

Analgesic Interaction between Intrathecal Midazolam and Glutamate Receptor Antagonists on Thermal-induced Pain in Rats

Tomoki Nishiyama, M.D., Ph.D.,* Laszlo Gyermek, M.D., Ph.D.,† Chingmuh Lee, M.D.,† Sachiko Kawasaki-Yatsugi, B.S.,‡ Tokio Yamaguchi, Ph.D.§

Background: Two major neurotransmitters, γ -aminobutyric acid (GABA) and the excitatory amino acid, glutamate, may be involved in nociception in the spinal cord. GABA and glutamate receptors may operate in concert to modify signals in the central nervous system. The purpose of this study was to investigate the spinal analgesic interaction between midazolam, a benzodiazepine-GABA_A receptor agonist, and two glutamate receptor antagonists on acute thermal nociception.

Methods: Sprague-Dawley rats were implanted with chronic lumbar intrathecal catheters and were tested for their tail withdrawal response by the tail flick test after intrathecal administration of saline, midazolam (1–100 μ g), AP-5 (1–30 μ g), or YM872 (0.3–30 μ g). AP-5 is an *N*-methyl-D-aspartate (NMDA) receptor antagonist and YM872 is an α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptor antagonist. The combination of midazolam and the other two agents were also tested by isobolographic analyses. Motor disturbance and behavioral changes were observed.

Results: Dose-dependent increases in the tail flick latency were observed with midazolam, AP-5, and YM872 with 50% effective dose values of 1.57 ± 0.34 (SEM) μ g, 5.54 ± 0.19 μ g, and 1.0 ± 0.22 μ g, respectively. A potent synergy in analgesia

with decreased behavioral changes and motor disturbance was obtained when combining midazolam with AP-5 or YM872.

Conclusions: Spinally administered midazolam and an NMDA- or an AMPA-receptor antagonist exhibited potent synergistic analgesia on acute thermal nociception in rats. Side effects, shown by behavioral changes and motor disturbance, decreased with the combination of the agents. These results point out an important direction for the study of acute nociception. (Key words: α -Amino-3-hydroxy-5-methylisoxazole-4-propionic acid; analgesia; midazolam; *N*-methyl-D-aspartate; spinal cord.)

TWO major neurotransmitters, γ -aminobutyric acid (GABA)¹ and the excitatory amino acid, glutamate, may be involved in nociception in the spinal cord. GABA is found in high concentration in the spinal cord.² Specific benzodiazepine receptors are associated with dorsal-horn systems in the spinal cord that encode pain related information.³ Benzodiazepine receptor agonists appear to increase the intrinsic efficacy of GABA at the GABA_A receptor coupling with benzodiazepine receptor by increasing the chloride conductance for a given GABA-ergic stimulus.⁴ Midazolam, a benzodiazepine derivative, depresses spinal nociceptive neurotransmission, as measured by changes in the nociception related slow ventral root potential.⁵ It is also reported that midazolam has spinally mediated analgesic effects in behavioral studies.^{3,6}

On the other hand, glutamates, excitatory amino acids, exist in primary afferents and interneurons.⁷ Ionotropic glutamate receptors in the spinal cord are well known to mediate nociception. They may be mainly classified into two classes: the *N*-methyl-D-aspartate (NMDA) receptors and the α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) receptors. NMDA receptor antagonists block the facilitated states of pain processing but have little effect on acute nociception.⁸ In contrast, the AMPA receptor antagonists have analgesic effects on acute nociception.^{9,10}

GABA and glutamate receptors may operate in concert

* Research Associate, Department of Anesthesiology, Harbor University of California, Los Angeles Medical Center, Torrance, California; and Instructor, Department of Anesthesiology, The University of Tokyo, Tokyo, Japan.

† Professor, Department of Anesthesiology, Harbor University of California, Los Angeles Medical Center, Torrance, California.

‡ Research Associate, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Tsukuba, Japan.

§ Manager, Institute for Drug Discovery Research, Yamanouchi Pharmaceutical Co., Tsukuba, Japan.

Received from the Department of Anesthesiology, Harbor University of California, Los Angeles Medical Center, Los Angeles, California. Submitted for publication December 21, 1998. Accepted for publication April 13, 1999. Supported by the fund of the Department of Anesthesiology, Harbor University of California, Los Angeles Medical Center. Presented at the 73rd Meeting of the International Anesthesia Research Society, Los Angeles, California, March 15, 1999.

