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SHORT COMMUNICATION

A comparison of midazolam and dexmedetomidine for the recovery of serotonin syndrome in rats

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Abstract Serotonin syndrome is a drug-related toxicity caused by excess serotonin within the central nervous system. We recently encountered a case of serotonin syndrome that developed in the early postoperative period that was successfully treated with intravenous dexmedetomidine. Although the prescriptive literature has commonly recommended sedation with benzodiazepines for controlling agitation in serotonin syndrome, the effectiveness of dexmedetomidine has also been reported in several clinical conditions. In the present study, we conducted a reverse translational experiment to compare the efficacy of dexmedetomidine and midazolam, at equi-sedative doses, on serotonergic toxicity-like responses in rats. Animals were subcutaneously injected with 0.75 mg/kg 8-OH-DPAT, a full 5-HT_{1A} agonist. 8-OH-DPAT-treated rats showed serotonin syndrome-like behaviors (low body posture, forepaw treading), hyperlocomotion, and decreased body temperature, which were completely inhibited by pretreatment with WAY 100635, a selective 5-HT_{1A} antagonist ($n = 8$). Intramuscular injection of midazolam (1.0 mg/kg) or dexmedetomidine (0.01 mg/kg), which comparably induced observable signs of sedation, was tested in the present study. Concomitant treatment with midazolam significantly attenuated the hyperlocomotion, but failed to affect traditional serotonin syndrome behaviors and body temperature in 8-OH-DPAT-treated rats ($n = 8$). On the other hand, concomitant treatment with dexmedetomidine significantly attenuated all of these parameters ($n = 8$). The present case and related reverse translational experiment demonstrate that dexmedetomidine may be more beneficial for the treatment of serotonin syndrome compared to the current recommended treatment with benzodiazepines.

Keywords Serotonin syndrome · Midazolam · Dexmedetomidine

Serotonin syndrome (SS) is an uncommon drug reaction associated with increased serotonergic activity in the central nervous system, which is caused by a high dose of proserotonergic agents alone or, more commonly, in combination [1, 2]. It is characterized by the presence of a triad, i.e., altered mental status, neuromuscular excitation, and autonomic stimulation. Since there are no specific laboratory tests, the diagnosis of SS is made clinically according to the widely accepted criteria of the Sternbach or the Hunter Criteria [2]. The differential diagnosis of SS includes anticholinergic syndrome, malignant hyperthermia, and neuroleptic malignant syndrome. The severity of the SS can be mild, moderate, or even life-threatening (e.g., metabolic acidosis, rhabdomyolysis, disseminated intravascular coagulation), so early diagnosis and appropriate management are necessary for recovery. The principal

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treatment for SS is to discontinue the use of any serotonergic agent and to provide supportive care. Benzodiazepines (e.g., diazepam and midazolam) are considered a mainstay of SS treatment, particularly as a therapy for agitation [1, 2]. However, direct evidence is lacking on its therapeutic effectiveness in the treatment of SS.

Phenylpiperidine opioids, such as fentanyl, remifentanil, tramadol, and methadone, are considered to have mild serotonin-reuptake inhibition properties and therefore may potentiate the risk of SS, especially in the perioperative period [2–4]. We recently also encountered a case of SS during the postoperative period caused by fentanyl in a patient chronically taking a selective serotonin reuptake inhibitor (for a detailed case description, see Supplementary Material 1). Notably, in the present case, the symptoms were successfully improved after administration of a sedative dose of dexmedetomidine, a centrally acting α_2 adrenergic agonist. Although it is difficult to separate this dexmedetomidine effect from the spontaneous resolution of SS, several case studies similarly demonstrated that dexmedetomidine can treat SS that developed in different clinical settings [5, 6]. In most of these cases, benzodiazepine failed to treat SS before the administration of dexmedetomidine. Furthermore, preclinical studies showed that activation of α_2 adrenoceptors located on the serotoninergic terminal axons inhibits the release of serotonin [7, 8]. Taken together, all of these data indicate that dexmedetomidine may be more effective and efficient for SS than conventional treatment with benzodiazepines. To assess this hypothesis, we conducted an experiment to compare the efficacy of dexmedetomidine and midazolam, at equisedative doses, on the SS-like responses observed in a rat model.

