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Analgesic properties of the novel compound M43068 in rat models of acute and neuropathic pain

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Abstract

We investigated the effects of 2-(4-hydroxybenzoyl)amino-2-methylpropionic acid (M43068), a novel analgesic agent, in rat models of acute and neuropathic pain. Oral M43068 (10–100 mg/kg) suppressed only the late phase of formalin-induced nociceptive behaviors. In the neuropathic pain model, oral M43068 (10–100 mg/kg) suppressed mechanical allodynia in the nerve-injured paw without affecting normal thresholds. On the other hand, i.v. M43068 (30 mg/kg) mainly suppressed the Aβ-fiber-mediated response with the Neurometer. I.c.v. pretreatment with the α_1-adrenoceptor antagonist, prazosin, or i.p. pretreatment with the γ-aminobutyric acid $(GABA)_B$ receptor antagonist, saclofen, abolished the M43068-induced antinociception. However, oral M43068 (30–300 mg/kg) had no influence on blood pressure and motor function, unlike the α_1-adrenoceptor and the $GABA_B$ receptor agonists. These data indicate that M43068 shows antinociceptive and anti-allodynic effects with reduced risks of side effects. It is suggested that the descending noradrenergic system is involved in the analgesic effects of M43068.

Keywords: Neuropathic pain; Allodynia; Formalin test; Antinociception; Neurometer; (Rat)

1. Introduction

Neuropathic pain is a pain syndrome caused by primary damage and/or by the dysfunction of the neurotransmission system connecting the peripheral to the central nervous system (CNS). Patients with neuropathic pain frequently complain of sensory abnormality, including increased response to noxious stimulus (hyperalgesia) and pain response to non-noxious stimulus (allodynia) (Woolf and Mannion, 1999). Although conventional analgesics such as opioids and nonsteroidal anti-inflammatory drugs, antidepressants or anticonvulsants are clinically used in the treatment of neuropathic pain, they are not very satisfactory because of their ineffectiveness and side effects. Thus, new therapies are needed for the treatment of painful neuropathy.

In order to obtain a novel analgesic compound, we used the formalin test (Dubuisson and Dennis, 1977; Tjølsen et al., 1992; Abbott et al., 1995) as a screening method and found 2-(4-hydroxybenzoyl)amino-2-methylpropionic acid, M43068 (Fig. 1), in the synthesized compounds in our laboratory. However, the mechanism of action of M43068 has not been elucidated.

In this study, we showed the efficacy of M43068 in the formalin test and a neuropathic pain model. We also examined the effects of M43068 on the current stimulus thresholds with the Neurometer device, which provides selective examination for subset of nerve fibers (Kiso et al., 2001; Koga et al., 2005). To clarify the mechanism of the M43068-induced antinociception, we investigated the involvements of monoamine and γ-aminobutyric acid (GABA) receptor subtypes (α_1, 5-hydroxytryptamine $(5\text{-HT})_{1A/1B}$, 5-HT_2, $GABA_A$ and $GABA_B$) in the antinociceptive effects of M43068 by using the specific antagonists. In addition, we investigated the side-effect profiles of M43068.

2. Materials and methods

2.1. Animals

All experiments were performed in accordance with the ethical guidelines of the International Association for the Study

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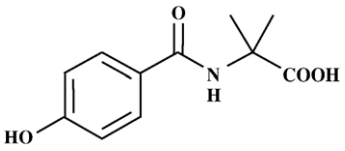

Fig. 1. Chemical structure of M43068.

of Pain (Zimmermann, 1983). In addition, all experimental procedures mentioned below were approved by the Institutional Animal Use Committee of our laboratory.

Male Wistar rats were obtained from Japan SLC and male Wistar Hannover rats from Charles River Japan. The rats were used at the age of 5–8 weeks for the experiments. They were kept in an air-conditioned and pathogen-free room with temperature of 23±2 °C and humidity of 55±10% on a regulated 12 h light/dark cycle. They had free access to standard laboratory chow (CE-2; Clea Japan) and drinking water. When the compound was given orally, the rats were fasted overnight with free access to drinking water.

