Anesthesiology
1998; 88:196–205

Characterization of the Effects of Gabapentin and 3-Isobutyl-γ-Aminobutyric Acid on Substance P-induced Thermal Hyperalgesia

Brett J. Partridge, Sandra R. Chaplan, M.D.,† Eiji Sakamoto, D.D.S.,‡ Tony L. Yaksh, PhD§*

Background: **The authors sought to characterize the pharmacologic characteristic and site of action of gabapentin (Neurontin) in a model of thermal hyperalgesia induced by intrathecal substance P administration.**

Methods: **Rats were prepared with long-term lumbar intrathecal catheters. Hind paw withdrawal latency was determined using a radiant heat stimulus focused through a glass surface onto the plantar surface of the paw.**

Results: **Within 5 min after intrathecal injection of substance P (30 nmol), hind paw withdrawal latency fell from 11 to 8 s. Gabapentin given intrathecally or intraperitoneally produced dose-dependent reversal of the thermal hyperalgesia, with complete reversal (ED_{100}) occurring at 163 μg for intrathecal and 185 mg/kg for intraperitoneal administration. *S*(+)-3-isobutyl-γaminobutyric acid, but not *R*(−)-3-isobutyl-γaminobutyric acid, also produced dose-dependent reversal of the intrathecal substance P-induced thermal hyperalgesia (intrathecal ED_{100}, 65 μg and intraperitonal ED_{100}, 31 mg/kg). The effects of intraperitoneally administered gabapentin and 3-isobutyl-γaminobutyric acid were reversed by intrathecal pretreatment with D-serine (100 μg) but not by L-serine. All effects were observed at doses that had little effect on motor function or spontaneous activity. Intrathecal N-methyl-D-aspartate (2 nmol) induced thermal hyperalgesia, which was blocked by gabapentin (100 mg/kg intraperitoneally) and *S*(+)-3-isobutyl-γaminobutyric acid (30 mg/kg intraperitoneally).**

Conclusions: **The structure–activity relationship and the stereospecificity noted after intrathecal delivery suggest that gabapentin and *S*(+)-3-isobutyl-γaminobutyric acid act at a common spinal locus to modulate selectively a facilitated state of nociceptive processing. (Key words: GABA; gabapentinoid; intrathecal; neurontin; NMDA; D-serine.)**

GABAPENTIN, first synthesized as a γ-aminobutyric acid (GABA) analogue with improved biologic stability and distribution, has been developed for clinical use as an anticonvulsant agent.[1] Several recent clinical case reports have suggested that gabapentin also is efficacious in treating human neuropathic pain.[2,3] Subsequent preclinical studies demonstrated that this agent, when given intrathecally, is able to reverse tactile allodynia-dose in a dose-dependent manner in the Chung model of neuropathy[4] and to reverse thermal hyperalgesia in the Bennett model of neuropathy after systemic and intrathecal administration,[5] the second phase of the formalin test after systemic delivery (Singh, personal communication, 1996), and the thermal hyperalgesia induced by thermal injury to the paw (Jun and Yaksh, unpublished data, 1997). Importantly, these effects have been observed at doses that appear to have little effect on behavior or motor function.

Functionally, the actions of gabapentin appear limited to those models of nociception that involve a facilitated state of processing. No changes in escape latencies have been seen with this agent in animal models using only short-term noxious stimuli, such as the hot plate or tail flick tests; effects have been manifested principally in the context of injury or inflammation.[1]

Many such models of facilitated processing are known to depend on an increase in the spinal release of glutamate and the subsequent activation of spinal N-methyl-D-aspartate (NMDA) receptors.[6,7] Gabapentin has been shown to have moderate inhibitory effects *in vitro* on branched-chain amino acid transferase (an enzyme that metabolizes several cytosolic amino acids to form glutamate).[8] Although some data suggest that gabapentin

* Summer Undergraduate Research Student.

† Assistant Professor of Anesthesiology.

‡ Anesthesiology Research Fellow. Current address: Kyushu Dental College, 2-6-1 Manazuru, Kokurakita-ku, Kitakyushu 803, Japan.

§ Professor of Anesthesiology; Vice-Chair for Research in Anesthesiology.

Received from the Department of Anesthesiology, University of California, San Diego, La Jolla, California. Submitted for publication March 14, 1997. Accepted for publication September 3, 1997. Performed in the Anesthesia Research Laboratory, University of California, San Diego. Supported in part by unrestricted funds from Parke Davis; National Institutes of Health grant NS01769 to Dr. Chaplan; and undergraduate summer support from the American Society of Pharmacology and Experimental Therapeutics to Mr. Partridge.

Address reprint requests to Dr. Yaksh: Department of Anesthesiology, University of California, San Diego, 9500 Gilman Drive, La Jolla, California, 92093-0818. Address electronic mail to: tyaksh@ucsd.edu

may reduce NMDA-evoked currents,‖ other studies have not shown effects at drug concentrations achieved *in vivo*.[9] Moreover, binding studies have reportedly shown no affinity of gabapentin for NMDA, α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid hydrobromide (AMPA) or nonstrychnine-sensitive glycine sites associated with the NMDA receptor.[10] It is noteworthy, however, that despite an apparent lack of affinity for the NMDA receptor complex, it has been reported that D-serine, an agent known to reverse the effects of drugs acting as antagonists at the nonstrychnine-sensitive glycine site, antagonizes the anticonvulsant effects of gabapentin.[11]

Although gabapentin is structurally similar to GABA, binding studies fail to show any affinity for either GABA-A or -B sites.[10] The compound has been shown to increase the rate of synthesis of GABA in several brain regions, however.[12] In addition, although gabapentin itself has no effect on the GABA transporter,[13] it may enhance extracellular levels of GABA released from astrocytes by reversal of the GABA transporter.[14] In contrast, in studies on the Chung model of neuropathy, the spinal delivery of either GABA-A or -B receptor antagonists, at doses capable of blocking the intrathecal effects of the respective agonists, did not reduce the antiallodynic effects of intrathecal gabapentin. These observations jointly appear to exclude an anticipated action at a conventional GABA site.[4]

Despite a lack of interaction at many sites, there appears to be a high-affinity binding site in the brain for gabapentin. Importantly, this gabapentin binding is displaced in a stereospecific fashion by *S*(+)-3-isobutyl-GABA but not by *R*(−)-3-isobutyl-GABA, and these agents show a similar activity profile as anticonvulsant agents.[15,16]

Although a supraspinal effect is not excluded, it is clear that the effects of gabapentin can be mediated by a spinal action. The dose required after spinal delivery is considerably lower than that required after systemic delivery; therefore, peripheral redistribution after spinal delivery appears unlikely to account for the antihyperalgesia conferred by this route of administration.

