# Antihyperalgesic and Side Effects of Intrathecal Clonidine and Tizanidine in a Rat Model of Neuropathic Pain

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*Background:* Although intrathecal clonidine produces pronounced analgesia, antinociceptive doses of intrathecal clonidine produce several side effects, including hypotension, bradycardia, and sedation. Intrathecal tizanidine, another $\alpha_2$-adrenergic agonist, has provided antinociception without producing pronounced hemodynamic changes in animal studies. However, it has been unclear whether antihyperalgesic doses of intrathecal clonidine and tizanidine produce hypotension and bradycardia in a neuropathic pain state. This study was designed to evaluate the antihyperalgesic effects and side effects of intrathecal clonidine and tizanidine in a rat model of neuropathic pain.

*Methods:* Male Sprague-Dawley rats were chronically implanted with lumbar intrathecal catheters, and the sciatic nerve was loosely ligated. After 21–28 days after surgery, the rats received intrathecal clonidine (0.3, 1.0, and 3.0 μg) and tizanidine (1.0, 2.0, and 5.0 μg), and the antihyperalgesic effects of thermal and mechanical stimuli were examined. In addition, the changes in blood pressure and heart rate, sedation level, and other side effects after intrathecal administration of drugs were recorded.

*Results:* The administration of 3.0 μg intrathecal clonidine or 5.0 μg tizanidine significantly reversed both thermal and mechanical hyperalgesia. The administration of 3.0 μg intrathecal clonidine, but not 5.0 μg tizanidine, significantly decreased mean blood pressure and heart rate and produced urinary voiding. A greater sedative effect was produced by 3.0 μg intrathecal clonidine than by 5.0 μg tizanidine.

*Conclusion:* The antihyperalgesic dose of intrathecal clonidine and the antinociceptive doses produced several side effects. Intrathecal tizanidine at the dose that reversed hyperalgesia would be preferable for neuropathic pain management because of absence of hypotension and bradycardia and lower incidence of sedation.

WE previously demonstrated that, although antinociceptive doses of intrathecal clonidine resulted in hypotension and bradycardia in normal animals, tizanidine, another $\alpha_2$-adrenergic agonist, provided antinociception in a manner similar to that of clonidine without producing hemodynamic changes.[1] However, it has been unknown whether the antihyperalgesic dose of intrathecal clonidine produces hypotension, bradycardia, and other side effects in a rat model of neuropathic pain. There are only a few studies that have examined the effect of intrathecal tizanidine on experimental neuropathic pain.[2–4] In these studies, the effect of intrathecal tizanidine to mechanical stimuli was examined, but the effect to heat stimuli was fully understood. In addition, there has been no study that has examined the hemodynamic effects and sedative effect at the antihyperalgesic dose.

In this study, we compared intrathecal clonidine with tizanidine on the antihyperalgesic effects to thermal and mechanical stimuli and on side effects, including hypotension and bradycardia, in a rat model of neuropathic pain.

## Materials and Methods

The protocol for this study was approved by Sapporo Medical University Animal Care and Use Committee (Sapporo, Japan). Experiments were conducted in male Sprague-Dawley rats (weight, 180–250 g; Japan SLC, Hamamatsu, Japan), which were housed individually in a temperature controlled (21 ± 1°C) room with a 12-h light–dark cycle and which were given free access to food and water.

### Surgical Procedures

All surgical procedures were performed under general anesthesia (3% isoflurane in oxygen). A polyethylene intrathecal catheter (PE-10; Clay Adams, NJ) was inserted 15 mm cephalad into the lumbar subarachnoid space at the L4–L5 intervertebrae, with the tip of the catheter located near the lumbar enlargement of the spinal cord to administer the drug intrathecally.[1] The catheter was tunneled subcutaneously and externalized through the skin in the neck region. The volume of dead space of the intrathecal catheter was 15 μl. In the experiments, we used only animals that showed normal behavior and motor function and that showed complete paralysis of the tail and bilateral hind legs after administration of 2% lidocaine, 10 μl, through the intrathecal catheter. Seven days after intrathecal catheter implantation, chronic constriction injury (CCI) was created as previously described.[5] Briefly, under general anesthesia, the left common sciatic nerve was exposed at the mid-thigh level. Four ligatures were loosely tied in approximately 1.0-mm intervals around the nerve just proximal to the trifurcation with 4-0 chromic gut suture. The wound was then closed.