2.2. Compounds

M43068 was synthesized in our laboratory. The following compounds were tested: maprotiline, R(+)-baclofen hydrochloride (Sigma Chemical Co.); fluvoxamine maleate, M6434 (Tocris Cookson Ltd.); 2,6-diisopropylphenol (propofol; Aldrich Chem. Co.). The following monoamine and GABA receptor antagonists were used: prazosin hydrochloride, pindolol, ketanserin tartrate, (-)-bicuculline methiodide and saclofen (Sigma Chemical Co.). The doses of these antagonists were determined according to the previous reports (Yokogawa et al., 2002; Nadeson and Goodchild, 1997; Shafizadeh et al., 1997). In all experiments, an equal volume of the vehicle was used as the control.

2.3. Intracerebroventricular (i.c.v.) injection

I.c.v. injection was performed according to the method of Malcangio et al. (1991). Briefly, under ether anesthesia, rats were grasped firmly behind the head. The hypodermic needle (21 gauge) was inserted 4 mm perpendicularly into the brain through the skull. The injection site was 2 mm to the left of the midline along a line drawn through the anterior base of the ears. In this study, ether anesthesia was carried out 35 min before the behavioral assessment. Under these conditions, the formalin-induced nociceptive behaviors of the ether-pretreated rats were not significantly different from those of normal rats, and there was little difference in the antinociceptive effects of M43068 between them.

2.4. The rat formalin test

The experiment was performed according to a modification of the method of Iyengar et al. (1997). Wistar rats were initially acclimated to the acryl cages (Muromachi Kikai, Tokyo, Japan) for 15 min prior to the formalin injection. Fifty microliters of 0.5% formaldehyde solution in saline was injected subcutaneously into the plantar surface of the rat's left hind paw. The observation of behaviors started immediately after the formalin injection and lasted for 45 min. Nociceptive behaviors were quantified by measuring the time spent licking/biting the injected paw with a stopwatch every 5 min. It was reported that the rodents showed two phases of nociceptive behaviors (Tjølsen et al., 1992); the recording of the early phase started immediately after the formalin injection and lasted for 10 min, the recording of the late phase started after the early phase and lasted for 35 min. The total time spent engaging in these behaviors was calculated in each phase.

M43068, maprotiline and fluvoxamine maleate were dissolved in 0.5 w/v% hydroxypropylmethylcellulose (HPMC), and then they were given orally (10 ml/kg) 30 min before the formalin injection (that is, 1 h before the late phase). Propofol or baclofen hydrochloride was dissolved in sterile saline, and then each compound was injected intraperitoneally (2 ml/kg) or subcutaneously (2 ml/kg) 15 min before the formalin injection. In addition, prazosin hydrochloride and ketanserin tartrate were dissolved in pure water containing 10 vol.% dimethylsulfoxide (DMSO). Pindolol was dissolved in sterile saline containing 1.2 mM HCl. These monoamine receptor antagonists were pretreated intracerebroventricularly (5 μl) 5 min before treatment with the compound. Bicuculline methiodide and saclofen were dissolved in sterile saline. These GABA receptor antagonists were pretreated intraperitoneally 15 min before treatment with the compound.

2.5. Chronic constrictive injury model of neuropathic pain

The surgical operation was performed according to the method of Bennett and Xie (1988). Wistar Hannover rats were anesthetized with sodium pentobarbital (45 mg/kg, i.p.). The skin on the lateral surface of the left thigh was incised after being disinfected with diluted chlorhexidine gluconate solution. A blunt dissection was made directly through the biceps femoris, exposing the left sciatic nerve and its three terminal branches. The common sciatic nerve was freed from adhering tissue, and four ligatures (3–0 chromic gut; Matsuda Ika Kogyo Co., Ltd., Tokyo, Japan) were tied loosely around it at intervals of 1 mm. And then, the muscle and skin were sutured. As a sham operation, the right sciatic nerve was isolated in the same way, but it was not ligated. After the operation, to minimize discomfort and painful mechanical stimulation, the rats were housed in the plastic cages with floors covered with soft bedding.

2.6. Mechanical allodynia

The mechanical allodynia was evaluated according to the method of Seltzer et al. (1990). The rats, 14–15 days after the operation, were placed individually in the plastic cages with mesh bottoms. The withdrawal thresholds to touch were measured with a set of von Frey filaments (Stoelting Company, Illinois, USA) by bending forces ranged from 0.69 to 28.84 *g*. Each filament was applied vertically to the mid-plantar skin in ascending order in a period of 3 s. At the thresholds, the rats

responded with a quick paw flick. When no response was observed, the force of the thickest filament (28.84 *g*) was assigned as the withdrawal threshold. The withdrawal thresholds were measured by a blind observer who did not know the treatment. M43068 was given orally.

