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# Antiemetic effects of N-3389, a newly synthesized 5-$HT_3$ and 5-$HT_4$ receptor antagonist, in ferrets

Masaru Minami [a,*], Toru Endo [a], Hiroyuki Tamakai [a], Takashi Ogawa [a], Naoya Hamaue [a], Masahiko Hirafuji [a], Yoshio Monma [a], Mitsuhiro Yoshioka [b], Koichiro Hagihara [c]

[a] *Department of Pharmacology, Faculty of Pharmaceutical Sciences, Health Sciences University of Hokkaido, 1757 Kanazawa, Ishikari-Tobetsu, Hokkaido 061-02, Japan*

[b] *Department of Pharmacology, Hokkaido University School of Medicine, Sapporo 060, Japan*

[c] *Pharmaceutical Research Center, Nisshin Flour Milling Co. Ltd., Saitama 356, Japan*

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**Abstract**

The antiemetic activity of N-3389 (*endo*-3,9-dimethyl-3,9-diazabicyclo[3,3,1]non-7-yl-1*H*-indazole-3-carboxamide dihydrochloride), a new 5-$HT_3$ and 5-$HT_4$ receptor antagonist, against cisplatin-, cyclophosphamide- and copper sulfate-induced emesis was investigated using ferrets. We also examined the effects of these agents on abdominal afferent vagus nerve activity in anesthetized ferrets. Both intraperitoneal (0.1–1.0 mg/kg) and oral (0.1–1.0 mg/kg) administration of N-3389 produced dose-dependent antiemetic effects. The time-course of cisplatin (10 mg/kg, i.p.)-induced emesis in another group of ferrets paralleled the increase in abdominal afferent vagus nerve activity induced by cisplatin (10 mg/kg, i.p.) and was inhibited by pretreatment with N-3389 (1.0 mg/kg, i.v.). Furthermore, the cisplatin (10 mg/kg, i.p.)-induced increase in abdominal afferent vagus nerve activity was markedly reduced by an additional injection of N-3389 (0.1–1.0 mg/kg, i.v.) in a dose-dependent manner. The antiemetic effects exhibited by N-3389 are probably due to the inhibition of 5-$HT_3$ and 5-$HT_4$ receptors on the abdominal afferent vagus nerves.

*Keywords:* N-3389; 5-$HT_3$ receptor antagonist; 5-$HT_4$ receptor antagonist; Antiemetic effect; Abdominal afferent vagus nerve activity; (Ferret)

## 1. Introduction

In the peripheral nervous system, the myenteric nerves are a therapeutic target for benzamide 5-$HT_4$ receptor agonists (Bockaert et al., 1994). These 5-$HT_4$ receptor agonists act as gastroprokinetic drugs, increasing gastric peristalsis (Bockaert et al., 1992). Both the mechanisms of action of 5-$HT_4$ receptor agonists and antagonists as well as the distribution of 5-$HT_4$ receptors may be useful therapeutic targets for the treatment of emesis. Although it is well known that 5-$HT_3$ receptor antagonists block anticancer drug-induced emesis in several species (Costall et al., 1986; Miner and Sanger, 1986; Hawthorn et al., 1988; Gylys et al., 1988; Smith et al., 1988; Bhandari et al., 1989; Endo et al., 1990; Matsuki et al., 1990), they are ineffective in the treatment of apomorphine-induced or copper sulfate-induced emesis and motion sickness.

We previously demonstrated that copper sulfate-induced emesis in ferrets is inhibited by a high oral dose of ondansetron (5 mg/kg), a 5-$HT_3$ receptor antagonist (Endo et al., 1991). Bhandari and Andrews (1991) have demonstrated in ferrets that copper sulfate-induced emesis is inhibited by a high dose of ICS205-930 ((3α-tropanyl)-1*H*-indole-3-carboxylic acid ester) (1 mg/kg, s.c.), but not by ondansetron or granisetron at the same dose. ICS205-930 has been reported to block 5-$HT_3$ receptors at low concentrations and 5-$HT_4$ receptors at high concentrations in vitro (Dumuis et al., 1988) and in vivo (Villalon et al., 1990; Sancilio et al., 1990).

Fukui et al. (1994) reported that oral administration of a 5-$HT_4$ receptor agonist, 5-methoxytryptamine (5-MT), caused vomiting at a dose of 100 mg/kg in dogs. Furthermore, emesis induced by either 5-MT or copper sulfate was inhibited by vagotomy or a high dose (1 mg/kg, i.v.) of ICS205-930. Their report indicated that the abdominal afferent vagus nerve and peripheral 5-$HT_4$ receptors play an important role in copper sulfate- and 5-MT-induced emesis. We previously reported that cisplatin, cyclophos-

* Corresponding author. Tel.: (81-1332) 31211; Fax: (81-1332) 31370.


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phamide and copper sulfate increase 5-HT levels in both the intestinal mucosa (Endo et al., 1991) and the area postrema (Endo et al., 1992). Furthermore, we demonstrated that abdominal vagotomy reduced the number of emetic episodes by 85% and simultaneously reduced 5-HT levels in the area postrema (Endo et al., 1992). It is proposed that the vomiting center receives input from the afferent discharge of vagal fibers, which evokes an emetic reflex.