### Behavioral Study

Behavioral testings were performed before CCI and 21–28 days after CCI when hyperalgesia had been estab-

lished.[5] Thermal nociceptive testing was conducted using an analgesimeter (Plantar test 7370; Ugo Basile, Italy). Radiant heat was applied on the plantar surface of each hind paw. The thermal nociceptive threshold was evaluated as paw withdrawal latency (PWL) from the heat source. Bulb intensity was adjusted so that the basal PWL was 9–11 s before CCI. Cutoff time was 20 s to avoid tissue damage. To examine the effect of intrathecal clonidine and tizanidine on thermal hyperalgesia, the rats received intrathecal clonidine (0.3, 1.0, or 3.0 $\mu$g) or tizanidine (1.0, 2.0, or 5.0 $\mu$g).

Mechanical hyperalgesia was assessed using a 5.07-g von Frey filament (111 mN) before CCI and before and 20 min after intrathecal drug administration. Animals were placed on a wire mesh platform and allowed to acclimate to their surroundings for a minimum of 30 min before testing. The filament was applied to the point of bending 10 times on the plantar surface of both hind paws, and the number of vigorous responses was recorded.[6] Data were expressed as percent response frequency. The rats received 3.0 $\mu$g intrathecal clonidine or 5.0 $\mu$g tizanidine.

To examine the sedative effect of intrathecal clonidine and tizanidine, the intensity of sedation was assessed at 10-min intervals using our modification of the scale proposed by Dowlatshahi and Yaksh.[7] This scale consists of five contents as follows: 0 = normal behavior, alert to the environment, standing or grooming; 1 = sitting quietly, sometimes standing or grooming; 2 = sitting quietly, no spontaneous movement, but moved if touched; 3 = no spontaneous movement, did not move when touched; 4 = loss of righting reflex, unresponsive. Scores were totaled over 60 min after drug administration (maximum score = 24; minimum score = 0).

### Blood Pressure and Heart Rate Measurement

In some neuropathic rats, a polyethylene catheter was placed in the left carotid artery under general anesthesia to examine the effects of intrathecal drug administration on blood pressure and heart rate. After the recovery period of 2 h, blood pressure was monitored and recorded before and after the administration of 3.0 $\mu$g intrathecal clonidine or 5.0 $\mu$g tizanidine during an awake, briefly restrained condition. Systolic and diastolic arterial pressures and heart rate were recorded, and mean arterial pressure was calculated.

### Drugs

Clonidine and tizanidine were purchased from Sigma (St. Louis, MO) and Sandoz (East Hanover, NJ), respectively. Intrathecal drug administration was accomplished using a microinjection syringe (Hamilton, Reno, NV) connected to the intrathecal catheter in awake, briefly restrained rats. Intrathecal drug administration was performed manually over a 10-s period in a single injection volume of 10 $\mu$l followed by a flush of 15 $\mu$l physiologic saline.

### Statistical Analysis

Paw withdrawal latencies, blood pressure, and heart rate were represented as mean ± SD. Sedation scores were represented as median (range). The frequency of vigorous responses was expressed as a percentage: (number of vigorous responses/number of total trials) × 100. Changes in the frequency of vigorous responses to mechanical stimuli were analyzed using a paired $t$ test. Changes in blood pressure and heart rate and changes in PWLs to the heat stimuli were analyzed using one-way analysis of variance followed by Dunnett test within a single group. Sedation scores were analyzed using the Mann–Whitney U test. A $P$ value less than 0.05 was considered to be statistically significant.

## Results

The mean basal PWL was 9.8 ± 1.1 s before CCI. On the 21–28 days after CCI, the mean PWL in the affected paw was significantly reduced to 5.4 ± 0.7 s. Figure 1, *A* shows the antihyperalgesic effect to thermal stimuli of