In the current studies, we sought to further characterize the actions of this family of agents by examining the dose-dependent spinal and systemic effects of (1) gabapentin, (2) the isomers of 3-isobutyl-GABA, and (3) D- and L-serine on the thermal hyperalgesia observed after the spinal delivery of substance P. This model was chosen because it reflects the activation of a spinal facilitatory cascade by a neurokinin (NK)-1 receptor leading to a behaviorally defined hyperalgesia that does not depend on a previous peripheral injury.[17] We further examined in a limited number of studies whether the hyperalgesia induced by intrathecal NMDA was similarly sensitive to the effects of gabapentin.

‖ Oles RJ, Singh L, Hughes J, Woodruff GN: The anticonvulsant action of gabapentin involves the glycine/NMDA receptor. Soc Neurosci Abstr 1995; 6:783

Materials and Methods

All animal surgery and testing procedures were approved by the Institutional Animal Care Committee of the University of California, San Diego (San Diego, CA).

Animals

Male Holtzmann Sprague-Dawley rats, weighing 300–400 g each, were housed separately after catheter implantation. They were allowed access to standard rat chow and water *ad libitum* and were maintained on a 12-h light/dark cycle. Rats were tested up to four times at 4–6-day intervals. Approximately 55 rats were used in this study.

Surgery

The surgical implantation of intrathecal catheters was performed under halothane anesthesia using a modification of the previously described technique.[18] Briefly, each rat was anesthetized under a mixture of 1–4% halothane in 50% O_2/air and shaved along the occiput and neck. The rat was then placed in a stereotaxic head holder, and the shaved skin was prepared with alcohol and Povidone iodine solution. To maintain anesthesia, 2% halothane was delivered through a face mask. An incision was made overlying the atlantooccipital junction, and the dura mater was exposed by blunt dissection. An incision was made in the dura, and a polyethylene catheter (PE-10), with a loose knot cemented with dental acrylic 8.4 cm from the end, was threaded caudally through the incision. The external end of the catheter was tunneled subcutaneously to exit atop the head. The catheter was then flushed with 10 μl of saline and plugged. The animals were allowed to recover for approximately 5 days after surgery before testing was begun. Motor function, body weight, and sensory threshold were assessed before testing, and those animals that showed impairment were killed.

Drugs

Drugs administered intrathecally in this study were substance P (molecular weight = 1,347; Peninsula Laboratories, Belmont, CA); NMDA (molecular weight = 147; Sigma Chemical, St. Louis, MO), gabapentin (1-[aminomethyl]cyclohexanacetic acid [Neurontin®]; molecular weight = 171; Parke-Davis, Ann Arbor, MI), *S*(+)-3-isobutyl-GABA (molecular weight = 157; Parke-Davis), and D-serine and L-serine (molecular weight = 105; Sigma Chemical). Stock solutions of substance P or NMDA were prepared in deionized water, lyophilized, stored at −70°C, and used immediately after reconstitution. Gabapentin was stored at 5°C in an opaque container. All drugs were dissolved in 0.9% sterile saline. Control experiments were performed with normal saline at volumes comparable to those of the drug solutions and injected intrathecally, intraperitoneally, or both, as required.

Injection

For intrathecal injection, the catheter was unplugged and connected to a gear-driven microinjection syringe via a length of calibrated PE-90 tubing. Drugs were injected in a 10-μl solution, followed by an additional 10 μl of saline flush.

For intraperitoneal injection, drugs were injected in a volume of 3 ml/kg. The same volume of normal saline was administered as a control. All drug doses were randomized.

Test Measures

Thermal Escape Latencies. The latency to hind paw withdrawal from a thermal stimulus was determined by exposing the plantar hind paw to radiant heat using a modified Hargreaves-type thermal testing device.[19] The apparatus used (University Anesthesiology Research and Development Group, University of California, San Diego) has been described elsewhere.[20] Briefly, rats were placed in individual enclosures on a glass plate maintained at 30°C. The radiant thermal stimulus, underneath the glass plate, was positioned directly under the hind paw. Activation of the bulb simultaneously activated a timer; both bulb and timer were immediately turned off by paw withdrawal or after 20 s (cutoff time). After acclimation, a measurement was taken for each hind paw to establish an average baseline latency (counted as time = 0). Measurements were then made at intervals after injection of intrathecal substance P. The mean of the response latencies from each paw was taken as the latency for that testing time. Data are presented as the escape latency in seconds. For analysis, the area under the time *versus* change-in-escape latency curve (AUC) was calculated between the intervals of 10 and 60 min after intrathecal substance P. The AUC was calculated using a program that computed the sum of the areas of a series of trapezoids created by the time-effect curves. The dimensions of the AUC are thus $\Delta s \cdot h$. As the response latencies were reduced during this interval, the AUC yielded a negative area. This value was used to define the magnitude of the thermal hyperalgesia.

General Behavior. Pinna twitching in response to touching a small probe to the meatus, blink in response to lightly touching the cornea, and the righting reflex (coordinated response made when the animal is placed on its back) were tested periodically to evaluate possible sedative effects.

Statistics

Raw hyperalgesia data are presented as the response latency in seconds (mean ± SEM). For statistical analysis of the magnitude of the hyperalgesia, the AUC was determined for the interval between 10 and 60 min after the injection of substance P; the 10-min delay was used to avoid including the initial increase in response latency that was typically observed immediately after injection of substance P. The AUC was used to construct dose-response curves with the computer programs of Tallarida and Murray.[21] These programs also were used to verify the parallelism of the dose-response curves. In the calculation of the complete reversal (ED_{100}), a complete response was defined as the dose of drug that returned the post-substance P response latency to the same value as the pre-substance P response latency. Comparisons of potency were made using ED_{100} values. Comparisons between groups were accomplished with repeated-measures or one-way analysis of variance. When significance was achieved, *post hoc* comparisons were performed using the Bonferroni-Dunn method. These statistical analyses were performed with Statview 4.5 for the Macintosh (Abacus Concepts, Inc., Berkeley, CA).

