

Comparison of Antiepileptic Drugs Tiagabine, Lamotrigine, and Gabapentin in Mouse Models of Acute, Prolonged, and Chronic Nociception

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ABSTRACT

Some antiepileptic drugs have been shown to be clinically effective in the treatment of neuropathic pain. This study determined whether the new antiepileptic drug tiagabine, a GABA uptake inhibitor, is efficacious in mice in a broad range of nociceptive tests (hot-plate, formalin, and dynorphin-induced chronic allodynia) and compared tiagabine's potency with two other antiepileptic drugs, gabapentin and lamotrigine. Intraperitoneally administered tiagabine, but not lamotrigine, gabapentin, or i.t. tiagabine, produced dose-dependent antinociception in the hot-plate test. A 5-min pretreatment with tiagabine (2–29 nmol i.t.) dose-dependently inhibited both the acute and late phase formalin behaviors; pretreatment with lamotrigine (4–265

nmol i.t.) inhibited only the late phase. In the formalin assay the GABA_A antagonist bicuculline reversed the acute phase antinociception, whereas the GABA_B antagonist saclofen reversed both the acute and late phase tiagabine-induced antinociception. Tiagabine administered i.p. but not i.t. dose-dependently reduced dynorphin-induced chronic allodynia for 120 min. Gabapentin and lamotrigine produced antinociception administered either i.t. or i.p. in a dose-dependent manner. Thus, we have shown that gabapentin and lamotrigine produced antinociception in two mouse models of pain, whereas tiagabine produced antinociception in all three mouse models of pain.

Enhancement of GABA neurotransmission may provide an approach to diminish the level of nociception in various pain states. Several studies have implicated GABA_A and GABA_B receptors in spinal nociceptive circuitry. GABA_A but not GABA_B receptor agonists inhibit *N*-methyl-D-aspartate-induced nociceptive behaviors (Aanonsen and Wilcox, 1989), whereas both GABA_A and GABA_B receptor agonists inhibit substance P-induced nociceptive behaviors (Hwang and Wilcox, 1989). Intrathecal administration of GABA receptor antagonists dose-dependently produced tactile allodynia (Yaksh, 1989), suggesting that inhibiting endogenous GABA can lead to an excited sensory state. Both GABA_A and GABA_B receptor agonists administered i.t. are antinociceptive in the acute and late phases of the formalin assay (Dirig and Yaksh, 1995; Kaneko and Hammond, 1997), and dose-dependently attenuate allodynia induced by peripheral injury (spinal nerve tight ligation) (Hwang and Yaksh, 1997). Peripheral nerve injury (Ibuki et al., 1997) and transient spinal cord ischemia (Zhang et al., 1994) result in a loss of

GABA expression in the spinal cord dorsal horn, suggesting that the loss of inhibitory GABA neurons may contribute to exaggerated sensory processing after nerve injury. Several antiepileptic drugs have been shown to have either a direct or indirect influence on the GABAergic transmission in the brain and antiepileptic drugs, such as gabapentin and lamotrigine, are therapeutic for the management of chronic pain (McQuay et al., 1995).

Gabapentin (GBP) is a structural analog of GABA whose mechanism of action is currently unknown, lacking affinity for both the GABA_A and GABA_B receptors and failing to inhibit GABA transaminase (Goldlust et al., 1995) or GABA uptake (Su et al., 1995), but possibly binding to a subunit common to most voltage-sensitive calcium channels in brain (Brown and Gee, 1998). Several studies have shown GBP to be antinociceptive. In the formalin test, GBP has been shown to inhibit the late phase but not the acute phase (Field et al., 1997; Shimoyama et al., 1997; Carlton and Zhou, 1998). In addition to its action in acute and inflammatory pain, GBP reverses hyperalgesia and allodynia observed after peripheral nerve injury (Xiao and Bennett, 1996; Hunter et al., 1997) and suppresses spontaneous ectopic discharges from

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ABBREVIATIONS: GBP, gabapentin; LTG, lamotrigine; TGB, tiagabine; DMSO, dimethyl sulfoxide; CI, confidence interval.

injured peripheral nerves (Chapman et al., 1998; Pan et al., 1999).

Lamotrigine (LTG) is a use-dependent inhibitor of voltage-activated sodium channels (Leach et al., 1986). It has been suggested that LTG's inhibition of voltage-activated sodium channels stabilizes the presynaptic neuronal membrane (Leach et al., 1986), thus preventing the release of excitatory neurotransmitters (Teoh et al., 1995), and inhibits sustained repetitive neuronal firing (Cheung et al., 1992). These qualities of LTG are suggestive of a drug having antinociceptive properties. LTG inhibited transient prostaglandin E-induced hyperalgesia and produced analgesia in streptozotocin-induced chronic hyperalgesia (Nakamura-Craig and Follenfant, 1995). Additionally, LTG reverses nerve injury-induced cold allodynia but not tactile allodynia (Hunter et al., 1997). LTG significantly reduced topical mustard oil but not brush-evoked dorsal horn neuronal activity (Blackburn-Munro and Fleetwood-Walker, 1997).

Tiagabine (TGB) [(*R*-)-*N*-(4,4-di(3-methylthien-2-yl)but-3-enyl) nipecotic acid hydrochloride], a newer antiepileptic drug approved by the Food and Drug Administration as an adjunctive therapy for partial seizures, is an inhibitor of neuronal and glial GABA uptake proteins (Nielsen et al., 1991). TGB increases the concentration of GABA in the brain (Fink-Jensen et al., 1992). Recently, it has been demonstrated that systemic administration of tiagabine produces antinociception in acute nociceptive tests in mice (Giardina et al., 1998; Ipponi et al., 1999) and nerve ligation-induced tactile allodynia in rats (Giardina et al., 1998). These past studies have examined TGB's efficacy when given systemically; however, neither study examined the efficacy after central drug administration. In the present study, we examine the nociceptive capability of TGB after central (intrathecal) as well as systemic (intraperitoneal) drug administration in mice. Additionally, we compared the antinociceptive efficacy of TGB to two other antiepileptic drugs (LTG and GBP) in a broad range of nociceptive tests in mice, ranging from acute (hot-plate) through tonic (formalin assay) to chronic (dynorphin-induced allodynia), and characterized which GABA receptors are involved in TGB-induced antinociception.