All experimental procedures were approved by the Kochi University Animal Experiment Committee. Male Sprague–Dawley rats (body weight, 200–250 g) were maintained on rat lab chow with a 12-h light–dark cycle and had ad libitum access to food and water. All experiments were conducted in an isolated room between 8:00 am and 1:00 pm. For the rat model of SS, male Sprague–Dawley rats were subcutaneously injected with 0.75 mg/kg 8-OH-DPAT (Sigma-Aldrich, St. Louis, MO, USA; dissolved in 0.9 % saline), a full 5-HT_{1A} agonist [9]. Intraperitoneal (i.p.) injection of an equivalent sedative dose of midazolam (1.0 mg/kg) or dexmedetomidine (0.01 mg/kg), selected based on our preliminary dose–response experiment (see Supplementary Material 2), was tested. To avoid the invasive procedure of catheterization and disturbance in

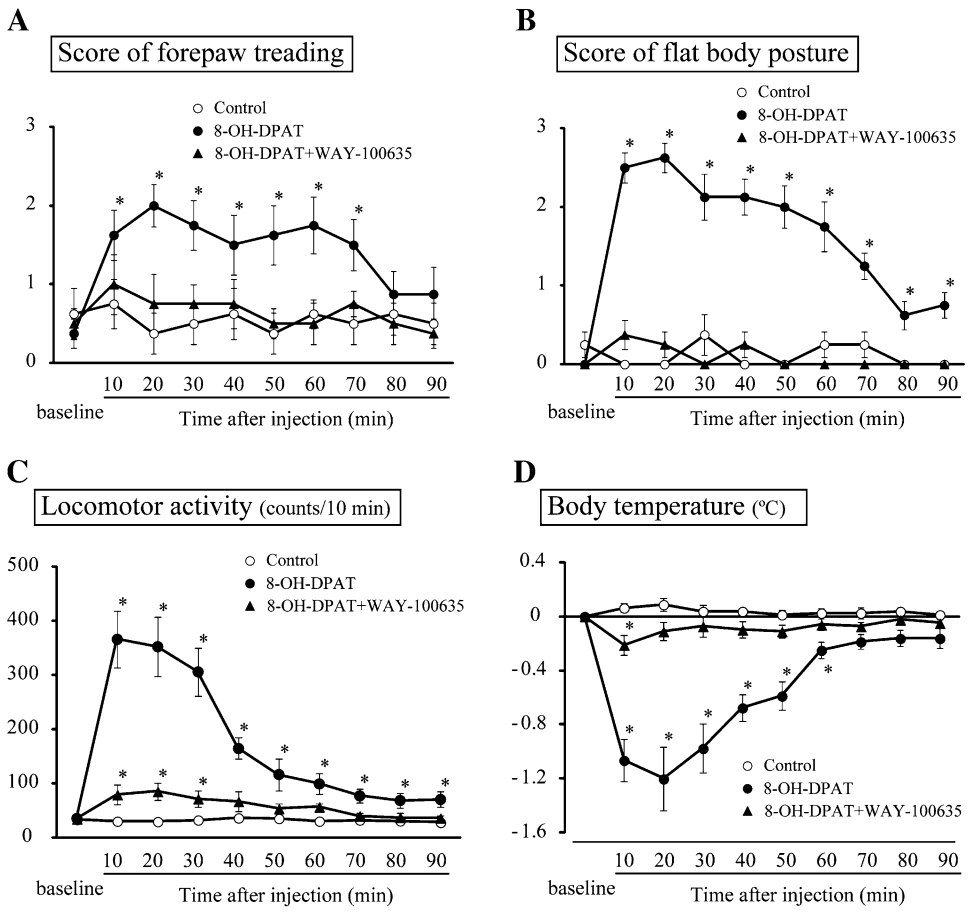

Fig. 1 Time course of 8-OH-DPAT-induced forepaw treading (**a**), flat body posture (**b**), locomotor activity (**c**), and body temperature (**d**) in the presence or absence of WAY-100635. Each parameter was scored at baseline and at 10-min intervals for 90 min after injection of 8-OH-DPAT or vehicle control. Each *vertical bar* represents the mean ± SD ($n = 8$ in each experimental group). *$p < 0.05$ vs. control rats

behavioral reactions, i.p. injection instead of an intravenous injection was used in the present study.