Address reprint requests to Dr. Nishiyama: 3-2-6-603, Kawaguchi, Kawaguchi-shi, Saitama, 332-0015, Japan. Address electronic mail to: nishiyamat-ane@h.u-tokyo.ac.jp

to regulate nociceptive signals in various regions of the brain.^{11,12} Because there were few reports of the relation of these two receptor systems in the spinal cord, we investigated the spinally mediated analgesic interaction of these two receptor systems on acute nociception using an intrathecally catheterized rat model.

Materials and Methods

Animal Preparations

The protocol was approved by the Research and Education Institute of Harbor-UCLA Medical Center. Sprague-Dawley rats (280–300 g; B. K. Universal, Fremont, CA) were implanted with chronic lumbar intrathecal catheters under halothane (2%) anesthesia according to the method described by Yaksh and Rudy.¹³ Briefly, an 8.5-cm polyethylene catheter (PE-10; Clay Adams, Parsippany, NJ) was advanced caudally through an incision in the atlantooccipital membrane, to the thoracolumbar level of the spinal cord. The external part of the catheter was tunneled subcutaneously to exit on the top of the skull and plugged with a 28-gauge stainless steel wire. Only rats with normal motor function and behavior 5 days after surgery were used.

Drugs and Administration

Midazolam (a benzodiazepine-GABA_A receptor agonist; Sigma, St. Louis, MO) 1, 3, 10, 30, and 100 μ g, and AP-5 (2-amino-5-phosphonovaleic acid, an NMDA receptor antagonist; Sigma) 1, 3, 10, and 30 μ g were dissolved in saline 10 μ l. YM872 ([2,3-Dioxo-7-(1H-imidazol-1-yl)-6-nitro-1,2,3,4-tetrahydro-1-quinolaliny] acetic acid), an AMPA receptor antagonist (Yamanouchi Pharmaceutical, Tsukuba, Japan) 10 mg was dissolved in 0.97 ml distilled water with 30 μ l 1 N NaOH to adjust pH to 7.3–7.5. Solutions of 0.3 (0.86), 1 (2.86), 3 (8.59), 10 (28.63), and 30 (85.89) μ g (nM) per 10 μ l were made using normal saline. After intrathecal drug injection, the catheter was flushed with a subsequent injection of 10 μ l of normal saline to clear the dead space of the catheter (7 ± 0.4 μ l, mean \pm SE). Microinjector syringes were used for all injections. In each dose group, eight randomly selected rats were used. Normal saline 10 μ l was injected in the control group.

Nociceptive Test: Tail Flick Test

Each rat was placed in a clear plastic cylindrical cage with its tail extended through a slot provided in the rear of the tube. Noxious stimulation was provided by a beam

of high-intensity light (Tail-flick Analgesia Meter 0570-001L, Columbus Instruments International, Columbus, OH) focused on the tail 2–3 cm proximal to the end. The response time was measured and defined as the interval between the onset of the thermal stimulation and the abrupt flick of the tail. The cutoff time in the absence of a response was set to 14 s to prevent tissue injury.

Behavioral and Motor Function Test

The general behavior (including agitation and allodynia), motor function, pinna reflex, and corneal reflex were examined. Their presence or absence was recorded. Agitation was judged to be present when the rat spontaneously vocalized or became restless. The presence of allodynia was examined by looking for agitation (escape or vocalization) evoked by lightly stroking the flank with a pencil. The stimulus was sufficient to move hair but not dent the skin. Motor function was evaluated by the placing/stepping reflex and by the righting reflex. The former was evoked by drawing the dorsum of either hind paw across the edge of the table. The latter was assessed by placing the rat horizontally with its back on the table, which normally gives rise to an immediate, coordinated turning of the body back to an upright position. Flaccidity was judged as a muscle weakness. Pinna and corneal reflexes were examined with a paper string.

Experimental Paradigm

The first series of experiments was performed to determine the dose dependency and time course of the analgesic actions of intrathecally administered midazolam, AP-5, and YM872 on acute thermal nociception. The tail flick test, behavioral test, and motor function test were performed before and 5, 10, 15, 30, 60, 90, 120 min after drug injection and at 1-h intervals until the response time returned to baseline (maximum 360 min).

