Antiemetic Effects of Serotonergic 5-HT_{1A}-Receptor Agonists in *Suncus Murinus*

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ABSTRACT—The antiemetic effects of six serotonergic 5-HT_{1A}-receptor agonists, 8-hydroxy-2-(di-n-propylamino)tetrarin (8-OH-DPAT), 4-{4-[4-(2-pyrimidinyl)piperazin-1-yl]butyl}-2,3,4,5-tetrahydro-1,4-benzoazepine-3,5-dione (SUN8399), buspirone, gepirone, ipsapirone and tandospirone, against motion sickness were investigated in *Suncus murinus*. Subcutaneous injection of all six agonists completely and dose-dependently suppressed motion-induced emesis. Pretreatment with 8-OH-DPAT or SUN8399 dose-dependently inhibited emesis elicited by nicotine (4.0 mg/kg, s.c.), veratrine (0.7 mg/kg, s.c.), cisplatin (20 mg/kg, i.p.) and copper sulfate (40 mg/kg, p.o.). These results suggest that serotonergic 5-HT_{1A}-receptor agonists are effective as anti-motion sickness drugs, and these drugs may block a common mechanism(s) for the emetic reflex of the suncus because the antiemetic effects of the 5-HT_{1A}-receptor agonists were exerted irrespective of the stimulus.

Keywords: 5-HT_{1A} receptor, Motion sickness, Emesis, Suncus, Cisplatin

The purposes of this study were several fold: 1) to determine if 8-OH-DPAT and buspirone prevent motion-induced emesis in the suncus, 2) if so, to determine if they have anti-motion sickness effects in common to those of other 5-HT_{1A}-receptor agonists, including ipsapirone, tandospirone, gepirone and a novel agonist, 4-{4-[4-(2-pyrimidinyl)piperazin-1-yl]butyl}-2,3,4,5-tetrahydro-1,4-benzoazepine-3,5-dione (SUN8399) (9), and 3) to investigate the inhibitory effects of the 5-HT_{1A}-receptor agonists against emetic drug-induced emesis.

MATERIALS AND METHODS

Experiments were performed on 3- to 6-month-old male *Suncus murinus* weighing 50–90 g. The animals were purchased from the Central Institute for Experimental Animals (Kanagawa) and housed in a temperature controlled room at 24±1 °C at The Animal Care Institute of The University of Tokyo. They were allowed free access to pellet chow (Central Institute for Experimental Animals) and tap water.

Experimental conditions were similar to those reported previously from our laboratory (7, 8, 10). Emetic stimuli employed were motion and four emetic drugs. For the experiment of motion sickness, animals were selected for susceptibility to motion sickness. Generally more than...
90% of the animals vomited during the motion stimulus. An interval longer than one week was kept between the motion tests to avoid the involvement of habituation to the motion stimulus. Motion sickness was elicited by reciprocal shaking (amplitude of 40 mm, frequency of 1.0 Hz, duration of 5 min). Each of the animals was placed in a transparent cage (10W x 15L x 12H cm) fixed on a reciprocal shaker (TAITEC R-30mini; Taiyo Scientific Industrial Co., Ltd., Saitama). After a 5-min acclimation, the motion stimulus was started. The number of vomiting episodes and the latency to the first vomiting were recorded for 5 min.

Emetic drugs used were subcutaneous injection of nicotine (4.0 mg/kg) and veratrine (0.7 mg/kg), oral administration of copper sulfate (40 mg/kg) and intraperitoneal injection of cisplatin (20 mg/kg). Each drug except for cisplatin was dissolved in sterile saline to an injection volume of 0.1 ml/50 g body weight. In the case of cisplatin, the pH of the solvent saline was adjusted to 4.0 using 0.1 N HCl. Then cisplatin was dissolved with this acidified saline, by sonication and warming to 50°C, in the final concentration of 2 mg/ml. After being cooled to 37°C, the solution was administered with an injection volume of 0.5 ml/50 g body weight. The number of vomiting episodes and the latency to the first vomiting were recorded for 30 min (nicotine, veratrine and copper sulfate) or 90 min (cisplatin).

