The Antinociceptive Effects of Midazolam on Three Different Types of Nociception in Mice

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Abstract. Antinociceptive effects of systemically administered midazolam remain controversial. The present study was performed to investigate its antinociceptive effects on different types of nociception in mice. Four different doses of midazolam (1, 3, 10, and 30 mg/kg) were administered intraperitoneally (i.p.). Saline was used as a control. The hot plate test, tail pressure test, acetic acid writhing test, the running wheel test, and the balance beam test were performed following the drug administration. In the hot plate test and tail pressure test, i.p. midazolam produced significant antinociceptive effects with the 50% effective dose (ED₅₀) of 3.46 mg/kg [confidence interval (CI), 1.99 – 6.01 mg/kg] and 3.52 mg/kg (CI, 2.77 – 4.47 mg/kg), respectively. In the acetic acid writhing test, i.p. midazolam also produced significant antinociceptive effects. In the running wheel test, no mice stopped running after saline or midazolam at 1, 3, or 10 mg/kg, but all mice stopped running 30 and 45 min after i.p. administration of midazolam at 30 mg/kg. In the balance beam test, 30 min after i.p. administration of saline or midazolam at 1, 3, and 10 mg/kg, all mice were able to stay on the beam for 90 s, none of them could with midazolam at 30 mg/kg. In conclusion, systemically administered midazolam had antinociceptive effects on acute thermal, acute mechanical, and acute inflammatory-induced nociception in mice. The antinociceptive potency of midazolam was the same for both acute thermal-induced nociception and mechanical-induced nociception.

Keywords: midazolam, antinociception, hot plate test, tail pressure test, acetic acid writhing test

Introduction

Benzodiazepines are generally known as sedative, anti-anxiety, hypnotic, and anticonvulsant drugs. They exert their pharmacological effects via interaction with γ-aminobutyric acid-A (GABAₐ) receptors, enhancing the action of inhibitory neurotransmitter GABA (1 – 3). Midazolam is commonly used as an adjunct to general anesthesia and has been shown to decrease the anesthetic requirements of volatile agents after intravenous (i.v.) administration in both animals (4) and humans (5, 6).

Some reports showed antinociceptive effects of benzodiazepines (7 – 9), while other findings did not support those observations (10 – 12). Both hyperalgesic (13) and antinociceptive (14) effects of systemically administered midazolam have been reported. These discrepancies may be due to the animal species used, the nature and intensity of the stimuli, or the routes of drug administration.

Different results may be ascribed to the action of midazolam on the spinal cord and/or brain. Lumbar intrathecal (i.t.) administration of midazolam produces antinociception (15, 16), while intracerebroventricular (i.c.v.) injection produces a hyperalgesic response (13). We already reported that intrathecally administered midazolam had antinociceptive effects on both acute thermally and inflammatory induced nociception, whereas intraperitoneally administered midazolam had antinociceptive effects on inflammatory induced nociception, but not on acute thermal nociception in Sprague Dawley
rats (17). The present study was performed to compare the effects of systemically administered midazolam on different types of nociception in mice.

**Materials and Methods**

**Animals and drugs**

This study was approved by the Laboratory Animal Care and Use Committee of the University of Tokyo. All experiments were performed in accordance with the guidelines of the Physiological Society of Japan regarding the care of experimental animals. Male ddY mice (SLC Japan, Hamamatsu), weighing 28–32 g were used. Mice were housed under controlled temperature (23 ± 2°C) and humidity (55 ± 10%) and habituated to the environment for at least 3 days before experiments. Animals were allowed to access food and water ad libitum. The mice were euthanized immediately after each experiment.

Midazolam (Sigma, Tokyo) was dissolved in normal saline to achieve solutions of 1, 3, 10, and 30 mg/kg for intraperitoneal (i.p.) administration. Normal saline was used as a control. The total injected volume was adjusted to 10 ml/kg in each mouse. A 1-mL syringe with a 26-G needle was used for i.p. injection.