To investigate the interaction between midazolam and AP-5 or YM872, an isobolographic analysis was used.¹⁴ The method is based on comparisons of dose ratios that are determined to be equieffective. First, the respective 50% effective dose (ED₅₀) values are determined from the dose-response curves of the agents alone. Subsequently, a dose-response curve is obtained by coadministration of the two drugs in a constant dose ratio based on the ED₅₀ values of the single agents. From the dose-response curve of the combined drugs, the ED₅₀ value of the mixture was calculated.

ANALGESIA OF MIDAZOLAM AND GLUTAMATE ANTAGONISTS

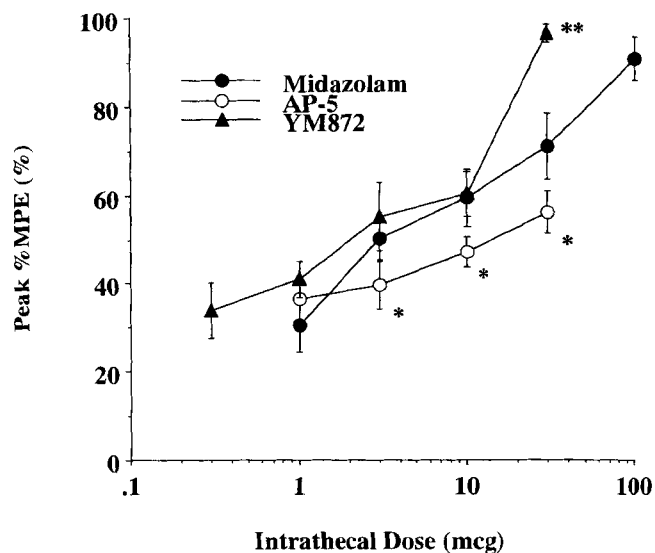


Fig. 1. Dose-response curves of intrathecal midazolam, AP-5 (NMDA antagonist), and YM872 (AMPA antagonist) on the tail flick latency expressed as the peak percentage maximum possible effect. Each point presents the mean \pm SEM of eight animals. * $P < 0.05$ versus the other two agents, ** $P < 0.01$ versus the other two agents.

Data Analysis and Statistics

Data were expressed as mean \pm standard error (SEM). Tail flick response latency was converted to percentage maximum possible effect (%MPE) according to the formula: %MPE = [(postdrug latency - baseline latency) / (cutoff time - baseline latency)] \times 100. ED₅₀ was calculated by a computer program, which was created in the laboratory of University of California, San Diego, according to Tallarida and Murray,¹⁵ as the dose that produces a value of 50% MPE.

To describe the magnitude of interaction between the agents, a total fractional dose value was calculated as follows: [(ED₅₀ dose of drug 1 in combination) / (ED₅₀ value for drug 1 alone)] + [(ED₅₀ dose of drug 2 in combination) / (ED₅₀ value for drug 2 alone)]. The values were normalized by assigning the ED₅₀ values of the agents given alone a value of 1. Values near 1 indicate an additive interaction, values greater than 1 imply an antagonistic interaction; values less than 1 indicate a synergistic interaction. To compare the theoretic additive point with experimentally derived ED₅₀, isobolographic analysis¹⁴ was used.

Differences between doses were analyzed with two-way analysis of variance followed by the Newman-Keuls test. Student *t* test was used to compare the calculated ED₅₀ values with the theoretic additive values. A *P* value less than 0.05 was considered statistically significant.

Results

Analgesic Effects of Midazolam, AP-5, and YM872

The baseline latency (before drug injection) in the tail flick test was 3.0 ± 0.2 s (mean \pm SE). Intrathecal administration of midazolam, AP-5, and YM872 resulted in dose-dependent increases in the tail flick latency (fig. 1). The ED₅₀ values were 1.57 ± 0.34 μ g, 5.54 ± 0.19 μ g, and 1.0 ± 0.22 μ g with midazolam, AP-5, and YM872, respectively.

