Studies for the Emetic Mechanisms of Ipecac Syrup (TJN-119) and Its Active Components in Ferrets: Involvement of 5-Hydroxytryptamine Receptors

Masayuki Hasegawa*, Toshinobu Sasaki, Koichi Sadakane, Masahiro Tabuchi, Yasunori Takeda, Masayuki Kimura and Yuichi Fujii

Research and Development Division, Tsumura & Co., 2, Rokubancho, Chiyoda-ku, Tokyo 102-8422, Japan

Received September 21, 2001 Accepted March 1, 2002

ABSTRACT—Ipecac syrup, prepared from a galentical ipecac, contains the nauseant alkaloids cephaeline and emetine. The involvement of receptors and serotonin- and dopamine-metabolizing enzymes in the emesis induced by ipecac syrup and these components was investigated. 1) In ferrets, the selective 5-HT₃-receptor antagonist ondansetron (0.5 mg/kg, p.o.) prevented each emesis induced by TJN-119 (0.5 mL/kg, p.o.), cephaeline (0.5 mg/kg, p.o.) and emetine (5.0 mg/kg, p.o.), but the intraperitoneal administration of the selective dopamine D₂-receptor antagonist sulpiride failed to significantly suppress the TJN-119, cephaeline and emetine-induced emesis at a dose of 0.1 mg/kg that blocked apomorphine-induced emesis. 2) In the receptor binding assays, cephaeline and emetine had a distinct affinity to 5-HT₃ receptor, but no or weak affinity to 5-HT₁A, 5-HT₁, nicotine, M₃, β₁, NK₁ and D₂ receptors. 3) Cephaeline and emetine did not affect activities of metabolic enzymes of 5-HT and dopamine (MAO-A, MAO-B, tryptophan 5-hydroxylase and tyrosine hydroxylase) in vitro. These results suggest that 5-HT₃ receptor plays an important role in the emetic action of TJN-119, cephaeline and emetine, and the 5-HT₄ receptor may be involved in their mechanisms.

Keywords: Ipecac syrup, Cephaeline, Emetine, Emesis, 5-HT receptor

Ipecac syrup, prepared from galentical ipecac, is an emetic used in the initial treatment of poisoning such as accidental ingestion. Ipecac syrup is listed in the Pharmacopoeia of several countries, including the USA, UK, Canada, Australia and France, where it is also available as an over-the-counter (OTC) drug. Ipecac syrup is listed in the 13th Pharmacopoeia of Japan (JP XIII) as a drug for in-hospital use.

TJN-119 (ipecac syrup) complies with the preparation specifications in JP XIII, and its pharmacologically active ingredients are alkaloids, cephaeline and emetine (Fig. 1). The emetic activity of cephaeline is approximately twofold more potent than that of emetine (1). These ingredients are considered to provoke emesis through their action on the gastrointestinal tract and vomiting center (2, 3). However, the detailed mechanism of action thereof is unknown. On the other hand, there is evidence for the involvement of some types of receptors in the development of emesis (4, 5). In this study, ferrets, an animal species that is scientifically appreciated as a model animal of emesis (6, 7), were used to examine the actions of a 5-HT₃ receptor antagonist and a dopamine D₂ receptor antagonist on emesis induced by TJN-119, cephaeline and emetine. Furthermore, the activities of cephaeline and emetine on the main neurotransmitter receptors and serotonin- and dopamine-metabolizing enzymes which are associated with the development of emesis were examined in vitro.

Fig. 1. Chemical structures of cephaeline and emetine.
MATERIALS AND METHODS

Test compounds

TJN-119 (Lot No.: GAHY) in the form of ipecac syrup as specified in the JP XIII and the Pharmacopoeia of USA (USP) was prepared by Tsumura & Co. (Tokyo). It contained cephaeline and emetine by 0.0763w/v% to 0.0787w/v% and 0.0467w/v% to 0.0477w/v%, respectively. Cephaeline (dihydrochloride, Lot No.: 960917-1, not less than 98% in purity) was isolated by Tsumura & Co. from Cephaelis acuminata. Emetine (dihydrochloride, Lot No.: AR01, not less than 99% in purity) was purchased via the Tokyo Kasei Kogyo Co., Ltd. (Tokyo). These compounds were dissolved in simple syrup (JP, 85% sucrose; Kowa, Osaka) to obtain a dosing volume of 0.5 mL/kg. In the in vivo study, these compounds were dissolved into purified water.