Results

Intraperitoneal Gabapentin and Intraperitoneal S(+)-3-Isobutyl GABA

Intraperitoneal doses of gabapentin and *S*(+)-3-isobutyl GABA up to 100 mg/kg and 30 mg/kg, respectively,

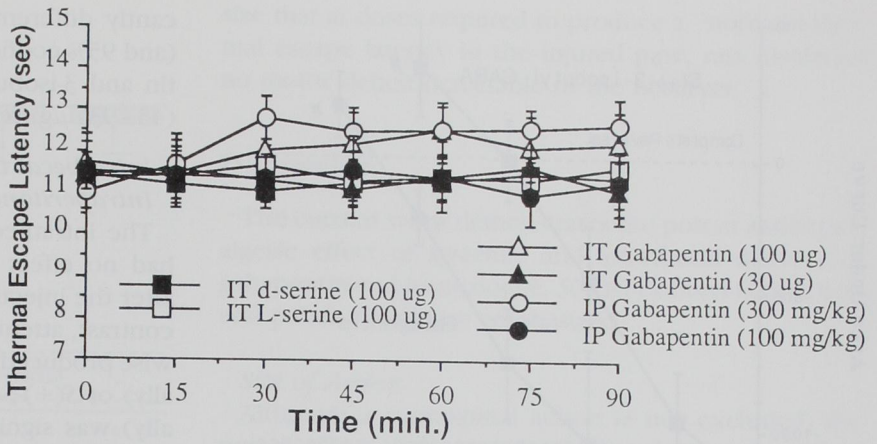


Fig. 1. Thermally evoked hind paw withdrawal latency measured before and after the intrathecal and intraperitoneal injections and the intrathecal injections of D-serine. These control experiments demonstrate that both of these compounds have little effect on withdrawal latency when administered alone.

did not change thermal response latencies and were without significant side effects (fig. 1). The intraperitoneal injection of 300 mg/kg of gabapentin caused some mild sedation and motor weakness. At this dose, there was a small increase in baseline thermal escape latency. Mortality was not observed, however, even at doses of gabapentin up to 500 mg/kg.

Effects of Intrathecal Substance P

In the absence of treatment, the mean latency to hind paw withdrawal was 11.3 ± 0.4 s (n = 50). The intrathecal injection of substance P (30 nmol) resulted in a brief period of agitation, with scratching of the flank and licking of the paws. Concurrent with this behavior, there was a brief increase in the thermal escape threshold that lasted approximately 10 min. By 30 min, the thermal escape latency had decreased relative to the pre-substance P latency to 7.7 ± 0.3 s (Δs = 3.6 ± 0.3 s). This reduced latency persisted for ≈40-50 min (fig. 2).

Intraperitoneal Gabapentin and 3-Isobutyl-GABA with Substance P

The intraperitoneal injection of either gabapentin (10-100 mg/kg; fig. 2) or *S*(+)-3-isobutyl-GABA (1-30 mg/kg; fig. 3) resulted in a dose-dependent reversal of the intrathecal substance P-induced lowering of the thermal escape latencies (fig. 2). Slopes of the respective dose-effect curves were statistically different from 0 ($P < 0.05$) and were not significantly different from parallel. Calculated ED_{100} doses (and 95% confidence intervals) for intraperitoneal gabapentin and *S*(+)-3-isobutyl GABA were 184 (91-363) and 31 (19-49) mg/kg, respectively.

Duration of Action of Intraperitoneal Gabapentin

To determine the time course of drug action, the just maximally effective dose of gabapentin (100 mg/kg in-

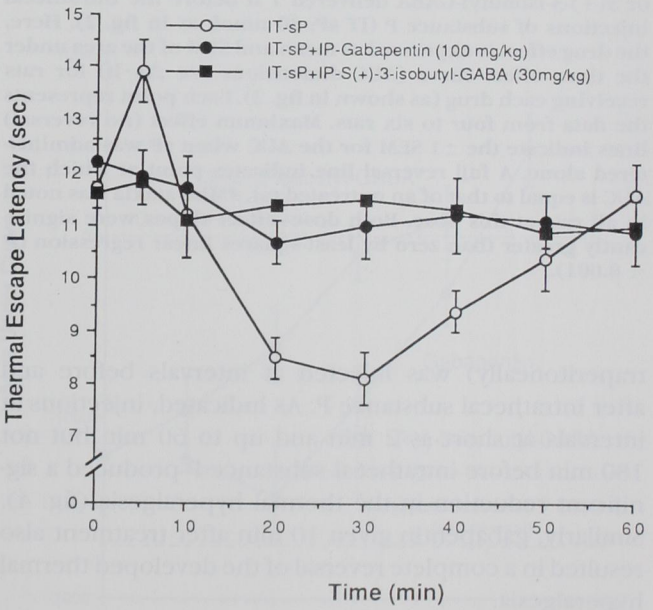


Fig. 2. Thermally evoked hind paw withdrawal latency measured before and after the intrathecal injections of substance P (IT sP) at time = 0 min. Gabapentin (100 mg/kg) or *S*(+)-3-isobutyl-GABA (30 mg/kg) was injected intraperitoneally 60 min before the delivery of the IT sP. Each line represents the mean ± SEM of six rats. Repeated-measures analysis of variance revealed a significant main effect for time for the group of rats receiving IT sP ($P > 0.001$). In contrast, there was no change in the escape latencies over time for groups receiving IT sP + gabapentin or IT sP + *S*(+)-3-isobutyl-GABA ($P > 0.10$). Therefore, at these doses, intraperitoneal gabapentin and *S*(+)-3-isobutyl-GABA prevented the hyperalgesia induced by IT sP.

