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Involvement of dopamine D_3 receptors in the area postrema in $R(+)$-7-OH-DPAT-induced emesis in the ferret

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Abstract

We investigated the possible involvement of dopamine D_3 receptors in $R(+)$-7-hydroxy-2-(N,N-di-n-propylamino)tetraline ($R(+)$-7-OH-DPAT)-induced emesis in the ferret. The $R(+)$enantiomer of 7-OH-DPAT (0.03–1 mg/kg, s.c.) caused emesis in a dose-dependent manner, whereas the $S(-)$enantiomer, even at 1 mg/kg s.c. failed to induce emesis. Quinpirole (0.1–1.0 mg/kg) and apomorphine (0.3 mg/kg, s.c. only) also elicited an emetic response. $S(-)$-Eticlopride, which has a high affinity for the dopamine D_3 receptor, antagonized $R(+)$-7-OH-DPAT (0.3 mg/kg, s.c.)-induced emesis (ID_{50} 1.4 μg/kg, s.c.). $R(+)$-7-OH-DPAT (0.1–1.0 μg) administered into the 4th cerebral ventricle dose dependently induced emesis within 1 min of dosing in ferrets. Intracerebroventricularly administered $S(-)$-eticlopride (0.01–1 μg) also inhibited the emesis induced by s.c. administration of $R(+)$-7-OH-DPAT. The emetic effect of $R(+)$-7-OH-DPAT was unaffected by abdominal vagotomy but was markedly reduced by ablation of the area postrema. These results suggest that dopamine D_3 receptors in the area postrema play an important role in $R(+)$-7-OH-DPAT-induced emesis in the ferret.

Keywords: Dopamine D_3 receptor; R-(+)-7-OH-DPAT (R-(+)-7-hydroxy-2-(N,N-di-n-propylamino)tetralin); Emesis; Area postrema; (Ferret)

1. Introduction

Molecular biological studies have recently identified a family of dopamine D_2-like receptors which include the D_2, D_3, and D_4 receptor subtypes (Giros et al., 1989; Monsma et al., 1989; Sokoloff et al., 1990; Van Tol et al., 1991). Among them, the novel dopamine D_3 receptor may, in the view of its high density in the mesolimbic dopaminergic projection field, play an important role in the pathogenesis of psychiatric disorders such as schizophrenia, depression and drug abuse (Bouthenet et al., 1991; Caine and Koob, 1993; Sokoloff et al., 1990).

Recently (±)-7-hydroxy-2-(N,N-di-n-propylamino)tetraline (7-OH-DPAT) has been reported as a high affinity and selective agonist at dopamine D_3 receptors expressed in transfected cell lines and it has been suggested that the $R(+)$enantiomer of 7-OH-DPAT is a dopamine D_3 receptor-selective isomer (Baldessarini et al., 1993; Damsma et al., 1993; Rivet et al., 1994). 7-OH-DPAT produces sniffing and yawning behaviour, hypothermia, and hypertensive effects in mice and rats (Ahlenius and Salmi, 1994; Damsma et al., 1993; Daly and Waddington, 1993; McElroy and Ward, 1995; Millan et al., 1994; Van den Buuse, 1993). More recently, Glavin (1995) reported that 7-OH-DPAT reduced basal gastric acid secretion and pepsin secretion in rats.

It is well known that apomorphine causes emesis in ferrets, dogs and humans (Andrews, 1990; Carpenter, 1989; King, 1990; Klein et al., 1970; Procter et al., 1978). Experiments with dogs have revealed that apomorphine-induced emesis may be mediated by dopamine D_2 receptors in the area postrema, the locus of the chemoreceptor trigger zone (Andrews, 1990; Harding et al., 1987). However, recent studies reported that apomorphine has equal affinity for dopamine D_2 and D_3 receptor sites (Seeman and Van Tol, 1994). Thus, it is not clear whether apomorphine-induced emesis is mediated by dopamine D_2 receptors and/or dopamine D_3 receptors.

The present study was carried out to investigate whether the selective dopamine D_3 receptor agonist, $R(+)$-7-OH-DPAT, elicited emesis in the ferret. Moreover, we investigated the site of dopamine D_3 receptors inducing the emetic response.

