Spinal GABA$_A$ and GABA$_B$ Receptor Pharmacology in a Rat Model of Neuropathic Pain

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Background: This study tests the hypothesis that loss of spinal activity of γ-aminobutyric acid (GABA) contributes to the allodynia and hyperalgesia observed after peripheral nerve injury.

Methods: Intrathecal catheters were implanted in male Sprague-Dawley rats. Antinociception was assessed by measuring withdrawal latency to immersion of the tail in a 52°C water bath. Nerve injury was produced by ligation of the L5 and L6 spinal nerves. Testing was performed 4–14 days after spinal nerve ligation, when tactile allodynia and thermal hyperalgesia were established. Tactile allodynia was quantitated using the threshold to withdrawal of the hind paw on probing with von Frey filaments. Thermal hyperalgesia was quantitated using the latency to withdrawal of the hind paw from radiant heat. Motor function was tested using a rotarod apparatus.

Results: Spinal administration of the GABA$_A$ receptor antagonist bicuculline or the GABA$_B$ receptor antagonist phaclofen produced tactile allodynia and thermal hyperalgesia in normal rats. The GABA$_B$ receptor agonist baclofen, administered spinally, produced antinociception in the tail-flick test, whereas the GABA$_A$ receptor agonist isoguvacine did not. Isoguvacine and baclofen each reversed tactile allodynia and thermal hyperalgesia produced by spinal nerve ligation. Baclofen but not isoguvacine prolonged thermal withdrawal latency in nerve-injured rats beyond preoperative values. Baclofen but not isoguvacine impaired motor function.

Conclusions: Pharmacologic inhibition of intrinsic GABA tone in normal rats resulted in tactile allodynia and thermal hyperalgesia, consistent with the hypothesis being tested. Exogenous administration of GABA agonists reversed spinal nerve ligation–induced allodynia and hyperalgesia, also consistent with this hypothesis. Isoguvacine produced specific antihyperalgesic and antiallodynic effects, whereas assessment of the effects of baclofen was complicated by motor dysfunction. Spinal GABA$_A$ agonists may provide a specific therapy for neuropathic pain.

IT has been hypothesized that one of the factors underlying the enhanced pain sensitivity observed in neuropathic pain states is the loss of activity of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) in the spinal cord.[1,2] If enhanced pain sensitivity is produced by loss of spinal GABAergic tone, spinal administration of GABA agonists might be predicted to be effective against neuropathy-induced allodynia and hyperalgesia.

The current study tests the hypothesis that loss of spinal GABA tone contributes to the allodynia and hy-

peralgesia observed after nerve injury by examining whether blockade of spinal GABA$_A$ and GABA$_B$ receptors is sufficient to lower tactile and thermal sensory thresholds. Yaksh[3] reported that intrathecal administration of the GABA$_A$ receptor–selective antagonist bicuculline resulted in increased responsiveness to tactile stimuli, demonstrating that loss of activity at the GABA$_A$ receptor is sufficient to produce tactile allodynia. Response to thermal stimuli was not tested. Spinal bicuculline was also shown to produce hypersensitivity of dorsal horn neurons, again suggesting the presence of a chronic inhibitory GABA tone in spinal cord.[4–7] Administration of the GABA$_B$ receptor antagonist CGP35348 resulted in pain-like responses to innocuous mechanical stimulation, demonstrating that loss of activity at the GABA$_B$ receptor is sufficient to produce tactile allodynia.[8] With the data presented in this article, we confirmed that blockade of GABA$_A$ or GABA$_B$ receptor tone produces tactile allodynia. We also tested the hypothesis that loss of GABA$_A$ or GABA$_B$ receptor tone increases sensitivity to thermal stimuli. Study of responses to both mechanical and thermal stimuli is important because thermal hyperalgesia has different spinal pharmacology and neural circuitry than does tactile allodynia.[9–11]