5-HT_{1A}-receptor agonists used were 8-hydroxy-2-(di-n-propylamino)tetrarin (8-OH-DPAT), buspirone (RBI, Natick, MA, USA), gepirone, ipsapirone, tandospirone and 4-[(4-(2-pyrimidinyl)piperazin-1-yl)butyl]-2,3,4,5-tetrahydro-1,4-benzoxazepine-3,5-dione (SUN8399; Suntory Ltd., Osaka). These 5-HT_{1A}-receptor agonists were dissolved in saline. Each drug solution was subcutaneously injected into the shoulder region of the animals 30 min before application of the emetic stimuli. The injection volume was adjusted to 0.1 ml/50 g body weight. ID_{50} values were calculated by Brownlee's up-and-down method (11).

RESULTS

Pretreatment with six 5-HT_{1A}-receptor agonists completely and dose-dependently prevented motion-induced emesis in the suncus (Table 1). In terms of ID_{50} values, 8-OH-DPAT was the most potent followed by ipsapirone, *Table 1. Effects of 5-HT_{1A}-receptor agonists on motion-induced emesis in *Suncus murinus*

| Agonists   | Dose (µg/kg) | No. of suncus vomiting/tested | No. of vomiting episodes | Latency (sec) | ID_{50} Values (µg/kg) |
|------------|--------------|-------------------------------|--------------------------|---------------|------------------------|
| 8-OH-DPAT  |              |                               |                          |               |                        |
| 11.3       | 1/1          | 4                             | 140                      | 36.7          |
| 22.6       | 4/4          | 5.0 ± 1.7                     | 157 ± 38                 |
| 45.2       | 1/5          | 1                             | 269                      |
| 90.4       | 0/1          | —                             | —                        |
| Ipsapirone |              |                               |                          |               |                        |
| 62.5       | 1/1          | 4                             | 259                      | 154           |
| 125        | 4/5          | 6.3 ± 2.0                     | 172 ± 27                 |
| 250        | 0/4          | —                             | —                        |
| SUN8399    |              |                               |                          |               |                        |
| 125        | 1/1          | 8                             | 126                      | 406           |
| 250        | 4/4          | 7.3 ± 1.9                     | 74 ± 19                  |
| 500        | 1/5          | 3                             | 100                      |
| 1000       | 0/1          | —                             | —                        |
| Tandospirone |            |                               |                          |               |                        |
| 500        | 3/3          | 6.3 ± 1.2                     | 51 ± 19                  | 1230          |
| 1000       | 2/4          | 4.10                          | 86 ± 26                  |
| 2000       | 1/2          | 10                            | 57                       |
| 4000       | 0/1          | —                             | —                        |
| Gepirone   |              |                               |                          |               |                        |
| 625        | 3/3          | 5.3 ± 1.8                     | 93 ± 42                  | 1340          |
| 1250       | 3/5          | 2.3 ± 0.9                     | 131 ± 23                 |
| 2500       | 0/2          | —                             | —                        |
| Buspirone  |              |                               |                          |               |                        |
| 500        | 2/2          | 3, 10                         | 279, 63                  | 1870          |
| 1000       | 2/3          | 8, 15                         | 69, 23                   |
| 2000       | 2/3          | 1, 7                          | 250, 96                  |
| 4000       | 1/3          | 1                             | 195                      |

ID_{50} values were calculated using the up-and-down method. Values for the number of vomiting episodes and the latency per vomiting animal are given as the mean ± S.E.M., but actual values are indicated when the number of vomiting animals was less than three.
SUN8399, tandospirone, gepirone and buspirone. The respective ID$_{50}$ values of these drugs were 36.7, 154, 406, 1230, 1340 and 1870 pg/kg. Flat body posture, which is a symptom of the serotonin syndrome, was observed when doses higher than 90.4 (8-OH-DPAT), 1000 (ipsapirone, SUN8399, tandospirone and gepirone) and 2000 (buspirone) pg/kg were used.

Since the 5-HT$_{1A}$-receptor agonists showed very potent prophylactic effects on motion-induced emesis, we tested whether these 5-HT$_{1A}$-receptor agonists block emesis induced by other emetic stimuli. The emetic stimuli employed were nicotine, veratrine, cisplatin and copper sulfate (Tables 2 and 3). The 5-HT$_{1A}$-receptor agonists tested were 8-OH-DPAT and SUN8399. Subcutaneous injection of 8-OH-DPAT and SUN8399 completely inhibited the emesis elicited by nicotine, veratrine, cisplatin and copper sulfate. The ID$_{50}$ values of 8-OH-DPAT against the emesis induced by nicotine, veratrine, cisplatin and copper sulfate were 46.7, 121, 857 and 286 μg/kg, respectively (Table 2). The respective ID$_{50}$ values of SUN8399 were 353, 515, 707 and 985 μg/kg (Table 3).