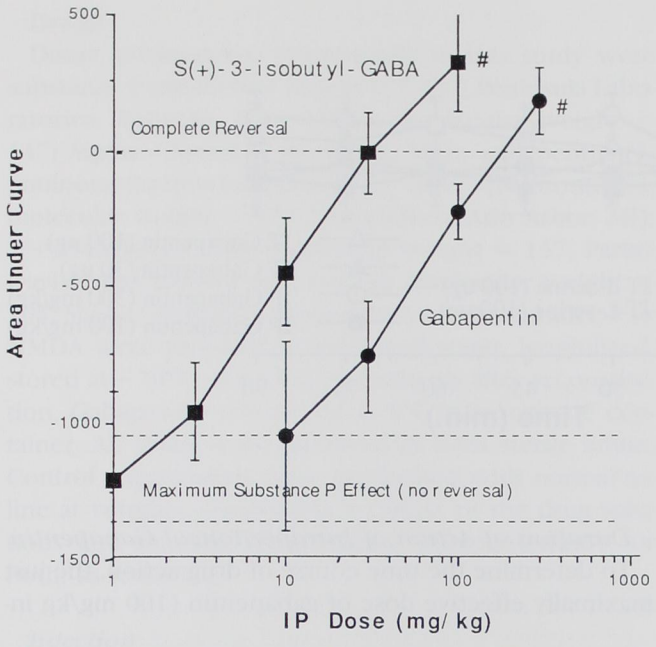


Fig. 3. Dose–effect curves for intraperitoneal (IP) gabapentin or *S*(+)-3-isobutyl-GABA delivered 1 h before the intrathecal injections of substance P (IT sP; 30 nmol; as in fig. 2). Here, the drug effect is expressed as mean and SEM of the area under the time–effect curve (AUC dimensions are Δs · h) for rats receiving each drug (as shown in fig. 2). Each point represents the data from four to six rats. Maximum effect (no reversal) lines indicate the ±1 SEM for the AUC when sP was administered alone. A full reversal line indicates point at which the AUC is equal to that of an untreated rat. #Mild ataxia was noted in all rats at this dose. Both dose–effect slopes were significantly greater than zero by least-squares linear regression ($P < 0.001$).

traperitoneally) was injected at intervals before and after intrathecal substance P. As indicated, injections at intervals at short as 2 min and up to 60 min but not 180 min before intrathecal substance P produced a significant reduction in the thermal hyperalgesia (fig. 4). Similarly, gabapentin given 10 min after treatment also resulted in a complete reversal of the developed thermal hyperalgesia.

Intrathecal Gabapentin and 3-Isobutyl-GABA

The intrathecal delivery of gabapentin (30–300 μg) and *S*(+)-3-isobutyl-GABA (1–30 μg) but not *R*(−)-3-isobutyl-GABA (30 μg) (figs. 5 and 6) produced a significant dose-dependent reversal of the thermal hyperalgesia. By themselves, however, these compounds caused no significant change from baseline (fig. 1). Slopes of the respective dose–effect curves were significantly different from 0 ($P < 0.05$) but not significantly different from parallel. Calculated ED_{100} doses (and 95% confidence intervals) for intrathecal gabapentin and 3-isobutyl-GABA were 165 (101–262) and 65 (45–93) μg, respectively.

Intrathecal D-*Serine or* L-*Serine and Intraperitoneal Gabapentin/S(+)-Isobutyl-GABA*

The intrathecal injections of D- or L-serine (100 μg) had no effect on thermal escape threshold alone or after the injection of intrathecal substance P (fig. 1). In contrast, attenuation of the thermal hyperalgesia otherwise produced by gabapentin (100 mg/kg intraperitoneally) or *S*(+)-3-isobutyl-GABA (30 mg/kg intraperitoneally) was significantly reversed by D-serine but not L-serine (fig. 7).

Effects of Intrathecal NMDA and Intraperitoneal Gabapentin

Intrathecal NMDA at 2 nmol evoked a short-lasting thermal hyperalgesia, the maximum effect of which was

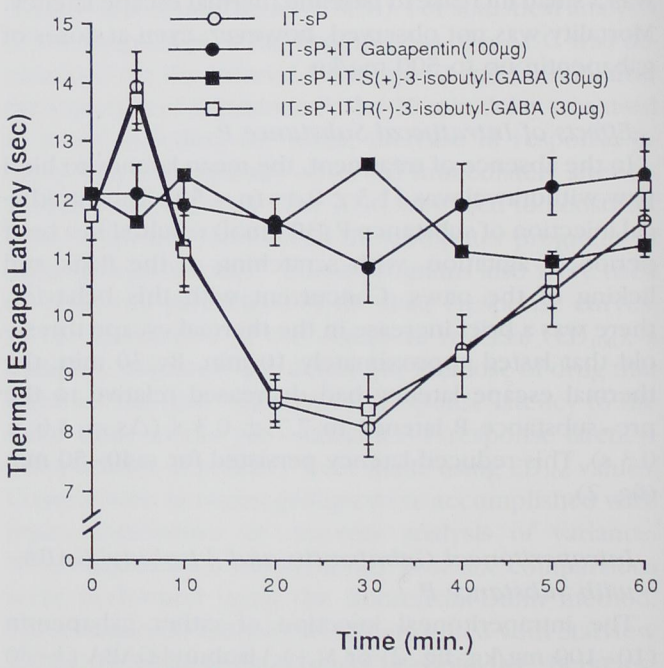


Fig. 4. Area under the time–effect curve (AUC; mean ± SEM) for thermally evoked hind paw withdrawal observed after the intrathecal injection of substance P (IT sP; 30 nmol) in groups of rats treated with intraperitoneal gabapentin (100 mg/kg) at 180, 60, 15, or 2 min before or 10 min after the injection of IT sP. The dashed lines indicate ±1 SEM of the effect produced by IT sP alone. One-way analysis of variance revealed a significant treatment effect between groups ($P < 0.001$). *Post hoc* comparisons indicated that 180 min before treatment was not different from IT sP + saline, whereas significant suppression of hyperalgesia was observed by treatment 60 to 2 min before and 10 min after IT sP ($P < 0.05$).

