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Comparison of the Antinociceptive Profiles of Gabapentin and 3-Methylgabapentin in Rat Models of Acute and Persistent Pain: Implications for Mechanism of Action

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Abbreviations: GBP, gabapentin; 3-MeGBP, 3-methylgabapentin; i.p., intraperitoneal; i.t. intrathecal; GABA, gamma-aminobutyric acid; SNL, spinal nerve ligation

ABSTRACT

The anticonvulsant gabapentin (GBP) has been shown to be effective for the treatment of neuropathic pain, although its mechanism of action remains unclear. A recent report has suggested that binding to the α2δ subunit of voltage gated calcium channels contributes to its antinociceptive effect, based on the stereoselective efficacy of two analogs: (1S,3R)3-MeGBP (IC50=42 nM) which is effective in neuropathic pain models, and (1R,3R)3-MeGBP (IC50>10000 nM) which is ineffective (Field et al., 2000). The present study was designed to further examine the profiles of GBP and 3-MeGBP in rat models of acute and persistent pain. Systemic administration of GBP or (1S,3R)3-MeGBP inhibited tactile allodynia in the spinal nerve ligation model of neuropathic pain, whereas (1R,3R)3-MeGBP was ineffective. The antiallodynic effect of GBP, but not (1S,3R)3-MeGBP, was blocked by i.t. injection of the GABAB receptor antagonist CGP52432. Systemic GBP or (1S,3R)3-MeGBP also inhibited the second phase of formalin-evoked nociceptive behaviors, whereas (1R,3R)3-MeGBP was ineffective. However, both (1S,3R)3-MeGBP and (1R,3R)3-MeGBP, but not GBP, inhibited first phase behaviors. In the carrageenan model of inflammatory pain, systemic GBP or (1R,3R)3-MeGBP failed to inhibit thermal hyperalgesia, whereas (1S,3R)3-MeGBP had a significant albeit transient effect. Systemic (1S,3R)3-MeGBP, but not GBP or (1R,3R)3-MeGBP, also produced an antinociceptive effect in the warm water tail withdrawal test of acute pain. These data demonstrate that GBP and 3-MeGBP display different antinociceptive profiles, suggesting dissimilar mechanisms of antinociceptive action. Thus, the stereoselective efficacy of 3-MeGBP, presumably related to α2δ binding, likely does not completely account for the mechanism of action of GBP.

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Gabapentin (GBP; Neurontin ®) is an anticonvulsant that has found increased utility for the treatment of clinical neuropathic pain. Although originally developed for the treatment of spasticity and epilepsy, recent attention has focused on the utility of GBP for the treatment of neuropathic pain based on its efficacy and minimal side effect profile in clinical trials (Rice and Maton, 2001). In rodent neuropathic pain models, GBP effectively attenuates thermal and mechanical hypersensitivity following peripheral nerve ligation (Hwang and Yaksh, 1997; Hunter et al., 1997; Xiao and Bennett, 1996). GBP has also been shown to inhibit thermal and mechanical hyperalgesia following carrageenan-induced inflammation (Field et al., 1997b; Lu and Westlund, 1999); however, other studies have reported limited effectiveness of GBP for inflammatory pain (Gould et al., 1997; Patel et al., 2001). Additionally, GBP inhibits spontaneous nociceptive behaviors and mechanical hyperalgesia produced by intra-plantar formalin or surgical incision, respectively (Field et al., 1997a, b). The antinociceptive effects of GBP in models of neuropathic, inflammatory and surgical pain appear to be selective for injury-induced hypersensitivity, since responses to acute noxious stimuli are unaffected (Field et al., 1997b; Hunter et al., 1997).

Despite growing interest in the analgesic properties of GBP, its mechanism of action remains unclear. Although a GABA analog, GBP does not bind GABAA or GABAB receptors, nor does it interact with GABA transporters (for review see Taylor et al., 1998). GBP has been shown, however, to increase brain extracellular GABA levels in both rat and human studies (Loscher et al., 1991; Petroff et al., 1996). This increased extracellular GABA is likely due to either directly stimulated GABA release (Gotz et al., 1993; Gu and Huang, 2002), or changes in GABA metabolism via effects on glutamic acid decarboxylase and/or GABA-transaminase (Goldlust et al., 1995). The notion that GBP increases extracellular GABA is consistent with it's effectiveness for neuropathic

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pain, since the pathology associated with this condition includes disruption of tonic inhibitory GABAergic transmission (Wiesenfeld-Hallin et al., 1997).

In addition to enhancing GABAergic transmission, it has been hypothesized that GBP modulates voltage-gated calcium channels, resulting in decreased neurotransmitter release. In support, GBP inhibits K^+-evoked excitatory amino acid neurotransmitter release in neocortical and trigeminal nucleus slices (Fink et al., 2000; Maneuf and McKnight, 2001). Additionally, GBP has been shown to inhibit voltage-gated calcium currents in dorsal root ganglia neurons (Sutton et al., 2002). GBP-mediated inhibition of voltage-gated calcium channels would result in a reduction of excitatory transmission in the spinal cord dorsal horn, consistent with an inhibition of spinal nociceptive transmission (Shimoyama et al., 2000).

