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Plasma Levels of Peptide YY Correlate with Cisplatin-induced Emesis in Dogs

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Abstract—The effect of cisplatin on plasma peptide YY (PYY) and 5-hydroxytryptamine (5-HT) concentrations was determined in conscious dogs (n = 6 per group) pretreated with either saline, or the 5-HT_3-receptor antagonists ondansetron or granisetron. Cisplatin (3·0 mg kg^{-1}, i.v.) caused emesis (18·8 ± 2·9 episodes; 75–284 min) and significantly increased the mean area under the curve (AUC) over a 6-h period of plasma PYY concentrations (7·4 ± 1·8 to 11·5 ± 3·7 ng) in all saline-pretreated dogs, whereas the mean AUC of plasma 5-HT concentrations did not significantly increase (34·7 ± 7·4 vs 35·6 ± 12·3 pM h). The concentrations of PYY correlated closely with the incidence of emesis (r = 0·99). In animals pretreated (36 min) with ondansetron (0·316 mg kg^{-1}, i.v.) or granisetron (0·316 mg kg^{-1}, i.v.), the number of cisplatin-induced emetic episodes was significantly ($P < 0·005$) decreased compared with control. In animals receiving cisplatin and pretreated with ondansetron, PYY concentrations were not significantly altered, whereas the mean AUC of plasma concentrations of 5-HT over 6 h increased (35·6 ± 12·3 to 82·3 ± 34·6 pM h; $P < 0·05$). In animals receiving cisplatin and pretreated with granisetron, plasma concentrations of 5-HT were not significantly altered, whereas the mean AUC of plasma PYY concentrations were significantly reduced compared with control (6·2 ± 1·7 vs 11·5 ± 3·7 ng h). Furthermore, in animals receiving ondansetron without cisplatin treatment, there was no change in the mean AUC of 5-HT or PYY concentrations, whereas the mean AUC of plasma 5-HT concentrations increased significantly (34·7 ± 7·4 to 68·6 ± 37·2 pM h; $P < 0·05$) in animals treated with granisetron alone. These studies indicate that plasma concentrations of PYY, and not 5-HT, correlate with cisplatin-induced emesis in dogs. Peptide YY may be an important mediator in cancer chemotherapy-induced emesis and other types of emesis.

The mechanism by which the anticancer drug cisplatin evokes nausea and vomiting remains uncertain, but recent studies have suggested the possible role of peptide YY (PYY) and 5-hydroxytryptamine (5-HT) in mediating this response (Harding & McDonald 1989; Cubeddu et al 1990). PYY, a 36-amino acid peptide, recently isolated (Tatemoto 1982), has been shown to cause emesis in conscious dogs after intravenous (i.v.) administration (Harding & McDonald 1989). Studies in our laboratory have shown that PYY (10·0–31·6 μg kg^{-1}, i.v.) induced emesis in ferrets in a dose-related manner (unpublished results). This emetic response was blocked by tropisetron (0·1 mg kg^{-1}, i.v.), but not by sulpiride (0·1 mg kg^{-1}, i.v.).

Considerable evidence exists linking PYY and 5-HT as mediators in the emetic response to cancer chemotherapeutic agents. Investigations of the emetic pathway by McCarthy & Borison (1984) showed that the emetic response to the anticancer drug cisplatin is mediated by a pathway involving the area postrema. Harding & McDonald (1989) demonstrated that the emetic response to PYY could be abolished by ablation of selective areas of the area postrema. Additionally, the concept of cisplatin-induced emesis being mediated through 5-HT_3 receptors on afferent nerves has been proposed (Andrews et al 1990; Cubeddu et al 1990).

Evidence from studies which have examined plasma concentrations of cisplatin, following the administration of this agent, argues against cisplatin acting directly on the area postrema. For example, Litterst et al (1976), showed that plasma concentrations of cisplatin rise immediately upon administration and are rapidly cleared. Significant tissue concentrations are seen within 10 min and remain for up to 12 days after a single dose. In contrast, the emesis induced by cisplatin is delayed for 1–6 h, despite high plasma and tissue concentrations of cisplatin during this period (Litterst et al 1976; Borison & McCarthy 1983). These studies suggest that the vomiting due to cisplatin therapy is mediated via an indirect mechanism and is not due to pharmacokinetics.