The administration of 8-OH-DPAT at doses higher than 1.6 mg/kg induced excitation and increased spontaneous motor activity in the suncus. At doses above 1000 μg/kg, SUN8399 increased the spontaneous motor activity, which lasted for 10 min.

**DISCUSSION**

Pretreatment with six 5-HT$_{1A}$-receptor agonists completely prevented motion-induced emesis. The present results support Lucot's findings that 8-OH-DPAT and buspirone block motion-induced emesis (3, 5), and they further show that the effects are common to 5-HT$_{1A}$-receptor agonists and are not restricted to their effects in cats. Because subcutaneous injection of all agonists caused the serotonin syndrome, the drugs must have acted at least on the central nervous system. The ID$_{50}$ values of agonists except for ipsapirone and gepirone were parallel to the affinity of binding at the 5-HT$_{1A}$ receptor (I. Hirotsu et al., per-
8-OH-DPAT, which has the strongest affinity among the six agonists, prevented motion sickness with the smallest ID$_{50}$ value. These results suggest that stimulation of 5-HT$_{1A}$ receptors by these agonists mainly suppressed the motion sickness, but the role of other receptors cannot be eliminated. Ipsapirone and gepirone, which have weaker affinities of binding at 5-HT$_{1A}$ receptors than buspirone and tandospirone, suppressed motion-induced emesis with a smaller ID$_{50}$ value than those of buspirone and tandospirone. Both ipsapirone and gepirone have stronger affinity of binding at the a$_2$-adrenoceptor than other agonists. It seems possible that a$_2$-adrenoceptors participate in the neural pathways of motion sickness. Xylazine, an a$_2$-adrenoceptor agonist, elicited emesis in cats, and yohimbine blocked xylazine-induced emesis. But yohimbine could not prevent motion sickness in the cat (12). Therefore, the role of a$_2$-adrenoceptors in motion sickness is not significant.

Antihistamines (e.g., diphenhydramine) are widely used as a main medication against motion sickness. We have already reported the effects of antihistamines on motion-induced emesis in Suncus murinus (8). The ID$_{50}$ values for antihistamines were 34 to 160 µmol/kg (T. Kaji et al., unpublished data). In this study, the ID$_{50}$ values of six 5-HT$_{1A}$-receptor agonists ranged between 36 to 1870 pg/kg or 0.2 to 4.9 pmol/kg. Therefore, 5-HT$_{1A}$ receptor agonists are more effective against motion-induced emesis than the antihistamines. Pretreatment with 8-OH-DPAT and SUN8399 also completely inhibited nicotine-, veratrine-, cisplatin and copper sulfate-induced emesis. In cats, nicotine acts on the central and peripheral nervous system (13), and veratrine stimulates nodose ganglions (14). Oral administration of copper sulfate stimulates the mucous membrane of the stomach. Peripheral 5-HT$_3$ receptors are involved in the cisplatin-induced emesis (10). These drugs appear to stimulate different neural pathways in the emetic reflex. It is possible that the antiemetic effects of 8-OH-DPAT and SUN8399 were caused

