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Antinociceptive Interaction of Intrathecal α_2-adrenergic Agonists, Tizanidine and Clonidine, with Lidocaine in Rats

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Background: **The intrathecal α_2-adrenergic agonist, clonidine, has been shown to have considerable antinociceptive effect, although clonidine causes hypotension and bradycardia. The combination of intrathecal clonidine and local anesthetics enhances analgesic effects, whereas the combination may cause marked hypotension and motor blockade, which may limit the clinical application of the combination. Tizanidine, another α_2-adrenergic agonist, has also provided antinociception without producing pronounced hemodynamic changes. This study was designed to evaluate the antinociceptive and hemodynamic interactions of tizanidine and clonidine with lidocaine.**

Methods: **Male Sprague Dawley rats were chronically implanted with lumbar intrathecal catheters. The tail-flick test was used to assess the thermal nociceptive threshold. The ability of intrathecal tizanidine, clonidine, lidocaine, or the combinations of α_2-adrenergic agonist and lidocaine to alter the tail-flick latency was examined. To characterize the antinociceptive interaction, the isobolographic analysis was applied. Additionally, the motor function, blood pressure and heart rate after intrathecal administration of drugs and combinations were also monitored.**

Results: **Intrathecal tizanidine, clonidine, or the combinations increased the tail-flick latency in dose- and time-dependent fashion without affecting motor function. The order potencies (dose producing a 50% of peak effect, in μg) of tizanidine and clonidine were 1.8 and 0.75, respectively. With isobolographic analysis, tizanidine with lidocaine and clonidine with lidocaine showed significantly synergistic antinociceptive interaction. Potency ratio analysis and fractional analysis also confirmed the synergistic interaction. At the doses in the combinations showing comparable antinociception, tizanidine with lidocaine, unlike clonidine with lidocaine, did not affect motor function or blood pressure.**

Conclusion: **The authors' results show that intrathecal tizanidine and clonidine synergistically interact with lidocaine so that the degree of antinociception to somatic noxious stimuli are enhanced. The antinociceptive synergistic interaction between tizanidine and lidocaine may be useful in clinical practice without affecting blood pressure, heart rate, or motor function. (Key words: Analgesia. Anesthetic techniques: intrathecal. Anesthetics, local: lidocaine. Interactions (drug): synergy. Pain. Sympathetic nervous system, α_2-adrenergic agonist: clonidine, tizanidine.)**

INTRATHECAL-EPIDURAL clonidine, an α_2-adrenergic agonist, produces potent analgesia and has played an important role in the control of acute and chronic pain. However, intrathecal-epidural clonidine induces hypotension and bradycardia.[1,2] Several investigators reported that intrathecal coadministration of clonidine and local anesthetics prolonged analgesic duration compared with local anesthetic alone in humans and animals.[3-5] Coadministration of clonidine and local anesthetics also caused marked hypotension and motor blockade, which may limit the clinical application of the combination.

Tizanidine, an another α_2-adrenergic agonist, has provided antinociception in a similar manner to clonidine, without producing pronounced hemodynamic changes, when intrathecally administered in animal studies.[6] Therefore, it might be possible that intrathecal coadministration of tizanidine and local anesthetics produces profound analgesia without affecting hemodynamics, but there is no information about the interaction between tizanidine and local anesthetics on antinociceptive and hemodynamic effects.

Determining optimum drug combinations that, at minimal doses, produce powerful analgesia with less side effects is of great interest for clinical pain management.

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In this study, we systematically evaluated the antinociceptive interactions of intrathecal tizanidine and clonidine with lidocaine in rats on antinociception using isobolographic analysis. The motor function, the blood pressure, and heart rate were also evaluated after the intrathecal administration of combined drugs.

Materials and Methods

The protocol for this study was approved by Sapporo Medical University Animal Care and Use Committee. Experiments were conducted in male Sprague-Dawley rats (weighing 200–250 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 given free access to food and water.

Animal Preparation and Surgical Procedure

During general anesthesia (halothane, 2%, 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, using a method described previously.[7] 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. At least 6 days of postsurgical recovery were allowed before animals were used in experiments. There was at least 1 week between successive experiments with any rat after intrathecal administration of drug, and each animal received, in total, two or three injections. In the experiments, we used only animals that showed normal behavior and motor function and that had showed complete paralysis of the tail and bilateral hind legs after administration of 2% lidocaine, 10 μl, through the intrathecal catheter. In another series of the experiment, a polyethylene catheter was placed in the tail artery to monitor blood pressure and heart rate before and after the intrathecal drug administration.

Nociceptive and Motor Function Test

Nociceptive Test. The tail-flick (TF) test was used to assess thermal nociceptive threshold. TF testing was used by monitored latency to withdrawal for the heat source (a 50-W projection lamp bulb) focused on a distal segment of the tail, using thermal analgesimeter (KN-205E; Natsume, Tokyo, Japan). The mean baseline TF latency in this experiment was 3.2 s (range, 3.1–3.6 s), and a cut-off time of 10.0 s was used to minimize damage to the skin of the tail.

Motor Function Test. To achieve a sensitive numeric index, motor function was assessed by bilaterally using the scale proposed by Penning and Yaksh.[8] This scale consists of four contents as follows: (1) the righting reflex, (2) the placing–stepping reflex, (3 and 4) the muscle tone of the upper and lower limbs (0 = absent, 1 = impaired, 2 = normal), the normal baseline aggregate score being 8 × 2 = 16. Muscle tone was graded as absent when the limb was flaccid with no detectable resistance to flexion–extension of the limb and as impaired when the limb was able to move but not support the rat's normal posture. Tone was graded as normal when the rat had no visible limb weakness (normal symmetric posture and gait).

Drugs

Intrathecal drug administration was accomplished by using a microinjection syringe (Hamilton, Reno, NV) connected to the intrathecal catheter in awake, briefly restrained rats. The drugs used in this experiment were tizanidine hydrochloride (Sandoz, East Hanover, NJ), clonidine hydrochloride (Sigma, St. Louis, MO), lidocaine hydrochloride (Fujisawa, Japan), and yohimbine hydrochloride (Sigma, St.Louis, MO). Drugs were freshly dissolved in sterile physiologic saline in concentrations that allowed intrathecal injections in 10-μl volumes. All drugs or drug combinations were administered manually over 10 s in a single injection volume of 10 μl followed by a flush of 15 μl physiologic saline.

