Hiroshima J. Med. Sci.
Vol. 62, No. 4, 83~89, December, 2013
HIJM 62–14

Long-term Administration of Fluvoxamine Attenuates Neuropathic Pain and Involvement of Spinal Serotonin Receptors in Diabetic Model Rats

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ABSTRACT

Diabetic neuropathic pain management is difficult even with non-steroidal anti-inflammatory drugs and narcotic analgesics such as morphine. Fluvoxamine, a class of selective serotonin reuptake inhibitors (SSRIs), is widely used to treat depression. Its analgesic effects are also documented for diabetic neuropathic pain, but they are limited because it is administered as a single-dose. In this study, we examined the time course of the antiallodynic effect of fluvoxamine in a rat model of diabetic neuropathic pain, which was induced by a single intraperitoneal administration of streptozotocin (75 mg/kg). In addition, the involvement of spinal serotonin (5-HT) receptors in long-term fluvoxamine treatment was studied by intrathecal administration of 5-HT receptor antagonists. In this study the development of mechanical hyperalgesia was assessed by measuring the hind paw withdrawal threshold using von Frey filaments. The results demonstrated that daily oral administration of fluvoxamine (10, 30, and 100 mg/kg) to diabetic rats from 3 to 8 weeks after streptozotocin administration resulted in a dose-dependent antiallodynic effect. The antiallodynic effect was sustained from 2 to 5 weeks after fluvoxamine administration. The antiallodynic effect of fluvoxamine in the diabetic rats was attenuated by WAY-100635 (a 5-HT_{1A} receptor antagonist) intrathecally administered 1 week after the onset of daily administration of fluvoxamine, whereas no significant attenuation was seen when the antagonist was administered 3 and 5 weeks after fluvoxamine administration. The antiallodynic effect of fluvoxamine was also attenuated by ketanserin (a 5-$HT_{2A/2C}$ receptor antagonist) and ondansetron (a 5-HT_3 receptor antagonist) intrathecally administered 1 and 3 weeks after the onset of daily fluvoxamine administration. However, no significant attenuation was observed when the antagonist was administered 5 weeks after fluvoxamine administration. This study demonstrated that daily oral administration of fluvoxamine can afford a sustained antiallodynic effect against streptozotocin-induced neuropathic pain. Furthermore, there appears to be a time-dependent relevance of different types of 5-HT receptors (5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3) to streptozotocin-induced diabetic neuropathic pain when treated with daily fluvoxamine.

Key words: *Fluvoxamine, Diabetes, Serotonin receptor antagonist, Hyperalgesia*

A large proportion of the population has diabetes, and 60% or more of the cases are associated with neuropathy and approximately 20% with neuropathic pain[3,21)]. In clinical practice, the management of diabetic neuropathic pain is difficult even with non-steroidal anti-inflammatory drugs (NSAIDs) and narcotic analgesics such as morphine[4)]. Furthermore, it has been reported that approximately 30% of patients with diabetes also suffer from depression[1,5)].

Selective serotonin reuptake inhibitors (SSRIs), a class of antidepressants, are widely used to treat depression because they have few adverse effects. Their analgesic effects in clinical studies[12,24)] and animal experiments[7,23)] are also documented; for example, the effect of fluvoxamine on acute pain[9)] as well as its influence on the pain relief effect of opioids[20)] has been studied. There are a few reports on the analgesic effect of fluvoxamine in neuropathic pain[11,14)], but they are limited to single-dose administration studies.

SSRIs strongly inhibit reuptake of the neurotransmitter serotonin (5-HT) at nerve endings. As a result, the amount of 5-HT in the synaptic clefts increases. Therefore, the accepted mechanism of their analgesic action is activation of the descending pain inhibitory pathway in the spinal cord. The primary site of action is believed to be

the spinal cord[6,27]. Three 5-HT receptor types in the spinal cord are involved in the antinociceptive effects of SSRIs[15,16]. There are a few reports on the involvement of 5-HT receptors in experimental models of neuropathic pain[10,20]. However, all these reports involve a single-dose administration of SSRI, and there has been no study on the involvement of 5-HT receptors in long-term treatment.