**Fig. 1. Antithermal hyperalgesic effects of intrathecal clonidine and tizanidine. (*A*) Time course of the withdrawal latencies to noxious heat stimuli after 3.0 $\mu$g intrathecal clonidine (*open squares*) and 5.0 $\mu$g tizanidine (*solid squares*) (n = 7 for each drug). (*B*) Dose–response effect of intrathecal clonidine (n = 6–8 for each dose). Pre = withdrawal latency before intrathecal drug administration; before CCI = withdrawal latency before the nerve ligation. Data are presented as mean ± SD. * and # indicate significant difference from withdrawal latency before intrathecal clonidine and tizanidine administration, respectively (*P* < 0.05). ** indicates significant difference from the withdrawal latency before intrathecal drug administration (the value at pre) (*P* < 0.05).**
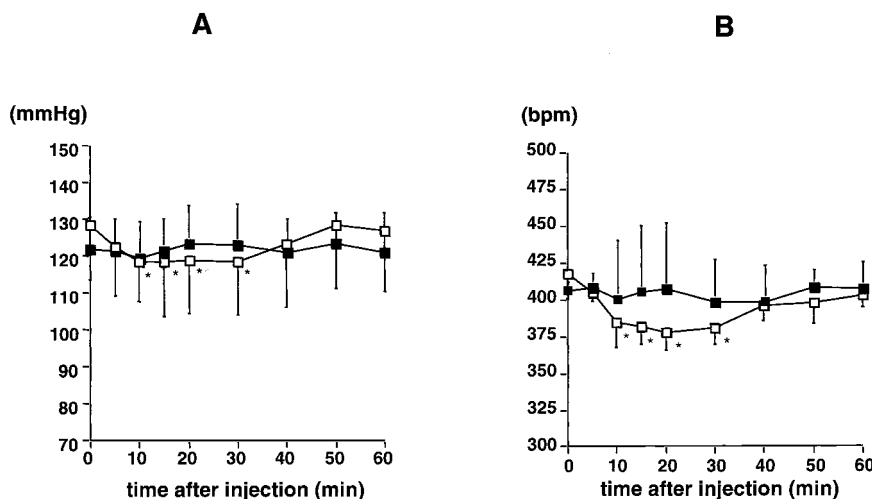
## A



## B
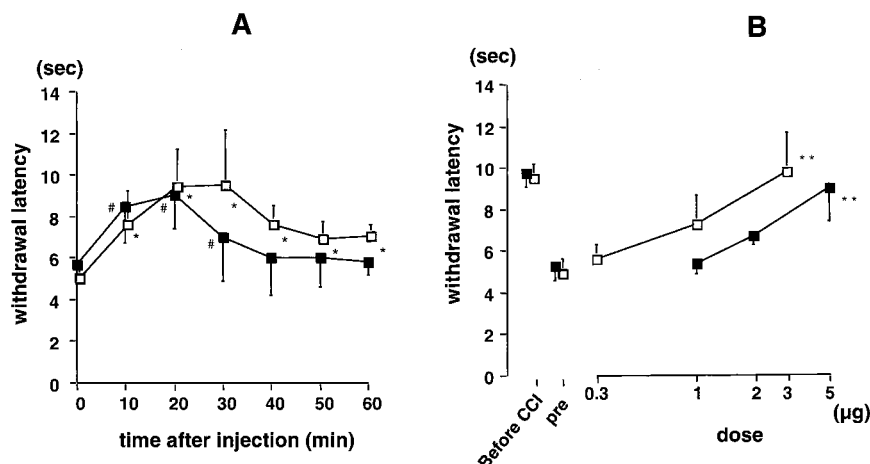


Fig. 2. Changes in mean arterial blood pressure and heart rate. (*A*) Time course of mean arterial blood pressure after 3.0 $\mu$g intrathecal clonidine (*open squares*) and 5.0 $\mu$g tizanidine (*solid squares*). (*B*) Time course of heart rate after 3.0 $\mu$g intrathecal clonidine (*open squares*) and 5.0 $\mu$g tizanidine (*solid squares*). * indicates a significant difference from the value before intrathecal drug administration (the value at time 0) (*P* < 0.05) (n = 6 for each drug).

3.0 $\mu$g intrathecal clonidine and 5.0 $\mu$g intrathecal tizanidine. The administration of 3.0 $\mu$g intrathecal clonidine and 5.0 $\mu$g tizanidine significantly prolonged the shortened PWL and reversed to the basal PWL level. The maximum antihyperalgesic effect of 3.0 $\mu$g intrathecal clonidine was observed 20–30 min after the injection, and that of intrathecal tizanidine was observed 10–20 min after injection. Figure 1, *B* shows the dose-response curve of the antihyperalgesic effect to thermal stimuli of intrathecal clonidine and tizanidine. Administering 1.0 $\mu$g clonidine tended to prolong the shortened PWL, but this was not statistically significant. Administering 2.0 $\mu$g tizanidine also tended to prolong the shortened PWL, but this was not statistically significant.

During mechanical testing, although the rats did not show any response to a 5.07-g von Frey filament before CCI, percent response frequency increased to 80 ± 8 and 80 ± 10% before intrathecal clonidine and tizanidine administration, respectively, on days 21–28 after CCI. Administering 3.0 $\mu$g intrathecal clonidine and 5.0 $\mu$g tizanidine significantly reduced percent response frequency to 20 ± 11 and 30 ± 12%, respectively.