Ligation of the L5 and L6 spinal nerves in rats produces a model of neuropathic pain that results in tactile allodynia, as manifested by increased sensitivity to probing of the hind paw with von Frey filaments, and thermal hyperalgesia, as manifested by decreased latency to withdrawal from a radiant heat source.[12,13] In the spinal nerve ligation (SNL) model, Hwang and Yaksh[14] demonstrated that allodynia was reversed by the GABA$_A$ receptor agonist muscimol and the GABA$_B$ receptor agonist baclofen. They also found that muscimol and baclofen resulted in dose-dependent hind limb motor weakness at doses greater than those required for antagonism of allodynia. Other studies using other models of neuropathic pain have also found GABA receptor agonists to reverse mechanical hyperalgesia.[15,16] Systemic administration of midazolam, which facilitates the action of GABA at the GABA$_A$ receptor, reduced A-δ– and C-fiber–evoked activity in dorsal horn neurons.[17] In the current experiments, we confirmed the effectiveness of intrathecal GABA$_A$ and GABA$_B$ receptor agonists in reversing tactile allodynia produced by SNL. In addition, we tested the hypothesis that GABA$_A$ and GABA$_B$ receptor agonists also reverse nerve injury–induced thermal hyperalgesia. Finally, we examined the effects of GABA receptor agonists on motor function.

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Methods

Animals

All procedures were approved by the University of Arizona Animal Care and Use Committee (Tucson, Arizona) and conformed to the policies, recommendations, and guidelines of the National Institutes of Health and the International Association for the Study of Pain.[18] Male Sprague-Dawley rats (Harlan, Indianapolis, IN) weighing 250–350 g at the time of testing were maintained in a climate-controlled room on a 12-h light–dark cycle and were allowed food and water *ad libitum*.

Implantation of Intrathecal Catheter

For intrathecal drug administration, rats were chronically implanted with catheters as described by Yaksh and Rudy.[19] Rats were anesthetized with halothane and placed in stereotactic head holders. The occipital muscles were separated from their insertion on the skull and retracted caudally to expose the cisternal membrane. Polyethylene tubing was passed caudally from the cisterna magna to the level of the lumbar enlargement. Animals were allowed to recover and were examined for evidence of neurologic injury. Animals with evidence of neuromuscular deficits were promptly euthanized.

Spinal Nerve Ligation Injury

The L5 and L6 spinal nerves were ligated as described by Kim and Chung.[12] During halothane anesthesia, the dorsal vertebral column was surgically exposed from L4 to S2. The L5 and L6 spinal nerves were exposed and tightly ligated distal to the dorsal root ganglion using 4-0 silk suture. The incisions were closed, and the animals were allowed to recover for at least 4 days. Testing of drug effects was performed 4–14 days after nerve injury. Rats that showed motor dysfunction or in which tactile allodynia did not develop (less than 2% of animals) were excluded from further testing.

Drug Administration

Isoguvacine hydrochloride, (±)-baclofen, (+)-bicuculline, and phaclofen were obtained from RBI/Sigma (Natick, MA). SCH50911 was obtained from Tocris Cookson Inc. (Ellisville, MO). All drugs were administered through the intrathecal catheter. Testing was performed 30 min after administration of drug or vehicle.

Nociceptive Testing

The tail was placed in a heated water bath maintained at 52°C. Latency to withdrawal was determined before drug administration and 30 min after drug injection. A 12-s cutoff was used to prevent tissue damage.

Testing of Allodynia and Hyperalgesia

The assessment of tactile allodynia (decreased threshold to paw withdrawal after probing with normally in- nocuous mechanical stimuli) consisted of testing the withdrawal threshold of the paw (ipsilateral to the side of surgery in nerve-injured animals) in response to probing with a series of calibrated von Frey filaments. Each filament was applied perpendicularly to the plantar surface of the paw of rats held in suspended wire-mesh cages. Withdrawal threshold was determined by sequentially increasing and decreasing the stimulus strength (the "up-and-down" method), and the data were analyzed using the nonparametric method of Dixon, as described by Chaplan *et al.*[13]

Thermal hyperalgesia of the hind paws was tested as described by Hargreaves *et al.*[20] Rats were allowed to acclimate within acrylic enclosures on a clear glass plate maintained at 30°C. A radiant heat source (high-intensity projector lamp) was focused onto the plantar surface of the hind paw. Activation of the heat source activated a timer that stopped when withdrawal of the paw was detected with a photodetector. A maximal cutoff of 40 s was used to prevent tissue damage.

Testing of Motor Function

Animals were trained to ambulate on a rotarod device (Columbus Instruments International, Columbus, OH) until all could remain on the device for a duration of 180 s at a speed of 10 revolutions per minute. They were again tested after drug administration, and the time they were able to remain on the device without falling was recorded. A maximal cutoff time of 180 s was used.