In this study, we investigated the time course of the antiallodynic effect of long-term fluvoxamine treatment in a rat model of diabetic neuropathic pain. Further, we studied the involvement of spinal 5-HT receptors in long-term fluvoxamine treatment by means of the intrathecal administration of 5-HT receptor antagonists.

MATERIALS AND METHODS

Animals

The experimental protocols were approved by the Institutional Animal Care Committee of Hiroshima University and were consistent with the guidelines of the Ethical Committee of the International Association for the Study of Pain[28]. The animals were 203 male Sprague-Dawley (SD) rats (body weight 200-250 g). The conditions in the animal room were as follows: temperature, 23°C ± 2°C; 12-hour light-dark cycle (lights turned on at 8 AM). The animals were allowed to consume food and water *ad libitum*.

Induction of diabetes

Streptozotocin (75 mg/kg; Sigma, St. Louis, MO) was dissolved in 1 ml of saline and intraperitoneally administered to 5-week-old rats. Intraperitoneally administered streptozotocin is known to ablate pancreatic ß cells and induce insulin-deficient diabetes[22]. Blood was sampled from the tail vein 1 week after streptozotocin treatment, and blood glucose levels were measured using a blood glucose meter (FreeStyle™, Nipro, Osaka, Japan). An animal with a blood glucose level of 350 mg/dl or above was defined as diabetic. Blood glucose was measured every week.

Behavioral assessment of streptozotocin-induced mechanical hyperalgesia

The development of mechanical hyperalgesia in the streptozotocin-treated rats was assessed by measuring the hind paw withdrawal threshold (mechanical thresholds: allodynia) using von Frey filaments (North Coast Medical, Inc., Morgan Hill, CA, USA). To familiarize the experimental animals with the environment, they were put in a plastic box placed on a metal mesh floor for 15 min before the start of the experiment. Then, a filament was pressed perpendicularly against the bottom of the hind paw until it bent, and the threshold value to cause brisk withdrawal or paw flinching was measured. The stimulation was performed with increasing filament thickness, and the thinnest filament that induced an escape response was noted. From a total of 6 measurements, the maximum and minimum values were excluded, and the mean of the remaining four values was used for calculations. From 1 week after streptozotocin treatment, the von Frey test was repeatedly performed every week, and animals with a pain threshold of 50% or less (with allodynia) relative to normal rats were defined as animal models of diabetic neuropathic pain (diabetic rats)[13,17]. Blood glucose levels and body weight were also measured every week.

Oral administration of fluvoxamine

Three weeks after streptozotocin treatment, the diabetic rats and age-matched normal rats were used for evaluation of the antiallodynic effect of fluvoxamine (Abbott, Tokyo, Japan), administered once a day orally. To avoid diurnal variation, all experiments were conducted at the same time range. Fluvoxamine (10, 30, and 100 mg/kg) or 2 ml of distilled water (non-treated control) was administered via a stomach tube. Fluvoxamine was resuspended in 2 ml of distilled water. Changes in allodynia were evaluated by the von Frey test prior to fluvoxamine administration and at 30, 60, 90, 120, 150, 180, 210, 240, and 360 min thereafter. In a parallel experiment, changes in allodynia over time were evaluated by the von Frey test prior to fluvoxamine administration and at 1, 2, 3, 4, and 5 weeks thereafter (*i.e.*, 3, 4, 5, 6, 7, and 8 weeks after streptozotocin treatment). In these daily experiments, to avoid the influence of fluvoxamine administered on the same day, the antiallodynic effect was measured before the administration of fluvoxamine.