**Hot plate test**

The hot plate test was performed at 55.0°C (ranging from 54.8°C to 55.2°C) using the MK-350 apparatus (Muromachi Kikai Co., Ltd., Tokyo) (18). Mice were preadapted to the test apparatus by putting each animal in the perspex box on the cold plate for 1–2 min on the day before testing. Mice were placed on the heated smooth surface, and the latency to licking, shaking of the limbs, or jumping was measured. Prior to drug administration, the nociceptive response of each mouse was measured three times with 30-min intervals. The first measurement was omitted and the mean of the next two measurements was employed as the baseline tail pressure threshold for each mouse.

The tail pressure threshold was measured by a single investigator. To evaluate the dose-dependent effect of midazolam, mice were divided into 4 groups to receive i.p. saline or i.p. midazolam at 1, 3, or 10 mg/kg (n = 8 in each group). The tail pressure threshold was measured before and 10, 30, 45, 60, 90, 120, and 150 min after i.p. drug injection. The test was terminated if a mouse did not show motor responses by 250 g to avoid tissue injury.

**Acetic acid writhing test**

The writhing activity was evaluated by the method of Hayashi and Takemori (20). The number of stretches or writhes (arching of the back, development of tension in the abdominal muscles, elongation of the body, and extension of the forelimbs) was counted for 10 min starting 5 min after the administration of 0.6% (v/v) acetic acid (10 ml/kg, i.p.). To evaluate the dose-dependent effect of midazolam, mice were divided into 4 groups to receive i.p. saline or i.p. midazolam at 1, 3, or 10 mg/kg (n = 8 in each group). The total injected volume was adjusted to 10 ml/kg in each mouse. Acetic acid 0.6% solution in a volume of 10 ml/kg was i.p. administered 30 min after saline or midazolam injection.

**Running wheel test**

The locomotor activity was tested with a running wheel (16 cm in diameter) with a ball-bearing axle (Doggyman. H.A. Co., Ltd., Osaka) (21). A force of 4 g on the circumference, midway between the top and bottom of the wheel, was sufficient to overcome inertia. The wheel could be turned in either direction. The system recorded each revolution of the wheel, and data were expressed as total number of revolutions per 5 min. To evaluate the effect of midazolam on locomotor activity, mice were divided into 5 groups to receive i.p. saline or i.p. midazolam at 1, 3, 10, or 30 mg/kg (n = 8 in each group). Just before drug administration, all mice were allowed time for habituation. After drug administration, mice were placed in the running wheels for 150 min. The number of wheel revolutions was recorded every 5 min.

**Balance beam test**

The effect of midazolam on muscle relaxing was...
tested with a balance beam (22). Balance was determined by measuring the time that mice were able to stay on a round wood beam. The cut off time was set at 90 s for all mice. The diameter of the beam was 15 mm. To evaluate the effect of midazolam on muscle relaxing, mice were divided into 5 groups to receive i.p. saline or i.p. midazolam at 1, 3, 10, or 30 mg/kg (n = 8 in each group). Before drug administration, all mice were allowed time for habituation. Mice were placed on the round wood beam 30 min after drug administration. The time that mice were able to stay on the beam was recorded.

Statistical analyses

Data are expressed as the mean ± S.E.M. Changes of the response time in the hot plate test, the tail pressure threshold in the tail pressure test, and the number of wheel revolutions in the running wheel test were analyzed with a two-way repeated measures analysis of variance (ANOVA), followed by the Fisher’s protected least significance difference test (PLSD) as a post hoc test. The number of contortions in the acetic acid writhing test, total amount of wheel revolutions in the running wheel test, and time on the beam in the balance beam test were analyzed with one-way ANOVA, followed by Fisher’s PLSD as a post hoc test. A P value less than 0.05 was considered to be significant.

The percentage of the maximum possible effect (% MPE) was calculated as follows: (post-drug latency – pre-drug latency at time 0) × 100 / (cutoff value – pre-drug latency at time 0). The 50% effective dose (ED50) and 95% confidence interval (CI) were calculated using the maximum effects in the hot plate test and in the tail pressure test. The ED50 of the hot plate test was calculated at 30 min after drug administration. The ED50 of the tail pressure test was calculated at 30 min after drug administration.