Actions of receptor antagonists on emesis induced by TJN-119, cephaeline and emetine

Animals: Male ferrets (Mustela putorius furo, L.), weighing 1.1 – 2.6 kg, were purchased from Marshall Farm (North Rose, NY, USA). They were individually housed in stainless steel cages (40 × 50 × 50 cm) under constant environmental conditions [room temperature: 22 ± 5°C, relative humidity: 60 ± 30%, and lightening: 12 h/day (7:00 to 19:00)]. Animals were given 50 g/day of cat chow (C.F.E.-2; Clea Japan Co., Ltd., Tokyo) and free access to water. To maintain the gastric content volume in each animal constant at the time of the study, animals were subjected to fasting for about 24 h from the day before administration and were fed with the relevant food (about 50 g) at 30 min prior to administration of the test compound.

Drugs: Ondansetron (Zofran Injection, 4 mg/2 mL; Glaxo Japan, Tokyo), a selective 5-HT3 receptor antagonist, was diluted with the simple syrup to twofold. (-)-Sulpiride (Sigma, St. Louis, MO, USA), a selective D2-receptor antagonist, was dissolved in a minimum amount of 0.1 N hydrochloric acid and prepared to volume with purified water. The volume of the dosing solution was established to be 0.5 mL/kg for these drugs.

Induction and measurement of emesis: TJN-119 (0.5 mL/kg), cephaeline (0.5 mg/kg) or emetine (5.0 mg/kg) was given orally. The animals were observed for 4 h after dosing to determine the latency and the numbers of retching and vomiting according to the method of Stable et al. (8). Retching was defined as a vomiting-like response without expulsion of any vomit. For ferrets that did not exhibit emesis, the latency period was taken as 240 min (i.e., observation time). Ondansetron (0.5 mg/kg, p.o.) or sulpiride (0.1 mg/kg, i.p.) was administered at 60 and 30 min prior to administration of the test compound, respectively. Based on a preliminary study, this dose level of ondansetron and sulpiride was established to inhibit completely cisplatin-induced emesis that develops via the 5-HT3 receptor and apomorphine-induced emesis that develops via the D2-receptor, respectively. The groups of animals that received the respective vehicle solution were considered to be the receptor antagonist nonadministration groups. Results are expressed as means ± S.E.M. in each group. The significance of differences between treatments was assessed by Mann-Whitney’s U-test. A P<0.05 was considered to be statistically significant.

Receptor binding studies

Materials: Materials were obtained from the following sources: A9L cells, (+)-butaclamol and MDL 72222 (RBI Inc., Natick, MA, USA); CHO cells, M1 receptor, β1 receptor, [3H]-spiperone, [3H]-[Sar9,Met(O2)11]-SP, [3H]-8-OH-DPAT, [3H]-cytisine hydrochloride, [3H]-QNB, [3H]-CGP-12177 and [3H]-BRL43694 (NEN Inc., Wilmington, DE, USA or Paris, France); guinea pig striate body and rat striate body (ABS Inc., Deforest, WI, USA); [3H]-GR113808 (Amersham Inc., Little Chalfont, UK); 8-OH-DPAT, serotonin hydrochloride, dopamine hydrochloride (Sigma); [Sar9,Met(O2)11]-SP (Bachem, Inc., Budendorf, Switzerland); N1E-115 cells, human placenta (Cerep Inc., Rueil-Malmaison, France); atropine sulfate, propanolol hydrochloride (Wako Pure Chemical Industry Co., Ltd., Osaka); nicotine bitartrate (Tokyo Kasei Kogyo Co., Ltd.). In the nicotine receptor binding study, the brain of male SD rats, purchased from Charles River Japan, Inc. (Tokyo), was homogenized and centrifuged at 25,000 × g, and the membrane fraction obtained was used.