2.7. Neurometer measurement of current stimulus threshold

The current stimulus thresholds with the Neurometer were measured according to the method of Kiso et al. (2001). A small electrode (GT100-30; Neurotron Inc., Baltimore, USA) for stimulation was attached to the right plantar surface of the rats. A skin patch dispersion electrode (TE174D; Fukuda Denshi, Tokyo, Japan) was attached to the left side of the back, where the hair had been removed with an animal hair clipper. The area was secured by being wrapped with gauze. In Ballman cages (Natsume, Tokyo, Japan), transcutaneous nerve stimuli of three sine-wave pulses at 2000, 250 and 5 Hz were applied to the plantar surface of the rats, in the mode of animal response test with the Neurometer CPT/C (Neurotron Inc.). The minimum intensity (mA) at which each rat vocalized was defined as the threshold, and at that point the stimulus was immediately stopped. The data were expressed as the means of the values obtained from three consecutive measurements. M43068 and maprotiline were dissolved in the mixture (3:3:4) of dimethylformamide (DMF), polyethylene glycol 200 (PEG200) and pure water, and then they were injected intravenously (1 ml/kg) 1 h before the measurement.

2.8. Side-effect profiles

2.8.1. Blood pressure

Mean blood pressure was measured by the tail-cuff method (BP98-A; Softron, Tokyo, Japan). Wistar rats were assigned to each group, based on the values assessed before the test. M43068 and α_1-adrenoceptor agonist, M6434, were given orally 1 h before the measurement.

2.8.2. Spontaneous locomotor activity

Spontaneous locomotor activity was measured using the Supermex (Muromachi Kikai, Tokyo, Japan). Wistar rats were assigned to each group, based on the values assessed the day before. The rats were placed into the acryl cages where photobeam breaks were recorded for 30 min. M43068 was given orally 1 h before the measurement, and the $GABA_B$ agonist, baclofen, was injected subcutaneously 30 min before that.

2.8.3. Motor coordination

Motor coordination was measured using the accelerating rotarod apparatus (model 7750; Ugo Basile, Camerio, Italy). Wistar rats were acclimatized to acceleration by 3 training sessions. The rats were assigned to each group, based on the values assessed the day before. They were placed onto the rotating rod, which increased in speed from 4 to 40 rpm over 5 min. The time required for the rat to fall from the rod was recorded, with a maximum cut-off of 300 s. M43068 was given orally 1 h before the measurement, and the $GABA_B$ agonist, baclofen, was injected subcutaneously 30 min before that.

2.9. Statistical analysis

Results were expressed as means ± S.E.M. Statistical analysis among multiple groups was performed with Dunnett's test. In the current stimulus threshold test, the thresholds before and after treatment with the compound were compared by Student's paired *t*-test. In the formalin test using the antagonist, comparisons between two groups were made by Student's *t*-test. For all the analyses, *P* values less than 0.05 were considered significant.

3. Results

3.1. Effects of M43068 in the rat formalin test

Fig. 2A shows the time-course of the nociceptive behaviors in the rat formalin test. M43068 (10–100 mg/kg, p.o.) did not show obvious effect on the nociceptive behaviors in the early phase. In contrast, it suppressed them in the late phase in a dose-dependent manner. Statistically significant effects in the late phase were observed at 30 and 100 mg/kg (Fig. 2B).

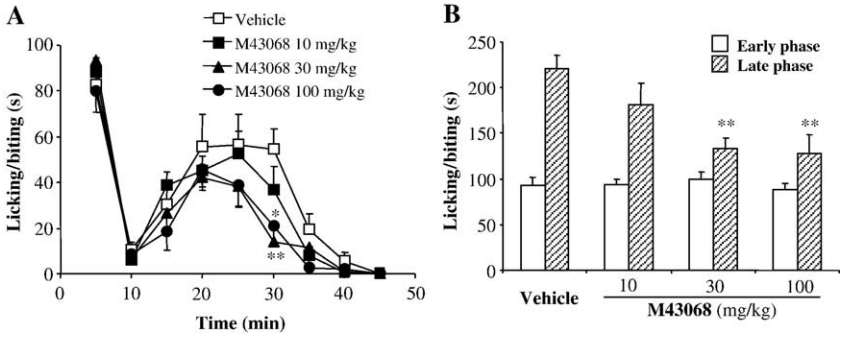

Fig. 2. Effects of M43068 on licking/biting behaviors in the rat formalin test. (A) Time-course of licking/biting behaviors. (B) Effects of M43068 on licking/biting behaviors in the early and late phases. Values are expressed as means ± S.E.M. of 5 animals. *: $P<0.05$, **: $P<0.01$ significantly different from the vehicle-treated group (Dunnett's test).