The discovery of the α2δ subunit of voltage-gated calcium channels as a high affinity binding site for GBP has further supported a role for voltage-gated calcium channels in it's antinociceptive action (Gee et al., 1996). Specific genes encoding three α2δ subtypes have been identified (α2δ-1, α2δ-2, α2δ-3), with α2δ-1 displaying the highest affinity for GBP (Marais et al., 2001). A specific role for α2δ in neuropathic pain was originally described by Luo et al. (2001), who found an increase in α2δ expression in the dorsal root ganglion ipsilateral to the peripheral nerve injury that corresponded to the development of tactile allodynia. Additionally, Luo et al. (2002) reported that gabapentin efficacy was only evident in specific rat neuropathic pain models in which increased α2δ expression was observed. Further evidence supporting a role for α2δ in the antinociceptive action of GBP was described in a recent study by Field et al. (2000), in which the authors utilized two GBP analogs that stereoselectively interact with α2δ: (1S,3R)3-MeGBP (IC_{50}=42 nM) and (1R,3R)3-MeGBP (IC_{50}>10000 nM). The results

demonstrated that while (1S,3R)3-MeGBP effectively reversed neuropathy-induced allodynia, (1R,3R)3-MeGBP was ineffective, supporting stereoselective efficacy related to α2δ binding. Given these results, the present series of experiments were designed to further compare the antinociceptive profiles of GBP and 3-MeGBP in order to better gauge the significance of the stereoselective efficacy of 3-MeGBP in terms of GBP action.

MATERIALS AND METHODS

Animal Care

Adult male Sprague Dawley rats (Harlan, San Diego) weighing 175-200 g were used in the experiments involving the spinal nerve ligation model, whereas rats weighing 275-300 g were used in the experiments involving the formalin model, carrageenan model, and warm water tail withdrawal test. Rats were housed 3 per cage, and those rats receiving indwelling intrathecal (i.t.) catheters were subsequently housed individually. All rats were maintained on a standard 12 hr light-dark cycle, and had free access to food and water. The experimental procedures described in the present study were approved by the Merck Institutional Animal Care and Use Committee and were performed in accordance with *The Guide for the Care and Use of Laboratory Animals.*

Surgical Procedures

L5/L6 spinal nerve ligation Rats were anesthetized with isoflurane (4-5% induction, 2-3% maintenance). Using aseptic technique, the left paraspinal muscles were dissected from the spinous processes at the levels of L4-S2, and the left L5 and L6 spinal nerves were isolated. Each spinal nerve was tightly ligated with a 4-0 silk suture distal to the dorsal root ganglion (Kim and Chung, 1992). Following spinal nerve ligation, the wound was sutured and the skin was closed with veterinarian grade cyanoacrylate.

Intrathecal (i.t.) catheter implantation 7 days following spinal nerve ligation, a subset of rats received an indwelling intrathecal catheter for spinal drug delivery. Rats were anesthetized as described above, the atlanto-occipital membrane was exposed, and a 33-gauge polyurethane catheter (ReCath Co., Allison Park, PA) was inserted into the

spinal subarachnoid space extending to the level of the lumbar enlargement. Following insertion of the catheter, the muscle was sutured and the skin was closed with veterinarian grade cyanoacrylate.

Spinal nerve ligation model

10-14 days following spinal nerve ligation, rats were placed in individual Plexiglas chambers on an elevated wire mesh where they were allowed to acclimate for 1 hr. Following the acclimation period, rats were tested for tactile allodynia by applying a series of calibrated von Frey filaments to the plantar aspect of the left hind paw ipsilateral to the site of nerve injury. The mean 50% withdrawal threshold (g.) was determined using the Dixon "up-down" test (Chaplan et al., 1994). Rats that displayed a pre-drug withdrawal threshold >4 g. were not considered allodynic and were excluded from the study. Following determination of pre-drug withdrawal thresholds, rats received systemic (i.p.) GBP, 3-MeGBP, or vehicle injection, and effects on tactile allodynia were determined over time by measuring hind paw withdrawal thresholds 30, 60, 90, 120 min post-injection. For the experiments examining the effects of CGP52432 on the antiallodynic action of systemic drugs, CGP52432 was injected i.t. immediately prior to the systemic injection. I.t. injection was performed using a 27 gauge needle connected to a Hamilton 50 μl syringe with PE50 tubing. 5 μl of CGP52432 or vehicle was delivered over a 30 sec period followed by a 5 μl flush of vehicle. At the conclusion of the experiment, i.t. catheter tip placement at the level of the lumbar enlargement was confirmed by injecting 5 μl 4% lidocaine, which produced a temporary hind limb paralysis.

Formalin model

Formalin-induced spontaneous nociceptive behaviors were recorded using an Automated Nociception Analyzer Instrument (Univ. of California San Diego). A small metal band was placed on the left hind paw of rats using a small amount of cyanoacrylate adhesive. Following a 30 min. acclimation period, rats received a s.c. injection of formalin (5%, 50 μl) into the plantar aspect of the left hind paw. Immediately following formalin injection, rats were placed in individual chambers containing an electromagnetic field. Spontaneous nociceptive behaviors consisting of flinching and shaking of the affected hind paw were recorded and quantified by a computer. The total number of nociceptive behaviors were quantified in two phases (Phase I, 0-5 min; Phase II, 20-40 min). Systemic (i.p.) GBP, 3-MeGBP, or vehicle was administered 60 min prior to s.c. formalin injection, such that effects on formalin-induced nociceptive behaviors were determined at the time of maximal efficacy (i.e. 60-100 min post-injection).

Carrageenan model

Rats were placed in individual Plexiglas chambers on top of a semitransparent horizontal surface. Thermal hind paw withdrawal latencies were measured by applying focused infra-red heat to the plantar aspect of the left or right hind paw and recording the time required to withdraw the hind paw from the noxious thermal stimulus (Stoelting, Wood Dale, IL). Withdrawal of the hind paw from the thermal stimulus terminated the test (recorded to the nearest 0.1 sec). The thermal stimulus was applied to each hind paw 3 times, and the average thermal hind paw withdrawal latency was determined from the last 2 latencies. A 21.5 sec. cut-off latency was used to avoid tissue damage. Following

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determination of pre-carrageenan withdrawal latencies, a carrageenan colloidal suspension was prepared (2% carrageenan in 0.9% saline), and rats were injected with carrageenan (50 µl) into the plantar aspect of the left hind paw. 3 hr following carrageenan injection, at the time of maximal hyperalgesia, rats were tested for post-carrageenan thermal withdrawal latencies, and rats subsequently received systemic (i.p.) injection of GBP, 3 Me-GBP, or vehicle. Effects on thermal withdrawal latencies were determined 30, 60, 90, 120 min post-drug injection.