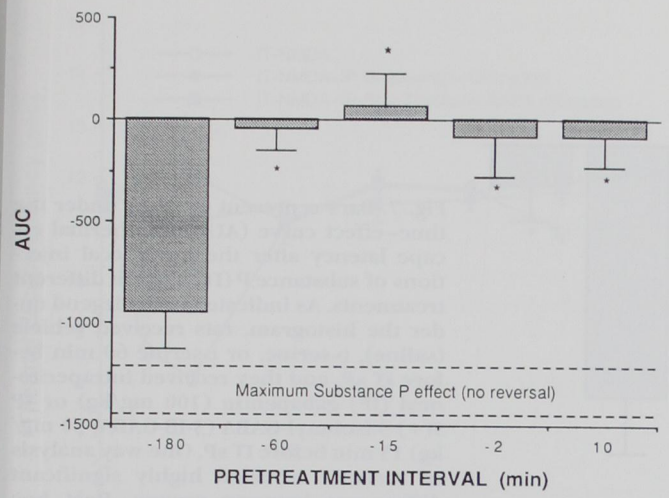


Fig. 5. Thermally evoked hind paw withdrawal latency measured before and after the intrathecal injections of substance P (IT sP) at time = 0 min. Gabapentin (100 μg), *S*(+)-3-isobutyl-GABA (30 μg), or *(R−)*-3-isobutyl-GABA (30 μg) was injected intrathecally 15 min before the delivery of the IT sP. Each line represents the mean ± SEM of six rats. One-way analysis of variance revealed a significant main effect for time for the group of rats receiving IT sP and IT sP + *R*(−)-3-isobutyl-GABA ($P > 0.001$). In contrast, there was no change in the escape latencies over time for groups receiving IT sP + gabapentin or IT sP + *S*(+)-3-isobutyl-GABA ($P > 0.10$). Therefore, at these doses, IT gabapentin and *S*(+)-3-isobutyl-GABA but not *R*(−)-3-isobutyl-GABA prevented the hyperalgesia induced by IT sP.

similar to that produced by intrathecal substance P, although the overall effect was shorter lasting. Pretreatment (at −60 min) with gabapentin (100 mg/kg intraperitoneally) or *S*(+)-3-isobutyl-GABA (30 mg/kg intraperitoneally) prevented the appearance of hyperalgesia (fig. 8).

Behavioral Effects

Gabapentin or *S*(+)-3-isobutyl-GABA had no effect on the righting response or pinna or blink reflexes at doses up to the maximum used after either intrathecal (fig. 2) or intraperitoneal (fig. 5) injection. Examination of ambulatory behavior revealed that there were no effects at intrathecal doses up to 30 or 100 μg or intraperitoneal doses of 30 or 100 mg/kg of *S*(+)-3-isobutyl-GABA and gabapentin, respectively. At the next higher dose by either route, animals receiving gabapentin or *S*(+)-3-isobutyl-GABA were clearly able to ambulate but typically displayed signs of weakness characterized by splaying of the hind paws. Even at these doses, the rats displayed a placing and stepping response, although it was less brisk than that observed at lower doses or in animals in the control group. It is important to emphasize that at doses required to produce a "normal" thermal escape latency in the injured paw, rats displayed no motor deficit detectable to the observer.

Discussion

The current work demonstrates the potent antihyperalgesic effect of systemic and intrathecal delivery of gabapentin and its analogue, *S*(+)-3-isobutyl-GABA. Several points should be emphasized.

Site of Action

Although a supraspinal action is not excluded, the potent effect of the spinally delivered agent compared with the systemic activity demonstrated in the current study and in other works[4,5] suggests that the spinal cord is a certain site of drug action. As is discussed further later, the effects of systemic gabapentin and 3-isobutyl-GABA were readily antagonized by intrathecally delivered D-serine. This antagonism by intrathecal injection

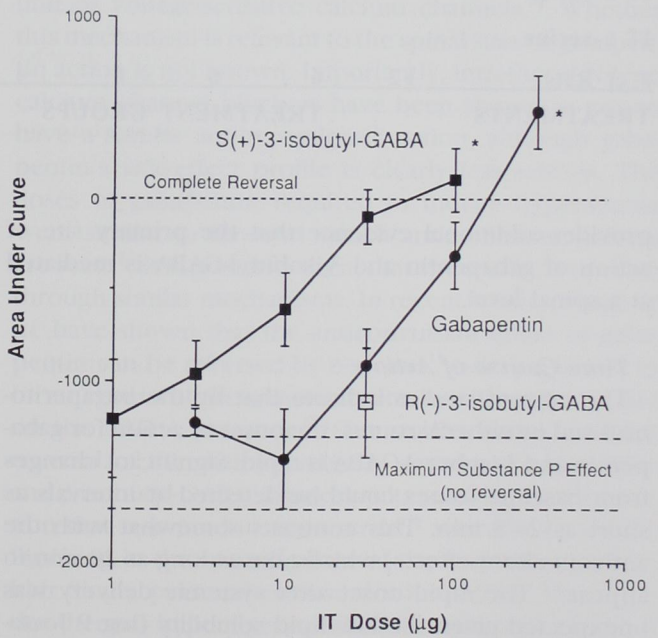
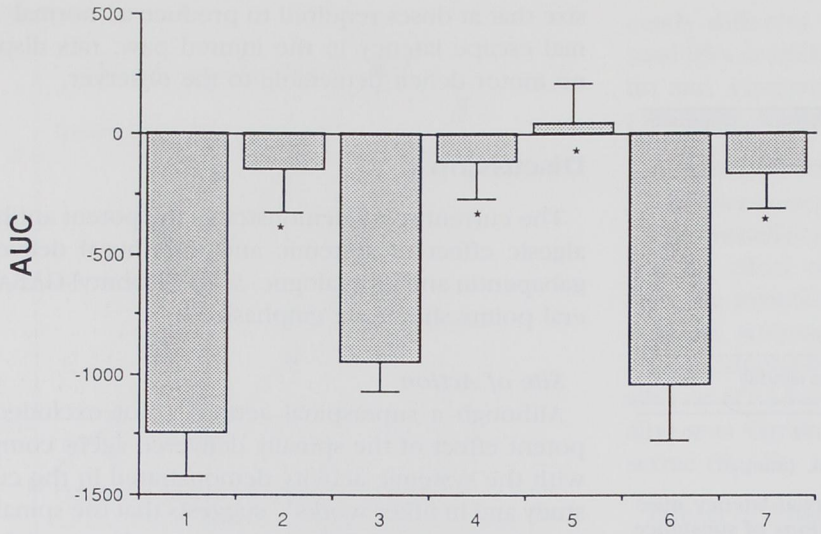


Fig. 6. Dose–effect curves for intrathecal gabapentin, *S*(+)-3-isobutyl-GABA or *R*(−)-3-isobutyl-GABA delivered 1 h before the intrathecal injections of substance P (IT sP; 30 nmol; as in fig. 5). Here, the drug effect is expressed as mean and SEM of the area under the time–effect curve (AUC; as shown in fig. 5) for rats receiving each drug. Each point represents the data from four to six rats. *Mild motor ataxia was noted in all rats at this dose. Calculation of least-squares linear regression revealed that dose–effect slopes for gabapentin and *S*(+)-3-isobutyl-GABA were significantly greater than zero ($P < 0.001$).