Methods: Receptor binding assays of cephaeline and emetine to the serotonin receptors (5-HT1A, 5-HT3 and 5-HT4), dopamine receptor (D2), tachykinin receptor (NK1), nicotine receptor (Nic), muscarine receptor (M4) and β adrenaline receptor (β1) were performed as described in the reported methods (9 – 14) according to the conditions listed in Table 1. In the presence of the test compound or the reference compound, the membrane and the tritium-labeled specific ligand were incubated for a given time, and the ligand-bound membrane was allowed to adsorb onto a glass fiber filter (GF/B; Packard, Zurich, Switzerland or Filtermat B; Wallac, France) by rapid vacuum filtration. Following washing of the filter, radioactivity was counted in a scintillation counter (Topcount, Packard; or Betaplate, Wallac). In reference to the maximum blood concentration after administration of the ipecac syrup to dogs at its pharmacological dose level (about 1 × 10^{-8} M in the case of cephaeline), the final concentration of the test drug was established to be 0.85 × 10^{-8} M to 1 × 10^{-8} M. The amount of specific binding was calculated by deducing the amount of nonspecific binding in the presence of nonradioactive ligand in excess from the total binding, and the ratio...
The Emetic Mechanisms of Ipecac Syrup

of the volume of binding in the test compound group to that in the nonaddition group was considered as the rate of inhibition. The 50% inhibition concentrations (IC_{50}) of the test drug and control drug to the 5-HT_4 receptor, and the Hill coefficient (nH) were calculated from the regression curve according to the method of nonlinear regression.

**Materials**

Materials were obtained from the following sources: clorgyline, deprenyl (BRI Inc.); [^3^H]-tyrosine (NEN Inc.); kynuramine benzylamineserotonin, 3-iodo-tyrosine (Sigma). In the study of the tryptophan 5-hydroxylase activity, the hippocampus of male SD rats purchased from Charles River Japan, Inc. was homogenized and centrifuged at 35,000 \times g; the supernatant obtained was used.

**Methods**

The activity of monoamine oxidase A and B (MAO-A and -B), tyrosine hydroxylase and tryptophan 5-hydroxylase was determined as described in the reported methods (15 – 18) according to the conditions listed in Table 2. The reaction products by MAO-A and -B were determined by spectrophotometry (DU70; Beckman, San Ramon, CA, USA). The reaction products by tyrosine hydroxylase were isolated with the cation exchange column, followed by the determination of radioactivity with a liquid scintillation counter (LS1701, Beckman). The reaction products by tryptophan 5-hydroxylase were determined by high-performance liquid chromatography (column: Eicompak MA-50DS and detector: ECD-200; Eicom, Tokyo). The final concentration of the test compound was

### Table 1. Experimental protocol for various receptor binding assays

| Receptor | Tissue source | Reference compound | Ligand | Nonspecific compound | Incubation condition |
|----------|---------------|--------------------|--------|----------------------|---------------------|
| 5-HT_{1A} | human recombinant (CHO cells) | serotonin | [^3^H]-8-OH-DPAT (0.3 nM) | 8-OH-DPAT (10 \mu M) | 60 min/22°C |
| 5-HT_3 | NIE-115 cells | serotonin | [^3^H]-BRL43694 (1.0 nM) | metoclopramide (100 \mu M) | 180 min/4°C |
| 5-HT_4 | guinea pig striatum | serotonin | [^3^H]-GR113808 (0.1 nM) | serotonin (30 \mu M) | 30 min/37°C |
| D_1 | human recombinant (A9L cells) | dopamine | [^3^H]-spiperone (0.3 nM) | (+)butaclamol (10 \mu M) | 60 min/22°C |
| NK_1 | human recombinant (CHO cells) | [Sar^9,Met(O^2)_{11}]-SP | [^3^H]-[Sar^9,Met(O^2)_{11}]-SP (0.5 nM) | [Sar^9,Met(O^2)_{11}]-SP (0.3 \mu M) | 60 min/22°C |
| Nicotinic | rat brain | nicotine bitartrate | [^3^H]-cytisine HCl (1.0 nM) | nicotine bitartrate (10 \mu M) | 75 min/4°C |
| MAO-A | human plasenta | clorgyline | kynuramine (0.15 mM) | 4-OH-quinoline | spectro-photometry | 30 min/30°C |
| MAO-B | rat brain | deprenyl | benzylamine (0.2 mM) | benzaldehyde | spectro-photometry | 30 min/30°C |
| Tyrosine hydroxylase | rat striatum | 3-iodotyrosine | [^3^H]-tyrosine (10 \mu M) | [^3^H]-H_2O | liquid scintillation | 40 min/37°C |
| Tryptophan 5-hydroxylase | rat hippocampus | p-chlorophenyl alanine | l-tryptophan (0.16 nM) | 5-hydroxy-l-tryptophan | HPLC with ECD | 10 min/37°C |

RT: room temperature.