Warm water tail withdrawal test

Rats were wrapped in a towel, and approximately half of the tail was submerged in a warm water bath held at a constant temperature of 52 ^{o} C. The time required to initiate the tail withdrawal reflex was recorded using a stopwatch and was designated as the tail withdrawal latency (recorded to the nearest 0.1 sec). A cut-off latency of 15 seconds was used to prevent tissue injury. Rats were tested once at each time point, and following determination of pre-drug withdrawal latencies, rats received systemic (i.p.) injection of GBP, 3-MeGBP, or vehicle. Effects on tail withdrawal latencies were determined 30, 60, 90, 120 min post-drug injection.

Data analysis and statistics

All behavioral experimental groups consisted of 5-8 rats. For all experiments the data were represented as mean ± SEM of the response. Statistical analysis of drug effect was performed by comparing post-drug response to pre-drug response (or vehicle in the formalin model experiments) using a one-way ANOVA with Dunnett's test for post hoc comparisons. For the experiments involving the spinal nerve ligation model, the 50%

inhibitory dose (ID_{50}) and 95% confidence limits were determined from the dose-response functions by comparing thresholds in drug treated rats to age-matched naïve control rats. The data were converted to % inhibition (% inhibition = [post-drug threshold – pre-drug threshold]/[naïve – pre-drug threshold] X 100), and a computer program was used to calculate the dose required to produce a 50% inhibition of the allodynic response at the time of maximal effect.

Drugs

The drugs used in the present experiments were gabapentin, (1S,3R)3-methylgabapentin, (1R,3R)3-methylgabapentin (Merck Research Laboratories); baclofen (Sigma Chemical Co., St. Lous, MO); and CGP52432 (3-[[(3,4-Dichlorophenyl)methyl]amino]propyl] diethoxymethyl)phosphinic acid; Tocris, Ellisville MO). All drugs were dissolved in 0.9% saline (pH ~7).

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RESULTS

Effects of GBP and 3-MeGBP on SNL-induced tactile allodynia.

SNL resulted in a decreased tactile withdrawal threshold (tactile allodynia) compared to age-matched naïve rats which was dose-dependently inhibited by systemic (i.p.) administration of GBP ($p < 0.05$; Fig. 1, 2A). The greatest dose of 100 mg/kg produced a complete inhibition of the tactile allodynia (113.1 ± 7.9 % inh.) at the time of maximal effect. The ID_{50} (95% C.L.) for this effect was 32.3 (25.6 – 40.9) mg/kg.

Similar to GBP, i.p. administration of (1S,3R)3-MeGBP dose-dependently inhibited SNL-induced tactile allodynia ($p < 0.05$; Fig. 1, 2B). The greatest dose of 100 mg/kg completely inhibited tactile allodynia (91.5 ± 19.0 % inh.) at the time of maximal effect. The ID_{50} (95% C.L.) for this effect was 58.1 (44.1 – 76.6) mg/kg. In contrast, i.p. administration of (1R,3R)3-MeGBP at a dose of 100 mg/kg had no effect on tactile withdrawal thresholds ($p > 0.05$; Fig. 1, 2B).

Effects of i.t. CGP52432 on the antiallodynic action of GBP and 3-MeGBP.

To examine a potential role for spinal GABAB receptors in the antiallodynic action of GBP and (1S,3R)3-MeGBP, the effect of i.t. injection of the GABAB receptor antagonist CGP52432 was determined. Systemic (i.p.) administration of the GABAB receptor agonist baclofen (6 mg/kg) inhibited SNL-induced tactile allodynia, and this antiallodynic effect was dose-dependently blocked by i.t. injection of CGP52432 immediately prior to baclofen injection ($p < 0.05$; Fig. 3A). The ID_{50} (95% C.L.) for blocking this effect was 0.25 (0.09 – 0.71) µg , and a complete block was achieved using the dose of 3.0 µg.

CGP52432 (3.0 μg) had no effect on tactile withdrawal thresholds alone (data not shown).

Systemic (i.p.) administration of GBP (60 mg/kg) inhibited SNL-induced tactile allodynia, and this antiallodynic action of GBP was completely blocked by i.t. injection of CGP52432 (3.0 μg) immediately prior to GBP injection ($p < 0.05$; Fig. 3B). In contrast, the antiallodynic action of systemically administered (1S,3R)3-MeGBP (60 mg/kg) was unaffected by i.t. injection of CGP52432 (3.0 μg) ($p > 0.05$; Fig. 3B).

Effects of GBP and 3-MeGBP on formalin-induced nociceptive behaviors.

Intra-plantar injection of formalin (5%, 50 μl) into the left hind paw resulted in spontaneous nociceptive behaviors consisting of flinching and shaking of the affected paw that were quantified into 2 phases (Phase I, 0-5 min; Phase II, 20-40 min). Systemic administration of GBP (100 mg/kg) 60 min prior to formalin injection produced a significant inhibition of nociceptive behaviors compared to vehicle in Phase II (45 ± 8 % inh., $p < 0.05$), but had no effect on Phase I behaviors (-18 ± 18 % inh., $p > 0.05$) (Fig. 4A).

