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Action of ondansetron and CP-99,994 to modify behavior and antagonize cisplatin-induced emesis in the ferret

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Abstract

The action of ondansetron (1 mg/kg, i.p.) and (+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine (CP-99,994; 10 mg/kg, i.p.) on spontaneous behavior and the emesis induced by cisplatin (10 mg/kg, i.p.) was studied in the ferret. Ondansetron was inactive to modify behavior, but CP-99,994 reduced spontaneous locomotor activity and lip licking by 48% ($P<0.01$) and 79% ($P<0.01$), respectively; CP-99,994 also abolished spontaneous burrowing activity ($P<0.05$). Treatment of animals with cisplatin induced an emetic response that was abolished by both ondansetron and CP-99,994 ($P<0.01$). However, cisplatin did not significantly modify other behavioral measures although animals that received CP-99,994, cisplatin, or CP-99,994 in combination with cisplatin exhibited more episodes of defecation than animals that received ondansetron ($P<0.05$). The action of CP-99,994 to modify behavior in this species is discussed in relation to animal models of nausea.

Keywords: Cisplatin; Nausea; Emesis; 5-HT_3 receptor; Tachykinin NK_1 receptor; Anti-emetic

1. Introduction

There are several major classes of drugs available for the treatment of nausea and emesis including antagonists acting at dopamine, histamine, muscarinic and 5-hydroxytryptamine$_3$ (5-HT_3) receptors (Sanger, 1993). These agents probably reduce nausea and emesis by blocking receptors on afferent inputs into the so-called "vomiting centre" conferring specificity against various emetic challenges. Tachykinin NK_1 receptor antagonists represent a new range of anti-emetic drug that probably prevent emesis at sites deeper in the vomiting reflex below the convergence of several afferent inputs (i.e. in the brainstem), that probably explains their remarkable spectrum of action in animals to prevent vomiting induced by a wide variety of challenges (Andrews and Rudd, 2004; Rupniak and Kramer, 1999; Tattersall et al., 1996).

Tachykinin NK_1 receptor antagonists are active in man to reduce chemotherapy-induced delayed emesis (Campos et al., 2001; Navari et al., 1999) and the post-operative emesis induced by surgery with anaesthesia (Diemunsch et al., 1999; Gesztesi et al., 2000). However, a control of chemotherapy induced acute nausea and emesis and nausea induced by surgery with anaesthesia is less impressive than predicted from animal studies (Andrews and Rudd, 2004; Gesztesi et al., 2000; Navari et al., 1999). It is also interesting that the tachykinin NK_1 receptor antagonist L-758, 298 (the pro-drug for the tachykinin NK_1 receptor antagonist, aprepitant) is inactive to prevent motion-induced nausea (Reid et al., 2000). It is a possibility that even if emesis is reduced, because tachykinin NK_1 receptors at points deep in the vomiting reflex are blocked, there still

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could be a sensation of nausea because of ongoing afferent activity in circuits that are connected to the forebrain (Andrews and Rudd, 2004).

Unfortunately, it is impossible to directly determine the action of drugs on nausea in animals, since they are unable to communicate their emotional status. However, there are a number of investigations using emetic drugs and ferrets where several drug-induced behaviors have been suggested as being analogous to nausea. For example, Bermudez et al. (1988) demonstrated an increase in the incidence of 'backward walking' and 'burrowing' after cisplatin administration that could be prevented by granisetron. In another study, emetic doses of radiation were associated with a reduction of locomotor activity that was differentially prevented by anti-emetic drugs (King and Landauer, 1990). A more detailed study focused on the frequencies of lip licking, mouth scratching, wet dog shakes and gagging that were associated with an emetic dose of loperamide; these behaviors were stereoselectively antagonized by the tachykinin NK_1 receptor antagonist ((+)-(2S,3S)-3-(2-methoxybenzylamino)-2-phenylpiperidine) (CP-99,994; Zaman et al., 2000). More complicated methods have also been used in the ferret where behavioral scores (e.g. of lip licking and backward walking, etc.) are aggregated or transformed to a point system to assess the effectiveness of anti-emetic agents to reduce drug-induced 'nausea' or 'prodromal' signs of emesis (Gonsalves et al., 1996; Hawthorn and Cunningham, 1990).