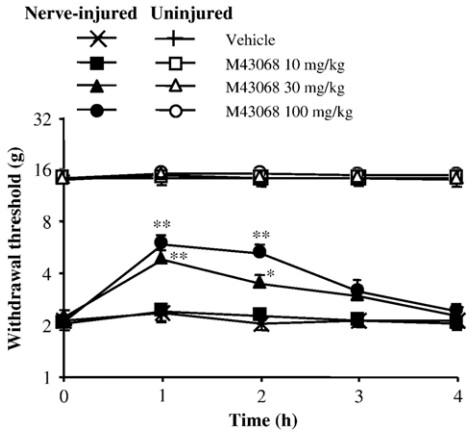

Fig. 3. Effects of M43068 on mechanical allodynia in the chronic constrictive injury model. After oral treatment with M43068, the withdrawal thresholds were measured at 1, 2, 3 and up to 4 h. Values are expressed as means ± S.E.M. of 10 animals. *: $P<0.05$, **: $P<0.01$ significantly different from the vehicle-treated group (Dunnett's test).

3.2. Effects of M43068 on mechanical allodynia

Fig. 3 shows the effects of M43068 on the withdrawal thresholds of the nerve-injured and uninjured paws in the chronic constrictive injury model. The withdrawal thresholds of the nerve-injured paw were obviously lower than those of the uninjured paw in the vehicle-treated group, indicating mechanical allodynia. M43068 (10–100 mg/kg, p.o.) suppressed this mechanical allodynia in the nerve-injured paw in a dose-dependent manner. Statistically significant effects were observed at 30 and 100 mg/kg. In the uninjured paw, M43068 showed no effect at any doses.

3.3. Effects of M43068 on the current stimulus threshold with the Neurometer

Fig. 4 shows the change of the current stimulus thresholds caused by the treatment with the compound using the Neurometer in rats. I.v. injection of the vehicle had little effect on the thresholds at 2000, 250 and 5 Hz (Fig. 4A). At M43068 30 mg/kg, i.v., the changes in thresholds at 2000, 250 and 5 Hz were 147 ± 13%, 123 ± 17% and 148 ± 32%, respectively. The threshold at 2000 Hz significantly increased, and that at 5 Hz tended to increase but it was not significant (Fig. 4B). On the other hand, at maprotiline 30 mg/kg, i.v., the changes in thresholds at 2000, 250 and 5 Hz were 133 ± 12%, 109 ± 13% and 85 ± 10%, respectively. Only the threshold at 2000 Hz significantly increased (Fig. 4C).

3.4. Involvements of monoamine receptors in M43068-induced antinociception

In the rat formalin test, the noradrenaline reuptake inhibitor, maprotiline (50 mg/kg, p.o.), significantly suppressed nociceptive behaviors in the late phase ($P<0.01$). The α_1-adrenoceptor antagonist, prazosin hydrochloride (1 μg, i.c.v.), significantly abolished this maprotiline-induced antinociception ($P<0.01$; data not shown). On the other hand, the 5-HT reuptake inhibitor, fluvoxamine maleate (30 mg/kg, p.o.), significantly suppressed nociceptive behaviors not only in the early phase ($P<0.01$) but also in the late phase ($P<0.01$). The 5-$HT_{1A/1B}$ receptor antagonist, pindolol (1.5 μg, i.c.v.), significantly abolished these fluvoxamine-induced antinociception in both phases ($P<0.05$ and $P<0.01$, respectively; data not shown). The 5-HT_2 receptor antagonist, ketanserin tartrate (6 μg, i.c.v.), significantly abolished the fluvoxamine-induced antinociception in the late phase ($P<0.01$; data not shown). In addition, these monoamine receptor antagonists alone did not modify nociceptive behaviors compared with those of the vehicle-treated group.

