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# The role of $D_2$ and $D_3$ dopamine receptors in the mediation of emesis in Cryptotis parva (the least shrew)

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**Summary.** This study introduces Cryptotis parva (the least shrew) as a new dopaminergic animal model of emesis. The potential emetogenic effects of a nonselective dopamine agonist [apomorphine], two $D_1$ agonists [SKF-38393 and SKF-82958], a $D_2$ preferring agonist [quinpirole], and two $D_3$-preferring agonists [7-(OH) DPAT and PD 128, 907] were investigated. Intraperitoneal administration of $D_1$ agonists failed to induce emesis. However, other agonists caused a dose-dependent increase in the percentage of animals vomiting as well as potentiating the mean frequency of emesis with the following $ED_{50}$ potency order: 7-(OH) DPAT < apomorphine < quinpirole < PD 128, 907. For antagonist studies a 2 mg/kg dose of these agonists were used to induce emesis. Thus, the inhibitory dose-response effects of a $D_2$-preferring [sulpride], a $D_3$-preferring [U 99194A] and combination of varying doses of these antagonists [sulpride + U 99194A] were evaluated on the ability of the cited agonists to produce vomiting. Sulpride decreased the number of shrews vomiting and the mean vomiting frequency produced by the cited agonists in a dose-dependent fashion with the following $ID_{50}$ order [apomorphine < PD 128, 907 < 7-(OH) DPAT < quinpirole]. By itself, U 99194A failed to significantly alter the emesis produced by any of the cited agonists, however, it potentiated (3–8 times) the antiemetic effects of sulpride both in reducing the number of shrews vomiting as well as decreasing the mean vomiting frequency with the following $ID_{50}$ order: PD 128, 907 < 7-(OH) DPAT < quinpirole. However, U 99194A attenuated the potent antiemetic effect of sulpride on the apomorphine-induced emesis. The results suggest that the tested agonists primarily activate dopamine $D_2$ receptors to induce emesis in the least shrew whereas activation of $D_3$ sites potentiate the vomiting action of $D_2$ dopamine receptors.

**Keywords:** Cryptotis parva, least shrew, apomorphine, 7-(OH) DPAT, quinpirole, PD 128, 907, sulpride, U99194A, dopamine $D_2$ receptor, dopamine $D_3$ receptor.

## Introduction

Emesis is a reflex that has developed to different degrees in different species and allows an animal to rid itself from ingested toxins. It is a complicated process and requires coordination by the vomiting center (VC) (Reviews: Brunton, 1996; Naylor and Rudd, 1996). The VC is a collection of recipient and effector nuclei that includes part of the nucleus tractus solitarius (NTS), the dorsal motor nucleus of the vagus nerve and the area of postrema. The VC receives input from the chemoreceptor trigger zone (CTZ), the vestibular apparatus, the brain cortical structures, and from the visceral afferents. It is believed that several neurotransmitters (acetylcholine, dopamine, histamine and serotonin) act via their specific receptors to induce emesis. Indeed, selective activation of dopaminergic $D_2$-, muscarinic $M_1$-, or serotonergic 5-$HT_3$-receptors in the CTZ induce emesis. In addition, both stimulation of the latter receptors and the histamine $H_1$ receptor in the NTS, can also produce vomiting.

Prior to 1990, the dopamine receptor population was considered to consist of two subtypes, $D_1$ and $D_2$ (Clark and White, 1987). Dopaminergic agonists such as apomorphine cause emesis in both animals and man (Andrews et al., 1990; Leslie et al., 1990). The $D_2$ receptor in the CTZ is thought to mediate the emetic action of apomorphine (Harding et al., 1987; King, 1990). More recently, the application of molecular techniques has led to the identification and cloning of genes for additional subtypes of $D_1$ and $D_2$ receptors (Levant, 1997; Missale et al., 1998). Thus, the $D_1$ receptor family consist of $D_1$ and $D_5$ sites, whereas the $D_2$ family comprises the $D_2$, $D_3$ and $D_4$ sites. Two relatively recent studies in dogs (Yoshida et al., 1995) and ferrets (Yoshikawa et al., 1996) have indicated that the $D_3$ dopamine receptor may also play an important role in the induction of emesis.

Although the dog and cat represent the most well investigated animal models of emesis, the utilization of these large animals is not cost effective and therefore alternative models have been found. Indeed, Japanese investigators have introduced a small animal (adult being 50–100 g in weight), the house musk shrew (Suncus murinus), as an experimental model for various emetic stimuli (Ueno et al., 1987; Torii et al., 1991a,b; Okada et al., 1994; Ito et al., 1995). The house musk shrew is endogenous to Asia and Africa. The least shrew (Cryptotis parva) is relatively smaller (adult weight 4–6 g) and lives in various ecological niches in Central and North America. Recently, the least shrew was introduced as a new serotonergic experimental model of emesis (Darmani, 1998). As with the house musk shrew (Torii et al., 1991a), the least shrew vomits in response to serotonergic 5-$HT_3$ receptor agonists (Darmani, 1998). Furthermore, in both species of shrews, 5-$HT_3$ receptor antagonists prevent emesis produced by the chemotherapeutic agent cisplatin (Torii et al., 1991b; Darmani, 1998). Unlike other animals (Reviews: Andrews et al., 1990; Leslie et al., 1990; King, 1990), the house musk shrew does not vomit in response to apomorphine administration (Ueno et al., 1987). However, our preliminary studies in the least shrew indicated that apomorphine is a potent emetogenic substance.

The purpose of the present study was two fold: 1) to introduce the least shrew as a new dopamine animal model of emesis; and 2) to pharmacologically characterize the dopaminergic receptors responsible for the production of emesis in this species. Because there is no selective agonist or antagonist available for $D_2$ or $D_3$ receptors (Levant, 1997), the emetic dose-response effects of a nonselective dopamine agonist [apormorphine], a $D_2$-[quinpirole] and two $D_3$-preferring agonists [7-(OH) DPAT and PD 128, 907] were investigated. In antagonist studies, the inhibitory dose-response effects of the $D_2$-preferring (sulpride) and the $D_3$-preferring (U 99194A) antagonists were investigated on the ability of a 2 mg/kg emetic dose of the cited dopaminergic agonists. In order to determine whether sulpride and U 99194A may produce synergistic action, the antiemetic effect of several combined doses of the latter antagonists were also investigated. Although there is no evidence that the $D_1$ family of receptors mediates emesis, the ability of two $D_1$ selective agonists (SKF-38393 and SKF-82958) (Seeman and Van Tol, 1994) to produce emesis in the least shrew were also examined.