### Table 2. Experimental protocol for various enzyme assays

| Enzyme | Tissue source | Reference compound | Substrate | Reaction product | Method of detection | Incubation condition |
|--------|---------------|--------------------|-----------|------------------|---------------------|---------------------|
| MAO-A  | human plasenta | clorgyline | kynuramine (0.15 mM) | 4-OH-quinoline | spectro-photometry | 30 min/30°C |
| MAO-B  | rat brain | deprenyl | benzylamine (0.2 mM) | benzaldehyde | spectro-photometry | 30 min/30°C |
| Tyrosine hydroxylase | rat striatum | 3-iodotyrosine | [^3^H]-tyrosine (10 \mu M) | [^3^H]-H_2O | liquid scintillation | 40 min/37°C |
| Tryptophan 5-hydroxylase | rat hippocampus | p-chlorophenyl alanine | l-tryptophan (0.16 nM) | 5-hydroxy-l-tryptophan | HPLC with ECD | 10 min/37°C |

MAO: monoamine oxidase.
established to be $0.85 \times 10^{-5}$ M to $1 \times 10^{-4}$ M on the basis of the same reasons as those in the receptor binding study.

The yield of the reaction products at each concentration of the test compound was compared with that in the test compound nonaddition groups, and the rates of enzymatic reaction inhibition were calculated. The 50% IC$_{50}$ of the control drug and nH were calculated from the regression curve according to the method of nonlinear regression (PRISM; Graph Pad, San Diego, CA, USA). Furthermore, a comparative study of tryptophan 5-hydroxylase was limitedly conducted at 3 concentrations, and the 50% inhibition concentration was not calculated.

**RESULTS**

*Actions of receptor antagonists on emesis induced by TJN-119, cephaeline and emetine*

The effects of ondansetron, a 5-HT$_3$-receptor antagonist, on emesis induced by TJN-119, cephaeline and emetine are shown in Table 3. All animals in the TJN-119 (0.5 mL/kg), cephaeline (0.5 mg/kg) and emetine (5.0 mg/kg) oral administration groups developed emesis. Preadministration of ondansetron at 0.5 mg/kg completely inhibited the development of retching and vomiting induced by TJN-119, cephaeline or emetine. On the other hand, sulpiride at 0.1 mg/kg slightly reduced the number of episodes of retching and vomiting induced by TJN-119, cephaeline or emetine. However, the reduction was not statistically significant, and sulpiride affected neither incidence nor latency to the first emetic response (Table 4).

*Receptor binding studies*

The inhibitory activities of cephaeline and emetine on the binding to various types of receptors are shown in Table 5. The two compounds showed inhibitory activity on the 5-HT$_4$ receptor, but no or weak inhibitory activity on the other at a concentration range of $0.85 \times 10^{-5}$ to $1 \times 10^{-4}$ M (the maximum inhibition rate of about 28% for 5-HT$_{1A}$). Each of the compounds exhibited inhibitory activity on the 5-HT$_3$ receptor at about $1 \times 10^{-6}$ M or higher concentrations and showed nearly 100% inhibition at a concentration of $1 \times 10^{-5}$ M. The IC$_{50}$ values of cephaeline and emetine are shown in Table 5. The two compounds showed inhibitory activity on the 5-HT$_3$ receptor at about $1 \times 10^{-6}$ M or higher concentrations and showed nearly 100% inhibition at a concentration of $1 \times 10^{-5}$ M. The IC$_{50}$ values of cephaeline

