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Modulation of emesis by fentanyl and opioid receptor antagonists in *Suncus murinus* (house musk shrew)

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Abstract

The anti-emetic mechanism of action of fentanyl to inhibit nicotine (5 mg/kg, s.c.)-induced emesis was investigated in *Suncus murinus*. The anti-emetic action of fentanyl (40 μg/kg, s.c.) was antagonised by the opioid receptor antagonists naltrexone (1 mg/kg, s.c.), naloxone (1 mg/kg, s.c.), M8008 (16*S*-methylcyprenorphine; 1 mg/kg, s.c.) and MR 2266 (5,9-diethyl-2-(3-furylmethyl)2′-hydroxy-7,7-benzomorphan; 1 mg/kg) but not by naloxone methylbromide (1 mg/kg, s.c.), naloxone methyliodide (1 mg/kg, s.c.), naltrindole (1 mg/kg, s.c.), DIPPA (2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)-ethyl]acetamide; 3 mg/kg, i.p.) or naloxonazine (35 mg/kg, i.p.). This indicates an involvement of μ_2-opioid receptors within the brain to mediate the anti-emetic effect of fentanyl. In other studies, naloxone 10–60 mg/kg, s.c. induced dose-related emesis but naltrexone was only emetic at 60 mg/kg, s.c. and naloxone methylbromide failed to induce emesis at doses up to 60 mg/kg, s.c. The emesis induced by a high dose of naloxone 60 mg/kg, s.c. was antagonized by CP-99,994 ((+)-(2*S*,3*S*)-3-(2-methoxybenzylamino)-2-phenylpiperidine; 3–30 mg/kg, i.p.), 8-OH-DPAT, ((±)-8-hydroxy-dipropylaminotetralin; 0.003–0.3 mg/kg, s.c.), buspirone (3 mg/kg, s.c.) and fluphenazine (1–3 mg/kg, i.p.) but not by naltrexone (1–30 mg/kg, s.c.), metoclopramide (0.3–3 mg/kg, i.p.), sulpiride (0.3–3 mg/kg, i.p.), domperidone (0.1–3 mg/kg, i.p.), ondansetron (0.3–3 mg/kg, i.p.), granisetron (0.3–3 mg/kg, i.p.), scopolamine (0.3–3 mg/kg, i.p.) or promethazine (0.3–3 mg/kg, i.p.). The data is discussed in relation to opioid receptor mechanisms moderating emesis and the identification of potential sites of drug action available to inhibit the emetic reflex. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Opioid; Anti-emesis; (*Suncus murinus*)

1. Introduction

Morphine and related analgesic opioid receptor agonists are invaluable drugs for the treatment of pain but have the potential to induce or contribute to nausea and vomiting when administered either as single agents, or as adjuncts to anaesthesia (Ventafridda, 1984; Watcha and White, 1992). Studies indicate that μ-, δ- and κ-opioid receptors located in the area postrema and/or nucleus tractus solitarius are involved in the emetic effects of the opioid receptor agonists (see Rudd and Naylor, 1995).

Some opioid receptor agonists, particularly those that act at μ-opioid receptors, have an important 'broad inhibitory' profile to antagonise emesis. For example, fentanyl and sufentanyl are highly selective for μ-opioid receptors and are the most potent anti-emetic opioid receptor agonists tested (Niemegeers et al., 1976; Barnes et al., 1991). Perhaps relevant to the anti-emetic mechanism of action is the observation that μ-opioid receptor may be subdivided into μ_1- and μ_2-sites. Activation of the high affinity μ_1-opioid receptor mediates analgesia whereas activation of the lower affinity μ_2-opioid receptor mediates respiratory depression and inhibition of gastric transit (Paul and Pasternak, 1988). While fentanyl has affinity for both μ_1- and μ_2-opioid receptor subtypes (Jang and Yoburn, 1991), it is not known if activation of both subtypes is required for anti-emesis.

The available evidence using a variety of agonists indicates that activation of μ-opioid receptors can inhibit the emetic reflex. Thus, it has been hypothesised that endogenous opioids could provide an activation or tone within the brain to inhibit emesis. Consistent with the hypothesis are observations that naloxone can potentiate emesis in ani-

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mals (Barnes et al., 1991; King and Weatherspoon, 1992) and man (Rowbotham et al., 1983; Kobrinsky et al., 1988) and is emetic per se when administered at high doses (McCarthy et al., 1974; Goldberg et al., 1976; Costello and Borison, 1977; Bhargava et al., 1981; Miller et al., 1987; Gupta et al., 1989).