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2. Materials and methods

2.1. Animals

Male albino ferrets (Marshall Res. Animal) weighing 1–1.6 kg and individually housed at 25 ± 1°C on a 12-h light/dark cycle were used in this study. They were given a standard cat diet and allowed free access to water. The animals were fasted overnight prior to all experiments.

2.2. Surgery

2.2.1. Cannulation for administration of drugs into the 4th cerebral ventricle

Ferrets were anesthetized with pentobarbital sodium (35–40 mg/kg, i.p.) and placed in a stereotaxic apparatus. The skull was exposed and a small hole drilled to allow the unilateral stereotaxic implantation of the guide cannula (23 gauge stainless steel tube, 22 mm length) in the 4th cerebral ventricle (1 mm above the area postrema) according to the method of Higgins et al. (1989). The coordinates for this area were: −17.00 mm anterior to bregma, −4.00 mm lateral to the midline and −13.00 mm at 20° from the vertical axis from skull surface. Cannulas were fixed to the skull with stainless steel screws and dental cement. Experiments were performed at least 4 days after surgery. To confirm the location of the cannula, the animals were killed with pentobarbital sodium after the experiments, and 1–2 μl of Evans blue solution was injected into the 4th cerebral ventricle via the indwelling cannula.

2.2.2. Vagotomy

Vagotomy was performed according to the method of Hawthorn et al. (1988). The animals were anesthetized with pentobarbital sodium. The dorsal and ventral vagal trunks coursing along the supradiaphragmatic oesophagus were ligated and sectioned. In addition the oesophageal serosa, at the level of the gastroesophageal junction, was incised and the hepatic branch of the ventral vagus was sectioned. The animals were allowed 7–14 days to recover from surgery prior to use in experiments.

2.2.3. Ablation of area postrema

The area postrema was destroyed electrolytically according to the method of Jovanovic-Micic et al. (1995). After pentobarbital sodium anaesthesia, a 4.5 mA d.c. electrolytic current was delivered to the area postrema for 1 min by a lesion-producing device (Stoelting Co., Chicago, IL, USA). The coordinates of the area postrema were as described above. The animals were allowed 10 days to recover from surgery prior to use in experiments. The ablation of the area postrema was verified by standard histological procedures.

2.3. Experimental protocols

Each dopamine receptor agonist was administered s.c. in a volume of 2 ml/kg in ferrets. For i.c.v. administration, test drugs were slowly administered in a volume of 10 μl over a period of 1 min through an injection cannula (27 gauge, 23 mm length). Two minutes after i.c.v. injection, the cannula was removed and a dummy cannula was inserted. In some experiments, *S*(−)-eticlopride or domperidone was administered 30 min (s.c.) or 3 min (i.c.v.) before *R*(+)-7-OH-DPAT (0.3 mg/kg, s.c.).

The animals were observed for 30 min (s.c.) or 15 min (i.c.v.) after each dopamine receptor agonist and the number of retches and vomits were recorded. In other experiments, *R*(+)-7-OH-DPAT was administered s.c. to vagotomized, ablated (area postrema) or sham-operated ferrets, and the animals were observed for 30 min as described above.

Table 1
Emetic effects of dopaminergic agents in ferrets

Treatment	Dose (mg/kg, s.c.)	Latency (min)	No. of retches	No. of emetic episodes	Emesis/ tested
R(+)-7-OH-DPAT	0.01	28.3 ± 1.1	0.7 ± 0.5	0	0/6
	0.03	9.9 ± 2.3	13.8 ± 3.8	3.33 ± 1.15	5/6
	0.1	4.7 ± 0.7	15.2 ± 3.7	4.00 ± 1.03	6/6
	0.3	2.3 ± 0.3	13.0 ± 2.9	4.44 ± 1.00	9/9
	1.0	2.2 ± 0.6	24.0 ± 4.2	6.50 ± 1.28	6/6
S(−)-7-OH-DPAT	1.0	> 30	0	0	0/4
Apomorphine	0.1	18.5 ± 4.2	4.0 ± 1.5	0.25 ± 0.25	1/4
	0.3	6.2 ± 0.6	13.5 ± 3.0	3.75 ± 1.11	4/4
	1.0	9.9 ± 5.1	4.6 ± 2.3	1.80 ± 0.97	3/5
Quinpirole	0.03	22.8 ± 3.1	2.6 ± 1.7	0.60 ± 0.60	1/5
	0.1	8.8 ± 1.1	7.0 ± 2.5	3.00 ± 1.14	4/5
	0.3	5.7 ± 0.9	14.2 ± 2.0	5.40 ± 1.33	5/5
	1.0	3.2 ± 1.0	22.8 ± 4.6	8.40 ± 2.68	5/5
SKF38393	1.0	> 30	0	0	0/4

Each value represents the mean ± S.E.M. Retching and emetic episodes were recorded for 30 min after administration of agents.