5-$HT_3$ receptors are located on the peripheral terminals of the vagal afferents as one of the sites of peripheral action (Richardson et al., 1985; Round and Wallis, 1986; Ireland and Tyers, 1987). 5-$HT_4$ receptors have been reported to be located in the central nervous system (CNS) as well as in peripheral tissues (Craig et al., 1990; Baxter et al., 1991; Elswood et al., 1991), including the vagus (Rhodes et al., 1992). The issue of the involvement of 5-$HT_4$ receptors in emesis is controversial. With regard to 5-HT release, Gebauer et al. (1993) suggested that stimulation of 5-$HT_4$ autoreceptors on enterochromaffin (EC) cells causes inhibition of 5-HT release in the guinea-pig intestine. In the rat, however, 5-MT induces 5-HT release from the isolated intestine (Minami et al., 1995a). With regard to the involvement of 5-$HT_4$ receptor agonists and antagonists in emesis, zacopride (a 5-$HT_3$ receptor antagonist and 5-$HT_4$ receptor agonist) is emetic when given alone. This effect was originally ascribed to 5-$HT_4$ receptor stimulation (Bhandari and Andrews, 1991). Fukui et al. (1994) reported that 5-MT induced a dose-dependent emesis as described above. However, 5-MT failed to increase the discharge of vagal afferent fibers in the rat in vitro (Tonini, 1995) and the selective 5-$HT_4$ receptor antagonist, GR125487 (1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl-5-fluoro-2-methoxy-1*H*-indole-3-carboxylate), failed to prevent zacopride-induced emesis in the ferret (Twissell et al., 1995).

We have synthesized a potent 5-$HT_3$ receptor antagonist, N-3389 (*endo*-3,9-dimethyl-3,9-diazabicyclo[3,3,1]non-7-yl-1*H*-indazole-3-carboxamide dihydrochloride), which also has affinity for 5-$HT_4$ receptors (Fig. 1). N-3389 exhibits potent 5-$HT_3$ receptor antagonist activity in a radioligand assay ($pK_i = 8.77$) and against 2-methyl-5-HT -induced bradycardia in rats ($ED_{50} = 0.73$ μg/kg, i.v.) (Hagihara et al., 1994). The activity is almost the same as that of selective 5-$HT_3$ receptor antagonists such as granisetron and ondansetron. N-3389 also inhibits 2-methyl-5-HT-induced contractions in longitudinal muscle myenteric plexus preparations of guinea-pig ileum ($IC_{50} = 3.2 \times 10^{-8}$ M) and its activity is more potent than that of granisetron and ondansetron. In addition, N-3389 ($10^{-7}$–$10^{-5}$M) inhibits both the 5-$HT_3$ receptor antagonist sensitive-phase and 5-$HT_4$ receptor antagonist sensitive-phase of contractions induced by 5-HT in longitudinal muscle myenteric plexus preparations of the guinea-pig ileum (Hagihara et al., 1994)

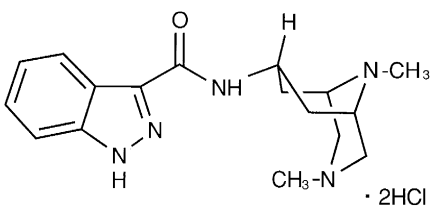


Fig. 1. Chemical structure of N-3389.

In this study, we investigated the action of N-3389 on both anticancer drug- and copper sulfate-induced emesis. We have directly examined abdominal afferent vagal nerve activity using an electrophysiological method reported previously (Yoshioka et al., 1992; Endo et al., 1995). The present experiments were designed to follow up on these observations by investigating the effect of emetic agents and the action of N-3389 on the rate of afferent discharge from abdominal vagus nerves. The time-course over which these agents induced emesis was also measured in another group of ferrets. Finally, we investigated whether abdominal afferent vagus nerve activity was mediated by 5-HT, 2-methyl-5-HT or 5-MT.

## 2. Materials and methods

### 2.1. Animals and behavioral experiments

Experiments were performed using ferrets weighing on average 1.4 (1.0–1.8) kg. Adult male fitch ferrets (*Mustela putorius furo L.*) were supplied by Marshall Research Animals (North Rose, NY, USA) and fed on cat chow (Purina Taiyo, Japan) and allowed water ad libitum.

The effects of N-3389 on the retching and vomiting induced by cisplatin, cyclophosphamide and copper sulfate were studied using the method of Stables et al. (1987). All animals moved freely in their cages. Following administration of one of the cytotoxic drugs, the ferrets were placed in individual cages and observed continuously for 6 h. N-3389 was administered orally or intraperitoneally at 0.01–5.0 mg/kg before the administration of cisplatin (10 mg/kg, i.p.), cyclophosphamide (200 mg/kg, i.p.) or copper sulfate (40 mg/kg, p.o.). The frequency of retching and vomiting, the latency to first emesis (retching) and its duration were carefully monitored.

### 2.2. Nerve recording and general surgical procedure

Ferrets were anesthetized with 500 mg/kg (i.p.) urethane and 50 mg/kg (i.p.) α-chloralose. After immobilization with gallamine triethiodide (10 mg/kg, i.v.), respiration was maintained through a tracheal cannula connected to a Harvard respirator (model 683, USA). By using an expired gas monitor (Nippondenki San-ei IH26, Tokyo, Japan), ventilation was adjusted to maintain end tidal $O_2$ and $CO_2$ at approximately 15% and 5.0%, respectively. Cisplatin and N-3389 were injected intraperitoneally. Other

Table 1
The inhibitory effects of N-3389 (i.p.) on cisplatin-induced emesis in the ferret

| Dose (mg/kg) | No. of animals retching/tested | No. of retching episodes | No. of vomiting episodes | Latency to retching (min) | Duration of retching (min) |
|---|---|---|---|---|---|
| Cisplatin (10) | 6/6 | 142.3 ± 15.7 | 14.3 ± 3.3 | 98.2 ± 6.4 | 183.2 ± 18.5 |
| N-3389 (0.01) + cisplatin (10) | 6/6 | 140.7 ± 40.2 | 15.7 ± 4.8 | 106.5 ± 8.8 | 189.7 ± 16.5 |
| N-3389 (0.1) + cisplatin (10) | 4/6 | 31.5 ± 11.2 [b] | 3.0 ± 1.2 | 264.5 ± 30.8 [b] | 80.3 ± 24.7 [b] |
| N-3389 (1.0) + cisplatin (10) | 5/6 | 19.0 ± 6.8 [b] | 0.8 ± 0.5 [b] | 289.5 ± 32.4 [b] | 21.4 ± 12.9 [b] |