Data Analysis

Reversal of allodynia was expressed as the percent of the maximum possible effect (MPE) using the formula:

$$\%MPE = (WT - CT)/(CO - CT),$$

where WT is the withdrawal threshold obtained experimentally, CT (control threshold) is the baseline value before drug administration, and CO is the cutoff value (15 g). Antinociception was also expressed as a percent of the MPE, calculated by the formula described. Reversal of thermal hyperalgesia was defined as return of paw withdrawal latency toward the values observed in nonoperated animals. Antinociception to thermal stimuli was defined as further increases in paw withdrawal latency beyond the baseline values of nonoperated animals.

Results

Intrathecal administration of the GABA$_A$ receptor–selective antagonist bicuculline decreased the threshold for withdrawal from tactile stimuli by 89%, resulting in tactile allodynia (fig. 1). The GABA$_B$ receptor–selective antagonist phaclofen decreased the threshold for withdrawal from tactile stimuli by 92%. Similarly, bicuculline

Fig. 1. Tactile allodynia produced by intrathecal bicuculline (*A*) or phaclofen (*B*). Thermal hyperalgesia produced by intrathecal bicuculline (*C*) or phaclofen (*D*). Data are expressed as mean ± standard error of the mean. N = 6 per group.
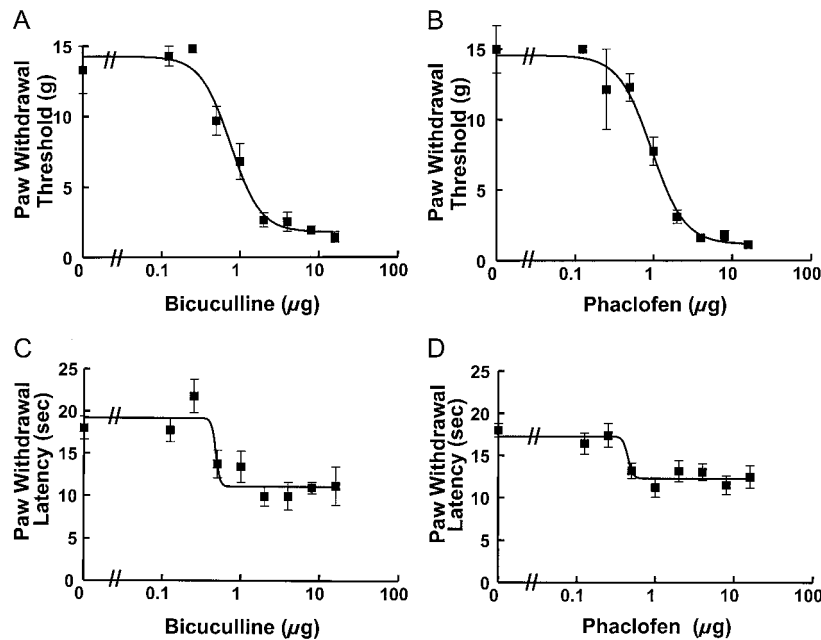
reduced the latency to withdrawal from a radiant thermal stimulus by 38%, resulting in thermal hyperalgesia. Phaclofen reduced thermal withdrawal latency by 36%.

Baseline tail-flick latency was 4.1 ± 0.7 s (mean ± SD). Intrathecal administration of the GABA_A receptor–selective agonist isoguvacine did not affect antinociception, as assessed by tail-flick latency (fig. 2). In contrast, intrathecal administration of the GABA_B receptor–selective agonist baclofen prolonged tail-flick latency to 100% of the MPE.

Ligation of the L5 and L6 spinal nerves resulted in tactile allodynia, manifested by a decrease in withdrawal threshold to tactile stimuli from 11 ± 4 to 1.9 ± 2.1 g (mean ± SD; $P < 0.05$, Student *t* test). Intrathecal administration of isoguvacine reversed this tactile allodynia to a maximum of 75% MPE (fig. 3). The effect of isoguvacine was completely ($> 80\%$) reversed by intrathecal bicuculline or phaclofen. Intrathecal administration of baclofen reversed tactile allodynia to a maximum of 90% MPE (fig. 4). This effect was reversed by intrathecal phaclofen (55% effect), SCH 50911 (85% effect), and bicuculline (70% effect).