Study Paradigms

Effects of Drugs in Nociceptive Test. To determine the dose effects and time courses of intrathecal tizanidine, clonidine, and lidocaine alone, rats received intrathecal tizanidine (0.5, 1.0, 2.5, or 5.0 μg), clonidine (0.2, 0.5, 1.0, 1.5, or 2.0 μg), or lidocaine (20, 50, 100, 200, 400, 600, or 1000 μg) in a random fashion after determination of baseline TF latencies. The latency were measured at 5, 10, 15, 20, 30, 45, and 60 min after the injections. The values were converted to percent maximum possible effect (MPE): %MPE = (postdrug value − baseline value) / (cut-off value − baseline value) × 100.

To determine the drug interaction between clonidine and lidocaine, or tizanidine and lidocaine, the isobolographic analysis was applied. After calculation of the

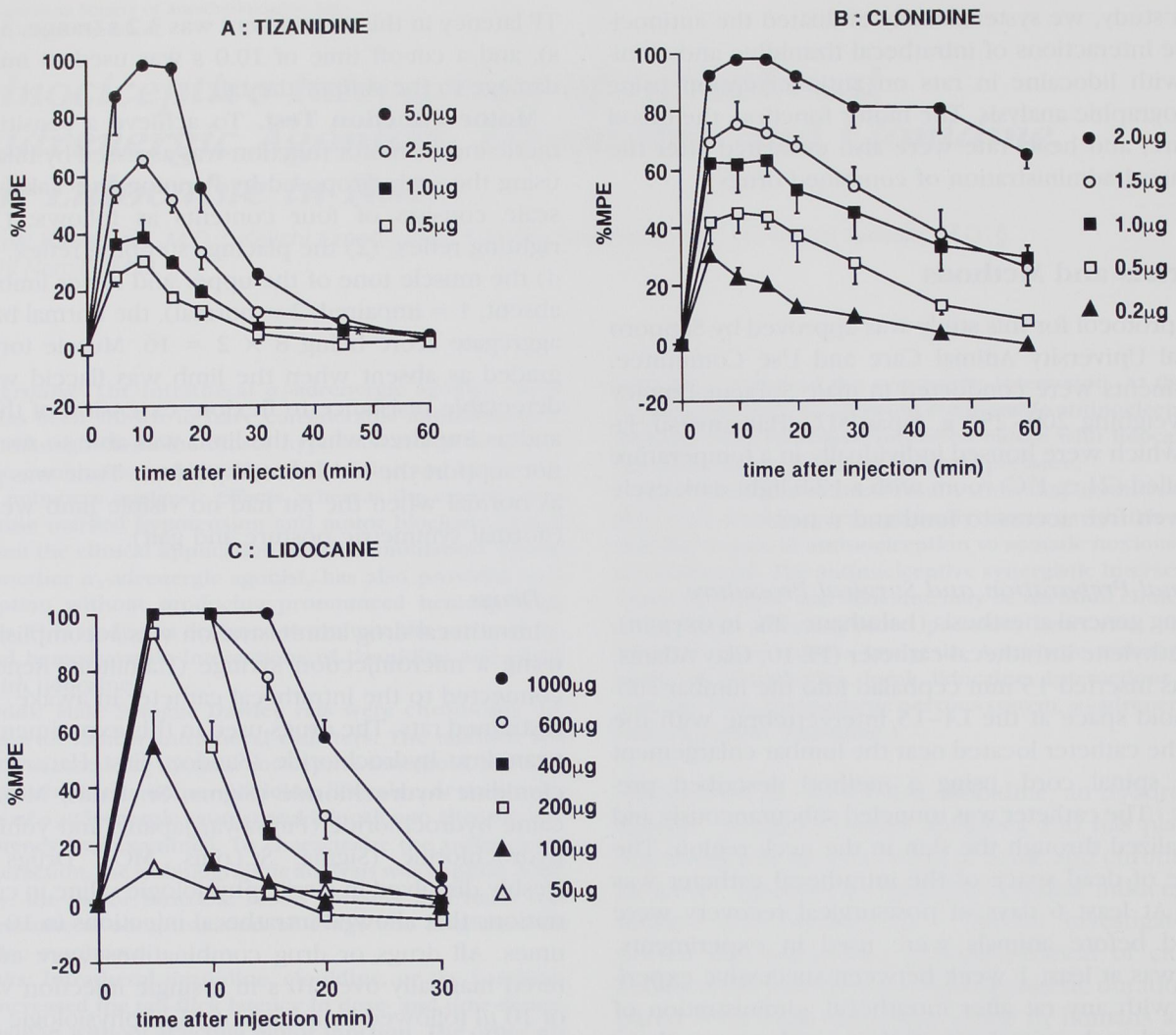

Fig. 1. Time courses of percent maximum possible effect (%MPE) in the tail-flick test after intrathecal administration of tizanidine (*A*), clonidine (*B*), and lidocaine (*C*). N = 8 for each group. Data are presented as means ± SEM.

dose producing a 50% of peak %MPE (ED_{50}) and 95% confidence interval (CI), ED_{50} fractions (1/2, 1/4, 1/6, 1/8, 1/12) of drug combinations of tizanidine and lidocaine (tizanidine/lidocaine), or clonidine and lidocaine (clonidine/lidocaine) were intrathecally administered. TF latencies were recorded at 5, 10, 15, 20, 30, 45, and 60 min after the injections, and the ED_{50} and 95% CI of each combination was established.

In addition, to examine if the antinociception of tizanidine and the antinociceptive interaction of α_2-adrenergic agonists and lidocaine is mediated through α_2-adrenergic action, some rats received 10 μg yohimbine intrathecally 15 min before the injection of tizanidine 5 μg, 1/2 ED_{50} tizanidine/lidocaine or 1/4 ED_{50} clonidine/lidocaine, which produced comparable antinociceptive effects in this study, and the latencies were measured for 60 min.

Effect of Drugs in Motor Function Test. Motor function was assessed at 5, 10, 15, 20, 30, 45, and 60 min after the drug injections at the doses used in the antinociceptive study. In separate groups of rats, to examine whether tizanidine potentiates the effects of lidocaine on motor function, a combination of tizanidine 5.0 μg with lidocaine 100 μg, 200 μg, or 400 μg was intrathecally administered, and motor function was evaluated 2, 5, 10, 15, and 20 min after injections.

