Chemotherapy-induced pica and anorexia
are reduced by common hepatic branch vagotony in the rat

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Running head: Reduced Pica in the rat following CHB vagotony

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ABSTRACT

Anti-cancer agents, such as cisplatin, induce vomiting, nausea, and anorexia. Cisplatin primarily acts on vagal afferents to produce emesis but little is known about how this drug generates nausea and anorexia. Electrophysiology indicates that cisplatin activates vagal afferents of the common hepatic branch (CHB). Rats lack an emetic response, but do ingest kaolin clay (a pica response) when made sick by toxins, and this behavior can be inhibited by anti-emetic drugs. It has been postulated that pica may serve as a proxy for emesis in the rat. The goal of this study was to assess the effect of CHB, ventral gastric (GAS), or celiac branch (CEL) vagotomies on pica and anorexia produced by cisplatin in the rat. The effects of apomorphine, a dopamine receptor agonist, which induces emesis via a central mechanism, were also assessed. Cisplatin-induced pica was suppressed by CHB vagotomy (a 61% reduction), but not by GAS and CEL vagotomy. Suppression of daily food intake and body weight following cisplatin treatment was also blunted by CHB ablation, but not by GAS or CEL vagotomy. No vagotomy condition exhibited altered apomorphine-induced pica. The results indicate that the CHB, which innervates primarily the duodenum, plays an important role in cisplatin-induced malaise. These data suggest that pica has sensory pathways similar to emetic systems since a vagotomy condition inhibited cisplatin-induced pica, but had no effect on apomorphine-induced pica. This investigation contributes to the delineation of the physiology of pica and neural systems involved in malaise in the non-vomiting rat.

Key Words: Nausea, Emesis, Pica, Vagus, Cisplatin
INTRODUCTION

Anti-cancer agents, such as cisplatin, stimulate nausea, vomiting, anorexia and behaviors indicative of malaise in several species (2; 4). Evidence in several animal models has shown that ablation of the vagus nerve can greatly attenuate or even block cisplatin-induced vomiting, indicating that an intact vagus is necessary to observe a maximal emetic response to this drug (1; 12; 13; 55). Cisplatin-induced emesis is caused in large part by promoting the release of serotonin (5-HT) from enteroendocrine cells within the gastrointestinal tract which activates 5-HT3 receptors located on vagal afferent fibers (for review see 50). Electrophysiological data demonstrate that cisplatin activates vagal afferent fibers in both the ferret (10) and the rat (27), an effect that can be blocked with a 5-HT3 receptor antagonist. Of particular interest to the current work are data from our laboratory reporting cisplatin-induced activation of the common hepatic branch (CHB) of the vagus, which contains afferents that innervate the gastrointestinal tract (primarily the duodenum), portal vein, and liver (43; 63). Specifically, cisplatin treatment produced excitation of the CHB through activation of the gastroduodenal sub-branch of the CHB, indicating that gastrointestinal, and not hepatic or portal vein, afferents were responsible for the stimulatory effects of cisplatin on the CHB (27). Note also that the duodenum of the dog appears to be the most sensitive region of the gastrointestinal tract for the stimulation of emesis by copper sulfate (29; 30).

Due to the nature of in vivo electrophysiology experiments, data on CHB activation by cisplatin provide insight into only short-term physiological events and does not extend to possible behavioral effects of cisplatin acting on the CHB. Indeed, very little is known about the neurobiological systems responsible for nausea and anorexia produced by cisplatin and other chemotherapy drugs (for review see 20; 21). It is not possible to directly measure the emetic
potential of such stimuli in the rat, as they lack a vomiting response. They do, however, ingest kaolin clay (a pica response) when made sick by toxins, including cisplatin (e.g., 37; 58; 61). Consumption of clay induced by toxicosis might represent an adaptive response to bind or dilute a toxin in the gastrointestinal tract and reduce its adverse effects. This behavior can be inhibited by anti-emetic drugs (e.g., 36; 52; 53; 58). In light of this relationship, it has been postulated that kaolin consumption may serve as a proxy for emesis in the rat (58). Although valuable, the large majority of reports assessing cisplatin-induced pica do not extend beyond 3 days post-cisplatin injection (e.g., 36; 52; 58), with only scant data extending up to 7 days post-treatment (54; 62). Examining a longer time course of behavior post-cisplatin is essential when considering possible comparisons to acute and delayed phases of emesis associated with chemotherapy-induced nausea and vomiting in humans (50).