The data represent the means ± S.E. from six animals. [b] Values are significantly different ($P < 0.01$) from cisplatin (10).

drugs were administered intravenously through a catheter inserted into the jugular vein. Rectal temperature was maintained between 37 and 38°C with a heating pad. The dorsal abdominal vagus nerve was exposed and sectioned under an operating microscope. Abdominal afferent vagus nerve activity was recorded from the peripheral cut end of the nerve with bipolar platinum-iridium wire electrodes according to the method of Yoshioka et al. (1992). The nerve was immersed in a pool of warm paraffin oil. Nerve activity was amplified, passed through a filter (time constant 6.7 ms and high-cut filter 1000 Hz) and displayed on a thermal array recorder (Nihon Kohden, RTA-1300M, Tokyo, Japan). The discharge rate counted every 10 s was analyzed after conversion of raw data to standard pulses by a window discriminator that could distinguish the discharge of afferent fibers from background noise. The histogram plot used in the present study holds each value for 10 s. There is a time lag between the histogram and real discharge, i.e., the histogram follows the actual nerve discharge after 10 s.

### 2.3. Drugs

In order to obtain a stable solution with comparable emetic activity, the cytotoxic drugs used in this study were commercial cisplatin (Nippon Kayaku, Tokyo, Japan) and cyclophosphamide (Shionogi, Osaka, Japan). Copper sulfate (Wako, Osaka, Japan) was dissolved in distilled water and administered intragastrically by stomach tube at a volume of 5.0 ml/kg.

The following drugs were used: 5-HT creatinine sulfate (Sigma, St. Louis, MO, USA); 2-methyl-5-HT (Research Biochemicals International, Natick, MA, USA); 5-MT hydrochloride (Sigma); N-3389 (Nisshin Flour Milling, Saitama, Japan). These drugs were dissolved in normal saline. An equal volume of physiological saline was administered to the control animals. Dose–response curves for 5-HT, 2-methyl-5-HT and 5-MT were made noncumulatively with a dose cycle of more than 10 min to avoid tachyphylaxis (Ginzel and Kattegoda, 1954).

### 2.4. Statistical analysis

All values are given as means ± S.E. The significance of differences between two mean values was assessed using Student's *t*-test. Analysis of variance, followed by the Bonferroni modified *t*-test (Wallenstein et al., 1980), was used to compare more than two groups. A $P < 0.05$ was considered to be statistically significant.

## 3. Results

### 3.1. Emetic effects of cisplatin, cyclophosphamide and copper sulfate and the antiemetic effects of N-3389

#### 3.1.1. Cisplatin-induced emesis in ferrets

We previously reported that cisplatin caused emesis at doses of 10 mg/kg (i.p.) (6 animals retching/6 tested) and

Table 2
The inhibitory effects of N-3389 (p.o.) on cisplatin-induced emesis in the ferret

| Dose (mg/kg) | No. of animals retching/tested | No. of retching episodes | No. of vomiting episodes | Latency to retching (min) | Duration of retching (min) |
|---|---|---|---|---|---|
| Cisplatin (10) | 8/8 | 126.0 ± 15.8 | 11.9 ± 3.0 | 104.8 ± 6.5 | 176.9 ± 14.3 |
| N-3389 (0.01) + cisplatin (10) | 6/6 | 123.7 ± 24.8 | 18.2 ± 4.3 | 107.0 ± 6.2 | 205.8 ± 20.0 |
| N-3389 (0.1) + cisplatin (10) | 6/6 | 43.8 ± 14.7 | 3.7 ± 1.6 | 191.8 ± 13.4 [a] | 113.8 ± 20.2 [a] |
| N-3389 (1.0) + cisplatin (10) | 3/6 | 2.0 ± 1.0 [b] | 0.3 ± 0.2 [b] | 316.7 ± 22.0 [b] | 4.7 ± 3.2 [b] |

The data represent the means ± S.E. from six to eight animals. Values are significantly different ([a] $P < 0.05$, [b] $P < 0.01$) from cisplatin (10), respectively.

Table 3
The inhibitory effects of N-3389 (i.p.) on cyclophosphamide-induced emesis in the ferret

| Dose (mg/kg) | No. of animals retching/tested | No. of retching episodes | No. of vomiting episodes | Latency to retching (min) | Duration of retching (min) |
|---|---|---|---|---|---|
| Cyclophosphamide (200) | 6/6 | 149.2 ± 22.2 | 22.0 ± 2.6 | 15.8 ± 0.5 | 236.5 ± 23.2 |
| N-3389 (0.1) + cyclophosphamide (200) | 6/6 | 78.3 ± 19.8 [a] | 8.7 ± 2.5 [a] | 167.8 ± 40.3 | 120.0 ± 44.1 [a] |
| N-3389 (1.0) + cyclophosphamide (200) | 4/6 | 24.1 ± 11.5 [b] | 2.0 ± 0.9 [b] | 298.3 ± 21.1 [b] | 40.0 ± 24.2 [b] |

The data represent the means ± S.E. from six animals. Values are significantly different ([a] $P < 0.05$, [b] $P < 0.01$) from cisplatin (10), respectively.