On the basis of the above results, we assessed the influence of these monoamine receptor antagonists on the antinociception of M43068 (30 mg/kg, p.o.) in the late phase. The antinociceptive effect of M43068 was abolished by i.c.v. pretreatment with prazosin (Fig. 5A), but not with pindolol (Fig. 5B) or ketanserin (Fig. 5C).

3.5. Involvements of GABA receptors in M43068-induced antinociception

In the rat formalin test, the anesthetic, propofol (30 mg/kg, i.p.), significantly suppressed nociceptive behaviors in the late phase ($P<0.01$). The $GABA_A$ receptor antagonist, bicuculline

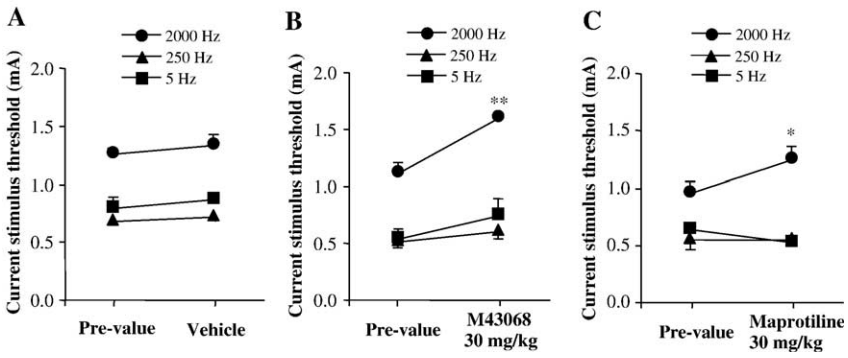

Fig. 4. Neurometer measurement of current stimulus threshold. (A) Effects of vehicle on the current stimulus thresholds with the Neurometer at 2000, 250 and 5 Hz. (B and C) Effects of M43068 (B) and maprotiline (C) on the current stimulus thresholds with the Neurometer at 2000, 250 and 5 Hz. Values are expressed as means ± S.E.M. of 5 animals. *: $P<0.05$, **: $P<0.01$ significantly different from the pre-value (Student's paired t-test).

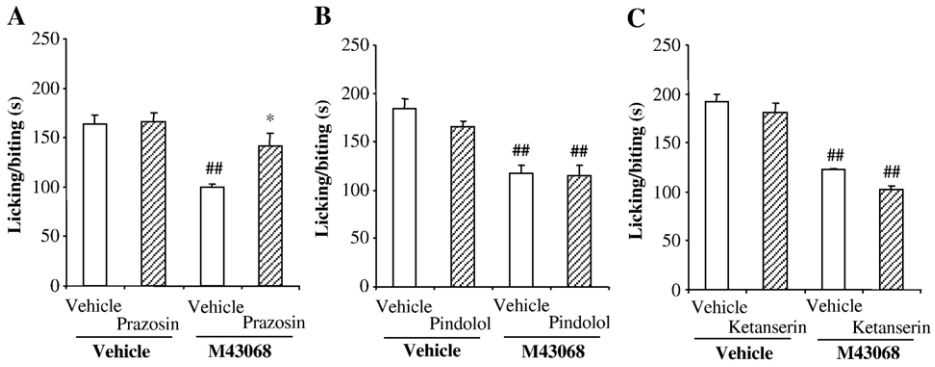

Fig. 5. Influence of i.c.v. pretreatment with monoamine receptor antagonists on the antinociception of M43068. (A) Influence of i.c.v. pretreatment with prazosin on the antinociception of M43068 in the late phase of formalin-induced nociceptive behaviors. (B and C) Influence of i.c.v. pretreatment with pindolol (B) and ketanserin (C) on the antinociception of M43068 in the late phase. Values are expressed as means ± S.E.M. of 5 animals. ##: $P<0.01$ significantly different from the vehicle/vehicle group (Student's *t*-test). *: $P<0.05$ significantly different from the vehicle/compound group (Student's *t*-test).

methiodide (0.5 mg/kg, i.p.), significantly abolished this propofol-induced antinociception ($P<0.01$; data not shown). On the other hand, the $GABA_B$ receptor agonist, baclofen hydrochloride (1.5 mg/kg, s.c.), significantly suppressed nociceptive behaviors in the late phase ($P<0.01$). The $GABA_B$ receptor antagonist, saclofen (11.2 mg/kg, i.p.), significantly abolished this baclofen-induced antinociception ($P<0.01$; data not shown). In addition, these GABA receptor antagonists alone did not modify nociceptive behaviors compared with those of the vehicle-treated group.

