

# Brain Serotonin Content Regulates the Manifestation of Tramadol-induced Seizures in Rats

## Disparity between Tramadol-induced Seizure and Serotonin Syndrome

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### ABSTRACT

**Background:** Tramadol-induced seizures might be pathologically associated with serotonin syndrome. Here, the authors investigated the relationship between serotonin and the seizure-inducing potential of tramadol.

**Methods:** Two groups of rats received pretreatment to modulate brain levels of serotonin and one group was treated as a sham control (n = 6 per group). Serotonin modulation groups received either para-chlorophenylalanine or benserazide + 5-hydroxytryptophan. Serotonin, dopamine, and histamine levels in the posterior hypothalamus were then measured by microdialysis, while simultaneously infusing tramadol until seizure onset. In another experiment, seizure threshold with tramadol was investigated in rats intracerebroventricularly administered with either a serotonin receptor antagonist (methysergide) or saline (n = 6).

**Results:** Pretreatment significantly affected seizure threshold and serotonin fluctuations. The threshold was lowered in para-chlorophenylalanine group and raised in benserazide + 5-hydroxytryptophan group (The mean  $\pm$  SEM amount of tramadol needed to induce seizures; sham:  $43.1 \pm 4.2$  mg/kg, para-chlorophenylalanine:  $23.2 \pm 2.8$  mg/kg, benserazide + 5-hydroxytryptophan:  $59.4 \pm 16.5$  mg/kg). Levels of serotonin at baseline, and their augmentation with tramadol infusion, were less in the para-chlorophenylalanine group and greater in the benserazide + 5-hydroxytryptophan group. Furthermore, seizure thresholds were negatively correlated with serotonin levels (correlation coefficient; 0.71,  $P < 0.01$ ), while intracerebroventricular methysergide lowered the seizure threshold ( $P < 0.05$  vs. saline).

**Conclusions:** The authors determined that serotonin-reduced rats were predisposed to tramadol-induced seizures, and that serotonin concentrations were negatively associated with seizure thresholds. Moreover, serotonin receptor antagonism precipitated seizure manifestation, indicating that tramadol-induced seizures are distinct from serotonin syndrome.

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TRAMADOL, one of the most frequently prescribed analgesics worldwide, has a multitude of pharmacological properties, acting as an agonist at opioid receptors, while inhibiting reuptake of serotonin (5-hydroxytryptamine [5-HT]) and noradrenaline.<sup>1-4</sup> However, along with its therapeutic actions, tramadol has been identified as a major cause of drug-induced seizures; about 8% of new cases over the last decade were due to tramadol exposure.<sup>5</sup> Although recent data are not available, a retrospective study revealed that 602 patients out of 557,004 cases reported to the California Poison Control System during a 2.5-yr period had suffered from tramadol-induced seizures.<sup>6</sup> In correspondence with the increasing sale of tramadol, the number of patients experiencing tramadol-induced seizures will likely rise.

Elucidating the pathogenesis of tramadol-induced seizures will help reduce the occurrence of such events. However, multiple factors underlie the potentiation of seizures, and several of tramadol's pharmacological features could individually influence seizure potency.<sup>7,8</sup> In general, alteration of brain monoamines and activation of opioid receptors

### What We Already Know about This Topic

- Tramadol, a commonly prescribed analgesic, is a significant cause of medication-induced seizures. The mechanism by which seizure threshold is reduced by tramadol is not known.
- In addition to  $\mu$ -receptor agonism, tramadol inhibits uptake of serotonin. Excessive serotonin levels lead to the serotonin syndrome that is characterized by seizures. However, the role of serotonergic neurotransmission in tramadol-induced seizures is not known.
- The effect of augmenting and antagonizing serotonergic neurotransmission on tramadol-induced seizures was evaluated.

### What This Article Tells Us That Is New

- Tramadol-induced seizure thresholds were reduced by serotonin depletion and increased by serotonin augmentation. Serotonin antagonists also reduced seizure threshold.
- The results suggest that tramadol-induced seizures are not related to serotonin uptake inhibition and that these seizures are distinct from the serotonin syndrome.

are known to modulate the development of epileptic episodes.<sup>9,10</sup> Further, Spiller *et al.*<sup>11</sup> suggested that inhibiting the

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reuptake of monoamines could be what is responsible for tramadol intoxication rather than its agonistic actions on the opioid receptor, since administration of an opioid receptor antagonist seems to aggravate seizures.<sup>11,12</sup>

5-Hydroxytryptamine is generally known to protect against seizures,<sup>13</sup> and selective serotonin reuptake inhibitors (SSRIs) are presumed to be preventative in various animal models of seizure.<sup>14–17</sup> To our knowledge, there is no direct evidence determining the role of 5-HT in the generation of tramadol-induced seizures; specifically, whether this therapeutic agent potentiates or protects against seizure activity. Serotonin syndrome (SS), caused by excessive amounts of 5-HT, is another adverse side effect of tramadol, with neuromuscular hyperactivity *i.e.*, clonus, myoclonus, and even seizures regarded as the major symptoms characterizing the disorder.<sup>18,19</sup> Furthermore, concomitant use of tramadol with drugs that affect 5-HT, such as SSRIs and tricyclic antidepressants (TCAs), is associated with a higher risk of developing seizure and/or SS.<sup>18,20–24</sup> These observations have led some to believe that tramadol-induced seizures may be one of the symptoms of SS. Thus, clarifying the role of 5-HT could aid in differentiating between tramadol-induced seizures and SS.

Interestingly, some antihistamines have been shown to relieve symptoms of SS.<sup>25</sup> This is surprising since antihistaminergic activity has generally been suggested to potentiate seizures<sup>5,26</sup>; however, antihistamines and histamine release inhibitors have been reported to attenuate the potential of tramadol to induce seizures.<sup>27</sup> Although no direct evidence has been proposed regarding histamine neuromodulation with tramadol, there may be connections between tramadol-induced seizure and histamine.

The aim of the current study was to investigate the role of 5-HT and histamine in the generation of tramadol-induced seizures. To accomplish this, we used intracerebral *in vivo* microdialysis to characterize the variation of brain 5-HT and histamine concentrations before, during, and after tramadol-induced seizures while simultaneously evaluating hemodynamics during all experiments. We also focused on the effect of 5-HT receptor antagonism on seizure threshold.

## Materials and Methods

### Animals

Upon approval from the Institutional Animal Care and Use Committee of our institution (Osaka City University Graduate School of Medicine, Osaka, Japan), 7- to 9-week-old male Sprague–Dawley rats weighing 240 to 300 g (Clea Inc., Osaka, Japan) were acquired for this study. Rats had free access to food and water and were housed in plastic cages under a 12h light–dark cycle. After probe implantation and intraarterial and venous cannulation, rats were housed individually in a cage. Experiments were carried out during the light cycle and after experiment completion, rats were euthanized with an intravenous overdose of pentobarbital (50 mg/kg).