Table 4
The inhibitory effects of N-3389 (p.o.) on cyclophosphamide-induced emesis in the ferret

| Dose (mg/kg) | No. of animals retching/tested | No. of retching episodes | No. of vomiting episodes | Latency to retching (min) | Duration of retching (min) |
|---|---|---|---|---|---|
| Cyclophosphamide (200) | 10/10 | 136.6 ± 16.7 | 19.7 ± 1.9 | 19.0 ± 1.7 | 230.0 ± 16.1 |
| N-3389 (0.1) + cyclophosphamide (200) | 7/7 | 104.4 ± 29.6 | 11.7 ± 3.7 | 109.4 ± 21.2 [a] | 155.6 ± 17.6 [a] |
| N-3389 (1.0) + cyclophosphamide (200) | 2/6 | 5.5 ± 3.5 [b] | 0 ± 0 [b] | 330.0 ± 21.4 [b] | 42.0 ± 4.0 [b] |

The data represent the means ± S.E. from six to ten animals. Values are significantly different ([a] $P < 0.05$, [b] $P < 0.01$) from cisplatin (10), respectively.

Table 5
The inhibitory effects of N-3389 (p.o.) on copper sulfate-induced emesis in the ferret

| Dose (mg/kg) | No. of animals retching/tested | No. of retching episodes | No. of vomiting episodes | Latency to retching (min) | Duration of retching (min) |
|---|---|---|---|---|---|
| $CuSO_4$ (40) | 6/6 | 68.8 ± 22.7 | 9.3 ± 2.7 | 5.2 ± 1.0 | 95.3 ± 29.7 |
| N-3389 (1.0) + $CuSO_4$ (40) | 5/6 | 24.7 ± 10.6 | 1.8 ± 0.5 | 99.3 ± 53.9 [a] | 12.8 ± 9.6 |
| N-3389 (5.0) + $CuSO_4$ (40) | 5/6 | 14.7 ± 6.2 [a] | 1.2 ± 0.5 [a] | 120.0 ± 53.5 [a] | 4.8 ± 3.3 [a] |

The data represent the means ± S.E. from six animals. Values are significantly different ([a] $P < 0.05$) from cisplatin (10).

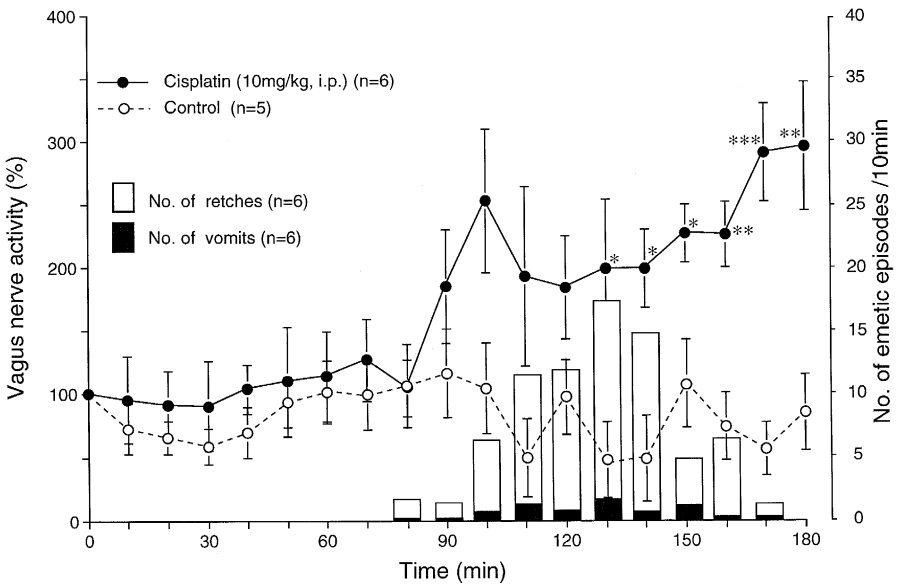


Fig. 2. Effects of cisplatin on abdominal vagus nerve activity and mode of emetic action in ferrets. Behavioral data obtained from a separate group of ferrets. Each data point represents the mean ± S.E. from five to six animals. For the column of emetic episodes $n = 6$, Significant differences ([*] $P < 0.05$, [**] $P < 0.01$, [***] $P < 0.001$) from control animals, respectively.

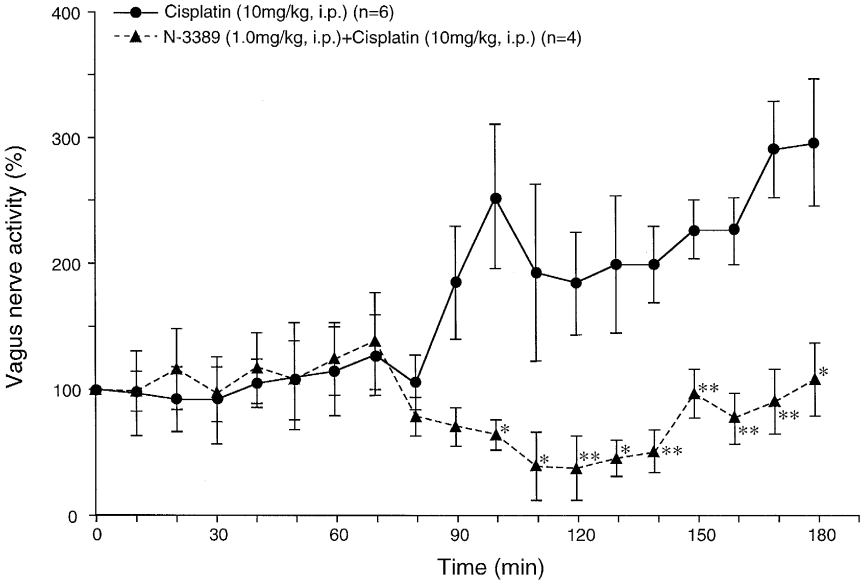


Fig. 3. Effects of pretreatment with N-3389 on cisplatin-induced changes in abdominal vagus nerve activity of anesthetized ferrets. Each data point represents the mean ± S.E. from four to six animals. Significant differences ( * $P < 0.05$, * * $P < 0.01$) from control animals, respectively.