TREATMENTS	TREATMENT GROUPS						
IT sP	+	+	+	+	+	+	+
IP Gabapentin		+	+	+			
IP 3-IB-GABA					+	+	+
IT D-serine			+			+	
IT L-serine				+			+
# of Rats	12	6	6	6	4	5	4

Fig. 7. Bars represent the area under the time–effect curve (AUC) for thermal escape latency after the intrathecal injections of substance P (IT sP) with different treatments. As indicated in the legend under the histogram, rats received vehicle (saline), D-serine, or L-serine 60 min before IT sP, and they received intraperitoneal (IP) gabapentin (100 mg/kg) or IP *S*(+)-3-isobutyl-GABA (3-IB-GABA; 30 mg/kg) 15 min before IT sP. One-way analysis of variance revealed highly significant differences between groups. *Post hoc* comparisons showed that, compared with IT sP alone, gabapentin and *S*(+)-3-butyl-GABA significantly reduced the negative AUC (hyperalgesia), and this was prevented in both cases by D-serine but not by L-serine (*$P < 0.05$ compared with IT sP alone).

provides additional evidence that the primary site of action of gabapentin and 3-isobutyl-GABA is mediated at a spinal level.

Time Course of Action

The current studies indicate that by the intraperitoneal and intrathecal routes, the onset of action for gabapentin and 3-isobutyl-GABA is rapid. Significant changes from baseline values could be detected at intervals as short as 2–5 min. This contrasts somewhat with the anticonvulsant effects, which take as long as 20 min to appear.[22] The rapid onset after systemic delivery was unexpected given the low lipid solubility (log P [octanol/water] = −1.2).[23] Gabapentin is actively transported into the bloodstream by an L-amino acid transporter in the gut.[24] L-Amino acid transporters also have been demonstrated to be present at the blood–brain barrier[25] and likely account in part for the immediate appearance of gabapentin in the brain[26,27] (as measured by intracranial microdialysis) after intraperitoneal delivery and thus for the rapid onset of action observed here. It is also interesting to note that gabapentin was effective even 10 min after treatment with substance P. This demonstrates that gabapentin, unlike many drugs, is effective before and after sensitization.

Intrathecal Substance P/NMDA-evoked Hyperalgesia

Spinal delivery of substance P and NMDA produces a thermal hyperalgesia by an action at NK-1 and NMDA sites, respectively.[17] Substance P and NMDA effects are attenuated by intrathecal delivery of cyclooxygenase[17] and nitric oxide synthase inhibitors.[28] These results suggest a positive feedback loop in which spinal prostanoids and nitric oxide enhance the excitability of the presynaptic terminal (see ref. 29). These findings are consistent with our work showing that that intrathecal substance P increases the gain of the function that relates response latency to stimulus intensity.[30] Although intrathecal NMDA receptor antagonism has not been reported to reverse the thermal hyperalgesia[17] of intrathecal substance P, recent studies have shown that

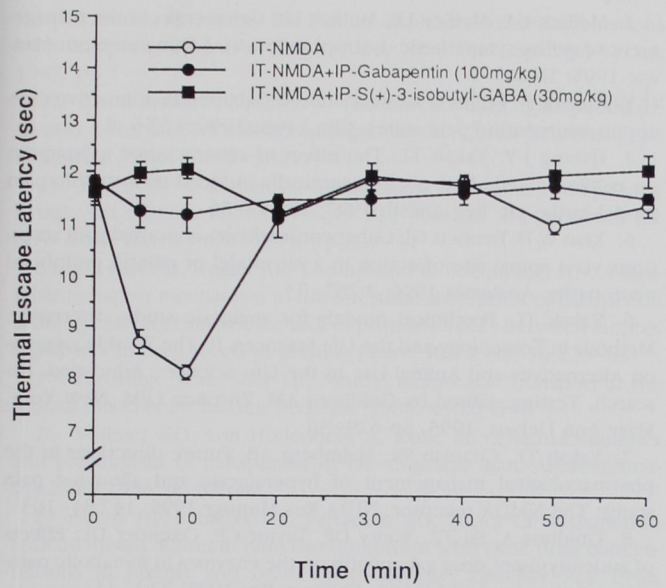


Fig. 8. Thermally evoked hind paw withdrawal latency measured before and after the intrathecal injections of N-methyl-D-aspartate (IT NMDA; 2 nmol) at time = 0 min. Gabapentin (100 mg/kg) was injected intraperitoneally 60 min before the delivery of the IT NMDA. Each line represents the mean ± SEM of six rats. Repeated measures analysis of variance revealed a significant main effect for time for the group of rats receiving IT NMDA ($P > 0.001$). In contrast, there was no change in the escape latencies over time for the group receiving IT NMDA + gabapentin. Therefore, at this dose, intraperitoneal gabapentin prevented the hyperalgesia induced by IT NMDA.

exogenous substance P increases spinal glutamate release[31,32] (Hua, Marsala, and Yaksh, unpublished data). This raises the possibility that at least a component of the thermal hyperalgesia might be mediated by an indirect action at one or another spinal NMDA or non-NMDA receptors.