Spinal nerve ligation of L5-L6 reduced withdrawal latency to radiant heat from 22 ± 4 to 12 ± 4 s (mean ± SD), indicating thermal hyperalgesia ($P < 0.05$, Student

t test). Intrathecal isoguvacine returned withdrawal latency to preoperative values (fig. 5). In contrast, intrathecal baclofen lengthened withdrawal latency beyond preoperative values, reaching the cutoff value of 40 s.

Intrathecal isoguvacine minimally decreased performance on the rotarod apparatus at antiallodynic and antihyperalgesic doses (fig. 6). In contrast, baclofen decreased duration on the rotarod apparatus by 86%.

Discussion

GABA_A and GABA_B receptor antagonists produced tactile allodynia and thermal hyperalgesia, confirming the hypothesis that loss of endogenous GABA tone leads to allodynia and hyperalgesia. Constitutive, endogenous spinal GABA activity may lead to tonic inhibition that prevents innocuous mechanical or thermal stimuli from being perceived as aversive or painful. Tonic inhibition is consistent with the high concentrations of GABA and GABA receptor–containing cells found in dorsal horn.[21,22] It is also consistent with electrophysiologic studies in which GABA_A receptor inhibition resulted in exaggerated spinal responses to afferent input[4–6] and with the observation that GABA_B receptor inhibition

Fig. 2. Thermal antinociception produced by intrathecal isoguvacine (*A*) or baclofen (*B*). Data are expressed as mean ± standard error of the mean. N = 6 per group. MPE = maximum possible effect.
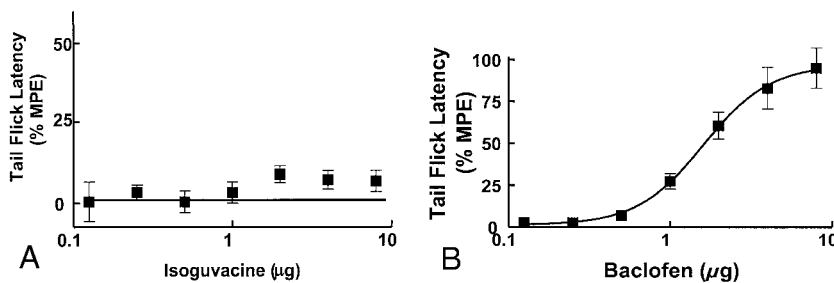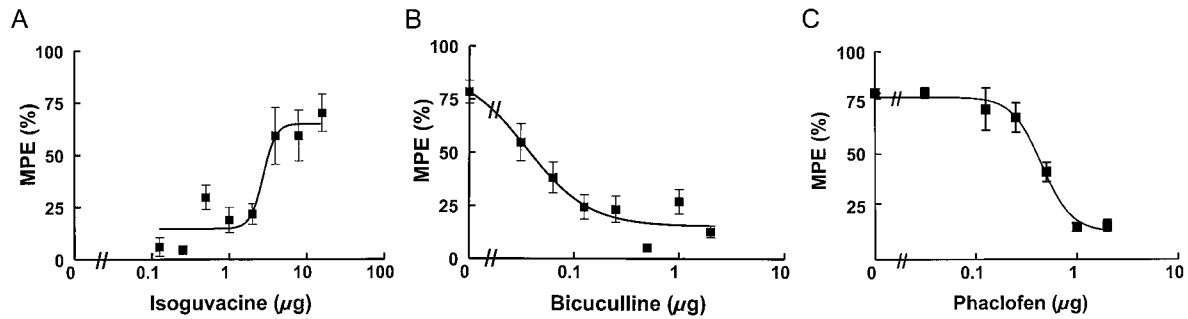
Fig. 3. Reversal of SNL-induced tactile allodynia by intrathecal isoguvacine (*A*). Reversal by bicuculline (*B*) or phaclofen (*C*) of the antiallodynia produced by 10 μg isoguvacine. Data are expressed as mean ± standard error of the mean. N = 6 per group. MPE = maximum possible effect.

increased substance P release, as assessed by receptor internalization.[23]