Effects of Drugs on Blood Pressure and Heart

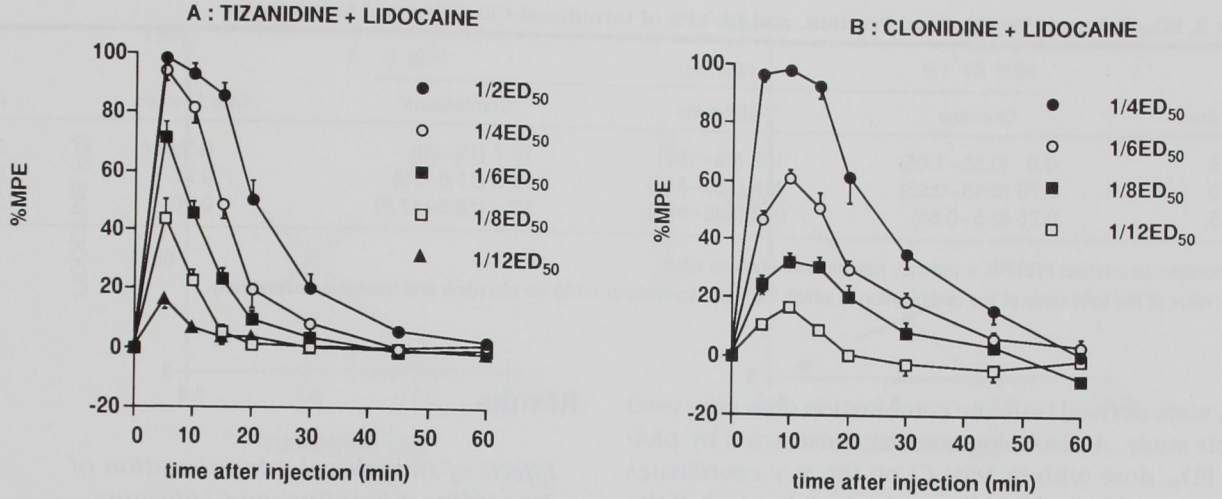

Fig. 2. Effects of intrathecal tizanidine/lidocaine (*A*) and clonidine/lidocaine (*B*) at the dose ratio of the 1:50 and 1:100, respectively. N = 8 for each group. Data are presented as means ± SEM.

Rate. The another series of experiment was performed to examine the effects of intrathecal drugs on blood pressure and heart rate. Blood pressure and heart rate were monitored and recorded before and after injections of each drug or the combinations in awake rats. Tizanidine, 5.0 μg, clonidine, 2.0 μg, lidocaine, 200 μg, 1/2 ED_{50} tizanidine/lidocaine, or 1/4 ED_{50} clonidine/lidocaine, which produced comparable antinociceptive effects in this study, were used. Systolic and diastolic arterial pressures and heart rate were recorded, and mean arterial pressure was calculated.

Statistical Analysis

All data were presented as the mean values ± SEM. The effects of intrathecal drugs on mean blood pressure and heart rate were presented as the change from the predrug baseline values. Changes in %MPE, mean arterial blood pressure, and heart rate after intrathecal injections were compared with baseline using a one-way analysis of variance (ANOVA) for repeated measures followed by Dunnett's test within a single group and were analyzed using a two-way ANOVA for repeated measures to assess the influence of management and time followed by Scheffe's F test for between-group comparison. To analyze the motor function data, the individual motor scores for each rat were cumulated, and individual comparisons were carried out with the Mann-Whitney U test. A P value < 0.05 was considered to be statistically significant.

To construct an isobolographic analysis for ED_{50}, using a least-squares regression analysis, ED_{50}s and their 95% CIs of drugs and the combinations were calculated. For the combination of tizanidine/lidocaine, clonidine/lidocaine, the total dose of the combined drugs was used for a least-regression analysis. Component doses of tizanidine/lidocaine and of clonidine/lidocaine for

Table 1. ED_{50} Values (95% CI), Total Fraction, and PR/FPR of Intrathecal Tizanidine and Lidocaine

Time (min)	ED_{50} (μg)			Total Fraction	PR/FPR
	Tizanidine	Lidocaine	Combination*		
5	2.1 (1.6–2.8)	102 (55–190)	15.8 (11.2–19.4)	0.30	3.4/1.7
10	1.8 (1.1–2.3)	215 (135–310)	19.9 (17.8–22.4)	0.31	3.3/2.2
15	2.2 (1.8–2.7)	600 (530–665)	30.6 (26.5–35.7)	0.32	3.1/1.4

CI = confidence interval; PR/FPR = potency ratio/fiducial potency ratio.

* ED_{50} value of the total dose of the combination in which the dose consists of 1:50 for tizanidine and lidocaine, respectively.

Table 2. ED_{50} Values (95% CI), Total Fraction, and PR/FPR of Intrathecal Clonidine and Lidocaine

Time (min)	ED_{50} (μg)			Total Fraction	PR/FPR
	Clonidine	Lidocaine	Combination*		
5	0.8 (0.55–1.05)	102 (55–190)	15.7 (12–23)	0.35	3.4/1.9
10	0.75 (0.45–0.95)	215 (135–310)	15.3 (12.5–18)	0.27	3.3/1.7
15	0.75 (0.5–0.85)	600 (530–665)	17 (16.5–17.5)	0.26	3.1/1.5

CI = confidence interval; PR/FPR = potency ratio/fiducial potency ratio.

* ED_{50} value of the total dose of the combination in which the dose consists of 1:100 for clonidine and lidocaine, respectively.

ED_{50} were derived from the combination dose ratio used in this study. An isobologram was constructed by plotting ED_{50} dose with its 95% CI on the x, y coordinates (x for tizanidine or clonidine and y for lidocaine). If the experimentally determined isobole (a point representing x, y coordinates for ED_{50}) decreased significantly below the theoretically additive isobole, the interaction between tizanidine/lidocaine or clonidine/lidocaine was defined as being supraadditive (synergistic). The theoretical isobole for the purely additive interaction was derived from an additive line and the combination dose ratio. The additive line was drawn by connecting the point indicating ED_{50} on the x-axis (tizanidine or clonidine alone) with that on the y-axis (lidocaine alone). The 95% CIs for the theoretical additive isobole were similarly acquired by connecting the 95% CIs on the x-axis with those on the y-axis.

For the statistical estimation of the difference between the experimental combination ED_{50} and the theoretically additive ED_{50}, potency ratio analysis was used.[9] The significant difference between the two isoboles can be determined by the relation of potency ratio (PR) and its fiducial potency ratio (FPR). The PR for the experimental isobole with theoretical isobole is defined as the ratio for each isobole. The FPR was obtained from a nomogram.[10] If PR is greater than FPR, the two isoboles are deemed to be significantly different from each other ($P < 0.05$).

To obtain a value for describing the magnitude of the interaction, a total fraction value was calculated as described by Roerig *et al.*[11] Total fraction values were calculated as follows: (ED_{50} of tizanidine or clonidine when injected with lidocaine)/(ED_{50} of tizanidine or clonidine alone) + (ED_{50} of lidocaine when injected with tizanidine or clonidine)/(ED_{50} of lidocaine alone).

Values near 1 indicate an additive interaction, and values of less than 1 imply a supraadditive interaction.

