GABA<sub>A</sub>-Receptor Blockade Reverses the Injury-Induced Sensitization of Nociceptor-Specific (NS) Neurons in the Spinal Dorsal Horn of the Rat

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INTRODUCTION

Persistent pain states, such as those induced by peripheral inflammation or neuropathy, are characterized by the development of hyperalgesia and allodynia (Treede et al. 1992). The sensory expressions of these phenomena include a switch in the modality of the sensation evoked by low-threshold mechanoreceptors from touch to pain (touch-evoked pain or mechanical allodynia) (LaMotte et al. 1991; Treede et al. 1992). In areas of hyperalgesia remote from an injury site (secondary or referred hyperalgesia) touch-evoked pain arises from an alteration in the central processing of impulses in low-threshold mechanoreceptors such that they become capable of accessing nociceptive neurons and pathways (LaMotte et al. 1991; Torebjörk et al. 1992).

The central changes responsible for the development of touch-evoked pain are thought to result from a state of spinal cord hyperexcitability known as central sensitization (Woolf and Salter 2000). One possible mechanism for sensitization is disinhibition, whereby a reduction in normally operating inhibitory systems results in the unmasking of low-threshold inputs to nociceptive neurons (Woolf et al. 1994). Most inhibitory systems in the spinal dorsal horn are mediated by γ-aminobutyric acid (GABA) or glycine and it is known that intrathecal administration of antagonists of these transmitters evokes a behavior interpreted as allodynia (Hammond 1997; Yaksh 1989). Blocking GABA-mediated inhibition reveals low-threshold inputs to nociceptive withdrawal reflexes (Sivilotti and Woolf 1994; Woolf et al. 1994) and facilitates low-threshold inputs of superficial dorsal horn neurons (Baba et al. 2003). Neuropathic lesions of the type that cause hyperalgesic states are also known to induce a loss of GABAAergic inhibition in the spinal dorsal horn (Coul et al. 2003; Drew et al. 2004; Moore et al. 2002).

Much of the work on the relation between GABA and touch-evoked pain has focused on the postsynaptic actions of GABA on dorsal horn neurons. The published data support the notion that an injury-induced reduction in GABA-mediated postsynaptic inhibition results in an enhanced excitability of nociceptive neurons leading to the generation of allodynic states (Baba et al. 2003; Moore et al. 2002). However, GABA also mediates presynaptic inhibition in the spinal dorsal horn a process brought about by GABAAergic depolarization of primary afferent terminals [primary afferent depolarization (PAD)] (Rudomin and Schmidt 1999; Schmidt 1971; Todd and Lochhead 1990). Primary afferent neurons show depolarizing responses to GABA because of the expression of the NKCC1 cation chloride cotransporter that maintains a high intracellular concentration of chloride ions, causing outward chloride currents when GABA<sub>A</sub> receptors are activated (Alvarez-Leefmans et al. 1998; Sung et al. 2000). Release of GABA by spinal interneurons induces a depolarization of the spinal endings of primary afferents that in turn decreases the amount of transmitter released by the afferents, thus reducing their postsynaptic efficacy (Rudomin and Schmidt 1999).

Inflammation and other forms of peripheral injury have been shown to enhance PAD to the point that spikes can be evoked in the primary afferent terminals (Willis 1999). These discharges, known as dorsal root reflexes (DRRs) can be detected antidromically (Lin et al. 2000; Rees et al. 1996; Sluka et al. 1995) but can also cause excitation of second-order neurons in the spinal cord (Cervero and Laird 1996a). Under normal conditions the costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked “advertisement” in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.
circumstances activation of low-threshold afferents causes PAD (and presynaptic inhibition) on nociceptive afferents. However, an enhancement of GABAAergic presynaptic activity caused by a peripheral injury or by neuropathy could lead to the excitation of nociceptive neurons by low-threshold afferents and thus provide a potential substrate for touch-evoked pain. In this way PAD, which is normally an inhibitory process, can be transformed into an excitatory one if the afferent depolarization is large enough to evoke spikes on the afferent terminals (Cervero and Laird 1996a).