Materials and Methods

Chemicals and Materials

Bicuculline and 2-hydroxysaclofen (saclofen) were purchased from Sigma/RBI (Natick, MA) and clonidine from Boeringer Ingelheim USA (Ridgefield, CT). Dynorphin A (1–17), and morphine were provided by National Institute on Drug Abuse (Bethesda, MD), tiagabine by Abbott Diagnostics (Abbott Park, IL), lamotrigine by Glaxo-SmithKline (Uxbridge, Middlesex, UK), and gabapentin by Parke-Davis (Ann Arbor, MI). Individual von Frey filaments were purchased from North Coast Medical, Inc. (San Jose, CA).

Animals

Male ICR mice (Harlan, Indianapolis, IN) weighing 20 to 30 g were used in all experiments. Mice were maintained in cages with free access to food and water and kept on a 12-h light/dark schedule in the University of Minnesota's Research Animal Resources facilities. The Institutional Animal Care and Use Committee approved these experiments.

Drug Preparation

Drugs were administered either i.t. in a volume of 5 μ l to awake mice, as described previously (Hylden and Wilcox, 1980) or i.p. in a volume of 200 μ l with a 27-gauge needle attached to a 1-ml disposable syringe. Drugs were diluted in 0.9% saline for all i.t. injections and systemic injections of GBP. For systemic studies of TGB and LTG, these drugs were first diluted in dimethyl sulfoxide (DMSO) and then further diluted with 0.9% saline. The maximum concentration of DMSO used in this study was 9% after the final drug dilution.

Experimental Design

These studies used mice and compared effects of drugs given i.p. and i.t. on three measures of nociception. The hot-plate assay tested for acute nociception, the formalin assay tested for tonic nociception, and the dynorphin-induced allodynia tested for the antiepileptic drug's effect in chronic nociception. Each mouse was only used once, which means that the mouse was tested with only one nociceptive method and received one drug.

Hot-Plate Assay. The hot-plate assay is a model for acute thermal nociception. This test, along with the tail-flick assay, is a standard test to determine the antinociceptive efficacy of a compound. Mice (281) were placed onto a 53°C metal plate, and latency to either licking of the hind paw or vertical jumping was determined. A maximum cutoff of 60 s was imposed to avoid tissue damage. At least 24 h after two baseline latencies were established, drugs were administered either intrathecally (0.1, 0.2, 7, 14, 29, and 73 nmol for TGB; 0.1, 1, 10, and 100 nmol for GBP; and 0.4, 4, 12, and 39 nmol for LTG) or intraperitoneally (1, 4, and 10 mg/kg for TGB; 10, 30, and 100 mg/kg for GBP; and 0.1, 1, 3, 10, and 100 mg/kg for LTG); then latencies to the hot-plate were measured at 10 (only with intrathecal), 30, 60, and 120 min.

Formalin Assay. The formalin test was used as a tonic model of nociception. Two phases of behavior follow injection of formalin into the hind paw. The first phase consists of intense licking and biting of the injected paw for the first 5 min followed by a period of little activity. The second phase spans from 15 to 30 min after the formalin injection and involves periods of licking and biting of the injected paw. The first phase is thought to be a model of acute chemical pain, whereas the second phase reflects a state of central sensitization driven by the presumed first phase involvement of excitatory amino acids (Coderre and Melzack, 1992). The testing environment was maintained at a minimum of 26°C. All together, 163 mice were used for the formalin experiments. Mice were placed into individual 2-liter beakers for at least 1 h before testing. Mice were injected with 20 μ l of 5% formalin into the dorsal right hind paw and returned to the beakers for observation. The amount of time spent licking and biting the injected paw and leg was recorded in 5-min intervals for 25 to 40 min after the formalin injection. TGB (2, 14, and 29 nmol) or LTG (4, 12, 39, and 265 nmol) were intrathecally administered 5 min before the intraplantar injection of formalin. In determining the mechanism of TGB-induced antinociception, bicuculline (1, 3, 10, or 20 pmol) or saclofen (0.01, 0.1, 1, 10, 100, or 900 nmol) was coadministered with TGB.

Dynorphin-Induced Allodynia. For chronic nociception, we used the dynorphin-induced chronic allodynia model. In this model, a single i.t. injection of 3 nmol of dynorphin induces allodynia and hyperalgesia for at least 100 days (Laughlin et al., 1997). Mechanical allodynia was determined with a set of von Frey filaments modified as described previously (Laughlin et al., 1997); these filaments are used to stimulate the dorsal side of the hind paw. The innocuous 2.44 (0.3–0.4 mN) filament was applied to the point of bending three times to the dorsal surface of the left and right hind paw for a total of six applications per mouse. This filament elicits paw withdrawal responses only in mice exposed to allodynia-inducing manipulations such as i.t. applied dynorphin (Laughlin et al., 1997). For testing, 247 mice were placed in individual 2-liter bedding-lined beakers and allowed to adjust to the surroundings for at least 1 h before all

behavioral testing. Allodynia was determined 1 to 3 days after the i.t. injection of dynorphin; thereafter, only allodynic mice (responding at least 50% to the 0.4 mN von Frey filament) were used to determine efficacy of the antiepileptic drugs. TGB (0.2, 7, or 24 nmol i.t.; 0.1, 1, or 10 mg/kg i.p.), LTG (4, 39, or 86 nmol i.t.; 0.1, 1, or 10 mg/kg i.p.), and GBP (0.1, 30, or 60 nmol i.t.; 0.1, 1, 10, or 100 mg/kg i.p.) were administered to allodynic mice. Nonallodynic mice (responding less than 50%) were removed from the study, so that approximately 10% of mice were removed from the study.

Statistical Analysis

Both nonparametric and parametric statistical tests were used to analyze the data. Both tests yielded the same results; only the parametric results are reported in the figures. All data were expressed as the mean \pm S.E.M. The allodynia data were converted to percentage of inhibition of baseline response according to: % inhibition = $100 \times (BR - TR)/BR$, where BR is baseline response to the 2.44 von Frey filament (possible 0 to 6 paw withdrawals) and TR is the test response to the same filament (possible 0 to 6 paw withdrawals). A mean and S.E.M. were calculated from these values. The data were analyzed using Student's *t* test only when two means were compared; otherwise, analysis of variance was used. Statistical differences between groups were further analyzed with Dunnett's test for multiple post hoc comparisons to a control. The Kruskal-Wallis nonparametric statistical analysis was performed on the data; this analysis produced the same results as the analysis of variance. *p* values of less than 0.05 were considered statistically significant. The ED₅₀ values [and 95% confidence interval (CI)] were calculated according to the method of Tallarida and Murray (1987).