### Surgical Preparation

Before surgery, rats were administered an intramuscular injection of cefazolin 25 mg/kg into their triceps muscle. Rats were then anesthetized with 3 to 5% sevoflurane (Abbvie Japan, Tokyo, Japan) in oxygen *via* a nose cone. For microdialysis, rats were implanted with an intracerebral guide cannula (7 mm in length and 0.5 mm in OD, AG-7; Eicom, Kyoto, Japan) 7 days before the experiment by using a stereotaxic frame (Narishige, Tokyo, Japan). Guide cannulas were fixed with dental cement and aimed at the posterior hypothalamus according to the following coordinates: 1.0 mm lateral and 4.0 mm posterior to bregma, and 7.0 mm ventral to dura.<sup>28</sup> Stainless-steel stylets were inserted into the guide cannulas to prevent obstruction. Rats were single-housed postoperatively, and allowed one week to recover. Rats were then anesthetized 2 days before microdialysis, and an intravenous catheter was inserted into their right jugular vein, while an arterial catheter was inserted into their right carotid artery. Catheters were filled with heparinized saline and routed subcutaneously to exit at the back of the neck.

Seven days before the start of the antagonist study, rats were implanted with guide cannulas (4 mm in length and 0.5 mm in OD, AG-4; Eicom) aimed at their lateral ventricle according to the coordinates: 2.0 mm lateral and 1.0 mm posterior to bregma, and 3.5 mm ventral to dura.<sup>28</sup> Intravenous catheters were inserted into their right jugular vein 2 days before the experiment.

### Drugs

The following drugs were used: Tramadol HCl (4 mg kg<sup>-1</sup> min<sup>-1</sup> intravenous injection; Sigma Aldrich Japan, Tokyo, Japan), 4-chloro-DL-phenylalanine methyl ester HCl (para-chloro-phenylalanine [PCPA]; 160 mg/kg, intraperitoneal injection; Sigma Aldrich Japan), benserazide HCl (30 mg/kg intraperitoneal injection; Sigma Aldrich Japan), 5-hydroxytryptophan (5-HTP; 30 mg/kg intraperitoneal injection; Sigma Aldrich Japan), methysergide maleate (40 µg/animal intracerebroventricular injection; Sigma Aldrich Japan). All compounds were dissolved in 0.9% NaCl. Volumes of intraperitoneally administered drugs were 1.0 ml/kg of body weight except for 5-HTP, which was 5.0 ml/kg because of its solubility to 0.9% NaCl. Volumes of intracerebroventricularly administered drugs were 10 µl/animal. The dosage of tramadol was determined from our preliminary study.

### Experiment 1: In Vivo Microdialysis and Hemodynamic Evaluation during Seizure Generation with Tramadol Group Allocation and Drug Pretreatment for 5-HT Modulation.

To deplete brain 5-HT, we used PCPA, an inhibitor of tryptophan hydroxylase, which is the rate-limiting enzyme in 5-HT synthesis.<sup>29</sup> Alternatively, we concomitantly administered 5-HTP along with benserazide, immediate precursors of 5-HT, and a peripheral aromatic amino acid decarboxylase inhibitor to increase 5-HT concentration in the central nervous system.<sup>30</sup>

Using the envelope method, rats were randomly allocated to each experimental group, which consisted of six total subjects. For the PCPA group, rats received PCPA (160 mg/kg) 72, 48, and 24 h before microdialysis. Dose and timing of PCPA administration has been previously described by Prinsen *et al.*<sup>31</sup> and was expected to decrease extracellular 5-HT levels by about 90% from baseline. For the benserazide + 5-HTP group, rats received benserazide (30 mg/kg) 120 min before and 5-HTP (30 mg/kg) 90 min before microdialysis. Details have been described by Baumann *et al.*<sup>30</sup> who successfully demonstrated that the combined administration of these two drugs resulted in the elevation of 5-HT without altering other parameters. Finally, the sham group of rats received no pharmacological pretreatment. An experimenter who was not involved in monitoring the seizure experiment performed the pretreatments.

**In Vivo Microdialysis.** On the morning of microdialysis testing, rats were brought to the laboratory and a microdialysis probe, which was confirmed to have a recovery rate of  $5 \pm 1\%$  before the experiment, was carefully inserted into the guide cannulas. The probe was connected to extension cannulas and was perfused using a microdialysis pump (ESP-32; Eicom) with Ringer's solution containing 147.0 mM NaCl, 4.0 mM KCl, and 2.3 mM  $\text{CaCl}_2$  at a constant flow rate of 2  $\mu\text{l}/\text{min}$ . Each rat was placed in a plastic cage for 90 min before drug intravenous injection so that they could acclimate to the surrounding environment. After constant perfusion for 90 min, the perfusate was collected every 5 min to determine basal dopamine, 5-HT, and histamine concentrations. Infusion of drugs *via* the venous catheter was concurrent with collection of the dialysate, which was diluted and directly measured using the following procedures.

**Analytical Procedure.** Dopamine and 5-HT concentrations were measured in dialysates using high-performance liquid chromatography (Eicompak PP-ODS II, 30 mm  $\times$  4.6 mm I.D.; Eicom) with electrochemical detection (HTEC-500; Eicom), as previously described by Ohmura *et al.*<sup>32</sup> The mobile phase, which consisted of 2.1 mM sodium 1-decan-sulfonate, 0.1 mM EDTA-2Na/0.1 M phosphate buffer (pH 6.0), and 2% (v/v) methanol, was pumped at a rate of 250  $\mu\text{l}/\text{min}$ . Concentrations of 5-HT and dopamine in the dialysate were measured using a pure graphite working electrode (WE-PG; Eicom) and a salt bridge Ag-AgCl reference electrode. The working potential was set at 450 mV, while the signal from the current-potential converter (the integrator output) was filtered with a low-pass in-line noise filter and integrated by a computerized data acquisition system using chromatography data software (Power-Chrom; AD-Instruments Pty Ltd., Castle Hill, New South Wales, Australia).

Histamine was analyzed by the high-performance liquid chromatography method coupled with a post-column ortho-phthalaldehyde derivatization of the target amines.<sup>33</sup> The high-performance liquid chromatography system consisted of an EP-700 pump system, a M505 auto-injector,

an EHA-500 reaction pump system (Eicom), and an RF-10AXL fluorescence detector (Shimadzu, Kyoto, Japan). The mobile phase consisted of methanol-0.1 M  $\text{NaH}_2\text{PO}_4$  (1:9, v/v) containing 170 mg/l sodium 1-octanesulfonate (flow rate, 0.5 ml/min). Histamine was separated on an analytical reverse-phase column (Eicompak SC-5ODS; 150 mm  $\times$  3.0 mm I.D.; Eicom), and the eluent line was connected by a T piece with a reagent line, which mixes 0.05% ortho-phthalaldehyde containing 2.5% 2-mercaptoethanol solution and 0.5 M  $\text{K}_2\text{CO}_3$  in a short reaction coil (9 m  $\times$  0.5 mm I.D.) at a flow rate of 0.2 ml/min (pump 1, 0.1 ml/min; pump 2, 0.1 ml/min). The eluent and basic ortho-phthalaldehyde solution were mixed in a reaction coil (3 m  $\times$  0.5 mm I.D.), while the separation and reaction procedures were performed at  $40.0^\circ \pm 0.1^\circ\text{C}$  in a CTO-10A column oven (Shimadzu). The fluorescence intensity was subsequently measured at 450 nm with excitation at 340 nm in a fluorescence detector. The chromatography data were analyzed in the same way as the 5-HT and dopamine data.