Previous studies in *Suncus murinus* have investigated the mechanisms and sites(s) of anti-emetic action of morphine and loperamide (Selve et al., 1994; Kakimoto et al., 1997). The first aim of the present studies was to determine the identity of the opioid receptor(s) involved in the anti-emetic action of fentanyl. To enable this, we selected nicotine to induce emesis with a mechanism of action involving the area postrema and/or the vestibular system (Laffan and Borison, 1957; Money and Cheung, 1983; Beleslin and Krstic, 1987) and a number of selective opioid receptor antagonists. The second aim of the studies was to investigate the emetic potential of the opioid receptor antagonists, naltrexone, naloxone and naloxone methylbromide (Fowler and Fraser, 1994) to establish a model of opioid receptor antagonist-induced emesis. A model of opioid receptor antagonist-induced emesis could be useful to identify anti-emetic mechanisms and treatments acting 'downstream' from the opioid receptor system within the emetic reflex.

2. Materials and methods

2.1. Animals

The experiments were performed on adult male or female *S. murinus* (30–85 g), bred at the Chinese University of Hong Kong. Prior to the experiments, they were housed in a temperature controlled room at 24 ± 1°C under artificial lighting, with lights on between 0700 h and 1730 h. They were allowed free access to water and pelleted cat chow (Feline Diet 5003, PMI® Feeds, USA). All experiments were conducted in accordance with the Animal Research Ethics Committee, The Chinese University of Hong Kong. Each animal was only used once.

2.2. Measurement of emesis

On the day of the experiment, the animals were transferred to clear perspex observation chambers for the assessment of emetic behavior as previously described (Rudd et al., 1999). Episodes of emesis were characterized by rhythmic abdominal contractions, which were either associated with the oral expulsion of solid or liquid material from the gastrointestinal tract (i.e., vomiting) or not associated with the passage of material (i.e., retching movements). Episodes were considered separate when an animal changed its location in the observation chamber, or when the interval between retching and/or vomiting exceeded 2 s. An assessment of emesis was made over a 30-min observation time following the administration of the potential emetic drug under investigation.

2.3. Anti-emetic action of fentanyl

Nicotine 5 mg/kg, s.c. was used to generate a retching and/or vomiting response. Fentanyl (10–40 μg/kg) or vehicle was administered subcutaneously 15 min prior to the injection of nicotine. In some studies, M8008 (16*S*-methylcyprenorphine; 1 mg/kg), MR 2266 (5,9-diethyl-2-(3-furylmethyl)2′-hydroxy-7,7-benzomorphan; 1 mg/kg), naltrindole (1 mg/kg), naltrexone (1 mg/kg), naloxone (1 mg/kg), naloxone methylbromide (1 mg/kg), naloxone methyliodide (1 mg/kg) or their respective vehicles, were administered subcutaneously 5 min prior to fentanyl injection. DIPPA (2-(3,4-dichlorophenyl)-*N*-methyl-*N*-[1*S*)-1-(3-isothiocyanatophenyl)-2-(1-pyrrolidinyl)ethyl]acetamide 3 mg/kg) or vehicle, was administered intraperitoneally 48 h prior to fentanyl. Naloxonazine (35 mg/kg) or vehicle, was administered subcutaneously 24 h prior to the injection of fentanyl. The doses of nicotine, fentanyl and the antagonists were selected on the basis of preliminary studies and previous investigation (Niemegeers, 1982; Hersom and Mackenzie, 1987; Paul and Pasternak, 1988; Barnes et al., 1991; Jang and Yoburn, 1991; Rudd and Naylor, 1992; Schwartz et al., 1997; Rudd et al., 1999).

2.4. Emetic mechanism of action of opioid receptor antagonists

Naltrexone (5–60 mg/kg), naloxone (5–60 mg/kg) and naloxone methylbromide (5–60 mg/kg) were administered subcutaneously to assess their emetic potential and to determine the optimum doses for use in the mechanism of action studies. The potential anti-emetic drugs metoclopramide (0.1–3 mg/kg), sulpiride (0.3–3 mg/kg), fluphenazine (0.1–3 mg/kg), domperidone (0.1–3 mg/kg), ondansetron (0.3–3 mg/kg), granisetron (0.3–3 mg/kg), scopolamine (0.3–3 mg/kg), promethazine (0.3–3 mg/kg) and CP-99,994 ((+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine); 1–30 mg/kg) were administered intraperitoneally as 30 min pretreatments; 8-OH-DPAT ((±)-8-hydroxy-dipropylaminotetralin; 0.003–0.3 mg/kg) and buspirone (0.03–3 mg/kg) were administered subcutaneously as 15-min pretreatments.

2.5. Data analysis

In each animal, the latency to retch and/or vomit following the administration of the respective emetogen and the total number episodes of retching and/or vomiting were calculated for the duration of the experiment. Latency data is expressed as the mean time (min) of only the animals that retched or vomited; all other data is expressed as the mean ± S.E.M. The significance of differences between treatments was assessed by a one-way analysis of variance (ANOVA) followed by a Fisher's Protracted Least Significant Difference (PLSD) test (Statview®, Abacus Concepts, USA) and is indicated as $^{a}P < 0.05$.