Results

Effects of Intrathecal Administration of Tizanidine, Clonidine, and Lidocaine

Tizanidine, clonidine, and lidocaine alone given intrathecally produced dose- and time-dependent antinociceptive effects in the TF test (fig. 1). The peak effects of tizanidine and clonidine were observed between 5 and 10 min. The duration of antinociceptive effects of clonidine was longer than that of tizanidine. The pretreatment with yohimbine significantly abolished the antinociceptive effect of tizanidine, 5.0 μg, (data not shown). Tizanidine and clonidine at the doses used in the current study did not affect motor function (data not shown).

Intrathecal lidocaine produced a rapid onset and a peak effect 5 min after administration and showed a shorter duration of action than tizanidine and clonidine (fig. 1). Lidocaine alone showed a significant decrease in motor function score in a dose-dependent manner. Although neither lidocaine 20 nor 50 μg produced any evidence of motor impairment, at the dose of lidocaine 100 μg, the rats showed mild impairment of hindlimb function that disappeared within 5 min after injection. At lidocaine, 200 μg, the rats had transient flaccid paralysis and recovered at 5 min. Lidocaine 400, 600, and 1000 μg also produced flaccid paralysis lasting for 5, 10, and 15 min, with full recovery observed at 15, 20, and 30 min, respectively (data of lidocaine 400 μg is shown in figure 6).

Effects of Intrathecal Coadministration of Tizanidine/Lidocaine or Clonidine/Lidocaine

Intrathecal tizanidine/lidocaine or clonidine/lidocaine produced dose- and time-dependent antinociceptive effects in the TF test (fig. 2). The ED_{50} values and 95% CIs for tizanidine, clonidine, lidocaine, and the combi-

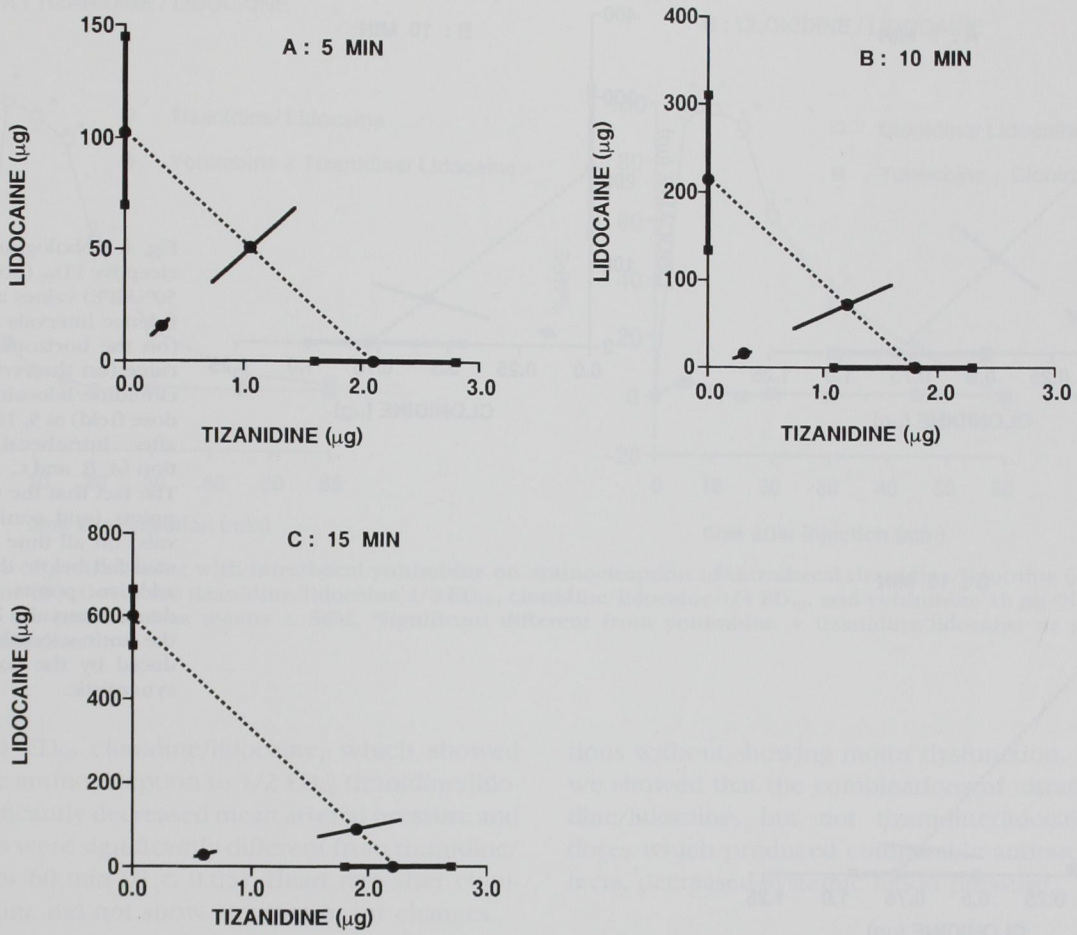

Fig. 3. Isobolograms of antinociceptive ED_{50} (dose producing 50%MPE) values and 95% confidence intervals for tizanidine (on the horizontal axis), lidocaine (on the vertical axis), or tizanidine/lidocaine (in the dose field) at 5, 10, and 15 min after intrathecal administration (*A, B,* and *C,* respectively). The heavy lines on the axes represent the confidence intervals for the single agents. The dashed diagonal line connecting the tizanidine and lidocaine ED_{50} values is the theoretical additive line, and the point on this line is the theoretical additive point (and confidence interval). The fact that the experimental points (and confidence intervals) on all time points fall below the theoretical additive points (and confidence intervals) indicates that the antinociceptive effect produced by the combination is synergistic.

nations at 5, 10, and 15 min after administration in TF test are summarized in tables 1 and 2. The experimentally derived ED_{50} and CI for the combination of tizanidine/lidocaine at 5 min plotted in the isobologram fell below the theoretically additive dose line, and the CIs of the experimental point and theoretical point did not overlap (fig. 3A). This result revealed a significant synergistic interaction between tizanidine and lidocaine at 5 min after intrathecal administration in the TF test ($P < 0.05$). This significant synergistic interaction was also confirmed by potency ratio analysis and total fraction (table 1). As illustrated in figures 3B and 3C, tizanidine/lidocaine also clearly showed synergistic interaction at 10 and 15 min after injection ($P < 0.05$). Likewise, the interaction between clonidine and lidocaine was found to be significantly synergistic at 5, 10, and 15 min after injection (fig. 4 and table 2) ($P < 0.05$).

Figure 5 shows the effect of yohimbine on antinociceptive interaction of tizanidine/lidocaine (fig. 5A) and clonidine/lidocaine (fig. 5B). Antinociceptive interactions of 1/2 ED_{50} tizanidine/lidocaine or 1/4 ED_{50} clonidine/lidocaine were significantly abolished by the pretreatment of intrathecal yohimbine 10 μg at 5, 10, 15, 20, and 30 min after the combinations administration ($P < 0.05$).