#### **Drug Administration and Evaluation of Seizure Activity.**

Seizures induced with tramadol infusion were monitored by two researchers who were blinded to the group allocation at the same time as microdialysis perfusion. Seizure activity was defined as the fifth stage of the modified Racine score (tonic-clonic seizures),<sup>34</sup> since tramadol-induced seizures typically manifest as generalized tonic-clonic.<sup>35–37</sup> We avoided evaluating electroencephalograms because this methodology may not accurately reflect seizure severity of tramadol-induced seizures.<sup>38</sup> Drug infusion was discontinued with seizure onset, and the latency to seizure was recorded to determine the amount of tramadol needed to induce the epileptic activity.

**Blood Pressure and Heart Rate Determination.** An extension of tubing from the arterial catheter was attached to a pressure transducer to record blood pressure on a polygraph (UB-104U; Unique Medical Co., Ltd., Tokyo, Japan) when rats were placed in plastic cages. Along with microdialysis and seizure activity experiments, we measured arterial blood pressure and heart rate (HR), which was calculated from the arterial pressure waveform using an on-line computer system (Unique Acquisition; Unique Medical Co., Ltd.). Mean arterial blood pressure and HR were obtained before the infusion (baseline value), and again 20 s before and 60 s after seizure manifestation (preconvulsion value/postconvulsion value, respectively). To validate the volume effect of infusion, 0.9% NaCl was infused at the same rate to the six rats making up the control group, which did not receive microdialysis. Since rats in the control group did not exhibit seizures, values at 9 and 11 min after infusion were substituted for the pre- and postconvulsion values, respectively.

#### **Experiment 2: Antagonist Study**

To demonstrate the direct involvement of 5-HT in seizure generation, 12 rats were randomly allocated to two groups; one group of rats ( $n = 6$ ) was given 0.9% NaCl

intracerebroventricularly (saline group), while the other group ( $n = 6$ ) was given 40  $\mu\text{g}$  of methysergide maleate, a nonselective 5-HT 1/2 receptor antagonist, intracerebroventricularly in a volume of 10  $\mu\text{l}$ <sup>39</sup> (methysergide group). On the day of the experiment, rats were brought to the laboratory and an intracerebroventricular port (AMI-4; Eicom) was attached to the guide cannula. Two hours after the procedure, test drugs and tramadol were administered through the intracerebroventricular port and the intravenous catheter, respectively. As in Experiment 1, 4 mg  $\text{kg}^{-1} \text{min}^{-1}$  of tramadol was infused until rats exhibited generalized tonic-clonic seizures. The amount of tramadol needed to induce these seizures was then compared between saline and methysergide groups.

### Data Calculations and Statistical Analysis for Both Experiments

The number of rats in each group was based on the results of our preliminary experiment ( $n = 6$  for each group); the difference of the mean amount of tramadol needed to induce seizures was about 20 mg/kg and the expected SD was 10, with an  $\alpha$  of 0.05 and a  $\beta$  of 0.80.

Amounts of 5-HT, dopamine, and histamine were calculated by comparing the peak heights of samples and standards. The minimal dose of tramadol (mg/kg) needed to induce a seizure was calculated from the latency and was used as an index of seizure threshold. Comparisons of these data between groups were performed with an unpaired  $t$  test and one-way ANOVA followed by the Holm–Sidak *post hoc* test. Extracellular 5-HT, dopamine, and histamine concentrations are expressed as pg/ $\mu\text{l}$  of sample. These data were analyzed by a two-way ANOVA with time as a repeated measure and with group as the independent factor. When there were significant interactions between these two variables, a simple effects test was performed (respective  $F$  values along with degrees of freedom are indicated). Comparisons between the variables at each time point within the same group were made by one-way repeated-measures ANOVA, and comparisons between pretreatment groups at the respective time points of measurement were made using one-way ANOVA. When there was a significant difference, we used the Holm–Sidak test as a *post hoc* analysis. We also utilized Spearman product-moment correlations. All analyses were performed using Sigma Plot ver. 11 (Systat Software Inc., San Jose, CA), and statistical significance for all analysis was set at  $P$  value less than 0.05. Data are presented as mean and SEM.

## Results

### Seizure Activity and Tramadol Threshold

Every rat manifested tonic-clonic seizures on infusion of tramadol and no infusion resulted in lethality. Furthermore, seizure threshold was significantly affected by pretreatment with PCPA or benserazide + 5-HTP. The mean  $\pm$  SEM latency to seizure was 647.2  $\pm$  63.3 s in the Sham group, 347.7  $\pm$  17.4 s in the PCPA group, and 891.7  $\pm$  101.3 s in the benserazide +

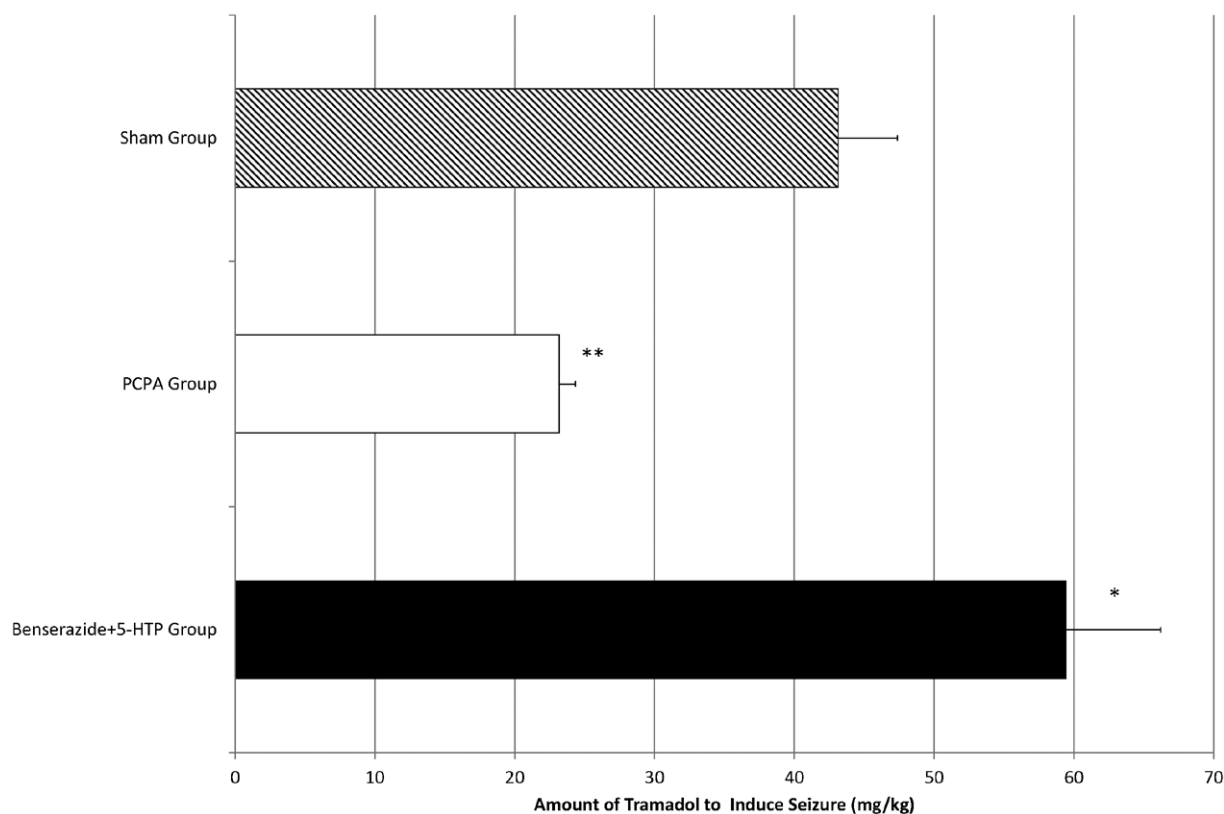
5-HTP group. Figure 1 shows that PCPA pretreatment significantly reduced the threshold of tramadol needed to induce seizures, while pretreatment with benserazide + 5-HTP increased the tramadol threshold for seizure induction.