### Table 3. Effects of SUN8399 on emetic drugs-induced emesis in Suncus murinus

| Emetic drug          | Pretreatment (µg/kg) | No. of suncus vomiting/tested | No. of vomiting episodes | Latency (min) | ID$_{50}$ values (µg/kg) |
|----------------------|----------------------|-------------------------------|--------------------------|---------------|--------------------------|
| Nicotine (4 mg/kg, s.c.) | saline               | 2/2                           | 18, 22                   | 5, 5          | 353                      |
|                      | SUN8399              |                               |                          |               |                          |
|                      | 50                   | 1/1                           | 19                       | 5             |                          |
|                      | 100                  | 2/2                           | 16, 10                   | 5, 8          |                          |
|                      | 200                  | 4/5                           | 5.8 ± 3.0                | 6.8 ± 1.7     |                          |
|                      | 400                  | 3/4                           | 1.0 ± 0.0                | 11.0 ± 1.0    |                          |
|                      | 800                  | 0/2                           | —                        | —             |                          |
| Veratrine (0.7 mg/kg, s.c.) | saline               | 1/1                           | 18                       | 5             | 515                      |
|                      | SUN8399              |                               |                          |               |                          |
|                      | 200                  | 1/1                           | 2                        | 9             |                          |
|                      | 400                  | 3/5                           | 5.7 ± 2.2                | 11.3 ± 3.5    |                          |
|                      | 800                  | 1/5                           | 2                        | 21            |                          |
|                      | 1600                 | 0/1                           | —                        | —             |                          |
| Cisplatin (20 mg/kg, i.p.) | saline               | 2/2                           | 8, 23                    | 48, 23        | 707                      |
|                      | SUN8399              |                               |                          |               |                          |
|                      | 250                  | 2/3                           | 8, 26                    | 65, 29        |                          |
|                      | 500                  | 4/5                           | 8.5 ± 2.1                | 51.5 ± 5.3    |                          |
|                      | 1000                 | 1/4                           | 2                        | 60            |                          |
|                      | 2000                 | 0/1                           | —                        | —             |                          |
|                      | 4000                 | 0/1                           | —                        | —             |                          |
| Copper sulfate (40 mg/kg, p.o.) | saline               | 2/2                           | 5, 8                     | 1, 1          | 985                      |
|                      | SUN8399              |                               |                          |               |                          |
|                      | 400                  | 1/1                           | 4                        | 2             |                          |
|                      | 800                  | 4/5                           | 1.3 ± 0.3                | 16.5 ± 3.8    |                          |
|                      | 1600                 | 0/4                           | —                        | —             |                          |

ID$_{50}$ values were calculated using the up-and-down method. Values for the number of vomiting episodes and the latency per vomiting animal are given as the mean±S.E.M., but actual values are indicated when the number of vomiting animals was less than three.
by action on common neural pathway(s) of the emetic reflex in the central nervous system, such as the so-called "vomiting center".

Another area of medicine in which effective antiemetic drugs are necessary is the emesis induced by chemotherapeutic agents and postoperative emesis (2). The possibility of using 5-HT₁A-receptor agonists as antiemetics against chemotherapy-induced emesis may be low because 5-HT₃-receptor antagonists are available. These antagonists are used clinically today. The antiemetic effect of 5-HT₁-receptor antagonists is selective against the emesis induced by chemotherapeutic agents, and these antagonists do not prevent emesis induced by motion, veratrine, nicotine or copper sulfate (10). It is reported that cisplatin increases the releases of 5-HT and 5-HIAA in guinea pig small intestine (15). 5-HT itself causes emesis that is blocked by a 5-HT₂-receptor antagonist in suncus (16). Both cisplatin- and 5-HT-induced emeses are completely prevented by surgical vagotomy (16, 17). Taken together, cancer chemotherapeutic agents probably cause the release of 5-HT from the enterochromaffin cells, and the released 5-HT stimulates 5-HT₃ receptors located on the vagal afferents. Then the impulses are transmitted directly or indirectly to the so-called "vomiting center" and cause emesis.

Against postoperative emesis, scopolamine, antihistamines and neuroleptic agents are widely used as premedicants; however, the effects of these drugs do not achieve a 100% protection against postoperative emesis (2). There is a need for development of effective antiemetic agents. The etiology of postoperative emesis is usually multifactorial (18). The main causes include inhalation anesthetic agents, opioid analgesics and distension of the stomach or intestines (19). In this study, 5-HT₁A-receptor agonists showed a wide spectrum of antiemetic effects. These results suggest that the 5-HT₁A-receptor agonists may be ideal antiemetic agents against postoperative emesis.

As mentioned above, in the case of cancer chemotherapeutics-induced emesis, 5-HT acts peripherally. In the present study, 5-HT₁A-receptor agonists showed an antiemetic effect that is exerted irrespective of the emetic stimulus. These results suggest that the stimulation of 5-HT₁A receptors in the central nervous system may cause the antiemetic effect. It is interesting to clarify the role of 5-HT in the brain with respect to emesis. If the antiemetic effects of 5-HT₁A-receptor agonists are due to the stimulation of presynaptic autoreceptors, the release of 5-HT will be decreased and activation of serotonergic neurons may be emetogenic. However, if the site of action is postsynaptic, serotonergic neurons may be inhibitory against emesis. Further investigations are necessary to clarify the physiological roles of endogeneous 5-HT in the central nervous system.

In conclusion, the results of the present study suggest that 5-HT₁A-receptor agonists are more effective as antimotion sickness drugs than is the medicine used in humans and that 5-HT₁A-receptor agonists can be used as a general antiemetic drug. It is possible that a 5-HT₁A receptor-mediated mechanism(s) is involved in the emetic reflex of the suncus.

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