In contrast to GBP, systemic administration of (1S,3R)3-MeGBP (100 mg/kg) 60 min prior to formalin produced a significant inhibition of nociceptive behaviors in Phase I (65 ± 8 % inh., $p < 0.05$) and Phase II (39 ± 8 % inh., $p < 0.05$) (Fig. 4B). Additionally, systemic administration of (1R,3R)3-MeGBP (100 mg/kg) significantly inhibited nociceptive behaviors in Phase I (77 ± 3 % inh., $p < 0.05$), but had no effect on behaviors in Phase II (-10 ± 12 % inh., $p > 0.05$) (Fig. 4B).

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Effects of GBP and 3-MeGBP on carrageenan-induced thermal hyperalgesia.

Intra-plantar injection of carrageenan into the left hind paw resulted in a decreased thermal paw withdrawal latency in the ipsilateral hind paw 3 hr following injection (post-carr) compared to pre-carr (Fig. 5A, B). No change in thermal withdrawal latency was observed for the contralateral paw (data not shown). Systemic administration of GBP (10-100 mg/kg) had no effect on carrageenan-induced thermal hyperalgesia ($p > 0.05$; Fig. 5A), and did not affect the thermal withdrawal latency for the contralateral paw (data not shown).

Systemic administration of (1S,3R)3-MeGBP (30, 100 mg/kg), on the other hand, transiently inhibited carrageenan-induced thermal hyperalgesia 30 min following injection (60.0 ± 29.0 % inh. at 30 mg/kg; 60.7 ± 16.6 % inh. at 100 mg/kg; $p < 0.05$) (Fig. 5B), but did not affect the thermal withdrawal latency for the contralateral paw (data not shown). Systemic (1R,3R)3-MeGBP had no effect on carrageenan-induced thermal hyperalgesia ($p > 0.05$; Fig. 5B), and did not affect the thermal withdrawal latency for the contralateral paw (data not shown).

Effects of GBP and 3-MeGBP on thermal tail withdrawal latency.

Systemic administration of GBP (100 mg/kg) had no effect on thermal tail withdrawal latency from a 52 º C warm water bath compared to pre-drug latency ($p > 0.05$; Fig. 6A).

In contrast, systemic administration of (1S,3R)3-MeGBP (100 mg/kg) produced an increase in tail withdrawal latency compared to pre-drug which was maximal 60 min

following injection (26.1 ± 2.0 % inh., $p < 0.05$) (Fig. 6B). Systemic administration of (1R,3R)3-Me-GBP (100 mg/kg) had no effect of thermal tail withdrawal latency compared to pre-drug ($p > 0.05$; Fig. 6B).

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DISCUSSION

The results from the present study demonstrate that GBP and 3-MeGBP have dissimilar antinociceptive profiles in models of acute and persistent pain, suggesting that their mechanisms of antinociceptive action differ. Preclinical studies have found that GBP effectively attenuates neuropathy-induced hyperalgesia and allodynia in rodent neuropathic pain models, at least in part, via a spinal site of action (Hwang and Yaksh, 1997; Hunter et al., 1997; Xiao and Bennett, 1996). It has been hypothesized that this antinociceptive action may be due to binding to the α2δ subunit of voltage-gated calcium channels, resulting in inhibition of calcium currents and neurotransmitter release (Fink et al., 2000; Gee et al., 1996; Maneuf and McKnight, 2001; Sutton et al., 2002). The synthesis of GBP analogs with varying affinities for α2δ has provided an additional strategy to examine the role of α2δ in the antinociceptive action of GBP (Bryans et al., 1998). Using this approach, Field et al. (2000) evaluated the antiallodynic efficacy of two stereoisomers displaying stereoselective binding to α2δ: (1S,3R)3-MeGBP (IC_{50} = 42 nM) and (1R,3R)3-MeGBP (IC_{50} > 10000 nM). The results from that study showed that (1S,3R)3-MeGBP, but not (1R,3R)3-MeGBP, effectively attenuated allodynia in the streptozocin and SNL models of neuropathic pain, supporting the notion that α2δ binding contributes to the antiallodynic action of GBP (IC_{50} = 140 nM). In the present study, systemic (1S,3R)3-MeGBP was found to attenuate SNL-induced tactile allodynia (ID_{50} = 58.1 mg/kg) in a dose range similar to GBP (ID_{50} = 32.3 mg/kg), whereas (1R,3R)3-MeGBP was ineffective (ID_{50} > 100 mg/kg). These data are in agreement with Field et al. (2000), and support the conclusion from that study that binding to α2δ contributes to the antiallodynic action of GBP in neuropathic pain models. Although these results do not unequivocally demonstrate that α2δ binding is responsible for gabapentin's action, recent reports have provided more direct evidence to support his notion. For example,

disruption of gabapentin binding to α2δ using ruthenium red or magnesium chloride selectively attenuates the antinociceptive effect of gabapentin in vivo (Cheng et al., 2003). Moreover, the antinociceptive efficacy of gabapentin has been shown to be completely absent in transgenic mice in which a single amino substitution on the α2δ subunit abolished gabapentin binding (Taylor 2004). These results, in addition to the stereoselective efficacy of 3-MeGBP, seem to support a role for α2δ binding in the antinociceptive action of GBP.