Table 1
The anti-emetic action of fentanyl (Fent) to inhibit nicotine-induced emesis (Nic; 5 mg/kg, s.c.) and the interaction with naltrexone (Naltrex; 1 mg/kg, s.c.), naloxone (Nal; 1 mg/kg, s.c.), naloxone methylbromide (Nal.Br; 1 mg/kg, s.c.) and naloxone methyliodide (Nal.I; 1 mg/kg, s.c.)

Treatment	Latency (min)	No. of episodes	Responders
Nic + Veh	5.4	24.0 ± 3.2	4/4
Nic + Fent 10	8.9	2.75 ± 1.7[a]	2/4
Nic + Fent 40	–	0.0 ± 0.0[a]	0/4
Nic + Veh	5.0	16.7 ± 2.6	3/3
Nic + Naltrex	4.8	36.0 ± 5.0[a,b]	3/3
Nic + Fent 40	–	0.0 ± 0.0[a]	3/3
Nic + Naltrex + Fent 40	6.1	16.3 ± 2.9[b]	3/3
Nic + Veh	5.4	17.8 ± 2.5	4/4
Nic + Nal	5.5	27.8 ± 6.1[a,b]	4/4
Nic + Nal.Br	7.9	23.0 ± 7.9[b]	3/3
Nic + Nal.I	5.1	16.5 ± 3.3[b]	4/4
Nic + Fent 40	5.2	1.8 ± 2.0[a]	1/5
Nic + Nal + Fent 40	7.0	21.5 ± 3.0[b]	4/4
Nic + Nal.Br + Fent 40	–	0.0 ± 0.0[a]	0/4
Nic + Nal.I + Fent 40	–	0.0 ± 0.0[a]	0/4

For the interaction studies, fentanyl was used at a dose of 40 μg/kg, s.c. (Fent 40). Significant differences relative to control values (Nic + Veh) are indicated as [a]$P < 0.05$; significant differences relative to Nic + Fent 40 treated animals are indicated as [b]$P < 0.05$ (ANOVA followed by a post-hoc Fisher's PLSD test).

2.6. Drugs

(−)-Nicotine di-D-tartrate (Research Biochemicals International), fentanyl citrate (Sigma), naloxone hydrochloride (Sigma), naltrexone hydrochloride (Sigma), naloxone methylbromide (GlaxoWelcome), naloxone methyliodide (Research Biochemicals International), metoclopramide hydrochloride (Sigma), scopolamine hydrochloride (Sigma), promethazine hydrochloride (Sigma), CP-99,994 (Pfizer), (±)-8-OH-DPAT hydrobromide (Research Biochemicals International), buspirone hydrochloride (Research Biochemicals International), fluphenazine hydrochloride (Research Biochemicals International), ondansetron dihydrochloride (GlaxoWelcome) and granisetron hydrochloride (SmithKline Beecham) were dissolved in distilled water. Naloxonazine (Research Biochemicals International), M8008 (GlaxoWelcome), MR2266 (GlaxoWelcome), domperidone (Research Biochemicals International) and (−)-sulpiride (Research Biochemicals International) were first dissolved with a few drops of 0.1 M hydrochloric acid and were made up in distilled water (the final pH was adjusted to 6 with 0.1 M NaOH). DIPPA (Tocris) was formulated in 25% (v/v) dimethylsulfoxide (Sigma). All drugs were administered in a volume of 2 ml/kg unless stated otherwise. Doses are expressed as the free base weight unless otherwise indicated.

Table 2
The role of μ-, δ- and κ-opioid receptors in the mechanism of anti-emetic action of fentanyl (Fent; 40 μg/kg, s.c.) to inhibit nicotine-induced emesis (Nic; 5 mg/kg, s.c.) in *S. murinus*

Treatment	Latency (min)	No. of episodes	Responders
Nic + Veh	5.7	18.5 ± 3.0	4/4
Nic + M8008	1.6	37.3 ± 9.2[a,b]	3/3
Nic + Fent	–	0.0 ± 0.0[a]	0/4
Nic + M8008 + Fent	3.5	24.3 ± 2.2[b]	3/3
Nic + Veh	5.5	20.3 ± 2.2	3/3
Nic + Nalt	6.4	12.7 ± 2.2[b]	3/3
Nic + Fent	–	0.0 ± 0.0[a]	0/3
Nic + Nalt + Fent	–	0.0 ± 0.0[a]	0/3
Nic + Veh	6.6	18.0 ± 1.4	4/4
Nic + MR2266	3.2	25.0 ± 6.0[b]	3/3
Nic + Fent	–	0.0 ± 0.0[a]	0/4
Nic + MR2266 + Fent	4.1	28.7 ± 10.3[b]	3/3
Nic + Veh	5.7	16.0 ± 2.3	4/4
Nic + DIPPA	4.7	16.0 ± 2.3[b]	3/3
Nic + Fent	–	0.0 ± 0.0[a]	0/3
Nic + DIPPA + Fent	15.6	0.3 ± 0.3[a]	1/4

M8008, naltrindole (Nalt) and MR 2266 were administered at a dose of 1 mg/kg, s.c. DIPPA was administered at a dose of 3 mg/kg, i.p. Significant differences relative to control values (Nic + Veh) are indicated as [a]$P < 0.05$; significant differences relative to Nic + Fent treated animals are indicated as [b]$P < 0.05$ (ANOVA followed by a post-hoc Fisher's PLSD test).