## Materials and methods

### *Animals and drugs*

Shrews (Cryptotis parva) were bred and maintained in the animal facilities of the Kirksville College of Osteopathic Medicine. Both male and female (4–6 g, 45–70 days old) shrews were used throughout the study. The animals were kept on a 14:10-h light-dark cycle at a room temperature of 21 ± 1°C in open-top clear polycarbonate cages (20 × 18 × 21 cm) lined with heated dry loam soil. Depending upon the size of the litter, 3–6 litter mates were housed per cage. A wooden nest box (5.5 × 5.5 × 9 cm) containing dry grass, a food bowl, and a lick tube water bottle were placed in each cage. Animals were fed twice daily. In the morning, 5–6 mealworms (Tenebrio sp) were given per animal, and in the evening each shrew was offered a 6-g mixture consisting of two-thirds dry cat food (PMI Nutrition Cat formula) and one third canned cat food (Kozy Kitten) in sufficient water to give the mixture a paste-like consistency. All animals received care according to the "Guide for the Care and Use of Laboratory Animals", DHHS Publication, Revised, 1985. The facilities are certified by the American Association of Accreditation of Laboratory Care. These studies were approved by the Institutional Animal Care and Use Committee of KCOM. The following drugs were purchased from Research Biochemicals Inc., Natick, MA: R(−)apomorphine HCl; R(+)-2-dipropylamino-8-hydroxy-1,2,3,4-tetrahydronaphthalene hydrobromide; (R(+)-7-hydroxy-DPAT HBr); (−)-quinpirole 2HCl; S(+)-PD 128, 907 HCl; S(−)-sulpride; (±)-SKF-38393 HCl; (±)-SKF-82958 HBr [(±)-chloro-APB hydrobromide], and U 99194A maleate. Sulpride was dissolved in distilled water with a 10 µl volume of 1/3 concentrated HCl which was then back titrated to pH 5 by the addition of NaOH. All other drugs were dissolved in distilled water. Agonists were administered intraperitoneally whereas the antagonists were injected via the subcutaneous route at a volume 10 ml/kg. Doses of drugs are expressed as their stated salts. All experiments were performed between 0900 and 1700 h.

### *Experimental protocols*

The present protocols were based upon our preliminary studies on the apomorphine-induced vomiting as well as our published findings on the ability of serotonergic agents to produce emesis (Darmani, 1998) and other behaviors in the least shrew (Darmani et al., 1994; Darmani and Zhao, 1998). On the test day, the animals were transferred to the experimental room and were allowed to acclimate for at least 1 h prior to

experimentation. The fume hood was turned on to produce a constant white noise during the experimental procedures. To habituate the shrews to the test environment, each animal was randomly selected and transferred to a 20 × 18 × 21 cm clean clear plastic holding cage and was offered 4 meal worms 30 min prior to experimentation. Then, different groups of shrews were injected intraperitoneally with either vehicle or varying doses of different $D_1$, $D_2$ or $D_3$ preferring agonists. Immediately following injection, each shrew was placed in the observation cage and the onset latency to first vomit as well as the frequency of vomiting (mean ± SEM) were recorded for each individual shrew for the next 30 min. The emetic agonists included: apomorphine (0, 0.1, 0.5, 2 and 4 mg/kg, n = 6–7 per group), quinpirole (0, 0.1, 0.5, 2 and 4 mg/kg, n = 6–7 per group), 7-(OH) DPAT (0, 0.1, 0.25, 0.5 and 2 mg/kg, n = 6–8 per group), and PD 128, 907 (0, 0.5, 1 and 2 mg/kg, n = 6–7 per group). The $ED_{50}$ of each agonist was then computed. $D_1$ agonists (SKF-38393 and SKF-82958) at doses 0.5, 2 and 4 mg/kg (n = 5–6 per group) failed to cause emesis under the above experimental conditions. To determine whether $D_2$- or $D_3$-receptor antagonist pretreatment can abolish agonist-induced emesis, different groups of shrews were injected subcutaneously with either vehicle or different doses (dose range 0.1–8 mg/kg, n = 6–9 per dose) of sulpride, U 99194A or a combination of varying doses of the latter two antagonists. Immediately after injection, the treated animals were offered 4 mealworms and 30 min later were injected with a 2 mg/kg emetic dose (i.p.) of either apomorphine, quinpirole, 7-OH DPAT or PD 128, 907. The emesis parameters were recorded for the next 30 min as described above. The $ID_{50}$ dose of each antagonist was then calculated.

### *Statistical analysis*

The data were analyzed by the Kruskal-Wallis nonparameteric one-way analysis of variance (ANOVA) and posthoc analysis by Dunn's multiple comparisons test. A p-value of $<0.05$ was necessary to achieve statistical significance. The $ED_{50}$ (the effective dose that produced 50% maximal frequency of emesis) and $ID_{50}$ (the inhibitory dose that attenuated the maximal vomiting frequency by 50%) were calculated by the use of a computerized program (Graph Pad InPlot, San Diego, CA).

## Results

The Kruskal-Wallis nonparametric ANOVA test indicated that intraperitoneal administration of apomorphine (0.1–4 mg/kg) in the least shrew caused significant enhancements in the mean frequency of emesis with an $ED_{50}$ of 0.72 ± 1.9 mg/kg ($kw_{4,29} = 20.42$, $p < 0.0004$) (Figs. 1A and 2A). Dunn's multiple comparisons test showed that relative to the vehicle injected control group, significant enhancements in the frequency of emesis occurred in the 2 ($p < 0.05$) and the 4 mg/kg ($p < 0.05$) treatment groups (Fig. 1A). Irrespective of the dose administered, animals that exhibited vomiting produced their first vomit within a couple of minutes of their injection. Apomorphine also caused a dose-dependent increase in the percentage of animals vomiting ($kw_{4,29} = 22.85$, $p < 0.0001$) (Fig. 2A). Dunn's multiple comparisons test indicated that significant enhancements in the percentage of animals vomiting occurred at the 0.5 ($p < 0.05$), 2 ($p < 0.01$) and 4 mg/kg ($p < 0.05$) doses of apomorphine. Although in this dose-response study one shrew in each of the apomorphine doses failed to vomit, our other studies indicate that apomorphine at 2 mg/kg dose can induce emesis in all shrews. For antagonist studies, a 2 mg/kg dose of apomorphine was used to induce vomiting. The $D_2$ preferring antagonist, sulpride (0.5–2 mg/kg), dose-dependently attenuated both the mean frequency of vomiting (Fig. 1B) and