### Extracellular Monoamine Concentrations in the Posterior Hypothalamus

Continuous infusion of tramadol resulted in marked increases in both 5-HT and dopamine concentrations (figs. 2 and 3). A two-way ANOVA demonstrated significant intergroup differences of these variables (5-HT:  $F[2,120] = 51.9$ ,  $P < 0.001$ , dopamine:  $F[2,120] = 9.3$ ,  $P < 0.01$ ), as well as differences between time points (5-HT:  $F[8, 120] = 11.2$ ,  $P < 0.001$ , dopamine:  $F[8,120] = 24.2$ ,  $P < 0.001$ ). Since significant interactions were detected between time and group factors (5-HT:  $F[16,120] = 6.2$ ,  $P < 0.01$ , dopamine:  $F[16,120] = 2.4$ ,  $P < 0.01$ ), we performed individual simple effects. We determined that 5-HT concentrations were significantly elevated from baseline values during the infusion of tramadol in all groups, except for values at 0 to 5 min in the benserazide + 5-HTP group (Sham group:  $F[8,40] = 18.3$ ,  $P < 0.001$ , PCPA group:  $F[8,40] = 18.3$ ,  $P < 0.001$ , benserazide + 5-HTP group:  $F[8,40] = 7.6$ ,  $P < 0.001$ ) (fig. 2). Both of the pretreatments significantly affected 5-HT levels at baseline as well as during tramadol infusion. A one-way ANOVA with a *post hoc* Holm–Sidak test revealed significant intergroup differences on respective time-points from baseline to 10 to 15 min, the period at which tramadol was infused ( $P < 0.05$ , respectively,  $F$  values are indicated in fig. 2). Regarding extracellular dopamine, a one-way ANOVA revealed that concentrations were comparable among groups at baseline and were similarly elevated during the period which tramadol infusion had been conducted (0 to 15 min). Within group difference at different time periods (Sham group:  $F[8,40] = 12.0$ ,  $P < 0.001$ , PCPA group:  $F[8,40] = 12.8$ ,  $P < 0.001$ , benserazide + 5-HTP group:  $F[8,40] = 7.0$ ,  $P < 0.001$ ) (intergroup differences with  $F$  values at each time-point; described in fig. 3) Baseline extracellular histamine concentrations were similar among groups and were not affected by tramadol infusions (fig. 4). Individual 5-HT concentrations at the time of seizure manifestation and the amount of tramadol used to induce seizures in each subject are plotted in figure 5, with a significant positive correlation between factors (correlation coefficient 0.71,  $P < 0.01$ , Spearman correlation).

### Hemodynamics during the Experiment

Hemodynamic changes during the experiment are presented in table 1. Similar hemodynamic alterations were observed in each group to which tramadol was administered. With tramadol infusion, HR gradually decreased to 60 to 80% of the baseline value of each rat at the time before the seizure, and recovered to almost 100% after the seizure. Mean blood pressure values were similar between groups before and after seizures. At the onset of seizure, almost all rats seemed to express



**Fig. 1.** Total amount of tramadol needed to induce seizures in each group. Each group consisted of six rats. Data are shown as means and bars indicate SEM. \* $P < 0.05$ , \*\* $P < 0.01$  compared with the value of the Sham group. A one-way ANOVA was used to detect statistical significance between groups and a Holm–Sidak *post hoc* test was performed. 5-HTP = 5-hydroxytryptophan; PCPA = para-chlorophenylalanine.

marked hypertension and tachycardia; however, we could not obtain proper arterial waveforms because they were markedly affected by seizure activity. Finally, the control group (infused with 0.9% NaCl) did not exhibit any change in mean blood pressure or HR during the infusion period.

### Effects of Serotonin Receptor Antagonism on Tramadol-induced Seizures

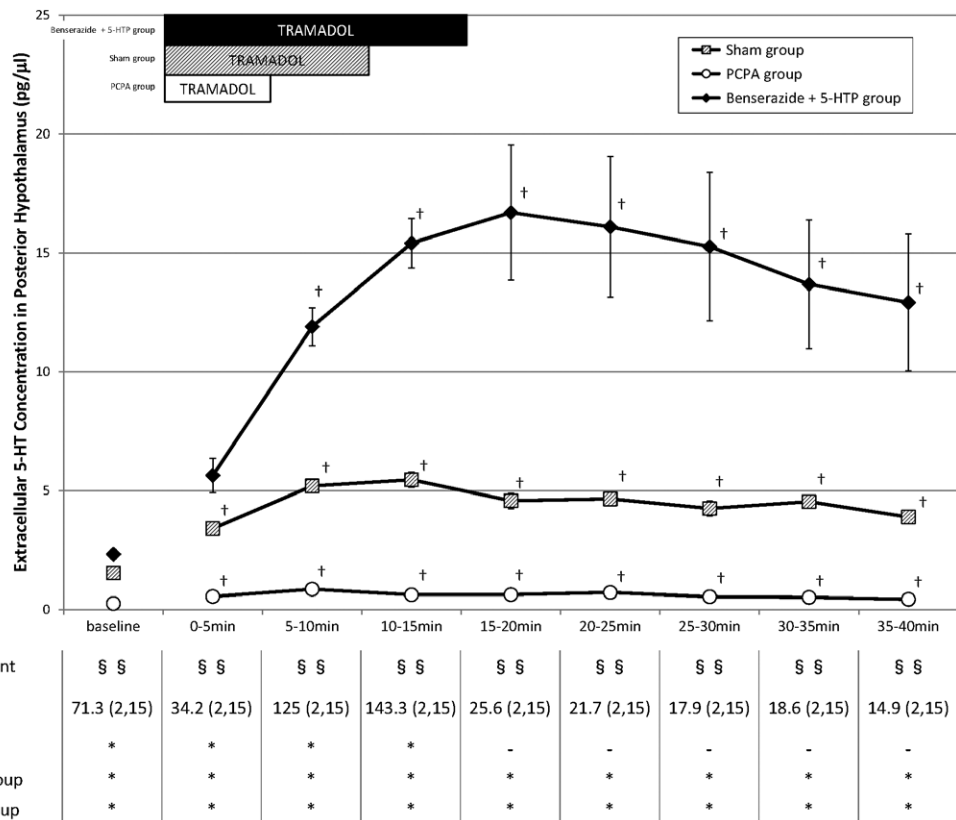
An intracerebroventricular administration of methysergide markedly potentiated seizure propagation compared to saline administration in rats ( $P < 0.05$ , Unpaired *t* test) (fig. 6). The mean  $\pm$  SEM amount of tramadol used to induce seizures was  $80.8 \pm 5.4$  mg/kg in saline groups, while that used in the methysergide group was  $60.1 \pm 4.2$  mg/kg.