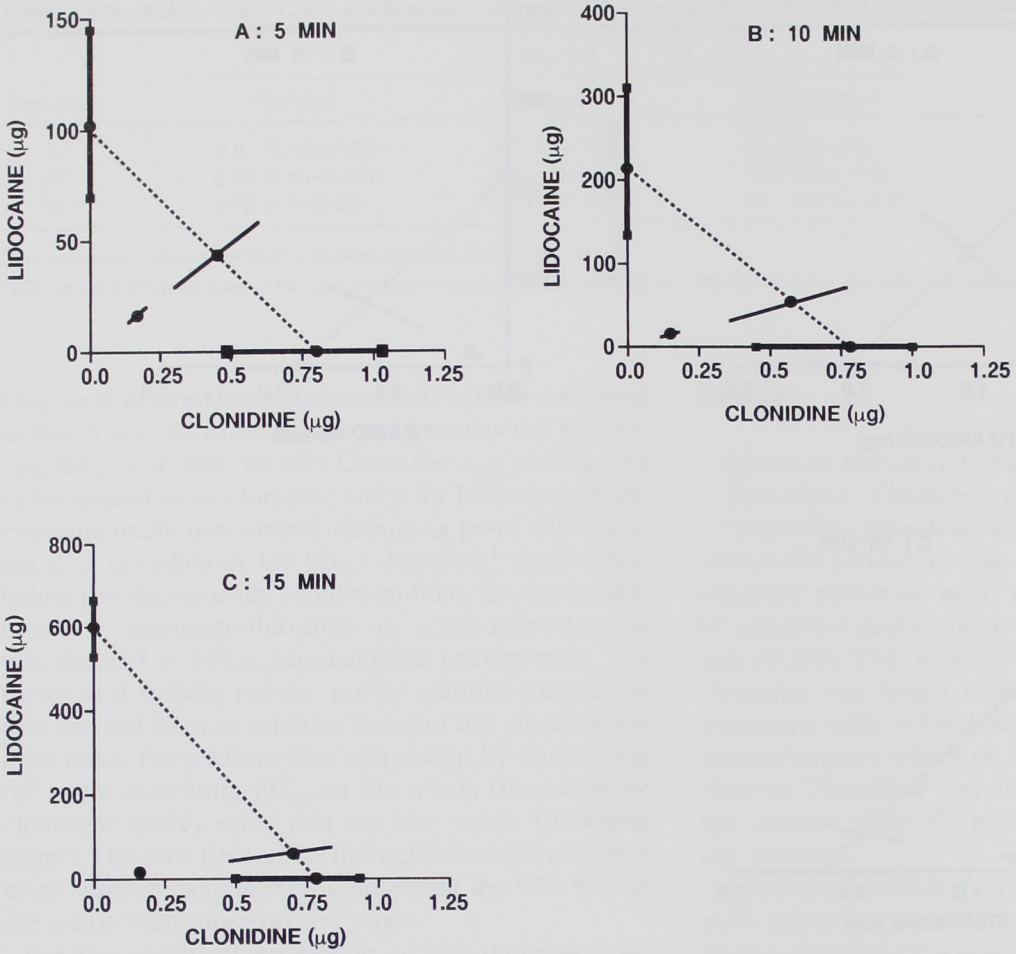

Fig. 4. Isobolograms of antinociceptive ED_{50} (dose producing 50%MPE) values and 95% confidence intervals for clonidine (on the horizontal axis), lidocaine (on the vertical axis), or clonidine/lidocaine (in the dose field) at 5, 10, and 15 min after intrathecal administration (*A*, *B*, and *C*, respectively). The fact that the experimental points (and confidence intervals) on all time points evaluated fall below the theoretical additive points (and confidence intervals) indicates that the antinociceptive effect produced by the combination is synergistic.

Tizanidine/lidocaine and clonidine/lidocaine at the doses used in the antinociceptive study did not show any changes in motor function scores (data not shown). Table 3 shows the cumulative motor function scores after intrathecal administration of lidocaine alone and the combination of tizanidine with lidocaine. The addition of tizanidine 5.0 μg to lidocaine 100, 200, and 400 μg produced no effect on the degree or the duration of the change in motor function scores induced by lidocaine alone. The time courses of the changes in the scores after lidocaine alone (400 μg) and tizanidine (5.0 μg)/lidocaine (400 μg) are presented in figure 6.

Effects of Intrathecal Administration of Tizanidine, Clonidine, Lidocaine, or the Combinations on Blood Pressure and Heart Rate

In unanesthetized rats, the predrug baseline mean arterial blood pressure and heart rate were 115.7 ± 3.2 mmHg and 363.5 ± 6.7 beats/min, respectively. Figure 7 showed the effects of intrathecal tizanidine, clonidine, and lidocaine alone on mean arterial blood pressure and heart rate at the doses that produced comparable antinociceptive effects. Clonidine, 2.0 μg and lidocaine 200 μg, significantly decreased mean arterial blood pressure compared with the predrug baseline value (fig. 7B and 7C). The depressor effects of clonidine and lidocaine were observed for 45 and 15 min, respectively. Clonidine also showed a decrease in heart rate 15–30 min after the intrathecal injection. In contrast, tizanidine, 5.0 μg, affected neither mean arterial blood pressure nor heart rate. Figure 8 showed the effects of intrathecal administration of tizanidine/lidocaine and clonidine/lidocaine on blood pressure and heart rate. The combination of 1/2 ED_{50} tizanidine/lidocaine affected neither mean arterial blood pressure nor heart rate during the study. On the other hand, the combina-