Interaction between Midazolam and NMDA Antagonist

Coadministration of midazolam and AP-5 intrathecally shifted a dose response curve to the left (fig. 2) and showed a significant increase in the thermal escape latency compared with the agents alone by an isobolographic analysis (fig. 3). The experimentally obtained ED₅₀ of the combination of midazolam and AP-5 was midazolam 0.21 ± 0.18 μ g and AP-5 0.75 ± 0.18 μ g. These doses were significantly lower than the theoretic additive doses (midazolam 0.79 ± 0.38 μ g and AP-5 2.77 ± 0.24 μ g). The total fractional dose value of the combination was calculated to be 0.27 ± 0.11 , which indicates a synergistic interaction.

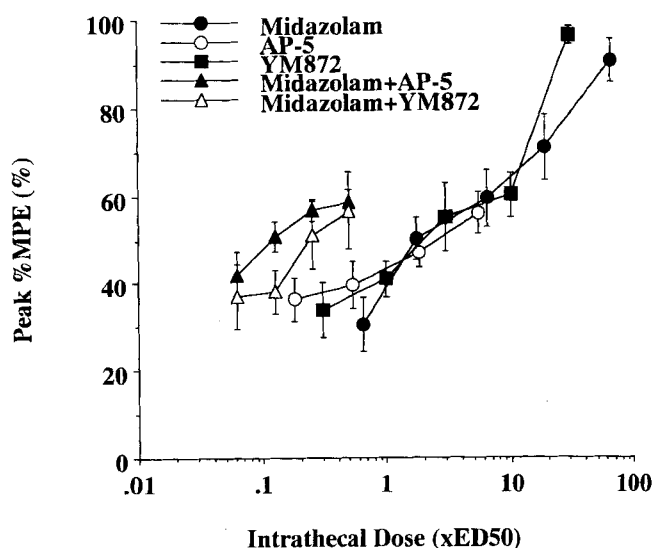


Fig. 2. Comparison of the dose-response curves of intrathecal midazolam, AP-5 (NMDA antagonist), YM872 (AMPA antagonist), midazolam plus AP-5, and midazolam plus YM872 on the tail flick latency expressed as the peak percentage maximum possible effect. Intrathecal dose is indicated as the percentage of ED₅₀. Each point presents the mean \pm SEM of eight animals.

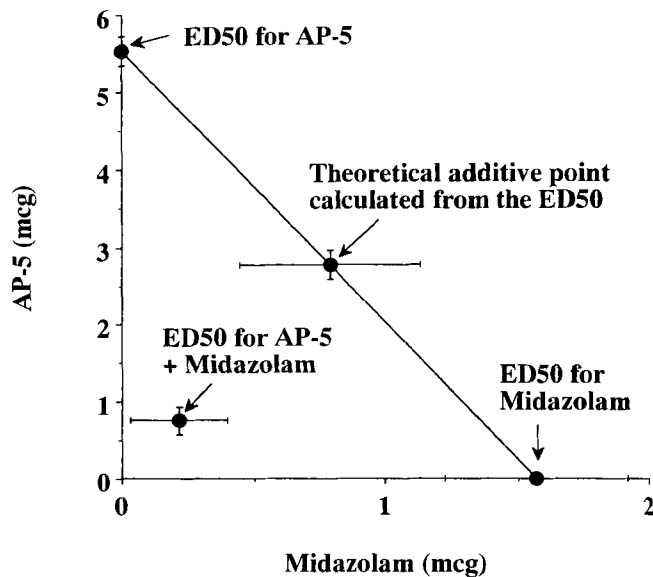


Fig. 3. Isobologram for the intrathecal interaction of midazolam and AP-5. Horizontal and vertical bars indicate SEM. The oblique line between the x-axis and y-axis is the theoretic additive line. The point in the middle of this line is the theoretic additive point calculated from the separate ED₅₀ values. The experimental point lies far below the additive line, indicating a significant synergism.

Interaction between Midazolam and AMPA Antagonist

Coadministration of midazolam and YM872 intrathecally shifted a dose-response curve to the left (fig. 2) and showed a significant increase in the thermal escape latency compared to the agents alone (fig. 4). The experimentally obtained ED₅₀ of the combination of midazolam and YM872 was midazolam $0.15 \pm 0.09 \mu\text{g}$ and YM872 $0.24 \pm 0.09 \mu\text{g}$. These doses were significantly lower than the theoretic additive doses (midazolam $0.79 \pm 0.38 \mu\text{g}$ and YM872 $0.5 \pm 0.22 \mu\text{g}$). The total fractional dose value of the combination was calculated to be 0.34 ± 0.12 , which indicates a synergistic interaction.

