Serotonin-Independent Model of Cisplatin-Induced Emesis in the Ferret

John A. Rudd[1], Celine H.K. Cheng[2] and Robert J. Naylor[2]

[1]*Department of Pharmacology, Faculty of Medicine, The Chinese University of Hong Kong, Shatin, N.T., Hong Kong*
[2]*Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, BD7 1DP, U.K.*

Received October 22, 1997 Accepted August 3, 1998

ABSTRACT—*para*-Chlorophenylalanine (PCPA, 100–200 mg/kg) was used as a pharmacological tool to characterize the 5-hydroxytryptamine (5-HT) involvement in the emesis occurring 24 hr after the administration of cisplatin (10 mg/kg) in the ferret. PCPA was effective to antagonize the initial 8 hr period of retching and vomiting, but potentiated the emesis that occurred during the remaining 8- to 24-hr observation period. Tissue samples removed from the brainstem at 24 hr post injection of cisplatin alone revealed an elevation of 5-HT, dopamine and homovanillic acid that was antagonized by the injection of PCPA. Cisplatin also induced increases in the urinary levels of 5-hydroxyindoleacetic acid that was similarly antagonized by PCPA. Results are discussed in terms of the relevance of 5-HT to the model of cisplatin (10 mg/kg)-induced emesis in the ferret compared to the problem of acute and delayed emesis in man. The residual or delayed phase of cisplatin-induced emesis may involve a 5-HT-independent mechanism.

Keywords: Cisplatin, Emesis, *p*-Chlorophenylalanine, Ferret, Delayed

Cisplatin-induced emesis in man is characterized by acute (the first 24 hr) and delayed (post-24 hr) phases of nausea and vomiting (1, 2). The acute, but not the delayed phase, is associated with increases in urinary 5-hydroxyindoleacetic acid (5-HIAA) levels (3, 4) and the emesis is almost completely prevented by the use of 5-hydroxytryptamine$_3$ (5-HT$_3$) receptor antagonists (5) or by pretreatment with the 5-hydroxytryptamine (5-HT) synthesis inhibitor *p*-chlorophenylalanine (PCPA) (6). This indicates the importance of 5-HT and the 5-HT$_3$ receptor in the mediation of the acute response. The control of the delayed emesis is considered to be more problematic with the 5-HT$_3$-receptor antagonists having a limited action (5, 7) and the course of emesis is not associated with increases in urinary 5-HIAA levels (4). Fortunately, however, the control of both the acute and delayed phases of emesis is improved by the concomitant use of 5-HT$_3$-receptor antagonists with corticosteroids such as dexamethasone (8, 9).

In most animal studies, the course of cisplatin (10 mg/kg)-induced emesis has been followed for 2–6 hr (10). The observation times have proved sufficient to effectively identify the anti-emetic activity of the 5-HT$_3$-receptor antagonists (10, 11), but appear less relevant to an understanding of the mechanisms of delayed emesis. In an attempt to increase the usefulness of the cisplatin (10 mg/kg)-induced emesis model in the ferret, we extended the observation time to 24 hr (12). Utilizing this extended observation period, we confirmed that whilst the early (1–6 hr) phase of emesis was antagonized by 5-HT$_3$-receptor antagonists, the later phase of emesis was partially resistant (13, 14).

The cisplatin (10 mg/kg)-induced emesis model in the ferret is used extensively to evaluate the potential of new anti-emetic drugs. However, it is of importance to the development of the model to examine the effect of PCPA treatment on the 24 hr emetic response induced by cisplatin to enable a comparison with the clinical data. The present studies were therefore designed to give an insight into the model's relevance regarding the ability to reflect the emesis seen in man. The studies also investigated the potential changes in monoamine levels in the dorsal vagal complex (DVC), gastrointestinal tract and urine at the end of the 24-hr observation period. The studies with PCPA may be useful to reveal a 5-HT-independent phase of emesis that may be relevant to the mechanism of delayed emesis in man.

MATERIALS AND METHODS

Male ferrets (UK bred, 0.75–1.3 kg) were housed individually at 22±1°C and had free access to food (SDS Diet "C" (E); Special Diet Services, Ltd., Chelmsford, UK) and water. Animals were injected intraperitoneally

once per day, for four days, with PCPA at 100 mg/kg, PCPA at 200 mg/kg or vehicle (1% Tween 80, 2 ml/kg). Cisplatin (10 mg/kg) or saline (0.9% w/v, 5 ml/kg) was administered intraperitoneally 4 hr after the last injection of PCPA or vehicle, and the animals were transferred to individual observation cages; emesis was recorded as previously described (15) and was expressed as retches plus vomits. At 24 hr post-injection of cisplatin or saline, animals were killed by an overdose of halothane and exsanguinated. A urine sample (obtained by direct puncture of the bladder) and a mid-ileal segment was immediately removed and frozen in liquid nitrogen. The brain was removed over ice and a transverse section through the brainstem, which contained the limits of the area postrema (i.e., 1- to 3-mm thickness caudal to rostral) was dissected. The area postrema and immediate underlying structures (to a depth of 1–1.5 mm, the DVC) was identified using a binocular light microscope (10× magnification) and removed and weighed before being frozen in liquid nitrogen. The remainder of the brainstem section was also weighed and frozen in liquid nitrogen. The mucosa of the ileum was dissected from the muscle and the tissues weighed.

