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Short communication

Central antiemetic effects of AS-8112, a dopamine D2, D3, and 5-HT_3 receptor antagonist, in ferrets

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Abstract

The involvement of a central mechanism in the antiemetic effect of AS-8112 ((*R*)-5-bromo-*N*-(1-ethyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-2-methoxy-6-methylamino-3-pyridinecarboxamide · 2 fumarate), a novel and potent dopamine D2, D3, and 5-HT_3 receptor antagonist, was investigated in ferrets. Intracerebroventricularly administered AS-8112 dose dependently inhibited *R*(+)-7-OH-DPAT (*R*(+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino) tetraline)-induced emesis (ID_{50}; 0.11 μg/kg, i.c.v.). In addition, AS-8112 (10 μg/kg, i.c.v.) significantly inhibited emesis induced by cisplatin. Ondansetron (10 μg/kg, i.c.v.) also inhibited cisplatin-induced emesis, but did not inhibit *R*(+)-7-OH-DPAT-induced emesis. *S*(−)-eticlopride (10 μg/kg, i.c.v.) did not inhibit emesis induced by cisplatin. However, racemic CP-99,994 ((±)-(2*S*, 3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine) (10 μg/kg, i.c.v.) inhibited both cisplatin- and *R*(+)-7-OH-DPAT-induced emesis. These results suggest that the antiemetic effects of AS-8112 are centrally mediated via dopamine D3 and 5-HT_3 receptors in ferrets.

Keywords: AS-8112 ((*R*)-5-bromo-*N*-(1-ethyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-2-methoxy-6-methylamino-3-pyridinecarboxamide · 2 fumarate); *R*(+)-7-OH-DPAT (*R*(+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino)tetraline); Cisplatin; Emesis; Area postrema; (Ferret)

1. Introduction

Over the last few decades, substantial evidence has been obtained indicating that central and peripheral serotonergic and central dopaminergic mechanisms play an important role in the regulation of emesis in humans, ferrets, and dogs.

The introduction of 5-HT_3 receptor antagonists into clinical practice in the early 1990s represented an important advance in the development of well-tolerated drugs specifically designed to prevent severe emesis caused by antineoplastic therapy. The mechanisms by which cytotoxic drugs elicit emesis in ferrets are mainly mediated via 5-HT_3 receptor activation. Accordingly, many studies have raised the possibility that cytotoxic drugs cause the release of 5-HT in the gastrointestinal tract and that the released 5-HT in turn stimulates abdominal visceral afferent nerves via 5-HT_3 receptors (Hawthorn et al., 1988; Andrews et al., 1990). Administration of ondansetron or other various 5-HT_3 receptor antagonists into the area postrema or the 4th cerebral ventricle has been reported to inhibit emesis induced by cisplatin (Higgins et al., 1989; Smith et al., 1988; Kamato et al., 1993). Moreover, radioligand binding studies have revealed that 5-HT_3 receptors are densely present in the area postrema, nucleus tractus solitarii, and dorsal motor nucleus of the vagus nerve, all of which are important sites for emetic responses (Kilpatrick et al., 1989; Waeber et al., 1989). Although the abdominal vagus is thought to be the major pathway for cytotoxic drug-induced emesis, stimulation of central 5-HT_3 receptors also plays an important role.

As for the role of central dopaminergic mechanisms in emesis, it is well known that dopamine D2 receptors in the area postrema play an important role in the regulation of emetic responses in humans, ferrets, and dogs (Andrews et al., 1990; Harding et al., 1987). Moreover, we have recently reported that *R*(+)-7-OH-DPAT (*R*(+)-7-hydroxy-2-(*N*,*N*-di-*n*-propylamino) tetraline), a selective dopamine D3 receptor agonist, elicits emesis in ferrets and dogs (Yoshida et al., 1995; Yoshikawa et al., 1996). Experiments with ferrets have also revealed that *R*(+)-7-OH-DPAT-induced emesis may be mediated by dopamine

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D3 receptors located in the area postrema, which is the locus of the chemoreceptor trigger zone (Yoshikawa et al., 1996). It can, therefore, be assumed that dopamine D2 and D3 receptors in the area postrema play an important role in the regulation of emesis in ferrets.