### Discussion

This study showed the protective role of 5-HT on tramadol-induced seizures. We have revealed a correlation between brain 5-HT concentration and the threshold of tramadol needed to induce a seizure. We also showed that extracellular 5-HT augmented by tramadol actually protected against seizure. Additionally, the administration of a serotonin receptor antagonist, methysergide, potentiated seizure generation. These findings strongly suggest that tramadol-induced

seizures are a distinctly different concept from SS. Interestingly, tramadol did not influence extracellular concentrations of histamine; consequently, histamine is not presumed to play a role in tramadol-induced epileptic activity.

Our study is unique in that we only used tramadol as a drug to induce seizure activity. Furthermore, its continuous infusion enabled proper time-course validation of the monoamine contribution to seizure development. Most of the previously reported experiments on tramadol-related seizures often used various epileptic models that could have affected the potency of tramadol to induce seizures, such as pentylenetetrazole<sup>27,40,41</sup> and maximal electroshock-induced seizures<sup>7,8,41</sup>; therefore, these previous studies may not be appropriate in qualifying the seizure potential of tramadol. Indeed, Bankstahl *et al.*<sup>41</sup> reported a discrepancy between seizure models in that tramadol reduced the threshold of pentylenetetrazole to induce seizures but increased that of maximal electroshock-induced seizures. Generally, 5-HT exerts a preventative role in seizure onset under various experimental conditions.<sup>13–15,17</sup> Moreover, administration of SSRIs, including fluoxetine, is related to seizure attenuation in some animal models of epilepsy.<sup>14–17</sup> For example, Yan *et al.*<sup>15</sup> found that the anticonvulsant effect of fluoxetine selectively correlated with an enhanced synaptic availability of 5-HT. Interestingly, the pharmacological action of tramadol on 5-HTergic neurotransmission resembles the

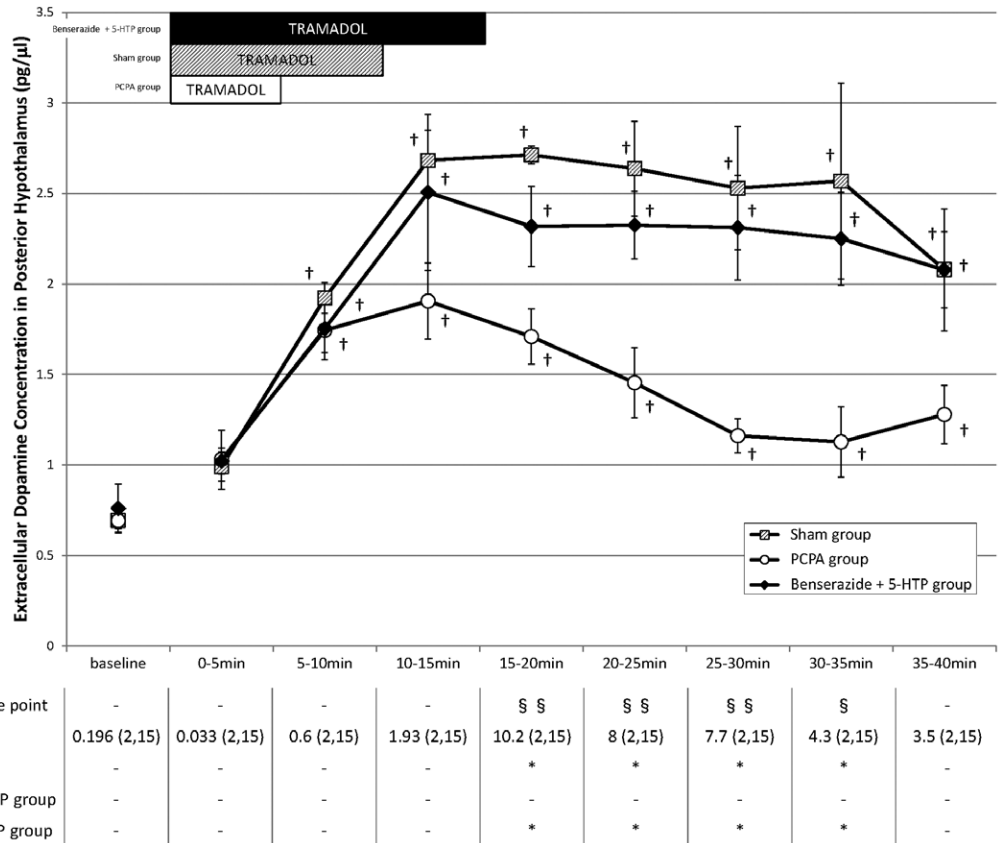


**Fig. 2.** Extracellular 5-hydroxytryptamine (5-HT) concentrations in the posterior hypothalamus of each group. Each group consisted of six rats. Data are shown as means and vertical bars indicate SEM. F values with degrees of freedom (numbers in the parenthesis) are indicated in some cases. Horizontal bars indicate the mean duration of tramadol infusion in each group. A two-way repeated-measures ANOVA demonstrated significant main effects in the difference among pretreatment groups ( $F[2,120] = 51.9, P < 0.001$ ) and between time-points ( $F[8,120] = 11.2, P < 0.001$ ). In addition, a significant interaction between groups and time factors was been detected ( $F[16,120] = 6.2, P < 0.001$ ). Significant simple effects were detected regarding both group and time factors. †Expresses  $P < 0.05$  compared with the baseline values of each group. (One-way repeated-measures ANOVA followed by a Holm–Sidak post hoc analysis.) The table under the figure indicates the intergroup differences at each time-point. §§Expresses  $P < 0.01$  difference between groups at each time-point detected by a one-way ANOVA, and F values with degrees of freedom are also indicated below. Post hoc analyses were demonstrated using the Holm–Sidak test, which indicated some significant intergroup differences between Sham and para-chlorophenylalanine (PCPA) groups, Sham and benserazide + 5-hydroxytryptophan (5-HTP) groups, and PCPA and benserazide + 5-HTP groups, respectively. \*Significant difference in mean 5-HT concentrations between the two groups.

actions of SSRIs.<sup>4</sup> Furthermore, lower baseline 5-HT levels are correlated with subsequent increases in generalized seizure duration and frequency.<sup>42</sup> Our primary finding was consistent with these previous reports and we assume that 5-HT was responsible for the seizure-resilient properties that we observed in this study.