Implantation of an intrathecal catheter

Diabetic rats (n = 115), who had received a daily oral dose of fluvoxamine (100 mg/kg) each week from 2 weeks after streptozotocin treatment, were anesthetized using intraperitoneal administration of 50 mg/kg pentobarbital, and an intrathecal catheter was implanted surgically. A polyethylene catheter (Intramedic PE-10, Becton Dickinson and Co., Franklin Lakes, NJ) was inserted such that the tip was positioned at the lumbar enlargement[26]. The catheter was withdrawn from the body from the back of the neck via a subcutaneous tunnel. The catheter tip was attached with a stainless-steel wire, and the musculature and skin were sutured with a 3-0 silk suture thread[13,17]. A recovery period of 1 week was allowed after the catheter insertion, and then the experiments were resumed. Animals exhibiting nerve damage or worsening of their general condition were excluded from subsequent experiments.

Intrathecal administration of 5-HT receptor antagonists

A total of 60 μg of WAY-100635 (5-HT_{1A} receptor antagonist; Abcam, Tokyo, Japan), 30 μg of ketanserin (5-$HT_{2A/2C}$ receptor antagonist; Wako, Tokyo, Japan), 30 μg of ondansetron (5-HT_3 receptor antagonist; Abcam, Tokyo, Japan), or 100% dimethyl sulfoxide (DMSO; Sigma, St. Louis, MO) was intrathecally administered to the diabetic rats that had received oral fluvoxamine (100 mg/kg daily). WAY-100635, ketanserin, and ondansetron were dissolved in 10 μl of 100% DMSO, and after the agents were administered, 10 μl of saline was added to flush the catheter. The 5-HT receptor antagonists were intrathecally administered at 1, 3, and 5 weeks after the onset of fluvoxamine administration (*i.e.*, 4, 6, and 8 weeks after streptozotocin treatment), and changes in allodynia were evaluated using the von Frey test.

Data analysis

All statistical tests were performed by means of two-way ANOVA, and the Bonferroni method was used for post hoc analysis. Values of $p < 0.05$ were considered statistically significant. All values were expressed as mean ± S.E.M.

RESULTS

Among the subjects, 175 of 188 (93%) rats exhibited high blood glucose levels 1 week after intraperitoneal streptozotocin treatment. Blood glucose levels of the diabetic rats and normal rats at the same week were 488 ± 9 mg/dl and 107 ± 7 mg/dl, respectively ($p < 0.001$). Blood glucose levels of the diabetic rats remained at or above 350 mg/dl up to 8 weeks after streptozotocin treatment and were significantly higher than those of the normal rats at the same week. Furthermore, increases in water consumption and urine excretion were seen in the diabetic rats. The average body weight of the diabetic rats at 6 weeks after streptozotocin treatment was 319 ± 18 g and that of the normal rats at the same week was 411 ± 14 g ($p < 0.001$). The weight gain of the diabetic rats beyond 6 weeks after streptozotocin treatment significantly diminished compared with the normal rats at the same week. A significant drop in mechanical thresholds in the von Frey test was observed in the diabetic rats 1 week after streptozotocin treatment, compared with the normal rats at the same week (Fig. 1).

A single oral administration of fluvoxamine to the diabetic rats 3 weeks after streptozotocin treatment resulted in a concentration-dependent antiallodynic effect. In the group to which 30 mg/kg of fluvoxamine was administered, a significant antiallodynic effect was observed 90 min after administration, when compared with the non-treated control group, and it was sustained for up to 150 min after administration. In the 100-mg/kg group, a significant antiallodynic effect was observed 30 min after fluvoxamine administration when compared with the non-treated control group and was sustained for up to 240 min after administration. The greatest antiallodynic effect in both 30-mg/kg and 100-mg/kg groups was achieved 120 min after administration. In the 30-mg/kg group, the antiallodynic effect gradually declined and returned to the baseline level 240 min after fluvoxamine administration. In the 100-mg/kg group, the antiallodynic effect gradually declined and returned to the baseline level 360 min after fluvoxamine administration (Fig. 2). Although we attempted to determine whether fluvoxamine had an analgesic effect on age-matched normal rats, it was shown that a dose of 100-mg/kg did not increase the paw-withdrawal threshold in response to applied mechanical stimuli (data not shown).