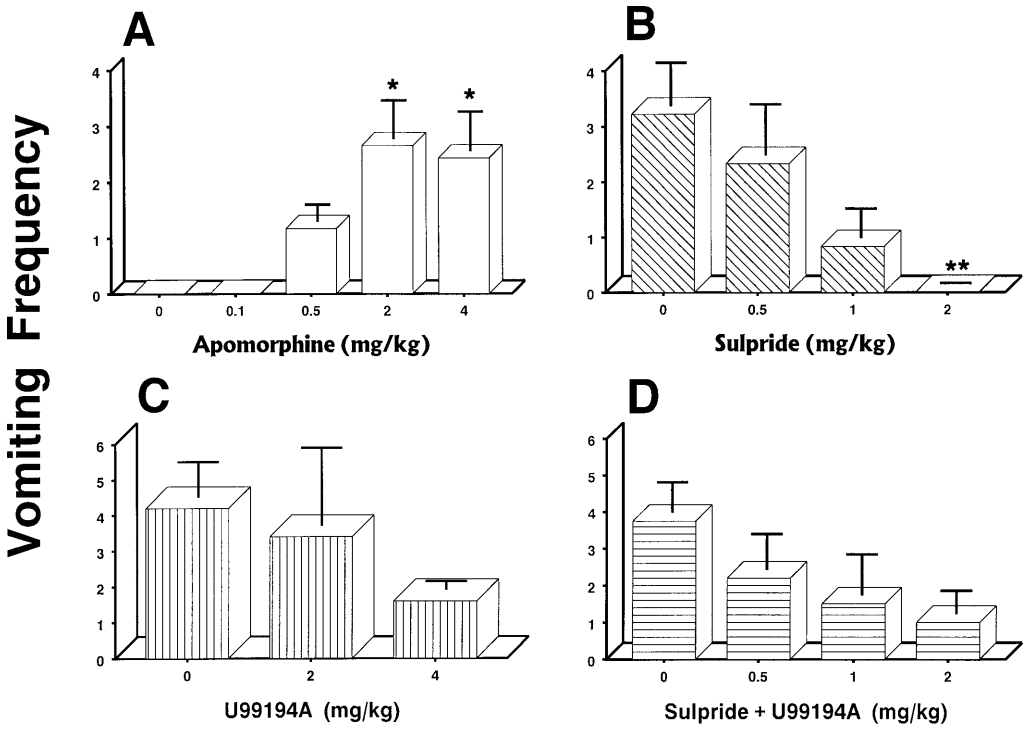


**Fig. 1.** Graph A represents the emetogenic dose-response effect of the cited doses of the nonselective dopamine receptor agonist apomorphine in potentiating the mean frequency of emesis (±S.E.M.) in the least shrew in 30 min. Graphs B, C and D respectively show the capacity of the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A); to attenuate the mean frequency (±S.E.M.) of emesis produced by a 2 mg/kg emetic dose of apomorphine. Antagonists were administeed i.p. 30 min prior to injection of apomorphine and the vomiting frequency was recorded for 30 min immediately following agonist injection. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

the percentage of animals vomiting (Fig. 2B) [($kw_{3,23} = 13.61$, $p < 0.003$) and ($kw_{3,23} = 17.36$, $p < 0.0006$) respectively]. The $ID_{50}$ of sulpride to reduce the frequency of apomorphine-induced emesis was computed to be 0.57 ± 1.6 mg/kg. Dunn's multiple comparisons test showed that significant reductions in both the vomiting frequency ($p < 0.01$) and the percentage of shrews vomiting ($P < 0.001$) occurred at the 2 mg/kg sulpride dose (Figs. 1B and 2B, respectively). Although the $D_3$ preferring antagonist, U 99194A (2–4 mg/kg), tended to decrease the frequency of apomorphine-induced emesis, however, at the doses tested, the reductions did not attain significance (Fig. 1C). U 99194A also failed to significantly attenuate the number of animals vomiting (Fig. 2C). The combination of varying doses of sulpride and U 99194A (0.5–2 mg/kg of each antagonist) also tended to reduce the emetic frequency, however, the reductions just failed to attain significance ($kw_{3,21} = 7.4$, $p < 0.06$) (Fig. 1D). On the other hand, the highest combined tested dose of sulpride and U 99194A (2 mg/kg), significantly ($p < 0.05$) blocked the

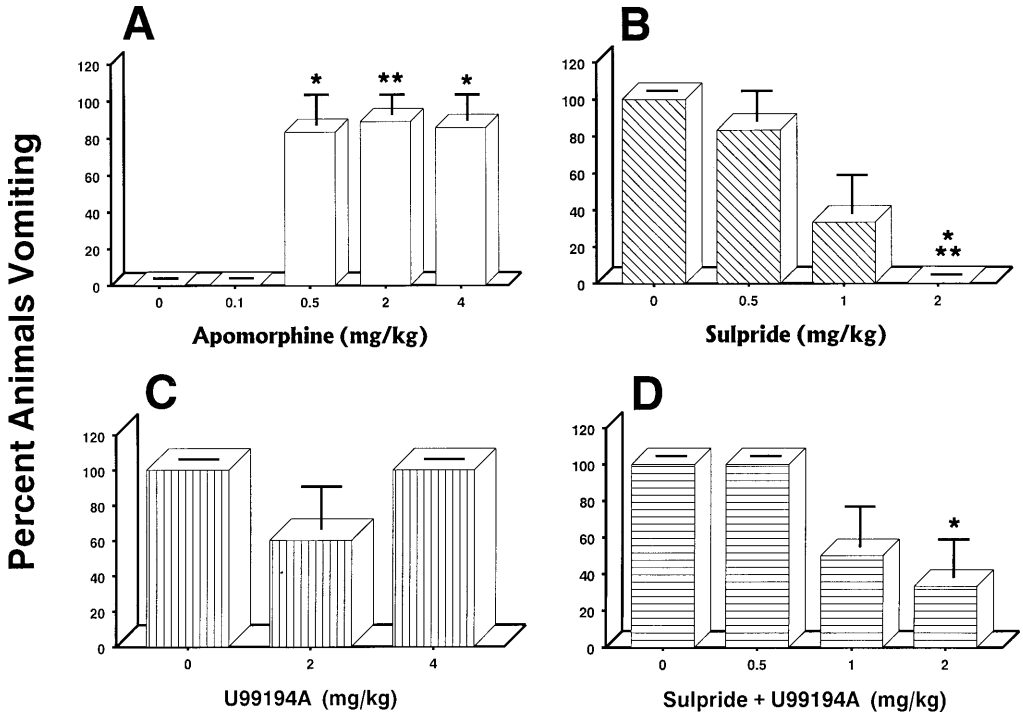


**Fig. 2.** Graph A shows the percentage of shrews vomiting (Mean ± S.E.M.) in response to intraperitoneal administration of the cited doses of the nonselective dopamine receptor agonist apomorphine in the 30 min observation period. Graphs B, C and D respectively represent the effects of 30 min prior treatment with the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A) to reduce the number of shrews vomiting (percent) in response to a 2 mg/kg emetic dose of apomorphine. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

percentage of shrews vomiting in response to the apomorphine injection (Fig. 2D) ($kw_{3,21} = 10.5$, $p < 0.015$).