We report here the study of responses of nociceptor-specific (NS) neurons in the superficial dorsal horn of the rat. These are neurons excited mainly or only by peripheral nociceptors (Christensen and Perl 1970) and are a key element of the pathway that transmits injury-related information leading to the development of hyperalgesic states (Cervero and Laird 1996b; Julius and Basbaum 2001; Mantyh and Hunt 2004). We have looked at the acquisition of low-threshold inputs by NS neurons (sensitization) after a sustained nociceptive input induced by peripheral application of capsaicin or mustard oil. The role of GABAA receptors in the unmasking of novel responses of NS neurons to low-threshold input was studied to test the hypothesis that presynaptic GABA release can contribute to the generation of touch-evoked pain. Some preliminary results were previously published in abstract form (Garcia-Nicas et al. 2003).

**Methods**

Experiments were conducted on 39 adult Wistar rats of either sex (body weights 240–350 g) anesthetized with pentobarbital sodium [initial dose of 50 mg/kg, administered intraperitoneally (ip); 10 mg · kg$^{-1}$ · h$^{-1}$ intravenous (iv) maintenance dose]. The level of anesthesia was maintained such that there were no reflex motor responses on application of noxious stimuli and corneal reflexes were absent. Pupillary constriction was also monitored and used as an indicator of adequate anesthesia. One catheter was placed in the left carotid artery for continuous arterial blood pressure recordings and another in the left jugular vein for injection of anesthetic. The trachea was cannulated to allow artificial ventilation and continuous end-tidal CO$_2$ recordings. Rectal temperature was kept constant at 38°C with a feedback-controlled electric blanket.

The left sciatic nerve was dissected and prepared for stimulation through bipolar silver electrodes. A laminectomy (T13 to L2) was performed to expose the spinal segments L4 to S1 and the animal was mounted on a rigid frame. A pool was made around the thoracolumbar laminectomy with the skin flaps and filled with agar. The exposed spinal cord was protected with mineral oil. Before nerve stimulation, the spinal cord was protected with mineral oil. Before nerve stimulation, the electrical threshold of the primary afferent volleys (about 3.3 N, area of stimulation 8.9 mm$^2$). Mechanical thresholds [in millinewtons (mN)] were established using a set of calibrated Von Frey monofilaments. The threshold and latency of the responses to electrical stimulation of the sciatic nerve were noted for both A fiber and C fiber volleys. The neurons were also characterized with thermal noxious stimulation: cold stimuli using a small piece of ice and heat stimuli using a metal bar heated at 55–57°C. Neurons were classified as Class 1 [or low threshold (LT)] if they responded only to low-intensity stimuli such as brush and touch, Class 2 [or wide dynamic range (WDR)] if they responded to both low-intensity and high-intensity stimuli such as pinch of skin folds and pin prick, and Class 3 [or nociceptor specific (NS)] if they responded only to high-intensity stimuli. Only Class 3 (NS) neurons with A and C fiber inputs from the sciatic nerve were selected for further analysis.

**Experimental design**

Once a NS neuron was characterized, the following experimental protocol was applied. Each series began with a 1-min recording of background activity. Then three mechanical stimuli of 20-s duration each were applied (brushing, gentle rubbing with a cotton swab, and pinch). The interstimulus interval was 20 s or the end of the previous response, whichever was the longest. Electrical stimuli were then applied to the sciatic nerve at A-fiber intensity (twofold the threshold with 0.1-ms pulses at 0.2 Hz) and at C-fiber intensity (40-fold the threshold with 1-ms pulses also at 0.2 Hz). Thermal noxious stimuli, cold and heat, were applied in the center of the receptive filed for 20–30 s.

The same protocol was applied before and after the induction of a peripheral injury discharge by the cutaneous application of either mustard oil or capsaicin. Chemical stimulation of the skin was applied inside the receptive field (RF) of each neuron but well away from the sites of mechanical stimulation. Capsaicin was injected intradermally (one or two doses of 20 µl each; 0.3%) and mustard oil was applied topically on the skin using filter-paper discs (24 mm$^2$) (one or two applications of 5 µl each; 50% in ethanol). The activity of the neuron was monitored after the chemical stimulation until recovery to the baseline activity before stimulation. After an interval of 40 min the neuron was characterized again with the same series of mechanical, electrical, and thermal tests as described above. The size of the receptive field was also mapped before and after the noxious chemical stimulation.