The brain tissues, ileal mucosa and muscle samples were homogenized over ice (Soniprep 150; MSE, Crawley, UK) in 200 μl of 0.2 M perchloric acid (containing 100 pg/μl *N*-methyl-5-HT) and centrifuged at 15,400 $\times g$ for 3 min (L8-7 ultracentrifuge; Beckman, Palo Alto, CA, USA). The urine samples were diluted 1 in 100 with 0.1 M perchloric acid (containing 100 pg/μl *N*-methyl-5-HT) and centrifuged at 15,400$\times g$ for 3 min. The supernatants of all the samples were assayed for 5-hydroxytryptophan (5-HTP), 5-HT, 5-HIAA, adrenaline (AD), noradrenaline (NA) and dopamine (DA) and its metabolites homovanillic acid (HVA) and dihydroxyphenylacetic acid (DOPAC) by high performance liquid chromatography with electrochemical detection as previously described (16). The urine samples were also analyzed for creatinine content using a commercially available colorimetric creatinine assay kit obtained from Sigma Diagnostics (Sigma Chemical, Dorset, UK). Data were subject to statistical analysis using a one way analysis of the variance (ANOVA) followed by a post-hoc Fisher's PLSD test.

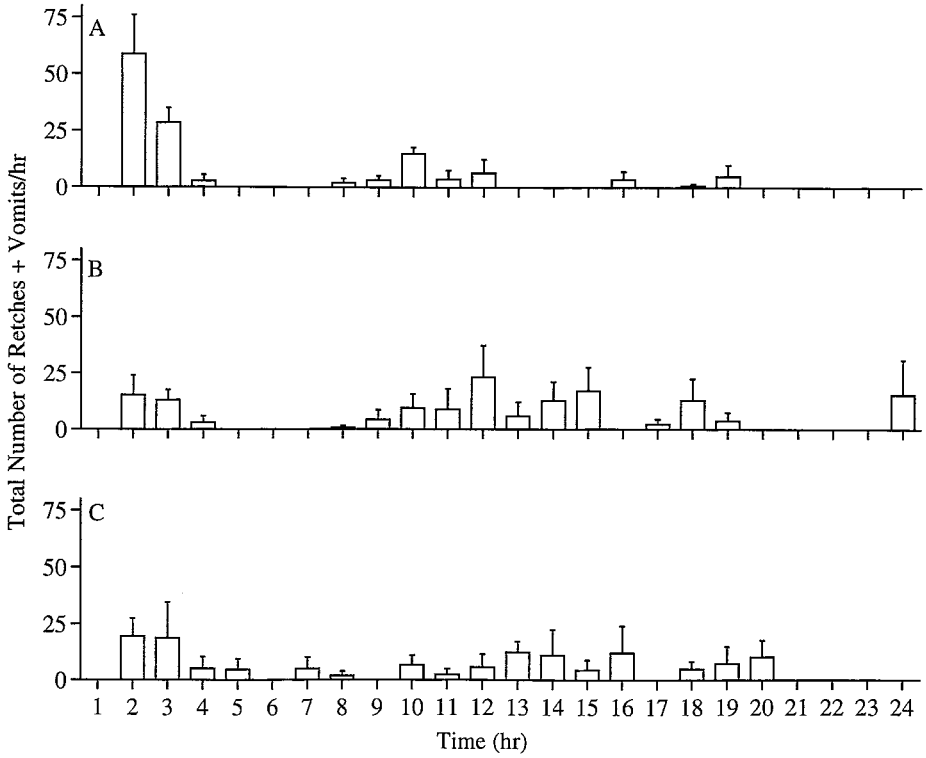

Fig. 1. The effect of Tween 80 (A); PCPA at 100 mg/kg, i.p. (B); or PCPA at 200 mg/kg, i.p. (C) on the profile of retching+vomiting induced by cisplatin (10 mg/kg, i.p.) in the ferret. PCPA (100–200 mg/kg) or 1% Tween 80 (Tween, 2 ml/kg) was administered intraperitoneally once per day for four days prior to the administration of cisplatin (10 mg/kg, i.p.). Cisplatin was administered 4 hr after the last dose of PCPA or Tween. Results represent the mean±S.E.M. of the total numbers of retches+vomits occurring in 1-hr time intervals post cisplatin injection at 0 hr (n=4).