The stereoselective efficacy of 3-MeGBP in the SNL model, however, does not appear to entirely relate to the mechanism of action of GBP, based on the differential role of spinal GABAB receptors in the actions of these compounds. In the present study, prior i.t. administration of the GABAB receptor antagonist CGP52432 blocked the antiallodynic effect of systemic GBP, but not (1S,3R)3-MeGBP, supporting a role for spinal GABAB receptors in the antiallodynic action of GBP, but not (1S,3R)3-MeGBP. This effect appears to involve a selective blockade of GABAB receptors, since the dose of CGP52432 used completely inhibited the antiallodynic action of the GABAB receptor agonist baclofen. Since GBP does not directly bind to GABAA or GABAB receptors, the GABAB receptor-mediated antiallodynic effect is likely the result of an indirect enhancement of spinal GABAergic transmission. GBP has been shown to stimulate GABA release in vitro and increase extracellular GABA levels in rat and human studies (Gotz et al., 1993; Loscher et al., 1991; Petroff et al., 1996). Additionally, neuropathy-induced hypersensitivity is known to involve disruption of tonic GABAergic transmission, and GABA agonists and metabolic inhibitors have been shown to be effective in neuropathic pain models (Giardina et al., 1998; Malan et al., 2002; Wiesenfeld-Hallin et al., 1997). Moreover, evidence for a role of GABAB receptors in the anticonvulsant action of GBP has been previously suggested (Cao et al., 2001). The ability of i.t.

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CGP52432 to block the antiallodynic action of GBP is consistent with a specific GBP-induced increase in extracellular spinal GABA which appears to be sufficient for its antiallodynic efficacy, and may not be related to α2δ binding.

In the present study, the antinociceptive actions of GBP and 3-MeGBP were additionally discriminated by different effects on formalin-induced spontaneous nociceptive behaviors. Intra-plantar injection of formalin is a commonly used model of acute inflammatory pain in which rodents display spontaneous nociceptive behaviors consisting of flinching/shaking of the affected hind paw in two distinct phases (Dubuisson and Dennis, 1977). The first and second phases are generally believed to reflect excitation of peripheral afferent nociceptors, and central sensitization, respectively (Puig and Sorkin, 1995; Dickenson and Sullivan, 1987; Yaksh et al., 2001). Consistent with previous reports (Field et al., 1997b), GBP was found to selectively attenuate second phase nociceptive behaviors in the present study, suggesting a specific inhibition of central sensitization. Moreover, (1S,3R)3-MeGBP, but not (1R,3R)3-MeGBP, attenuated second phase nociceptive behaviors, demonstrating a stereoselective effect of 3-MeGBP on central sensitization consistent with α2δ binding. Interestingly, both (1S,3R)3-MeGBP and (1R,3R)3-MeGBP, but not GBP, were found to attenuate first phase nociceptive behaviors as well. The observation that both 3-MeGBP stereoisomers were equipotent in inhibiting first phase behaviors suggests that these compounds have additional mechanisms of antinociceptive action specific to inhibition of nociceptor activity that are not related to α2δ binding. Different mechanisms have been shown to be involved in phase I and phase II nociceptive behaviors, based on the differential pharmacology associated with these behaviors. For example, while second phase behaviors are selectively attenuated by NMDA receptor antagonists, cyclooxygenase inhibitors, and nitric oxide synthase inhibitors, first and second phase

behaviors are attenuated by opioids, GABA agonists, and calcium channel blockers (for review see Yaksh et al., 2001). Although the results from the present study do not identify a specific mechanism of action of 3-MeGBP, these data further demonstrate that diverse mechanisms contribute to the antinociceptive effects of 3-MeGBP, at least some of which are not attributable to α2δ binding.

In addition to the formalin model, different antinociceptive profiles for GBP and 3-MeGBP were observed in the present study using the carrageenan model of inflammatory pain. Carrageenan is seaweed extract which produces a localized inflammation and thermal hyperalgesia following intra-plantar injection (Hargreaves et al., 1988). In the present study, both GBP and (1R,3R)3-MeGBP were found to be ineffective in inhibiting carrageenan-induced thermal hyperalgesia, whereas (1S,3R)3-MeGBP produced a short-lived antihyperalgesic effect. Previous studies examining the effects of GBP on inflammation-induced hyperalgesia have reported somewhat inconsistent results. For example, although GBP has been shown to inhibit thermal hyperalgesia following intra-plantar carrageenan injection (Field et al., 1997b), GBP was found to be ineffective or only minimally effective in other inflammatory pain models (Gould et al., 1997; Patel et al., 2001). It is unclear why the present results are somewhat in conflict with those reported by Field et al. (1997b), although differences in carrageenan concentration, route of GBP administration, and times of behavioral testing may explain this inconsistency. Nevertheless, the observation that (1S,3R)3-MeGBP, but not GBP, was effective in inhibiting carrageenan-induced hyperalgesia suggests that this effect is not mediated by α2δ binding, and supports the notion that the mechanisms of action of these compounds differ.

Consistent with previous reports, GBP was found to be ineffective in inhibiting behavioral responses to an acute noxious stimulus in the present study. The lack of effect of GBP in the warm water tail withdrawal test supports the notion that the antinociceptive action of GBP is related to specific mechanisms associated with the sensitized state following injury (Field et al., 1997b; Hunter et al., 1997; Maneuf and McKnight, 2001). Interestingly, (1S,3R)3-MeGBP, but not (1R,3R)3-MeGBP produced an inhibition of the warm water tail withdrawal reflex. The different effects observed with these stereoisomers are likely not due to their stereoselective binding to α2δ, since GBP was ineffective. Moreover, that (1R,3R)-3MeGBP inhibited first phase formalin behaviors but was ineffective in the tail withdrawal test suggests a specific action following persistent, but not acute, nociceptor activation. These results further demonstrate that the mechanism of action for GBP cannot be fully explained by the stereoselective efficacy of 3-MeGBP.