When fluvoxamine was orally administered daily to the diabetic rats from 3 weeks after streptozotocin treatment, a concentration-dependent antiallodynic effect was observed. In the group in which 30 mg/kg of fluvoxamine was administered, a significant antiallodynic effect was observed when compared with the non-treated control group 2 weeks after the initial fluvoxamine administration (*i.e.*, 5 weeks after streptozotocin treatment) and was sustained up to 5 weeks after initial administration (*i.e.*, 8 weeks after streptozotocin treatment). In the 100-mg/kg group, a significant antiallodynic effect was observed, when compared with the non-treated control group, as early as 1 week after the initial fluvoxamine administration (*i.e.*, 4 weeks after streptozotocin treatment) and

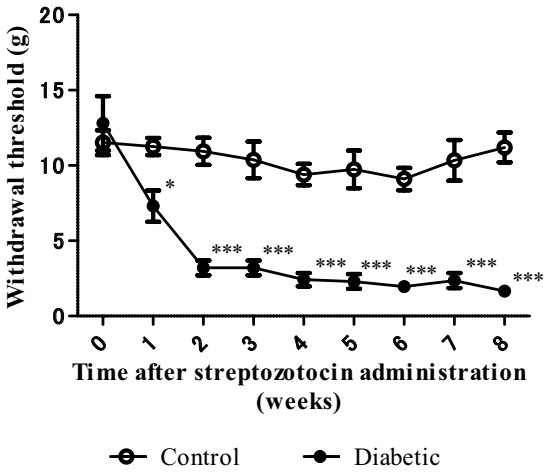

Fig. 1. Changes in withdrawal thresholds of the hind paw in diabetic and control animals.

The time course of mechanical hyperalgesia was assessed after intraperitoneal administration of streptozotocin or saline. Data are presented as mean ± S.E.M. Control: n = 5; diabetic rats: n = 5; *$p < 0.05$, ***$p < 0.001$.

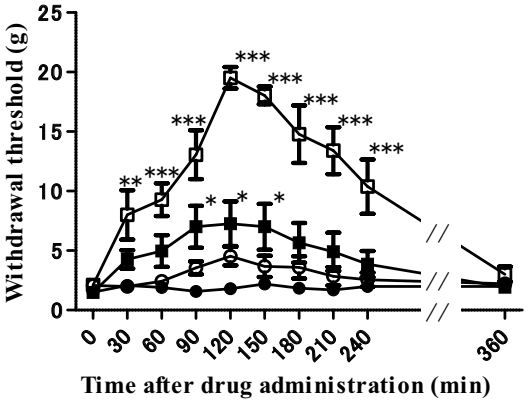

Fig. 2. The time course of the effects of a single oral administration of fluvoxamine on withdrawal thresholds of the hind paw in diabetic animals (n = 5).

The antinociceptive effects were determined 0, 30, 60, 90, 120, 150, 180, 210, 240, and 360 min after administration of fluvoxamine. Data are presented as mean ± S.E.M. *p < 0.05, **p < 0.01, ***p < 0.001, compared with the respective baseline of the fluvoxamine 0-mg/kg group at the corresponding time points.

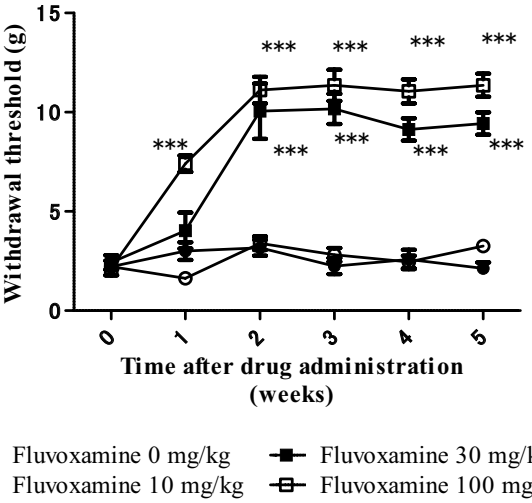

Fig. 3. The time course of the effects of daily oral administration of fluvoxamine on withdrawal thresholds of the hind paw in diabetic animals (n = 6 each, fluvoxamine).