3. Results

3.1. The ability of fentanyl to antagonise nicotine-induced emesis in S. murinus

The dose–response relationship for nicotine to induce emesis in the *S. murinus* has been established in previous studies in these laboratories (Rudd et al., 1999). In the present studies, nicotine 5 mg/kg, s.c. induced 11–24 episodes of retching and/or vomiting, with an onset of action of approximately 5.6 min. A low dose of fentanyl 10 μg/kg, s.c. antagonised significantly nicotine-induced emesis by 87.7% and delayed emesis by 3.5 min ($P < 0.05$). The higher dose of 40 μg/kg, s.c. completely prevented the emetic response ($P < 0.05$; Table 1).

3.2. The effect of opioid receptor antagonists to modify the anti-emetic action of fentanyl

The experimental design necessitated that groups of animals received nicotine plus vehicle or naltrexone or one

Table 3
The role of the role μ_1-opioid receptor in the mechanism of anti-emetic action of fentanyl (Fent; 40 μg/kg, s.c.) to inhibit nicotine-induced emesis (Nic; 5 mg/kg, s.c.) in *S. murinus*

Treatment	Latency (min)	No. of episodes	Responders
Nic + Veh	5.8	15.0 ± 4.9	3/3
Nic + Nal.Z	4.6	11.0 ± 1.4[b]	3/3
Nic + Fent	–	0.0 ± 0.0[a]	0/3
Nic + Nal.Z + Fent	–	0.0 ± 0.0[a]	0/3

Naloxonazine (Nal.Z) was administered as a 24-h pretreatment at a dose of 35 mg/kg, s.c. Significant differences relative to control values are indicated as [a]$P < 0.05$; significant differences relative to Nic + Fent treated animals are indicated as [b]$P < 0.05$ (ANOVA followed by a post-hoc Fisher's PLSD test).

Table 4
The emetic potential of naltrexone (Naltrex), naloxone (Nal) and naloxone methylbromide (Nal.Br) in *S. murinus*

Treatment	(mg/kg)	Latency (min)	Episodes	Responders
Veh	0	–	0.0±0.0	0/5
Naltrex	5	–	0.0±0.0	0/5
Naltrex	10	–	0.0±0.0	0/5
Naltrex	20	–	0.0±0.0	0/5
Naltrex	30	–	0.0±0.0	0/5
Naltrex	60	7.6	6.8±5.0[a]	2/4
Veh	0	–	0.0±0.0	0/4
Nal	5	–	0.0±0.0	0/4
Nal	10	2.6	3.3±1.8	2/3
Nal	20	23.8	0.4±0.4	1/5
Nal	40	8.9	6.0±2.7[a]	4/5
Nal	60	7.1	9.2±2.2[a]	5/5
Veh	0	–	0.0±0.0	0/3
Nal.Br	10	–	0.0±0.0	0/3
Nal.Br	20	–	0.0±0.0	0/3
Nal.Br	40	–	0.0±0.0	0/3
Nal.Br	60	–	0.0±0.0	0/3

All drugs or respective vehicles (Veh) were administered subcutaneously. Significant differences relative to control vehicle treated animals are indicated as [a]$P < 0.05$ (ANOVA followed by a post-hoc Fisher's PLSD test).

Table 5
The lack of effect naltrexone (Naltrex; 1–30 mg/kg) to modify naloxone (Nal; 60 mg/kg)-induced emesis in *S. murinus*

Treatment (mg/kg)	Latency (min)	Episodes	Responders
Nal + vehicle	10.9	10.7±2.3	3/3
Nal + Naltrex 1	12.6	7.0±3.0	4/4
Nal + Naltrex 3	7.4	9.0±4.0	3/4
Nal + Naltrex 10	11.2	2.5±1.2	3/4
Nal + Naltrex 30	4.5	8.3±0.9	4/4

Naltrexone was administered 15 min prior to the injection of naloxone. All drugs were administered subcutaneously. There were no significant differences relative to control vehicle treated animals ($P > 0.05$).

of the other opioid receptor antagonists. Out of the antagonists tested only DIPPA (3 mg/kg, i.p.) induced emesis during the pretreatment period. The emesis occurred in eight out of eight animals at 5.1 ± 0.6 min post-administration and comprised 11.5 ± 1.8 episodes; the vehicle for DIPPA (DMSO) induced one episode 3.1-min post-administration in only one out of eight animals tested.