Unfortunately, a potential weakness with most of the studies conducted to date in the ferret is that the action of anti-emetic drugs has not been thoroughly investigated alone for their capacity to modify normal behavior, before progressing to evaluate their action on emetogen-induced behavioral changes. Thus, it is possible that the effect of anti-emetics on the putative measures of nausea has been overestimated. In the present studies, therefore, we investigate the action of selected anti-emetic drugs to affect drug-induced emesis and associated behaviors, taking into account any action of the anti-emetics, per se, to affect basal animal activity. Specifically, the studies investigate the action of ondansetron (Butler et al., 1988) and CP-99,994 (Mclean et al., 1993), as respective 5-HT_3 receptor and tachykinin NK_1 receptor antagonists, to alter behavior and their potential to modify behavior and emesis induced by the chemotherapeutic drug, cisplatin (Rosenberg, 1985).

2. Methods

2.1. Animals

Castrated male ferrets weighing between 1.0 and 2.5 kg were used in the studies. They were obtained from a reputable breeder in New Zealand and were housed in a temperature-controlled room (24±1 °C) in the Laboratory Animal Services Centre, The Chinese University of Hong Kong. Artificial lighting was provided between 06:00 and 18:00 h, with humidity being maintained at 50±5%. Water and dry pelleted cat chow (Feline Diet 5003, PMI® Feeds, St. Louis, USA) were available ad libitum. All experiments were conducted under the licence provided by the Government of the Hong Kong SAR and the Animal Research Ethics Committee, The Chinese University of Hong Kong.

2.2. Behavioral observation

Ferrets were transferred to opaque Perspex observation chambers (50×50×50 cm) illuminated to 15±1 lx. The image of each animal was captured by an overhead camera (Panasonic WV-CP460/P; Panasonic, Yokahoma, Japan) and the analog-video signal was converted to digital by a frame grabber and calculations of movement made using EthoVision Color Pro software (Version 2.3; Noldus Information Technology, Costerweg, Netherlands) running on a personal computer. Using this approach, it was possible to determine the spontaneous movement (locomotor activity: m) of each animal. Other behaviors were recorded manually and included episodes of (1) retching and/or vomiting, (2) lip licking, (3) burrowing, (4) rearing, (5) curling up, (6) backward walking and (7) defecation. The experiment started when the animals were introduced into the observation chamber. However, in order to reduce artifacts that might be induced by exposing the animals to a novel environment, the ferrets were first allowed to habituate in a Perspex box (50×50×50 cm) that was identical to the experiment observation chamber. This was done for 2-h periods on three consecutive days, prior to the start of the experiment. On the day of the experiment, animals were also allowed a 30-min habituation period in the Perspex box before drug administration, where behavioral data was collected (see below).

2.3. Administration of drugs

Ondansetron (1 mg/kg), CP-99,994 (10 mg/kg), or their vehicle (0.5 ml/kg, saline 0.9% w/v), was administered

Table 1
Behavioral activity of ferrets during the 30-min habituation period immediately prior to the administration of vehicle or drugs

Behavior	Pre-vehicle	Pre-ondansetron	Pre-CP-99,994
Distance travelled (m)	56.9±7.5	52.3±9.2	49.3±7.4
Lip licking	6.8±1.5	4.1±1.3	7.0±.18
Backward walking	1.4±1.1	0.9±0.3	2.9±1.6
Burrows	3.4±1.6	2.8±1.5	2.4±1.5
Rears	14.4±3.1	14.6±3.8	11.3±2.4
Curl ups	2.1±0.9	0.9±0.24	1.3±0.5
Defecations	0.3±0.1	0.4±0.2	0.2±0.1