In man, delayed vomiting occurs sporadically up to 120 h after dosing, although the most severe incidence of vomiting is seen during the initial 24 h after administration of cisplatin (Kris et al 1985). Additionally, plasma and tissue concentrations of cisplatin show no correlation with the latency or severity of this emetic response. This evidence suggests that there may be a secondary factor mediating the emesis caused by cisplatin. Such a factor could elicit emesis by acting either directly on the area postrema, or by activating visceral afferent nerves.

We hypothesize that PYY, or 5-HT, or both are possible secondary mediators of cisplatin-induced emesis. The aim of the present study was to determine whether plasma concentrations of PYY or 5-HT are altered following cisplatin treatment in dogs, and if concentrations of these substances correlate with the emetic response.

Materials and Methods

Compounds

Ondansetron, granisetron (Institute of Organic Chemistry, Syntex Research, Palo Alto, CA, USA) and aprotonin (Calbiochem, La Jolla, CA, USA) were made soluble in 0·9% NaCl (saline). Cisplatin (*cis*-platinum(II)diammine dichloride, Sigma Chemical Co., St Louis, MO) was dissolved in 70°C distilled water.

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Experimental procedures

Animal experiments. The study was approved by the Institutional Care and Use Committee of Syntex Research. Adult, female beagle dogs, 7·0–14·0 kg, were used. Food and water were freely available. Each dog was randomly assigned to one of six treatment groups (n = 6/group).

On the day of the study, baseline blood samples were obtained from all dogs, 72 and 36 min (t = −72 and t = −36) before dosing with test compound or vehicle. Immediately following the 36-min (t = −36) baseline blood sample, each animal was dosed intravenously via a cephalic vein with either vehicle (0·5 mL kg^{-1}), ondansetron (0·316 mg kg^{-1}), or granisetron (0·316 mg kg^{-1}) according to its assigned treatment group. Thirty-six minutes later (t = 0), another blood sample was obtained from each animal. Immediately following this sample, each animal received either vehicle (0·5 mL kg^{-1}), or cisplatin (3 mg kg^{-1}) intravenously according to its assigned treatment group. After recording the time of cisplatin or vehicle injection, each animal was observed for 5 h for time to onset of each retching and emetic episode. An emetic episode was defined as the successful evacuation of stomach contents. Blood samples were obtained from all animals at 18, 36, 72, 108, 144, 180, 216, and 288 min after cisplatin or vehicle administration. After the 5-h observation period, all animals treated with cisplatin were killed by lethal intravenous barbiturate injection.

Blood sample collections. Blood samples (5 mL) were collected via jugular venipuncture to determine plasma 5-HT and plasma PYY concentrations. All samples were collected in chilled 10-mL syringes with 20 gauge hypodermic needles. Samples were immediately transferred to sterile 5-mL Vacutainer tubes (Becton Dickinson, Rutherford, NJ, USA) containing 0·04 mL 15% EDTA and 0·03 mL aprotonin solution (19 700 K units mL^{-1}) and placed on ice. Thirty minutes after collection, all blood tubes were centrifuged at 900 *g* and 4°C for 25 min. The upper two-thirds of the plasma portion was transferred to amber 1·5-mL microtubes and stored at −70°C until analysed.

Analytical methods. Plasma concentrations of PYY were determined by radioimmunoassay (RIA) using an RIA kit (Peninsula Laboratories, Belmont, CA, USA). In brief, initial RIA reactions were set up between known standard concentrations of PYY, unknown samples, and rabbit anti-PYY serum, and incubated overnight. Tubes for total binding and non-specific binding were included. The following day ^{125}I-PYY was added to all reactions and incubated overnight. On the third day, anti-rabbit IgG serum and normal rabbit serum were added to all reactions, incubated at room temperature for 2 h, and a pellet was recovered after centrifugation and aspiration of the supernatant. The pellet from each reaction tube was counted on a gamma counter. The sensitivity of the assay was 9·1 pg/tube. The cross-reactivity with other structurally similar peptides (e.g. neuropeptide Y, human pancreatic polypeptide, vasoactive intestinal peptide) was <0·01%. The range for the inter-assay precision was 1·42 with a coefficient of variation of 7·51%.

Plasma concentrations of 5-HT were determined by enzyme immunoassay (EIA) using an EIA kit (Amac Inc., Westbrook, ME, USA). This assay makes use of the competition of acylated 5-HT and acetylcholinesterase coupled to 5-HT for an antibody fixed to the inner surface of wells of a microtitre plate. Briefly, standard concentrations and unknown samples of 5-HT were acylated. Acylated standard and unknown samples were then incubated with the 5-HT-acetylcholine esterase conjugate in cylindrical wells of a microtitre plate. The microplate wells were then aspirated and washed. Substrate was added to each well and the microplate was incubated to allow for conversion of substrate to product. The reaction was stopped and the absorbance of each well was read at 405–414 nm compared with blank wells. The sensitivity of this assay was 0·5 nM with cross-reactivity for structurally similar compounds (i.e. acylated tryptamine, 5-hydroxyindoleacetic acid, and 5-hydroxytryptophan) less than 0·03%. The range for the inter-assay precision was 8·35 with a coefficient of variation of 5·10%.