| Treatment | No. of animals (emesis/tested) | No. of emesis (times/4 h) | Latency to emesis (min) |
|-----------|-------------------------------|--------------------------|------------------------|
|           |                               | retching | vomiting | retching | vomiting |
| TJN-119 + Vehicle | 4/4 | 35.0 ± 9.8 | 3.5 ± 1.3 | 35.3 ± 14.7 | 38.3 ± 11.0 |
| + Ondansetron | 0/4 | 0.0 ± 0.0* | 0.0 ± 0.0* | 240.0 ± 0.0* | 240.0 ± 0.0* |
| Cephaeline + Vehicle | 4/4 | 28.8 ± 13.5 | 2.5 ± 1.0 | 38.3 ± 7.8 | 39.3 ± 8.2 |
| + Ondansetron | 0/4 | 0.0 ± 0.0* | 0.0 ± 0.0* | 240.0 ± 0.0* | 240.0 ± 0.0* |
| Emetine + Vehicle | 4/4 | 24.5 ± 9.1 | 2.3 ± 1.5 | 41.8 ± 0.5 | 47.0 ± 6.5 |
| + Ondansetron | 0/4 | 0.0 ± 0.0* | 0.0 ± 0.0* | 240.0 ± 0.0* | 240.0 ± 0.0* |

*All drugs were administered orally. Ondansetron (0.5 mg/kg) was pretreated 60 min before the administration of TJN-119 (0.5 mL/kg), cephaeline (0.5 mg/kg) or emetine (5.0 mg/kg). *Values represent the mean ± S.D. *P<0.05, significantly different from the respective control values. *If an animal either failed to exhibit retching or vomiting, then the latency to onset of emesis was taken as 240 min (i.e., the observation period).

| Table 4. Effect of sulpiride on TJN-119-, cephaeline- and emetine-induced emesis in ferrets |
| Treatment | No. of animals (emesis/tested) | No. of emesis (times/4 h) | Latency to emesis (min) |
|-----------|-------------------------------|--------------------------|------------------------|
|           |                               | retching | vomiting | retching | vomiting |
| TJN-119 + Vehicle | 4/4 | 23.8 ± 2.2 | 2.0 ± 0.8 | 41.0 ± 6.8 | 41.3 ± 6.6 |
| + Sulpiride | 4/4 | 15.3 ± 5.6 | 1.5 ± 0.6 | 38.0 ± 4.8 | 40.3 ± 3.1 |
| Cephaeline + Vehicle | 4/4 | 12.5 ± 3.7 | 1.3 ± 0.5 | 43.3 ± 10.9 | 43.8 ± 11.2 |
| + Sulpiride | 4/4 | 8.0 ± 2.2 | 1.0 ± 0.0 | 47.3 ± 13.9 | 48.5 ± 13.7 |
| Emetine + Vehicle | 4/4 | 20.5 ± 5.4 | 1.5 ± 0.6 | 45.8 ± 5.3 | 49.3 ± 8.5 |
| + Sulpiride | 4/4 | 15.8 ± 3.1 | 1.0 ± 0.0 | 40.8 ± 11.1 | 41.8 ± 9.8 |

*TJN-119 (0.5 mL/kg), cephaeline (0.5 mg/kg) and emetine (5.0 mg/kg) were administered orally. Sulpiride (0.1 mg/kg) was intraperitoneally pretreated 30 min before administration of these drugs. *Values represent the mean ± S.D.
and emetine for the 5-HT<sub>4</sub> receptor were 1.35 × 10<sup>-6</sup> and 2.16 × 10<sup>-6</sup> M, respectively.

**Actions on serotonin- and dopamine-metabolizing enzymes**

The effects of cephaeline and emetine on the in vitro enzymatic activity are shown in Table 5. The two compounds showed no definite inhibitory activity or accelerating activity on all enzymes examined at a concentration range of 0.85 × 10<sup>-5</sup> to 1 × 10<sup>-4</sup> M.

| Table 5. Effects of cephaeline and emetine on the emesis-associated receptors and enzymes |
|---------------------------------|---------------------------------|---------------------------------|
|                                  | Cephaeline                      | Emetine                        |
| Receptor binding                | 0.9 × 10<sup>-5</sup> M         | 1 × 10<sup>-4</sup> M          |
| 5-HT<sub>1A</sub>              | 17.2                           | ND                             |
| 5-HT<sub>3</sub>               | 16.9                           | ND                             |
| 5-HT<sub>4</sub>               | 94.5                           | ND                             |
| D<sub>2</sub>                  | 6.1                            | ND                             |
| NK<sub>1</sub>                 | 6.3                            | ND                             |
| Nicotinic                      | 4.6<sup>a</sup>                | 2.4                            |
| M<sub>3</sub>                  | 0<sup>a</sup>                  | 12.7                           |
| β<sub>1</sub>                  | 0<sup>a</sup>                  | 0<sup>a</sup>                  | 4.7<sup>b</sup>
| Reference compound             |                                |                                |
| IC<sub>50</sub> (M)            | 1 × 10<sup>-4</sup> M          |
| β<sub>1</sub>                  |                                |                                |
| IC<sub>50</sub> (M)            | 1 × 10<sup>-4</sup> M          |
| Hill coefficient               | 1 × 10<sup>-4</sup> M          |