The antinociceptive effects were determined 0, 1, 2, 3, 4, and 5 weeks after administration of fluvoxamine. Data are presented as mean ± S.E.M. ***p < 0.001, compared with the respective baseline of the fluvoxamine 0-mg/kg group at the corresponding time points.

was sustained up to 5 weeks after initial administration (*i.e.*, 8 weeks after streptozotocin treatment) (Fig. 3). Although we attempted to determine whether fluvoxamine had an analgesic effect on age-matched normal rats, it was shown that a dose of 100-mg/kg did not increase the paw-withdrawal threshold in response to applied mechanical stimuli (data not shown).

When the 5-HT_{1A} receptor antagonist WAY-100635 was intrathecally administered to the diabetic rats 1 week after daily oral administration of 100 mg/kg fluvoxamine, the antiallodynic effect was significantly suppressed, compared with the control group, which had been administered DMSO. When the antagonist was administered 3 or 5 weeks after fluvoxamine administration, the antiallodynic effect was slightly suppressed, but the difference was not statistically significant (Fig. 4A). When the 5-$HT_{2A/2C}$ receptor antagonist ketanserin or the 5-HT_3 receptor antagonist ondansetron was intrathecally administered 1 week or 3 weeks after fluvoxamine administration, the antiallodynic effect was significantly suppressed, compared with the control group, which had been administered DMSO. Administration of the antagonists 5 weeks after fluvoxamine administration slightly suppressed the antiallodynic effect, but the effect was not statistically significant (Fig. 4B and C).

DISCUSSION

In this study we demonstrated a dose-dependent antiallodynic effect of single oral administration of fluvoxamine to diabetic rats with neuropathic pain. In a previous report on intrathecal administration of fluvoxamine to diabetic rats with neuropathic pain, an antiallodynic effect was observed 60 min after administration[11]. In the present study, we observed the antiallodynic effect only at 90-120 min after administration. This is attributable to the different administration routes: oral administration resulted in a slow but sustained effect. In this study, single oral administration of fluvoxamine resulted in a transient antiallodynic effect, whereas repeated (daily) administration produced a stable antiallodynic effect.

The site of action of 5-HT relevant to the analgesic effect of SSRI treatment is considered to be the spinal cord[6,27]. At least 3 types of 5-HT receptors (5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3 classes) in the spinal cord are involved in the antinociceptive effect of SSRIs[15,16]. According to pharmacological studies on selective 5-HT agonists, the different types of 5-HT receptors in the spinal cord play different roles. Previous studies on 5-HT_1 receptors have shown that the antinociceptive effect of 5-HT intrathecally administered after painful thermal stimuli is diminished by 5-HT_{1A} and 5-HT_{1B} receptor antagonists[25]. Another study has shown that the 5-HT_{1A} receptor antagonist WAY-100635 attenuates the antinociceptive effect of 5-HT_{1A} receptor agonists[8], suggesting that 5-HT_1 receptors are involved in antinociception against acute pain. On the other hand, when the SSRI fluoxetine and the 5-$HT_{1A/1B}$ receptor antagonist pindolol were administered to mice with diabetic neuropathic pain 5 weeks after streptozotocin treatment, the antinoci-