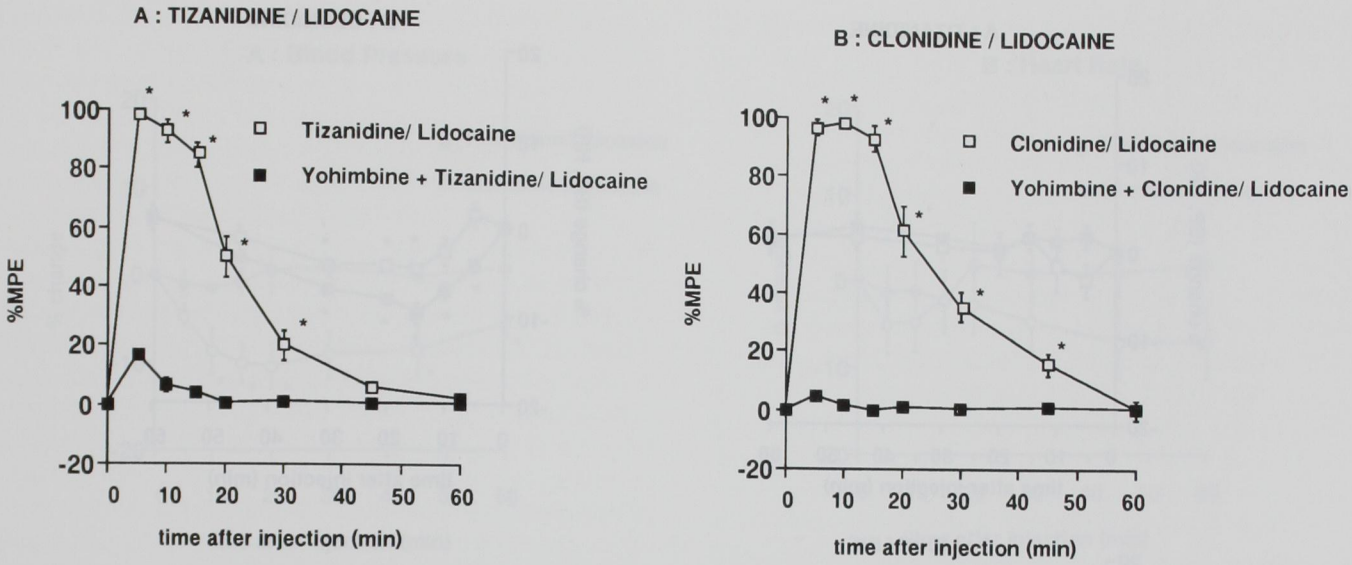

Fig. 5. Effects of pretreatment with intrathecal yohimbine on antinociception of intrathecal tizanidine/lidocaine (*A*) and clonidine/lidocaine (*B*). Doses are tizanidine/lidocaine 1/2 ED_{50}, clonidine/lidocaine 1/4 ED_{50}, and yohimbine 10 μg. N = 5 for each group. Data are presented as means ± SEM. *Significant different from yohimbine + tizanidine/lidocaine or yohimbine + clonidine/lidocaine.

tion of 1/4 ED_{50} clonidine/lidocaine, which showed comparable antinociception to 1/2 ED_{50} tizanidine/lidocaine, significantly decreased mean arterial pressure and the changes were significantly different from tizanidine/lidocaine for 60 min ($P < 0.05$). Heart rate after clonidine/lidocaine did not show any significant changes.

Discussion

The current study clearly showed that intrathecal combinations of clonidine/lidocaine and tizanidine/lidocaine showed synergistic antinociceptive interactions without showing motor dysfunction. In addition, we showed that the combinations of intrathecal clonidine/lidocaine, but not tizanidine/lidocaine, at the doses which produced comparable antinociceptive effects, decreased systemic blood pressure.

Table 3. Motor Function Scores after Intrathecal Injection of Lidocaine Alone and in Combination with Tizanidine 5.0 μg

Lidocaine Dose (μg)	Score		*P*
	Lidocaine Alone	Combination	
100	76.7 ± 0.8	76.3 ± 0.7	NS
200	69.5 ± 0.6	69.0 ± 0.5	NS
400	55.8 ± 1.0	54.0 ± 2.4	NS

Values are the mean ± SEM of the cumulative motor function score (normal score = 80). The cumulative score was obtained by the summation of motor function scores at 2, 5, 10, 15, and 20 min after the drug administration.
NS = not significant.

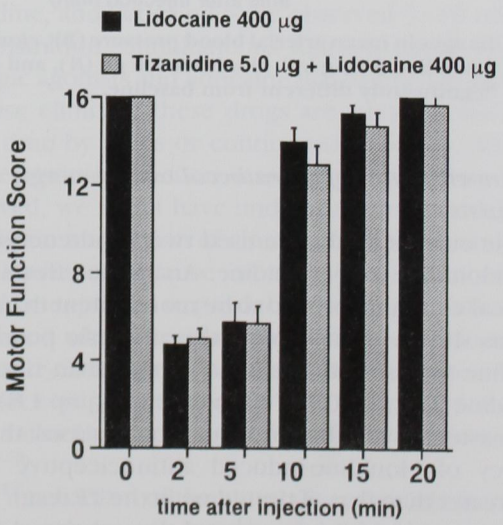

Fig. 6. Changes in motor function scores after intrathecal administration of lidocaine alone and tizanidine/lidocaine. N = 6 for each group. Data are presented as means ± SEM.