Therefore, the first goal (Experiment 1) of the current study was to assess the effects of selective CHB vagotomy on pica and anorexia produced by cisplatin in the rat, including a period of multiple days (10 days post-injection). We hypothesized that CHB vagotomy would attenuate pica, indicative of reduced malaise following cisplatin treatment, due in part to a lack of duodenal vagal input activated by cisplatin treatment. In order to systematically assess the effects of selective vagal branch vagotomy on cisplatin-induced malaise, our second goal (Experiment 2) was to evaluate the effects of removing vagal signaling arising from other sites of the gastrointestinal tract, namely part of the stomach and distal intestine. In this study, rats were either selectively vagotomized by severing the ventral gastric (i.e., removing part of the gastric input) or celiac branches (i.e., removing distal intestinal input) of the vagus. In order to examine whether selective vagotomies affect the animals’ general capacity to ingest kaolin, we also examined (in Experiments 1 and 2) the effects of apomorphine, a dopamine receptor agonist
shown to induce pica (58) and vomiting (31). Evidence indicates that apomorphine induces emesis by acting upon central receptor sites in the area postrema (e.g., 3; 16; 31).

MATERIALS AND METHODS

Subjects and chemicals used for induction of pica

One hundred and four male Sprague Dawley rats (Charles River, IN) were housed individually in mesh-floored stainless-steel hanging cages (8” x 9.5” x 8”) and maintained in a temperature-controlled vivarium, with a 12:12-h light-dark cycle (lights on at 0600). Animals were handled daily for two weeks prior to the onset of experiments. Tap water, powdered rat chow (Purina 5001), and pelleted kaolin clay (Research Diets, Inc) were available ad libitum throughout experiments, except where indicated otherwise. For all experiments, powdered rat chow (~100 g) was available from open-topped glass jar attached to the front panel of the animal cage. Kaolin pellets (~50 g) were placed in a standard small animal food hopper hung from the back side of the animal cage. Hoppers or jars, as well as spilled contents from each, were weighed daily to calculate intakes. Spillages from chow or kaolin were easily separately by hand or brush due to the visual contrast of the two substances (white kaolin clay versus brown chow). Protocols used were approved by the Monell Chemical Senses Center Institutional Animal Care and Use Committee.

The doses of cisplatin (6 mg/ml/kg body weight, ip; Sigma), and apomorphine (10 mg/ml/kg body weight, ip; Sigma) were chosen based on previous studies showing reliable induction of pica and suppression of feeding (36; 58). Cisplatin and apomorphine solutions were
prepared in 0.9% saline, and 0.9% saline was used for control injections (1 ml/kg body weight, ip).

**Vagotomy Surgeries**

Following an overnight fast, animals were anesthetized with sodium pentobarbital (50 mg/kg/ml: ip; Sigma) and vagotomies were performed according to previously established procedures using a thermal cautery (14; 26). Animals were randomly assigned to undergo one of four conditions: 1) CHB vagotomy, 2) accessory celiac branch plus dorsal celiac branch (CEL) vagotomy, 3) ventral gastric branch (GAS) vagotomy, or 4) a sham-operation. Briefly, ~4 cm midline incision was made in the abdomen and the stomach was gently manipulated to clearly reveal the desired vagal branch to be severed (46). In CHB vagotomized animals, the esophagus was moved to the left side of the abdomen to reveal the CHB extending from the esophagus to the liver hilus, and the CHB was then quickly transected. In the CEL condition, the accessory celiac and dorsal celiac branches were severed as they descend dorsally from the ventral and dorsal trunks, respectively. In the GAS group, the ventral vagal trunk was located at the gastroesophageal border and cauterized distal to the convergence of both the CHB and accessory celiac branches of the ventral trunk. Note that vagal branches of the rat are routinely visualized in physiological experiments from our laboratory (23; 24; 27). Sham-operated rats experienced laparotomy and gentle lifting of the stomach, but cauterization was not applied to any tissue. The peritoneum and abdominal muscles were sealed with silk sutures and the abdominal skin incision was closed using wound clips, which were removed 7 days post-surgery. Post-surgical analgesic (Buprenorphine, 0.5 mg/kg) and anti-biotic (Gentamicin, 1 mg/kg) were administered.
twice daily for 2 and 3 days following surgery, respectively. Rats were allowed a minimum of five days to recover before experimentation.

Vagotomy Verification

Visual verification of vagotomy is not reliable because of growth of connective tissue at the surgical site within the peritoneal cavity during recovery. Another method is to inject into the peritoneal cavity a retrograde neuronal tracer, which is transported to cell columns of the dorsal motor nucleus (DMN), corresponding to different branches of the vagus. Verification of CHB vagotomy is not possible using this method because there are very few motor fibers in this branch that would allow retrograde labeling in the DMN (26; 44; 45). However, a follow-up study using four naïve rats not used in the behavioral study was conducted to determine if CHB vagotomy might lead inadvertent destruction of vagal fibers of the ventral trunk. One week after CHB vagotomy these rats were given two 0.5 ml intraperitoneal injections of 0.1% Fluoro-Gold (Fluorochrome, LLC Denver, CO). Similarly, immediately following the last experimental day in Experiment 2, all sham, GAS or CEL vagotomized rats were injected with Fluoro-Gold. Four to five days after injection with the retrograde tracer rats were given a lethal injection of sodium pentobarbital (50 mg) and transcardially perfused using a 0.2M phosphate buffer solution, followed by 4% paraformaldehyde and 2% acrolein in 0.2M phosphate buffer (pH 7.4). Hindbrains were removed, stored in fixative for 4 h, and then cryoprotected in sucrose. Thirty-μm sections were processed for Fluoro-Gold immunoreactivity according to a slightly modified immunohistochemical procedure previously described (14; 22). The modifications include a normal donkey serum blocking step, Fluoro-gold primary (1:40k; Lot # 05023816), and a donkey anti-rabbit secondary (Jackson Immuno). Sections were placed on microscope slides, air dried,
coated with DPX mountant (Fluka, Ronkonkoma, NY), cover slipped, viewed with a microscope (Zeiss Axiostar Plus), and imaged using a digital camera (Scion CFW-1312C). Fluoro-Gold staining was analyzed by ImageJ software (NIH; http://rsb.info.nih.gov/ij/) to assess the density of staining (gray level) in the left and right DMN relative to the adjacent NTS (higher percentage values indicate darker staining relative to the NTS). See Figure 1 for representative DMN images collected from the hindbrain.