The $D_2$ preferring agonist, quinpirole (0.1–4 mg/kg), also produced emesis in the least shrew in a dose-dependent pattern (Figs. 3A and 4A). Kruskal-Wallis test indicated that both the quinpirole-induced mean vomiting frequency ($ED_{50} = 0.83 \pm 1.68$ mg/kg) ($kw_{4,26} = 21.01$, $p < 0.0003$), and the percentage of animals vomiting ($kw_{4,26} = 21.53$, $p < 0.0002$) were significantly increased. Dunn's multiple comparisons test showed that significant enhancements in both of quinpirole-induced vomiting parameters occurred at the 2 ($p < 0.01$) and 4 mg/kg ($p < 0.01$) doses (Figs. 3A and 4A). Sulpride (0.5–8 mg/kg) pretreatment dose-dependently attenuated both the quinpirole (2 mg/kg)-induced vomiting frequency ($ID_{50} = 2.45 \pm 2.5$ mg/kg) ($kw_{5,37} = 23.67$, $p < 0.0003$) and the percent of shrews vomiting ($kw_{5,37} = 27.47$, $p < 0.0001$). However, significant attenuations were only seen at the highest tested dose of sulpride (8 mg/kg, $p < 0.05$) for the vomiting frequency (Fig. 3B), and at 4 ($p < 0.05$) and 8 mg/kg ($p < 0.01$) doses for the percentage of

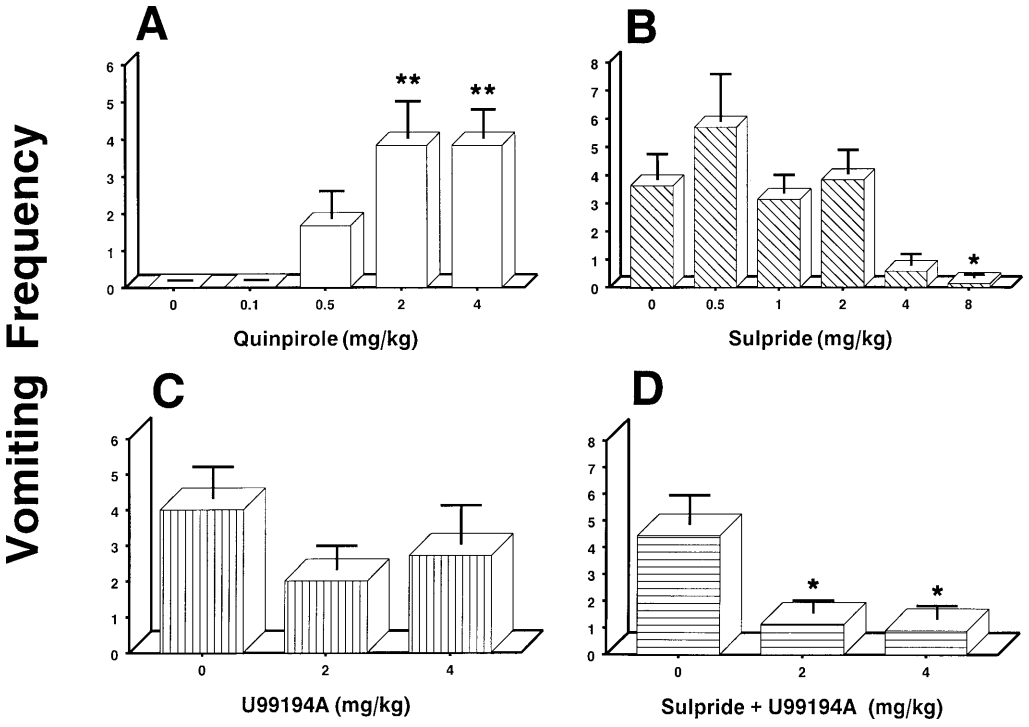


**Fig. 3.** Graph A represents the emetogenic dose-response effect of the cited doses of the dopamine $D_2$ preferring agonist quinpirole in potentiating the mean frequency of emesis (±S.E.M.) in the least shrew in 30 min. Graphs B, C and D respectively show the capacity of the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A); to attenuate the mean frequency (±S.E.M.) of emesis produced by a 2 mg/kg emetic dose of quinpirole. Antagonists were administeed i.p. 30 min prior to injection of quinpirole and the vomiting frequency was recorded for 30 min immediately following agonist injection. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

animals vomiting (Fig. 4B). U 99194A failed to significantly reduce either the quinpirole-induced mean vomiting frequency (Fig. 3C) or the percent of shrews vomiting (Fig. 4C). However, a combination of sulpride and U 99194A potently blocked both the vomiting frequency ($ID_{50}$ = 0.76 ± 1.2 mg/kg) ($kw_{2,20} = 9.39$, $p < 0.009$) and the number of shrews vomiting in response to quinpirole injection ($kw_{2,20} = 6.19$, $p < 0.045$) (Figs. 3D and 4D, respectively). Indeed, Dunn's multiple comparisons test showed that significant reductions in the vomiting frequency occurred at the 2 ($p < 0.05$) and 4 mg/kg ($p < 0.05$) combined doses of these antagonists (Fig. 3D), whereas a significant prevention of emesis in a large percentage of shrews was seen at the 4 mg/kg combined dose only (Fig. 4D, $p < 0.05$).

The $D_3$ preferring agonist, 7-OH DPAT (0.1–2 mg/kg), also produced emesis in the least shrew in a potent manner (Figs. 5A and 6A). Indeed, 7-OH DPAT caused a dose-dependent increase in the frequncy of emesis with an

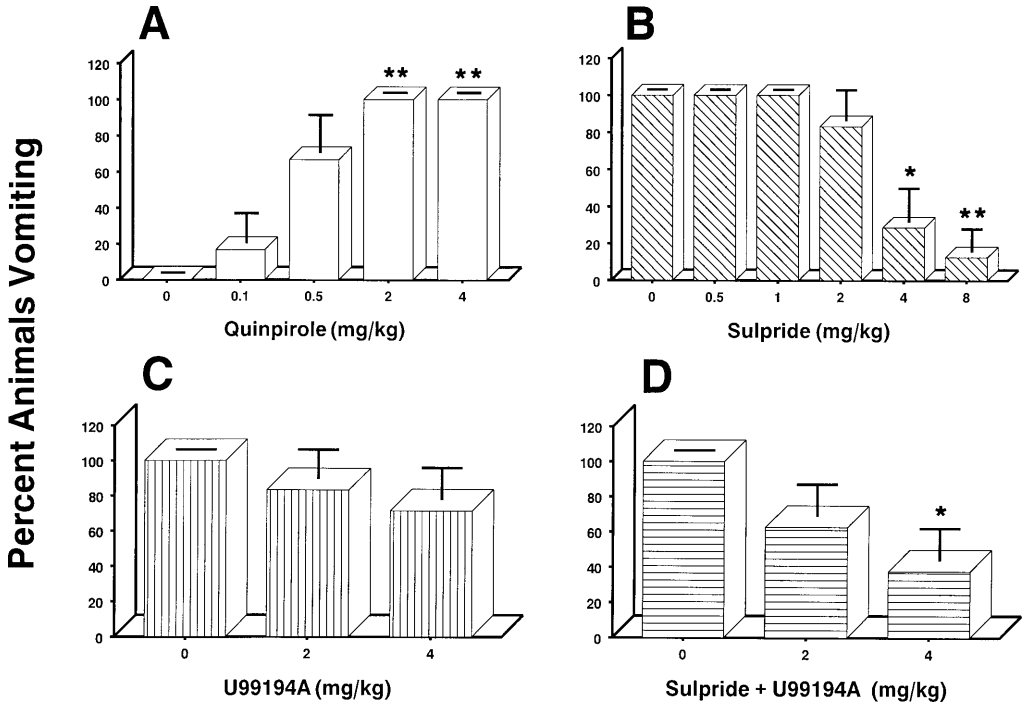


**Fig. 4.** Graph A shows the percentage of shrews vomiting (Mean ± S.E.M.) in response to intraperitoneal administration of the cited doses of the dopamine $D_2$ preferring agonist quinpirole in the 30 min observation period. Graphs B, C and D respectively represent the effects of 30 min prior treatment with the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A) to reduce the number of shrews vomiting (percent) in response to a 2 mg/kg emetic dose of quinpirole. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