Results

Hot plate test

Antinociceptive effects, as demonstrated by significant increases in response time compared with saline, were observed after the i.p. administration of midazolam at 3 and 10 mg/kg, but not at 1 mg/kg. Dose-dependent antinociceptive effects were observed 10, 30, 45, 60, and 90 min after i.p. midazolam (Fig. 1A). The ED50 was 3.46 mg/kg (CI, 1.99 – 6.01 mg/kg) (Fig. 1B).

Tail pressure test

In the tail pressure test, the baseline tail pressure threshold was between 50 and 80 g on average. Antinociceptive effects, as demonstrated by significant increases in tail pressure threshold compared with saline, were observed after i.p. administration of midazolam at 3 and 10 mg/kg, but not at 1 mg/kg. Dose-dependent antinociceptive effects were observed 10, 30, 45, 60, 90, and 120 min after i.p. midazolam (Fig. 2A). The ED50 was 3.52 mg/kg (CI, 2.77 – 4.47 mg/kg) (Fig. 2B).

Acetic acid writhing test

Antinociceptive effects, as demonstrated by significant decreases in number of contortions compared with saline, were observed after i.p. administration of midazolam at 3 and 10 mg/kg, but not at 1 mg/kg (Fig. 3).
Running wheel test

In the running wheel test, no mice stopped running after saline, or midazolam at 1, 3, and 10 mg/kg, but all mice stopped running 30 and 45 min after i.p. administration of midazolam at 30 mg/kg (Fig. 4A). Total amount of wheel revolutions with midazolam at 10 and 30 mg/kg was significantly lower than that with midazolam at 1 and 3 mg/kg, and total amount of wheel revolutions with midazolam at 1 mg/kg was significantly higher than that in the control group (Fig. 4B).

Balance beam test

In the balance beam test, 30 min after i.p. administration of saline or midazolam at 1, 3, and 10 mg/kg, all mice could stay on the beam for 90 s, but none of the mice given midazolam (30 mg/kg) could stay on the beam (Fig. 5).

Discussion

In the present study, we demonstrated that intraperitoneally administered midazolam had antinociceptive effects in the hot plate test, tail pressure test, and acetic acid writhing test in mice. The mean score and standard deviation for the saline-treated controls were determined for the hot plate test and the tail pressure test. In order to standardize comparisons across the two tests, the maximal antinociceptive effect (i.e., the cutoff value) for each test was defined as 4 standard deviations greater than the mean for the control group, according to the method by Michael et al. (23). The ED$_{50}$ was 3.46 mg/kg (CI, 1.99 – 6.01 mg/kg) in the hot plate test and 3.52 mg/kg (CI, 2.77 – 4.47 mg/kg) in the tail pressure test. There was no significant difference between the ED$_{50}$ values of the thermal and mechanical nociceptive tests.

Anxiolytic, muscle relaxing, and sedative effects of midazolam may alter the responses in nociceptive tests in different directions, thus explaining some of the conflicting results. Several different tests have been used to evaluate sensorimotor impairment induced by benzodiazepines (24, 25). In this study, the running wheel test and the balance beam test were used to provide a reliable and sensitive behavioral assay for the effects of midazolam in mice. Orii et al. reported that midazolam (1, 5, and 10 mg/kg) did not induce any detectable reduction in motor response using Fischer rats (26). In the present study, although the number of wheel revolutions with midazolam at 10 mg/kg was significantly lower than that in the control group 10 and 30 min after i.p. drug administration, mice were still
able to run on the wheel. However, all mice stopped running 30 and 45 min after i.p. administration of midazolam at 30 mg/kg (Fig. 4A). In the balance beam test, 30 min after i.p. administration of saline or midazolam at 1, 3, and 10 mg/kg, all mice could stay on the beam for 90 s, but no mouse could with midazolam at 30 mg/kg (Fig. 5). Therefore, we did not use 30 mg/kg for the nociceptive test to minimize the effects of sedation, reduced locomotor activity, or muscle relaxation, and we determined the maximum dose of midazolam as 10 mg/kg for the nociceptive test.