**GABA$\alpha$ blockade**

The effects of the GABA$\alpha$-receptor antagonists bicuculline and picrotoxin were tested on the responses of the neurons after sensitization. Cumulative doses of picrotoxin (0.5, 1 mg/kg; dissolved in saline) or bicuculline (0.03, 0.1, 0.3, 1 mg/kg; dissolved in saline) were previously published in abstract form (Garcia-Nicas et al. 2003).
were administered systemically (iv). In another group of animals different cumulative doses of bicuculline were applied directly over the spinal cord (0.03, 0.1, and 0.3 μg; volume of doses 50 μl; dissolved in saline). In both cases, each dose was given at 10-min intervals. The stimulation of the receptive fields began 3 min after each administration. The threshold of the electrically evoked A-fiber volley was also noted after every dose as were the responses to electrical stimulation of the sciatic nerve at twofold the threshold.

**Histological methods**

At the end of each experiment, the recording electrode was removed and replaced with one containing 4% Pontamine Sky Blue in 0.5 M sodium acetate solution. Three marks were made at 500-μm intervals by iontophoretic deposition of dye in the track in which the tested neuron had been recorded. The spinal cord was removed postmortem, frozen, and cut at 60-μm transverse sections using a cryostat. The recording sites were calculated from these marks recovered and locations reconstructed for 58 neurons. By depth measurements, 65 neurons (81%) were successfully recovered and locations reconstructed for 58 neurons (Fig. 1). Forty-five recording sites (78%) were located in the deeper layers of the dorsal horn. Dye marks were transferred to a paper at the end of the experiment. The areas of the RFs were measured with a digitalizing tablet connected to a computer.

The responses to the various stimuli were calculated as the total number of action potentials evoked during the 20 s of stimulation or until the end of the evoked response. Data are shown as means ± SE. Statistical analysis was performed on the raw data using one-way ANOVA for repeated measures with post hoc Dunnett’s multiple comparison tests where significant main effects or interactions were seen, or paired t-test, as appropriate. Fisher’s exact test was used for analysis of proportions. Values of \( P < 0.05 \) were taken as statistically significant.

**RESULTS**

**Sample of neurons**

Results were obtained from a sample of 80 NS (or Class 3) neurons. By depth measurements, 65 neurons (81%) were recorded in the superficial dorsal horn and 15 neurons (19%) were located in the deeper layers of the dorsal horn. Dye marks were successfully recovered and locations reconstructed for 58 neurons (Fig. 1). Forty-five recording sites (78%) were located in Laminae I and II and 13 (22%) in the deeper Laminae of the dorsal horn (V and VI).

All NS neurons responded vigorously to intense mechanical stimulation of their RFs (Fig. 2) and gave inconsistent responses to light mechanical stimulation. The mechanical threshold for superficial and deep NS neurons had a median of 8 mN and their RFs had a mean area size of 185 ± 79 mm². The mean A-fiber electrical threshold was 3.7 ± 0.4-fold the threshold of primary afferents (T) and the mean latency was 4.7 ± 0.1 ms.

Six deep neurons were tested with noxious cold stimulus and none was excited; eight deep neurons were tested with noxious heat stimulus: four neurons showed an excitatory response and four were not excited.

Fifty-eight of 63 superficial NS neurons tested and all 14 deep NS neurons in which a C-fiber response was tested showed a C-fiber input. The mean A-fiber electrical threshold was 3.7 ± 0.4-fold the threshold of primary afferents (T) and the mean latency was 4.7 ± 0.1 ms.