Data represent the mean±S.E.M. of 13–16 observations. There were no significant differences between the animals scheduled to receive saline (Pre-vehicle), ondansetron (1 mg/kg, i.p.; Pre-ondansetron) or CP-99,994 (10 mg/kg, i.p.; Pre-CP-99,994) ($P>0.05$, one way ANOVA, or Kruskal–Wallis test, as appropriate).

intraperitoneally at time=0 (t=0). Animal behavior was then recorded for 30 min before the intraperitoneal injection of cisplatin (10 mg/kg) or vehicle (10 ml/kg, saline 0.9% w/v). Animals were then observed for a further 120 min. All treatments were randomized and administered following a Latin square design.

2.4. Data analysis

Differences between the behavior of the vomiting control animals and the ondansetron or CP-99,994 treated animals was analyzed by various techniques. For spontaneous locomotor activity, the distance travelled data was first Log_{10} transformed and analyzed using a one-way analysis of variance (ANOVA) followed by pre-planned contrasts of specified means (SuperANOVA version 1.11, Abacus Concepts, Berkeley, CA, USA). The total lip licking, curling up, burrowing, backward walking, rearing and defecatory episodes and latency data were analyzed using a Kruskal–Wallis test followed by a Dunn's multiple comparison test (GraphPad Prism version 4.00, GraphPad Software, San Diego, CA, USA). When an animal failed to retch or vomit, a latency value equal to the test period observation time (i.e. 120 min) was used to perform the statistical analysis. The retching+vomiting data was only analyzed during the test periods (i.e. following emetogens, or vehicle) and differences between treatment groups was assessed by a one-way ANOVA followed by a Fisher's PLSD test (StatView version 5.0.1, SAS Institute, Cary, NC, USA). Results are expressed as the mean±S.E.M. unless otherwise stated. In all cases, difference between treatment groups were considered significant when P<0.05.