Mechanism of Action

GABAergic Receptor. Gapapentin and 3-isobutyl-GABA are GABA analogues. They have no reported affinity for the GABA-A or GABA-B binding sites.[22] It has been shown, however, that gabapentin can increase the extracellular levels of GABA in striatal slice preparations[33] and evoke a nonsynaptic release of GABA from glial cells.[14] Given that gabapentin may increase spinal GABAergic tone, its actions after spinal delivery are consistent with the ability of GABA-A and GABA-B receptor agonists to reduce the second phase of the formalin test,[34] the allodynia in the Chung model of neuropathy, and the thermal hyperalgesia in the Bennett model of neuropathy.[35] In the work with the Chung model of neuropathy, however, the effects of gabapentin were not antagonized by doses of bicuculline and CGP 35348, which were able to reverse selectively the effects of muscimol and (GABA-A) and baclofen (GABA-B), respectively.[4,35]

Glutamatergic Receptor. The several animal models noted previously (formalin test, second phase; allodynia in the Chung model of neuropathy; thermal hyperalgesia in the Bennett model of neuropathy), in which gabapentin has been shown to display a significant effect, reflect diverse underlying mechanisms. Common to all, however, is the role played by spinal NMDA receptors (see ref. 7). The interaction of gabapentin with the NMDA receptor appears to be indirect. Binding studies failed to indicate an affinity of gabapentin for NMDA, nonstrychnine-sensitive glycine sites, or non-NMDA sites.[23] Gabapentin has been reported not to alter NMDA channel responses.[9] Moreover, as with the GABAergic system, the lack of prominent motor effects renders it unlikely that the primary effects reflect a simple antagonism of the NMDA site.

Gabapentin has been shown to bind to the α_2/δ subunit of voltage-sensitive calcium channels.[36] Whether this mechanism is relevant to the spinal sites of gabapentin action is not known. Importantly, intrathecal N-type calcium channel blockers have been shown in rats to have a similar antihyperalgesic action, although gabapentin's side-effect profile is clearly less serious. The doses of gabapentin required to induce hyperalgesia were similar to those at which anticonvulsant effects are seen.[22] It is possible, then, that these effects operate through similar mechanisms. In recent studies, Singh *et al.* have shown that the anticonvulsant effect of gabapentin can be reversed by D-serine.[11] D-Serine is said to be an agonist at the nonstrychnine-sensitive glycine site on the NMDA receptor.[37] Our studies show a similar reversal of the antihyperalgesic activity of gabapentin and 3-isobutyl-GABA. These results confirm the observation that D-serine but not L-serine[38] was able to reduce flinching in the second phase of the formalin test (Lakhbir Singh, personal communication, 1996) and block the thermal hyperalgesia induced by thermal injury to the paw (Jun and Yaksh, unpublished data, 1997). The results also suggest that gabapentin and 3-isobutyl-GABA may be acting at the nonstrychnine-sensitive glycine site on the NMDA receptor; however, binding studies have failed to show any effect of gabapentin at this site. After intrathecal delivery, D-serine alone was reported to facilitate the thermally evoked tail flick.[39] In the current study, however, neither D-serine nor L-serine

altered the thermal escape response in healthy rats nor in rats rendered hyperalgesic by intrathecal substance P. The reason for this difference is not known.

The efficacy of gabapentin in several models of hyperalgesia, including hyperalgesia induced by intrathecal substance P and NMDA, suggests a common mechanisms associated with the generation of a facilitated state of processing. It also implies that gabapentin is not acting as a direct antagonist at the NK-1 or NMDA receptors.

Site specificity of gabapentin may be in doubt, but there are accumulating data to suggest that there is a specific mode of action. Gabapentin has been shown to bind to cellular plasma membranes with high nanomolar affinity. Its binding is displaced in a stereospecific fashion by *S*(+)-3-isobutyl-GABA but not by the stereoisomer *R*(−)-3-isobutyl-GABA. This stereoselectivity in binding is also reflected in the biologic activity of this family of agents. Therefore, as an anticonvulsant agent, *S*(+)-3-isobutyl-GABA is approximately 4 to 10 times more potent than gabapentin, whereas the stereoisomer *R*(−)-3-isobutyl-GABA is inactive.[19,20] The current studies reveal a similar structure-activity relationship for the spinal antihyperalgesic effects, with *S*(+)-3-isobutyl-GABA being approximately three times more potent than gabapentin as an antihyperalgesic agent, whereas *R*(−)-3-isobutyl-GABA is without effect at the highest dose examined. Moreover, the effects of both active agents are reversed by intrathecal D-serine. These data jointly suggest that the gabapentinoids, gabapentin and *S*(+)-3-isobutyl-GABA, may share a common, if as-yet undefined, mechanism of action.

The current study reveals a potent, dose-dependent, and stereospecific antihyperalgesic effect for gabapentin and 3-isobutyl-GABA. Comparison of the doses required for spinal versus systemic delivery, and the ability of intrathecal D-serine to reverse the effects of these agents given systemically, suggest the likely importance of a common spinal site of action. These results are in accord with a growing body of preclinical and clinical literature suggesting that gabapentin may exert potent effects on a variety of anomalous pain states in which facilitated spinal processing is induced by tissue or nerve injury.

The authors thank Dr. Michael Rafferty for his suggestions and critical reading of this manuscript.

References

1. Taylor CP: Emerging perspectives on the mechanism of action of gabapentin. Neurology 1994;44:S10-6; discussion S31-2

2. Mellick GA, Mellicy LB, Mellick LB: Gabapentin in the management of reflex sympathetic dystrophy [letter]. J Pain Symptom Manage 1995; 10:265-6

3. Rosner H, Rubin L, Kestenbaum A: Gabapentin adjunctive therapy in neuropathic pain states. Clin J Pain 1996; 12:56-8

4. Hwang J-Y, Yaksh TL: The effect of subarachnoid gabapentin on tactile-evoked allodynia in a surgically induced neuropathic pain model in the rat. Reg Anesth 1997; 22:249-56

5. Xiao W-H, Bennett GJ: Gabapentin relieves abnormal pain sensations via a spinal site of action in a rat model of painful peripheral neuropathy. Analgesia 1996; 2:267-73

6. Yaksh TL: Preclinical models for analgesic study, Alternative Methods in Toxicology and the Life Sciences, II. The World Congress on Alternatives and Animal Use in the Life Sciences: Education, Research, Testing. Edited by Goldberg AM, Zutphen LFM. New York, Mary Ann Liebert, 1995, pp 629-36

7. Yaksh TL, Chaplan SR, Malmberg AB: Future directions in the pharmacological management of hyperalgesic and allodynic pain states: The NMDA receptor. NIDA Res Monogr 1995; 147:84-103

8. Goldlust A, Su TZ, Welty DF, Taylor CP, Oxender DL: Effects of anticonvulsant drug gabapentin on the enzymes in metabolic pathways of glutamate and GABA. Epilepsy Res 1995; 22:1-11