2.4. Statistical analysis

The results are expressed as means ± S.E.M. A one-way analysis of variance was followed by the Mann-Whitney U-test. The significance level for all tests was set at $P < 0.05$. The ID_{50} values of the test drugs (doses causing 50% inhibition of the number of emetic episodes elicited by *R*(+)-7-OH-DPAT) were determined by Probit analysis.

2.5. Drugs

The drugs used in the experiments were: *R*(+), *S*(−)-7-OH-DPAT hydrobromide, *S*(−)-eticlopride, and domperidone, which were synthesized at Dainippon Pharmaceutical Co., quinpirole hydrochloride and 1-phenyl-2,3,4,5,-tetrahydro-(1*H*)-3-benzazepine-7,8-diol hydrochloride (SKF38393) (Research Biochemical), apomorphine hydrochloride and cisplatin (Sigma Chemical Co.). The enantiomeric purity of 7-OH-DPAT was determined to be > 99% enantiomeric excess on the basis of high-performance liquid chromatograms.

R(+) and *S*(−)-7-OH-DPAT, *S*(−)-eticlopride, quinpirole and SKF38393 were dissolved in saline solution. Apomorphine was dissolved in distilled water. Domperidone was dissolved in 0.1% lactic acid/saline. Doses of drugs are expressed in terms of the free base.

3. Results

3.1. Emetic effect of dopamine D_3 receptor agonists

Table 1 shows the emetic effects of subcutaneous administration of various dopaminergic agents in the ferret. *R*(+)-7-OH-DPAT (0.03–1 mg/kg, s.c.) caused emesis in a dose-dependent manner (Table 1). The frequency distribution of retches and emetic episodes after *R*(+)7-OH-DAPT (0.3 mg/kg, s.c.) is shown in Fig. 1. The highest frequency occurred over the 5–10 min time bins and no retching or vomiting occurred after 25 min. In contrast, the

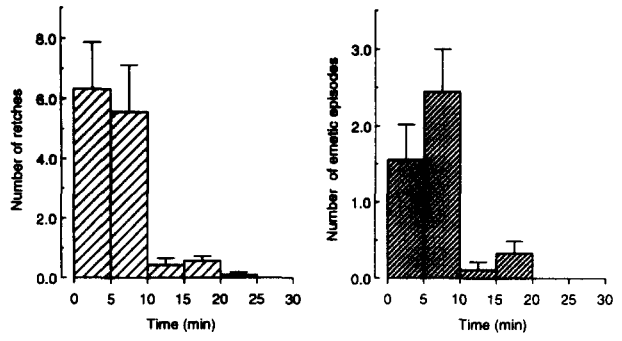

Fig. 1. Profile of retches or emesis after *R*(+)-7-OH-DPAT (0.3 mg/kg, s.c.) in ferrets. The *x*-axis represents bins of 5 min. The *y*-axis denotes the mean ± S.E.M. number of retches or emesis observed for each 5-min bin ($n = 9$ animals).

S(−)-isomer of 7-OH-DPAT, even at 1 mg/kg s.c., failed to cause retching and emesis in all ferrets tested ($n = 4$). Quinpirole (0.03–1 mg/kg, s.c.) also induced emesis, the potency being less than that of *R*(+)-7-OH-DPAT. Although apomorphine (0.1–1 mg/kg, s.c.) also caused retching and emetic responses, the effects were not dose-dependent. Namely, retching and vomiting occurred in all animals tested at a dose of 0.3 mg/kg apomorphine, but the potency was less than that of *R*(+)-7-OH-DPAT or quinpirole. However, at the highest dose (1 mg/kg, s.c.) of apomorphine only 3 out of 5 animals showed an emetic response (Table 1). SKF38393, a dopamine D_1 receptor agonist, at 1 mg/kg did not induce retch or emesis in any ferrets ($n = 4$).