Results

Role of Antiepileptic Drugs in Acute Thermal Nociception. The effect of three antiepileptic drugs on acute thermal nociception was tested using the hot-plate test. Intrathecal administration of TGB (0.1, 0.2, 2, 7, 14, 29, and 73 nmol), LTG (0.4, 4, 12, and 39 nmol), and GBP (0.1, 1, 10, and 100 nmol) had no effect on hot-plate latencies at 10, 30, 60, or 120 min after injection (data not shown). Systemically administered TGB (1, 4, and 10 mg/kg) dose-dependently increased latencies compared with a saline-treated group (Fig. 1). The 4-mg/kg dose of TGB induced antinociception for 30

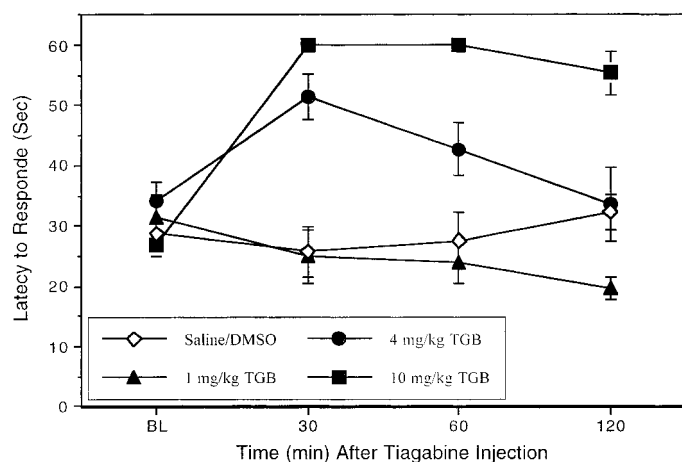


Fig. 1. Systemically administered tiagabine induced thermal analgesia in the hot-plate test. Neither saline + DMSO (open diamonds) nor 1 mg/kg TGB (closed triangles) had an effect, whereas 4 mg/kg TGB (closed circles) produced analgesia for 30 min, and 10 mg/kg (closed squares) produced analgesia for at least 120 min. The mean \pm S.E.M. represents the time (seconds) to lick hind paw or jump vertically; *n* = 9 to 10 mice/group.

min; the antinociception induced by 10 mg/kg TGB lasted at least 120 min, whereas 1 mg/kg TGB had no effect (Fig. 1). Unlike TGB, systemically administered LTG (0.1, 1, 3, 10, and 100 mg/kg) and GBP (10, 30, and 100 mg/kg) had no effect on hot-plate latencies at 30, 60, and 120 min (data not shown).

Role of Antiepileptic Drugs in Tonic Nociception. Intrathecal pretreatment with TGB dose dependently decreased formalin-induced behaviors; specifically 29 nmol reduced both acute and late phase behaviors, 14 nmol of TGB inhibited only the acute phase, and 2 nmol of TGB had no effect compared with the saline control group (Figs. 2 and 3). Unlike TGB, pretreatment with LTG (4–265 nmol) inhibited only the late phase (Fig. 3). For the acute phase, 12 nmol of LTG significantly enhanced the formalin-induced behaviors, whereas 4, 39, and 265 nmol of LTG had no effect (Fig. 3A). For the late phase, TGB inhibited the formalin-induced nociceptive behaviors with an ED₅₀ value of 13 nmol (95% CI = 5–31 nmol), and for LTG the ED₅₀ was 28 nmol (95% CI = 3–274 nmol). To determine whether TGB prevented the development of central sensitization or acted as an analgesic similar to morphine, we administered TGB after the acute phase. TGB (29 nmol i.t.) administered 5 min after the intraplantar injection of formalin still reduced the late phase nociceptive behaviors in a manner similar to that of the pretreatment (Fig. 4).

GABA_A and GABA_B receptor antagonists were coadministered with TGB to determine whether the GABA receptors play a role in TGB-induced antinociception. The GABA_A antagonist bicuculline (1–20 pmol) or the GABA_B antagonist saclofen (0.01–900 nmol) was coadministered i.t. with 29 nmol of TGB 5 min before intraplantar injection of formalin. For bicuculline, only 3 pmol of bicuculline significantly reversed TGB-induced antinociception in the acute phase (Fig. 5A), and none of the bicuculline doses had any effect on TGB-induced late phase antinociception (Fig. 5B). A 5-min pretreatment with only 20 pmol (i.t.) of bicuculline (no TGB) significantly increased the acute phase behaviors

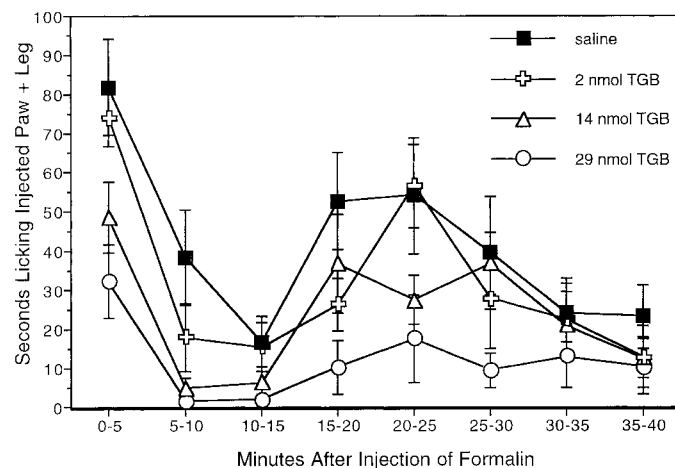


Fig. 2. Tiagabine dose-dependently inhibited formalin-elicited behaviors. TGB was administered i.t. 5 min before intraplantar injection of formalin. A dose of 29 nmol of TGB (open circles) reduced both the acute and late phases, whereas 14 nmol of TGB (open triangles) only inhibited the acute phase and 2 nmol of TGB (open crosses) had no effect. The mean \pm S.E.M. represents the amount of time (seconds) the mice spent licking and biting the injected paw and leg; *n* = 7 to 10 mice/group.