Behavior and Motor Function

Midazolam $\geq 3 \mu\text{g}$ (each one rat in 3, 10, 30, and 100 μg) and AP-5 $\geq 10 \mu\text{g}$ (one in 10 μg and two in 30 μg) induced agitation and allodynia. Motor disturbances (tested by the placing/stepping reflex and by the righting reflex) occurred with midazolam $\geq 30 \mu\text{g}$ (two rats in 30 μg and six in 100 μg), AP-5 $\geq 10 \mu\text{g}$ (two in 10 μg and three in 30 μg) or YM872 $\geq 10 \mu\text{g}$ (three in 10 μg and four in 30 μg). Flaccidity was seen in the rats with midazolam $\geq 30 \mu\text{g}$ (one in 30 μg and two in 100 μg),

or YM872 $\geq 10 \mu\text{g}$ (two in 10 μg and six in 30 μg). AP-5 30 μg induced loss of pinna reflex (two rats). In contrast, combination of midazolam and AP-5 induced no observable side effects. Allodynia was seen with midazolam 0.2 μg plus YM872 0.125 μg (one rat), and loss of righting reflex occurred with midazolam 0.8 μg plus YM872 0.5 μg (one rat). The combinations of midazolam and AP-5 or YM872 displayed fewer side effects than the equipotent doses of the individual agents (table 1). No rats showed paralysis in this study.

Discussion

We found that intrathecally administered midazolam (benzodiazepine-GABA_A receptor agonist), AP-5 (NMDA receptor antagonist) and YM872 (AMPA receptor antagonist) produced dose-dependent increases in tail flick latency. Midazolam showed synergistic analgesic effects with both AP-5 and YM872.

In the dorsal horn of the spinal cord, GABA receptors mediate presynaptic inhibition on the primary afferent terminals.¹⁶ At these endings, GABA produces a mild depolarization of the primary afferents and thereby reduces the release of the excitatory transmitter onto the second-order neurons in the spinal cord.¹⁷ Binding sites

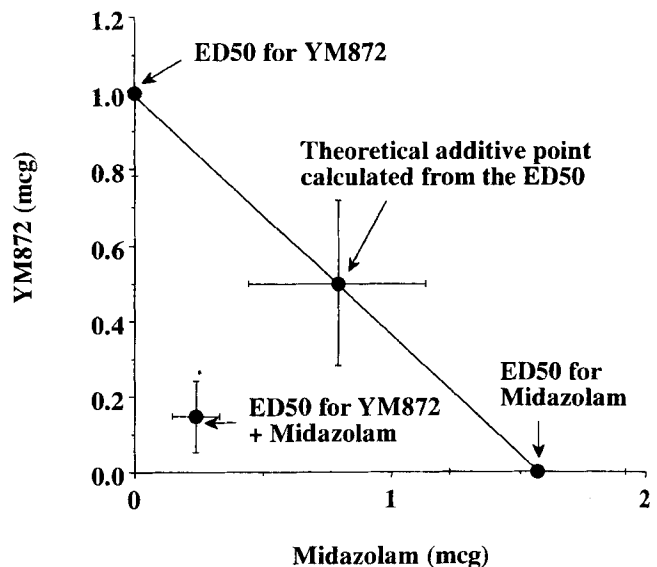


Fig. 4. Isobologram for the intrathecal interaction of midazolam and YM872. Horizontal and vertical bars indicate SEM. The oblique line between the x-axis and y-axis is the theoretic additive line. The point in the middle of this line is the theoretic additive point calculated from the separate ED₅₀ values. The experimental point lies far below the additive line, indicating a significant synergism.

ANALGESIA OF MIDAZOLAM AND GLUTAMATE ANTAGONISTS

Table 1. Side Effects with the Comparable Doses

	Saline (control)	Midazolam 3 μ g	AP-5 10 μ g	YM872 1 μ g	Midazolam 0.2 μ g + AP-5 0.69 μ g	Midazolam 0.2 μ g + YM872 0.125 μ g
Agitation	0	0	1	0	0	0
Allodynia	0	1	1	0	0	1
Loss of righting reflex	0	1	2	0	0	0
Loss of placing and stepping reflex	0	0	1	0	0	0
Flaccidity	0	0	0	0	0	0
Loss of corneal reflex	0	0	0	0	0	0
Loss of Pinna reflex	0	0	0	0	0	0