3.2. Effects of dopamine receptor antagonists on *R*(+)-7-OH-DPAT-induced emesis

The dopamine D_2 and D_3 receptor antagonists, *S*(−)-eticlopride and domperidone, were used to prevent the emesis produced by *R*(+)-7-OH-DPAT (0.3 mg/kg, s.c.). As shown in Table 2, *S*(−)-eticlopride (0.3–10 μg/kg) dose relatedly prevented *R*(+)-7-OH-DPAT-induced emesis; the latency was prolonged and the number of retches

Table 2
Effects of *S*(−)-eticlopride and domperidone on *R*(+)-7-OH-DPAT-induced emesis in ferrets

Treatment	Dose (mg/kg, s.c.)	Latency (min)	No. of retches	No. of emetic episodes	Emesis/ tested
Control		2.3 ± 0.3	13.0 ± 2.9	4.44 ± 1.00	0/9
S(−)-Eticlopride	0.0003	11.7 ± 5.8	11.3 ± 3.9	3.33 ± 1.26	2/6
	0.001	10.5 ± 3.3 [a]	12.3 ± 2.5	2.75 ± 0.92	3/8
	0.003	9.6 ± 1.7 [b]	7.5 ± 2.2	1.50 ± 0.62 [a]	2/6
	0.01	> 30 [b]	0 [b]	0 [b]	6/6
Domperidone	0.003	5.5 ± 1.2	10.8 ± 3.1	2.33 ± 0.92	1/6
	0.01	19.7 ± 4.2 [b]	4.2 ± 2.0 [a]	1.17 ± 0.54 [a]	3/6
	0.03	25.9 ± 2.8 [b]	3.2 ± 2.0 [a]	0.67 ± 0.42 [b]	4/6
	0.1	> 30 [b]	0 [b]	0 [b]	6/6

Each value represents the mean ± S.E.M. *S*(−)-eticlopride or domperidone was injected 30 min before *R*(+)-7-OH-DPAT (0.3 mg/kg, s.c.). Retching and emetic episodes were recorded for 30 min after administration of *R*(+)-7-OH-DPAT. [a] $P < 0.05$. [b] $P < 0.01$, significantly different from control group.