CHB animals in the control study showed no significant change in labeling between the left and right DMN (t(3) = 1.4, p = 0.26; see Fig. 1 for representative image), indicating that CHB vagotomy does not lead to destruction of vagal fibers in the ventral trunk of the vagus. In the left DMN, GAS vagotomized rats showed significantly lighter staining relative to sham rats (6.1 ± 1.2 %, GAS, versus 25.9 ± 3.0 %, Sham; t(34)=6.2, p<0.05), consistent with ablation of ventral gastric fibers (36); however, CEL vagotomized rats (25.1 ± 2.7 %) were not significantly different from sham (p>0.05, t-test). In the right DMN, GAS (23.6 ± 2.4 %) and CEL vagotomized rats (24.5 ± 3.6 %) were not significantly different from sham rats (23.9 ± 2.4 %; ps>0.05, t-tests). Note that at least a 40% reduction in staining in the left DMN relative to the right DMN was used as a criterion for determining completeness of GAS vagtomy and all GAS animals met this criterion. It is likely that CEL vagotomies are unable to be adequately visualized due to the small number of immunoreactive cells arising from the CEL branches. Indeed, reports indicate that sham-operated versus celiac vagotomized animals are not greatly different in the levels of DMN staining (see Fig.1 in ref. 44). However, in the current study, CEL vagotomized rats did exhibit behavioral deficits indicative of neuronal damage (see Results).
Experiment 1: Effects of CHB vagotomy

All animals surpassed pre-surgical body weight at 2 days post-surgery. Six days following recovery of pre-surgical body weight, body weight, kaolin, food and water intakes were measured every 24 h at 0900 h. Animals were run in two groups to control for order of testing with cisplatin and apomorphine—group 1 (N = 20) was conducted with cisplatin testing prior to apomorphine testing and group 2 (N = 19) was conducted in reverse order. Prior to tests with cisplatin or apomorphine animals were blocked based on body weight (BW) and randomized for injections. There were 9 to 10 animals in each of the four conditions (sham/saline, sham/drug, CHB/saline, and CHB/drug). There were no significant differences in BW between sham (BW = 412 ± 6.0, n = 20) and CHB vagotomized (BW = 418 ± 5.5, n = 19) animals prior to testing (p>0.05). Three days of baseline measures were collected prior to testing with cisplatin or apomorphine, and ten and three days of data were collected after injection with cisplatin and apomorphine, respectively.

Experiment 2: Effects of GAS and CEL vagotomies

All animals surpassed pre-surgical body weight at 2 days post-surgery. Five days following recovery of pre-surgical body weight, body weight, kaolin, food and water intakes were measured every 24 h at 1000 h. Animals were run in two groups (group 1, N = 29; group 2, N = 32) but, unlike Experiment 1, only one order of drug treatment was used, apomorphine followed by cisplatin, due to the observed long-term deleterious effects of cisplatin in CEL and GAS rats; this prevented complete body weight and food intake recovery in cisplatin-treated rats.
(see Results). Prior to tests with cisplatin or apomorphine animals were blocked based on body weight (BW) and randomized for injections. There were 9 to 10 animals in each of the four conditions (sham/saline, sham/drug, CHB/saline, and CHB/drug). BWs were not significantly different between sham (BW = 399 ± 4.0, n = 19), GAS vagotomized (BW = 394 ± 6.7, n = 20), CEL vagotomized (BW = 398.8 ± 6.6, n = 22) animals prior to testing (p>0.05). Three days of baseline measures were collected prior to testing with cisplatin or apomorphine, and ten and three days of data were collected after injection with cisplatin and apomorphine, respectively.