On the basis of the above results, we assessed the influence of these GABA receptor antagonists on the antinociception of M43068 (30 mg/kg, p.o.) or maprotiline (50 mg/kg, p.o.) in the late phase. The antinociceptive effect of M43068 was abolished by i.p. pretreatment with saclofen (Fig. 6B), but not with bicuculline (Fig. 6A). Interestingly, the antinociceptive effect of maprotiline was also abolished by i.p. pretreatment with saclofen (Fig. 6C).

3.6. Influence of M43068 on blood pressure and motor function

Fig. 7A shows the influence of M43068 and the α_1-adrenoceptor agonist, M6434, on mean blood pressure. M43068 (30–300 mg/kg, p.o.) showed no influence on mean blood pressure, but M6434 (2 mg/kg, p.o.) significantly increased it. Thus, M43068 had an action profile different from that of M6434.

Fig. 7B and 7C show the influence of M43068 and the $GABA_B$ receptor agonist, baclofen, on spontaneous locomotor activity and time on rod. M43068 (30–300 mg/kg, p.o.) showed no influence on spontaneous locomotor activity and time on rod, but baclofen (5 mg/kg, s.c.) significantly reduced them. Thus, M43068 had action profiles different from those of baclofen.

4. Discussion

In this study, a novel compound, M43068, showed analgesic actions in the formalin test and the neuropathic pain model. Interestingly, in the latter model, oral M43068 suppressed mechanical allodynia in the nerve-injured paw without affecting the withdrawal thresholds in the uninjured paw. Taken together with the results that M43068 had no influence on motor function, M43068 selectively suppressed mechanical allodynia induced by neuropathy.

Non-noxious stimulation is transmitted through low-threshold, large-diameter myelinated fibers such as Aβ-fibers.

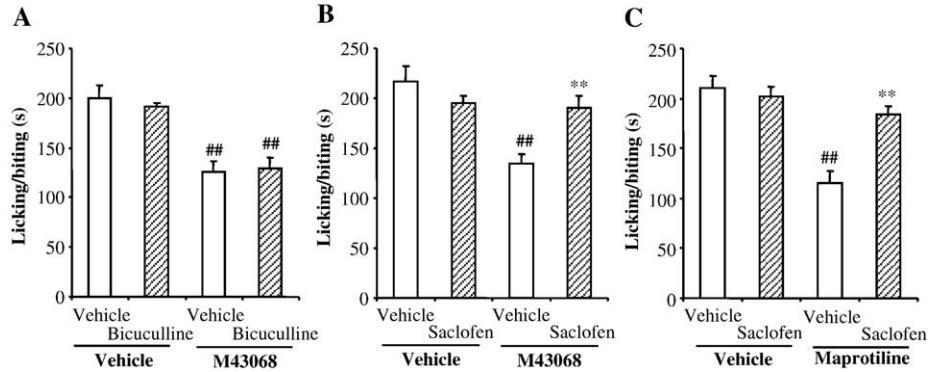

Fig. 6. Influence of i.p. pretreatment with GABA receptor antagonists on the antinociception of M43068. (A and B) Influence of i.p. pretreatment with bicuculline (A) and saclofen (B) on the antinociception of M43068 in the late phase of formalin-induced nociceptive behaviors. (C) Influence of saclofen on the antinociception of maprotiline in the late phase. Values are expressed as means ± S.E.M. of 5 animals. ##: $P<0.01$ significantly different from the vehicle/vehicle group (Student's *t*-test). **: $P<0.01$ significantly different from the vehicle/compound group (Student's *t*-test).