RESULTS

The animals that were injected with 1% Tween 80 (the vehicle for PCPA) for 4 days followed by cisplatin exhibited 114.5±25.9 retches+vomits; 68.2±6.6% of the response occurred within the first 8 hr (Fig. 1). Animals that were treated with 1% Tween 80 for 4 days failed to develop a retching and/or vomiting response when subsequently injected with saline (cisplatin vehicle). Pretreatments with PCPA at 100 and 200 mg/kg, i.p. significantly reduced the percentage of cisplatin-induced retching and vomiting that occurred during the first 8 hr period by 70% and 55%, respectively ($P<0.05$). In addition, the latency of onset of cisplatin to induce emesis in control

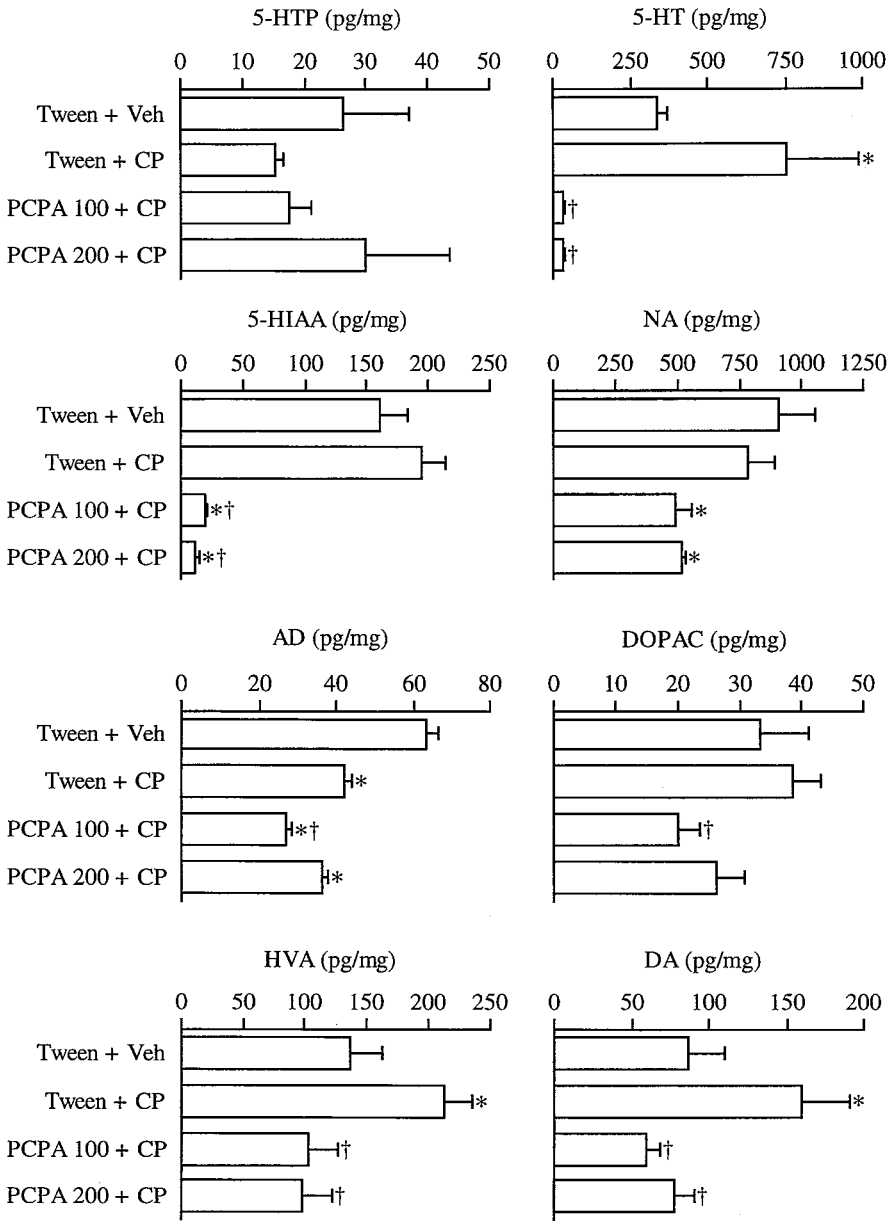

Fig. 2. DVC catecholamine and indoleamine levels at 24 hr post cisplatin (10 mg/kg, i.p.) or cisplatin (10 mg/kg, i.p.) and PCPA (100 or 200 mg/kg, i.p.) co-administration. PCPA (100–200 mg/kg) or 1% Tween 80 (Tween, 2 ml/kg) was administered intraperitoneally once per day for four days prior to the administration of cisplatin (CP; 10 mg/kg, i.p.) or saline (Veh; 5 ml/kg, i.p.). CP or Veh was administered 4 hr after the last dose of PCPA or Tween, and samples were taken 24 hr after the injection of CP or Veh. Significant differences in the levels of monoamines between Tween+Veh-treated animals and drug-treated animals are indicated as $^{*}P<0.05$; significant differences between Tween+CP- and drug-treated animals are indicated as $^{\dagger}P<0.05$ (one way ANOVA followed by a Fisher's PLSD test). Data represent the mean±S.E.M. of 4 determinations.

animals was also increased from 1.4±0.1 hr to 2.2±0.3 ($P<0.05$) and 1.8±0.1 hr ($P>0.05$), respectively. However, the cisplatin-induced retching and vomiting in the animals treated with 100 and 200 mg/kg of PCPA were potentiated during the subsequent 8- to 24-hr period by 207% and 92%, respectively ($P<0.05$, see Fig. 1).