**Statistical Analysis**

Food, water, and kaolin intake data are expressed as means ± SEM. In initial comparisons, no differences were noted between groups in each experiment and data were pooled for subsequent analyses. BW data are presented as percent change from baseline prior to treatment by the following equation: % BW Change = [Daily Body weight following injection/(Mean of BW 1 and 2 days prior to injection)]*100. Appropriate three-way repeated measures ANOVAs were performed for behavioral measures with Vagotomy (Sham or CHB; Sham, GAS, or CEL), Injection (saline or cisplatin; saline or apomorphine), and Time (experimental days) as main factors. Post-hoc analyses were performed using Fisher’s Least Significance Difference (LSD) tests where applicable. Differences were considered statistically significant if p<0.05. Statistical analyses were computed with Statistica (Version 6. Tulsa, OK). For water consumption analysis, measures that indicated spillage, i.e., in excess of 100 ml of water intake, were excluded. This criterion resulted in removing two animals and five other time point measures from different animals from the analysis of water intake. These omissions were distributed across the experimental conditions. For data in Figure 9, one-sample t-tests were
used to determine if mean percentage values were significantly different from zero. Percentage values were computed by using the following formula: (individual animal cisplatin effect in a vagotomy condition – mean saline effect in the vagotomy condition)/(mean cisplatin effect in sham condition – mean saline effect in sham condition)* 100.

RESULTS

Experiment 1: Effects of CHB vagotomy

Cisplatin-induced pica. ANOVA results showed a significant Vagotomy x Injection x Time interaction \[F(12, 420)=5.97, p<0.00001\]. Figure 1 shows results from post-hoc analyses revealing significant increases in kaolin intake following cisplatin treatment compared to saline treatment on days 1, 2, 4 and 8-10 in sham-operated rats, but only on days 1 and 2 in CHB vagotomized animals (ps<0.05). It was also observed that CHB vagotomized cisplatin-treated rats exhibited significantly lower intakes of kaolin compared to cisplatin-treated sham rats on post-injection days 1 and 4 (ps<0.05).

Cisplatin-induced reduction in body weight. We did not observe a significant 3-way Vagotomy x Injection x Time interaction on BW change nor a Vagotomy x Injection effect (ps > 0.05), however significant Vagotomy x Time \[F(10, 350)=2.87, p<0.002\] and Injection x Time \[F(10, 350)=29.82, p<0.0001\] interactions were detected. Post-hoc analyses showed significant body weight loss due to cisplatin compared to saline injection in sham-operated and CHB vagotomized rats from days 1-10 post-injection (p<0.05), while weight loss in sham-operated rats treated with cisplatin was significantly greater than in CHB vagotomized cisplatin-treated rats from days 2-10 (ps<0.05; Fig. 2, top panel).
Cisplatin-induced reduction in food intake. A significant Vagotomy x Injection x Time interaction was noted for food intake [F(12, 420)=2.60, p<0.003]. In post-hoc analyses, food intake in sham-operated rats was significantly reduced following cisplatin treatment compared to saline injection in both sham-operated and CHB vagotomized rats from day 1-10 (ps<0.05), however in CHB-vagotomized animals, this reduction was significantly less than in cisplatin-treated sham animals from days 1-6 (ps<0.05; Fig. 2, middle panel).

Cisplatin-induced changes in water consumption. A significant Vagotomy x Injection x Time interaction was noted for water intake [F(12, 396)=7.80, p<0.00001]. A post-hoc assessment showed a significant reduction in water intake in sham rats treated with cisplatin compared to saline injection on day 2 (p<0.05; Fig. 2, lower panel). There was a significant increase in water intake after cisplation treatment compared to saline injection in cisplatin-treated sham and CHB vagotomized rats on days 4 and 5, as well as increased intake only in cisplatin-treated sham rats compared to saline injected controls from post-injection days 6-10 (ps<0.05).

Apomorphine-induced pica, body weight change, food intake, and water intake. ANOVA assessments revealed no significant Vagotomy x Injection x Time, Vagotomy x Injection, nor Vagotomy x Time interactions (ps>0.05). A significant Injection x Time interaction effect [F(5, 175)=117.27, p<0.0001] did occur, however no main effect for Vagotomy was observed (p>0.05). Post-hoc comparisons shown in Figure 3 reveal kaolin intake to be increased similarly in both sham operated and CHB-vagotomized rats on day 1 post-treatment (ps<0.05). Apomorphine had no significant effect on body weight change, food intake, or water intake in any treatment group (data not shown).
Experiment 2: Effects of GAS and CEL vagotomy

Cisplatin-induced pica. Results of ANOVA testing showed no significant Vagotomy x Injection x Time, Vagotomy x Injection, nor Vagotomy x Time interactions (ps>0.05). A significant Injection x Day interaction was observed for cisplatin-induced kaolin intake [F(12, 660)=15.56, p<0.0001]. There was no main effect of vagotomy (p>0.05). Post hoc analyses revealed significant increases in pica on day 1, 2 in all cisplatin-treated groups, regardless of vagotomy condition (ps<0.05; Fig. 4). In sham-operated rats, cisplatin also increased intake on days 4 and 6. In contrast, CEL vagotomized animals showed a longer-term pica response to cisplatin, showing additional increases in intake on days 4, 6, 8, and 10, while GAS vagotomized rats showed additional increased pica on day 10 (p<0.05). CEL vagotomized cisplatin-treated animals also showed increased intake on day 8 relative to cisplatin-injected sham controls (p<0.05).