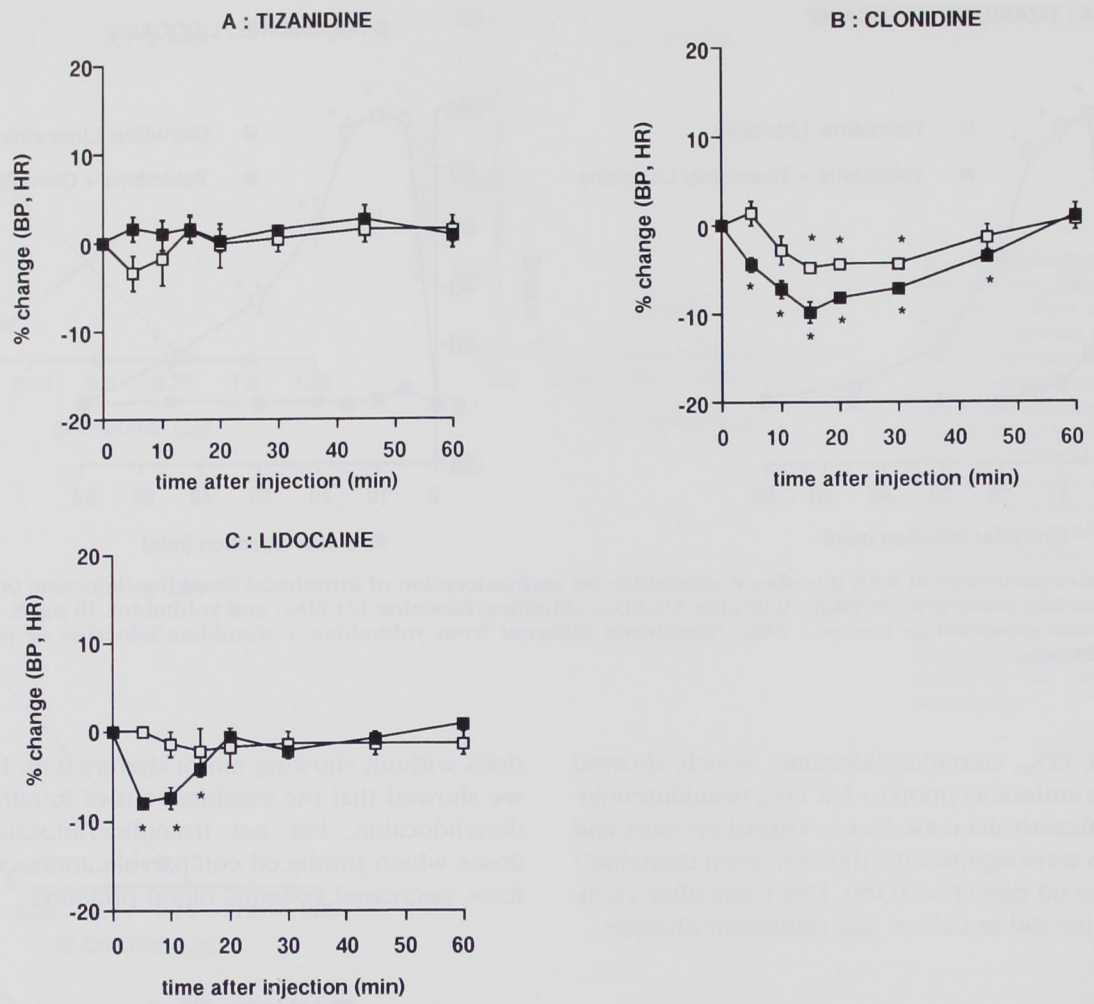

Fig. 7. Changes in mean arterial blood pressure (BP, *closed square*) and heart rate (HR, *open square*) after intrathecal administration of tizanidine 5.0 μg (*A*), clonidine 2.0 μg (*B*), and lidocaine 200 μg (*C*). Data are presented as means ± SEM. N = 6 for each group. *Significantly different from baseline.

Antinociception of Intrathecal α_2-adrenergic Agonists

In the current study, we used two α_2-adrenergic agonists, clonidine and tizanidine. Analgesic effects of intrathecal clonidine proved to be more potent than tizanidine as shown in this study; the analgesic potency of clonidine was about 2.4 times greater than tizanidine (clonidine ED_{50} = 0.75; tizanidine ED_{50} = 1.8). This is consistent with the previous observations that the potency of clonidine-induced antinociceptive action was greater than that of tizanidine in the TF test,[12] acetic acid-induced writhing test,[12] and the colorectal distension test.[13] In addition, this study showed that the duration of clonidine-induced analgesic effect was longer than that of tizanidine. Although clonidine is similar to tizanidine in protein-binding affinity, clonidine has a higher affinity to the α_2-adrenergic receptor by about 3 times and a higher lipophilicity and larger pKa than tizanidine.[14] These may contribute to the difference in the analgesic potency and duration between clonidine and tizanidine.

Although oral tizanidine is classified therapeutically as a muscle relaxant, it does not suppress monosynaptic and polysynaptic reflexes when given intrathecally.[6] Previous reports suggested that tizanidine produced the antinociceptive effects in TF test at doses levels that were considerably lower than those which would be expected to show muscle relaxant action.[6,15] These anti-

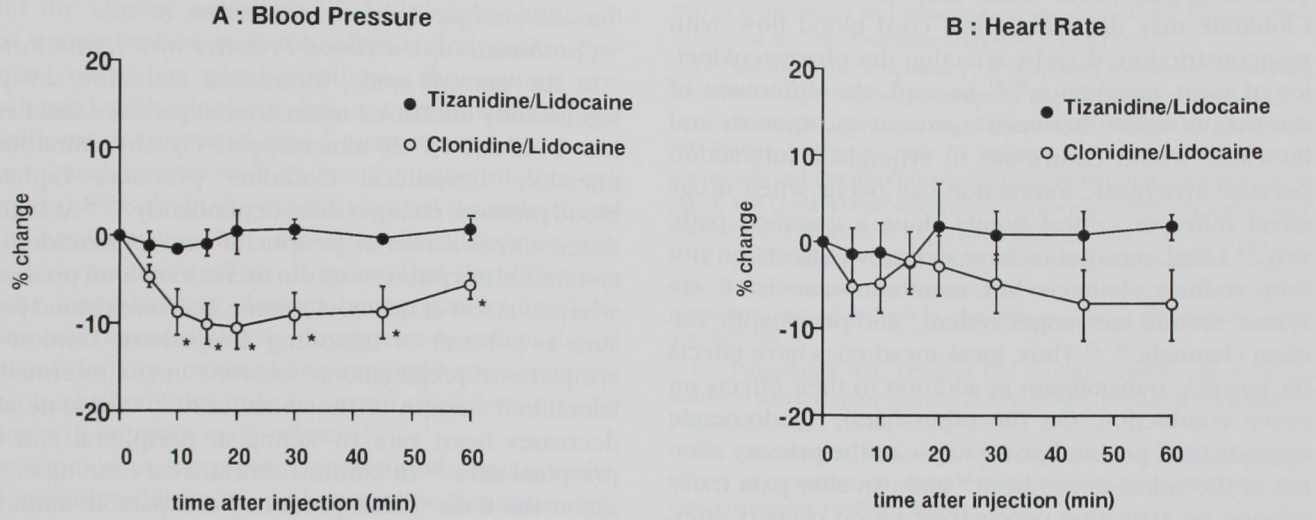

Fig. 8. Effects of intrathecal tizanidine/lidocaine (*closed circle*) and clonidine/lidocaine (*open circle*) on mean arterial blood pressure (*A*) and heart rate (*B*). Data are presented as means ± SEM. N = 6 for each group. *Significantly different from tizanidine/lidocaine at individual times.

nociceptive effects also were reversed by α_2-adrenergic agonist, yohimbine.[6] Consistent with these reports, we showed that intrathecal tizanidine at doses used did not affect motor function and that pretreatment with yohimbine abolished the antinociception of tizanidine. Thus, tizanidine-induced prolongation of TF latency in the current study is likely a result of the antinociceptive effect mediated through α_2-adrenergic action, not a result of motor dysfunction.

Antinociceptive Interaction of Intrathecal α_2-adrenergic Agonists and Lidocaine

Several lines of studies have shown that intrathecal or epidural clonidine potentiated analgesic effect of local anesthetics in humans.[3-5] Additionally, Glynn *et al.*[16] suggested that the combination of epidural clonidine and lidocaine had supraadditive interaction in patients with chronic pain, although there were no systematic studies that showed the pharmacologic nature of antinociceptive interaction between α_2-adrenergic agonists and local anesthetics. The important results obtained in this study clearly showed that intrathecal tizanidine/lidocaine and clonidine/lidocaine produced a synergistic antinociceptive interaction in the TF test without affecting motor function. The magnitude of the antinociceptive interaction, *e.g.*, total fraction values, also less than 1, indicated a synergistic interaction. Further, the degree of synergism in clonidine/lidocaine and tizanidine/lidocaine were comparable.