7 mg/kg (2/3) but not at 5 mg/kg (Endo et al., 1990). In this study, the inhibitory effect of N-3389 given 30 min before an injection of 10 mg/kg cisplatin was tested in ferrets. Both intraperitoneal (i.p.) (Table 1) and oral administration (p.o.) (Table 2) of N-3389 (0.01–1.0 mg/kg) produced dose-dependent antiemetic effects against cisplatin-induced emesis. As shown in Table 1, N-3389 markedly delayed the onset of cisplatin-induced emesis and significantly reduced its frequency and duration. An oral 1.0 mg/kg dose of N-3389 prior to cisplatin injection (Table 2) was followed by less vomiting than was observed after an intraperitoneal dose (Table 1).

### *3.1.2. Cyclophosphamide-induced emesis in ferrets*

The results for the emesis induced by cyclophosphamide (200 mg/kg, i.p.) are summarized in Tables 3 and 4. Intraperitoneal injection (Table 3) and oral administration (Table 4) of N-3389 given 30 min before cyclophosphamide also reduced the number of emetic episodes in a dose-dependent manner. Oral administration of N-3389

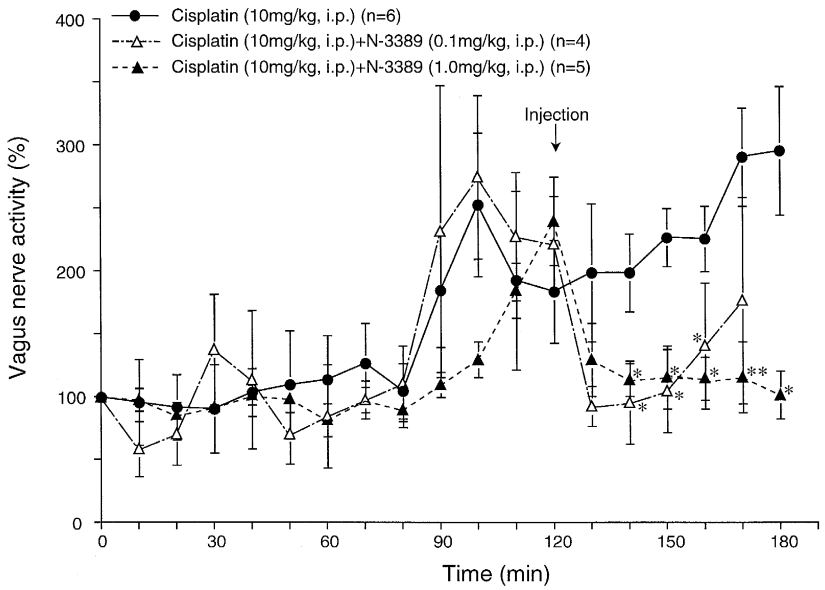


Fig. 4. Effects of N-3389 on increased abdominal vagus nerve activity induced by cisplatin in anesthetized ferrets. Each data point represents the mean ± S.E. from four to six animals. Significant differences ( * $P < 0.05$, * * $P < 0.01$) from control animals, respectively.