Assessments of locomotor activity and SS behaviors were conducted inside a transparent Plexiglas chamber (45 × 45 × 40 cm). All subjects were individually habituated in the test environment for at least 2 h prior to the test. Spontaneous locomotor activity was measured using pairs of 16 photo beams positioned 10 cm apart and 5 cm from the floor of the cage. The total accumulated counts of horizontal beam crosses were recorded for every 10 min. Two distinct types of behavior, flat body posture (the abdomen resting close to the cage floor) and forepaw treading (alternating bilateral forward-and-backward movements of both forepaws), which are well-characterized components of SS in rodents [9], were assessed by an observer blind to drug treatment. Behaviors were scored for each 10-min time period of the 90-min test, according to previous literature, as follows: 0 = absent; 1 = occasional; 2 = frequent; and 3 = constant. In a separate experiment, body temperature (rectal probe inserted 5 cm) was measured using copper/constantan thermocouples in conjunction with a digital thermometer, and recorded at baseline and at 10-min intervals for 90 min following 8-OH-DPAT injection. The environmental temperature was maintained at 23 ± 0.5 °C, and was monitored by a thermal probe placed inside the experimental chamber. The results are presented as the mean ± SD. Data were analyzed by two-way repeated measures ANOVA among groups, followed by one-way ANOVAs among groups over ten time points with Bonferroni's correction. Values $p < 0.05$ were considered statistically significant.

In confirmation of previous studies, 8-OH-DPAT-treated rats showed well-established responses commensurate with SS: increase in forepaw treading (Fig. 1a) and flat body posture (Fig. 1b), hyperlocomotion (Fig. 1c), and decreased body temperature (Fig. 1d). All of these 8-OH-DPAT-induced responses were almost completely inhibited by 10-min pretreatment with a serotonin 5-HT_{1A} receptor antagonist, WAY-100653 (0.5 mg/kg, i.p.).

Next, the effects of a sedative dose of midazolam and dexmedetomidine on 8-OH-DPAT-induced responses were investigated. Concomitant treatment with midazolam failed to inhibit both of two specific SS-like behaviors, forepaw treading (Fig. 2a) and flat body posture (Fig. 2b), as well as hypothermia (Fig. 2d). Although non-specific hyperlocomotive response was significantly attenuated (Fig. 2c), this may be related to the sedative effect of midazolam. However, concomitant treatment with dexmedetomidine resulted in significant attenuation of all 8-OH-DPAT-induced responses: forepaw treading (Fig. 2a), flat body