In subsequent experiments, the anti-emetic action of fentanyl 40 μg/kg, s.c. was reversed to control values by naltrexone 1 mg/kg, i.p. ($P < 0.05$; Table 1), naloxone 1 mg/kg, i.p. ($P < 0.05$; Table 1), M8008 1 mg/kg, i.p. ($P < 0.05$; Table 2) and MR2266 1 mg/kg, i.p. ($P < 0.05$; Table 2), but not by naloxone methylbromide 1 mg/kg, i.p. ($P > 0.05$; Table 1), naloxone methyliodide 1 mg/kg, i.p. ($P > 0.05$; Table 1), naltrindole 1 mg/kg, i.p. ($P > 0.05$; Table 2), DIPPA 3 mg/kg, i.p. ($P > 0.05$, Table 2) or naloxonazine 35 mg/kg, s.c. ($P > 0.05$; Table 3). Further analysis of the data revealed that as single treatments naltrexone 1 mg/kg, s.c., naloxone 1 mg/kg, s.c. and M8008 1 mg/kg, s.c. potentiated significantly nicotine-induced emesis by 115.7% ($P < 0.05$; Table 1), 56.2%

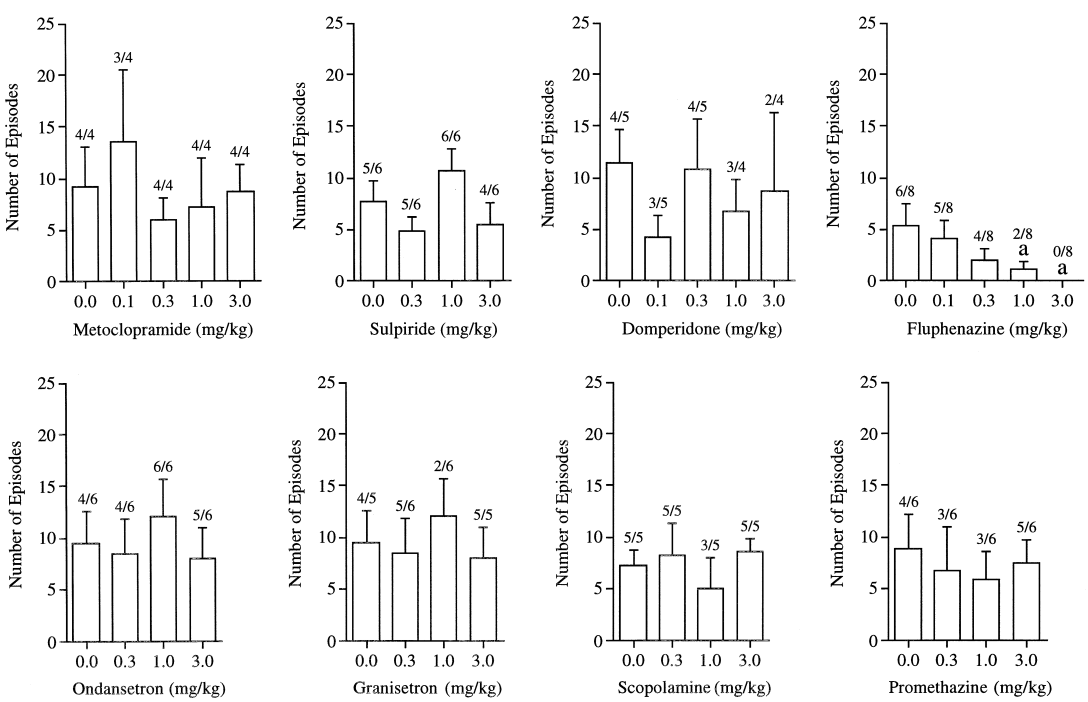

Fig. 1. The effect of dopamine receptor antagonists and other agents on naloxone (60 mg/kg, s.c.)-induced emesis in *S. murinus*. Values are the means ± S.E.M. of 4–8 determinations. The number of animals retching and/or vomiting out of the number of animals tested is indicated as a 'fraction' for each treatment group. Significant differences from vehicle treatment are indicated as [a]$P < 0.05$ (one-way ANOVA followed by a post-hoc Fisher's PLSD test).