Our finding that the GABA$_A$ receptor–selective antagonist bicuculline produces tactile allodynia when administered spinally to the rat is consistent with previous studies.[3,5,24,25] The production of thermal hyperalgesia by bicuculline supports the hypothesis of Yamamoto and Yaksh,[1] who showed that bicuculline enhanced the thermal hyperalgesia produced by chronic constriction injury and hypothesized that the lesion and its exaggeration by a GABA$_A$ antagonist suggest mechanistic homology along a spectrum of progressive severity. The production of tactile allodynia and thermal hyperalgesia by bicuculline is consistent with the finding that mice lacking the β3 subunit of the GABA$_A$ receptor exhibit tactile allodynia and thermal hyperalgesia.[26] Bicuculline-mediated thermal hyperalgesia is not predicted by the results of an electrophysiologic study in which GABA$_A$ receptor inhibition resulted in an exaggerated response to low but not high threshold afferent input,[4] but it is consistent with the results of other electrophysiologic studies

in which GABA$_A$ receptor inhibition resulted in increased responsiveness to both low and high threshold afferent input.[6,27]

These observations with spinal GABA antagonists demonstrate that loss of GABA activity at spinal GABA$_A$ and GABA$_B$ receptors is sufficient to lower mechanical and thermal sensory thresholds. There is evidence that endogenous GABA concentrations may be reduced after peripheral nerve injury. Loss of GABA-like immunoreactivity has been reported after chronic constriction injury of the sciatic nerve.[2] Primary afferent depolarization, a GABA receptor–mediated effect, is decreased after chronic constriction injury.[28] There is also evidence that GABA receptor number or function may be altered after peripheral nerve injury. The sensitivity of dorsal roots to GABA was decreased after sciatic axotomy or crush injury.[29] In contrast, bicuculline increased C-fiber–mediated activity in dorsal horn neurons in spinal nerve–ligated rats but not in control animals, suggesting an increased GABAergic inhibitory tone in spinal cord after nerve injury.[7] Direct examinations of GABA receptor
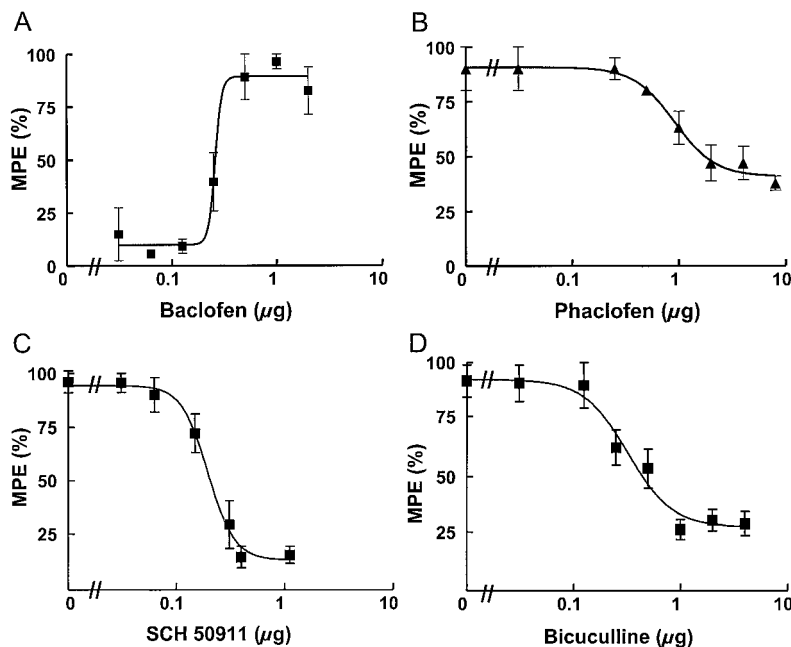

Fig. 4. Reversal of spinal nerve ligation–induced tactile allodynia by intrathecal baclofen (*A*). Reversal by phaclofen (*B*), SCH 50911, (*C*) or bicuculline (*D*) of the antiallodynia produced by 1 μg baclofen. Data are expressed as mean ± standard error of the mean. N = 6 per group. MPE = maximum possible effect.