In contrast to our findings and the discussed literature, Raffa *et al.*<sup>10</sup> reported that the seizure potency of tramadol was not affected by reserpine pretreatment, which nonspecifically depletes 5-HT. Along with its deleterious effects on 5-HT, reserpine induces depletion of various brain catecholamines that themselves may affect seizure potency, and induce hypotension,<sup>43</sup> which may have a negative effect on seizures. To omit the possible influence of our various pretreatment regimens on hemodynamics, we measured hemodynamic changes during the experiment and found that alterations in hemodynamic values were similar among

groups. Mean blood pressure was unaffected by the infusion of tramadol, and the reduction in HR that we observed during tramadol administration was observed in each group. More specifically, HR gradually dropped to about 70 to 80% of its baseline value before seizure onset, however, this was not a consequence of volume load because the same infusion rate of 0.9% NaCl did not result in any hemodynamic change. At seizure onset, on the other hand, tachycardia was observed in all of the tramadol-infused rats. Tachycardia is regarded as one of the risk factors associated with tramadol-induced seizures.<sup>6</sup> However, as the authors of the previous report mentioned, it was not clear whether tachycardia was present before the seizures they induced or if it occurred subsequent to seizures. The results of our experiment indicate that the latter incident may be the case. Moreover, this suggests that seizures induced by tramadol are not a consequence of hemodynamic changes.

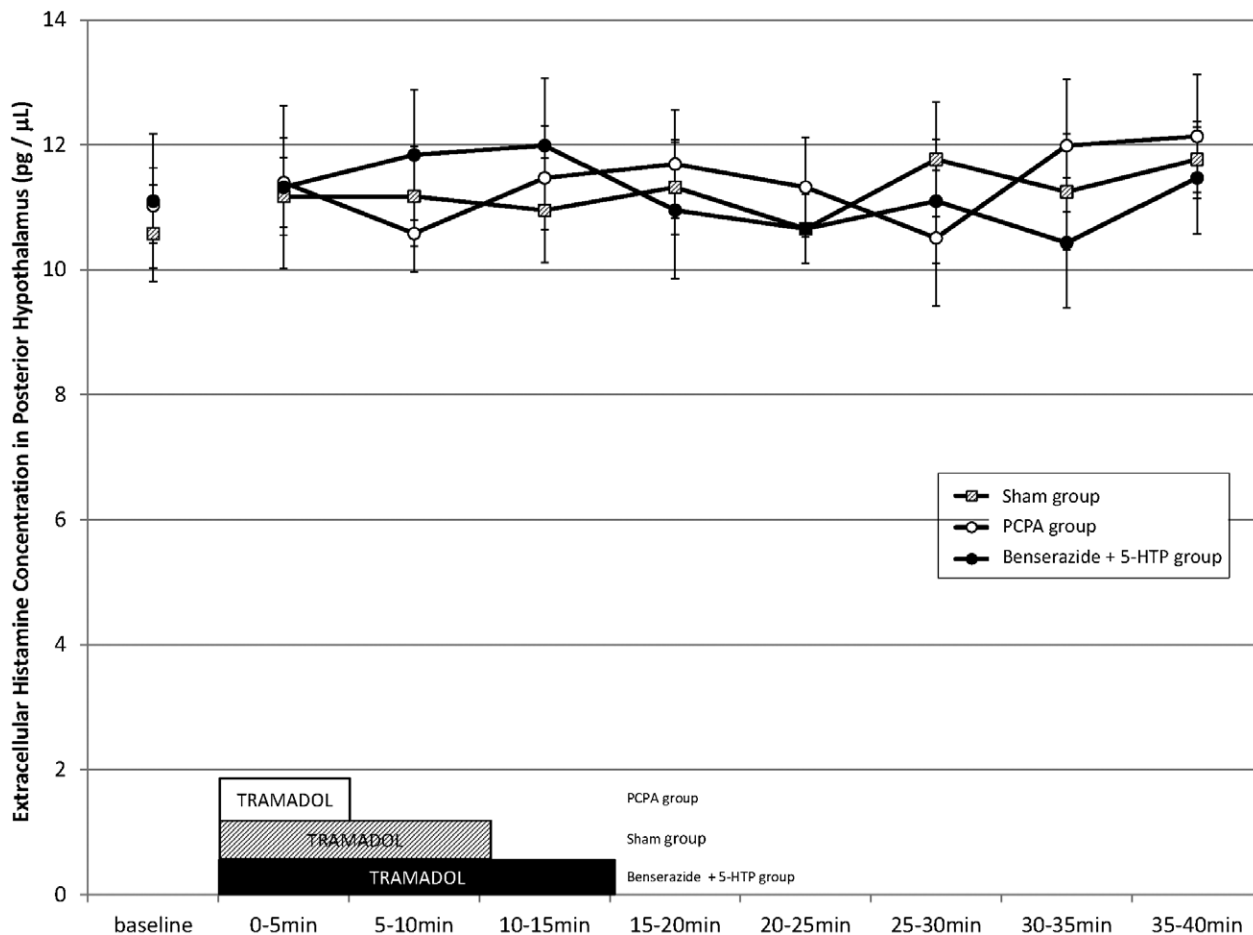


**Fig. 3.** Extracellular dopamine concentrations in the posterior hypothalamus of each group. Each group consisted of six rats. Data are shown as means and vertical bars indicate SEM. F values with degrees of freedom (numbers in the parenthesis) are indicated in some cases. Horizontal bars indicate the mean duration of tramadol infusion in each group. A two-way repeated-measures ANOVA demonstrated significant main effects in the difference among pretreatment groups ( $F[2,120] = 9.3, P < 0.01$ ) and between time-points ( $F[8,120] = 24.2, P < 0.001$ ). In addition, a significant interaction between groups and time factors was detected ( $F[16,120] = 2.4, P < 0.01$ ). Significant simple effects were detected regarding both group and time factors. †Expresses  $P < 0.05$  compared with the baseline values of each group. (One-way repeated-measures ANOVA followed by a Holm–Sidak post hoc analysis.) The table under the figure indicates the intergroup differences at each time-point. §,§§Express  $P < 0.05$  and  $P < 0.01$  differences between groups at each time-point detected by a one-way ANOVA, and F values with degrees of freedom are also indicated below. Post hoc analyses were demonstrated with the Holm–Sidak test, which indicated some significant intergroup differences between Sham and para-chlorophenylalanine (PCPA) groups, Sham and benserazide + 5-hydroxytryptophan (5-HTP) groups, and PCPA and benserazide + 5-HTP groups, respectively. \*Significant difference in mean dopamine concentrations between two groups.