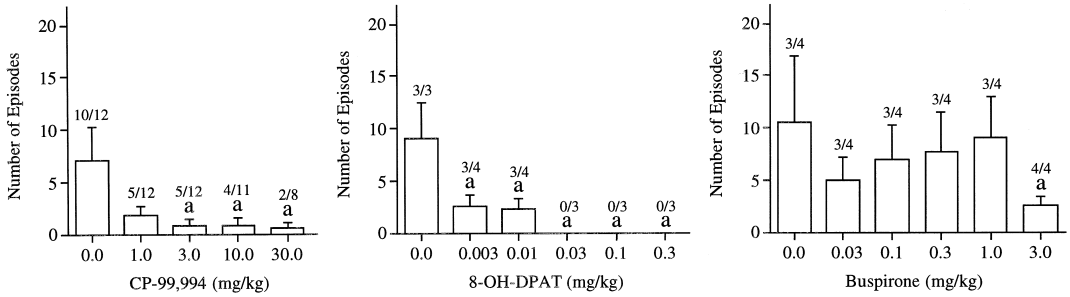

Fig. 2. Antagonism of naloxone (60 mg/kg, s.c.)-induced emesis by CP-99,994, 8-OH-DPAT and buspirone in *S. murinus*. Values are the means ± S.E.M. of 3–12 determinations. The number of animals retching and/or vomiting out of the number of animals tested is indicated as a 'fraction' for each treatment group. Significant differences from vehicle treatment are indicated as $^{a}P < 0.05$ (one-way ANOVA followed by a post-hoc Fisher's PLSD test).

($P < 0.05$; Table 1) and 101.6% ($P < 0.05$; Table 2), respectively, and M8008 1 mg/kg, s.c. (Table 2), MR2266 1 mg/kg, s.c. (Table 2) and naloxonazine 1 mg/kg, s.c. (Table 3) reduced the latency to onset of nicotine-induced emesis by 4.1, 3.4 and 1.2 min, respectively. Conversely, naloxone methylbromide 1 mg/kg, s.c. delayed the onset of nicotine-induced emesis by 2.5 min (Table 1); the other antagonists produced small (less than 1 min) changes in the latency to onset of emesis.

3.3. Emetic mechanism of naltrexone and naloxone and effect of their interaction

An extensive dose-range was used to investigate the emetic potential of naltrexone, naloxone and naloxone methylbromide. In these studies, naltrexone only induced emesis in two out of four animals (6–21 episodes, latency 5.8–9.4 min) at the high-dose of 60 mg/kg, s.c; lower doses were ineffective ($P < 0.05$; Table 4). In contrast, naloxone induced emesis in two out of three animals (4–6 episodes, latency 1.3–3.8 min) at doses as low as 10 mg/kg, s.c. and produced a consistent emetic response in five out of five animals (6–18 episodes, latency 4.9–10.8 min) at a dose of 60 mg/kg, s.c. ($P < 0.05$; Table 4). Naloxone methylbromide did not induce emesis at doses up to 60 mg/kg, s.c. ($n = 3$, Table 4).

We decided to investigate the emetic mechanism of action of naloxone 60 mg/kg, s.c. The emetic response was not antagonised significantly by metoclopramide 0.1–3 mg/kg, i.p., sulpiride 0.3–3 mg/kg, i.p., domperidone 0.1–3 mg/kg, i.p., ondansetron 0.3–3 mg/kg, i.p., granisetron 0.3–3 mg/kg, i.p., scopolamine 0.3–3 mg/kg, i.p. promethazine 0.3–3 mg/kg, i.p. or naltrexone 1–30 mg/kg, s.c. ($P > 0.05$; Fig. 1 and Table 5). However, fluphenazine 0.1–3 mg/kg, i.p.(Fig. 1), CP-99,994 1–30 mg/kg, i.p. and 8-OH-DPAT 0.003–0.03 mg/kg, s.c. (Fig. 2) produced a dose-related inhibition of emesis. The maximum reductions ranged from 91 to 100% and were significant at some of the doses tested ($P < 0.05$). Buspirone reduced significantly ($P < 0.05$) naloxone-induced emesis by 76% at the highest dose of 3 mg/kg, i.p. (Fig. 2).

In another set of experiments, we decided to investigate the potential of naltrexone to synergise with naloxone to induce emesis. In these experiments, naltrexone 30 mg/kg, s.c. or vehicle was administered 15 min prior to a lower dose of naloxone 30 mg/kg, s.c. but no significant interaction was detected ($P > 0.05$; Table 6).

Table 6
The lack of effect naltrexone (Naltrex; 30 mg/kg) to potentiate naloxone (Nal; 30 mg/kg)-induced emesis in *S. murinus*

Treatment	Latency (min)	Episodes	Responders
Veh + Veh	–	0.0 ± 0.0	0/6
Naltrex + Veh	–	0.0 ± 0.0	0/6
Veh + Nal	15.0	$4.6 \pm 1.5^{a,b}$	5/7
Naltrex + Nal	3.5	$4.3 \pm 2.1^{a,b}$	3/6

Naltrexone was administered 15 min prior to the injection of naloxone. All drugs were administered subcutaneously. Significant differences relative to vehicle + vehicle treated animals (Veh + Veh) are indicated as $^{a}P < 0.05$; significant differences between Naltex + vehicle (Natrex + Veh) treated animals are indicated as $^{b}P < 0.05$ (ANOVA followed by a post-hoc Fisher's PLSD test).