$ED_{50}$ of 0.39 ± 1 mg/kg ($kw_{4,27} = 17.7$, $p < 0.001$). Dunn's multiple comparisons test showed that a significant potentiation ($p < 0.05$) in the vomiting frequency occurred at the 2 mg/kg dose (Fig. 5A). Administration of 7-OH DPAT also caused a dose-dependent increase in the number of animals vomiting ($kw_{4,27} = 17.47$, $p < 0.001$) and a significant effect was also seen at the 2 mg/kg dose ($p < 0.05$) (Fig. 6A). Relative to the vehicle-treated control group, sulpride pretreatment (2–8 mg/kg), dose-dependently attenuated both the frequency of 7-OH DPAT (2 mg/kg)-induced emesis ($ID_{50} = 2.1 \pm 1.8$ mg/kg) ($k_{3,23} = 10.78$, $p < 0.01$) as well as the percentage of shrews vomiting ($kw_{3,23} = 13.11$, $p < 0.004$) (Figs. 5B and 6B). Significant reductions for both vomiting parameters were seen at the 8 mg/kg sulpride dose ($p < 0.01$). Although the $D_3$ preferring antagonist, U 99194A (2–4 mg/kg), tended to attenuate the frequency of 8-OH DPAT-induced emesis, the reductions did not attain significance (Fig. 5C). Likewise, it failed to reduce the number of shrews vomiting in response to 7-OH DPAT injection (Fig. 6C). However, combination of various doses of sulpride and U 99194A (0.1–2 mg/kg),

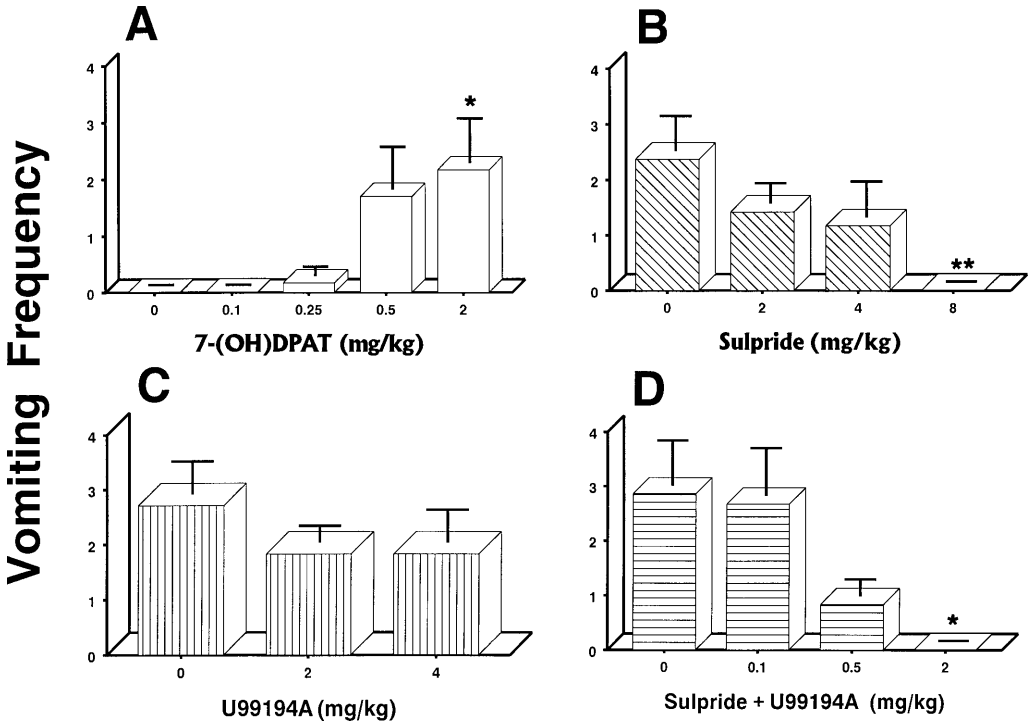


**Fig. 5.** Graph A represents the emetogenic dose-response effect of the cited doses of the dopamine $D_3$ preferring agonist 7-(OH) DPAT in potentiating the mean frequency of emesis (±S.E.M.) in the least shrew in 30 min. Graphs B, C and D respectively show the capacity of the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A); to attenuate the mean frequency (±S.E.M.) of emesis produced by a 2 mg/kg emetic dose of 7-(OH) DPAT. Antagonists were administeed i.p. 30 min prior to injection of 7-(OH) DPAT and the vomiting frequency was recorded for 30 min immediately following agonist injection. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

potently attenuated the vomiting frequency ($ID_{50}$ = 0.26 ± 1.46 mg/kg) ($kw_{3,21}$ = 11.45, $p < 0.01$) (Fig. 5D) as well as reducing the percentage of shrews vomiting in response to 7-OH DPAT administration ($k_{3,21}$ = 11.95, $p < 0.008$) (Fig. 6D). Dunn's multiple comparisons test showed that significant attenuations for both parameters occurred at the 2 mg/kg combined antagonist dose ($p < 0.05$) (Figs. 5D and 6D).

The emetic effects of the second tested $D_3$ preferring agonist PD 128, 907 is presented in Figs. 7A and 8A. Administration of PD 128, 907 (0.5–2 mg/kg) increased both the mean vomiting frequency ($ED_{50}$ = 0.94 ± 1 mg/kg) ($kw_{3,21}$ = 11.95, $p < 0.008$) as well as the percentage of animals vomiting in a dose-dependent manner ($kw_{3,21}$ = 10.36, $p < 0.01$). Significant enhancements for both vomiting parameters occurred at the 2 mg/kg dose ($p < 0.05$) (Figs. 7A and 8A). Sulpride (2–4 mg/kg) pretreatment dose-dependently reduced both the mean vomiting frequency ($ID_{50}$ = 0.73 ± 2.6 mg/kg) ($kw_{3,24}$ = 18.26, $p < 0.0004$) as well as the number of shrews vomiting ($kw_{3,24}$ = 18.51, $p < 0.003$) in