Before intrathecal drug administration, the sedation scores were 1 in all tested rats. After the administration of 3.0 $\mu$g intrathecal clonidine and 5.0 $\mu$g tizanidine, the ranges of the scores at each time point were 1–3 and 1–2, respectively. The total sedation score for 60 min after intrathecal clonidine was 12.5 (range, 11–14) and was significantly higher than that after intrathecal tizanidine (8 [7–8]). In the rats that received 3.0 $\mu$g intrathecal clonidine but not 5.0 $\mu$g tizanidine, urinary voiding was observed.

Figure 2, *A* shows the effects of 3.0 $\mu$g intrathecal clonidine and 5.0 $\mu$g tizanidine on mean arterial blood pressure. The baseline mean arterial blood pressures were 128.2 ± 14.4 and 123.3 ± 8.6 mmHg before intrathecal clonidine and tizanidine, respectively, and were not significant different between the two groups. Clonidine but not tizanidine significantly decreased

mean arterial blood pressure 10–30 min after the intrathecal injection, compared with the predrug baseline value. The baseline heart rates were 415.0 ± 10.2 and 408.5 ± 9.8 beats/min before intrathecal clonidine and tizanidine, respectively, and were not significantly different between the two groups. Clonidine but not tizanidine also significantly decreased heart rate 10–30 min after intrathecal injection (fig. 2, *B*).

## Discussion

The current study showed that intrathecal clonidine and tizanidine reversed thermal and mechanical hyperalgesia in a CCI model. Intrathecal clonidine but not tizanidine decreased mean blood pressure and heart rate at the doses that produced comparative antihyperalgesic effects. In addition, intrathecal clonidine caused urinary voiding and produced a more profound sedative effect compared with intrathecal tizanidine.

In our results, intrathecal tizanidine and clonidine reversed both thermal and mechanical hyperalgesia. We evaluated mechanical hyperalgesia by the number of vigorous responses to repeated application of the von Frey filament (111 mN).[6] However, because the response frequency to the filament resulted in no withdrawal response in normal animals, the mechanical stimulus we used may be innocuous rather than noxious. Therefore, an increased rate of vigorous responses to the von Frey filament after CCI observed in the current study may represent mechanical allodynia. Similar to the antinociceptive potency, the antihyperalgesic potency of clonidine was likely to be greater than that of tizanidine in the current study. Leiphart *et al.*[2] reported that intrathecal tizanidine had no effect on thermal hyperalgesia to noxious heat stimuli in a CCI model. Interestingly, intrathecal tizanidine had no effect on withdrawal latency to noxious heat stimuli in sham-operated or nonoperated rats and the tail-flick test in nonoperated rats in

their study. However, several investigators have shown intrathecal tizanidine-induced antinociceptive effects to noxious thermal stimuli through the activation of $\alpha_2$-adrenergic receptors.[1,8,9]

In the current study, the antihyperalgesic dose of 3.0 $\mu$g intrathecal clonidine was associated with side effects, including hypotension, bradycardia, sedation, and urinary voiding. The hypotensive effect of intrathecal clonidine would be caused by the inhibition of sympathetic outflow at sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord[10] and the supraspinal effects *via* systemic absorption.[11] In addition, higher doses of intrathecal clonidine increase blood pressure as a result of direct action at peripheral $\alpha_2$-adrenergic receptors.[10] Clonidine also decreases heart rate by acting at peripheral and supraspinal sites that produce sympathetic inhibition and parasympathetic stimulation.[12,13] Sedation and urinary voiding observed in the current study also reflect systemic effects of clonidine. Experimental data show that the sedative–hypnotic effect of $\alpha_2$-adrenergic agonists is caused by actions primarily in the locus ceruleus.[14] $\alpha_2$-Adrenergic agonist-induced diuresis is involved in the direct action on the juxtaglomerular apparatus[15] and the release of arterial natriuretic factor.[16] On the other hand, in the rats that received 5.0 $\mu$g intrathecal tizanidine, which produced antihyperalgesic effects comparable with those seen with 3.0 $\mu$g clonidine, only very light sedation was observed. Hypotension, bradycardia, and apparent urinary voiding were not observed. The difference in the incidence of side effects between clonidine and tizanidine may be caused by the lipophilicity of these drugs. Clonidine has a higher lipophilicity than tizanidine.[17] Because of the high lipophilicity of clonidine, intrathecally administered clonidine is well absorbed systemically. A lower incidence of the side effects of intrathecal tizanidine may reflect that intrathecally administered tizanidine poorly diffuses to the intermediolateral cell column, which is deeper from the spinal cord surface than the substantia gelatinosa, and is poorly absorbed into the systemic circulation because of its low lipophilicity. However, higher doses of intrathecal tizanidine produce hypotensive effects.[9]

Taken together with the experimental data previously reported, intrathecal clonidine, at not only the antinociceptive dose but also at the antihyperalgesic dose, produces hypotension, bradycardia, and other side effects caused by systemic absorption, whereas intrathecal tizanidine does not. This evidence may show the superiority of intrathecal tizanidine, compared with clonidine, in human neuropathic pain management. In the current study, we performed the experiment in animals because clinically available tizanidine solution for intrathecal administration has not been developed. Intrathecal tizanidine has already been shown to have no neurotoxicity.[18] Our results would also encourage the development of clinically available tizanidine solution for epidural and/or intrathecal administration and a clinical trial of intrathecal tizanidine in the clinical setting, especially during a neuropathic pain state.