**Responses to capsaicin or mustard oil**

The responses of 37 NS neurons to sensitizing agents [mustard oil (MO) or capsaicin (CAP)] were analyzed. Twenty-three neurons were tested with CAP and 14 with MO. The agents were applied to locations inside the RF of the neuron but remote to the points where mechanical sensitivity was tested. In 18 neurons MO or CAP was applied to a different digit from the site of application of MO or CAP was applied to locations inside the RF of the neuron but remote to the points where mechanical sensitivity was tested. The responses to irritants were very variable, ranging from intense (Fig. 2, A and B) to no response at all. The mean response evoked by CAP was 1,623 ± 360 spikes (range: 15 to 6,255 spikes) and by MO was 985 ± 309 spikes (range: 0 to 4,726 spikes) but the differences between these responses were not significant (unpaired t-test, \( P = 0.12 \)). Responses lasted for ±5 min after the applications.

**Data analysis**

The contours of the RFs of the neurons were traced on the skin with colored pens before and after chemical stimulation. These contours were transferred to a paper at the end of the experiment. The areas of the RFs were measured with a digitalizing tablet connected to a computer.
Sensitization after CAP or MO application

After CAP or MO applications the neurons were retested. A neuron was considered to be sensitized if it showed consistent responses to low-threshold mechanical stimulation of the RF and could be excited by electrical stimulation of the sciatic nerve at Aβ-fiber intensity (2T). The complete process of sensitization was followed in 28 NS neurons. Only one NS neuron was recorded and only one application of the sensitizing agent was carried out in each of these 28 experiments. Of these 28 neurons, 20 showed sensitization (Fig. 2, A). Eight did not. Sensitization was characterized by consistent responses to low-intensity mechanical stimuli as well as enhanced responses to more intense stimuli (Fig. 2, A and B) and eight did not. Sensitization was characterized by consistent responses to low-intensity mechanical stimuli as well as enhanced responses to more intense stimuli (Fig. 2, A and B).

The relation between sensitization, location of the neuron in the dorsal horn, and sensitizing agent was also studied. Most sensitized neurons (16 of 20) were located in the superficial dorsal horn (Figs. 3A and 4) and most nonsensitized (five of eight) neurons were located in the deep dorsal horn (Figs. 3A and 4). This difference was found to be statistically significant (Fisher’s exact test, \( P = 0.0089 \)). On the other hand, CAP was more likely to induce sensitization (16 of 18 neurons, Fig. 3B) than MO (four of ten neurons, Fig. 3A), a statistically significant difference (Fisher’s exact test, \( P = 0.0028 \)).

All sensitized neurons showed a significant increase in their responses to low-intensity mechanical stimuli after application of the sensitizing agent (Fig. 3, C and D; \( P < 0.001 \) for both brush and stroke). The mechanical thresholds of the neurons on stimulation of their RFs showed a significant decrease after application of the sensitizing agents. The median mechanical threshold for sensitized NS neurons was 181 mN before and 45.3 mN after application of the irritants (\( P = 0.007 \)). However, no statistically significant differences were found regarding superficial or deep location in the dorsal horn (Fig. 3C) or type of sensitizing agent (Fig. 3D). Although fewer deep NS neurons were sensitized and CAP was more likely to induce sensitization, once a neuron was sensitized the magnitude of its responses to low-intensity mechanical stimuli was similar in all groups (Fig. 3, C and D).

The size of the RFs of the neurons was measured before and after CAP or MO applications. The eight nonsensitized neurons did not show changes in RF size. Of the 20 sensitized neurons, four increased the RF size by an average of 64% (116 ± 19 mm²) and the remaining 16 did not show RF increases.

Relationship between sensitization and responses to CAP or MO

For the 28 neurons fully studied for sensitization, the relationship between the magnitude of the response to the sensitizing agent and the presence or absence of sensitization was analyzed. As described above the actual responses of the neurons to the sensitizing agents were very variable and it was hypothesized that there could be a relationship between the magnitude of the response to the sensitizing agent and the development of sensitization. However, no such relationship was found (see Fig. 5). The range of responses in both groups (sensitized and nonsensitized neurons) was found to be similar; sensitized neurons gave mean responses of 1,711 ± 398.7 spikes (median 827 spikes) and nonsensitized neurons gave mean responses of 1,354 ± 577.9 spikes (median 603 spikes). Moreover, some neurons did not respond at all to the sensitizing agent and were sensitized and others gave vigorous responses to the agent and showed no sensitization (Fig. 5).