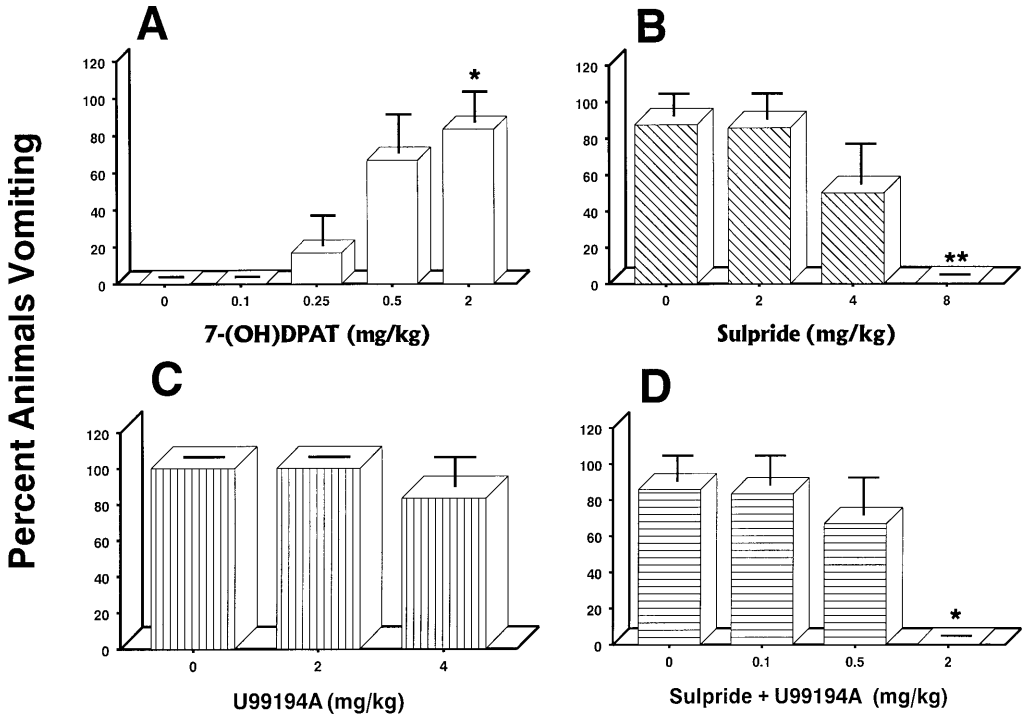


**Fig. 6.** Graph A shows the percentage of shrews vomiting (Mean ± S.E.M.) in response to intraperitoneal administration of the cited doses of the dopamine $D_3$ preferring agonist 7-(OH) DPAT in the 30 min observation period. Graphs B, C and D respectively represent the effects of 30 min prior treatment with the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A) to reduce the number of shrews vomiting (percent) in response to a 2 mg/kg emetic dose of 7-(OH) DPAT. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

response to PD 128, 907 (2 mg/kg) administration (Figs. 7B and 8B, respectively). Dunn's multiple comparisons test showed that significant blockade of both emesis parameters occurred at 3 mg/kg or greater doses of sulpride (significance range, $p < 0.01$–$0.001$). Pretreatment with U 99194A (2–8 mg/kg) failed to significantly reduce both the frequency of PD 128, 907-induced vomiting (Fig. 7C) as well as the percentage of animals vomiting (Fig. 8C). However, the combination of various doses of sulpride and U 99194A (0.5–2 mg/kg) more potently blocked both the vomiting frequency ($ID_{50}$ = 0.14 ± 1.32 mg/kg) ($kw_{3,25} = 16.74$, $p < 0.0008$), and the number of animals vomiting in response to PD 128, 907 administration ($kw_{3,25} = 10.99$, $p < 0.012$) (Figs. 7D and 8D). Dunn's multiple comparisons test showed that significant reduction in vomiting frequency occurred at the 0.5 ($p < 0.05$), 1 ($p < 0.05$) and 2 mg/kg ($p < 0.01$) combined doses of the antagonists (Fig. 7D). However, a significant reduction in the percent of animals vomiting was only apparent at the 2 mg/kg combined dose (Fig. 8D). Intraperitoneal administration of $D_1$

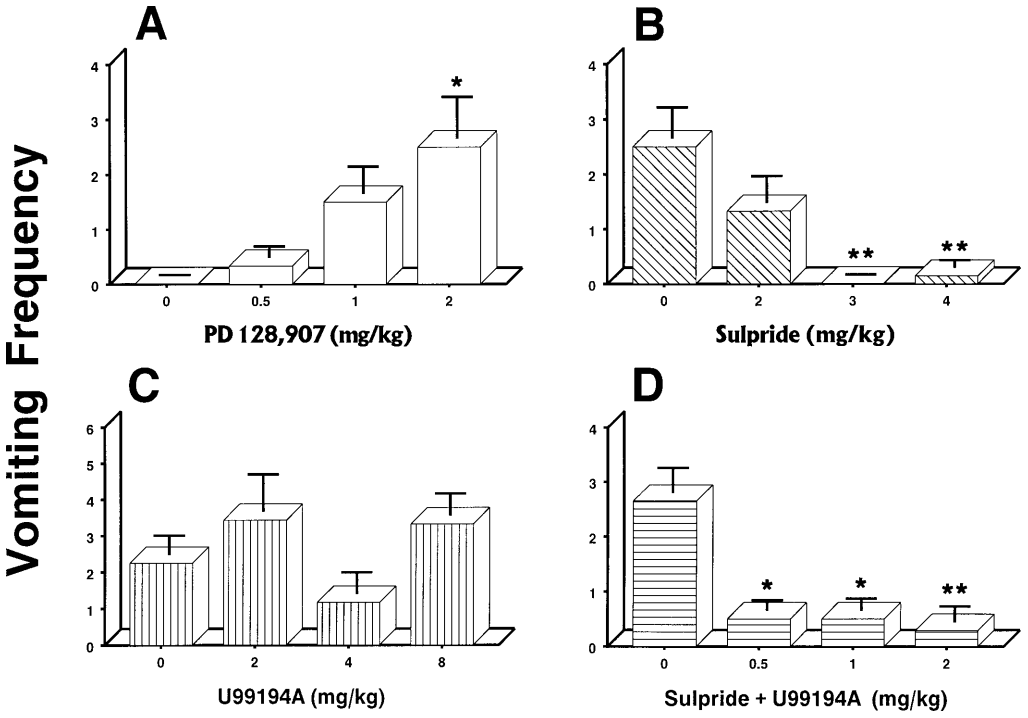


**Fig. 7.** Graph A represents the emetogenic dose-response effect of the cited doses of the dopamine $D_3$ preferring agonist PD 128, 907 in potentiating the mean frequency of emesis (±S.E.M.) in the least shrew in 30 min. Graphs B, C and D respectively show the capacity of the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A); to attenuate the mean frequency (±S.E.M.) of emesis produced by a 2 mg/kg emetic dose of PD 128, 907. Antagonists were administeed i.p. 30 min prior to injection of PD 128, 907 and the vomiting frequency was recorded for 30 min immediately following agonist injection. *$p < 0.05$ and **$p < 0.01$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

agonists (SKF-38393 and SKF-82958) failed to induce emesis in any animal at the following doses: 0.5, 2 and 4 mg/kg.