For cephaeline and emetine, results are expressed as a percent inhibition of specific binding or enzyme activity (mean value, n = 3–4). ND: not determined. *The values are the results from experiments at 1.0 × 10<sup>-4</sup> M. *Hill coefficient. MAO: monoamine oxidase, Tyr-Hyd: tyrosine hydroxylase, Try-5-Hyd: tryptophane 5-hydroxylase.

DISCUSSION

In this study, ondansetron, a 5-HT<sub>3</sub> receptor antagonist, completely inhibited emesis which was induced by TJN-119 and its active ingredients, cephaeline and emetine. On the other hand, sulpiride, a D<sub>2</sub> receptor antagonist, little inhibited these emeses. Furthermore, the binding profile to the main neurotransmitter receptors associated with the development of emesis (5-HT<sub>1A</sub>, 5-HT<sub>3</sub>, 5-HT<sub>4</sub>, D<sub>2</sub>, NK<sub>1</sub>, Nic, M<sub>3</sub>, and β<sub>1</sub>) was examined. Consequently, cephaeline and emetine were evidenced to have a distinct affinity to the 5-HT<sub>3</sub> receptor. These results suggested that the 5-HT receptor is deeply involved in the development of emesis.

It is generally considered that ipecac syrup-induced emesis is due to direct stimulation of the local ileal mucosa by ipecac syrup or the stimulation of CTZ by the its emetic ingredients which were absorbed into blood (2, 3). However, details of the mechanism are still unknown. Recently, Endo et al. (19) found that TJN-119 increases the abdominal afferent vagus nerve activity and tends to increase the ileal 5-HT content in the ferret. Furthermore, T. Endo et al. (unpublished) showed that TJN-119 induced significant increases in 5-HT release from the intestine isolated from rat and ferret. Enhancement of the afferent vagus nerve activity and an increase in 5-HT release from the intestine also have been observed similarly in emesis induced by an anticancer agent, cisplatin (20, 21). 5-HT, which is released from intestinal enterochromaffin cells (EC cells) by cisplatin, may stimulate the abdominal afferent vagal nerves via 5-HT<sub>3</sub> receptor. The vomiting center receives input from the afferent discharge of the vagal fibers, which evokes the emetic reflex (20, 22, 23). Ondansetron may exert antiemetic activity through blockade of the 5-HT<sub>3</sub> receptor in the terminals of the nerve fibers of the abdominal afferent vagus (24). Based on the result in this study that ondansetron completely inhibited TJN-119-induced emesis and on information provided by Endo et al. about enhancement of the afferent vagus nerve activity and an increase in 5-HT release from the intestine, TJN-119 is presumed to induce the release of 5-HT from EC cells which stimulates...
the afferent vagus nerve via the 5-HT₄ receptor, thus provoking emesis. Furthermore, a similar mechanism of development is also applicable for emesis induced by its active ingredients, cephaeline and emetine.

TJN-119 is considered not to affect the 5-HIAA/5-HT ratio (19). The result of this study that cephaeline and emetine showed no in vitro activity on 5-HT-metabolizing enzyme is considered to support this fact.

The 5-HT release from EC cells is affected by various humoral and neuronal factors. 5-HT₃, Nic, M₁ and β₁ receptor agonists are considered to act to accelerate the 5-HT release, while M₁, α₂, histamine H₂, GABA, VIP and other receptor agonists are considered to inhibit 5-HT release. The 5-HT₄ receptor also is involved in the 5-HT release from EC cells (25). Gebauer et al. (26) reported that a selective 5-HT₄ receptor agonist, 5-methoxytryptamine (5-MT), inhibited the 5-HT release the guinea pig perfused gut preparation; however, this information was obtained under nonphysiological conditions in the presence of tetrodotoxin. On the other hand, Minami et al. (27) discovered that 5-MT dose-dependently enhances the 5-HT release in the untreated isolated rat gut preparation. In this study, the IC₅₀ values of cephaeline and emetine in the 5-HT₄ receptor were 1.35 × 10⁻⁶ and 2.16 × 10⁻⁶ M, respectively. On the other hand, the maximum blood concentrations of cephaeline and emetine after oral administration of TJN-119 at a dose level where they surely provoke emesis in dogs (1.0 mL/kg) were about 1 × 10⁻⁶ M and about 1 × 10⁻⁷ M, respectively. Therefore, the possibility that these active ingredients were bound to the 5-HT₄ receptor and provoked the 5-HT release from EC cells is admissible.