Analysis of tissue dissected from the DVC revealed a significant increase ($P<0.05$) in the levels of 5-HT (125.6%, $P<0.05$), DA (83.3%, $P<0.05$) and HVA (56.7%, $P<0.05$) following treatment with cisplatin alone (Fig. 2). Conversely, the levels of 5-HTP and AD were reduced by approximately 40% ($P>0.05$) and 33%,

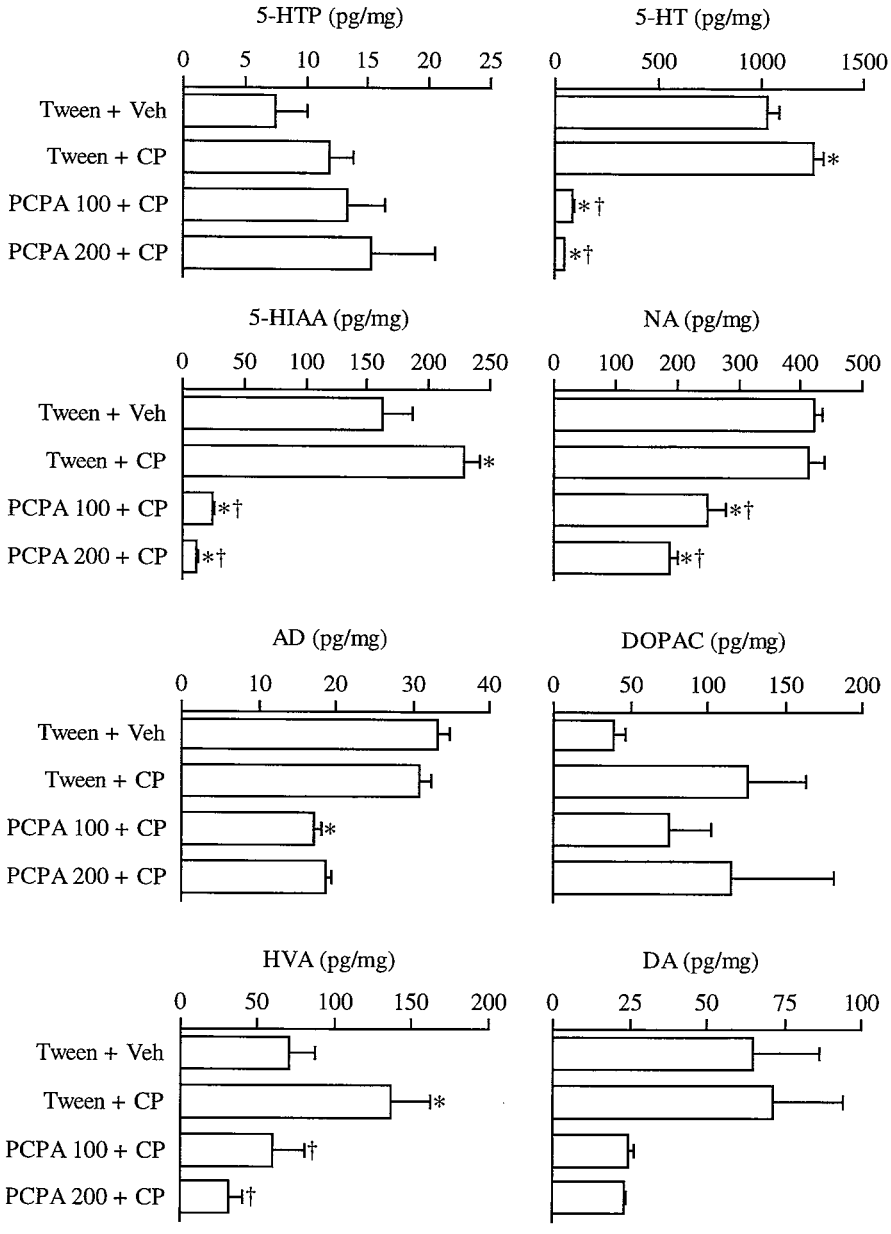

Fig. 3. Brainstem section (minus DVC) catecholamine and indoleamine levels at 24 hr post cisplatin (10 mg/kg, i.p.) or cisplatin (10 mg/kg, i.p.) and PCPA (100 or 200 mg/kg, i.p.) co-administration. PCPA (100–200 mg/kg) or 1% Tween 80 (Tween, 2 ml/kg) was administered intraperitoneally once per day for four days prior to the administration of cisplatin (CP; 10 mg/kg, i.p.) or saline (Veh; 5 ml/kg, i.p.). CP or Veh were administered 4 hr after the last dose of PCPA or Tween, and samples were taken 24 hr after the injection of CP or Veh. Significant differences in the levels of monoamines between Tween+Veh-treated animals and drug-treated animals are indicated as $^{*}P<0.05$; significant differences between Tween+CP- and drug-treated animals are indicated as $^{\dagger}P<0.05$ (one way ANOVA followed by a Fisher's PLSD test). Data represent the mean±S.E.M. of 4 determinations.

respectively ($P<0.05$). Changes were also evident in the remainder of the brainstem section, with the levels of 5-HT, 5-HIAA and HVA being significantly elevated by 22.4%, 41.0% and 92.0%, respectively ($P<0.05$); no other significant changes were recorded (Fig. 3). A combination treatment of PCPA (100 or 200 mg/kg) with cisplatin prevented the observed increases in 5-HT and HVA in both the DVC and brainstem section (the levels of 5-HT and 5-HIAA were at least 90% below that of the control vehicle-treated animals). Furthermore, the combined treatment of PCPA (100 or 200 mg/kg) with cisplatin also had the effect to reduce AD and HVA levels in the DVC and brainstem by approximately 40–80% ($P<0.05$) below the values recorded for the control (1% Tween 80)- and saline (cisplatin vehicle)-treated animals.