## Discussion

In comparison with the well-investigated house musk shrew (see introduction), the least shrew appears to be a more versatile animal model of emesis since the former shrew species do not vomit in response to a wide dose-range of apomorphine (0.1–100 mg/kg) (Ueno et al., 1987). In the least shrew, apomorphine seems to be a relatively potent emetogenic substance ($ED_{50}$ = 0.72 mg/kg), which rapidly and in a dose-dependent manner increases both the emetic episodes as well as the percentage of shrews vomiting. The severity of apomorphine-induced emesis varies among different species (King, 1990). Apomorphine appears to be a relatively less potent emetogenic substance in the least shrew than in man, dog or ferret (Andrews et al., 1986; King, 1990;

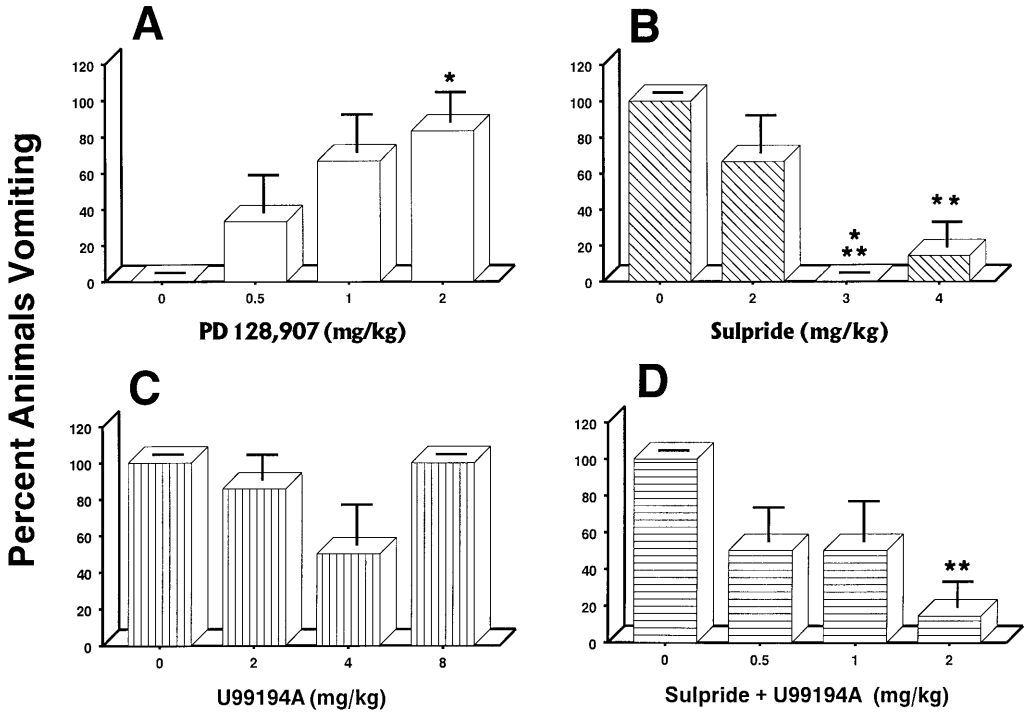


**Fig. 8.** Graph A shows the percentage of shrews vomiting (Mean ± S.E.M.) in response to intraperitoneal administration of the cited doses of the dopamine $D_3$ preferring agonist PD 128, 907 in the 30 min observation period. Graphs B, C and D respectively represent the effects of 30 min prior treatment with the cited doses of either a $D_2$ preferring antagonist (sulpride), a $D_3$ preferring antagonist (U 99194A), or a combination of varying doses of these antagonists (sulpride + U 99194A) to reduce the number of shrews vomiting (percent) in response to a 2 mg/kg emetic dose of apomorphine. *$p < 0.05$, **$p < 0.01$ and ***$p < 0.001$ indicate significant differences relative to corresponding controls by Dunn's multiple comparisons test

Yoshida et al., 1995; Yoshikawa et al., 1996). The relative greater metabolic activity of shrews (Churchfield, 1990) probably accounts for the reduced emetic potency of apomorphine in this species. However, the ferret exhibits a narrow bell-shaped dose-response effect to apomorphine which may limit its usefulness in such studies (Andrews et al., 1986; Yoshikawa et al., 1996). Apomorphine possesses significant affinity for all known dopamine receptors (Levant, 1997; Seeman and Van Tol, 1994; Tice et al., 1994). Dopamine $D_1$ and $D_5$ receptors are unlikely to be involved in the production of emesis since the potent and selective $D_1/D_5$ agonist, SKF-38393 (Seeman and Van Tol, 1994; Tice et al., 1994), does not induce emesis in both dogs (Yoshida et al., 1995) or ferrets (Yoshikawa et al., 1996). Moreover, in the present study SKF-38393 and its analog SKF-82958 also failed to cause emesis in the least shrew. Currently, there is no selective agonist or antagonist available to distinguish between the members of the $D_2$ receptor family. Receptor ligand studies indicate that sulpride preferentially binds to $D_2$ sites and has no affinity for either $D_1$ or $D_5$ sites (Levant, 1997; Seeman and Van Tol, 1994; Sokolof et al.,

1992). However, sulpride binds $D_3$ sites with a 2–8 fold lower affinity as well as minimally binding to $D_4$ sites. On the other hand, U 99194A is considered as a preferential antagonist of the $D_3$ site since it possesses a 20-fold lower affinity for the $D_2$ receptor (Haadsma-Svensson and Svensson, 1998). In the present study, sulpride potently and in a dose-dependent manner reduced both the mean vomiting frequency and the number of shrews vomiting in response to a 2 mg/kg dose of apomorphine. Moreover, sulpride at 2 mg/kg, completely prevented the induced vomiting response in all animals tested. The $D_3$ preferring antagonist U 99194A did not alter the apomorphine-induced vomiting symptoms to a significant degree. The combination of various doses of sulpride and U 99194A were less robust in attenuating the apomorphine-induced vomiting symptoms. Indeed, unlike the 2 mg/kg dose of sulpride, the 2 mg/kg combined dose of sulpride and U 99194A failed to completely block the induced vomiting. Since at present truly selective ligands for $D_2$ and $D_3$ receptors are not yet available, it is difficult to precisely describe why U 99194A attenuates the antiemetic action of sulpride on the apomorphine-induced emesis. However, it is known that apomorphine possesses up to 50-fold lower affinity for $D_3$- than for both $D_1$- and $D_2$-dopamine receptors (Seeman and Van Tol, 1994) . Furthermore, U 99194A possesses partial agonist action at these sites. Indeed, U 99194A has been shown to produce partial substitution in rats trained to discriminate either d-amphetamine or cocaine from saline (Baker et al., 1998) as well as producing certain $D_2$- and $D_1$-like behaviors (Clifford and Waddington, 1998). Moreover, high doses of U 99194A can displace tritiated $D_2$ ligands (Walters et al., 1994). A combination of these factors are probably responsible for the attenuation of the antiemetic action of sulpride by U 99194A. Thus, it seems that apomorphine induces emesis in the least shrew mainly via the activation of $D_2$ sites.