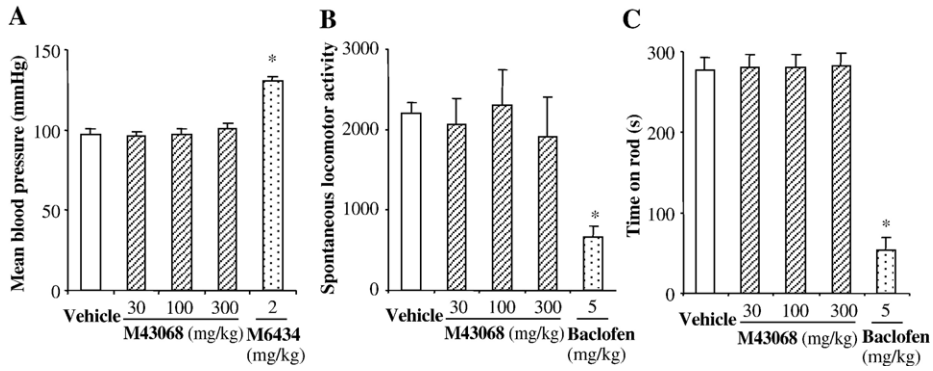

Fig. 7. The side-effect profiles of M43068 in normal rats. (A) Influence of M43068 and M6434 on blood pressure in conscious rats. (B and C) Influence of M43068 and baclofen on spontaneous locomotor activity (B) and time on rod (C) in normal rats. Values are expressed as means ± S.E.M. of 5 animals. *: $P<0.05$ significantly different from the vehicle-treated group (Dunnett's test).

In the normal state, Aβ-fibers terminate in the deeper laminae (III–V) of the dorsal horn. However, peripheral nerve injury triggers the sprouting of Aβ-fibers from their original termination into the substantia gelatinosa (lamina II) (Okamoto et al., 2001; Kohno et al., 2003), which is a region critical for modulating nociceptive information (Kumazawa and Perl, 1978; Yoshimura and Jessell, 1989). The information about non-noxious stimuli appears to be misinterpreted by the nervous system as the nociception. Thus, the sprouting of Aβ-fibers is considered to be important for the development of mechanical allodynia. On the other hand, the Neurometer device has been reported to be useful for the evaluation of fiber-selective blockade by drugs (Kiso et al., 2001; Oda et al., 2005). Three sine-wave pulses (2000, 250 and 5 Hz) produced by the Neurometer provide selective stimulation for three subsets of nerve fibers (Aβ-, Aδ- and C-fibers, respectively) with different diameters. Recently, Koga et al. (2005) have provided electrophysiological evidence that the sine-wave stimulation could differentiate the changes in thresholds of Aβ-, Aδ- and C-afferent fibers by combining data obtained from 2000, 250 and 5 Hz stimuli, using intracellular and in vivo patch-clamp recordings. Therefore, we examined the effects of M43068 on the current stimulus thresholds with the Neurometer in rats. I.v. M43068 significantly suppressed the Aβ-fiber-mediated response and tended to suppress the C-fiber response. These results suggest that the Aβ-fiber-inhibiting action of M43068 contributes to its anti-allodynic effect.

The formalin test is a valid model for clinical pain because of the connection to tissue injury. Especially, nociceptive behaviors in the late phase are related to a facilitated pain processing (hyperalgesia) associated with inflammation (Dubuisson and Dennis, 1977; Tjølsen et al., 1992; Abbott et al., 1995). In this study, M43068 showed antinociceptive effect in this late phase. On the other hand, M43068 mainly suppressed the function of Aβ-fibers, which are considered to be a little concerned with the transmission of nociceptive signals (Koga et al., 2005). The reason for this discrepancy may be that the formalin test is more sensitive than most classic tests such as the hot plate and the tail-flick test (Le Bars et al., 2001) or M43068 has the inhibitory effect on C-fibers which play an important role in transmitting nociception. In addition, we have confirmed that oral M43068 at 100 mg/kg showed no effect in the hot plate test (55 °C; data not shown).

Monoamines such as noradrenaline and 5-HT are implicated in enhancing endogenous analgesic mechanism via the descending inhibitory pain pathway in the CNS. Therefore, we investigated the involvements of monoamine receptor subtypes in the brain according to the previous studies (Otsuka et al., 2001; Yokogawa et al., 2002). The antinociceptive effect of M43068 was abolished by i.c.v. pretreatment with the α_1-adrenoceptor antagonist, but not with the 5-$HT_{1A/1B}$ or 5-HT_2 receptor antagonist. These data suggest that α_1-adrenoceptors, which are known to be rich in the CNS (Zhong and Minneman, 1999), play a dominant role in the M43068-induced antinociception. Yokogawa et al. (2002) have demonstrated that the activation of central α_1-adrenoceptor contributes to the antinociception. These reports support our results regarding the contribution of α_1-adrenoceptors in the CNS to the antinociception.