To summarize, although the specific mechanism(s) of action for GBP and 3-MeGBP remain unclear, indirect activation of spinal GABAB receptors appears to be sufficient for the antiallodynic effect of GBP, but not 3-MeGBP, in the SNL model. The results from the formalin studies demonstrate that the 3-MeGBP stereoisomers have multiple mechanisms of antinociceptive action, both unrelated and possibly related to α2δ binding. Additionally, the stereoselective effects of 3-MeGBP in the carrageenan model and tail withdrawal test do not appear to be related to α2δ binding, and thus the role of α2δ binding in the action of GBP remains ambiguous. The different profiles of GBP and 3-MeGBP in pain models suggest that multiple mechanisms likely contribute to their antinociceptive effects, including modulation of calcium channels via α2δ binding, modulation of GABAergic transmission, and possibly additional unidentified

mechanisms. The degree to which these mechanisms are necessary and/or sufficient for the actions of these compounds remains to be elucidated.

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FOOTNOTES

1. Supported by Merck & Co.

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FIGURE LEGENDS

Figure 1. Chemical structures of gabapentin, (1S,3R)3-methylgabapentin and (1R,3R)3-methylgabapentin.

Figure 2. (A) Dose-dependent inhibition of SNL-induced tactile allodynia following systemic (i.p.) injection of GBP or vehicle (0.9% saline). (B) Dose-dependent inhibition of SNL-induced tactile allodynia following systemic (i.p.) injection of (1S,3R)3-MeGBP, but not (1R,3R)3-MeGBP. SNL (pre-drug) resulted in decreased withdrawal thresholds compared to naïve rats. The data are represented as the mean ± SEM of the 50% withdrawal threshold (Chaplan et al., 1994) over time following systemic injection. * significant increase in withdrawal threshold compared to pre-drug (one-way ANOVA, $p < 0.05$).

Figure 3. (A) Dose-dependent inhibition of the antiallodynic effect of systemic baclofen (6 mg/kg) by i.t. administration of the GABAB receptor antagonist CGP52432 immediately prior to baclofen. CGP52432 (0.3, 3 μg) significantly attenuated the antiallodynic response to baclofen (one-way ANOVA, $p < 0.05$). (B) Inhibition of the antiallodynic effect of systemic GBP (60 mg/kg), but not (1S,3R)3-MeGBP (60 mg/kg), by i.t. administration of the GABAB receptor antagonist CGP52432 immediately prior to systemic injection. The data are represented as the mean ± SEM of the 50% withdrawal threshold over time following systemic injection. CGP52432 significantly attenuated the antiallodynic response to GBP (one-way ANOVA, $p < 0.05$).

Figure 4. (A) Inhibition of formalin-evoked spontaneous nociceptive behaviors by systemic GBP (100 mg/kg). (B) Inhibition of formalin-evoked spontaneous nociceptive

behaviors by systemic (1S,3R)3-MeGBP and (1R,3R)3-MeGBP (100 mg/kg). The data are represented as the mean ± SEM of the total number of nociceptive behaviors 0-5 min (Phase I) and 20-40 min (Phase II) following formalin injection. * significant inhibition of nociceptive behaviors compared to 0.9% saline vehicle (one-way ANOVA, $p < 0.05$).

Figure 5. (A) Lack of effect of systemic GBP on carrageenan-induced thermal hyperalgesia. (B) Inhibition of carrageenan-induced thermal hyperalgesia following systemic (1S,3R)3-MeGBP, but not (1R,3R)3-MeGBP. Intra-plantar carrageenan resulted in decreased thermal paw withdrawal latencies three hours following injection (post-carr). The data are represented as the mean ± SEM of the thermal paw withdrawal latency over time following systemic injection. * significant increase in thermal withdrawal latency compared to post-carr (one-way ANOVA, $p < 0.05$).

Figure 6. (A) Lack of effect of systemic GBP on the tail withdrawal reflex from a 52 ° C warm water bath. (B) Inhibition of the tail withdrawal reflex by systemic (1S,3R)3-MeGBP, but not (1R,3R)3-MeGBP. The data are represented as the mean ± SEM of the tail withdrawal latency over time following systemic injection. * significant increase in tail withdrawal latency compared to pre-drug (one-way ANOVA, $p < 0.05$).

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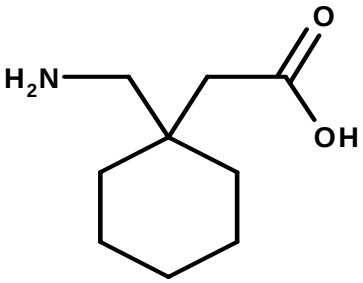
Fig. 1



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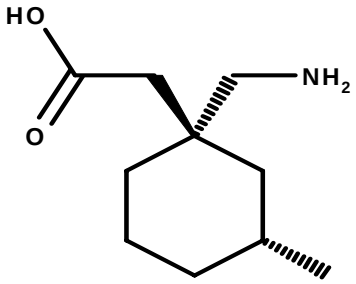

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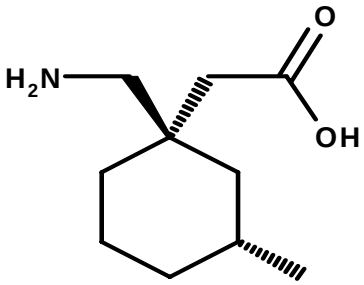

(1R,3R)3-MeGBP

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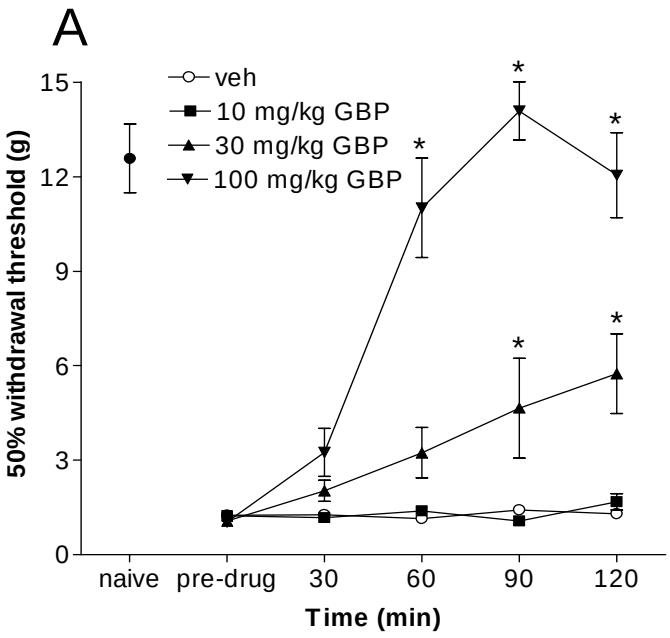
Fig. 2