Analysis of the tissue dissected from the gastrointestinal tract revealed no significant changes ($P>0.05$) in the levels of 5-HTP, 5-HT or 5-HIAA in either the ileal muscle or mucosa following treatment with cisplatin (Table 1). However, treatment with PCPA (100–200 mg/kg) in combination with cisplatin resulted in 60–97% reductions in the levels of 5-HT and 5-HIAA in both tissues when compared to the control vehicle-treated animals; some of the reductions reached statistical significance ($P<0.05$, Table 1). Cisplatin treatment also increased significantly the levels of 5-HIAA in the urine by 174% ($P<0.05$) compared to control (1% tween 80)- and saline (cisplatin vehicle)-treated animals and the increase was similarly prevented by combination of cisplatin with PCPA (100 and 200 mg/kg, $P<0.05$).

DISCUSSION

The dose of cisplatin (10 mg/kg) used in the present studies in the ferret has been widely used to induce an emetic response to mimic the severe vomiting response that occurs in man (10). Many investigators have used the ferret model to investigate the anti-emetic effectiveness of various drugs, and additional studies have looked at the anatomical pathways that are likely to be involved (17). The ferret model has most recently been used to evaluate the anti-emetic potential of the tachykinin NK_1-receptor antagonists to suppress emesis (18, 19). However, whilst the use of the model has clear benefits, it is possibly inadequate to assess the anti-emetic potential of drugs to prevent chemotherapy-induced delayed emesis (14). The limitations of the ferret model motivated the use of extended observation periods during the present studies.

We have used PCPA as a pharmacological tool to characterize the emetic response that occurs in the ferret during the 24 hr period following cisplatin at 10 mg/kg. PCPA is known to inhibit 5-HT synthesis (20, 21) and to reduce acute cisplatin-induced emesis in humans (6) and the 4-hr emetic response induced by cisplatin (10 mg/kg) in animals (16, 22, 23). In the clinical study, PCPA was administered in doses of 6 g daily (approximately 85 mg/kg/day) for up to 3 days prior to the start of chemotherapy and was effective to reduce urinary 5-HIAA levels by 60–70% (6). In the present studies, the regimens of PCPA used (up to 200 mg/kg/day for 3 days) produced comparable reductions of urinary 5-HIAA levels (an 85% reduction was recorded) to indicate a similarity in the reduction of 5-HT function.

The doses of PCPA used in the clinical studies were reported to produce tiredness, lightheadedness, dizziness, loss of balance, headache and nausea prior to the start of the chemotherapy. In the present studies, PCPA (200 mg/kg) induced transient emesis in three out of four animals during the pretreatment schedule that comprised three to four episodes of retching and vomiting (data not shown), and all the animals exhibited mild sedation. The doses of PCPA used in the present study are reported to

Table 1. The effect of cisplatin or cisplatin and PCPA (100 or 200 mg/kg, i.p.) co-administration on monoamine levels in the ileum and urine of the ferret

Treatment	Ileal muscle			Ileal mucosa			Urine
	5-HTP	5-HT	5-HIAA	5-HTP	5-HT	5-HIAA	5-HIAA
Tween + Veh	13.4±4.4	84.9±33.7	198.9±44.3	20.0±3.6	122.1±62.1	227.8±61.3	1.0±0.1
Tween + CP	11.6±1.7	71.6±45.3	205.0±55.4	23.5±7.1	37.8±15.5	171.6±37.1	2.7±0.2*
PCPA 100 + CP	9.3±1.1	5.6±1.4	41.4±11.6*†	13.1±3.2	13.9±3.4*	80.6±31.5*†	1.0±0.5†
PCPA 200 + CP	8.1±3.1	2.5±0.5	27.5±8.5*†	23.3±10.0	6.4±3.1*	12.6±3.6*†	0.1±0.0†

Tissue 5-HTP, 5-HT and 5-HIAA levels are in pg/wet weight; urinary 5-HIAA levels are expressed as μg/mg creatinine. PCPA (100–200 mg/kg) or 1% Tween 80 (Tween, 2 ml/kg) was administered intraperitoneally once per day for four days prior to the administration of cisplatin (CP; 10 mg/kg, i.p.) or saline (Veh; 5 ml/kg, i.p.). CP or Veh was administered 4 hr after the last dose of PCPA or Tween and samples were taken 24 hr after the injection of CP or Veh. Significant differences in the levels of monoamines between Tween+Veh-treated animals and drug-treated animals are indicated as *$P<0.05$; significant differences between Tween+CP- and drug-treated animals are indicated as †$P<0.05$ (one way ANOVA followed by a Fisher's PLSD test). Data represent the mean±S.E.M. of 4 determinations.

produce 70–90% reductions in central 5-HT levels (16). Whilst higher doses of PCPA (400 mg/kg) can produce a complete reduction of central 5-HT levels it is associated with marked sedation (16). We considered it inappropriate to use the higher doses since they would loose specificity of action against the 5-HT systems and would not be directly comparable with the clinical use of the compound.