Cisplatin-induced reduction in body weight. ANOVA showed no significant Vagotomy x Injection x Time, Vagotomy x Injection, nor Vagotomy x Time interactions for alterations in body weight (ps>0.05). There was a significant Injection x Time interaction for both body weight change [F(10, 550)=38.51, p<0.0001], however no main effect of vagotomy was shown (p>0.05). Post-hoc results shown in Figure 5 depict body weight loss in all cisplatin-treated groups from days 1-10 (ps<0.05). CEL vagotomized rats exhibited significantly greater body weight loss than cisplatin-treated sham controls on days 7-10, while GAS cisplatin-treated rats showed significantly increased weight loss compared to sham-operated animals treated with cisplatin on days 5-10 (ps<0.05; Fig., upper panel).

Cisplatin-induced reduction in food intake. ANOVA showed no significant Vagotomy x Injection x Time, Vagotomy x Injection, nor Vagotomy x Time interactions for food intake
There was a significant Injection x Time interaction for food intake [F(12, 660)=40.24, p<0.0001], however no main effect of vagotomy was observed (p>0.05). All cisplatin-treated groups significantly decreased intake from days 1-10 (ps<0.05; Fig. 5, lower panel). CEL vagotomized rats exhibited significantly greater suppression of food intake than cisplatin-treated sham controls on days 2-4 and 6-10, while GAS cisplatin-treated rats showed significantly lower food intakes compared to sham-operated animals treated with cisplatin on days 3-8 (ps<0.05).

**Cisplatin-induced changes in water consumption.** ANOVA showed no significant Vagotomy x Injection x Time, Vagotomy x Injection, nor Vagotomy x Time interactions for water intake (ps>0.05). However, there was a significant Injection x Time interaction [F(12, 600)=11.44, p<0.0001], and no significant main effect of Vagotomy (p>0.05). Post-hoc analyses depicted in Figure 6 show increased water intake in sham-operated rats on days 6, 7, 9 and 10, as well as in GAS vagotomized rats treated with cisplatin on days 7, 9 and 10 (ps<0.05), while no increase was observed in CEL vagotomized rats. In contrast, water intake was suppressed in CEL and GAS vagotomized rats after cisplatin on days 1-4 post-injection, while intake decreased in sham-operated rats only on day 2 (ps<0.05). CEL vagotomized rats also displayed significantly decreased water intake on days 3, 9 and 10 relative to sham animals treated with cisplatin, while GAS vagotomized rats showed greater reductions in water intake compared to cisplatin injected sham rats on days 3 and 4 post-injection (ps<0.05).

**Apomorphine-induced pica, body weight change, food intake, and water intake.**

Evaluations by ANOVA showed no significant Vagotomy x Injection x Time, Vagotomy x Injection, nor Vagotomy x Time interactions (ps>0.05). A significant Injection x Time interaction was shown [F(5,215)=117.29, p<0.0001], however a lack of Vagotomy main effect
was seen (p>0.05). Post-hoc data shown in Figure 7 demonstrate an increase among all apomorphine-treated groups on day 1 post-treatment, with no difference between vagotomy conditions (p<0.05). As in the previous set of experiments, apomorphine did not significantly alter body weight, food intake, or water intake in any apomorphine treatment group (data not shown).

**DISCUSSION**

The current results indicate an important role for the CHB, which innervates primarily the duodenum (43; 63), in cisplatin-induced malaise. Ablation of the CHB led to diminished pica (61% less over 10 days post-injection; Fig. 9), as well as decreased reductions in food intake and body weight, compared to control animals following a single treatment with cisplatin. In contrast, selective GAS and CEL vagotomies did not reduce the adverse effects of cisplatin. In fact, CEL vagotomized animals showed a trend toward more long-lasting pica (Fig. 9), as well as more prominent daily reductions of water intake, indicating that this surgery potentiates the deleterious effects of cisplatin on ingestive behavior and overall health.

These data clearly show that pica can be reliably elicited in all groups of vagotomized rats (CHB, GAS, or CEL). When treated with apomorphine, all vagotomized and sham-operated rats exhibited pica similar to previously published data (58). The presence of unaltered apomorphine-induced pica in the selectively vagotomized groups suggests parallel neural mechanisms to those capable of eliciting emesis, independent of vagal activation, in emetic species (3; 16; 31). Thus, our findings of reduced pica due to cisplatin in CHB-vagotomized rats are not likely due to a generalized mechanical or behavioral deficit following vagotomy surgery.
What is the role of the common hepatic branch in cisplatin-induced pica?