In the clinical setting, it is sometimes difficult to distinguish tramadol-induced seizures from SS, since neuromuscular symptoms of SS include clonus, myoclonus, and even seizures.<sup>18,19</sup> We confirmed that 5-HT elevation resulted in an anticonvulsive effect and that 5-HT depleted rats were more susceptible to seizures. Moreover, a nonselective 5-HT antagonist methysergide, which may be a potent SS reliever,<sup>44</sup> promoted seizure generation. These findings indicate that tramadol-induced seizures are distinctly different from SS, and that neuromuscular symptoms of SS are qualitatively different from tramadol-induced seizures. This is clinically of importance that, when we face seizure-like symptoms with tramadol user, therapeutics for one syndrome probably exacerbate the other.

SS tends to arise in patients concomitantly taking TCAs or SSRIs with tramadol compared to those with tramadol

monotherapy.<sup>24,45,46</sup> There can be no doubt that TCAs or SSRIs in addition to tramadol, synergistically augment neuronal 5-HT, which accounts for the occurrence of SS; however, as we have already discussed, the same trend has been reported with cases of tramadol-induced seizures.<sup>20–24</sup> This seems to be contradictory to our current findings on the anticonvulsive effect of 5-HT, though this discrepancy could be explained by the influence of drug metabolism and characteristics of the tramadol user. Drug–drug interactions are speculated to be associated with the risk of developing tramadol-related seizures,<sup>47</sup> as is much more evident in cases of SS.<sup>24,45,46</sup> Interestingly, tramadol, TCAs, and SSRIs are all commonly metabolized by cytochrome P450 2D6 (CYP2D6),<sup>48</sup> therefore, there may be some competition for enzymatic reaction when these agents are taken concomitantly. Furthermore, TCAs and SSRIs are known



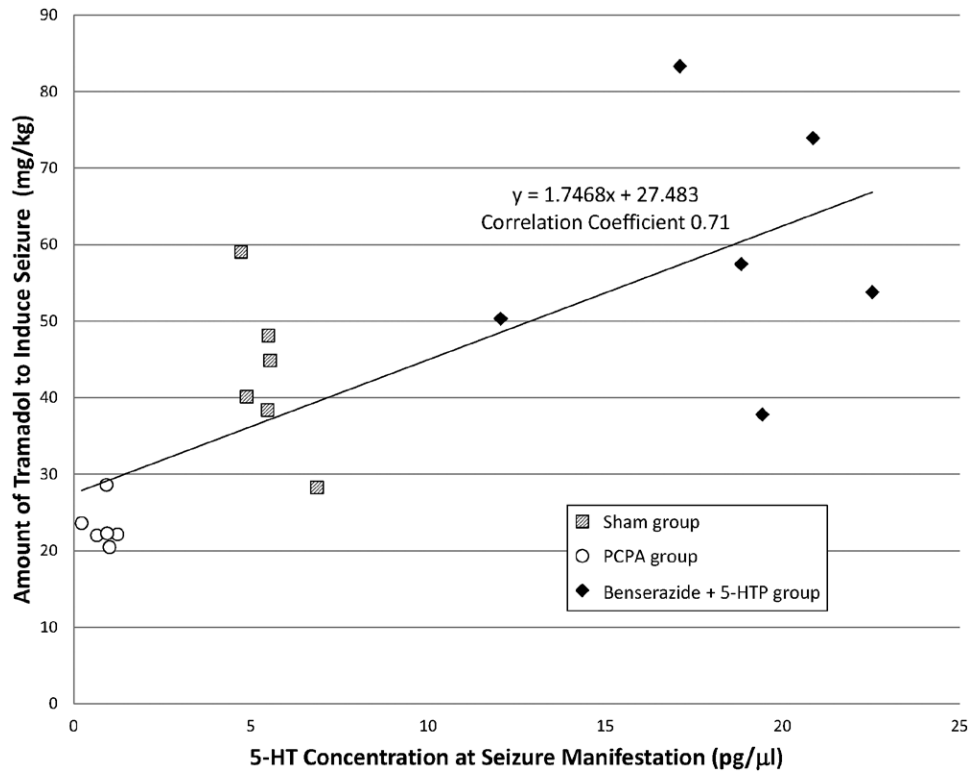
**Fig. 4.** Extracellular histamine concentrations in the posterior hypothalamus of each group. Each group consisted of six rats. Data are shown as means and vertical bars indicate SEM. Horizontal bars indicate the mean duration of tramadol infusion in each group. A two-way repeated-measures ANOVA did not detect a significant effect of time and intergroup group difference in histamine concentration. 5-HTP = 5-hydroxytryptophan; PCPA = para-chlorophenylalanine.

to inhibit the activity of CYP2D6.<sup>49</sup> These interactions may result in the insufficient metabolism of these drugs, yielding extremely high levels of tramadol, overwhelming the anticonvulsive property of 5-HT, and thereby provoking seizures. This is consistent with observations that the occurrence of tramadol-induced seizures is dose-independent and that the dose that precipitates seizures differs greatly among individuals.<sup>6,20,36,37</sup> Gardner *et al.*<sup>22</sup> suggested that a small subset of the population is sensitive to seizures induced by tramadol because of a genetic polymorphism of CYP2D6, resulting in individuals who are poor metabolizers of tramadol. Aside from this small population, it remains that patients who are prescribed tramadol along with antidepressants are at higher risk of developing tramadol-related seizures. Moreover, it has been revealed that tramadol users are more likely to have been exposed to drugs that interact with tramadol, such as CYP2D6 substrates and inhibitors.<sup>22</sup> Furthermore, patients with major depression have been proven to be six-fold more likely to have an unprovoked seizure than the general population.<sup>50</sup> Interestingly, most documented cases of tramadol overdose are in relation to suicide attempts or

patients with mental disorders, a population of individuals that may already be susceptible to tramadol intoxication.<sup>6,12</sup> Eventually, our results indicated that SSRI can theoretically relieve tramadol induced seizure by 5-HT augmentation, however, its administration seems to affect drug metabolism and sometimes militated against seizure threshold. In addition, it should be noticed that a concomitant administration of SSRI with tramadol will probably cause SS, so that we do not recommend SSRI as a reliever of tramadol-induced seizure.

Based on the observation that antihistamines such as cyproheptadine can relieve symptoms of SS,<sup>25</sup> and that histamine plays a possible role in tramadol-induced seizure,<sup>27</sup> we measured both histamine as well as 5-HT in the current study. We selected the posterior hypothalamus as the region of monoamine measurement since this area is rich in histaminergic neurons,<sup>51,52</sup> and is thus an appropriate area to evaluate the possible effect of tramadol on histamine.<sup>53</sup> Furthermore, this was also a reasonable area for confirming 5-HT modulations using pretreatments, because 5-HTergic innervations from the raphe nucleus distribute over the entire





**Fig. 5.** Correlation between seizure threshold and 5-hydroxytryptamine (5-HT) concentration at the time of seizure manifestation. Seizure threshold was defined as the minimum amount of tramadol needed to induce seizure (mg/kg). An overall n = 18 was used in this analysis. Spearman correlation revealed a significant correlation between these factors (correlation coefficient: 0.71, P < 0.01). 5-HTP = 5-hydroxytryptophan; PCPA = para-chlorophenylalanine.