Fig. 5. Reversal of spinal nerve ligation–induced thermal hyperalgesia by intrathecal isoguvacine (*A*) or baclofen (*B*). Data are expressed as mean ± standard error of the mean. N = 6 per group.
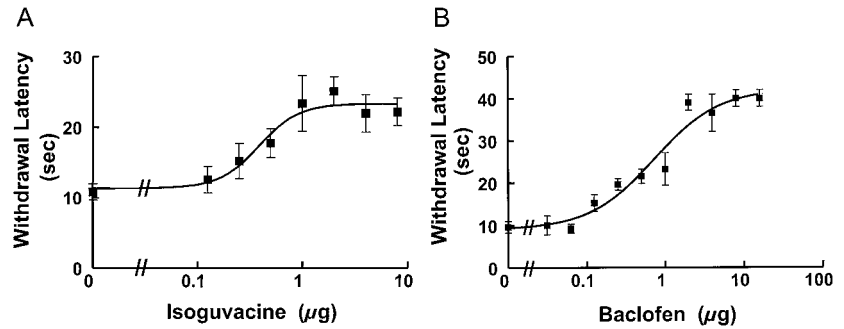
number have also yielded conflicting results. GABA$_B$ receptor binding was decreased after sciatic neurectomy, whereas GABA$_A$ receptor binding was increased.[30] In contrast, messenger RNA for the GABA$_A$ receptor was decreased in dorsal root ganglion after L5 SNL.[31]

If nerve injury–induced tactile allodynia and thermal hyperalgesia are produced by loss of endogenous GABA tone, GABA$_A$ agonist, GABA$_B$ agonist, or both should reverse these signs of increased nociceptive sensitivity. In our studies, both isoguvacine and baclofen relieved allodynia and hyperalgesia, consistent with this prediction. Relief of allodynia by spinal GABA$_A$ and GABA$_B$ agonists is consistent with previous studies. Baclofen and muscimol reversed touch-evoked allodynia produced by L5–L6 SNL and mechanical hyperalgesia produced by chronic constriction injury.[14,15] In contrast to findings in the SNL and chronic constriction injury models, intrathecal baclofen did not alleviate pain behaviors in a model of sciatic nerve injury produced by photochemically induced ischemia.[16]

Thermal hyperalgesia produced by peripheral nerve injury seems to have a different spinal pharmacology and neural circuitry than does tactile allodynia. For example, thermal hyperalgesia is much more responsive to intrathecal morphine than is tactile allodynia.[9] The afferent signal encoding tactile allodynia seems to be encoded by large, myelinated primary afferent fibers, whereas the signal encoding thermal hyperalgesia is carried by small, high-threshold unmyelinated fibers.[10] Thermal hyperalgesia seems to be mediated by pathways intrinsic to the spinal cord, whereas tactile allodynia requires the contribution of supraspinal sites.[11] Therefore, examination of both modalities is important when examining the spinal pharmacology of the nerve-injured state.

The ability of GABA receptor agonists to diminish behavioral responses to high-threshold stimuli has been demonstrated,[15,32–38] implying that pathways encoding high-threshold nociceptive input contain GABA receptor. The presence of GABA receptors on pathways encoding high-threshold input provides a mechanism for endogenous spinal GABA to act tonically to down-regulate thermal responsiveness. Disinhibition of these pathways by GABA antagonists would result in thermal hyperalgesia.

Isoguvacine exerted a specific antihyperalgesic effect at the doses used. That is, it relieved thermal hyperalgesia but did not affect thermal nociception. Previous studies using higher doses have shown antinociceptive effects of isoguvacine after intrathecal or epidural administration.[39,40] The reversal of hyperalgesia but not nociception at the doses used in this study may be because a smaller number of GABA$_A$ receptors need to be activated to reverse hyperalgesia than to produce antinociception.

The effects of GABA receptor agonists on nerve injury–induced tactile allodynia were blocked by the appropriate GABA receptor antagonists. The effects of baclofen on tactile allodynia were incompletely prevented by the GABA$_B$ receptor–selective antagonist phaclofen. However, the GABA$_B$ receptor–selective antagonist SCH50911 fully reversed the effect of baclofen, suggesting that the partial reversal by phaclofen may have been due to incomplete antagonism of baclofen at the GABA$_B$ receptor. Bicuculline and phaclofen have been reported to be selective for the GABA$_A$ and GABA$_B$ receptors, respectively. However, after nerve injury, we were not able to demonstrate significant receptor selectivity of these compounds. Bicuculline was only 10-fold more