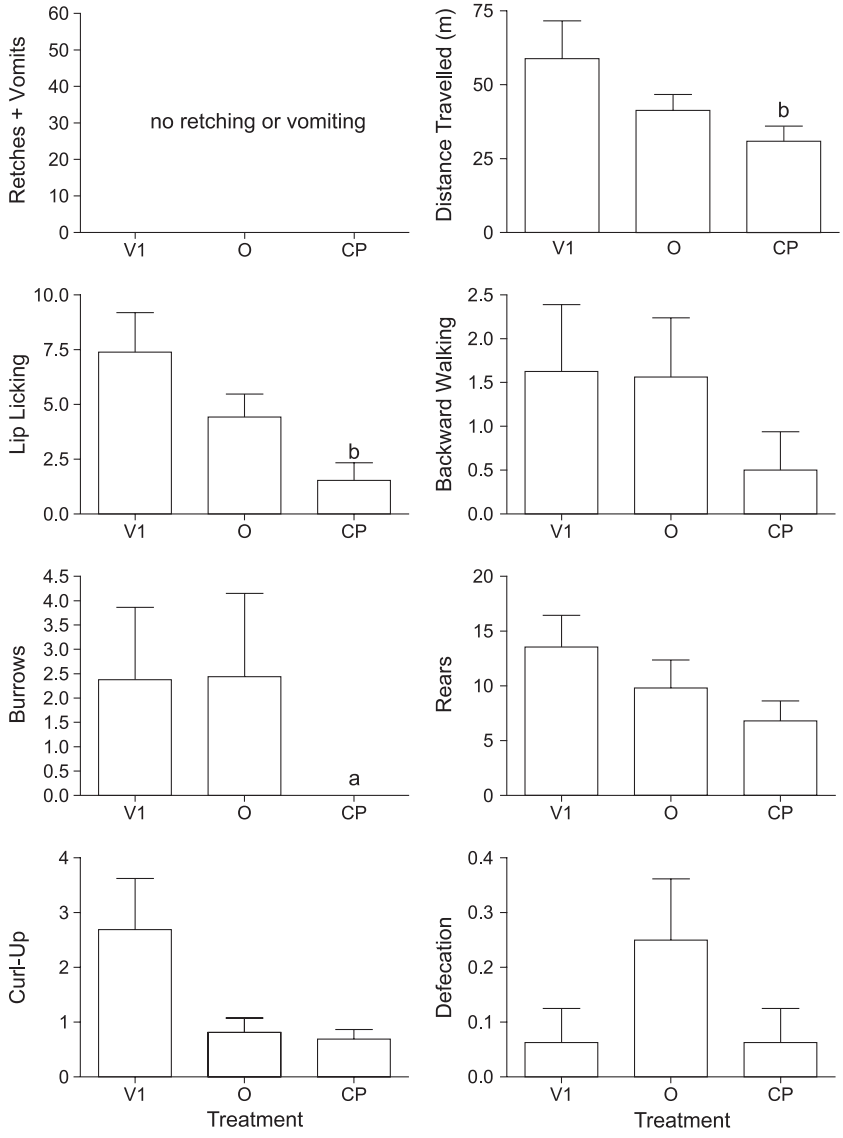

Fig. 1. Behavioral repertoire of the ferret following the administration of saline (V1: 0.9% w/v; 1 ml/kg), ondansetron (O: 1 mg/kg) or CP-99,994 (CP: 10 mg/kg). Results represent the mean±S.E.M. of 13–16 observations during a 30-min observation time. Significant differences relative to saline treated animals are indicated as [a]P<0.05, [b]P<0.01 (one way ANOVA, or Kruskal–Wallis test, followed by appropriate post hoc tests).

3. Results

3.1. Basal activity of the ferrets during the habituation period

During the 30-min habituation period, animals travelled approximately 53 m with approximately 2 episodes of backward walking, 3 episodes of burrowing, and 13 episodes of vertical rears; they had approximately 1 episode where they curled up (Table 1). The ferrets also exhibited approximately 6 episodes of lip licking and only a few animals exhibited defecation (combined group averages were approximately 0.3; see Table 1). There were no significant differences between any of the behavioral parameters that were measured prior to drug/vehicle administration (Table 1; $P>0.05$). No retching or vomiting was observed during the habituation period.

3.2. Effect of anti-emetics on ferret behavior

The administration of ondansetron, CP-99,994, or vehicle was not associated with retching or vomiting (Fig. 1). However, CP-99,994 (10 mg/kg, i.p.) reduced significantly the distance travelled by the ferrets (controls travelled approximately 58 m during the 30-min observation time) by approximately 48% ($P<0.01$) and also reduced lip licking episodes (controls exhibited approximately seven episodes) by approximately 79% ($P<0.01$) and prevented burrowing