The $D_2$ preferring agonist quinpirole also dose-dependently increased both the number of shrews vomiting and the mean frequency of emesis with an $ED_{50}$ (0.83 mg/kg) similar to apomorphine. Quinpirole also produces vomiting in both dogs (Yoshida et al., 1995) and ferrets (Yoshikawa et al., 1996). Relative to the least shrew, the latter animals appear to be more sensitive to quinpirole. Unlike it's potent antagonism of apomorphine-induced emesis, sulpride was less effective in blocking quinpirole (2 mg/kg)-induced vomiting in the least shrew. Indeed, although sulpride at 4–8 mg/kg doses significantly blocked both of quinpirole-induced emetic parameters, complete blockade was not achieved even at 8 mg/kg. In a similar fashion, large doses of sulpride (>50 mg/kg) is required to significantly block the ability of a small dose of quinpirole (25.5 μg/kg) to induce hypolocomotion, a $D_3$-mediated effect (Storey et al., 1995). As in the case of apomorphine-induced emesis, U 99194A (2–4 mg/kg) also failed to significantly alter quinpirole (2 mg/kg)-induced emesis. However, U 99194A in combination with sulpride, more potently blocked quinpirole-induced vomiting frequency at 2 mg/kg, and at 4 mg/kg it also significantly attenuated the number of animals vomiting. This synergism indicates that quinpirole probably produces emesis via the activation of both $D_2$ and $D_3$ sites.

The $D_3$ preferring agonist, 7-(OH) DPAT, dose-dependently increased the number of shrews vomiting as well as potentiated the mean vomiting frequency with an $ED_{50}$ of 0.39 mg/kg. Relative to the least shrew, in dogs (Yoshida et al., 1995) and ferrets (Yoshikawa et al., 1996), 7-(OH) DPAT is a more potent emetogenic agent. Although sulpride (2–8 mg/kg) attenuated 7-(OH) DPAT-induced emetic parameters in a dose-dependent manner, significant and complete blockade was only observed at the 8 mg/kg dose. As with the cases of apomorphine and quinpirole, the $D_3$ preferring antagonist U 99194A also failed to antagonize 7-(OH) DPAT (2 mg/kg)-induced emesis. However, it significantly and dose-dependently potentiated the ability of sulpride to suppress the induced vomiting. Thus, a 2 mg/kg combined dose of sulpride and U 99194A completely and potently blocked 7-(OH) DPAT-induced emesis with an eight fold lower $ID_{50}$ (0.26 mg/kg) relative to sulpride alone. This synergistic antiemetic action suggest important roles for both $D_2$ and $D_3$ dopamine receptors in the ability of 7-(OH) DPAT to produce vomiting. Furthermore, the $D_2/D_3$ antagonist (−) eticlopride (Levant, 1997), can potently block emesis produced by 7-(OH) DPAT in both dogs (Yoshida et al., 1995) and ferrets (Yoshikawa et al., 1996), whereas $D_1$ (SCH 23390) and $D_4$ (clozapine) antagonists failed to modify the vomiting response.

The second $D_3$ preferring agonist, PD 128, 907 (Pugsley et al., 1995; Routledge et al., 1996) , also dose-dependently increased the percentage of shrews vomiting as well as potentiating the mean frequency of vomiting ($ED_{50}$ = 0.94 mg/kg). However, PD 128, 907 appears to be more than two fold less potent than 7-(OH) DPAT. Likewise, this agent is two times less potent than 7-(OH) DPAT in another function (mitogenesis) of $D_3$ receptors (Griffon et al., 1996). To our knowledge, the emetic action of PD 128, 907 has not yet been investigated in other animal models of emesis. Relative to 7-(OH) DPAT-induced emesis, vomiting produced by a 2 mg/kg dose of PD 128, 907 was more potently blocked by sulpride ($ID_{50}$ = 0.93 mg/kg) which further reflects PD 128, 907's weaker emetic action. Indeed, 3 mg/kg sulpride was necessary to nearly completely prevent emesis produced by PD 128, 907; whereas 8 mg/kg sulpride was required for the case of the same dose of 7-(OH) DPAT. Although U 99194A by itself failed to significantly block PD 128, 907-induced (2 mg/kg) emesis, combined doses of sulpride and U 99194A (0.5–2 mg/kg) more potently ($ID_{50}$ = 0.14 mg/kg) reduced the frequency of the induced vomiting relative to the administration of these antagonists by themselves. Furthermore, this combination is 5 times more effective in blocking emesis produced by PD 128, 907 than by 7-(OH) DPAT. It is also of interest to note that U 99194A (1 mg/kg) by itself can only partially prevent the motor effects of very low doses of apomorphine (28 μg/kg) and PD 128, 907 (42 μg/kg) in monkeys (Blanchet et al., 1997). Moreover, PD 128, 907 causes activation of $D_2$ sites in $D_3$ mutant mice (Koeltzow et al., 1998). Thus, it seems that PD 128, 907 also activates both $D_2$ and $D_3$ sites to cause vomiting.

Overall, the present study supports a pivotal role for the dopamine $D_2$ receptor in the mediation of emesis in the least shrew. Since relative to the administration of each antagonist alone, combination of varying doses of $D_2$ and $D_3$ preferring antagonists, more potently blocks emesis produced by

several $D_2$ and $D_3$ preferring agonists, this potentiation reveals a significant synergistic emetic role for the $D_3$ site. Thus, it appears that both $D_2$- and $D_3$-dopamine receptors are involved in vomiting and simultaneous activation of both sites produce a greater degree of vomiting relative to each receptor being stimulated alone. The inability of U 99194A to prevent emesis produced by $D_3$ preferring agonists suggest: 1) a weak antagonist or $D_2$ partial agonist nature of U 99194A (see earlier); 2) $D_3$ preferring agonists possess significant efficacy at the $D_2$ site, activation of which leads to emesis; or 3) a combination of these effects. Autoradiographic studies implicate the functional involvement of $D_2$, $D_3$ and possibly $D_4$ sites in emesis since these receptors are concentrated (Hyde et al., 1996) in several brain loci that control vomiting (see introduction). Indeed, ablation of the area postrema markedly attenuates the ability of apomorphine and 7-(OH) DPAT to induce emesis in the ferret (Harding et al., 1987; Yoshikawa et al., 1996). The clarification of an emetic role for the $D_4$ site awaits development of selective agonists and antagonists. However, it is known that the $D_4$ antagonist clozapine does not block 7-(OH) DPAT-induced vomiting in both ferrets (Yoshikawa et al., 1996) and dogs (Yoshida et al., 1995). In addition, clozapine is ineffective in preventing PD 128, 907-induced vomiting in the least shrew (data not given). In summary, this study reveals the roles of dopamine $D_2$ and $D_3$ receptors in vomiting and validates the least shrew as a new dopaminergic animal model of emesis.

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