Recently we have reported that AS-8112 ((*R*)-5-bromo-*N*-(1-ethyl-4-methylhexahydro-1*H*-1,4-diazepin-6-yl)-2-methoxy-6-methylamino-3-pyridinecarboxamide · 2 fumarate) is a novel and potent dopamine D2, D3, and 5-HT_3 receptor antagonist with broad antiemetic effects (Yoshikawa et al., 1998). However, the sites of AS-8112 antiemetic action have not been identified. In the present study, we investigated the involvement of central sites in the effect of AS-8112 against *R*(+)-7-OH-DPAT- and cisplatin-induced emesis in ferrets.

2. Methods and materials

2.1. Animals

Male albino ferrets (Marshall Res. Animal) weighing 1.0–1.3 kg and individually housed in a room kept at 22–25 °C under a 12-h light/dark cycle were used in this study. They were given a standard cat diet (70–80 g/animal, Purina®) and allowed free access to water. The animals were fasted overnight prior to all experiments.

2.2. Drugs and solutions

The drugs used in the experiments were AS-8112, ondansetron hydrochloride, *S*(−)-eticlopride, *R*(+)-7-OH-DPAT hydrobromide and racemic CP-99,994 ((±)-(2*S*, 3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine) (synthesized at Dainippon Pharmaceutical, Suita, Japan), metoclopramide hydrochloride, and cisplatin (Sigma, St. Louis, USA). The enantiomeric purity of AS-8112, *S*(−)-eticlopride and *R*(+)-7-OH-DPAT was determined as > 99% enantiomeric excess on the basis of high-performance liquid chromatograms.

AS-8112, metoclopramide, ondansetron, *S*(−)-eticlopride, CP-99,994, *R*(+)-7-OH-DPAT, and cisplatin were dissolved in saline. All doses are expressed in terms of the free base.

2.3. Surgical procedure and experimental protocols

The surgical method of cannulation for drug administration into the 4th cerebral ventricle was carried out as previously reported (Yoshikawa et al., 1996). For i.c.v. administration, each drug was slowly administered in a volume of 10 μl/kg over a period of 1 min through an injection cannula. Two minutes after i.c.v. injection, the cannula was removed and a dummy cannula was inserted instead. In the experiments of *R*(+)-7-OH-DPAT-induced emesis, test drugs or saline (control) was administered 3 min before *R*(+)-7-OH-DPAT (0.3 mg/kg, s.c.), and the latency to the first emetic response, the number of retches, and the number of emetic episodes were recorded for 30 min after injection of *R*(+)-7-OH-DPAT. In the experiments of cisplatin-induced emesis, test drugs were administered immediately after the first emetic episode induced by cisplatin (10 mg/kg, i.v.), and the number of emetic episodes was recorded for 60 min after test drug administration.

2.4. Statistical analysis

Results are expressed as mean ± S.E.M. Significant differences were evaluated using the nonparametric Dunnett's multiple comparison test or the Wilcoxon rank sum test. The significance level was set at $P < 0.05$, and test

Table 1
Effects of AS-8112 and other test drugs administered (i.c.v.) into the 4th cerebral ventricle on *R*(+)-7-OH-DPAT-induced emesis in ferrets

Test drugs	Dose (μg/kg, i.c.v.)	Latency (min)	No. of retches	No. of emetic episodes	Emesis/tested*
Saline		3.8 ± 0.7	24.8 ± 2.4	4.93 ± 0.54	14/14
AS-8112	0.01	5.2 ± 1.2	23.0 ± 2.7	4.00 ± 0.86	6/6
	0.1	9.4 ± 1.9[a]	14.2 ± 2.0	2.83 ± 0.17	6/6
	1	24.4 ± 3.5[c]	1.3 ± 1.0[c]	0.50 ± 0.34[c]	2/6
Metoclopramide	1	8.1 ± 3.1	19.8 ± 6.5	3.83 ± 1.19	5/6
	10	14.9 ± 5.1	8.5 ± 3.1[a]	1.83 ± 0.65[a]	4/6
	100	13.8 ± 5.0[a]	5.0 ± 3.1[b]	1.00 ± 0.45[b]	4/6
CP-99,994	1	13.9 ± 5.1	11.3 ± 5.9	2.17 ± 1.11	3/6
	10	15.0 ± 6.0	8.2 ± 3.5[a]	1.83 ± 0.70[a]	4/6
Ondansetron	10	4.9 ± 2.2	18.3 ± 3.3	3.83 ± 1.01	6/6

Each value represents the mean ± S.E.M. Saline or test drug was injected 3 min before *R*(+)-7-OH-DPAT (0.3 mg kg^{-1}, s.c.). Animals were observed for 30 min after *R*(+)-7-OH-DPAT administration. Statistically significant difference from the control group is indicated by [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, nonparametric Dunnett's multiple comparison test.