Cisplatin is thought to produce acute emesis largely through stimulation of 5-HT release from enteroendocrine cells that activates 5-HT$_3$ receptors located on vagal afferent fibers in the gastrointestinal tract (for review see 4; 39; 50). Pica is arguably a proxy for emesis in the rat (58) and cisplatin-induced pica is substantially inhibited by treatment with 5-HT$_3$ receptor antagonists (36; 58). Furthermore, cisplatin activates the CHB and this response is blocked by a 5-HT$_3$ receptor antagonist (27). Duodenal input is the likely source of this effect, because lesion of the gastrointestinal sub-branch of the CHB blocks the electrophysiological response of the CHB to cisplatin treatment (27).

The current data support an important role for CHB vagal afferents in the generation of cisplatin-induced pica. Note that the CHB is mostly sensory, of the ~3,000 fibers 73% are sensory, 7% are motor, and 20% are adventitial (fibers that pass between the CHB and ventral gastric branch) (47; 48). Ablation of the CHB led to a 67% reduction in cisplatin-induced pica during the 24 h post-injection and a 55% reduction for days 2 to 10 (Fig. 9). The sum of all past and current data leads to the hypothesis that cisplatin and possibly other toxins activate enteroendocrine cells of the duodenum, release 5-HT, and stimulate vagal fibers of the CHB containing 5-HT$_3$ receptors—a toxin detection pathway that can then generate pica. Indeed, work in the dog suggests that the duodenal region of the gastrointestinal tract is especially sensitive to the emetogenic stimulus of copper sulfate (29; 30).

As CHB vagotomy did not completely block cisplatin-induced pica, other pathways must also be at work to mediate pica in this surgical condition. It is possible that spinal afferents or central effects of humoral factors may play a role in cisplatin-induced pica following CHB vagotomy. Vagotomy produces neural plasticity in the peripheral nervous system (35); thus, the
operative pathways for cisplatin-induced pica might be somewhat different after surgery. For example, vagal afferent activation from cisplatin in the mid-jejunum is 5-HT₃ receptor dependent but then changes to a 5-HT₃ independent mechanism after vagotomy, possibly involving spinal afferents (19). Furthermore, data from the ferret shows that cisplatin-induced emesis is greatly reduced following subdiaphragmatic vagotomy, but only completely blocked when combined with ablation of the greater splanchnic nerves (17). There are also reports of elevated 5-HT and substance P levels in both the blood (9; 18; 38) and in isolated ileal preparations (11) following cisplatin treatment, suggesting the possibility that the CNS, e.g., the area postrema, could be activated directly to produce pica after cisplatin treatment.

*How do other vagal branches contribute to cisplatin-induced pica?*

Ideally, performing total subdiaphragmatic vagotomy would the first-step in assessing the role of the vagus in cisplatin-induced pica in the rat. However, due to well-known sequelae of reduced gastrointestinal motility, basal food intake, as well as the usually required use of a liquid maintenance diet following surgery (14; 32), total subdiaphragmatic vagotomy was avoided as an experimental condition in these studies. Importantly, in a small pilot study we observed that total subdiaphragmatic vagotomized animals exhibited deficits in their ability to consume pelleted kaolin after injection with apomorphine (10 mg/kg; ip) (unpublished results). These results are difficult to interpret, but might be related to reduced gastric clearance and motility in this surgical condition (32). Other possibilities to assess the role of vagal signaling in cisplatin-induced pica include the use of perivagal capsaicin (49) or unilateral rhizotomy plus vagotomy (57) to lesion vagal afferent fibers, however, neither of these procedures offers a complete ablation of vagal afferent fibers (see 7; 25).
In contrast to results of CHB vagotomy, CEL vagotomized rats showed a trend indicative of increased cisplatin-induced pica relative to sham controls, 57% over days 2 to 10, while GAS vagotomized animals showed little difference from sham rats (Fig. 9). It is not clear from our data why CEL vagotomy would possibly induce a lasting increase in kaolin intake, however the similarity in responses relative to sham rats on day 1 post-cisplatin injection (Fig. 9) suggests that it is not the initial phase of malaise that is altered, but rather CEL vagotomy might induce delayed effects on pica consumption not seen in sham animals treated with cisplatin.

Previous electrophysiological work showed that mid-jejunal mesenteric fibers, likely of vagal origin, are sensitive to cisplatin (19). Anatomical data indicate that jejunal vagal fibers are mostly derived from the celiac branches of the vagus (6; 63), and the current study indicates that intact vagal celiac fibers are not necessary for cisplatin-induced pica. It is also possible that cisplatin’s effects on delayed gastric emptying and gastric stasis may be exacerbated following these vagotomies, leading to increased malaise and a longer recovery time following cisplatin injection (34; 36).