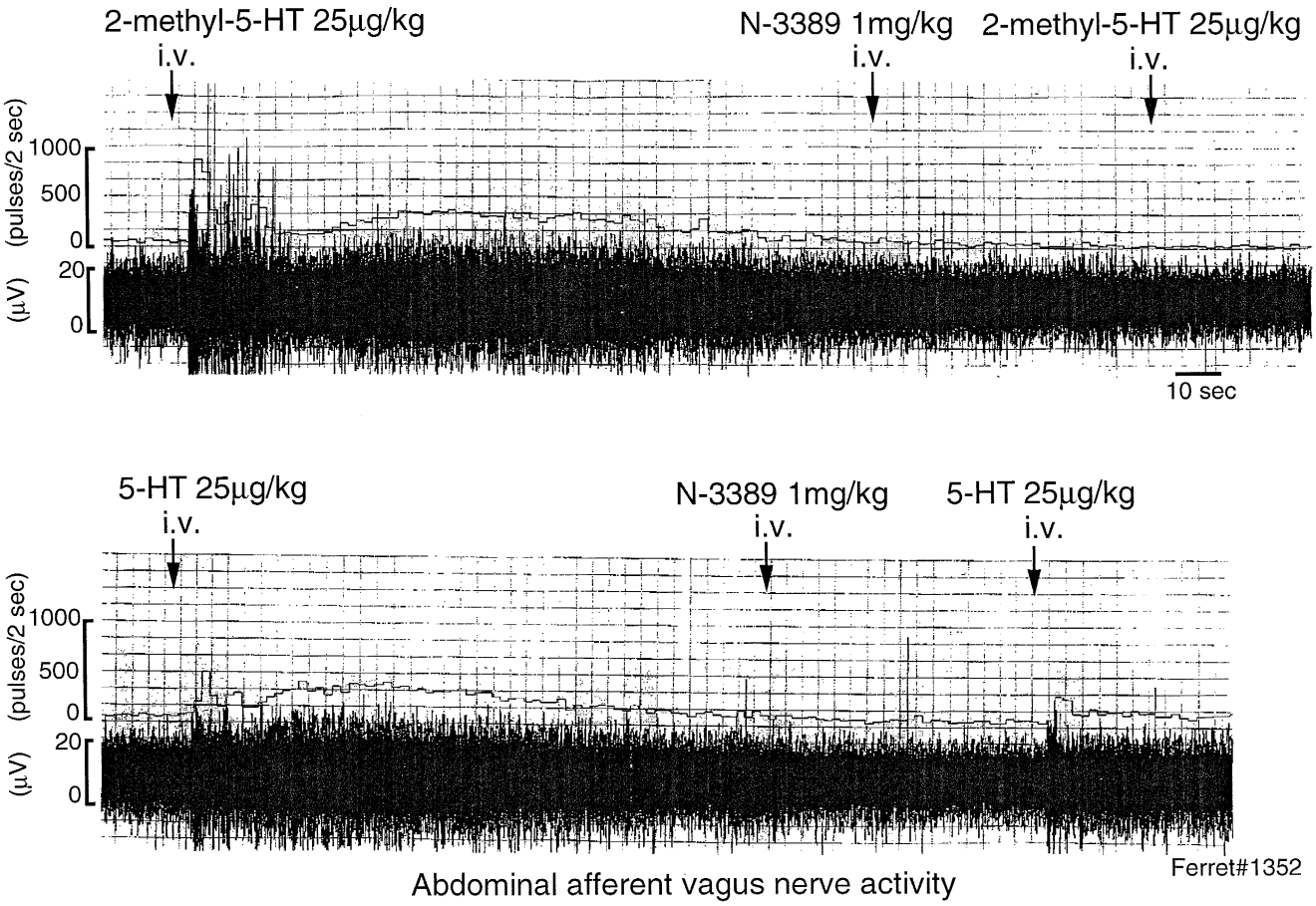


Fig. 5. Effects of N-3389 on the 2-methyl-5-HT- or 5-HT-induced increase of abdominal vagus nerve activity in an anesthetized ferret. Traces are the actual records of abdominal vagus nerve activity in the same animal.

was more effective in reducing the cyclophosphamide-induced emesis than was intraperitoneal administration.

*3.1.3. Copper sulfate-induced emesis in ferrets*

We previously reported that copper sulfate at doses of 20 mg/kg (3/6) and 40 mg/kg (6/6) produced dose-related increases in the number of emetic episodes (Endo et al., 1991). The emetic episodes induced by copper sulfate are summarized in Table 5. Copper sulfate at a dose of 40 mg/kg produced retching and vomiting within 10 min and this continued infrequently. N-3389 halved the number of retching and vomiting episodes induced by copper sulfate. At 5.0 mg/kg, N-3389 produced a significant reduction in the number of vomiting episodes.

*3.2. Effects of N-3389 on abdominal afferent vagus nerve activity*

*3.2.1. Effects of N-3389 on cisplatin-induced abdominal afferent vagus nerve activity in anesthetized ferrets*

Cisplatin (10 mg/kg, i.p.) produced a significant increase in abdominal afferent vagus nerve activity (Fig. 2). The time-course of cisplatin-induced emesis in another group of ferrets paralleled the time-course of afferent nerve activity induced by cisplatin. Pretreatment with N-3389 significantly (Fig. 3) inhibited the increase of the afferent nerve activity induced by cisplatin. Furthermore, the cisplatin-induced increase in afferent nerve activity was significantly reduced by injection of N-3389 (arrow at injection in Fig. 4) in a dose-dependent manner.

Table 6
Effects of intravenous injection of 5-hydroxytryptamine (5-HT), 2-methyl-5-HT or 5-methoxytryptamine (5-MT) on abdominal vagus nerve activity in anesthetized ferrets

| Compound | Dose (μg/kg, i.v.) | % increase from pre-injection level |
|---|---|---|
| Vehicle | | 104.7 ± 8.8 ($n = 6$) |
| 5-HT | 25 | 297.5 ± 43.6 [b] ($n = 7$) |
| | 50 | 303.2 ± 45.2 [c] ($n = 5$) |
| 2-methyl-5-HT | 25 | 209.9 ± 17.1 [c] ($n = 6$) |
| | 50 | 288.5 ± 17.4 [c] ($n = 6$) |
| 5-MT | 25 | 199.3 ± 26.8 [b] ($n = 5$) |
| | 50 | 285.9 ± 14.9 [c] ($n = 4$) |

The data represent the means ± S.E. from four to seven animals. Values are significantly different ([a] $P < 0.01$, [b] $P < 0.001$) from cisplatin (10), respectively. In order to avoid tachyphylaxis, a minimum of 10 min was allowed between injection of each dose.

*3.2.2. Effects of 5-HT, 2-methyl-5-HT and 5-MT on abdominal afferent vagus nerve activity in anesthetized ferrets*

Intravenous bolus injection of 5-HT (25.0–50.0 μg/kg, i.v.) produced a dose-dependent increase in abdominal afferent vagus nerve activity. The maximum response induced by 5-HT (50 μg/kg, i.v.) was estimated to be $301.2 \pm 24.1\%$ ($n = 5$) the pre-injection level (Table 6). Vehicle (normal saline) did not mimic the 5-HT effects. 2-Methyl-5-HT (25.0–50.0 μg/kg, i.v.) also produced a dose-dependent increase in abdominal afferent vagus nerve activity. The maximum response induced by 2-methyl-5-HT (50 μg/kg, i.v.) was estimated to be $291.5 \pm 15.4\%$ ($n = 5$) the pre-injection level. The pattern of evoked discharge induced by 2-methyl-5-HT was similar to that evoked by 5-HT. Although 5-MT (25.0–50.0 μg/kg, i.v.) increased abdominal afferent vagus nerve activity, the afferent nerve response to 5-MT was less than that to 5-HT.

The traces shown in Fig. 5 are the actual records of abdominal vagus nerve activity from the same ferret. Intravenous injection of N-3389 at a dose of 1.0 mg/kg completely blocked the 2-methyl-5-HT (25 μg/kg, i.v.)-induced increase in abdominal afferent vagus nerve activity (upper trace of Fig. 5). Concerning the 5-HT (25 μg/kg, i.v.)-induced increase in abdominal afferent vagus nerve activity, a resistant component was observed after injection of N-3389 (1.0 mg/kg)(lower trace of Fig. 5).