4. Discussion

4.1. Anti-emetic mechanism of action of fentanyl

We have used a number of selective opioid receptor antagonists to identify the receptors involved in the anti-emetic action of fentanyl in *S. murinus*. The δ/μ receptor antagonist M8008 (Sitsapesan and Parratt, 1989; McIntosh et al., 1992) but not the δ-opioid receptor antagonist naltrindole (Portoghese et al., 1988) or the irreversible selective κ-opioid receptor antagonist DIPPA (Schwartz et al., 1997), was capable of reversing the anti-emetic action. The data generated indicate that the anti-emetic mechanism is likely to involve μ- but not δ- or κ-opioid receptors. The contribution of the μ-opioid receptor to the mechanism was also hypothetically strengthened by the activity of MR2266, a non-selective κ/μ-opioid receptor antagonist (Leslie, 1987; Calcagnetti et al., 1990; Pournaghash and Riley, 1993) to reverse the anti-emetic action.

An action of fentanyl at μ-opioid receptors to inhibit emesis is logical since the agonist has approximately 700

times greater affinity for μ- than δ- or κ-opioid receptors (Leslie, 1987) and the effect was evident at doses as low as 10 μg/kg: the anti-emetic potency is consistent with data generated from anti-emetic studies in the ferret (Barnes et al., 1991) and dog (Blancquaert et al., 1986). However, to further characterize an involvement of the μ-opioid receptors in the anti-emetic action of fentanyl, we used naloxonazine, an irreversible selective μ_1-opioid receptor antagonist (Paul and Pasternak, 1988). Naloxonazine was ineffective to reverse the action of fentanyl to inhibit emesis to infer that the anti-emetic mechanism of fentanyl involves the μ_2-opioid receptor. Unfortunately, the finding suggests that it may be difficult to dissociate the anti-emetic effects of fentanyl from its potential to induce respiratory depression. The potential side effects may therefore preclude the development of selective μ_2-opioid receptor agonists for use as anti-emetics in man.

4.2. Site of anti-emetic action of fentanyl

Previous investigations have revealed that many opioid receptor agonists are anti-emetic when injected into the cerebroventricular system suggesting that an anti-emetic site of action resides in the brain (Costello and Borison, 1977; Beleslin et al., 1981; Kakimoto et al., 1997). In our studies, the anti-emetic action of fentanyl was clearly reversed by naltrexone and naloxone but not by the quaternary naloxone derivatives naloxone methylbromide and naloxone methyliodide. Both derivatives have poor penetration into the central nervous system (Brown and Goldberg, 1985) and their inability to antagonize the action of fentanyl suggests that the peripheral opioid receptors are not important to the anti-emetic mechanism and indirectly support the central site of action. The precise location(s) of the opioid receptors that mediate the anti-emetic action of fentanyl is unknown. However, since fentanyl has a broad inhibitory action to antagonize emesis (Barnes et al., 1991) the site of action is likely to be at a point at which information is integrated within the emetic circuit, or at an output essential to the physical process of retching and/or vomiting. Such a site could be the nucleus tractus solitarius and this is also the proposed site of action of the substance P tachykinin NK_1 receptor antagonists to exert a 'broad' antagonism of emesis (Watson et al., 1995; Tattersall et al., 1996; Rudd et al., 1999). Certainly, this seems reasonable since μ_2- but not μ_1-opioid receptors are present in the nucleus tractus solitarius and the brain area is not accessed easily by quaternary opioid receptor antagonists (Moskowitz and Goodman, 1985).

4.3. Emetic mechanism of action of opioid receptor antagonists

It is perhaps worthy of consideration that the doses of naloxone, naltrexone and M8008 that prevented the anti-emetic actions of fentanyl had no effect in their own right to induce emesis. This may suggest that under normal conditions there is no existing basal tone on the μ_2-opioid receptor to oppose emesis. However, when nicotine was used to induce emesis all three antagonists markedly enhanced the number of emetic episodes. This could indicate that nicotine may trigger the release of endogenous opioids that may normally oppose emesis. Certainly both the area postrema and nucleus tractus solitarius contain a variety of different endogenous opioids such as leu-encephalin, met-encephalin and β-endorphin and their receptors (Van Giersbergen et al., 1992; Leslie and Reynolds, 1993; Fowler and Fraser, 1994). While this hypothesis is attractive, we have no explanation as to why DIPPA was emetic in its own right. Further studies with kappa receptor agonists would be required to characterize the mechanisms involved. In our attempt to mimic earlier investigations to completely remove the hypothesized endogenous inhibitory tone from emetic reflex we used naltrexone, naloxone and naloxone methylbromide at doses up to 60 mg/kg. The high-doses of naloxone (>5 mg/kg) required to induce emesis were found to be consistent with previous studies in other species following a peripheral administration (Goldberg et al., 1976; Costello and Borison, 1977; Miller et al., 1987) and the lack of effect of naloxone methylbromide to induce emesis was predicted from its earlier failure to reverse the anti-emetic effect of fentanyl. However, it was surprising that naltrexone was much less potent to induce emesis than naloxone considering that they both have a similar affinity and selectivity for opioid receptors (Chang and Cuatrecasas, 1981; Leslie, 1987). The low potency of naltrexone to induce emesis may indicate that opioid receptors are not exclusively involved in the emetic mechanism of action of naloxone. Certainly, naltrexone had no effect to modify the emetic action of naloxone and if they were acting via the same receptor we should have expected to see an additive interaction to induce emesis.