**What is the contribution of the vagus to cisplatin-induced alterations of body weight, food intake, and water consumption?**

CHB vagotomy did not affect daily food intake, or body weight prior to injections, which is consistent with previous data (59). Food intake and body weight in GAS and CEL rats tended to be lower than controls. Body weight decrease following cisplatin injection is likely the result of decreased food intake. The mechanism for chemotherapy-induced anorexia is poorly understood but the current results suggest that anorexia and pica produced by cisplatin treatment are at least partially related. The average daily intakes of food and kaolin should be inversely
related, suggesting a lower level of malaise, i.e., when food intake is increased kaolin intake should be reduced. This relationship exists in the current study, e.g., the average negative correlation (Pearson’s R) in Experiment 1 between food and kaolin intake was -0.28 ± 0.11 for cisplatin-treated sham rats and -0.24 ± 0.14 for cisplatin-treated CHB vagotomized animals.

Water intake in sham rats (Fig. 3 and 7) following cisplatin treatment was significantly decreased at two days post-injection, which could be associated with the decrease in food intake. By 4 to 6 days post-injection with cisplatin water consumption was significantly greater than saline injected animals, which has been reported elsewhere (15; 37). Increased water consumption might be the result of well known effects of cisplatin on nephrotoxicity, with associated polyuria (8; 15), and diarrhea (5). Cisplatin did not change water intake in CHB vagotomized rats. Although the lack of a reduction of water intake after cisplatin treatment might be associated with reduced malaise with CHB vagotomy, the absence of a stimulation of drinking is more difficult to explain. It is possible that CHB vagotomy, and also CEL vagotomy (Fig. 7), reduces the diarrhea produced by cisplatin treatment. Additionally, significant decreases in water intake in GAS and CEL rats for multiple days (days 1 to 4; Fig. 7) immediately following cisplatin treatment were not seen in sham rats, which might indicate a higher degree of malaise.

**Does cisplatin-induced malaise in rats parallel effects in humans?**

It is well established that cisplatin is highly emetogenic in humans, and this drug is placed at the top of the list for emetic liability among cancer chemotherapy agents (33). Anti-emetic treatments may differ depending on efficacy for the acute (up to 1 day post-treatment) and delayed (1-5 days post-treatment) phases of emesis associated with cisplatin, and other
chemotherapy treatments (50). 5-HT₃ receptor antagonists largely control the severity of acute phase emesis, while the NK₁ receptor antagonists appear most useful for treatment of delayed chemotherapy induced vomiting (28). The current data show increased pica in intact rats most markedly within the first and second days post-injection, as well as a re-appearance of significant kaolin intake on the fourth day following cisplatin treatment, but also on days 6 to 10 (Fig. 2 and 5). A few recent reports have assessed pica beyond 72 hours post-stimulus and show evidence of a biphasic time response to cisplatin over several days (54; 62). Together with our findings, this evidence may support the notion that pica in the rat may be an applicable model for not only acute emetic behaviors, but show relevance to delayed models as well. We are unaware of any reports assessing either 5-HT₃ or NK₁ antagonists on pica behavior in the rat longer than 72 hours post-cisplatin treatment.

Although cancer itself plays an important role in anorexia and cachexia (muscle wasting), chemotherapy seems to also be an important factor contributing to body weight loss and an overall diminished quality of life in cancer patients (64). Increasing appetite in order to stimulate food intake in these patients is highly desirable. Indeed, a number of agents known to be involved in the control of food intake, such as ghrelin (40) and endocannabinoids (41), appear to offer potential clinical efficacy in stimulating energy intake in patients who exhibit anorexia following chemotherapy, and notably these agents are also anti-emetic (e.g., 51; 56). Our findings of attenuated food intake suppression in cisplatin-treated CHB vagotomized animals are intriguing, as it may be both an indicator of reduced malaise, but also increased appetite or disrupted signaling of feedback cues involved in the control of food intake.

**Perspectives and Significance**
The current work is the first to show effects of peripheral nerve lesions on pica behavior in the rat. Here, we have used cisplatin to induce pica because it is one of the most commonly investigated emetogenic stimuli in animal studies of emesis (2). Previous data have shown lesioning of central sites, such as the amygdala or hippocampus, to affect motion-induced pica in the rat (60). Cisplatin may also induce changes in Fos expression in multiple brain regions, including the nucleus of the solitary tract and amygdala (22). Taken together, the current results clearly show a reduction in displayed ingestive behaviors indicative of cisplatin-induced sickness following CHB vagotomy in the rat. This phenomenon is likely due to decreased sensory input as a result of blunted vagal activation. These data also suggest that pica has sensory pathways similar to emetic systems in other species since a vagal lesion, in this instance CHB vagotomy, reduced cisplatin-induced pica, but had no effect on apomorphine-induced pica.