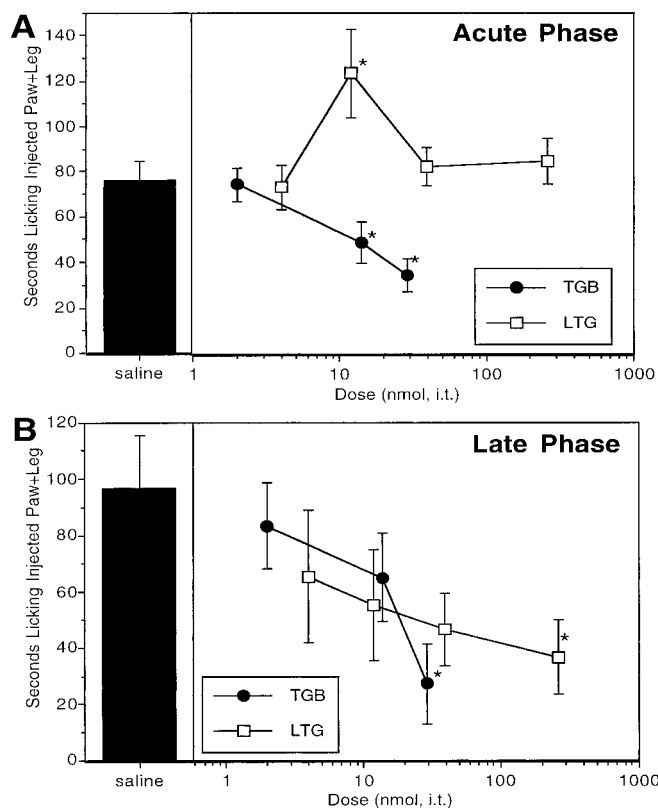


Fig. 3. Tiagabine and lamotrigine dose-response curves for the formalin assay. A, acute phase (0–5 min) of the formalin assay. TGB (2–29 nmol i.t.; closed circles) dose dependently reduced behaviors. LTG (4–265 nmol i.t.; open squares) did not inhibit the acute phase behaviors. B, late phase (15–25 min) of the formalin assay. TGB (closed circles) and LTG (open squares) dose dependently reduced late phase responses. The mean \pm S.E.M. represents the amount of time (seconds) the mice spent licking and biting the injected paw and leg. *, $p < 0.05$ from the saline control group (black column); $n = 7$ to 11 mice/group, except $n = 18$ for saline group (no differences were observed between the two saline groups for the TGB experiment and the LTG experiment, so they were combined).

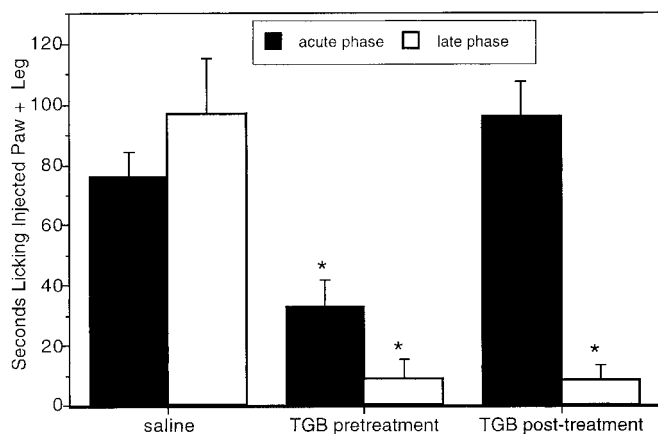


Fig. 4. Effect of pre- versus post-treatment with tiagabine in the formalin assay. A 5-min pretreatment with TGB (29 nmol i.t.) reduced both the acute and late phases of the formalin assay. A 5-min post-treatment (after intraplantar injection of formalin) with TGB (29 nmol i.t.) reduced late but not acute phase behaviors. The mean \pm S.E.M. represents the amount of time (seconds) the mice spent licking and biting the injected paw and leg. *, $p < 0.05$ from the saline group; $n = 8$ to 13 mice/group.

with no effect on the late phase (Fig. 5). In the acute phase for saclofen, only 0.1 nmol of saclofen significantly reversed TGB-induced antinociception; 0.01, 1, 10, and 100

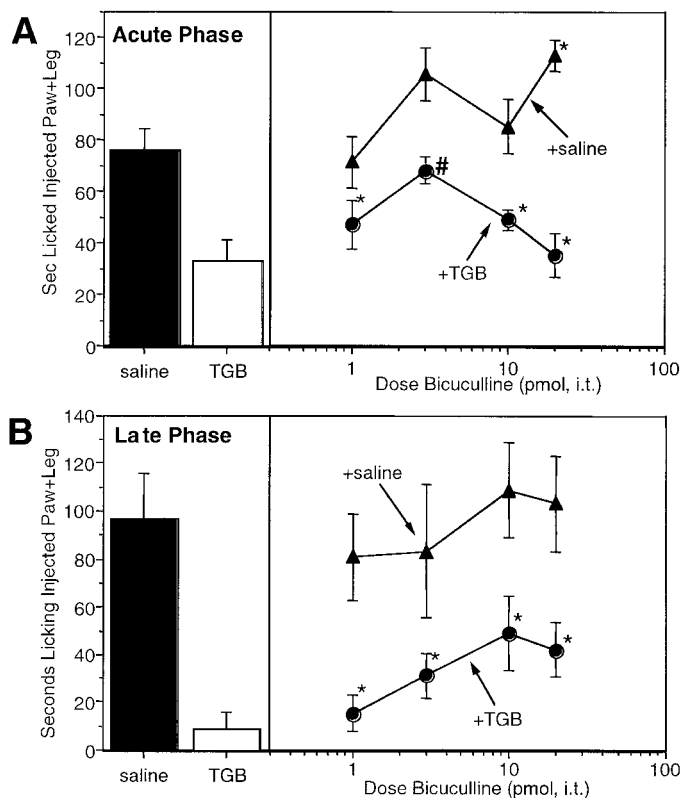


Fig. 5. Effect of bicuculline on tiagabine-induced antinociception in the formalin assay. The GABA_A antagonist bicuculline (1–20 pmol) was co-administered i.t. with 29 nmol TGB (closed circles) or saline (closed triangles) 5 min before intraplantar formalin. A, acute phase (0–5 min) of the formalin assay. Only 3 pmol of bicuculline (closed circles) significantly reversed TGB-induced antinociception, and 20 pmol of bicuculline itself (closed triangles) significantly increased acute phase formalin behaviors. B, late phase (15–25 min) of the formalin assay. Bicuculline had no effect on late phase formalin behaviors (closed triangles) or on TGB-induced antinociception (closed circles). The mean \pm S.E.M. represents the amount of time (seconds) the mice spent licking and biting the injected paw and leg. *, $p < 0.05$ from saline (black column); #, $p < 0.05$ from 29 nmol of TGB (white column); $n = 8$ to 13 mice/group.