Since it was first introduced in the USA in 1986, midazolam has become the most frequently used sedative agent within and outside the operating room. Occasionally, rather than sedating and calming the patient, midazolam may precipitate hostility, rage, and even physical violence, necessitating the restraint of such patients until these responses (termed ‘paradoxical’) fade spontaneously (27, 28). The present study demonstrated that in the running wheel test, the total number of wheel revolutions with midazolam at 1 mg/kg was significantly higher than that in the control group. Observations on the effect of midazolam reported in several previous studies support this possibility. First, Ricou et al. reported that rectal midazolam as a premedication in children could cause paradoxical reactions such as agitated excitement, restlessness, irritation, lack of co-operation, disorientation or confusion, emotional crying, and visual disturbances (29). Second, Fulton et al. reported that after premedication with midazolam at 5 mg (titrated slowly to sedation), the patient (a 62-year-old man) was swinging his arms and shaking his head (30). Third, the appearance of paradoxical phenomena were often reported after 4 – 7 mg midazolam, a dose that may under-sedate the ordinary patient and lead to the induction of restlessness and other signs of brain agitation by yet unspecified stimuli (31).

The hot plate test is a method to evaluate supraspinal antinociceptive effects, and it reflects activity in thermally sensitive afferent fibers and activity of A\(\delta\) and C fibers (32). The tail pressure test was considered to be a supraspinally-integrated response, and it reflects activity in mechanically sensitive afferent fibers and activity of A\(\delta\) and C fibers (19, 33). The difference between the hot plate test and the tail pressure test is the type of stimulation. In the present study, there was no significant difference between the ED\(_{50}\) of the thermal nociceptive
test and that of the mechanical nociceptive test. Moreover, the duration of the antinociceptive effect of midazolam in the hot plate test was almost equal to that in the tail pressure test. Therefore, the effects of midazolam on thermally sensitive afferent fibers and those on mechanically sensitive afferent fibers might be equipotent.

Rosland et al. reported that in the hot plate test, intraperitoneally administered midazolam with doses up to 1 mg/kg did not induce any significant effects in male albino mice (Bom:NMRI) weighing 25–35 g (34). Higher doses of midazolam (2 mg/kg), however, significantly increased the response latency (34). These results are consistent with our study. Systemically administered midazolam reduces Aδ fiber–evoked responses of the neurons of the dorsal horn of the spinal cord and also reduces the C fiber–mediated activity in a spinal nerve ligation model of neuropathic pain, most likely acting at spinally located benzodiazepine receptors (35). Therefore, systemically administered midazolam may have actions at the level of the spinal cord.

In the acetic acid writhing test, both central and peripheral antinociception could be evaluated, and many investigators recommend it as a simple screening method for the effects of drugs on inflammatory-induced nociception (36). This test consists of characteristic visceromotor stretching or writhing, an alternating abdominal flexion-extension. Acetic acid has been demonstrated to produce an acute peritoneal inflammation (37). The acetic acid induced nociceptive response may involve both direct stimulation of the nociceptive afferent fibers due to the pH reduction and the synthesis of inflammatory mediators (38). In the present study, midazolam at 3 and 10 mg/kg produced a significant decrease in the number of writhing episodes. The acetic acid writhing test possesses high sensitivity because narcotic analgesics are effective with ten times lower doses in the writhing test than doses effective in the hot plate test or tail flick test (39). Our results might suggest that the acetic acid writhing test also possesses high sensitivity to midazolam.

In conclusion, systemically administered midazolam had antinociceptive effects on acute thermal, acute mechanical, and acute inflammatory induced nociception in mice. The antinociceptive potency of midazolam was the same for both acute thermal-induced nociception and mechanical-induced nociception.

References

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