GABA is implicated in enhancing endogenous analgesic mechanism in the downstream of the descending noradrenergic pathway (Baba et al., 2000), and it is also detected in a variety of peripheral organs (Tanaka, 1985). Taken together with the observation that M43068 showed no central action, we have speculated that the GABA receptors in the spinal cord and/or peripheral nerves might be involved in its antinociception. Therefore, we investigated the involvements of GABA receptor subtypes in them according to previous studies (Ballal et al., 1996; Sabetkasai et al., 1999; Asahi and Yonehara, 2001). The antinociceptive effect of M43068 was abolished by systemic pretreatment with the $GABA_B$ receptor antagonist, but not with the $GABA_A$ receptor antagonist. Interestingly, the antinociceptive effect of the noradrenaline reuptake inhibitor, maprotiline, was also abolished by the $GABA_B$ receptor antagonist. These data suggest that $GABA_B$ receptors play a dominant role in both M43068- and maprotiline-induced antinociception. In respects of the antagonisms by both α_1-adrenoceptor and $GABA_B$ receptor antagonists, the analgesic properties of M43068 were similar to those of maprotiline. Furthermore, maprotiline as well as M43068 mainly increased the threshold at 2000 Hz with the Neurometer. These results led us to the hypothesis that the target molecule of M43068 is related

to α_1-adrenoceptor or noradrenaline. However, oral M43068 (30–300 mg/kg) showed no influence on blood pressure in conscious rats, unlike the α_1-adrenoceptor agonist. In addition, M43068 had no binding activity for several transporters (noradrenaline, 5-HT, dopamine, GABA, etc.) and receptors (adrenergic, 5-HTergic, dopaminergic, GABAergic, glutamate, opiate, etc.) even at 300 μM in the standardized assay by MDS Pharma Services (data not shown). Further studies are required to elucidate the mechanism of action of M43068.

Rosenstein et al. (1989) and Ferraro et al. (1993) have shown that noradrenaline increases endogenous GABA outflow from cortex slices and synaptosomes by activating α_1-adrenoceptors rather than α_2-adrenoceptors on the GABAergic neurons. Baba et al. (2000) have also investigated how the descending noradrenaline-containing fiber from the brain stem inhibits nociceptive transmission at the spinal level. It follows that noradrenaline enhances GABAergic and glycinergic inhibitory postsynaptic current through the activation of α_1-adrenoceptors in the substantia gelatinosa. Anatomic studies show high concentration of noradrenaline-containing terminals in the substantia gelatinosa of the spinal cord (Westlund et al., 1982, 1983). Thus, the activation of these noradrenergic neurons enhances the inhibitory neurons such as GABA- and glycine-containing neurons, which are endowed with α_1-adrenoceptors. Taken together, it is suggested that M43068 inhibits nociceptive transmission by enhancing these inhibitory neurons via α_1-adrenoceptors.

GABA has been reported to suppress the transmission of both sensory information and nociception by activating $GABA_B$ receptors on large and fine afferent fibers (Yang et al., 2001). These findings are supported by a previous study demonstrating that spinal baclofen reduced Aβ-, Aδ- and C-fiber evoked responses of spinal dorsal horn neurons (Sokal and Chapman, 2003). On the other hand, glycine has been shown to be associated with large myelinated fiber-mediated response (Sorkin and Puig, 1996). The blockage of spinal glycine receptors with i.t. strychnine produces a tactile-evoked allodynia, and i.t. glycine reverses it (Sherman and Loomis, 1995). These findings are supported a previous study that glycine-like immunoreactivity is observed presynaptically on the terminals from Aβ-afferent fibers in synapses (Todd et al., 1991). Thus, GABA and glycine would be involved in regulating the function of Aβ-fibers. Taken together, we would speculate that M43068 showed higher effects on Aβ-fibers than on Aδ- or C-fibers by enhancing the inhibitory neurons such as GABAergic and glycinergic neurons.

In summary, M43068 showed antinociceptive and anti-allodynic effects in rat models of sustained and neuropathic pain. Although the antinociceptive effect of M43068 was abolished by the α_1-adrenoceptor and $GABA_B$ receptor antagonists, it had had no influence on blood pressure and motor function, unlike the α_1-adrenoceptor and $GABA_B$ receptor agonists. It is suggested that M43068 acts by enhancing the inhibitory neurons via the descending noradrenergic system. Thus, M43068 might belong to a novel class of analgesics, and it is expected to be useful for the treatment of neuropathic pain.

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