We decided to dissect the emetic mechanism of action of the naloxone (60 mg/kg, s.c.). The $5\text{-}HT_3$ receptor antagonists ondansetron (Butler et al., 1988) and granisetron (Sanger and Nelson, 1989), the muscarinic receptor antagonist, scopolamine (Hulme et al., 1990) and the histamine receptor antagonist, promethazine (Pearlman, 1976) failed to antagonize emesis. The data suggest that naloxone is unlikely to induce emesis by causing a release of 5-HT, acetylcholine or histamine to activate $5\text{-}HT_3$, muscarinic or histamine receptors, respectively.

The role of dopamine in the emetic mechanism of action of high-dose naloxone is less clear. The selective dopamine D_2 receptor antagonists metoclopramide, sulpiride, and domperidone were used at doses known to prevent apomorphine-induced emesis (Niemegeers, 1982; Costall et al., 1990) but failed to antagonize naloxone-induced emesis. In contrast, fluphenazine, a non-selective dopamine D_1/D_2 receptor antagonist (Christensen et al., 1985), dose-dependently antagonized the emetic response. Taken together, the results may implicate dopamine D_1 receptors in the emetic mechanism of action of naloxone.

However, SKF 38393 (*R*(+)-7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-(1*H*)-3-benzazepine), a reasonably selective D_1 receptor agonist, is only weakly emetic in *S. murinus* (Matsuki et al., 1992) and SCH-23390 (*R*(+)-2,3,4,5-tetrahydro-3-methyl-5-phenyl-(1*H*)-3-benzazepine-7-ol), another selective D_1 receptor agonist, fails to induce emesis in the dog (Yoshida et al., 1995). The precise mechanism of action of fluphenazine to inhibit naloxone-induced emesis is uncertain but is useful to question the relevance of the model to detect novel 'broad inhibitory' anti-emetic drugs. Thus, if naloxone had acted specifically via an opioid receptor, to remove an hypothesized endogenous opioid tone that is mimicked by fentanyl, then fluphenazine should not have been identified as being active. The rationale is based on the fact that fluphenazine is not a 'broad inhibitory' anti-emetic agent, failing to prevent the emesis induced by intragastric copper sulphate or morphine in the ferret (Costall et al., 1990; Rudd, unpublished data) and nicotine in the *S. murinus* (Rudd, unpublished data).

In the course of our studies we also investigated the anti-emetic potential of a number of 'broad inhibitory' anti-emetic drugs to prevent naloxone-induced emesis. The tachykinin NK_1 receptor antagonist, CP-99,994 (Mclean et al., 1993) and the 5-HT_{1A} receptor agonists 8-OH-DPAT and buspirone (Hoyer, 1992), were effective to antagonize the emetic response. The data may have been expected since the drugs can provide a general 'broad inhibition' of emesis through mechanisms involving the nucleus tractus solitarius and associated structures (Bountra et al., 1993; Okada et al., 1994; Lucot, 1995; Watson et al., 1995; Rudd et al., 1999).

4.4. General conclusions

Fentanyl was found to inhibit nicotine-induced emesis by a mechanism that probably involves μ_2-opioid receptors located in the central nervous system. The ability of moderate doses of naltrexone, naloxone and M8008 to potentiate nicotine-induced emesis supports the existence of an endogenous inhibitory opioid tone in the mechanisms modulating the emetic reflex. Nevertheless, the emesis induced by higher doses of naloxone probably involves mechanisms other than or in addition to those involving opioid receptors. The activity of CP-99,994, 8-OH-DPAT and buspirone to antagonize naloxone-induced emesis suggest an involvement of NK_1 and 5-HT_{1A} receptors in the emetic mechanism. However, naloxone-induced emesis does not involve an activation 5-HT_3, muscarinic or histamine receptors. Curiously, however, fluphenazine was active to antagonize naloxone-induced emesis but other dopamine receptor antagonists were ineffective. Further studies would be necessary to fully resolve the anti-emetic action of fluphenazine. A model of naloxone-induced emesis does not seem useful to use alone to detect novel 'broad inhibitory' anti-emetic drugs.

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