Statistical analysis

In each group, the data were expressed as means ± s.d. of six animals. The emetic data were subjected to a Dunnett's *t*-test for comparing all treatment groups with vehicle control. Mean plasma PYY and mean plasma 5-HT values were obtained for each treatment group and time period. An area under the curve (AUC) was calculated using the trapezoid rule to obtain an estimated total amount of plasma PYY and plasma 5-HT for each animal over the 6-h period. Mean AUC for PYY and 5-HT were obtained for each treatment group.

A correlation analysis was performed for data from animals treated with cisplatin (vehicle + cisplatin) to measure if there was an association between the latency and incidence of emetic episodes and plasma PYY concentrations.

Mean plasma concentrations of PYY and 5-HT were analysed separately. A repeated measures analysis of variance was used to obtain overall tests for treatment, time, and treatment by time interaction. A one-way analysis of variance was used to obtain pairwise comparisons of interest at each time point (Kirk 1982). Mean AUC were also analysed using a one-way analysis of variance. If any overall tests were significant, then pairwise comparisons were not adjusted; otherwise pairwise comparisons were adjusted for the number of comparisons made to account for multiple comparisons using Fisher's least significant difference strategy and Dunn's procedure (Kirk 1982).

Results

Table 1 shows the results of intravenous cisplatin on emetic episodes and area under the curve (AUC) of plasma concentrations of PYY and 5-HT. Pretreatment (36 min) with ondansetron (0·316 mg kg^{-1}, i.v.) or granisetron (0·316 mg kg^{-1}, i.v.) significantly decreased ($P < 0·05$) the number of emetic episodes caused by cisplatin, as compared with vehicle. In vehicle-treated animals receiving cisplatin, the AUC of plasma PYY concentrations increased significantly ($P < 0·05$), whereas the AUC of plasma 5-HT was not significantly altered when compared with vehicle treatment alone. In animals receiving ondansetron treatment before

Table 1. Plasma PYY and 5-HT area under the curve (AUC) and emetic episodes following administration of cisplatin. An AUC was calculated to obtain total amounts of PYY and 5-HT for each treatment group over a 6-h period. Emetic episodes were counted for each animal in each group. Mean (±s.d.) of six groups of six animals.

Treatment group	Peptide YY AUC (ng h)	5-HT AUC (pM h)	Emetic episodes
Vehicle + vehicle	7·4 ± 1·8	34·7 ± 7·4	0·0 ± 0·0
Vehicle + cisplatin	11·5 ± 3·7[a]	35·6 ± 12·3	18·8 ± 2·9[c]
Ondansetron + vehicle	6·4 ± 3·7	41·3 ± 13·5	0·0 ± 0·0
Ondansetron + cisplatin	8·4 ± 2·7	82·3 ± 34·6[b]	3·5 ± 5·0[d]
Granisetron + vehicle	5·4 ± 1·9	68·6 ± 37·2[a]	0·0 ± 0·0
Granisetron + cisplatin	6·2 ± 1·7[b]	46·5 ± 28·6	0·8 ± 1·3[d]

[a] $P < 0.05$ vs vehicle + vehicle (analysis of variance). [b] $P < 0.05$ vs vehicle + cisplatin (analysis of variance). [c] $P < 0.005$ vs vehicle + vehicle (Dunnett's t-test). [d] $P < 0.005$ vs vehicle + cisplatin (Dunnett's t-test).

cisplatin, the AUC of plasma concentrations of PYY was not significantly changed, although the AUC of plasma concentrations of 5-HT increased significantly ($P < 0.05$), when compared with cisplatin treatment alone. In animals which were pretreated (36 min) with granisetron before cisplatin, the AUC of plasma concentrations of PYY was significantly decreased ($P < 0.05$), whereas the AUC of plasma concentrations of 5-HT did not significantly change, when compared with the group treated with cisplatin alone.

Plasma PYY concentrations increased after cisplatin administration (Fig. 1), and were correlated with emetic incidence (correlation coefficient r = 0·99, $P < 0.02$, slope = 7·65).