Values are the number of rats that showed each side effect. Total number of rats tested in each group is 8.

for benzodiazepine are in lamina II of the dorsal horn.¹⁸ Radioligand binding assays and electrophysiologic studies showed the linkage of the benzodiazepine sites to the GABA_A receptor complex in the spinal cord.^{19,20} Enhancement of presynaptic inhibition might be a possible mechanism for the action of midazolam, because benzodiazepines are known to increase GABA transmission *via* their specific binding site collocated with the GABA_A receptor.²¹ Benzodiazepines were reported not to block the transmission of sensory impulses through nerve fibers.²²

Yanez *et al.*²³ reported that intrathecally administered midazolam 20–60 μ g produced dose-dependent antinociception on thermally induced pain and larger doses (60–100 μ g) induced motor dysfunction. These published data are similar to those of our current study, in which midazolam 1–100 μ g induced dose-dependent analgesia and doses higher than 30 μ g induced motor dysfunction. In the study of Bahar *et al.*⁶, 75 μ g intrathecal midazolam induced sleep. However, 100 μ g did not induce sleep in our study. We did not apply higher doses of midazolam because of the limitation of its solubility in saline and because the 30 and 100 μ g doses already induced motor dysfunction.

NMDA receptors are involved in the wind-up phenomena of deep dorsal-horn cells evoked by C-fiber activation.²⁴ NMDA receptor antagonists are therefore the most efficacious against the continuously stimulated state of nociception, induced for example by formalin.⁸ Generally, they are inefficacious on acute nociception,¹¹ although some studies^{25,26} have shown analgesic effects on acute thermal stimuli. In the present study, AP-5 (NMDA receptor antagonist) produced dose-dependent analgesic effects on acute thermal stimulus, although the ED₅₀ value was relatively high. In a previous study, AP-5 had only weak analgesic effects at the maximum usable dose in the hot-plate test.²⁷ Considering these results together, NMDA antagonists might have some analgesic

effects on acute nociception depending on the experimental settings.

AMPA receptors are found throughout all superficial laminae of the dorsal horn pre- and postsynaptically.^{28,29} These receptors are thought to mediate the acute excitation from primary afferent fibers to dorsal horn neurons evoked by high intensity stimuli. Intrathecal application of AMPA receptor antagonists produces dose-dependent antinociception on acute pain in animal models.^{27,30} The results of the present study are consistent with these previous studies.^{22,30}

No single agent of these classes (benzodiazepines, NMDA, or AMPA receptor antagonists) administered alone is effective enough to block nociception without any adverse effects. One reason is that pain is not mediated by a single receptor or a single neurotransmitter. The other is that the receptors and neurotransmitters mediating pain are also connected to other neuronal networks in the central nervous system that may induce adverse effects. Thus, combination of agents acting through different mechanisms may be one of the best ways to arrive at better analgesic methods.

The present study showed a significant synergistic antinociception between midazolam, a benzodiazepine-GABA_A receptor agonist, and AP-5, an NMDA receptor antagonist, or YM872, a new AMPA receptor antagonist, on acute thermal stimulation. We used only the tail flick test. To confirm the results of the present study, further investigation using other methods is necessary. Aanonson *et al.*¹² reported that GABA_A receptor agonist inhibited behavioral effects of NMDA, quisqualic acid, and kainic acid. Only in the presence of NMDA, did GABA_A receptor agonist have antinociceptive effect in the tail flick test. GABA produces a mild depolarization of the primary afferents and thereby reduces the release of the excitatory transmitter onto the second-order neurons in the spinal cord.¹⁷ The GABA_A receptor might have some functional coupling with glutamate receptor.

With regard to the side effects, no paralysis was seen in this study. Therefore, we considered tail flick latency not to be affected by motor dysfunction. Midazolam plus AP-5 and midazolam plus YM872 decreased behavioral changes and motor dysfunction and enhanced analgesic effects. These combinations could enhance the therapeutic efficacy of the acute pain treatment and safety. However, one of the important concerns in applying the results to clinical pain management is toxicity of the agents. There are still some controversies surrounding the neurotoxicity of intrathecal midazolam.^{31,32} Current formulations of NMDA receptor antagonists are also neurotoxic.³³ AMPA receptor antagonists have poor water solubility and nephrotoxicity.³⁴ YM872 is a new AMPA receptor antagonist, which is much more water-soluble than the other formulations of AMPA receptor antagonists.³⁵ YM872 had no neurotoxicity in cat,³⁶ rat and monkey brains in toxicologic studies (unpublished data). However, there are no studies investigating the toxicity of YM872 on the spinal cord. Therefore, further studies of their toxicity and of new compounds should be performed before applying the results to humans.