Blockade of GABA\(_A\) receptors

The effects of two GABA\(_A\)-receptor antagonists (picrotoxin and bicuculline) were studied on the sensitized NS neurons.
described above. Intravenous picrotoxin was tested in five neurons, intravenous bicuculline in three, and local spinal bicuculline in 11 neurons. The administration of the antagonists reversed the sensitization of the neurons by reducing or abolishing the novel low-threshold responses caused by the application of MO or CAP (Fig. 6).

The five neurons tested with picrotoxin (iv) showed responses to low-intensity mechanical stimulation of their RFs (brush and cotton bud stroking) after the application of CAP or MO. These responses were significantly reduced after the administration of the antagonist (Fig. 7A; brush $P = 0.038$; stroke $P = 0.041$).

Bicuculline (iv) was tested in only three sensitized NS neurons and therefore the data were not analyzed statistically. However, the neurons showed a reduction of the novel low-intensity mechanical inputs and of the enhanced high-intensity drives after the administration of the antagonist.

The effects of bicuculline were also tested by direct spinal application of the antagonist on the responses of 11 sensitized NS neurons. Six of these neurons showed a significant reduction or abolition of the novel low-threshold inputs revealed by the application of the sensitizing agents (Fig. 6; brush $P < 0.001$; stroke $P = 0.0058$). These effects were significant at all doses used (Fig. 7B).

Sensitized neurons also showed enhanced responses to high-intensity mechanical stimuli (pinch) and most of these responses were reduced after the administration of the GABA$_A$ antagonists (Fig. 6). However, as a population this reduction was not significant ($P = 0.11$).

The effects of direct spinal application of bicuculline on the responses evoked by A$\beta$-fiber stimulation (2T) of the sciatic nerve were also studied. Before application of the sensitizing agents to the RFs the neurons did not show any response to stimulation of afferent A$\beta$-fibers but after the application all of them showed a novel A$\beta$-fiber response (Fig. 8). These responses were reduced by the spinal application of the lowest dose of bicuculline from a mean of $3.54 \pm 0.35$ spikes/stimulus after CAP or MO to $1.45 \pm 0.23$ spikes/stimulus (59% reduction) (Fig. 8).

**DISCUSSION**

Two main observations are reported here: 1) the confirmation that NS neurons in the spinal dorsal horn can be sensitized (i.e., acquire low-threshold inputs and enhance their excitability) by a sustained afferent discharge in peripheral nociceptors and 2) that this sensitization can be reduced or reversed by GABA$_A$ receptor antagonists, thus suggesting a positive role for GABA in the generation of the sensitization process.

**NS neurons and allodynia**

Our study has focused on the properties of NS neurons, a class of dorsal horn cell identified more than 35 years ago (Christensen and Perl 1970) and known to play key roles in the transmission of injury-related information and in the development of hyperalgesic states (Craig 2004; Craig and Burton 1981; Hunt and Mantyh 2001; Mantyh and Hunt 2004; Mantyh et al. 1997; Nichols et al. 1999). NS neurons (also known as Class 3 or high-threshold neurons) have been described in many animal species, including rats, and are characterized by their excitatory responses to a variety of noxious stimuli with...
little or no response to innocuous stimulation (Cervero 1986, 1995). NS neurons are predominantly located in the most superficial layers of the dorsal horn (Laminae I and II), although they can also be found, in smaller numbers, in the deeper laminae. Whether the two groups of NS neurons, superficial and deep, are similar in all respects or represent different functional classes remains to be established (Laird and Cervero 1989, 1990).

NS neurons have been identified as candidates to mediate hyperalgesic states, and especially allodynic states, because of the possibility that they may acquire low-threshold inputs after a period of intense noxious stimulation. The rationale for this interpretation is that NS neurons would normally signal pain and that, when they are activated by low-threshold stimuli, the result would be allodynia, that is, pain evoked by an innocuous stimulus. Acquisition of a low-threshold input may be functional (i.e., disinhibition of an already existing connection) or anatomical (i.e., sprouting of low-threshold afferent terminals and formation of new connections) (Woolf et al. 1992; but see Hughes et al. 2003). In the present study we have focused on the functional approach and have examined the expression of low-threshold inputs by NS neurons after a period of noxious stimulation of their RFs.