We constructed the isobolograms for several time points to determine the nature of the interaction throughout the time-effect course. Well-timed administration is commonly used for isobolographic analysis with drugs that have a significant time lag in peak effect.[9] In the current study, the peak effects of tizanidine, clonidine, and lidocaine were observed 5 - 10 min after administration. Simultaneous administration of α_2-adrenergic agonists and lidocaine was applied in this study because clinically these drugs are administered at the same time by bolus or continuous injection. Although a synergistic interaction was found at all time points observed, we might have underestimated the degree of synergistic interaction of clonidine/lidocaine and tizanidine/lidocaine because simultaneous administration would be expected to produce a lesser peak effect than well-timed administration[17] and because the fixed dose ratio of α_2-2adrenergic agonists and lidocaine we used was not equipotent at some time points and the ratio could influence the interaction results in synergistic effect.[18,19]

The mechanism of the antinociceptive interaction between α_2-adrenergic agonists and lidocaine remains to be elucidated. Possible mechanisms of the synergy are as follows. First, change in the pharmacokinetics of

each drug may occur when they are coadministered. Clonidine may decrease spinal cord blood flow with vasoconstriction, thereby affecting the pharmacokinetics of local anesthetics.[20,21] Second, the difference of the sites of action between α_2-adrenergic agonists and lidocaine would contribute to synergistic interaction because synergistic interaction can occur when drugs affect different critical points along a common pathway.[22] Local anesthetics have extensive effects on not only sodium channels but membrane-associated enzymes, second messenger system,[9] and presynaptic calcium channels.[23-25] Thus, local anesthetics have effects on synaptic transmission in addition to their effects on nerve conduction. On the other hand, α_2-adrenergic agonists bind pre- and postsynapse to the primary afferent in the spinal dorsal horn[26] and can alter pain transmission by activating presynaptically on primary afferent fibers to release neurotransmitters[27,28] and can act postsynaptically to hyperpolarize dorsal horn wide dynamic range neurons.[29] The α_2-adrenergic agonists also activates K^+ channels and inhibits voltage-dependent Ca^{2+} channels.[30]

Although tizanidine has muscle relaxant property, tizanidine/lidocaine had no effect on motor function at the doses employed in the current study. Tizanidine, 5.0 μg, also did not potentiate the change in motor function scores induced by lidocaine alone. In addition, antinociceptive interactions of tizanidine/lidocaine or clonidine /lidocaine were abolished by the pretreatment of an α_2-adrenergic antagonist, yohimbine. These results indicated that the synergistically antinociceptive interactions of tizanidine/lidocaine observed were likely mediated by α_2-adrenergic action but by neither muscle relaxant property nor other pharmacologic actions of tizanidine.

Previous studies showed that intrathecal coadministration of clonidine and local anesthetic prolonged the duration of motor blockade and sensory blockade, compared with local anesthetic alone.[3-5] The enhancement of motor blockade is an undesirable effect for clinical pain management. In the current study, however, intrathecal tizanidine and clonidine potentiated the antinociceptive effects of intrathecal lidocaine without eliciting motor blockade. This finding suggested that an appropriate combination of clonidine/lidocaine or tizanidine/lidocaine could produce enhanced antinociception without impaired motor function, resulting in the production of a clinically available analgesia.

Effects of Intrathecal Administration of α_2-adrenergic Agonists, Lidocaine, or the Combinations on Blood Pressure and Heart Rate

In the present study, intrathecal clonidine, 2.0 μg, significantly decreased mean arterial pressure and heart rate 5-45 and 15-30 min, respectively, after intrathecal injection. Intrathecal clonidine produces biphasic blood pressure changes dose-dependently.[31,32] At higher doses, direct action at peripheral vasoconstrictors *via* systemic absorption results in increased blood pressure, whereas at lower doses, clonidine decreases blood pressure as a result of inhibiting sympathetic outflow at sympathetic preganglionic neurons in the intermediolateral cell column of the spinal cord.[32] Clonidine also decreases heart rate by acting at peripheral and supraspinal sites.[30] In contrast, intrathecal tizanidine, 5.0 μg, at the dose which produced comparable antinociception with clonidine, 2.0 μg, affected neither blood pressure nor heart rate in the current study. Similar to the results in our study, McCarthy *et al.*[6] showed that intrathecal tizanidine did not caused a significant change in blood pressure in rats. Because tizanidine is poor lipophilicity, it could be speculated that this reflected an inability to diffuse to the intermediolateral cell column, which is deeper from the spinal cord surface than substantia gelatinosa, and an inability to absorb into the systemic circulation.

Recent evidence suggests that hypotensive actions, but not analgesic effects, of α_2-adrenergic agonists may be attributable not only to α_2-adrenergic receptor binding but also to imidazoline (I) receptor binding.[33] I_1 subtype of imidazoline receptor is characterized by a high affinity for clonidine and thought to mediate central blood pressure lowering effects. Tizanidine also binds to the imidazoline receptor with approximately 20 times higher affinity than α_2-adrenergic receptor.[34] Kroin *et al.*[31] suggested that intrathecal clonidine and tizanidine produced hypotension and bradycardia and that these hemodynamic effects might be mediated, in part, by the imidazoline receptor in dogs. Although the intermediolateral cell column of the rat spinal cord exhibited the low-to-medium density of imidazoline receptor binding sites, its subtype is an I_2-receptor, not an I_1-receptor.[35] Thus, it is unclear whether the hemodynamic actions of intrathecal clonidine and tizanidine are a result of imidazoline receptor activation in the spinal cord.

In human studies, marked hemodynamic changes have been shown after administration of intrathecal

clonidine combined with local anesthetics, and this may limit the clinical usefulness.[3-5] Additionally, although oral premedication with tizanidine and clonidine prolonged tetracaine spinal anesthesia, the patients premedicated with clonidine but not tizanidine showed significant decreases in blood pressure and heart rate after spinal anesthesia.[36] In confirmation of this evidence, the results in this study indicated that intrathecal clonidine/lidocaine but not tizanidine/lidocaine produced hypotensive effect. It seems that the difference of the hemodynamic changes between clonidine/lidocaine and tizanidine/lidocaine appears to reflect the effects of clonidine or tizanidine itself on hemodynamics. That is, the hemodynamic actions of clonidine and lidocaine will enhance each other.

In conclusion, the present study showed that intrathecal clonidine/lidocaine and tizanidine/lidocaine produced synergistic antinociceptive interaction without apparently impairing motor function. Clonidine/lidocaine but not tizanidine/lidocaine did affect blood pressure. These findings may be important in clinical pain management because an appropriate combination of tizanidine and lidocaine will be able to provide improved analgesia and at the same time minimize the adverse effects.

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