nmol of saclofen reduced TGB-induced antinociception; and 900 nmol of saclofen had no effect (Fig. 6A). Similar to the acute phase, 0.1 nmol of saclofen significantly reversed TGB-induced late phase antinociception, whereas 0.01, 1, 10, and 100 nmol of saclofen reduced the TGB-induced antinociception, and 900 nmol of saclofen had no effect (Fig. 6B).

Role of Antiepileptic Drugs in Chronic Thermal Nociception. Intrathecal administration of morphine (0.3–1 nmol) and clonidine (0.01–1 nmol) both dose dependently attenuated the dynorphin-induced allodynia (Fig. 7). Neither saline nor 0.03 nmol of morphine had an effect on mechanical allodynia, whereas 0.3 nmol of morphine was effective for 10 min and 1 nmol of morphine was effective for at least 60 min (Fig. 7A). Both 0.01 and 0.1 nmol of clonidine produced antinociception for 10 min and 1 nmol of clonidine for 30 min; saline had no effect (Fig. 7B). For inhibition of allodynia in this model of chronic pain, clonidine had a greater potency than morphine [clonidine had an ED₅₀ of 5.3 pmol (95% CI = 1.1–25.9)]. Morphine had an ED₅₀ of 120 pmol (95% CI = 50–250) (Table 1) and produced a longer lasting effect (Fig. 7).

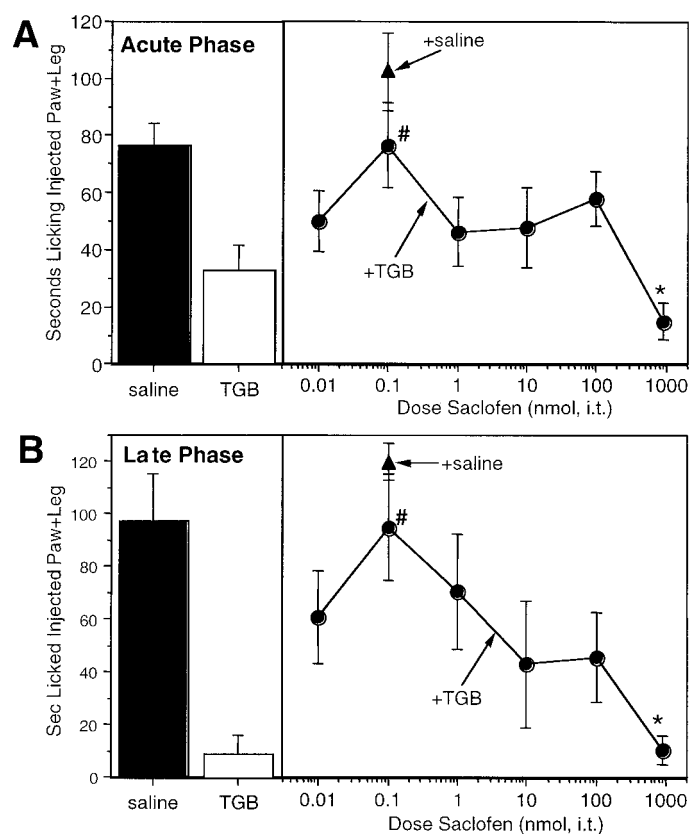


Fig. 6. Effect of saclofen on tiagabine-induced antinociception in the formalin assay. The GABA_B antagonist saclofen (0.01–900 nmol) was coadministered i.t. with 29 nmol of TGB (closed circles) or saline (closed triangles) 5 min before intraplantar formalin. A, acute phase (0–5 min) of the formalin assay. Saclofen dose dependently inhibited TGB-induced antinociception (closed circles); 0.1 nmol of saclofen significantly reversed, whereas 0.01, 1, 10, and 100 nmol of saclofen reduced, and 900 nmol of saclofen had no effect on TGB-induced antinociception. Saclofen (0.1 nmol; closed triangles) had no effect on the acute phase. B, late phase (15–25 min) of the formalin assay. Saclofen dose dependently inhibited TGB-induced antinociception (closed circles); 0.1 nmol of saclofen significantly reversed, whereas 0.01, 1, 10, and 100 nmol of saclofen reduced, and 900 nmol of saclofen had no effect on TGB-induced antinociception. Saclofen (0.1 nmol; closed triangles) had no effect on late phase behaviors. The mean \pm S.E.M. represents the amount of time (seconds) the mice spent licking and biting the injected paw and leg. *, $p < 0.05$ from saline (black column); #, $p < 0.05$ from 29 nmol of TGB (white column); $n = 8$ mice/group, except $n = 6$ for the 10 nmol of saclofen + TGB group, and $n = 13$ for the saline group.

Intrathecal administered TGB (2, 7, and 24 nmol) had no effect on the dynorphin-induced allodynia (Fig. 8A). In contrast, both LTG (4, 39, and 86 nmol i.t.) and GBP (0.1, 30, and 60 nmol i.t.) dose-dependently reduced dynorphin-induced allodynia for at least 120 min (Fig. 8, B and C). Both 39 and 86 nmol of LTG had an effect at 30 min, but only 39 nmol of LTG inhibited allodynia through 120 min (Fig. 8B). The effect of 60 nmol of GBP was delayed until 45 min and lasted until 120 min (Fig. 8C). GBP was more potent than LTG; however, both drugs were less potent than morphine and clonidine (Table 1). Systemically administered TGB (0.1, 1, and 10 mg/kg), LTG (0.1, 1, and 10 mg/kg), and GBP (0.1, 1, 10, and 100 mg/kg) all dose and time dependently reduced dynorphin-induced allodynia for at least 120 min (Fig. 9). For systemic administration, TGB was the most potent followed by GBP; LTG was least potent (Table 1).