Fig. 6. Effects of intrathecal isoguvacine (*A*) or baclofen (*B*) on ambulation. Data are expressed as mean ± standard error of the mean. N = 6 per group.
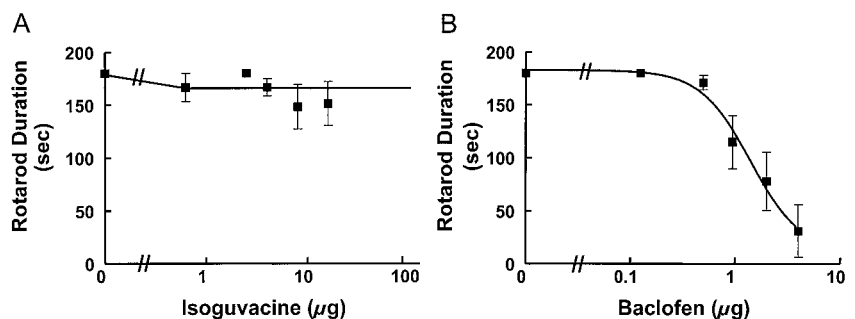
potent in reversing the actions of isoguvacine than in reversing the actions of baclofen. Similar doses of phaclofen were required to reverse the actions of isoguvacine and baclofen. Because these antagonists decrease withdrawal threshold or latency when administered alone, the apparent blockade of agonist actions may have been due to direct allodynic or hyperalgesic actions of biculline or phaclofen, rather than to blockade of the antiallodynic or antihyperalgesic of isoguvacine and baclofen at GABA receptors. With respect to agonist selectivity, our results show that isoguvacine acted only at the GABA$_A$ receptor because it did not produce the antinociceptive or motor effects observed with baclofen.

Analysis of antinociception, antiallodynia, and antihyperalgesia produced by baclofen is complicated by motor effects of the drug. Baclofen impaired performance on the rotarod apparatus in the antinociceptive, antiallodynic, and antihyperalgesic dose ranges, suggesting that motor dysfunction may have impaired withdrawal from the evocative stimuli, thereby mimicking antinociception, antiallodynia, and antihyperalgesia. Impairment of performance on the rotarod apparatus could be due to sensory dysfunction, sedation, loss of motor coordination, or motor weakness. The motor nature of these effects is supported by the qualitative observation that intrathecal baclofen–treated animals seemed to have motor weakness of the hind limbs

Our finding that isoguvacine did not produce significant motor dysfunction is in contrast to previous studies showing that the GABA$_A$ agonist muscimol produced motor weakness at analgesic doses.[34,36] This difference suggests that the motor effects of muscimol may have been caused by a mechanism not mediated by the GABA$_A$ receptor. Our results are consistent with those of Hwang and Yaksh[14] who, using a four-point scale of motor deficit scoring, did not observe motor deficits with antiallodynic doses of muscimol. Our results with baclofen are in contrast to those of Hwang and Yaksh,[14] who found that antiallodynic doses of baclofen did not produce visible motor deficits. This may be explained if rotarod testing is a more sensitive test of motor dysfunction than is the observational scale. The lack of specific antiallodynic and antihyperalgesic effects of baclofen at doses less than those producing motor dysfunction is consistent with the limited effectiveness of baclofen in many clinical cases of neuropathic pain.

These data support the hypothesis that neuropathic pain may result from a loss of GABA receptor–mediated inhibition of somatosensory pathways. Our results provide the first demonstration that GABA$_A$ and GABA$_B$ receptor–selective antagonists produce thermal hyperalgesia and that GABA$_A$ and GABA$_B$ receptor–selective agonists reverse the thermal hyperalgesia produced by peripheral nerve injury. In parallel, they confirm previous work demonstrating that GABA$_A$ and GABA$_B$ receptor antagonists agonists produce tactile allodynia and

that GABA$_A$ and GABA$_B$ receptor agonists reverse the tactile allodynia produced by peripheral nerve injury. Study of responses to both mechanical and thermal stimuli is crucial because thermal hyperalgesia has different spinal pharmacology and neural circuitry than does allodynia.[9–11] In addition, our studies used a sensitive, reproducible test of motor function to compare the antiallodynic, antihyperalgesic, and motor effects of GABA receptor agonists.

The specific antiallodynic and antihyperalgesic effects of isoguvacine, which lacks effects on acute nociception or motor function at antiallodynic and antihyperalgesic doses, suggest that GABA$_A$ agonists may be efficacious in the treatment of neuropathic pain without sensory or motor side effects.

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