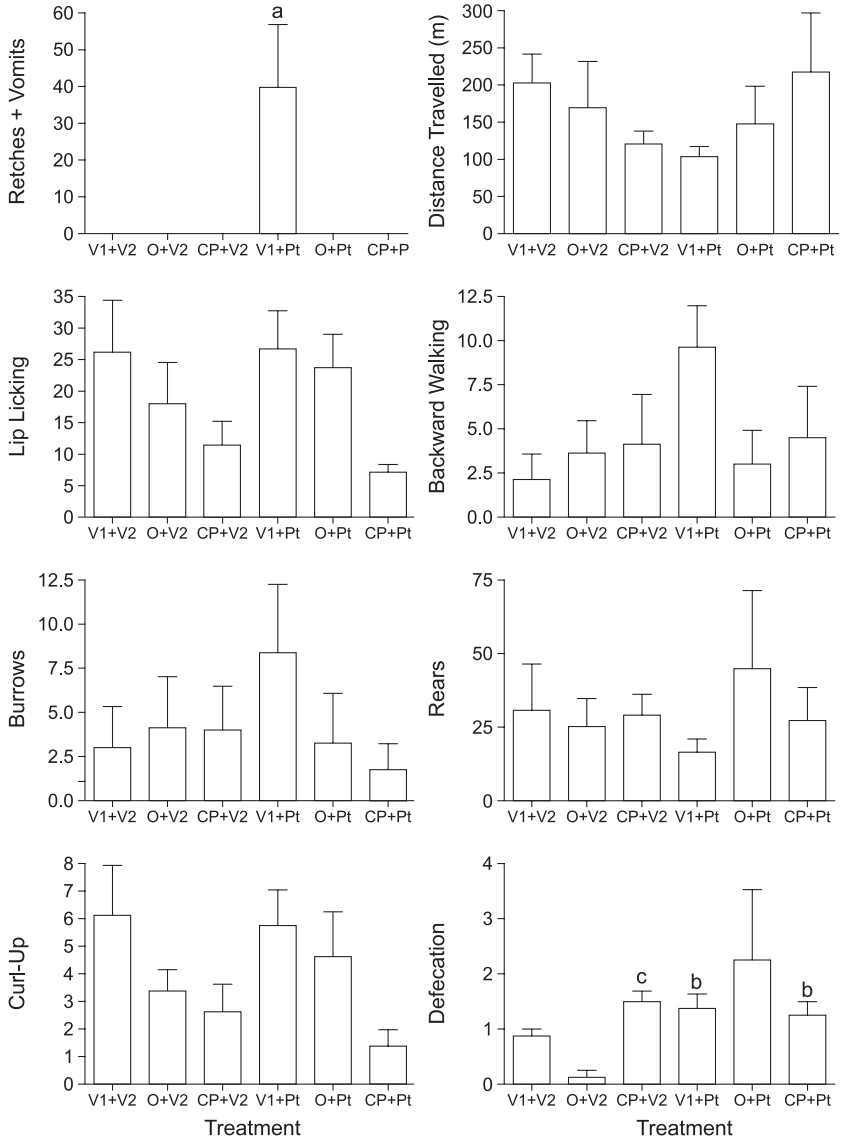

Fig. 2. Behavioral repertoire of the ferret following treatment with anti-emetic drugs and/or cisplatin. Ondansetron (O: 1 mg/kg, i.p.), CP-99,994 (CP: 10 mg/kg, i.p.), or vehicle (V1: saline 1 ml/kg, i.p.) was administered 30 min prior to the injection of cisplatin (Pt: 10 mg/kg, i.p.) or vehicle (V2: saline 0.9% w/v, 10 ml/kg, i.p.). Data was collected for 120-min post cisplatin or V2 administration and results represent the mean±S.E.M. of six to eight determinations. Significant differences relative to V1+V2 treated animals are indicated as $^{a}P<0.001$ (one-way ANOVA followed by a Fisher's PLSD test), significant differences relative to O+V2 treated animals are indicated as $^{b}P<0.05$, $^{c}P<0.01$ (Kruskal–Wallis test followed by a Dunn's multiple comparison test).

(controls exhibited approximately two episodes) in all animals (Fig. 1; $P<0.05$). Ondansetron did not have any significant action to modify behavior (Fig. 1; $P>0.05$).

3.3. Ferret behavior following cisplatin or vehicle administration after the anti-emetic/vehicle treatment

Following a 30-min pretreatment with CP-99,994, ondansetron, or vehicle, the animals then received cisplatin (10 mg/kg) or its vehicle (saline; V2—see Fig. 2); the administration of the cisplatin vehicle (V2) was not associated with retching or vomiting during the 120-min observation period. However, the cisplatin control animals exhibited approximately 40 retches+vomits (Fig. 2; $P<0.01$) following a latency of 99.4±4.8 min. Ondansetron and CP-99,994 both prevented retching and vomiting induced by cisplatin (Fig. 2; $P<0.01$). There were no differences in distance travelled, or in the frequencies lip licking, backward walking, burrowing, rearing, or curling up (including time spend curled up; data not shown) between the respective treatment groups (Fig. 2; $P>0.05$). However, animals that received CP-99,994, cisplatin, or CP-99,994 in combination with cisplatin, exhibited more episodes of defecation than animals that received ondansetron (Fig. 2; $P<0.05$).