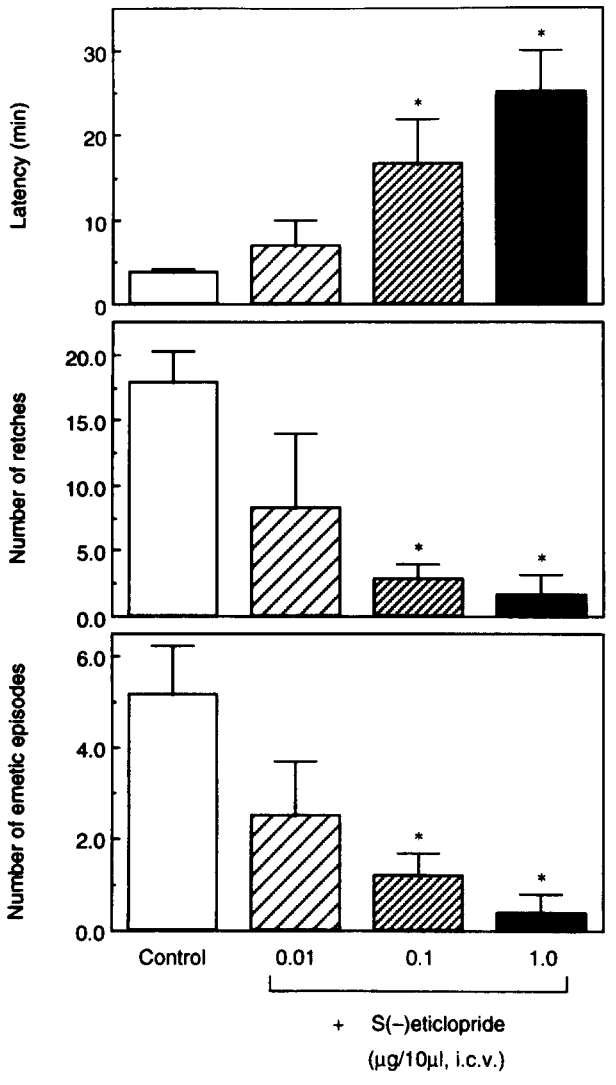

Fig. 2. Effect of $S(-)$-eticlopride administered into the 4th cerebral ventricle on $R(+)$-7-OH-DPAT-induced emesis in ferrets. Columns represent the means ± S.E.M. $S(-)$-eticlopride was injected 3 min before $R(+)$-7-OH-DPAT (0.3 mg/kg, s.c.). Retching and emetic episodes were recorded for 30 min after administration of $R(+)$-7-OH-DPAT. * $P <$ 0.01, significantly different from control group.

and emetic episodes was decreased. The ID_{50} value, the dose causing 50% inhibition of the number of emetic episodes, of $S(-)$-eticlopride was 1.4 (0.48–4.3) μg/kg, s.c. Domperidone (3–100 μg/kg) also inhibited $R(+)$-7-OH-DPAT-induced emesis with an ID_{50} value of 2.6 (0.3–25.3) μg/kg, s.c.. The anti-emetic effect of $S(-)$-eticlopride was 2-fold more potent than that of domperidone. Furthermore, the emetic responses induced by quinpirole (0.3 mg/kg, s.c.) or apomorphine (0.3 mg/kg, s.c.) were completely inhibited by $S(-)$-eticlopride at 0.01 mg/kg, s.c. (data not shown). Clozapine or ondansetron at 1 mg/kg s.c. did not significantly inhibit the emesis induced by $R(+)$-7-OH-DPAT at 0.3 mg/kg s.c. (data not shown).

3.3. Emetic effect of i.c.v. injection of 7-OH-DPAT

Table 3 shows the emetic effects of 7-OH-DPAT administered into the 4th cerebral ventricle in ferrets. When administered i.c.v., vehicle (10 μl saline) failed to induce retches or emesis in any ferrets ($n = 6$). $R(+)$-7-OH-DPAT (1 μg) administered into the 4th cerebral ventricle caused retches and emesis within 1–2 min and the effect lasted for 10–12 min. The emetic effects of $R(+)$-7-OH-DPAT were shown in a dose-dependent manner. In contrast, at 10 μg of $S(-)$isomer of 7-OH-DPAT only 1 out of 5 animals showed a few retches but no emetic episodes (Table 3). Quinpirole (1–10 μg) administered i.c.v. also induced emesis, but the potency was less than that of $R(+)$-7-OH-DPAT.

3.4. Antagonistic effect of i.c.v. S(−)-eticlopride on R(+)-7-OH-DPAT-induced emesis

We examined the pretreatment effect of i.c.v. $S(-)$-eticlopride on $R(+)$-7-OH-DPAT-induced emesis in ferrets. When administered i.c.v., $S(-)$-eticlopride (0.01–1 μg/animal) alone did not cause retch or emesis in any ferrets. Pretreatment with i.c.v. $S(-)$-eticlopride (0.01–1 μg) dose dependently inhibited the number of retches and emesis induced by $R(+)$-7-OH-DPAT (0.3 mg/kg, s.c.) with an ID_{50} value of 8.9 (0.4–186.1) ng (Fig. 2).

3.5. Effect of vagotomy or ablation of the area postrema on R(+)-7-OH-DPAT-induced emesis

$R(+)$-7-OH-DPAT at 0.3 mg/kg s.c. induced emesis in sham-operated animals as in the control animals described

Table 3
Emetic effects of $R(+)$, $S(-)$-7-OH-DPAT and quinpirole administered into the 4th cerebral ventricle in ferrets

Treatment	Dose (μg/animal)	Latency (min)	No. of retches	No. of emetic episodes	Emesis/ tested
Saline		> 15	0	0	0/6
$R(+)$-7-OH-DPAT	0.1	12.2 ± 2.8	1.4 ±1.4	0.40 ± 0.40	1/5
	0.3	6.0 ± 2.8	11.2 ±4.6	3.67 ± 1.73	6/6
	1.0	1.2 ± 0.2	15.8 ±2.9	4.00 ± 0.89	5/5
$S(-)$-7-OH-DPAT	10	12.5 ± 2.5	0.60 ± 0.6	0	0/5
Quinpirole	1.0	8.3 ± 2.5	6.0 ±2.4	2.29 ± 0.94	4/7
	10	2.7 ± 0.3	19.0 ±4.9	5.67 ± 1.67	3/3

Each value represents the mean ± S.E.M. Retching and emetic episodes were recorded for 15 min after administration of agents.