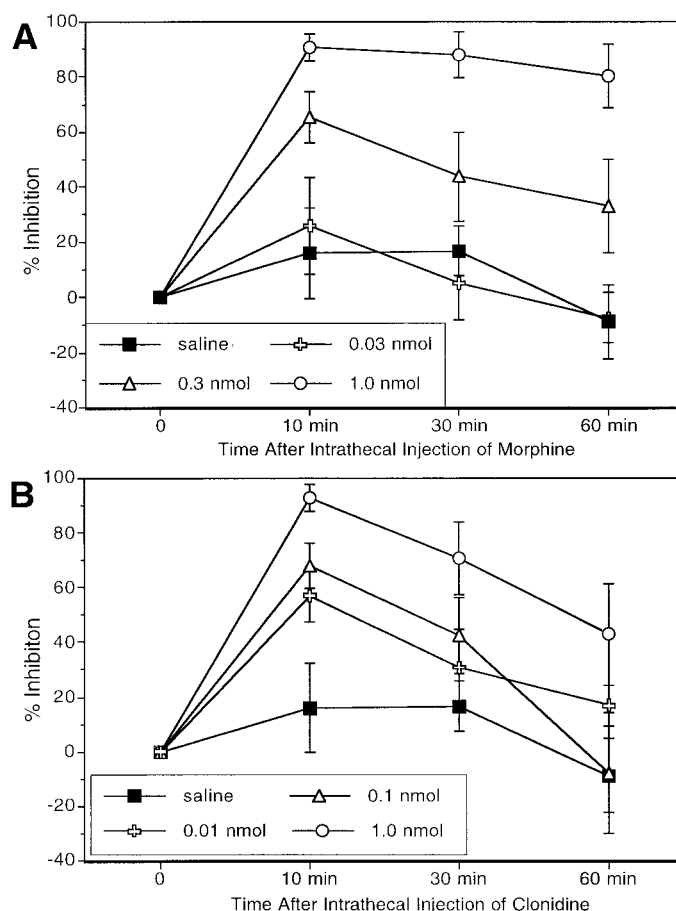


Fig. 7. Effect of intrathecally administered morphine and clonidine on dynorphin-induced allodynia. Intrathecally administered morphine (0.3–1 nmol; open symbols) (A) and clonidine (0.01–1 nmol; open symbols) (B) dose and time dependently reduced dynorphin-induced allodynia for 10 to 60 min. Administration of saline (closed squares) had no effect on the mechanical allodynia. The mean \pm S.E.M. represents the percentage of inhibition of the response to the 0.4 mN von Frey filament; $n = 6$ to 9 mice/group.

TABLE 1
Effect of antiepileptic drugs on dynorphin-induced allodynia

Drug	Intrathecal ED ₅₀ (95% CI)	Intraperitoneal ED ₅₀ (95% CI)
	nmol	mg/kg
TGB	No effect	1.6 (0.3–7.6)
LTG	73.9 (24.4–223.5)	20.1 (0.5–785.8)
GBP	31.8 (4.8–210.0)	2.5 (0.6–11.4)
Morphine	0.12 (0.05–0.25)	
Clonidine	0.0053 (0.0011–0.0259)	

Discussion

The present study demonstrates that the antiepileptic drug TGB produces antinociception in three mouse models of pain (Table 2). TGB, administered i.p. but not i.t. dose dependently produced antinociception in the hot-plate test. A 5-min pretreatment with TGB (i.t.) dose dependently inhibited both acute and late phase formalin behaviors, and post-treatment with 29 nmol of TGB was still able to inhibit the late phase formalin behaviors. The TGB-induced antinociception in the formalin test was reversed by coadministration with GABA antagonists: saclofen (GABA_B antagonist) reversed inhibition of both the acute and late phase behaviors,