## 4. Discussion

In ferrets, N-3389, a new 5-$HT_3$ and 5-$HT_4$ receptor antagonist, significantly reduced in a dose-dependent manner the number of emetic episodes induced by cisplatin, cyclophosphamide and copper sulfate.

Our findings suggest that N-3389 may be an orally active 5-$HT_3$ and 5-$HT_4$ receptor antagonist (Tables 2 and 4). These results also suggest a peripheral site of N-3389 action in the upper gastrointestinal tract. Furthermore, 5-HT is known to be present in large amounts in EC cells in the vicinity of vagus afferent nerves (Minami et al., 1995b). 5-$HT_3$ receptors are known to be located on vagus nerves and abdominal afferent vagus nerves. The release of 5-HT from the gut is central to evoking emesis (Racké et al., 1995). Schwörer et al. (1991) reported that cisplatin increased the release of 5-HT from EC cells in the small intestine. We previously reported that abdominal vagotomy significantly inhibits the cisplatin-, cyclophosphamide- and copper sulfate-induced increase in 5-HT levels in the area postrema in the ferret (Endo et al., 1992). Furthermore, vagotomy inhibits cisplatin-induced emesis by 85% (Endo et al., 1992) and copper sulfate-induced emesis by 88% (Fukui et al., 1994).These reports suggest that the vagus afferent nerve is one of the common pathways of the emetic response induced by cytotoxic agents.

Drugs acting on 5-HT receptors have a potential role in the treatment of emesis (Costall and Naylor, 1990; Sanger, 1990). The difficulties in assessing the physiological role of 5-HT in the intestine include the presence of multiple receptor subtypes and multiple sites of action for 5-HT (Sanger and Gaster, 1994). Stimulation of 5-$HT_4$ receptors by 5-MT is known not to produce any effect at 5-$HT_3$ receptors (Fozard, 1990; Bockaert et al., 1992). As shown in Fig. 5, 5-HT produced an increase in abdominal afferent vagus nerve activity while N-3389 significantly reduced the increase of afferent nerve activity induced by 5-HT (Fig. 5).

It has been shown that 5-$HT_4$ receptors are present on rat isolated vagus nerves (Rhodes et al., 1992; Bley and Eglen, 1993). Although afferent vagus nerves are considered to have polymodal properties, depolarization of the vagus nerves by 5-HT may be mediated predominantly by 5-$HT_3$ receptors since, in the rat vagus nerve, the depolarization affecting 5-$HT_4$ receptors was 13–19% of the maximum evoked by 5-HT (Rhodes et al., 1992). As shown in Table 6, the response of vagus nerves to 5-HT is predominantly mediated via 5-$HT_3$ receptors in ferrets. The presence of 5-$HT_4$ receptors on vagus nerves in addition to 5-$HT_3$ receptors could explain an involvement of these receptors in the mechanism by which emesis is evoked in ferrets by copper sulfate and other agents (Bhandari and Andrews, 1991; Fukui et al., 1994). Yoshioka et al. (1992) reported that 5-$HT_4$ receptors are not involved in the 5-HT-induced increase in afferent cervical vagus nerve activity. Although there may be many reasons for this discrepancy, it is conceivable that the main reason is the difference in nerves used, i.e., abdominal and cervical afferent vagus nerves. The cervical vagus nerves include large numbers of pulmonary afferents from the lung stretch receptors. The stretch receptors will be activated during the inspiratory phase. 5-HT also activates the stretch receptors indirectly via 5-$HT_2$ receptors on the vascular system in the lung. In addition to stretch receptors, 5-$HT_3$ receptors activate other afferent vagus nerves including C-fibers. Under these experimental conditions, cervical afferent discharge includes so many components that 5-$HT_4$ receptor-mediated afferent discharges from the gastrointestinal tract might be masked by or compete with stretch receptor-mediated discharges. In the present study, we focused our attention on abdominal afferent vagus nerves because of the site of action of antiemetic drugs. In this context, the methods we used may be much more useful for studying antiemetic drugs. We recently reported that intraperitoneal administration of 5-MT (10–30 mg/kg) produced a dose-dependent increase in kaolin intake (pica) in rats (Tamakai et al., 1996). Furthermore, following intravenous administration, the 5-MT (25 μg/kg)-induced increase in abdominal afferent vagus activity in anesthesized rats was inhibited by GR113808 (1-[2-[(methylsulfonyl)amino]ethyl]-4-piperidinyl]methyl-1-methyl-1*H*-indole-3-carboxylate), a selective 5-$HT_4$ receptor antago-

nist, but not by granisetron and ondansetron (Tamakai et al., 1996). These results suggest that abdominal afferent vagus nerve activity might be increased by the administration of 5-$HT_4$ receptor agonists and inhibited by antagonists of 5-$HT_4$ receptors.

Cisplatin and copper sulfate increased abdominal afferent vagus nerve activity in anesthetized ferrets. This increase in afferent nerve activity agreed well with the mode of emetic response induced by these emetics. Pretreatment with N-3389 significantly inhibited the cisplatin-induced increase in vagus nerve activity (Fig. 3). Also, the increased vagus nerve activity induced by cisplatin was significantly inhibited by N-3389 in a dose-dependent manner (Fig. 4). We recently reported that ondansetron (1 mg/kg, i.v.) produced a transient inhibition of the increased vagus nerve activity induced by cisplatin (Endo et al., 1995). The N-3389 (1 mg/kg, i.v.)-induced inhibition of the increase in afferent vagus activity was maintained throughout the experiment (Fig. 4). In order to assess the contribution of 5-$HT_3$ and 5-$HT_4$ receptor antagonism to the antiemetic effect, we compared the results obtained with those in the literature from our group using 5-$HT_3$ receptor antagonists on their own in the ferret. As far as the anti-emetic effects on cisplatin-induced emesis in ferrets are concerned, no significant difference was observed in terms of the frequency of retching and vomiting among N-3389, ondansetron and granisetron. As shown in Table 7, the duration of retching induced by cisplatin was significantly reduced by N-3389, in accordance with this study (Fig. 4), but not by ondansetron or granisetron. As shown in Table 6, the 5-MT-induced increase in afferent vagus activity was approximately 60% of that induced by 5-HT. Furthermore, N-3389 completely blocked the 2-methyl-5-HT-induced increase in abdominal vagus nerve activity but N-3389 did not completely inhibit the 5-HT-induced increase in abdominal vagus nerve activity (Fig. 5). These results suggest that abdominal vagus nerve activity involves 5-$HT_3$ and other 5-HT receptors. Therefore, 5-$HT_3$ receptor antagonist and 5-$HT_4$ receptor antagonist might have an additive effect to 5-$HT_3$ receptor antagonism in abdominal afferent vagus nerves.