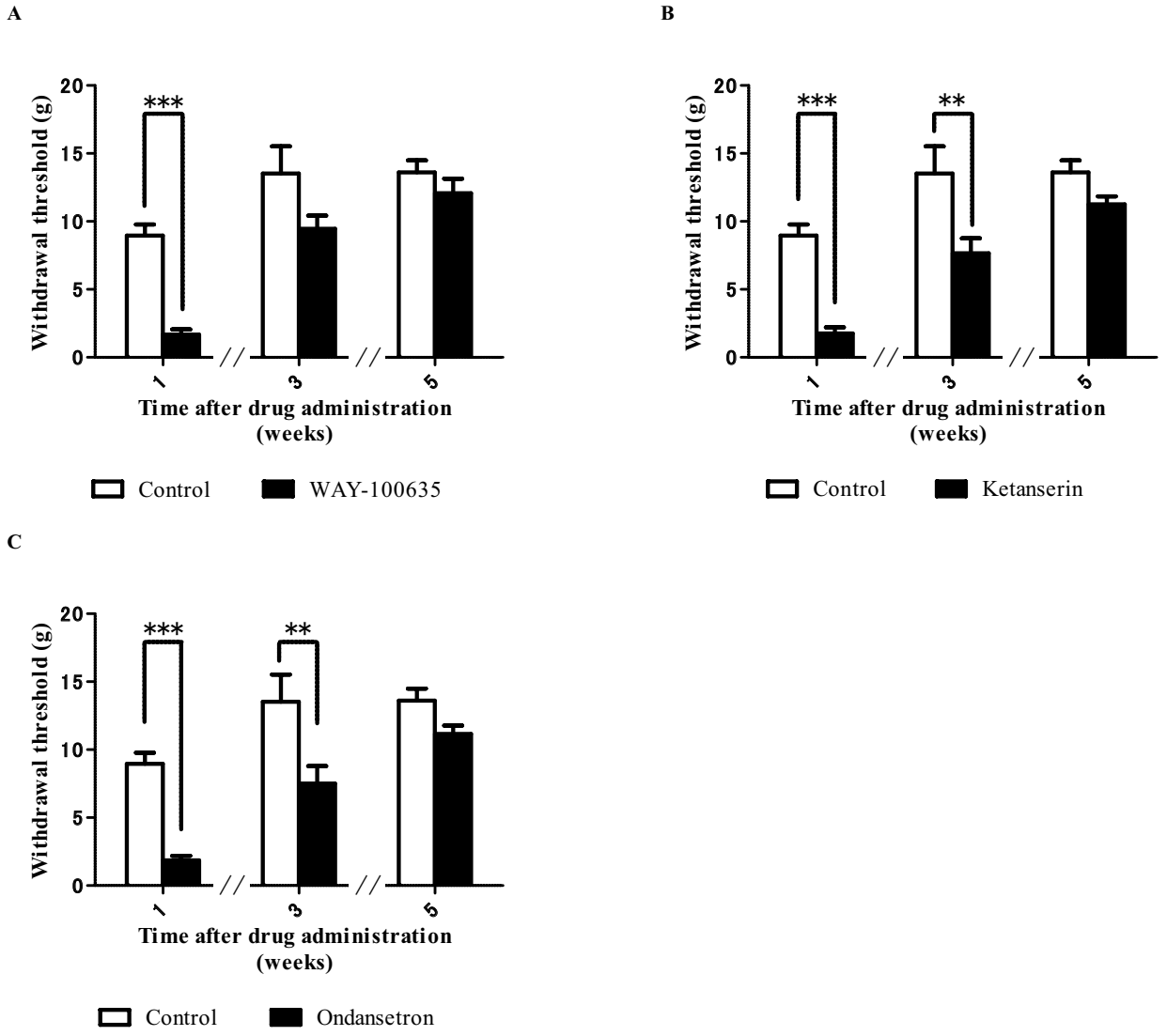

Fig. 4. The effects of intrathecal administration of 5-HT receptor antagonists on the antihyperalegic effect of 100 mg/kg of fluvoxamine.

The threshold was determined 1, 3, and 5 weeks after fluvoxamine administration. Animals received intrathecal administration of 60 µg WAY-100635 (A; 5-HT_{1A} receptor antagonist), 30 µg ketanserin (B; 5-$TH_{2A/2C}$ receptor antagonist), 30 µg ondansetron (C; 5-HT_3 receptor antagonist), or the control (DMSO), and the threshold was determined 90 min later. Data are presented as mean ± S.E.M. Control: n = 6; each 5-HT receptor antagonist: n = 6; **p < 0.01, ***p < 0.001 compared with the control at the corresponding time points.

ceptive effect was not attenuated[2]. It has also been reported that no changes occurred in the antinociceptive effect when fluvoxamine and WAY-100635 were administered to mice with neuropathic pain 7 days after partial sciatic nerve ligation[10]. Therefore, it appears that the relevance of 5-HT_{1A} receptors to chronic pain is not appreciable. However, in the present study, the 5-HT_{1A} receptor antagonist intrathecally administered significantly suppressed the antiallodynic effect compared with the control group only when the antagonist was administered 1 week after fluvoxamine administration. Our results suggest that 5-HT_{1A} receptors in the spinal cord are involved in antinociception at a relatively early stage after streptozotocin treatment.