A determination of the indoleamine and catecholamine content of various tissues at the end of the 24-hr observation period revealed that cisplatin was capable of inducing changes in neurotransmitter and metabolite levels in the brain, but was less effective to cause detectable changes in the ileum. Indeed, cisplatin did not induce changes in the levels of 5-HT, 5-HIAA or 5-HTP in either the mucosa or muscle of the ileum, but PCPA treatment and cisplatin was effective to reduce the levels of 5-HIAA. The samples were obviously removed from the animals at a time when emesis had essentially subsided, and the alterations in tissue levels may not ideally reflect changes that can be associated with triggering or contributing to the observed emetic response. Moreover, in our studies we can not rule out the possibility that the reductions were caused solely by PCPA treatment. However, changes in 5-HT levels in the ileum have been observed during the early 6-hr phase of cisplatin-induced emesis in animals (24, 25), and this may suggest that the 5-HT levels in the ileum can be rapidly replenished and appear unchanged at the time of sampling in the present studies. Such a function seems likely since cisplatin is reported to increase the activity of tryptophan hydroxylase, the rate limiting enzyme for the synthesis of 5-HT and the activity of monoamine oxidase in the ileum (26).

Whilst we were unable to detect changes in the levels of 5-HT, 5-HIAA and 5-HTP in the ileum, we were able to detect a 174% increase in 5-HIAA in the urine at 24 hr after cisplatin administration. The changes may reflect a gross release of 5-HT in the body, which does not necessarily relate to release from a single site such as the gastrointestinal tract. Since the ferrets urinary frequency was not monitored, it is not possible to associate the elevation of urinary 5-HIAA levels with a specific phase of the emetic response. In man, an approximate 300% increase in urinary levels has been reported to occur 4–6 hr after cisplatin administration and correlated well with emesis (27). However, 24 hr after cisplatin administration (a time at which the emesis had subsided), the urinary 5-HIAA levels had returned to normal (27). It is interesting, however, that cisplatin-induced emesis in the dog, is not associated with increases in urinary levels of 5-HIAA (28). The origin of 5-HIAA in the clinical study was hypothesized to arise from the metabolism of released 5-HT from the enterochromaffin cells of the gastrointestinal tract; the hypothesis was strengthened by observations of increased plasma chromogranin A, a marker of enterochromaffin function (29).

The present studies also determined that cisplatin induced marked increases in the levels of 5-HT in the brainstem, particularly in the DVC and that such increases were antagonized by PCPA. In an earlier study, when tissue samples were removed at approximately 2 hr post injection of cisplatin, the levels of 5-HT in the area postrema were unchanged (16). Again, the present studies used a different sample interval and the tissue removed was the collective mass of the area postrema, nucleus tractus solitarius and dorsal motor nucleus of the vagus nerve (i.e., the DVC). Certainly, central 5-HT and 5-HT_3 receptors can be considered to be important in the mediation of the early emesis induced by cisplatin (30), but it is unclear why elevated levels of 5-HT in the DVC are not associated with intense emesis at the time of sampling. A possible explanation could be that 5-HT would normally act with other substances (either additively or synergistically) in the brainstem to cause emesis and that the other substances are no longer elevated. Certainly, 5-HT- or selective 5-HT_3-receptor agonists when injected alone centrally are not potent to induce emesis to tentatively support the hypothesis (30).

Cisplatin also had actions to increase the levels of DA and HVA in the DVC, but reduced the levels of AD. It may be pertinent that no significant changes in the levels of DA or NA were recorded in the remaining brainstem section and that elevations of DA and HVA in the area postrema have previously been reported at the time of cisplatin-induced emesis. However DA, AD, NA and HVA levels were all reduced (but not abolished) by combination of PCPA with cisplatin, suggesting that the neurotransmitters are not likely to play a major role in the PCPA-resistant phase of emesis.

Certainly, whilst PCPA treatment was effective to antagonize the early emetic response, it was not completely effective and apparently potentiated the intensity of emesis in the subsequent 8- to 24-hr period. This may indicate that reducing endogenous levels of 5-HT in the ferret may remove an endogenous inhibitory tone that will normally suppress emesis in the later phase of the 24-hr model. This may relate to an agonist action at 5-HT_{1A} receptors, which is known to reduce emesis in the cat (31) and *Suncus murinus* (32), although such effects are less consistent in the ferret (33). However, such conclusions may be incautious since protecting the animals against the initial retching and vomiting response may reduce fatigue to enable the animals to develop a later and more intense response (14). Certainly, the present studies suggest that a major component of the residual emetic response is unlikely to be mediated via 5-HT.

There is evidence to suggest that there is plasticity in the organization of the emetic reflex. For example, studies have demonstrated a reorganization of the emetic response to radiation following surgical lesion of the abdominal vagus nerves to produce delayed emesis (34). Using PCPA, it is possible that we have chemically lesioned a 5-HT pathway involved in emesis control to produce a reorganization of the emetic reflex and have also inadvertently revealed a 5-HT-independent delayed emetic response. Further studies would need to be performed to ascertain the relevance of the 5-HT-independent mechanism of action of the residual or delayed emesis to the side-effects of chemotherapy in man.