In conclusion, intrathecal coadministration of midazolam (a benzodiazepine-GABA_A receptor agonist) with AP-5 (an NMDA receptor antagonist) or midazolam with YM872 (an AMPA receptor antagonist) produced significant synergistic analgesia with decreased side effects on acute thermal nociception measured by tail flick test. These results suggest a functional coupling of benzodiazepine-GABA_A receptors with NMDA and AMPA receptors in acute nociception in the spinal cord.

The authors thank Dr. Ang Ji, Dr. Young-moon Cho, and Nguyen B. Nguyen for their assistance.

References

1. Buckett WR: Induction of analgesia and morphine potentiation by irreversible inhibitors of GABA-transaminase. *Br J Pharmacol* 1980; 68:129-30
2. Buckett WR: Irreversible inhibitors of GABA transaminase induce antinociceptive effects and potentiate morphine. *Neuropharmacology* 1980; 19:715-22
3. Goodchild CS, Serrao JM: Intrathecal midazolam in the rat: Evidence for spinally-mediated analgesia. *Br J Anaesth* 1987; 59:1563-70
4. Sawynok J: Gabaergic mechanisms of analgesia: An update. *Pharmac Biochem Behav* 1987; 26:463-74
5. Feng J, Kendig JJ: Synergistic interactions between midazolam and alfentanil in isolated neonatal rat spinal cord. *Br J Anaesth* 1996; 77:375-80
6. Bahar M, Cohen ML, Grinshpon Y, Chanimov M: Spinal anaesthesia with midazolam in the rat. *Can J Anaesth* 1998; 44:208-15
7. Battaglia G, Rustioni A: Coexistence of glutamate and substance P in dorsal root ganglion cells of the rat and monkey. *J Comp Neurol* 1988; 177:302-12
8. Näsström J, Karlsson U, Post C: Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. *Eur J Pharmacol* 1992; 212:21-9
9. Dougherty PM, Palecek J, Paleckova V, Sorkin LS, Willis WD: The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal and electrical stimuli. *J Neurosci* 1992; 12:3025-3041
10. Hunter JC, Singh L: Role of excitatory amino acid receptors in the mediation of the nociceptive response to formalin in the rat. *Neurosci Lett* 1994; 174:217-221
11. Aanonsen LM, Wilcox GL: Nociceptive action of excitatory amino acids in the mouse: Effects of spinally administered opioids, phencyclidine and sigma agonists. *J Pharmacol Exp Ther* 1987; 243:9-19
12. Aanonsen LM, Wilcox GL: Muscimol, γ -aminobutyric acid_A receptors and excitatory amino acids in the mouse spinal cord. *J Pharmacol Exp Ther* 1989; 248:1034-8
13. Yaksh TL, Rudy TA: Chronic catheterization of the spinal subarachnoid space. *Physiol Behav* 1976; 17:1031-6
14. Tallarida RJ, Porreca F, Cowan A: Statistical analysis of drug-drug and site-site interactions with isobologram. *Life Sci* 1989; 46:947-61
15. Tallarida RJ, Murray RB: *Manual of Pharmacologic Calculations with Computer Programs*, ed 2.. New York. Springer-Verlag, 1987
16. Malcangio M, Bowery NG: GABA and its receptors in the spinal cord [Review]. *TIPS* 1996; 17:457-62
17. Haefely WE: Benzodiazepines. *Int Anesth Clinics* 1988; 26:262-72
18. Faull RLM, Villiger JW: Benzodiazepine receptors in the human spinal cord: A detailed anatomical and pharmacological study. *Neuroscience* 1986; 17:791-802
19. Unnerstall JR, Kuhar MJ, Niehoff DL, Palacios JM: Benzodiazepine receptors are coupled to a subpopulation of GABA receptors: Evidence from a quantitative autoradiographic study. *J Pharmacol Exp Ther* 1981; 218:797-804
20. Nistri A, Berti C: Influence of benzodiazepines of GABA-evoked responses of amphibian brain and spinal neurons in vitro. *Neuropharmacology* 1984; 23:851-2
21. Schofield PR, Darlison MG, Fujita N, Burt DR, Stephenson FA, Rodriguez H, Rhee LM, Ramachandran J, Reale V, Glencorse TA, Seeburg PH, Barnard EA: Sequence and functional expression of the GABA A receptor shows a ligand gated receptor super-family. *Nature* 1987; 328:221-7
22. Edwards M, Serrao JM, Gent JP, Goodchild CS, Chir B: On the mechanism by which midazolam causes spinally mediated analgesia. *ANESTHESIOLOGY* 1990; 73:273-7
23. Yanez A, Sabbe MB, Stevens CW, Yaksh TL: Interaction of midazolam and morphine in the spinal cord of the rat. *Neuropharmacology* 1990; 29:359-64
24. Dickenson AH, Sullivan AF: Differential effects of excitatory amino acid antagonists on dorsal horn nociceptive neurones in the rat. *Brain Res* 1990; 506:31-9
25. Kristensen JD, Karlsten R, Gordh T, Berge OG: The NMDA antagonist 3-(2-carboxypiperazine-4-yl) propyl-1-phosphonic acid (CPP) has antinociceptive effect after intrathecal injection in the rat. *Pain* 1994; 56:59-67
26. Murray CW, Cowan A, Larson AA: Neurokinin and NMDA antag-