The 5-HT release from EC cells also is provoked by cytotoxic substances or irritant substances. In emesis induced by cisplatin or x-ray, the radicals produced are considered to provoke the 5-HT release. The 5-HT release from EC cells also is provoked by the afferent vagus nerve (4, 32), and NK₁ receptor distinctly, which led us to consider the possibility that the 5-HT₄ receptor is involved in the mechanism of TJN-119-induced 5-HT release from EC cells.

REFERENCES

1 Manno BR and Manno JE: Toxicology of ipecac: a review. Clin Toxicol 10, 221 – 242 (1977)
2 Borison HL and Wang SC: Physiology and pharmacology of vomiting. Pharmacol Rev 5, 193 – 230 (1953)
3 Weaver JE and Griffith JF: Induction of emesis by detergent ingredients and formulations. Toxicol Appl Pharmacol 14, 214 – 220 (1969)
4 Andrews PLR, Rapeport WG and Sanger GJ: Neuropharmacology of emesis induced by anti-cancer therapy. Trends Pharmacol Sci 9, 334 – 341 (1988)
5 Minami M, Endo T and Hiraiifuji M: Role of serotonin in emesis. Folia Pharmacol Jpn (Nippon Yakurigaku Zasshi) 108, 233 – 242 (1996) (text in Japanese with English abstract)
6 Florczyk AP, Schurig JE and Brandner WT: Cisplatin-induced emesis in the ferret: a new animal model. Cancer Treat Rep 66, 187 – 189 (1982)
7 Yamashita M, Tanaka J, Chagi K, Takeda S, Kurishara T, Takeda Y and Fujii Y: Vomiting induction by ipecac syrup in dogs and ferrets. J Toxicol Sci 22, 409 – 412 (1997)
8 Stable R, Andrews PLR, Bailey HE, Costall B, Gunning SJ, Hawthron J, Naylor RJ and Teyers MB: Antiemetic properties of 5-HT₁-receptor antagonist, GR38032F. Cancer Treat Rev 14, 333 – 336 (1987)
9 Mulheron JG, Casanas SJ, Arthur JM, Garnovskaya MN, Gettys TW and Raymond JR: Human 5-HT₁ₐ receptor expressed in insect cells activates endogenous Gα₁-like G protein(s). J Biol Chem 269, 12954 – 12962 (1994)
10 Hoyer D and Neijt HC: Identification of serotonin 5-HT₁ receptors: recognition sites in membranes of N1E-115 neuroblastoma cells by radioligand binding. Mol Pharmacol 33, 303 – 309 (1988)
11 Grossman CJ, Kilpatrick GJ and Bunce KT: Development of a radioligand binding assay for 5-HT₄ receptors in guinea-pig and rat brain. Br J Pharmacol 109, 618 – 624 (1993)
12 Anderson DJ and Arneric SP: Nicotinic receptor binding of [³H]cytisine, [³H]nicotine and [³H]methylcarbamylcholine in rat brain. Eur J Pharmacol 253, 261 – 267 (1994)
The Emetic Mechanisms of Ipecac Syrup

Cascieri MA, Ber E, Fong TM, Sadowski S, Bansal A, Swain C, Seward E, Frances B, Burns D and Strader CD: Characterization of the binding of a potent, selective, radioiodinated antagonist to the human neurokinin-1 receptor. Am Soc Pharmacol Exp Ther 42, 458 – 463 (1992)

Grandy DK, Marchionni MA, Makan H, Stofko RE, Alfano M, Burke-howie KJ, Bunzow JR, Server AC and Civelli O: Cloning of the cDNA and gene for a human D2 dopamine receptor. Biochemistry 36, 9762 – 9766 (1989)