9. Rock DM, Kelly KM, Macdonald RL: Gabapentin actions on ligand-and voltage-gated responses in cultured rodent neurons. Epilepsy Res 1995; 16:89-98

10. Taylor CP: Mechanism of action of new anti-epileptic drugs, New Trends in Epilepsy Management: The Role of Gabapentin. Edited by Chadwick D. London Royal Society of Medicine Services. 1993

11. Singh L, Field MJ, Ferris P, Hunter JC: The antiepileptic agent gabapentin (Neurontin) possesses anxiolytic-like and antinociceptive actions that are reversed by d-serine. Psychopharmacology 1996; 127:1-9

12. Loscher W, Honack D, Taylor CP: Gabapentin increases aminooxyacetic acid-induced GABA accumulation in several regions of rat brain. Neurosci Lett 1991; 128:150-4

13. Su TZ, Lunney E, Campbell G, Oxender DL: Transport of gabapentin, a gamma-amino acid drug, by system 1 alpha-amino acid transporters: A comparative study in astrocytes, synaptosomes, and CHO cells. J Neurochem 1995; 64:2125-31

14. Kocsis JD, Honmou O: Gabapentin increases GABA-induced depolarization in rat neonatal optic nerve. Neurosci Lett 1994; 169:181-4

15. Taylor CP, Vartanian MG, Andruszkiewicz R, Silverman RB: 3-alkyl GABA and 3-alkylglutamic acid analogues: Two new classes of anticonvulsant agents. Epilepsy Res 1992; 11:103-10

16. Taylor CP, Vartanian MG, Yuen PW, Bigge C, Suman-Chauhan N, Hill DR: Potent and stereospecific anticonvulsant activity of 3-isobutyl GABA relates to in vitro binding at a novel site labeled by tritiated gabapentin. Epilepsy Res 1993; 14:11-5

17. Malmberg AB, Yaksh TL: Hyperalgesia mediated by spinal glutamate or substance-P receptor blocked by spinal cyclooxygenase inhibition. Science 1992; 257:1276-9

18. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976; 17:1031-6

19. Hargreaves K, Dubner R, Brown F, Flores C, Joris J: A new and sensitive method for measuring thermal nociception in cutaneous hyperalgesia. Pain 1988; 32:77-88

20. Dirig DM, Yaksh TL: Differential right shifts in the dose-response curve for intrathecal morphine and sufentanil as a function of stimulus intensity. Pain 1995; 62:321-8

21. Tallarida RJ, Murray RB: Manual of pharmacologic calculations with computer programs. 2nd edition. New York, Springer-Verlag, 1987, pp 1-95

22. Bloms-Funke P, Loscher W: The anticonvulsant gabapentin decreases firing rates of substantia nigra pars reticulata neurons. Eur J Pharmacol 1996; 316(2-3):211-8

23. Taylor CP: Gabapentin: Mechanisms of action, Antiepileptic Drugs. 4th edition. Edited by Levy RH, Mattson RH, Meldrum BS. New York, Raven Press, 1995, pp 829-41

24. Stewart BH, Kugler AR, Thompson PR, Bockbrader HN: A saturable transport mechanism in the intestinal absorption of gabapentin is the underlying cause of the lack of proportionality between increasing dose and drug levels in plasma. Pharm Res 1993; 10:276-81

25. Pardridge WM, Choi TB: Neutral amino acid transport at the human blood-brain barrier. Fed Proc 1986; 45:2073-8

26. Vollmer KO, von Hodenberg A, Kolle EU: Pharmacokinetics and metabolism of gabapentin in rat, dog and man. Arzneimittelforschung 1986; 36:830-9

27. Welty DF, Schielke GP, Vartanian MG, Taylor CP: Gabapentin anticonvulsant action in rats: Disequilibrium with peak drug concentrations in plasma and brain microdialysate. Epilepsy Res 1993; 16:175-81

28. Malmberg AB, Yaksh TL: Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain 1993; 54:291-300

29. Yaksh TL, Malmberg AB: Central pharmacology of nociceptive transmission, Textbook of Pain. 3rd edition. Edited by Wall P, Melzack R. Edinburgh, Churchill Livingstone, 1994, pp 165-200.

30. Dirig DM, Yaksh TL: Thermal hyperalgesia in rat evoked by intrathecal substance P at multiple stimulus intensities reflects an increase in the gain of nociceptive processing. Neurosci Lett 1996; 220:1-4

31. Maehara T, Suzuki H, Yoshioka K, Otsuka M: Substance P-evoked release of amino acid transmitters from the newborn rat spinal cord. Regul Pept 1993; 46:354-6

32. Skilling SR, Harkness DH, Larson AA: Experimental peripheral neuropathy decreases the dose of substance P required to increase excitatory amino acid release in the CSF of the rat spinal cord. Neurosci Lett 1992; 139:92-6

33. Gotz E, Feuerstein TJ, Lais A, Meyer DK: Effects of gabapentin on release of gamma-aminobutyric acid from slices of rat neostriatum. Arzneimittelforschung 1993; 43:636-8

34. Dirig DM, Yaksh TL: Intrathecal baclofen and muscimol, but not midazolam, are antinociceptive using the rat-formalin model. J Pharmacol Exp Ther 1995; 275:219-27

35. Hwang J-H, Yaksh TL: The effect of spinal GABA receptor agonists on tactile-evoked allodynia in a surgically induced neuropathic pain model in the rat. Pain 1997; 70:15-22

36. Gee, NS, Brown JP, Dissanayake VU, Offord J, Thurlow R, Woodruff GN: The novel anticonvulsant drug, gabapentin (Neurontin), binds to the alpha2delta subunit of a calcium channel. J Biol Chem 1996; 271:5768-76

37. Kemp JA, Leeson PD: The glycine site of the NMDA receptor—five years on. Trends Pharmacol Sci 1993; 14:20-5

38. Brugger F, Wicki U, Nassenstein-Elton D, Fagg GE, Olpe HR, Pozza MF: Modulation of the NMDA receptor by D-serine in the cortex and the spinal cord, in vitro. Eur J Pharmacol 1990; 191:29-38

39. Kolhekar R, Meller ST, Gebhart GF: N-methyl-D-aspartate receptor-mediated changes in thermal nociception: Allosteric modulation at glycine and polyamine recognition sites. Neuroscience 1994; 63:925-36