ANALGESIA OF MIDAZOLAM AND GLUTAMATE ANTAGONISTS

onists (but not a kainic acid antagonist) are antinociceptive in the mouse formalin test. *Pain* 1991; 44:179-85

27. Nishiyama T, Yaksh TL, Weber E: Effects of intrathecal NMDA and non-NMDA antagonists on acute thermal nociception and their interaction with morphine. *ANESTHESIOLOGY* 1998; 89:715-22

28. Furuyama T, Kiyama H, Sato K, Park HT, Maeno H, Takagi H, Tohyama M: Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with specific reference to nociception. *Mol Brain Res* 1993; 18:141-51

29. Tölle TR, Berthele A, Schadrack J, Zieglgangersberger W: Involvement of glutamatergic neurotransmission and protein kinase C in spinal plasticity and the development of chronic pain. *Prog Brain Res* 1996; 110:193-206

30. Advokat C, Rutherford D: Selective antinociceptive effect of excitatory amino acid antagonists in intact and acute spinal rats. *Pharmacol Biochem Behav* 1995; 51:855-60

31. Serrao JM, Mackenzie JM, Goodchild CS, Gent JP: Intrathecal midazolam in the rat: An investigation of possible neurotoxic effects. *Eur J Anaesthesiol* 1990; 7:115-22

32. Svensson BA, Welin M, Gordh T Jr, Westman J: Chronic sub-arachnoid midazolam (Doromicum) in the rat: Morphological evidence of spinal cord neurotoxicity. *Reg Anesth* 1995; 20:426-34

33. Olney JW, Labruyere J, Wang G, Wozniak DF, Price MT, Sesma MA: NMDA antagonist neurotoxicity: Mechanism and prevention. *Science* 1991; 254:1515-8

34. Xue D, Huang Z-G, Barnes K, Lesiuk HJ, Smith KE, Buchan AM: Delayed treatment with AMPA, but not NMDA, antagonists reduces neocortical infarction. *J Cereb Blood Flow Metab* 1994; 14:251-61

35. Kohara A, Okada M, Tsutsumi R, Ohno K, Takahashi M, Sasamata SM, Shishikura J, Inami H, Sakamoto S, Yamaguchi T: In-vitro characterization of YM872, a selective, potent and highly water-soluble α -amino-3-hydroxy-5-methylisoxazole-4-propionate receptor antagonist. *J Pharm Pharmacol* 1998; 50:795-801

36. Takahashi M, Ni JW, Yatsugi KS, Toya T, Yatsugi S, Sasamata SM, Koshiya K, Shishikura J, Sakamoto S, Yamaguchi T: YM872, a novel selective α -amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor antagonist, reduces brain damage after permanent focal cerebral ischemia in cats. *J Pharmacol Exp Ther* 1998; 284:467-73