N-3389 is a 5-$HT_4$ receptor antagonist but its affinity at this receptor was weaker than at the 5-$HT_3$ receptor. Furthermore, N-3389 prevented 5-MT-induced relaxation of rat esophageal tunica muscularis mucosa precontracted with carbacol, producing a $pA_2$ value for N-3389 of 7.03 (Hagihara, personal communication). This activity was more potent than that of ICS205-930 (Baxter et al., 1991) and almost equal to that of SDZ205-557 (2-methoxy-4-amino-5-chlorobenzoic acid 2-(diethylamino)ethyl ester) (Eglen et al., 1993) and DAU6285 (*endo*-6-methoxy-8-methyl-8-azabicyclo [3.2.1] oct-3-yl-2,3-dihydro-2-oxo-1*H*-benzimidazole-1-carboxylate hydrochloride) (Waikar et al., 1993), but weaker than that of newly developed selective 5-$HT_4$ receptor antagonists such as SB207710 ((1-butyl-4-piperidinylmethyl)-8-amino-7-iodo-1,4 benzodioxan-5-carboxylate) (McLean and Coupar, 1995). It has been suggested that N-3389 is an antagonist of both 5-$HT_3$ and 5-$HT_4$ receptors, although the effect on the 5-$HT_3$ receptor is over ten times more potent than that on the 5-$HT_4$ receptor. The 5-$HT_4$ receptor antagonism exhibited by N-3389 may be mild compared with its antiemetic activity. Further study is needed to clarify the involvement of 5-$HT_4$ receptor antagonism in emesis by using selective 5-$HT_4$ receptor antagonists such as SB207710 and GR125487.

In the intestine, the EC cell has been proposed as a 'detector' which responds to a range of stimuli and releases 5-HT to discharge afferent vagus nerves (Minami et al., 1995b). In our previous study, tissue 5-HT concentrations increased in the ileal mucosa after cisplatin and copper sulfate administration (Endo et al., 1990). Cisplatin produced a release of 5-HT from isolated ileal mucosa preparations in guinea pigs (Schwörer et al., 1991), cats (Milano et al., 1991) and ferrets (Endo et al., 1993). The mechanisms involved in the regulation of 5-HT release from the intestinal mucosa are very complex (Racké and Schwörer, 1991). We reported that 5-MT induced a dose-dependent increase in 5-HT release from rat isolated ileum (Minami et al., 1995a). Our experiments were performed without the use of tetrodotoxin because we were exploring the potential clinical use of 5-$HT_4$ receptor antagonists.

Table 7
Effects of N-3389, ondansetron and granisetron on cisplatin (10 mg/kg, i.p.)-induced emesis. Comparison of duration of retching (min) after administration of N-3389, ondansetron and granisetron (percentage change from cisplatin control)

| Dose (mg/kg, i.p.) | Duration of retching (min) | Ref. |
|---|---|---|
| Cisplatin (10) | 183.2 ± 18.5 ($n = 6$) | |
| N-3389 (1.0) + cisplatin (10) | 21.4 ± 12.9 [b] ($n = 6$) (11.7%) | |
| Cisplatin (10) | 132.3 ± 41.9 ($n = 6$) | Endo et al., 1990 |
| Ondansetron (1.0) + cisplatin (10) | 24.5 ± 18.9 ($n = 7$) (18.5%) | |
| Cisplatin (10) | 224.6 ± 14.1 ($n = 7$) | Endo et al., 1992 |
| Granisetron (1.0) + cisplatin (10) | 46.5 ± 14.4 ($n = 6$) (20.7%) | |

The data represent the means ± S.E. from six to seven animals. Values are significantly different ([b] $P < 0.01$) from cisplatin (10).

Gebauer et al. (1993) suggest that stimulation of 5-$HT_4$ autoreceptors on EC cells causes inhibition of 5-HT release in the vascularly perfused guinea-pig intestine in the presence of tetrodotoxin. Tetrodotoxin can abolish neuronally mediated effects on 5-HT release (Gebauer et al., 1993). This discrepancy may be caused by the use of different methods and species differences. The data of Gebauer et al. (1993) were obtained from vascularly perfused guinea-pig small intestine in the presence of tetrodotoxin, while our data were obtained from rat isolated small intestine without tetrodotoxin. In the central nervous system, stimulation of 5-$HT_4$ receptors results in an increase in cAMP levels and inhibition of voltage-dependent potassium channels (Fagni et al., 1992). The general belief that 5-$HT_4$ receptors are coupled to adenylate cyclase has been challenged. Bley and Eglen (1993) failed to find an increase in tissue cAMP generated by 5-$HT_4$ receptor activation in guinea-pig ileum or rat vagus nerve. However, others have reported that 5-$HT_4$ receptors are linked to adenylate cyclase in guinea-pig ileum (Galzin and Delahaya, 1992). Further study is needed to elucidate the involvement of 5-$HT_4$ receptors in 5-HT release in the small intestine.

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