Weyler W and Salach JI: Purification and properties of mitochondrial monoamine oxidase type A from human placenta. J Biol Chem 260, 13199 – 13207 (1985)

Salach JI: Monoamine oxidase from beef liver mitochondria: simplified isolation procedure, properties, and determination of its cysteinyl flavin content. Arch Biochem Biophys 192, 128 – 137 (1979)

Nagatsu T, Levitt M and Udenfriend S: A rapid and simple radioassay for tyrosine hydroxylase activity. Anal Biochem 9, 122 – 126 (1964)

Johnson M, Elayan I, Hanson GR, Foltz RL and Lim HK: Effects of 3,4-dihydroxyamphetamine and 2,4,5-trihydroxyamphetamine, two metabolites of 3,4-methylenedioxyamphetamine, on central serotoninergic and dopaminergic systems. J Pharmacol Exp Ther 261, 447 – 453 (1992)

Endo T, Nemoto M, Ogawa T, Takahashi M, Hamaue N, Hiraufi M, Fukushima Y and Minami M: Pharmacological aspects of ipecac syrup (TJN-119)-induced emesis in ferrets. Res Commun Mol Pathol Pharmacol 108, 187 – 200 (2000)

Endo T, Takahashi M, Minami M, Yoshioka M, Saito H and Parvez SH: Changes in thefferent abdominal vagal nerve activity induced by cisplatin and copper sulfate in the ferret. Biogenic Amines 11, 399 – 407 (1995)

Schworer H, Racke K and Kilbinger H: Cisplatin increases the release of 5-hydroxytryptamine (5-HT) from the isolated vascu

of 5-HT3 receptors. Naunyn Schmiedeb ergs Arch Pharmacol 344, 143 – 149 (1991)

Milano S, Simon C and Grelot L: In vitro release and tissue levels of ileal serotonin after cisplatin-induced emesis in the cat. Clin Auto Res 1, 275 – 280 (1991)

Endo T, Minami M, Monma Y, Yoshioka M, Saito H and Parvez SH: Vagotomy and ondansetron (5-HT3 antagonist) inhibited the increase of serotonin concentration induced by cytotoxic drugs in the area postrema of ferrets. Biogenic Amines 9, 163 – 175 (1992)

Racke K and Schworer H: Regulation of serotonin release from the intestinal mucosa. Pharmacol Res 23, 13 – 25 (1991)

Gebauer A, Merger M and Kilbinger H: Modulation by 5-HT3 and 5-HT4 receptors of the release of 5-hydroxytryptamine from the guinea-pig small intestine. Naunyn Schmiedeb ergs Arch Pharmacol 347, 137 – 140 (1993)

Minami M, Tamakai H, Ogawa T, Endo T, Hamaue N, Hiraufi M, Yoshioka M and Blower PR: Chemical modulation of 5-HT3 and 5-HT4 receptors affects the release of 5-hydroxytryptamine from the ferret and rat intestine. Res Commun Mol Pathol Pharmacol 89, 131 – 142 (1995)

Torii Y, Mutoh M, Saito H and Matsuki N: Involvement of free radicals in cisplatin-induced emesis in Rombus murinus. Eur J Pharmaco 248, 131 – 135 (1993)

Endo T, Minami M, Monma Y, Yoshioka M, Saito H, Toshimitsu Y and Parvez SH: Effect of ondansetron, a 5-HT3 antagonist, on copper sulfate-induced emesis in the ferret. Biogenic Amines 8, 79 – 86 (1991)

Pellini EJ and Wallace GB: The pharmacology of emetine. Am J Med Sci 152, 325 – 336 (1916)

Watson JW, Gonsalves SF, Fossa AA, Mclean S, Seeger T, Obach S and Andrews PLR: The anti-emetic effects of CP-99,994 in the ferret and the dog: role of NK1 receptor. Br J Pharmaco 115, 84 – 94 (1995)

Minami M, Endo T, Kikuchi K, Ihira E, Hiraufi M, Hamaue N, Monma Y, Sakurada T, Tan-no K and Kisara K: Antiemetic effects of sendide, a peptide tachykinin NK1 receptor antagonist, in the ferret. Eur J Pharmaco 363, 49 – 55 (1998)

Andrews PLR and Hawthorn J: The neurophysiology of vomiting. Baillieres Clin Gastroenterol 2, 141 – 168 (1988)