons after high-intensity stimulation, glutamate can overspill at the synapse to activate presynaptic inhibitory mGluRs. This use-dependent activation of mGluRs may influence the strength of synaptic transmission in the spinal dorsal horn (Scanziani et al. 1997). We found that the inhibitory effect of both capsaicin and bradykinin on the frequency of GABAergic sIPSCs in lamina II neurons was completely blocked by specific group II and group III mGluRs antagonists LY341495 and CPPG. Because either antagonist alone only partially reduced the inhibitory effect of capsaicin and bradykinin on GABAergic sIPSCs, both group II and group III mGluRs must be involved. Because bradykinin also decreased the frequency of GABAergic sIPSCs in a population of lamina II neurons, those non-TRPV1–expressing afferent terminals can also inhibit spinal GABAergic tone. Collectively, our results suggest that increased nociceptive input leads to endogenous glutamate release from glutamatergic interneurons in the dorsal horn, which activates both group II and group III mGluRs expressed on GABAergic interneurons (to function as heteroreceptors) to reduce GABAergic transmission. Capsaicin-induced reduction in GABAergic tone and increase in glutamatergic input can act in concert to facilitate nociceptive transmission in the spinal cord.

The mGluR2/3 is present at the afferent terminals in the spinal superficial dorsal horn (Jia et al. 1999; Tang and Sim...
Furthermore, two subtypes of group III mGluRs—mGluR4 and mGluR7—are located in the rat spinal dorsal horn (Azkue et al. 2001; Ohishi et al. 1995). Immunocytochemical labeling studies suggest that group II mGluRs appear to be in the inner zone of lamina II, but group III mGluRs are mainly in the lamina I and the outer zone of lamina II (Jia et al. 1999; Ohishi et al. 1995; Tang and Sim 1999). In our study, group II mGluRs that mediated the inhibitory effect of capsaicin and bradykinin on sIPSCs appeared to occur mostly in the outer lamina II neurons. Also, the group III mGluRs that mediated the decrease in sIPSCs by capsaicin and bradykinin were predominantly in the inner lamina II neurons. However, the histological evidence does not necessarily conflict with the results of our electrophysiological study. It is important to note that the GABAergic interneurons expressing group II and group III mGluRs may be located at a distance from the recorded lamina II neurons. Although group II and group III mGluRs may be present on glutamatergic, GABAergic, or glycineric neurons in the neuroanatomical studies, we focused on only mGluRs on GABAergic neurons in the present study. Because iGluR antagonists abolished the inhibitory effect of capsaicin, the source of glutamate that stimulates group II and group III mGluRs on GABAergic interneurons is probably released from glutamatergic interneurons rather than from primary afferents. If glutamate released directly from primary afferents was involved in activation of group II and group III mGluRs on GABAergic interneurons, the inhibitory effect of capsaicin on sIPSCs would have been observed in the presence of iGluR antagonists. Furthermore, the finding that capsaicin had no significant effect on GABAergic mIPSCs in most lamina II neurons is also consistent with the involvement of glutamatergic interneurons in the spinal dorsal horn.

Although we focused primarily on the mechanism responsible for the depression of GABAergic transmission by increased nociceptive inflow, we observed that primary afferent stimulation produced a stimulatory effect on synaptic GABA release to a subpopulation of lamina II neurons. This effect was especially prominent in PTX-treated rats. However, in the presence of CNQX and MK-801, capsaicin increased GABA release in only a few lamina II neurons recorded from PTX-treated rats. These data suggest that iGluRs expressed on GABAergic interneurons are also involved in the stimulatory effect of capsaicin on synaptic GABA release. Consistent with these data, it was previously shown that electrical stimulation of primary afferents elicits GABAergic IPSCs primarily through non-NMDA receptors (Yoshimura and Nishi 1995). Nevertheless, we observed that the stimulatory effect of capsaicin was not blocked by iGluR antagonists in a small group of lamina II neurons. Thus neurotransmitters other than glutamate, such as substance P (Vergnano et al. 2004) or ATP (Hugel and Schlichter 2000), may be responsible for this action. Alternatively, group I mGluRs on GABAergic interneurons (Dang et al. 2002; Jia et al. 1999; Tao et al. 2000) may mediate part of this capsaicin effect. Thus glutamate released from the primary afferents may increase GABAergic input to some lamina II neurons, which could modulate nociception during acute stimulation of primary afferents. It is uncertain how increased synaptic GABA release influences nociceptive transmission in the spinal dorsal horn. Precisely which phenotype of recorded postsynaptic neurons receiving GABAergic input will ultimately dictate their contribution to modulation of nociceptive information. This heterosynaptic interaction may be important for dynamic integration of sensory signals at the spinal levels, through the lamina II along subsequent projection pathways. An important observation is that both the increase-type and the decrease-type lamina II neurons in response to primary afferent stimulation do not overlap. Had this been the case, group II and group III mGluR antagonists would have reversed the bradykinin effect, although we never observed such a response. The dual effect of capsaicin and bradykinin on GABAergic input to two separate populations of lamina II neurons suggests a rather complex mechanism of integrating nociceptive inflow at the spinal level. The major limitation of this study is that the phenotype of the recorded postsynaptic neurons was not characterized. The spinal lamina II has excitatory and inhibitory (both GABAergic and glycineric) interneurons. Characterizing the phenotype of the postsynaptic neuron would be important in explaining the differential effect of capsaicin on synaptic GABA release.

In summary, the findings from our study provide new evidence for the interaction between glutamatergic and GABAergic synapses in the spinal dorsal horn after stimulation of the nociceptive primary afferents. Distinct populations of dorsal horn interneurons are involved in the integration of nociceptive information from primary afferents. As illustrated in Fig. 9, the group II and group III mGluRs located on the
somatodendritic and/or terminal sites of GABAergic interneurons are critically involved in the inhibition of GABAergic input to dorsal horn neurons after stimulation of the primary afferents. Glutamate released from primary afferents can heterosynaptically reduce GABAergic input to dorsal horn neurons by group II and group III mGluRs. Therefore increased glutamatergic input is directly linked to the reduction of GABAergic tone in the spinal dorsal horn and the reduction of glutamatergic input is directly linked to the reduction of GABAergic interneurons by group II and group III mGluRs. Therefore increased input to dorsal horn neurons after stimulation of the primary afferents are critically involved in the inhibition of GABAergic somatodendritic and/or terminal sites of GABAergic interneurons.

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