In the absence of cisplatin, administration of vehicle, ondansetron, or granisetron did not significantly alter baseline plasma concentrations of PYY (Fig. 2a). Pretreatment with ondansetron and granisetron, followed by cisplatin, significantly inhibited the increase in plasma PYY concentrations ($P < 0.05$) at the last four time points (144, 180, 216, and 288 min; Fig. 2b).

One point of clarification should be noted. The AUC of plasma PYY concentrations for ondansetron pretreatment followed by cisplatin was not significantly different from cisplatin treatment alone (Table 1). However, as stated above, ondansetron pretreatment followed by cisplatin significantly inhibited ($P < 0.05$) plasma PYY concentrations from rising at the last four time points measured (144, 180, 216, 288 min; Fig. 2b).

Cisplatin treatment did not significantly alter baseline concentrations of plasma 5-HT in vehicle-pretreated animals (Fig. 3a). A significant difference ($P < 0.05$) was seen at 108 min post-dose between the granisetron plus vehicle group vs vehicle control group (Fig. 3a). At 144 min a significant difference ($P < 0.05$) was seen between the ondansetron plus cisplatin group vs the cisplatin treatment group (Fig. 3b).

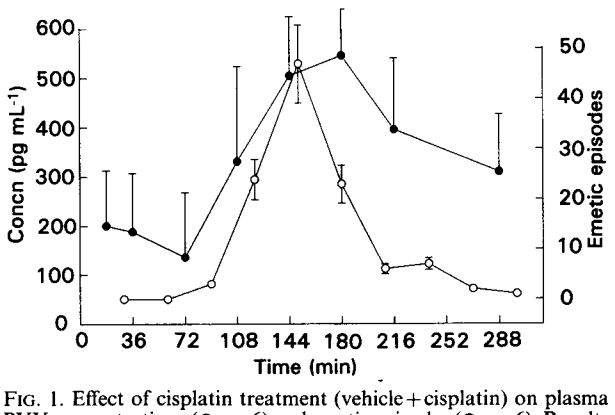

FIG. 1. Effect of cisplatin treatment (vehicle + cisplatin) on plasma PYY concentrations (● n = 6) and emetic episodes (○ n = 6). Results are means ± s.d. Each data point for the emetic episodes represents the emetic episodes that occurred during the preceding 30 min.

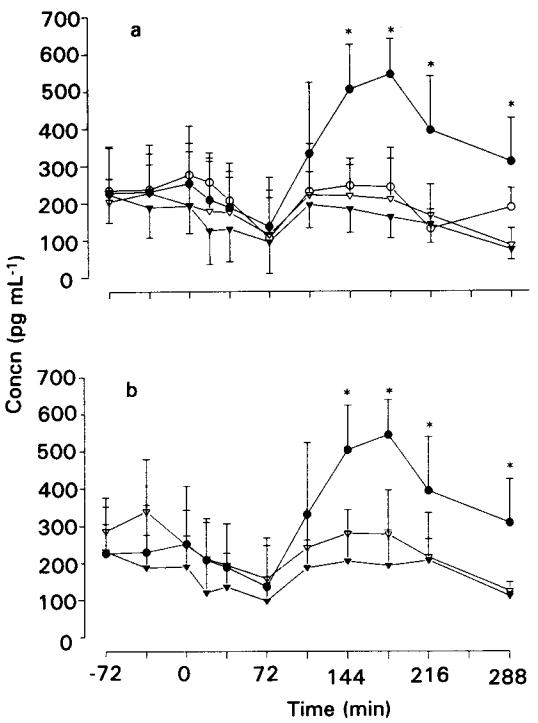

FIG. 2. a. Effect of vehicle (○ vehicle + vehicle), cisplatin (● vehicle + cisplatin), ondansetron (▽ ondansetron + vehicle), and granisetron (▼ granisetron + vehicle) treatment on plasma PYY concentrations in dogs. * $P < 0.05$ compared with vehicle alone. b. Effect of cisplatin treatment alone (● vehicle + cisplatin), and ondansetron (▽ ondansetron + cisplatin) and granisetron (▼ granisetron + cisplatin) before cisplatin, on plasma PYY concentrations in dogs. Results are means ± s.d. of six animals. There were significant differences between vehicle + cisplatin vs ondansetron + vehicle and granisetron + vehicle, at the last four time points, 144, 180, 216, 288 min (* $P < 0.05$ using a one-way analysis of variance at each time point).