This investigation is important because it helps to define neural systems involved in malaise, which can significantly impact feeding behavior in patients with chronic disease, such as cancer and AIDS, who receive potent drug treatments. Also of particular relevance is the long standing discussion of correctly interpreting the effects of chemicals that reduce food intake as through a satiating action, rather than a product of illness. This is an important challenge in research using the rat and mouse, which are non-vomiting species. In humans, chemotherapy–induced anorexia and cachexia often accompany symptoms of nausea and vomiting and current treatment directions are beginning to consider both anti-emetic treatments as well as appetite stimulating compounds to improve patient health. Delineation of the physiology of pica should greatly aide in the separation of neural systems responsible for the control of feeding behavior from those producing malaise and how they might interact.
ACKNOWLEDGMENTS

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Figure Legends

**Fig. 1.** Representative dorsal motor nucleus (DMN) staining produced by intraperitoneal injections of a retrograde neuronal tracer (Fluoro-gold) after sham surgery, common hepatic branch vagotomy (CHB), ventral gastric branch vagotomy (GAS), and vagotomy of the celiac branches (CEL). These sections were collected at approximately –13.92 relative to Bregma 42). Bar = 200 µm. * = loss of labeling in left DMN, indicating destruction of ventral gastric motor fibers.

**Fig. 2.** Effects of common hepatic branch (CHB) vagotomy on cisplatin-induced pica. Cisplatin (6 mg/kg) or saline was injected (ip) after three days of baseline measurements. * = p<0.05, saline versus cisplatin. † = p<0.05, sham-operated versus CHB vagotomy cisplatin conditions.

**Fig. 3.** Effects of CHB vagotomy on cisplatin-induced alterations of body weight, food intake, and water consumption. Cisplatin (6 mg/kg) or saline was injected (ip) after three days of baseline measurements. a = p<0.05, saline versus cisplatin, both surgical conditions. b = p<0.05, saline versus cisplatin, both surgical conditions, and sham-operated versus CHB vagotomy cisplatin conditions. c = p<0.05, saline versus cisplatin in sham animals, and sham-operated versus CHB vagotomy cisplatin conditions. * = p<0.05, saline versus cisplatin, sham-operated condition.
**Fig. 4.** Effects of CHB vagotomy on apomorphine-induced pica. Apomorphine (10 mg/kg) or saline was injected (ip) after three days of baseline measurements. * = p<0.05, saline versus apomorphine.

**Fig. 5.** Effects of celiac (CEL; accessory plus dorsal branches) or ventral gastric (GAS) branch vagotomy on cisplatin-induced pica. Cisplatin (6 mg/kg) or saline was injected (ip) after three days of baseline measurements. * = p<0.05, saline versus cisplatin. † = p<0.05, sham-operated versus CEL vagotomy cisplatin conditions.

**Fig. 6.** Effects of CEL or GAS vagotomy on cisplatin-induced alterations in body weight and food intake. Cisplatin (6 mg/kg) or saline was injected (ip) after three days of baseline measurements. a = p<0.05, saline versus cisplatin, all surgical conditions. b = p<0.05, saline versus cisplatin, all surgical conditions, and sham-operated versus GAS vagotomy cisplatin conditions. c = p<0.05, saline versus cisplatin, all surgical conditions, and sham-operated versus CEL vagotomy cisplatin conditions. d = p<0.05, saline versus cisplatin, all surgical conditions, sham-operated versus GAS vagotomy cisplatin conditions, and sham-operated versus CEL vagotomy cisplatin conditions.

**Fig. 7.** Effects of CEL or GAS vagotomy on cisplatin-induced alterations in water intake. Cisplatin (6 mg/kg) or saline was injected (ip) after three days of baseline measurements. * = p<0.05, saline versus cisplatin. † = p<0.05, sham-operated versus GAS or CEL vagotomy cisplatin conditions.
**Fig. 8.** Effects of CEL or GAS vagotomy on apomorphine-induced pica. Apomorphine (10 mg/kg) or saline was injected (ip) after three days of baseline measurements. * = p<0.05, saline versus apomorphine.

**Fig. 9.** Percent change in pica in each vagotomy condition (CHB, GAS, and CEL) relative to cisplatin-treated sham-operated animals. Acute phase represents 24 h after a single injection of cisplatin (6 mg/kg; ip) and the delayed period is the sum of kaolin intake over the next 2 to 10 days. The total (solid bar) shows the sum effect (all 10 days) of a vagotomy condition on cisplatin-induced pica, e.g., CHB vagotomy reduced cisplatin-induced pica by 61% over 10 days. * = p<0.05, one sample t-test, mean versus expected value of zero.
Reference List


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<tr>
<th>Time (days)</th>
<th>Sham-operated</th>
<th>CHB Vagotomized</th>
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* denotes statistically significant difference compared to baseline.
† denotes statistically significant difference compared to Sham-operated group.

Kaolin intake (g) vs. Time (days)
Sham-operated

GAS Vagotomized

CEL Vagotomized

Kaolin intake (g)

Injection

Time (days)

* Saline

* Cisplatin
Sham-Operated

GAS Vagotomized

CEL Vagotomized

Water intake (ml)

Time (days)

Injection
Vagotomy

% Change in kaolin intake

-100  -50   0    50    100

Acute (day 1)
Delayed (days 2 - 10)
Total (days 1 - 10)

% Change in kaolin intake

CHB  GAS  CEL

Vagotomy

*  *  *