4. Discussion

Previous studies in the ferret (and other species) failed to predict the inability of tachykinin NK_1 receptor antagonists to prevent motion-induced nausea in man, or their lower than expected anti-emetic efficacy during the early 5-HT_3 receptor antagonist sensitive component of chemotherapy-induced emesis (see Introduction). Therefore, the present studies were conducted in an attempt to further characterize ferret behavior that may be relevant to the future development of other classes of novel drugs to prevent *both* nausea and emesis.

Although most groups have not evaluated (or quantified) basal ferret activity, it does seem clear that when an animal retches and/or vomits, there is a temporally close association with the expression of other behaviors such as lip licking, burrowing or chewing activity (Bermudez et al., 1988; Milano et al., 1995; Watson et al., 1995). However, the design of our studies demonstrated that cisplatin alone was inactive to modify significantly the basal activity of the ferrets, although the level of backward walking and burrowing activity was almost increased significantly. We may have not been able to register significant elevations of behavior in our studies since we terminated the experiments slightly earlier than others because we were interested in harvesting the animals' brains for further analysis (not discussed in this manuscript). Nevertheless, our measurements were made after approximately 40 retches+vomits, where we may have expected the animals to be experiencing nausea, and where the doses of ondansetron and CP-99,994 were effective to totally abolish the emetic response confirming a role for 5-HT_3 receptors and tachykinin NK_1 receptors in the emetic reflex (Naylor and Rudd, 1996).

It is interesting that in the 'emetogen test period' of the present studies we could only detect changes in the frequency of defecation between animals receiving ondansetron alone and those receiving cisplatin alone, CP-99,994 alone, or cisplatin in combination CP-99,994; the clinical significance of the findings are unknown. What should be stated, however, is that if our experiments had been designed only to compare the action of (i) cisplatin plus vehicle, (ii) cisplatin plus ondansetron and (iii) cisplatin plus CP-99,994, we may have concluded following statistical analysis (i.e. using ANOVA with one less treatment group) that cisplatin does indeed induce emesis, lip licking, backward walking, burrowing and curling up activity that would have been reduced significantly by CP-99,994, with ondansetron reducing emesis, backward walking and burrowing activity. From such a closed analysis (i.e. missing out appropriate controls, where basal activity can be established), we may have over enthusiastically, and perhaps also inappropriately, concluded that we had observed a reduction of cisplatin-induced emesis *and* signs of 'nausea'.

In other studies, using a halothane-anaesthetized ferret model, the tachykinin antagonists sendide, spantide and CP-96,345 were highly effective to block emesis induced by an intra-duodenal administration of hypertonic saline and reduced the associated salivation and licking movements (Davison et al., 1995). Further, CP-99,994 has been previously reported to stereospecifically reduce the lip licking and mouth scratching behavior associated with emesis induced by loperamide in the conscious ferret; these reductions were seen at doses of CP-99,994 that abolished emesis (Zaman et al., 2000) and in a urethane anaesthetized ferret model, CP-99,994 prevented retching and licking and swallowing evoked by electrical stimulation of the abdominal vagal afferents (Watson et al., 1995). Conversely, CP-122,721, a structurally related analogue of CP-99,994, was inactive to modify lip licking and backwards walking activity associated with emesis induced by either loperamide or copper sulphate (Gonsalves et al., 1996). Yet, in the latter study, the effect of the CP-122,721 was measured at a dose that reduced retching by only 50% where the animals are still clearly retching and vomiting making it difficult to compare the studies.