In conclusion, PCPA was effective to antagonize cisplatin-induced increases in the levels of 5-HT in the brain and also affected 5-HT and 5-HIAA levels in the ileum when combined with cisplatin. The changes in 5-HT levels were also reflected as changes in urinary levels of 5-HIAA to suggest a role of altered 5-HT function that may occur during cisplatin-induced emesis. Certainly, the use of PCPA has highlighted an important difference between the mechanism of cisplatin-induced emesis in the ferret and man. PCPA was highly effective to reduce the acute emesis (measured over a 24-hr period) in humans (6), but in the present studies in the ferret, it was only active to antagonize emesis for approximately 8 hr. Importantly, the PCPA data indicate that the ferret 10 mg/kg-induced emesis model is unlikely to be ideally representative of the mechanisms that may be active during the acute phase of emesis in man. Our other studies with dexamethasone have also shown that the model is also not likely to reflect mechanisms occurring during delayed emesis (14). When the results with PCPA and dexamethasone are taken together, it is clear that the ferret model used in the present studies is inappropriate for studying the mechanisms that may be involved in acute and delayed emesis. Certainly, the mechanisms underlying the residual PCPA resistant response in the ferret is unknown and may be due to plasticity of the emetic reflex; the relevance of the 24-hr model and the use of cisplatin at a dose of 10 mg/kg must therefore remain questionable. Alternative animal models, using lower doses of cisplatin, have recently been developed using the piglet and ferret (12, 35) to more accurately mimic the acute and delayed emesis seen in man. The ferret model seems particularly useful based on the sensitivity of the acute and delayed emesis to ondansetron and dexamethasone (36). An assessment of the effect of PCPA on the emesis in the new models should prove useful to test the validity of the models.

REFERENCES

1 Kris MG, Gralla RJ, Clark RA, Tyson LB, O'Connell JP, Wertheim MS and Kelsen DP: Incidence, course, and severity of delayed nausea and vomiting following the administration of high-dose cisplatin. J Clin Oncol **3**, 1379–1384 (1985)

2 Martin M: The severity and pattern of emesis following different cytotoxic agents. Oncology **53**, Suppl **1**, 26–31 (1996)

3 Cubeddu LX, Hoffmann IS, Fuenmayor NT and Malave JJ: Changes in serotonin metabolism in cancer patients: its relationship to nausea and vomiting induced by chemotherapeutic drugs. Br J Cancer **66**, 198–203 (1992)

4 Cubeddu LX and Hoffmann IS: Participation of serotonin on early and delayed emesis induced by initial and subsequent cycles of cisplatinum-based chemotherapy: effects of antiemetics. J Clin Pharmacol **33**, 691–697 (1993)

5 Butcher ME: Global experience with ondansetron and future potential. Oncology **50**, 191–197 (1993)

6 Alfieri AB and Cubeddu LX: Treatment with *para*-chlorophenylalanine antagonises the emetic response and the serotonin-releasing actions of cisplatin in cancer patients. Br J Cancer **71**, 629–632 (1995)

7 Gebbia V, Cannata G, Testa A, Curto G, Valenza R, Cipolla C, Latteri MA and Gebbia N: Ondansetron versus granisetron in the prevention of chemotherapy-induced nausea and vomiting. Results of a prospective randomized trial. Cancer **74**, 1945–1952 (1994)

8 Chevallier B, Marty M and Paillarse JM: Methylprednisolone enhances the efficacy of ondansetron in acute and delayed cisplatin-induced emesis over at least three cycles. Ondansetron Study Group. Br J Cancer **70**, 1171–1175 (1994)

9 Roila F, Tonato M and Del Favero A: Prevention of chemotherapy-induced emesis: the state of the art. Dig Dis **11**, 343–353 (1993)

10 Naylor RJ and Rudd JA: Mechanisms of chemotherapy/radiotherapy-induced emesis in animal models. Oncology **53**, Suppl **1**, 8–17 (1996)

11 Rudd JA, Bunce KT and Naylor RJ: The interaction of dexamethasone with ondansetron on drug-induced emesis in the ferret. Neuropharmacology **35**, 91–97 (1996)

12 Rudd JA, Jordan CC and Naylor RJ: Profiles of emetic action of cisplatin in the ferret: a potential model of acute and delayed emesis. Eur J Pharmacol **262**, R1–R2 (1994)

13 Rudd JA and Naylor RJ: Effects of 5-HT_3 receptor antagonists on models of acute and delayed emesis induced by cisplatin in the ferret. Neuropharmacology **33**, 1607–1608 (1994)

14 Rudd JA and Naylor RJ: The actions of ondansetron and dexamethasone to antagonise cisplatin-induced emesis in the ferret. Eur J Pharmacol **322**, 79–82 (1997)

15 Rudd JA, Jordan CC and Naylor RJ: The action of the NK_1 tachykinin receptor antagonist, CP 99,994, in antagonizing the acute and delayed emesis induced by cisplatin in the ferret. Br J Pharmacol **119**, 931–936 (1996)

16 Barnes JM, Barnes NM, Costall B, Naylor RJ and Tattersall FD: Reserpine, *para*-chlorophenylalanine and fenfluramine antagonise cisplatin-induced emesis in the ferret. Neuropharmacology **27**, 783–790 (1988)