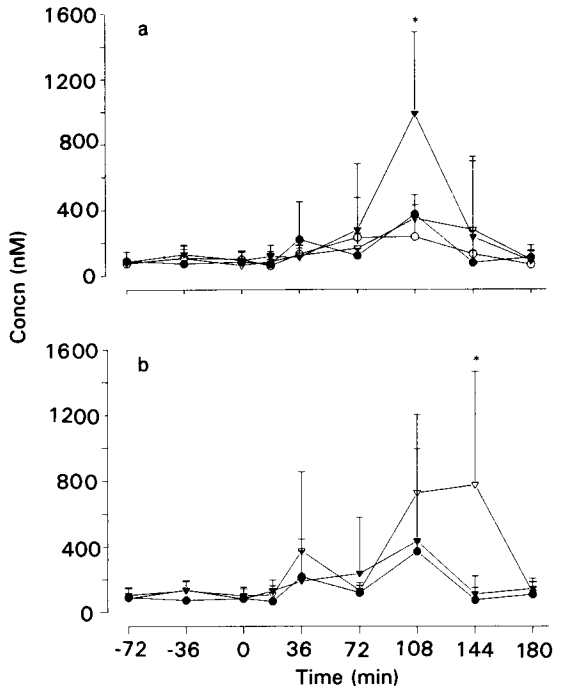

FIG. 3.a. Effect of vehicle (○ vehicle + vehicle), cisplatin (vehicle + cisplatin), ondansetron (▽ ondansetron + vehicle), and granisetron (▼ granisetron + vehicle) treatment on plasma 5-HT concentrations in dogs. Results are means ± s.d. of six animals. There was a significant difference between the granisetron + vehicle group, and the other groups tested, at 108 min (* $P < 0.05$ using a one-way analysis of variance at each time point). b. Effect of cisplatin treatment alone (● vehicle + cisplatin), and ondansetron (▽ ondansetron + cisplatin) and granisetron (▼ granisetron + cisplatin) before cisplatin, on plasma 5-HT concentrations in dogs. Results are means ± s.d. of six animals. There was a significant difference between the ondansetron + cisplatin group, and the other groups tested, at 144 min (* $P < 0.05$ using a one-way analysis of variance at each time point).

Discussion

Anticancer drugs, such as cisplatin, cause emesis which is blocked by 5-HT_3-receptor antagonists, suggesting the involvement of 5-HT_3 receptors. However, the sequential cascade of biological events following cisplatin administration, which involves 5-HT_3-receptor activation, has not been clearly elucidated. As discussed previously, recent studies have implicated possible secondary mediators. We hypothesized that a secondary factor (PYY or 5-HT) may be involved in mediating the emesis due to anticancer drugs. Thus, the purpose of the present study was to determine what effect, if any, cisplatin, ondansetron, and granisetron have on plasma concentrations of PYY and 5-HT in conscious dogs.

The present findings clearly demonstrated that cisplatin chemotherapy significantly increased plasma concentrations of PYY, without significantly altering plasma concentrations of 5-HT. Additionally, plasma PYY concentrations correlated closely with the incidence and severity of emesis. Furthermore, the 5-HT_3-receptor antagonists, ondansetron and granisetron, inhibited plasma PYY concentrations from rising following treatment with cisplatin.

In man and in dogs, greater than 80% of the 5-HT in the body is localized within enterochromaffin cells of the mucosal layer of the gastrointestinal tract (Erspamer & Testini 1959; Resnick & Gray 1961). The main metabolite of 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), has been shown to be a marker of gastrointestinal 5-HT content and turnover (Erspamer & Testini 1959; Bertaccini 1960). In discussing the present results, it is important to note that it is the 5-HT within the wall of the gastrointestinal tract, not the 5-HT found in platelets, that plays an active role in cisplatin-induced emesis (Cubeddu et al 1992). In both man and dogs, PYY is predominately localized in endocrine cells of the ileal, colonic, and rectal mucosa (Adrian et al 1985; Taylor 1985).

Our hypothesis is based on a model that cytotoxic drugs induce emesis by acting indirectly (i.e. by causing the release of secondary mediators). These secondary factors act on either afferent nerves or directly on the area postrema. The present finding that plasma concentrations of PYY increased following cisplatin administration is consistent with our hypothesis and the previous report by Harding & McDonald (1989) that PYY is emetogenic. Plasma concentrations of 5-HT, however, did not increase significantly. This result is in contrast to the findings of Cubeddu et al (1990) which indicated that the main metabolite of 5-HT, 5-HIAA, increases following cisplatin therapy in patients. One explanation for this apparent inconsistency is variation in metabolism of 5-HT between man and dogs. It is possible that 5-HT concentrations did increase following cisplatin treatment, but due to rapid metabolism did not attain concentrations high enough to be detected by our assays. 5-HIAA concentrations could be measured in future studies to clarify this point.