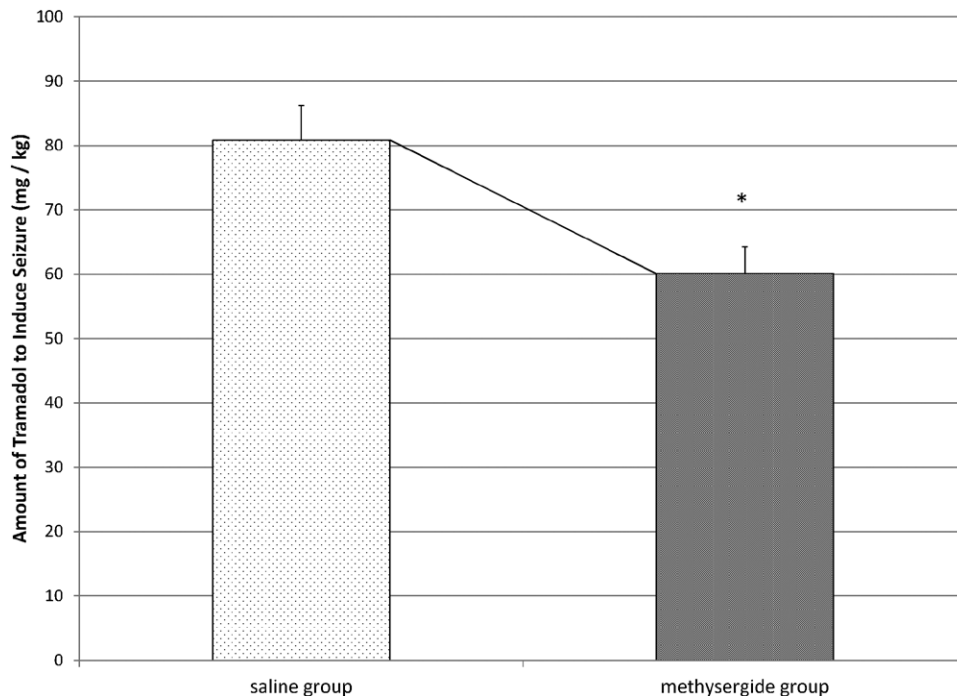
**Table 1.** Hemodynamics before and after Seizures in Each Group

|   | Baseline Value          | Preconvulsion Value      | Postconvulsion Value    |
|---|-------------------------|--------------------------|-------------------------|
|   | Mean Blood Pressure/HR  | Mean Blood Pressure/HR   | Mean Blood Pressure/HR  |
| <b>Tramadol infusion</b>                  |                         |                          |                         |
| Sham group                                | 125 (5)/397.5 (23.1)    | 117.5 (7.2)/287 (5.9)    | 126 (5.1)/387.2 (18.4)  |
| PCPA group                                | 122.2 (2.4)/362 (15.8)  | 123 (4.8)/267.2 (11.6)   | 133.8 (4.8)/343 (17.2)  |
| Benserazide + 5-HTP group                 | 113.8 (1.9)/381.3 (9.7) | 120.8 (2.5)/310.5 (11.4) | 124.2 (3.4)/364.7 (20)  |
| <b>Control group (0.9% NaCl infusion)</b> |                         |                          |                         |
|   | 120.7 (1.3)/377 (6.5)   | (9 min after infusion)   | (11 min after infusion) |

Each group consisted of six rats. Data are shown as mean (SEM). Unit for blood pressure was expressed as mmHg and HR as beats/minutes. A two-way repeated-measures ANOVA was used. There was no intergroup difference in either blood pressure or HR between tramadol-infused groups. 5-HTP = 5-hydroxytryptophan; HR = heart rate; PCPA = para-chlorophenylalanine.

forebrain (including the hypothalamus).<sup>54–56</sup> To date, there has been no reported pharmacological effect of tramadol on brain histamine, although Rehani *et al.*<sup>27</sup> showed that the administration of antihistamines can attenuate the pentylenetetrazole-induced seizure potentiating effect of tramadol. More specifically, this group suggested that tramadol exerted a seizurogenic effect on pentylenetetrazole-treated mice, possibly through an opioid receptor-dependent release of histamine. However, this finding was contrary to the widely accepted observation that antihistaminergic agents produce seizure activity in human and animals.<sup>5,26</sup> Moreover, the histamine-releasing potency of tramadol has proven to be much

smaller than that of morphine<sup>57</sup>; hence, it is not likely that opioid-induced histamine release would have a major effect on seizure development with tramadol. In the current study, we demonstrated that hypothalamic histamine concentrations remained unchanged throughout the measurement period. Furthermore, it should be noted that we avoided using antihistamines since they have been shown to potentiate seizures<sup>5,26</sup> and could likely enhance the seizure potency of tramadol.<sup>22,24</sup> Our findings indicate that tramadol has no pharmacological action on extracellular histamine and, despite the previous report discussed above,<sup>27</sup> brain histamine does not play a major role in tramadol-induced seizures.



**Fig. 6.** Effect of intracerebroventricular administration of either methysergide or saline on seizure threshold in normal rats. Both groups consisted of six rats. Data are shown as means and bars indicate SEM. \* $P < 0.05$  compared with saline group values. An unpaired  $t$  test was used to detect statistical significance between groups.

Finally, we measured dopamine concentration for fear that it might be affected by pretreatment.<sup>58</sup> Dopamine may influence seizure potency through its activation of various dopamine receptors.<sup>9,59</sup> Tramadol inhibits dopamine uptake, however, our understanding of how dopamine contributes to the pharmacological effect of tramadol remains limited.<sup>2,60</sup> In the current study, dopamine was similarly elevated among all three groups during tramadol infusion, suggesting that dopamine was not responsible for the difference in the seizure threshold observed between groups. However, it remains possible that elevations of neuronal dopamine can affect the seizure-inducing potential of tramadol, and further study involving specific dopamine modulation like this experiment may help in our understanding of this issue.

In conclusion, to our knowledge, this is the first study to provide evidence of the relationship between extracellular 5-HT and the seizure-inducing potential of tramadol. We found that depletion of 5-HT resulted in a lowered threshold for seizure induction, whereas elevated 5-HT raised this threshold. Furthermore, serotonergic receptor antagonism lowered the threshold. Accordingly, SS, which is caused by excessive 5-HT, is undoubtedly different from tramadol-induced seizures. Thus, in the clinical setting, we should be careful when a tramadol user manifests seizure-like symptoms, since the therapeutics for SS will aggravate tramadol-induced seizures. We also showed that tramadol does not elevate brain histamine content, and thus, histamine is likely not involved in the development of tramadol-induced seizures.

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### Competing Interests

The authors declare no competing interests.

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