## References

1. Kawamata T, Omote K, Kawamata M, Iwasaki H, Namiki A: Antinociceptive interaction of intrathecal $\alpha_2$-adrenergic agonists, tizanidine and clonidine, with lidocaine in rats. ANESTHESIOLOGY 1997; 87:436–48

2. Leiphart JW, Dills CV, Zikel OM, Kim DL, Levy RM: A comparison of intrathecally administered narcotic and nonnarcotic analgesics for experimental chronic neuropathic pain. J Neurosurg 1995; 82:595–9

3. Ono N, Kroin JS, Penn RD, Paice JA: Effects of intrathecal nonnarcotic analgesics on chronic tactile allodynia in rats: Alpha 2-agonists versus somatostatin analog. Neurol Med Chir 1997; 37:6–10

4. Vrinten DH, Adan RAH, Groen GJ, Gispen WH: Chronic blockade of melanocortin receptors alleviates allodynia in rats with neuropathic pain. Anesth Analg 2001; 93:1572–7

5. Bennett GJ, Xie YK: A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain 1988; 33:87–107

6. Stone LS, Vulchanova L, Riedl MS, Wang J, Williams FG, Wilcox GL, Elde R: Effects of peripheral nerve injury on alpha-2A and alpha-2C adrenergic receptor immunoreactivity in the rat spinal cord. Neuroscience 1999; 93:1399–407

7. Dowlatshahi P, Yaksh TL: Differential effects of two intraventricularly injected alpha 2 agonists, ST-91 and dexmedetomidine, on electroencephalogram, feeding, and electromyogram. Anesth Analg 1997; 84:133–8

8. Kroin JS, McCarthy RJ, Penn RD, Lubenow TR, Ivankovich AD: Intrathecal clonidine and tizanidine in conscious dogs: Comparison of analgesic and hemodynamic effects. Anesth Analg 1996; 82:627–35

9. McCarthy RJ, Kroin JS, Lubenow TR, Penn RD, Ivankovich AD: Effect of intrathecal tizanidine on antinociception and blood pressure in the rat. Pain 1990; 40:333–8

10. Solomon SE, Brody MJ, Gebhart GF: Pharmacological characterization of alpha adrenoceptors involved in the antinociceptive and cardiovascular effects of intrathecally administered clonidine. J Pharmacol Exp Ther 1989; 251:27–38

11. Jarrott B, Conway EL, Maccarrone C, Lewis SJ: Clonidine: Understanding its disposition, sites and mechanism of action. Clin Exp Pharmacol Physiol 1987; 14:471–9

12. Scriabine A, Stavorski J, Wenger HC, Torchiana ML, Stone CA: Cardiac slowing effects of clonidine (St 155) in dogs. J Pharmacol Exp Ther 1970; 171:256–64

13. Maze M, Tranquilli W: Alpha-2 adrenoceptor agonists: Defining the role in clinical anesthesia. ANESTHESIOLOGY 1991; 74:581–60

14. Correa-Sales C, Rabin BC, Maze M: A hypnotic response to dexmedetomidine, an alpha 2 agonist, is mediated in the locus coeruleus in rats. ANESTHESIOLOGY 1992; 76:948–52

15. Smyth DD, Umemura S, Yang E, Pettinger W: Inhibition of rennin release by $\alpha$ adrenoceptor stimulation in the isolated perfused rat kidney. Eur J Pharmacol 1987; 140:33–8

16. Chen M, Lee J, Huang BS, Grekin RJ, Malvin RL: Clonidine and morphine increase atrial natriuretic peptide secretion in anesthetized rats. Proc Soc Exp Biol Med 1989; 191:299–303

17. Takayanagi I, Konno F, Kusunoki M: Some pharmacological effects of tizanidine on smooth muscle organs and $\chi_2$ adrenoceptor. Gen Pharmacol 1985; 16:501–3

18. Ochs G, Tonn LJ, Toyka K: Distribution, tolerability and tissue compatibility of intrathecal tizanidine in the sheep. Acta Anaesthesiol Scand 1998; 42:786–93