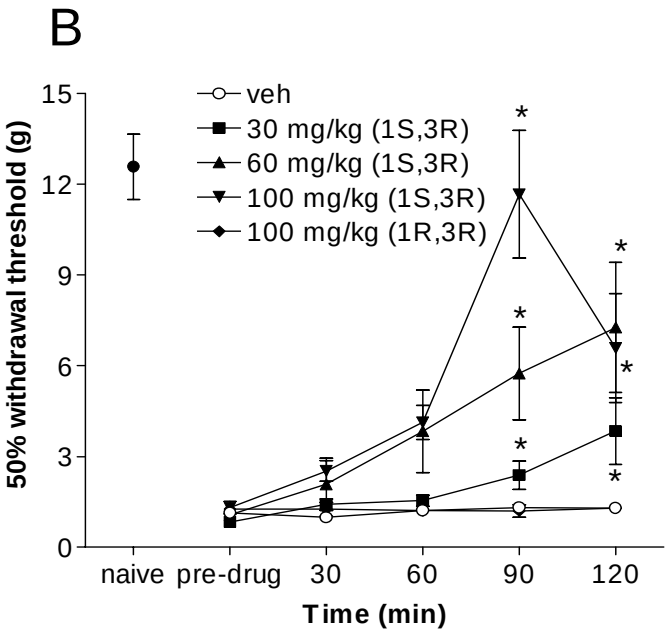


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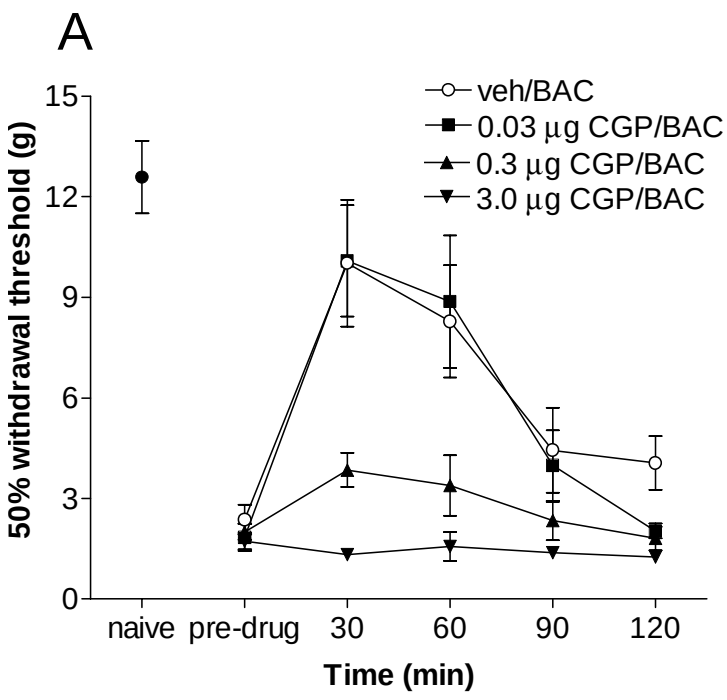
Fig. 3