17 Andrews PLR, Rapeport WG and Sanger GJ: Neuropharmacology of emesis induced by anti-cancer therapy. Trends Pharmacol Sci **9**, 334–341 (1988)

18 Gardner CJ, Armour DR, Beattie DT, Gale JD, Hawcock AB, Kilpatrick GJ, Twissell DJ and Ward P: GR205171: a novel antagonist with high affinity for the tachykinin NK_1 receptor, and potent broad-spectrum anti-emetic activity. Regul Pept **65**, 45–53 (1996)

19 Tattersall FD, Rycroft W, Francis B, Pearce D, Merchant K, MacLeod AM, Ladduwahetty T, Keown L, Swain C, Baker R, Cascieri M, Ber E, Metzger J, MacIntyre DE, Hill RG and Hargreaves RJ: Tachykinin NK_1 receptor antagonists act centrally to inhibit emesis induced by the chemotherapeutic agent cisplatin in ferrets. Neuropharmacology **35**, 1121–1129 (1996)

20 Koe K and Weissman A: *p*-Chlorophenylalanine: a specific depletor of brain serotonin. J Pharmacol Exp Ther **154**, 499–516 (1966)

21 Weber LJ: *p*-Chlorophenylalanine-induced depletion of gastrointestinal 5-hydroxytryptamine. Biochem Pharmacol **19**, 2169–2172 (1970)

22 Fukui H, Yamamoto M and Sato S: Vagal afferent fibres and peripheral 5-HT_3 receptors mediate cisplatin-induced emesis in dogs. Jpn J Pharmacol **59**, 221–226 (1992)

23 Preziosi P, D'Amato M, del Carmine R, Martire M, Pozzoli G and Navarra P: The effect of 5-HT_3 receptor antagonists on cisplatin-induced emesis in the pigeon. Eur J Pharmacol **221**, 343–350 (1992)

24 Fukui H, Yamamoto M, Ando T, Sasaki S and Sato S: Increase in serotonin levels in the dog ileum and blood by cisplatin as measured by microdialysis. Neuropharmacology **32**, 959–968 (1993)

25 Stables R, Andrews PLR, Bailey HE, Costall B, Gunning SJ, Hawthorn J, Naylor RJ and Tyers MB: Antiemetic properties of the 5-HT_3-receptor antagonist, GR38032F. Cancer Treat Rev **14**, 333–336 (1987)

26 Minami M, Endo T, Nemoto M, Hamaue N, Hirafuji M, Monama Y, Yajima T, Yoshioka M and Saito H: How do toxic emetic stimuli cause 5-HT release in the gut and brain? *In* Serotonin and the Scientific Basis of Anti-emetic Therapy, Edited by Reynolds DJM, Andrews PLR and Davis CJ, pp 68–76, Oxford Clinical Communications, Oxford (1995)

27 Cubeddu LX, Hoffmann IS, Fuenmayor NT and Finn AL: Efficacy of ondansetron (GR 38032F) and the role of serotonin in cisplatin-induced nausea and vomiting. N Engl J Med **322**, 810–816 (1990)

28 Nakajima Y, Yamamoto K, Yamada Y, Sawada Y and Iga T: Effect of cisplatin on the disposition of endogenous serotonin and its main metabolite, 5-hydroxyindole-3-acetic acid, in rats and dogs. Biol Pharm Bull **19**, 318–322 (1996)

29 Cubeddu LX, O'Connor DT, Hoffmann I and Parmer RJ: Plasma chromogranin A marks emesis and serotonin release associated with dacarbazine and nitrogen mustard but not with cyclophosphamide-based chemotherapies. Br J Cancer **72**, 1033–1038 (1995)

30 Higgins GA, Kilpatrick GJ, Bunce KT, Jones BJ and Tyers MB: 5-HT_3 receptor antagonists injected into the area postrema inhibit cisplatin-induced emesis in the ferret. Br J Pharmacol **97**, 247–255 (1989)

31 Lucot JB and Crampton GH: Buspirone blocks cisplatin-induced emesis in cats. J Clin Pharmacol **27**, 817–818 (1987)

32 Okada F, Torii Y, Saito H and Matsuki N: Antiemetic effects of serotonergic 5-HT_{1A}-receptor agonists in *Suncus murinus*. Jpn J Pharmacol **64**, 109–114 (1994)

33 Rudd JA and Naylor RJ: Modulation of emesis by 5-HT_{1A} receptors. Pathophysiology **1**, 267–268 (1994)

34 Andrews PLR, Davis CJ, Bingham S, Davidson HIM, Hawthorn J and Maskell L: The abdominal visceral innervation and the emetic reflex: pathways, pharmacology, and plasticity. Can J Physiol Pharmacol **68**, 325–345 (1990)

35 Milano S, Blower P, Romain D and Grelot L: The piglet as a suitable animal model for studying the delayed phase of cisplatin-induced emesis. J Pharmacol Exp Ther **274**, 951–961 (1995)

36 Rudd JA and Naylor RJ: An interaction of ondansetron and dexamethasone antagonizing cisplatin-induced acute and delayed emesis in the ferret. Br J Pharmacol **118**, 209–214 (1996)