Our findings and those of Cubeddu et al (1990) suggest three hypothetical models for analysis. Cisplatin may act directly on endocrine cells of the gastrointestinal tract, causing damage and subsequent release of PYY which in turn mediates the emesis. The fact that ondansetron and granisetron suppressed PYY concentrations from rising argues against this model. It is unlikely that a receptor antagonist would prevent direct damage by cisplatin. These findings also rule out the possibility that 5-HT_3-receptor activation occurs subsequent to PYY release. 5-HT may be released following cisplatin administration which in turn mediates the release of PYY (via a 5-HT_3 receptor). Although 5-HT concentrations did not rise following cisplatin administration, ondansetron and granisetron inhibited the increase in PYY concentrations following cisplatin. This suggests that 5-HT_3-receptor activation is involved in this pathway, and occurs at a step which is upstream to the site of PYY release.

The third possibility is that PYY may be released simply as a consequence of emesis, and thus blocking the emetic response (i.e. with a 5-HT_3 antagonist) would prevent plasma concentrations of PYY from rising.

The second model can explain the involvement of 5-HT and also the delayed onset and sustained recurrence of emesis for up to 120 h following cisplatin administration (Kris et al 1985). Schworer et al (1991) have shown that cisplatin, at concentrations which cause emesis during anticancer therapy in man, increases the release of 5-HT from the enterochromaffin cells of the small intestine of the guinea-pig. This effect was shown to be mediated by a sequence of events which involved release of acetylcholine and stimulation of 5-HT_3 receptors. We therefore hypothesize that 5-HT is released from enterochromaffin cells stimulated by cisplatin,

and mediates the release of PYY via activation of 5-HT_3 receptors. Plasma concentrations of PYY would then be required to rise to a certain threshold level before emesis can occur. This would be consistent with the delayed onset of emesis (1–3 h in man and in dogs). The incidence of persistent vomiting episodes, which occur sporadically 24–120 h after cisplatin treatment, could be explained by the need for PYY to be synthesized before further release could occur.

The effects of the 5-HT_3-receptor antagonists, ondansetron and granisetron, on plasma 5-HT concentrations were less clear than the effects on plasma PYY concentrations. Granisetron alone, and ondansetron in combination with cisplatin, significantly increased the AUC of 5-HT concentrations, whereas ondansetron treatment alone or granisetron administered with cisplatin did not significantly alter plasma 5-HT concentrations (as measured by AUC). These results should be viewed with caution since the variability at the particular time points where 5-HT concentrations were increased was very high. In addition, the elevations in the AUC causing significance for both of these treatment groups were due to increases at only one time point for both granisetron (108 min) and ondansetron (144 min). Another possible explanation for these inconsistent findings is that several subtypes of 5-HT_3 receptors may exist (Richardson et al 1985). Thus, ondansetron and granisetron could have different actions based on the subtype present in the tissue or cell in question.

Our data provide evidence that links PYY and 5-HT to emesis. Previous studies have demonstrated the importance of abdominal visceral afferent nerves and the area postrema in the emetic pathway (Borison & McCarthy 1983; Andrews et al 1990). Harding & McDonald (1989) have shown that the PYY emetic response could be abolished by ablation of selective regions of the area postrema. Additionally, Smith et al (1988) demonstrated that the potent 5-HT_3 antagonist zacopride, given intra-cerebroventricularly immediately following the onset of emesis produced by intravenous cisplatin, completely blocked the emetic response in cats. It is known that high-affinity binding sites for the ^{125}I-PYY ligand are localized in the area postrema and related nuclei of the dog medulla oblongata (Leslie et al 1988). Specific 5-HT_3 receptors have been shown to exist in high densities in these same regions (Higgins et al 1989). These experiments illustrate the importance of both visceral afferent nerves and the area postrema in the emetic response to cisplatin. They are also consistent with what has been demonstrated in the present study.

In conclusion, our results strongly implicate PYY as an endogenous mediator of cisplatin-induced emesis, and are consistent with the concept that PYY release occurs via a 5-HT_3 receptor-mediated pathway. The results, however, do not rule out the possibility that PYY is released simply as a consequence of emesis.

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