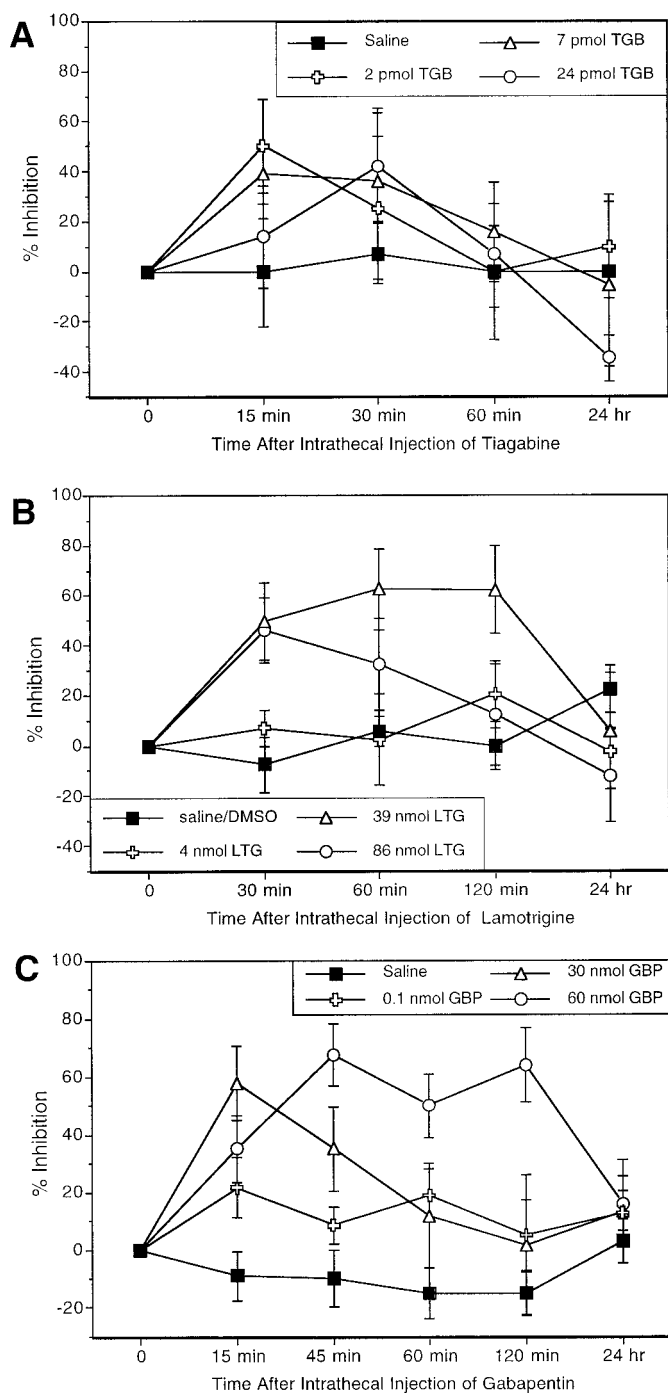


Fig. 8. Effect of intrathecally administered tiagabine, lamotrigine, and gabapentin on dynorphin-induced mechanical allodynia. A, intrathecally administered TGB (0.2–24 nmol; open symbols) had no effect on dynorphin-induced allodynia. B, intrathecally administered LTG (4–86 nmol; open symbols) dose-dependently reduced dynorphin-induced allodynia for 30 to 120 min. C, intrathecally administered GBP (0.1–60 nmol; open symbols) dose-dependently reduced dynorphin-induced allodynia for 15 to 120 min. For each drug treatment, the control injection of saline (closed squares) did not produce any significant changes in the level of allodynia. The mean \pm S.E.M. represents the percentage of inhibition from the response to the 0.4 mN von Frey filament; $n = 6$ to 10 mice/group.

whereas bicuculline (GABA_A antagonist) reversed only inhibition of the acute phase. Finally, we examined the antinociceptive potential of TGB in dynorphin-induced allodynia. Systemically, but not centrally (i.t.), administered TGB dose

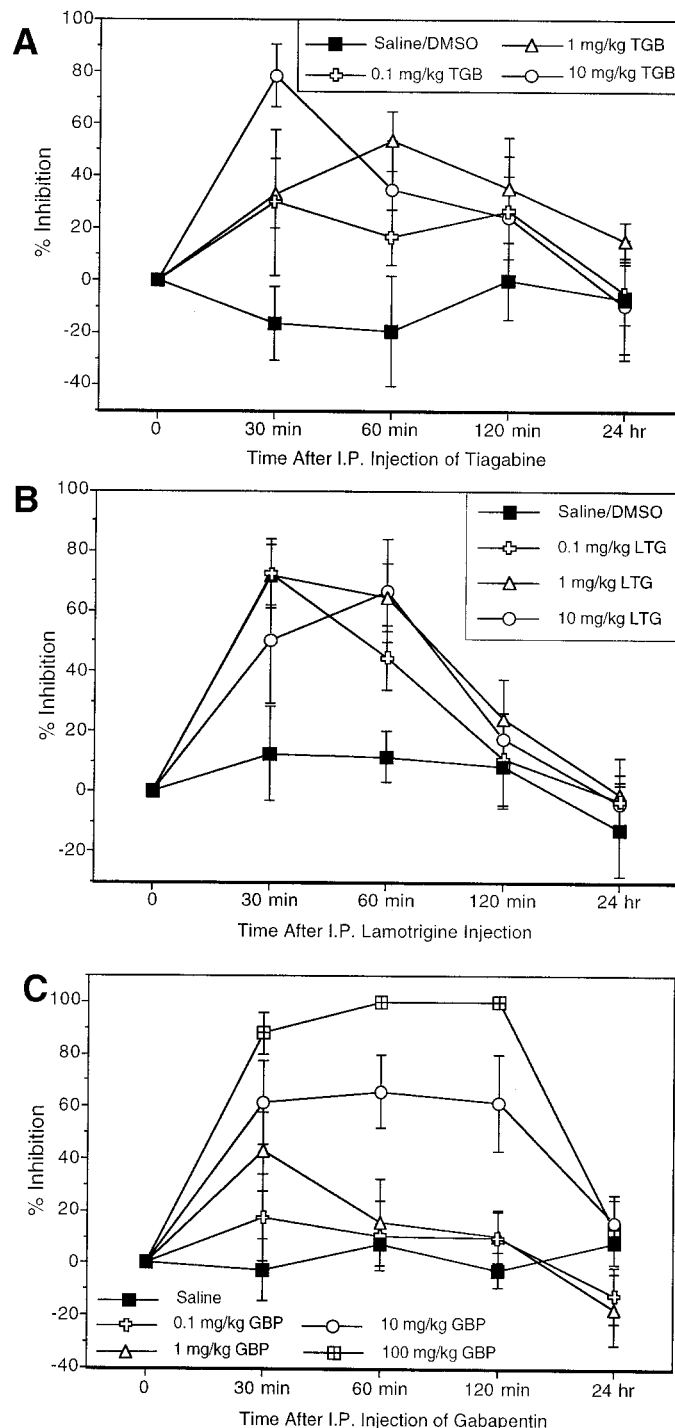


Fig. 9. Effect of systemically administered tiagabine, lamotrigine, and gabapentin on dynorphin-induced mechanical allodynia. Intraperitoneally administered TGB (0.1–10 mg/kg) (A), LTG (0.1–10 mg/kg) (B), and GBP (0.1–100 mg/kg) (C) all dose-dependently reduced dynorphin-induced allodynia for 30 to 120 min. For each drug treatment, the control injection of saline + DMSO (closed squares in A and B) and saline (closed squares in C) did not produce any significant changes in the level of allodynia. The mean \pm S.E.M. represents the percentage of inhibition from the response to the 0.4 mN von Frey filament; $n = 5$ to 12 mice/group.

independently attenuated established dynorphin-induced allodynia, a model of neuropathic pain, for 30 to 120 min. Thus, systemically administered TGB was effective in the acute (hot-plate) and chronic (dynorphin-induced allodynia) noci-

TABLE 2
Summary results of antiepileptic drugs in nociceptive mouse models

	Hot Plate		Formalin		DYN Allodynia		Clinical Efficacy
	i.t.	i.p.	Early	Late	i.t.	i.p.	
TGB	No	Yes	Yes	Yes	No	Yes	?
GBP	No	No	No*	Yes*	Yes	Yes	Yes
LTG	No	No	No	Yes	Yes	Yes	Yes/no

DYN, dynorphin.