It should be noted, however, that we were careful in our studies to expose the ferrets on several occasions to the observation chambers, but even using this approach they were highly active and mobile on the day of the experiment. We found the ferrets had a low level of lip licking activity that was relatively constant across the habituation, pretreatment and test periods and as part of their normal exploratory repertoire, the ferrets would burrow, rear and occasionally exhibit backward walking that was not necessarily associated with attempts to defecate. To our knowledge, only one

previous study has deliberately examined the normal behavioral repertoire of the ferret where the animals also exhibited lip licking behavior and episodes of defecation and tenesmus, but the behaviors were not quantified (Bermudez et al., 1988). Nevertheless, these behaviors are clearly present in normal animals and it is therefore difficult for us to conclude their presence alone is indicative of nausea. Perhaps one of the major findings of the present studies was the differential action of CP-99,994, but not ondansetron, to modify basal lip licking activity. It is possible that CP-99,994 has disrupted an endogenous activation of tachykinin NK_1 receptors in the ferret that normally drives salivation (Ekstrom et al., 1988), and that a basal rate of lip licking is required to aid swallowing of excess saliva. This hypothesis is feasible considering that substance P is a potent sialogouge and there are probably tachykinin NK_1 receptors in salivary glands (Cellier et al., 1996; Holzer and Holzer-Petsche, 1997). Given this, we believe that the ability of CP-99,994 to have an action on normal lip licking activity may complicate an interpretation of its action when used in combination with emetogens.

Our studies also clearly revealed the potential of CP-99,994, but not ondansetron, to decrease spontaneous locomotor activity, with concomitant reductions of spontaneous burrowing that may also be considered as motor events (as can lip licking). In the rat, tachykinin NK_1 and 5-HT_3 receptor antagonists are not particularly active to modify basal locomotor activity (van der Hoek and Cooper, 1994; Vasar et al., 1993) and the 5-HT_3 receptor antagonist, batanopride, also fails to reduce locomotor activity in the ferret (King and Landauer, 1990). There are reports, however, that in the cisplatin treated ferret, central administration (intra-dorsal vagal complex) of CP-99,994, and its less active enantiomer, causes motor impairment (Tattersall et al., 1996), but in our studies it is important to note that CP-99,994 did not affect backward walking, rearing, curling up activity, or the frequency of defecation, suggesting a mechanism that is more intricate than an action to cause generalized motor depression or sedation. We feel that it is necessary and important to mention other information that is relevant to the interpretation of behavioral data in emesis experiments conducted by other workers. For example, it is interesting that in the piglet, chewing activity and increases in heart rate are tightly coupled to the temporal appearance of emesis, but these events are only seen in approximately 60% of animals having an emetic episode (Milano et al., 1995). Yet these behaviors are relied on heavily as representing nausea (Grélot et al., 1998; Grélot et al., 1996; Milano et al., 1995). Does this mean that 40% of the time, the animals that do not vomit do not experience nausea, or are these behavioral measures misleading? Similarly, lip licking activity can occur prior to retching and/or vomiting (or fictive retching/vomiting) in anaesthetized (Davison et al., 1995; Watson et al., 1995) or decerebrate animals (Fukuda et al., 1999; Furukawa et al., 1998) where the forebrain does not have a controlling influence (see above). It is possible, therefore, that some autonomic/motor changes are programmed events arising from a close controlled association with inputs/outputs of the nucleus tractus solitarius and/or 'vomiting centre', rather than a manifestation (i.e. output) of activity resulting from a sensation of nausea that is envisaged to occur in the forebrain (Andrews and Rudd, 2004; Fukuda et al., 1999). We believe that other approaches are therefore necessary to study mechanisms involved in nausea.

In conclusion, the present studies have been important to reveal the normal behavioral repertoire of the ferret under laboratory conditions where CP-99,994 was active to selectively reduce lip licking and spontaneous locomotor activity but ondansetron was relatively silent. Nevertheless, the anti-emetic action of both ondansetron and CP-99,994 to inhibit cisplatin-induced emesis was confirmed but no evidence was found for cisplatin to modify other behaviors. We believe that apart from emesis, none of the behaviors that we measured could be used as an index of 'nausea behavior' and care should be taken in interpreting data from similar experiments. Perhaps future studies should examine the behavioral repertoire of emetic drugs at doses below the threshold to activate the emetic reflex. This approach may reveal behaviors more closely linked to nausea, rather than those expressed and/or tightly coupled to the physical processes of emesis.

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