Anjaneyulu et al have shown that the antinociceptive effect was attenuated when fluoxetine and the 5-$HT_{2A/2C}$ receptor antagonist ritanserin were administered to diabetic mice with neuropathic pain 5 weeks after streptozotocin treatment[2]. It has also been reported that significant suppression of the antinociceptive effect occurred when fluvoxamine and ketanserin were administered to mice with neuropathic pain 7 days after partial sciatic nerve ligation[10]. The present study also showed a significant suppression of antiallodynic effect, compared with the control group, when the 5-$HT_{2A/2C}$ receptor antagonist ketanserin was intrathecally administered 1 or 3 weeks after fluvoxamine administration. Based on these results, we suggest that 5-$HT_{2A/2C}$ receptors in the spinal cord are

involved in antinociception up to an intermediate stage, such as 5 weeks after streptozotocin treatment.

Nozaki et al observed strong inhibition of the antinociceptive effect when fluvoxamine and ondansetron were administered to mice with neuropathic pain 6 weeks after nerve ligation[20]. It has also been reported that inhibition of the antinociceptive effect tended to become weaker when fluvoxamine and ondansetron were administered to mice with neuropathic pain 7 days after the sciatic nerve ligation[10]. On the other hand, Anjaneyulu et al reported that there was no change in the antinociceptive effect when fluoxetine and ondansetron were administered to diabetic mice with neuropathic pain 5 weeks after streptozotocin treatment[2]. In the present study, we observed significant antagonism against the antiallodynic effect, compared with the control group, when the 5-HT_3 receptor antagonist ondansetron was intrathecally administered 1 or 3 weeks after fluvoxamine administration. These contradictions may result from variations in the experimental conditions among the studies, *i.e.*, fluvoxamine administration on consecutive days in this study as opposed to single SSRI administration in the study by Anjaneyulu et al. These results suggest that 5-HT_3 receptors in the spinal cord are involved in antinociception up to an intermediate stage, such as 5 weeks after streptozotocin treatment.

In this study, attenuation of the antiallodynic effect was almost undetectable in all groups in which WAY-100635, ketanserin, or ondansetron was administered intrathecally 5 weeks after fluvoxamine administration. These data suggest that the involvement of 5-HT receptors in the spinal cord diminishes when fluvoxamine is orally administered daily to diabetic rats with neuropathic pain beyond a certain length of time. Nagata et al reported that the antiallodynic effect was observed when the SSRI paroxetine was administered to rats with neuropathic pain, but this antiallodynic effect was not antagonized by WAY-100635, ketanserin, or ondansetron[18]. Because we observed the antiallodynic effect of fluvoxamine 5 weeks after administration in this study, we suggest that receptors or neurotransmitters other than 5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3 in the spinal cord may be involved in this effect. However, the precise molecular mechanisms remain unknown and require future studies. It was reported that fluvoxamine showed high affinity for $sigma_1$ receptors, but in the present study, the involvement of these receptors was not clear[19].

In conclusion, we demonstrated that daily oral administration of fluvoxamine resulted in a sustained antiallodynic effect against streptozotocin-induced mechanical hyperalgesia. Moreover, the results suggest that the involvement of 5-HT receptors (5-HT_{1A}, 5-$HT_{2A/2C}$, and 5-HT_3) in the spinal cord can change over time when fluvoxamine is administered daily for the treatment of the streptozotocin-induced neuropathic pain.

ACKNOWLEDGEMENTS

We would like to express our appreciation to the staff of the animal experiment facilities of Hiroshima University for their kind assistance.

(Received October 15, 2013)
(Accepted November 28, 2013)

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