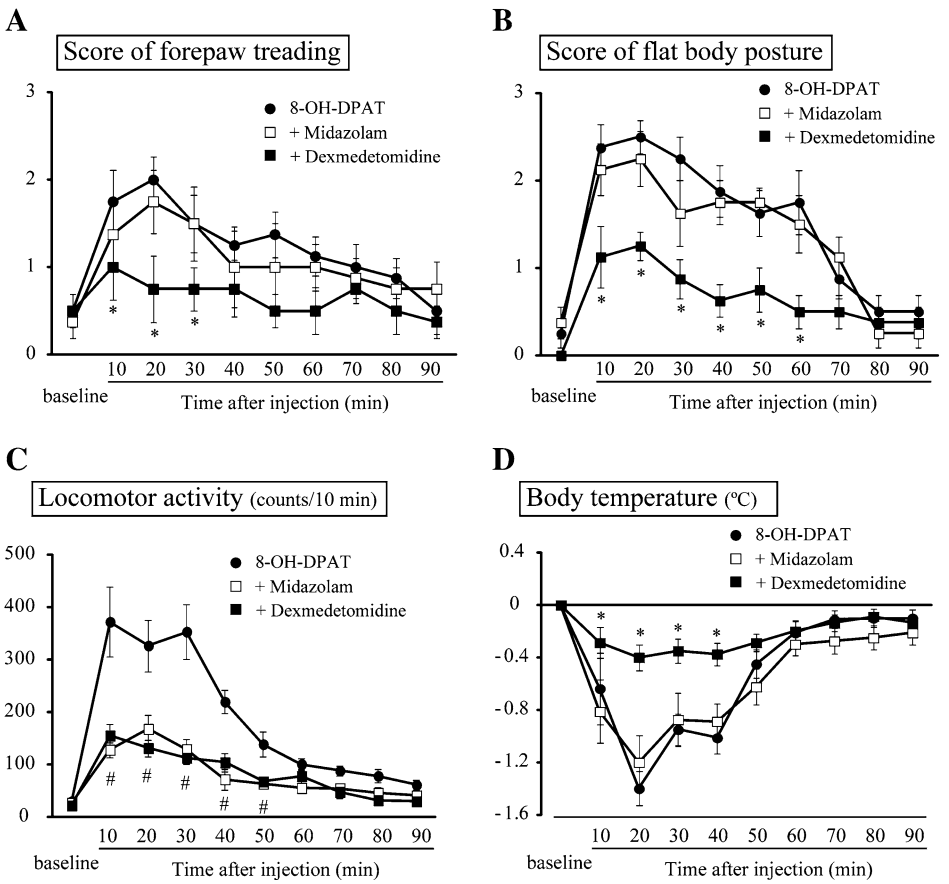

Fig. 2 Effects of concomitant treatment with midazolam or dexmedetomidine on 8-OH-DPAT-induced forepaw treading (**a**), flat body posture (**b**), locomotor activity (**c**), and body temperature (**d**). Each parameter was scored at baseline and at 10-min intervals for 90 min after injection of 8-OH-DPAT alone or concomitant with either midazolam or dexmedetomidine. Each *vertical bar* represents the mean ± SD ($n = 8$ in each experimental group). *$p < 0.05$; dexmedetomidine-treated rats compared with 8-OH-DPAT-treated rats. #$p < 0.05$; dexmedetomidine or midazolam-treated rats compared with 8-OH-DPAT-treated rats

posture (Fig. 2b), hypothermia (Fig. 2d), and hyperlocomotion (Fig. 2c).

The present preclinical findings may provide relevant support for the present case described in the Supplementary text: i.e., sedative dose of dexmedetomidine could treat SS. These findings are consistent with results from a mice transgenic model showing that dexmedetomidine can reduce L-5-hydroxytryptophan-induced serotonin toxicity via adrenaline α_{2C} receptors [10]. More importantly, these results also demonstrate that midazolam, at equi-sedative dose of dexmedetomidine, failed to show anti-SS effects in this model. Nishijima et al. [11] demonstrated that another common benzodiazepine, diazepam, dose-dependently prolongs mortality in a rat model of SS. Therefore, a higher dose of benzodiazepines may be required to achieve beneficial efficiency for 5-HT syndrome, while its usage is often limited by respiratory depression. Meanwhile, dexmedetomidine confers a sedation and analgesic effect without respiratory depression and shorter half-life after intravenous dosing, making it easier to titrate [12]. Therefore, dexmedetomidine may be more appropriate for the recovery of SS, compared to conventional treatment with benzodiazepines, especially during the post-surgical period. Further clinical studies may be needed to evaluate the efficacy of dexmedetomidine for SS.

As an antidote to SS, several anti-serotonergic agents, such as chlorpromazine and cyproheptadine, have also been reportedly used in clinical practice [2]. However, chlorpromazine may cause many adverse and toxic effects, including hypotension, seizure, and dystonic reactions, thus requiring caution, especially in severe cases [1, 2]. Furthermore, cyproheptadine is not available intravenously, and thus has limited use during the early postoperative period [2]. Although a previous study showed that dexmedetomidine may inhibit serotonin release in rat rostral raphé nuclei [8], its effects on serotonergic signaling pathways remain underinvestigated. Future research should include studies to determine whether dexmedetomidine exerts an antidote's potency on SS.

In conclusion, we encountered a case of SS during the postoperative period in which a successful resolution of symptoms was observed after administration with dexmedetomidine. Furthermore, our translational experiments indicate that a sedative dose of dexmedetomidine, but not midazolam, could recover the 8-OH-DPAT-induced SS-like responses in rats. Although benzodiazepines are commonly recommended in the treatment of SS, the results of our animal experiment imply that dexmedetomidine is more effective compared to benzodiazepines.

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