* Literature only, not studied here.

ceptive tests, whereas centrally (i.t.) administered TGB was effective in the formalin assay.

The first part of this study examined the ability of the three antiepileptic drugs to inhibit responses in the hot-plate test, which evaluates acute nociceptive function. Only systemically administered TGB was effective in this test. Systemically administered LTG or GBP had no effect, as did i.t. administered TGB, LTG, and GBP. Our results agree with previous acute nociceptive studies on normal rodents in which LTG had no effect in hot-plate or tail-flick tests (Nakamura-Craig and Follenfant, 1995), and GBP had no effect in paw pinch (Field et al., 1997), heat-evoked paw withdrawal (Field et al., 1997), or tail-flick tests (Hunter et al., 1997; Shimoyama et al., 1997). However, in agreement with our study, both 3 and 10 mg/kg TGB produced antinociception in the hot-plate test after systemic administration in mice (Giardina et al., 1998; Ipponi et al., 1999). These results suggest that antiepileptic drugs have little or no effect on most measures of normal transient nociceptive signaling, but rather inhibit sensitized signaling associated with allodynia and hyperalgesia. This interpretation is supported by the fact that GBP had no effect on normal afferent fiber activity, but inhibited the ectopic discharge activity associated with peripheral nerve injury (Pan et al., 1999).

The second part of this study examined the ability of TGB and LTG in the formalin test, which is a model of acute chemical pain (acute phase) and tonic nociception involving central sensitization (late phase). Several studies have previously demonstrated the ability of GBP to inhibit the late but not the acute phase of the formalin assay when administered systemically (Field et al., 1997) or centrally (Shimoyama et al., 1997). Similarly, in the present study, both TGB and LTG dose-dependently inhibited late phase formalin behaviors, but only TGB inhibited the acute phase. Thus, TGB may be affecting acute chemical nociception as it did thermal nociception in the hot-plate test. For inhibition of the late phase, TGB and LTG produced comparable dose-response curves. Unlike TGB, LTG actually enhanced rather than inhibited the acute phase formalin behaviors. Similar enhancing effects have been demonstrated with LTG, which facilitates C-fiber-evoked windup and after discharge of dorsal horn neurons (Chapman et al., 1997).

TGB is an inhibitor of neuronal and glial GABA uptake proteins. Thus, we examined whether activation of the GABA_A and/or GABA_B receptors was involved in the antinociceptive effect of TGB. In the acute phase of the formalin assay, both the GABA_A and GABA_B receptor antagonists dose-dependently reversed TGB-induced antinociception. In the late phase of the formalin assay, only the GABA_B receptor antagonist reversed TGB-induced antinociception. Thus, central administration of TGB results in elevated GABA levels, leading to the activation of both GABA_A and GABA_B

receptors in the acute phase; activation of GABA_B receptors was apparently involved in the late phase of the formalin test. These results are consistent with previous demonstrations that tiagabine-induced acute antinociception is inhibited by a GABA_B receptor antagonist (Ipponi et al., 1999).

The late phase of the formalin assay is believed to derive from acute phase primary afferent activity and is considered to be a model of central sensitization (Coderre and Melzack, 1992). Drugs that are considered analgesics, such as morphine, suppress the afferent input throughout the time course of the behavior and inhibit the late phase behaviors when given as either a pre- or post-treatment (Yamamoto and Yaksh, 1992). On the other hand, drugs that inhibit the development of central sensitization, such as *N*-methyl-D-aspartate receptor antagonists, are only capable of inhibiting the late phase behaviors when given as a pretreatment and have no effect when given as post-treatment (Coderre and Melzack, 1992). In this study, we have shown that TGB inhibited the late phase behaviors when given as either a pre- or post-treatment, suggesting that TGB has antinociceptive activity like that of morphine, suppressing afferent input rather than blocking the development of central sensitization.

The third part of this study examined the efficacy of the three antiepileptic drugs in dynorphin-induced allodynia, which was used as a pharmacological model of neuropathic pain. Previous studies have shown that LTG (Nakamura-Craig and Follenfant, 1995; Hunter et al., 1997), TGB (Giardina et al., 1998), and GBP (Xiao and Bennett 1996; Hunter et al., 1997) reversed behavioral signs of neuropathic pain in animal models. In this study, systemically administered TGB was the most potent antiepileptic drug to reduce dynorphin-induced allodynia (Table 1), followed by GBP and LTG; GBP, on the other hand, had the longest duration of action (120 min) compared with TGB and LTG (30–60 min). By the i.t. route, GBP was more potent than LTG in attenuating dynorphin-induced allodynia, whereas TGB was without effect on dynorphin-induced allodynia. Both GBP and LTG were less potent than morphine and clonidine (Table 1). Systemic administration of morphine is also more potent than systemic TGB (Giardina et al., 1998). That systemic but not central administration of TGB was efficacious in dynorphin-induced allodynia suggests that TGB evidently exerts its potent antiallodynic effect at a nonspinal site. This outcome could be due to the presence of a TGB metabolite. TGB is metabolized by the 3A isoform subfamily of hepatic cytochromes P450 to 5-oxo-TGB, which is further glucuronidated. There are also thought to be other unidentified metabolites because only 2% of the parent drug is excreted after administration of [¹⁴C]TGB to humans (Bopp et al., 1992).

In conclusion, we have shown that TGB produces antinociception in the hot-plate test, formalin assay, and dynor-

phin-induced allodynia model of neuropathic pain. Both GABA_A receptors (acute phase) and GABA_B receptors (acute and late phase) are involved in TGB-induced antinociception in the formalin test. This study presents the first comparison between systemic and intrathecal administration of TGB. In both the hot-plate assay and dynorphin-induced allodynia test, TGB was only effective when administered systemically; i.t. administration lacked any effect in these two tests. On the other hand, i.t.-administered TGB was effective in the formalin assay. The reason for this difference in efficacy across routes of administration remains unclear. Further research is required to determine the mechanisms involved in the systemic versus spinal activity profile of TGB. This study also presents the first comparison of TGB's antinociceptive efficacy to that of other antiepileptic drugs. Of the three antiepileptic drugs, TGB was the only drug efficacious in the hot-plate test. TGB was as effective as LTG in the formalin test, and TGB was the most potent systemically administered antiepileptic drug in the dynorphin-induced chronic allodynia test.

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