Sensitization of NS neurons

Persistent noxious stimulation of the periphery can sensitize NS neurons (Laird and Cervero 1989; Woolf et al. 1994), a process that is expressed by increases in RF size, by enhancement of the responses to noxious stimuli, and—most important—by the expression of novel responses to innocuous stimuli (Cervero et al. 1992; Laird and Cervero 1989; Simone et al. 1989, 1991; Woolf et al. 1994). However, it appears that NS neurons are more resistant to sensitization than WDR neurons and therefore that the mechanism of the enhanced excitability that characterizes sensitization may be different for NS neurons than for WDR cells (Cervero 1995; Laird and Cervero 1989; Woolf et al. 1994).

We used capsaicin (CAP) and mustard oil (MO) applied to the RFs of the neurons to generate sensitization. Both compounds are known to selectively excite nociceptors (Gee et al. 1996; Kenins 1982; Lynn 1990; Russell and Burchiel 1984) through the activation of TRP receptors (Bautista et al. 2006; Caterina et al. 1997). It is also known that the afferent discharges evoked by CAP or MO are responsible for the sensitization of dorsal horn neurons (Cook et al. 1987; Dougherty and Willis 1992; LaMotte et al. 1991; Simone et al. 1991; Woolf and Thompson 1991; Woolf et al. 1994). In our experiments, sensitization of NS neurons was expressed by an enhanced responsiveness to noxious stimulation of the RF and by the acquisition of novel responses to low-intensity stimuli including the demonstration of an Aβ-fiber afferent input.

**FIG. 5.** Responses of sensitized and nonsensitized NS neurons to their sensitizing agents. Note the similar range of responses in both groups irrespective of whether the neuron was sensitized. Horizontal lines indicate the mean response value in each group.

**FIG. 6.** Representative example of the reversal of sensitization of a NS neuron induced by GABA<sub>δ</sub>-receptor blockade. Diagrams at the top indicate the RF of the neuron, the site of application of the sensitizing agent (MO), and the location of the recording site in the superficial dorsal horn. Histograms show the neuronal responses to brushing (br) and stroking (str) for 20 s and to pinching the RF before (left histograms) and after (middle histograms) application of MO and after local application on the spinal cord of 0.3 µg of bicuculline (right histograms).
Superficial NS neurons were more likely to sensitize than deep NS neurons, although the magnitude of the sensitization was similar in both types of cell. Intradermal CAP was also more likely to generate neuronal sensitization than MO; this could be explained either by the activation of different types of nociceptors by the two agents or by differences in the spread and peripheral availability of the two compounds in the skin. In any case, sensitization of NS neurons by either agent was robust and very well defined.

**Heterosynaptic sensitization of NS neurons**

Previous reports described sensitization of dorsal horn neurons, including spinothalamic projection neurons, after intradermal injections of CAP (Dougherty and Willis 1992; Simone et al. 1989). Our results are in line with these descriptions in terms of the responses of the sensitized neurons to low- and high-intensity mechanical stimuli but we also noticed that the sensitization process appeared to be independent of whether the neuron responded to the sensitizing agent.

We found that NS neurons gave a wide range of responses to the sensitizing agents, both CAP and MO, from no response at all to very intense discharges. Yet, the intensity of these responses and even whether a response was observed were not predictive of the ability of the neuron to sensitize. Some neurons with intense responses to CAP or MO were not sensitized and some neurons were sensitized, not having responded at all to the sensitizing agents. This shows that not all NS neurons are able to sensitize, even after intense nociceptive activation, and also that the sensitization process may involve heterosynaptic connections (Clarke and Harris 2001; Thompson et al. 1993; Woolf et al. 1994) and nonconventional forms of neurotransmission (Dougherty and Willis 1991; Dougherty et al. 1994).