* Number of animals.

drug ID_{50} values (doses causing 50% inhibition of the number of emetic episodes elicited by *R*(+)-7-OH-DPAT) were determined by the method of logit analysis.

3. Result

3.1. Antagonistic effects of AS-8112 and other test drugs on R(+)-7-OH-DPAT-induced emesis

The effects of AS-8112, metoclopramide, CP-99,994, and ondansetron administered (i.c.v.) into the 4th cerebral ventricle on *R*(+)-7-OH-DPAT (0.3 mg/kg, s.c.)-induced emesis in ferrets are shown in Table 1. When administered alone, AS-8112 and other test drugs did not cause retching or emesis in ferrets. AS-8112 (0.01–1 μg/kg, i.c.v.) dose dependently prevented *R*(+)-7-OH-DPAT-induced emesis, as evidenced by a prolonged latency and a decrease in the number of retches and emetic episodes compared to those in the control group. The ID_{50} value (95% confidence limits) of AS-8112 was 0.11 (0.042–0.30) μg/kg, i.c.v. Metoclopramide (1–100 μg/kg, i.c.v.) also inhibited *R*(+)-7-OH-DPAT-induced emesis with an ID_{50} value of 6.0 (1.1–34) μg/kg, i.c.v. Furthermore, CP-99,994 (10 μg/kg, i.c.v.) significantly decreased the number of retches and emetic episodes. In contrast, ondansetron at 10 μg/kg, i.c.v. did not inhibit the emesis induced by *R*(+)-7-OH-DPAT.

3.2. Antagonistic effects of AS-8112 and other test drugs on cisplatin-induced emesis

The effects of AS-8112, ondansetron, CP-99,994, and *S*(−)-eticlopride administered (i.c.v.) into the 4th cerebral ventricle on cisplatin-induced emesis in ferrets are shown in Table 2. Intravenously administered cisplatin (10 mg/kg) caused an emetic response in all animals, with the first emetic episode taking place 68 ± 2 min after cisplatin injection. During the period of 0–30 min after test drug administration, AS-8112 and CP-99,994 at 10 μg/kg, i.c.v. significantly decreased the number of emetic episodes, and ondansetron at 10 μg/kg, i.c.v. blocked completely the emetic response. During the rest of the observation period (30–60 min), AS-8112 and CP-99,994 at 10 μg/kg, i.c.v. also significantly decreased the number of emetic episodes, but ondansetron did not inhibit the emetic response. A dopamine D2 and D3 receptor antagonist, *S*(−)-eticlopride (10 μg/kg, i.c.v.), which reduced emesis induced by *R*(+)-7-OH-DPAT (Yoshikawa et al., 1996), did not inhibit the emesis induced by cisplatin during the observation period.

Table 2
Effects of AS-8112 and other test drugs administered (i.c.v.) into the 4th cerebral ventricle on cisplatin-induced emesis in ferrets

Test drugs	Dose (μg/kg, i.c.v.)	Number of animals	No. of emetic episodes	
			0–30 min	30–60 min
Saline		7	6.43 ± 0.78	4.71 ± 0.57
AS-8112	1	6	5.00 ± 0.52	4.33 ± 0.61
	10	6	2.00 ± 0.45[b]	1.83 ± 0.65[a]
Ondansetron	1	6	5.00 ± 0.58	4.33 ± 0.76
	10	6	0[c]	3.00 ± 0.97
CP-99,994	10	6	2.83 ± 0.75[b]	2.50 ± 0.56[a]
S(−)-eticlopride	10	6	6.00 ± 1.06	3.17 ± 0.95

Each value represents the mean ± S.E.M. Saline or test drug was administered immediately after the first emetic episode induced by cisplatin (10 mg/kg, i.v.). Animals were observed for 60 min after administration of test drugs. Statistically significant difference from the control group is indicated by [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$, nonparametric Dunnett's multiple comparison test (AS-8112 and ondansetron) or the Wilcoxon rank sum test (CP-99,994 and S(−)-eticlopride).

4. Discussion

This study demonstrates that the antiemetic effects of AS-8112 against emesis-induced by *R*(+)-7-OH-DPAT or cisplatin are centrally mediated via dopamine D3 and 5-HT_3 receptors in ferrets. Intracerebroventricular administration of AS-8112 dose dependently inhibited the emetic responses induced by *R*(+)-7-OH-DPAT and significantly decreased the number of emetic episodes induced by cisplatin.