Table 4
Effect of vagotomy on the emetic responses to R(+)-7-OH-DPAT or cisplatin in ferrets

Drug	Treatment	Latency (min)	No. of retches	No. of emetic episodes
R(+)-7-OH-DPAT 0.3 mg/kg, s.c.	Sham operation	6.2 ± 2.0	12.2 ± 3.0	3.20 ± 0.86
	Vagotomy	3.1 ± 1.3	17.1 ± 3.6	4.14 ± 1.18
Cisplatin 10 mg/kg, i.p.	Sham operation	74.0 ± 3.2	–	13.6 ± 1.9
	Vagotomy	99.2 ± 9.1 [a]	–	4.0 ± 1.1 [b]

Each value represents the mean ± S.E.M. Retching and emetic episodes were recorded for 30 min after administration of R(+)-7-OH-DPAT and for 3 h after cisplatin. [a] $P < 0.05$. [b] $P < 0.01$, significantly different from sham operation group.

above (Table 1). In vagotomized ferrets, R(+)-7-OH-DPAT at 0.3 mg/kg s.c. induced emesis; the latency to the first emetic episodes was 3.1 ± 1.3 ($n = 4$) min, and the number of retches and emetic episodes was 17.1 ± 3.6 and 4.14 ± 1.18, respectively (Table 4). These values did not differ significantly from those of the sham-operated animals. The emesis induced by cisplatin (10 mg/kg, i.p.) was significantly reduced by vagotomy, as shown in Table 4.

In ferrets with a lesion of the area postreama, the onset of the retches and emesis induced by R(+)-7-OH-DPAT (0.3 mg/kg, s.c.) was markedly and the number of retches and emetic episodes was reduced (Table 5). These values were significantly different from those of the sham-operated animals. Furthermore, the emesis induced by cisplatin (10 mg/kg, i.p.) was also significantly reduced by ablation of the area postrema, as shown in Table 5. Histological sections confirmed nearly complete removal of the area postrema, with partial damage to the nucleus tractus solitarii, the area immediately subjacent to the area postrema.

4. Discussion

This study is the first to demonstrate that the selective dopamine D_3 receptor agonist, the R(+)enantiomer of 7-OH-DPAT, caused emesis in ferrets. Namely, subcutaneous administration of R(+)-7-OH-DPAT dose relatedly caused retching and emetic episodes within 3–4 min after dosing with a short duration of action. Interestingly, the S(−)enantiomer of 7-OH-DPAT at higher doses failed to induce emesis in the ferrets used. The result was consistent with those obtained by Damsma et al. (1993) and Rivet et al. (1994), who reported that S(−)-7-OH-DPAT had about 40–70-fold less affinity for dopamine D_3 receptors than R(+)-7-OH-DPAT. Moreover, the affinity of R(+)-7-OH-DPAT for the dopamine D_3 receptor was approximately 60–200-fold higher than that for the D_2 receptor (Baldessarini et al., 1993; Damsma et al., 1993; Rivet et al., 1994). Thus, these findings suggest that dopamine D_3 receptors may be involved in R(+)-7-OH-DPAT-induced emesis in ferrets. Furthermore, other dopamine receptor agonists, quinpirole and apomorphine, also induced emesis in ferrets. The potency of the agonists examined, R(+)-7-OH-DPAT > quinpirole > apomorphine, agrees well with the rank order of their affinities for dopamine D_3 receptor sites (Lévesque et al., 1992). Quinpirole, like R(+)-7-OH-DPAT, was reported as a preferential ligand of dopamine D_3 vs. D_2 receptors (Lévesque et al., 1992; Sokoloff et al., 1990). As described in the Introduction, however, apomorphine has been shown to be a non-selective dopamine receptor agonist which has equally high affinity for dopamine D_2, D_3 and D_4 receptors genetically transfected into cell membrane preparations (Seeman and Van Tol, 1994). As a result of this indiscriminate binding, it is difficult to assign a role for individual dopamine D_2-like receptor subtypes (D_2, D_3 and D_4) in the emetic effect of apomorphine. The availability of selective agonists for dopamine D_2 or D_4 receptor subtypes may prove useful in advancing our understanding of the functional role of individual dopamine receptor subtypes in emesis. However, the results from the present studies using 7-OH-DPAT or quinpirole in ferrets suggest that the dopamine D_3 receptor may be, at least in part, involved in the control