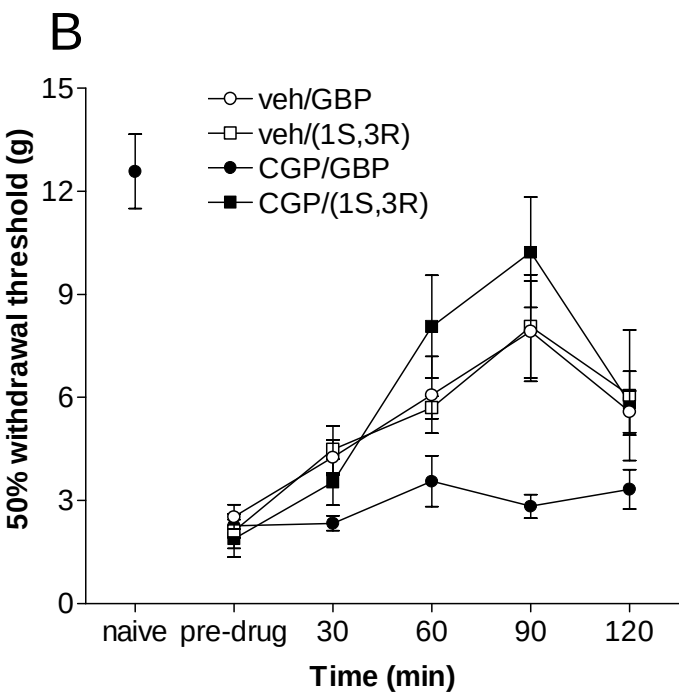


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Fig. 4

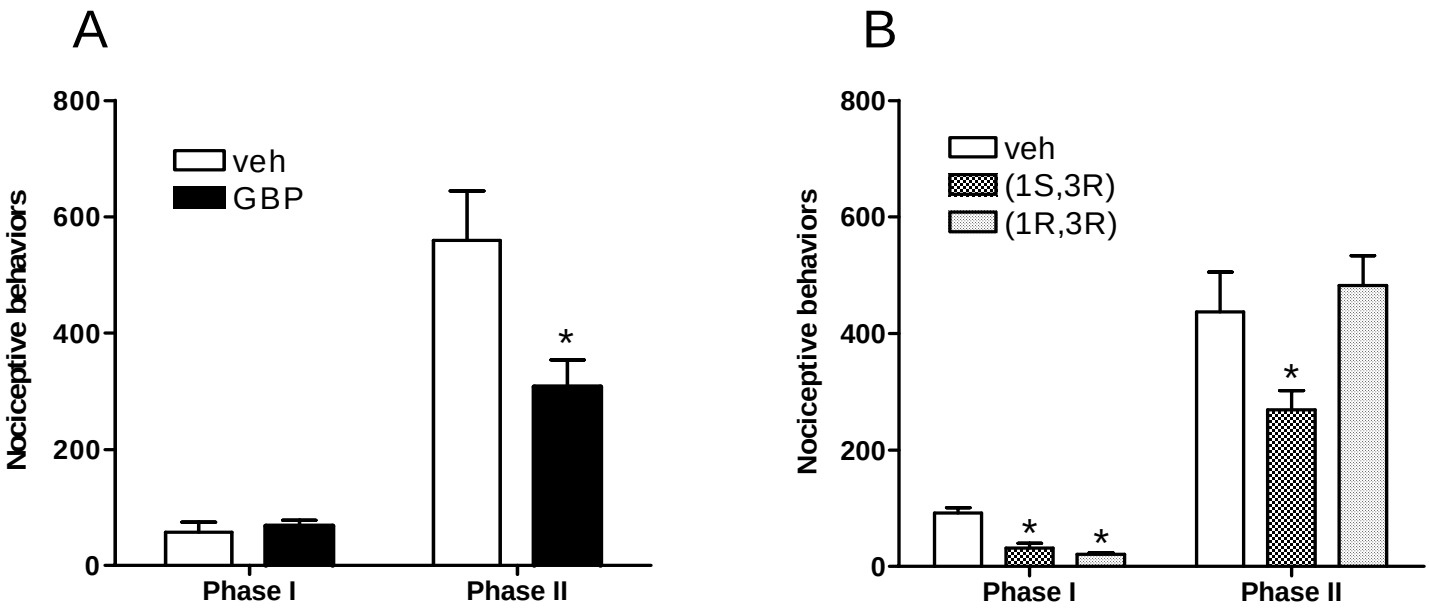

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Fig. 5

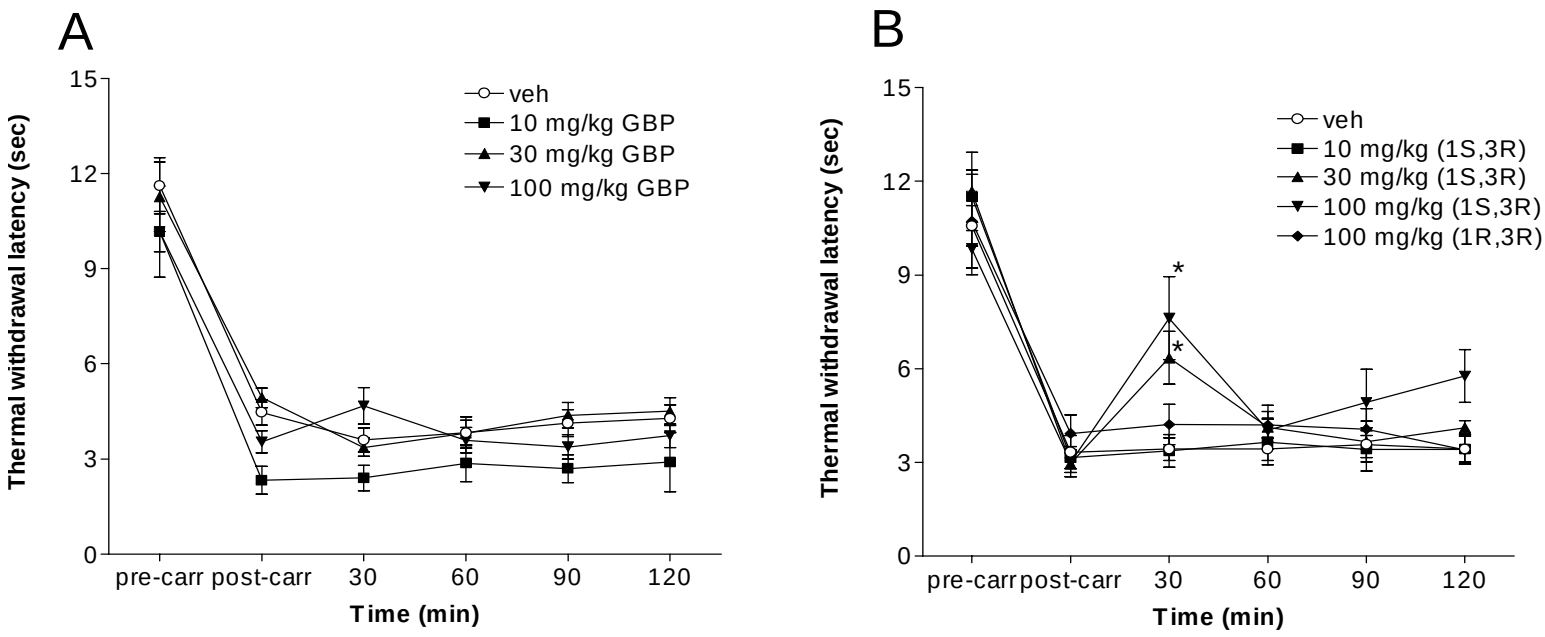

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Fig. 6