We have previously reported that AS-8112 has high and selective affinity for dopamine D2, D3, and 5-HT_3 receptors (Yoshikawa et al., 1998). In addition, the affinity of AS-8112 for the dopamine D3 receptor is about 60 times higher than that of metoclopramide, and its affinity for the 5-HT_3 receptor is equal to that of ondansetron, a selective 5-HT_3 receptor antagonist (data not shown). In the present study, the antiemetic effect of AS-8112 against emesis-induced by *R*(+)-7-OH-DPAT was about 55 times more potent than that of metoclopramide. The potency of AS-8112 and metoclopramide in preventing emesis induced by *R*(+)-7-OH-DPAT correlated well with their affinity for the dopamine D3 receptor. We have previously reported that *R*(+)-7-OH-DPAT-induced emesis is mediated via dopamine D3 receptors located in the area postrema in ferrets (Yoshikawa et al., 1996). In addition, it is clear that the antagonistic effect of AS-8112 on 5-HT_3 receptors is not involved in its antiemetic effects, because ondansetron, a selective 5-HT_3 receptor antagonist, had no effect on *R*(+)-7-OH-DPAT-induced emesis. These results indicate that the antiemetic effect of AS-8112 on *R*(+)-7-OH-DPAT-induced emesis is centrally mediated via the dopamine D3 receptor located in the area postrema.

As for the cisplatin-induced emesis, the antiemetic effect of AS-8112 was slightly less potent but slightly longer lasting than that of ondansetron. Cisplatin-induced emesis in ferrets is mainly mediated by stimulation of abdominal visceral afferent nerves via 5-HT_3 receptors (Hawthorn et al., 1988; Andrews et al., 1990). However, central 5-HT_3

receptors, which are mainly located in the area postrema, are also important in emetic responses, since the administration of ondansetron or other 5-HT_3 receptor antagonists into the area postrema or the 4th cerebral ventricle inhibits emesis induced by cisplatin (Higgins et al., 1989; Smith et al., 1988; Kamato et al., 1993). Moreover, we have previously reported that cisplatin-induced emesis can be partially reduced by abdominal vagotomy but is almost completely abolished by ablation of the area postrema (Yoshikawa et al., 1996). In this study, during the time period 0–30 min after administration of ondansetron at 10 μg/kg, i.c.v., none of the ferrets showed an emetic response to cisplatin. These findings suggest that the initial phase of cisplatin-induced emesis, at least in part, is mediated by stimulation of central 5-HT_3 receptors in the area postrema. In addition, cisplatin-induced emesis cannot be mediated via dopamine D2 or D3 receptors because *S*(−)-eticlopride, a dopamine D2 and D3 receptor antagonist, failed to inhibit this emesis in the present study. These results indicate that 5-HT_3 receptors located in the area postrema are involved in the antiemetic effect of AS-8112 on cisplatin-induced emesis.

Recently, tachykinin NK_1 receptor antagonists such as CP-99,994, GR203040, and L-742,694 have been shown to have broad antiemetic effects mediated via tachykinin NK_1 receptors located in the nucleus tractus solitarii (Longmore et al., 1997). In this study, CP-99,994 (10 μg/kg, i.c.v.) significantly inhibited the emesis induced by *R*(+)-7-OH-DPAT or cisplatin. These results are in accordance with those of a previous study showing that CP-99,994 (1 mg/kg, s.c.) equally prevented emesis induced by cisplatin (10 mg/kg, i.p.), apomorphine (0.2 mg/kg, s.c.), and other drugs in the ferret (Watson et al., 1995). In addition, in the present study, the antiemetic effect of CP-99,994 on *R*(+)-7-OH-DPAT-induced emesis was about 10 times less than that of AS-8112, although the effect of CP-99,994 on cisplatin-induced emesis was as potent as that of AS-8112. This observation may be explained by different sites of antiemetic action between the two drugs. The antiemetic effect of AS-8112 may be mainly mediated via a direct action on central dopamine D3 or 5-HT_3 receptors in ferrets. In contrast, many studies suggest that the mechanism by which CP-99,994 inhibits emetic reflex pathways induced by various emetogens is mediated via tachykinin NK_1 receptors in the nucleus tractus solitarii (Longmore et al., 1997). Thus, further studies are needed to elucidate the difference in mechanisms between the two drugs.

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