Table 5
Effect of ablation of the area postrema (AP) on the emetic responses to R(+)-7-OH-DPAT or cisplatin in ferrets

Drug	Treatment	Latency (min)	No. of retches	No. of emetic episodes
R(+)-7-OH-DPAT 0.3 mg/kg, s.c.	Sham operation	3.7 ± 0.5	17.9 ± 2.3	5.14 ± 1.08
	Ablation of AP	21.7 ± 5.4 [a]	1.6 ± 1.0 [b]	0.20 ± 0.20 [b]
Cisplatin10 mg/kg, i.p.	Sham operation	74.0 ± 3.2	–	13.6 ± 1.9
	Ablation of AP	159.2 ± 12.8 [b]	–	0.8 ± 0.5 [b]

Each value represents the mean ± S.E.M. Emetic episodes were recorded for 30 min after administration of R(+)-7-OH-DPAT and for 3 h after cisplatin. [a] $P < 0.05$. [b] $P < 0.01$, significantly different from sham operation group.

of emesis since these two compounds are highly selective dopamine D_3 receptor agonists.

There are no reports whether dopamine D_1 receptor agonists can induce emesis in experimental animals. In the present experiments, the selective dopamine D_1 receptor agonist, SKF38393, did not cause emesis at higher doses in ferrets. Lévesque et al. (1992) reported that the affinity of 7-OH-DPAT for the dopamine D_1 receptor was approximately 10000-fold lower than that for the D_3 receptor. These results clearly indicate that dopamine D_1 receptors are probably not involved in *R*(+)-7-OH-DPAT-induced emesis in ferrets. Pretreatment with the dopamine D_2 and D_3 receptor antagonists, *S*(−)-eticlopride and domperidone, inhibited the retching and emetic episodes induced by *R*(+)-7-OH-DPAT in ferrets. However, pretreatment with clozapine, a dopamine D_4 receptor antagonist, did not inhibit the emesis induced by *R*(+)-7-OH-DPAT in ferrets, and 7-OH-DPAT has 1000-fold lower affinity for dopamine D_4 receptors than for dopamine D_3 receptors (Lévesque et al., 1992). Therefore, these results indicate that *R*(+)-7-OH-DPAT-induced emesis is not mediated via the dopamine D_4 receptor subtype. In addition, the recent study of Yoshida et al. (1995) showed that *R*(+)-7-OH-DPAT induced emesis in dogs as well as in ferrets, whereas SKF38393 failed to induce emesis. Furthermore, the emesis induced by *R*(+)-7-OH-DPAT was found to be abolished by *S*(−)-eticlopride but not by SCH23390 or clozapine in dogs. These observations suggest that *R*(+)-7-OH-DPAT produces its emetic responses mediated via dopamine D_3 receptor sites in both the ferret and the dog.

There is considerable evidence that various subtypes of 5-HT receptors mediate the emetic response. Selective 5-HT_3 receptor antagonists such as granisetron and ondansetron inhibit emesis induced by cancer chemotherapeutic agents and radiation in ferrets and dogs (Andrews, 1990; King, 1990; Yoshida et al., 1992). In a binding study, *R*(+)-7-OH-DPAT had no affinity for 5-HT_3 receptor sites (unpublished observation). Furthermore, ondansetron at a high dose failed to inhibit *R*(+)-7-OH-DPAT-induced emesis in ferrets. However, it is well known that 2-aminotetralins and related compounds including 7-OH-DPAT have affinity for 5-HT_{1A} receptors. Zhuang et al. (1993) reported that 7-OH-DPAT had about 30-fold less affinity for 5-HT_{1A} receptors than for dopamine D_3 receptors. Recently, a broad antiemetic action has been reported to result from stimulation of 5-HT_{1A} receptors by agonists (Lucot and Crampton, 1989; Okada et al., 1994). Although 7-OH-DPAT has a moderate affinity for 5-HT_{1A} receptor sites, *R*(+)-7-OH-DPAT-induced emesis is probably not mediated via 5-HT_{1A} receptors. Thus, it is suggested that 5-HT receptor subtypes are not involved in *R*(+)-7-OH-DPAT-induced emesis in ferrets.