**Sensitization of NS neurons and GABA**

Previous studies focused on the actions of inhibitory neurotransmitters in the spinal dorsal horn (Harvey et al. 2004) and on the downregulation of GABA activity as a way to generate disinhibition and neuronal sensitization (Moore et al. 2002; Scholz et al. 2005; Torsney and MacDermott 2006). GABA disinhibition can indeed lead to neuronal hyperexcitability but in this study we focused on the presynaptic actions of GABA, where its role as an inhibitory neurotransmitter is expressed by depolarization of primary afferent terminals and not by hyperpolarization (Price et al. 2005).

Presynaptic inhibition in the spinal cord is mediated by axo–axonic synapses between GABAergic interneurons and the dorsal horn terminals of primary afferent fibers (Rudomin and Schmidt 1999; Schmidt 1971). This mechanism is known to play a key role in the control of all afferent signals at the first synaptic relay. A key element of this process is the fact that GABA can produce depolarizations of primary afferent terminals and not by hyperpolarization (Price et al. 2005).

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terminals, including nociceptors (Lin et al. 2000; Rudomin and Schmidt 1999). Primary afferent terminals express GABA\textsubscript{A} receptors (Labrakakis et al. 2003) and are postsynaptic to axonal projections of GABAergic spinal interneurons (Todd and Lochhead 1990; Todd and McKenzie 1989). We have proposed that under conditions that generate tactile allodynia the normal A\textbeta{}-fiber induction of PAD on the nociceptive terminals of fine afferent fibers is enhanced to the point that the depolarization can now generate spike activity in the afferent terminals (Cervero and Laird 1996a). These spikes are conducted antidromically as dorsal root reflexes (DRRs), causing local vasodilatation (Garcia-Nicas et al. 2001) but can also be conducted orthodromically causing A\textbeta{}-fiber excitation of nociceptive neurons and thus touch-evoked pain. This mechanism provides a potential way for a GABA-mediated inhibitory process to be transformed into an excitatory one. Interestingly, both excitation and inhibition are mediated by depolarization and the switch between inhibition (PAD) and excitation (DRRs) is only a reflection of the intensity of the depolarization.

In this study we have shown that the novel A\textbeta{} input acquired by NS neurons when they become sensitized is reduced or abolished by GABA\textsubscript{A} antagonists. A plausible explanation for this observation is that the new low-threshold drive is the result of excessive PAD of A\textbeta{} afferents onto the terminals of the nociceptive afferents that activate NS neurons leading to the generation of spike activity. The administration of GABA\textsubscript{A} antagonists would return PAD to more normal levels and reduce or eliminate the A\textbeta{} drive. The effects of GABA antagonists are not likely to be postsynaptic because this ought to produce more, and not less, excitation (Kontinen et al. 2001). We also know that the effects are selective for GABA because administration of strychnine, a glycine antagonist, does not affect the expression of A\textbeta{}-evoked DRRs after sensitization (Garcia-Nicas et al. 2002 and unpublished observations). The lack of an obvious dose–response relationship of the various doses of GABA\textsubscript{A} antagonists used in our study suggests that the effects could be maximal at the lowest doses used or be attributable to a removal of postsynaptic inhibition at higher doses.

A role for PAD in the sensitization of NS neurons?

That pain could result from central interactions between low-threshold mechanoreceptors and nociceptors by means of a mechanism involving PAD was the central proposal of the Gate Control Theory of pain mechanisms (Melzack and Wall 1965). Multiple lines of evidence indicate that PAD is enhanced in hyperalgesic states leading to the generation of spike activity. The administration of GABA\textsubscript{A} antagonists would return PAD to more normal levels and reduce or eliminate the A\textbeta{} drive. The effects of GABA antagonists are not likely to be postsynaptic because this ought to produce more, and not less, excitation (Kontinen et al. 2001). We also know that the effects are selective for GABA because administration of strychnine, a glycine antagonist, does not affect the expression of A\textbeta{}-evoked DRRs after sensitization (Garcia-Nicas et al. 2002 and unpublished observations). The lack of an obvious dose–response relationship of the various doses of GABA\textsubscript{A} antagonists used in our study suggests that the effects could be maximal at the lowest doses used or be attributable to a removal of postsynaptic inhibition at higher doses.

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