Apomorphine is known to produce its emetic effect via the chemoreceptor trigger zone in the area postrema of the brainstem (Carpenter, 1990). For example, it has been shown that apomorphine injected into the area postrema can induce emesis in ferrets and dogs (Harding et al., 1987; King, 1990). In addition, the ablation of the area postrema causes inhibition of apomorphine-induced emesis in dogs (Carpenter, 1990; Harding et al., 1987). From these reports, it is suggested that the emetic effect of apomorphine is mediated by central dopaminergic mechanisms. We therefore felt it necessary to investigate the site of dopamine D_3 receptors for inducing the emetic response to *R*(+)-7-OH-DPAT in ferrets. In the present study, when administered into the 4th cerebral ventricle, *R*(+)-7-OH-DPAT and quinpirole caused emesis immediately (1–2 min after dosing), whereas *S*(−)-7-OH-DPAT failed to induce emesis as it did after s.c. administration. In our study, when administered i.c.v. the emetic effect of *R*(+)-7-OH-DPAT was more than 100-fold more potent than when the drug was given by s.c. injection. Furthermore, *S*(−)-eticlopride administered into the 4th cerebral ventricle dose relatedly and potently prevented the retching and emetic episodes induced by s.c. administration of *R*(+)-7-OH-DPAT with an ID_{50} value of 8.9 ng/animal. Based on ID_{50} values, i.c.v. administration of *S*(−)-eticlopride was about 100-fold more potent than s.c. administration in inhibiting the *R*(+)-7-OH-DPAT-induced emesis. Therefore, these findings indicate that *R*(+)-7-OH-DPAT can produce its emetic effect by an action on central dopamine D_3 receptors in ferrets.

To confirm further the role of central sites, we examined the effect of ablation of the area postrema or vagotomy on *R*(+)-7-OH-DPAT-induced emesis in ferrets. In the present study, the destruction of the area postrema markedly abolished the emetic response to s.c. administration of *R*(+)-7-OH-DPAT in ferrets. It appears therefore that the chemoreceptor trigger zone is essential to the emetic actions of *R*(+)-7-OH-DPAT in ferrets. This result was supported by the previous finding that ablation of the area postrema prevents the emesis induced by centrally acting agents such as apomorphine, loperamide and nicotine (Bhandari et al., 1992; Beleslin and Krstic, 1986; Carpenter, 1990). It is known that the vagal nerve also plays an important role in the emesis induced by peripherally acting stimuli such as copper sulfate and cytotoxic drugs (Andrews, 1990). For example, the emetic response evoked by cisplatin, a cytotoxic agent, has been shown to be affected by abdominal vagotomy in ferrets (Hawthorn et al., 1988). In the present experiment, abdominal vagotomy, which markedly reduces the emetic response to cisplatin, had no effect on *R*(+)-7-OH-DPAT-induced emesis in the ferret. These findings strongly indicate that *R*(+)-7-OH-DPAT exerts its emetic effect through dopamine D_3 receptors located in the area postrema. However, it remains to be confirmed whether dopamine D_3 receptors present in the area postrema are involved in *R*(+)-7-OH-DPAT-induced emesis in ferrets.

In conclusion, the dopamine D_3 receptor agonist, *R*(+)-7-OH-DPAT, administered both peripherally and centrally, caused emesis in ferrets. In addition, the emetic

response induced by both s.c. and i.c.v. administration of *R*(+)-7-OH-DPAT was strongly inhibited by *S*(−)-eticlopride or domperidone. Furthermore, ablation of the area postrema completely reduced the emesis induced by s.c. administration of *R*(+)-7-OH-DPAT in ferrets. These studies suggest that the dopamine D_3 receptors in the area postrema play an important role in the regulation of emesis in ferrets. Furthermore, the selective dopamine D_3 receptor antagonist might be useful as a new antiemetic agent.

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