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

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De Jonghe BC, Horn CC. Chemotherapy-induced pica and anorexia are reduced by common hepatic branch vagotomy in the rat. Am J Physiol Regul Integr Comp Physiol 294: R756–R765, 2008. First published January 9, 2008; doi:10.1152/ajpregu.00820.2007. — Anticancer 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 antiemetic 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 or ventral gastric (Gas) or celiac (Cel) branch 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 nonvomiting rat.

Anticancer 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 Ref. 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 present 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 subbranch 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). It should also be noted 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).

Because of the nature of in vivo electrophysiology experiments, data on CHB activation by cisplatin provide insight only into short-term physiological events and do 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 Refs. 20, 21). It is not possible to directly measure the emetic potential of such stimuli in the rat, because they lack a vomiting response. They do, however, ingest kaolin clay (a pica response) when made sick by toxins, including cisplatin (see, e.g., Refs. 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 antiemetic drugs (see, e.g., Refs. 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 after cisplatin injection (e.g., Refs. 36, 52, 58), with only scant data extending up to 7 days after treatment (54, 62). Examining a longer time course of behavior after 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 present 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 after 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. 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 selectively vagotomized by severing either the ventral gastric (i.e., removing part of the gastric input) or celiac
MATERIALS AND METHODS

Subjects and Chemicals Used for Induction of Pica

One hundred and four male Sprague-Dawley rats (Charles River) were housed individually in mesh-floored stainless steel hanging cages (8 in. × 9.5 in. × 8 in.) and maintained in a temperature-controlled vivarium with a 12:12-h light-dark cycle (lights on at 0600). Animals were handled daily for 2 wk before the onset of experiments. Tap water, powdered rat chow (Purina 5001), and pelleted kaolin clay (Research Diets) were available ad libitum throughout experiments, except where otherwise indicated. For all experiments, powdered rat chow (~100 g) was available from a 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 and jars, as well as spilled contents from each, were weighed daily to calculate intakes. Spillages from chow or kaolin were easily separately by hand because of the visual contrast of the two substances (white intakes. Spillages from chow or kaolin were easily separately by hand because of the visual contrast of the two substances (white

Vagotomy Surgeries

After an overnight fast animals were anesthetized with pentobarbital sodium (50 mg·kg⁻¹·ml⁻¹·ip; Sigma), and apomorphine (10 mg·ml⁻¹·kg·body wt⁻¹·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 wt ip).

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 with 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 naive rats not used in the behavioral study was conducted to determine whether CHB vagotomy might lead to inadvertent destruction of vagal fibers of the ventral trunk. One week after CHB vagotomy these rats were given two 0.5-ml intra-peritoneal injections of 0.1% Fluoro-Gold (Fluorochrome, Denver, CO). Similarly, immediately after 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 pentobarbital sodium (50 mg) and transcardially perfused with a 0.2 M phosphate buffer solution, followed by 4% paraformaldehyde and 2% acrolein in 0.2 M phosphate buffer (pH 7.4). Hindbrains were removed, stored in fixative for 4 h, and then cryoprotected in sucrose. Thirty-micrometer 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 no. 05023816), and a donkey anti-rabbit secondary (Jackson Immuno). Sections were placed on microscope slides, air dried, coated with DPX mountant (Fluka, Ronkonkoma, NY), coverslipped, viewed with a microscope (Zeiss Axiosiostar Plus), and imaged with a digital camera (Scion CFW-12C). 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 nucleus of the solitary tract (NTS) (higher percentage values indicate darker staining relative to the NTS). See Fig. 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-vagotomized rats \([t(3) = 6.2, P < 0.05]\), consistent with ablation of ventral gastric fibers (36); however, Cel-vagotomized rats \([t(3) = 2.7%]\) were not significantly different from sham-vagotomized rats \([P < 0.05, t\text{-test}]. \) In the right DMN, Gas \([t(3) = 2.4\%]\) and Cel \([t(3) = 3.0\%]\) were not significantly different from sham-vagotomized rats \([P > 0.05, t\text{-tests}]. \) It should be noted 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 vagotomy, and all Gas animals met this criterion. It is likely that Cel vagotomies are unable to be adequately visualized because of 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 levels of DMN staining (see Fig. 1 in Ref. 44). However, in the present study, Cel-vagotomized rats did exhibit behavioral deficits indicative of neuronal damage (see RESULTS).

Experiment 1: Effects of CHB Vagotomy

All animals surpassed presurgical body weight at 2 days after surgery. Six days after recovery of presurgical body weight, body weight and 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 before apomorphine testing, and
Experiment 2: Effects of Gas and Cel Vagotomies

All animals surpassed presurgical body weight at 2 days after surgery. Five days after recovery of presurgical body weight, body weight and 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, because of 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). Before tests with cisplatin or apomorphine animals were blocked based on body weight and randomized for injections. There were 9 or 10 animals in each of the 4 conditions (sham-saline, sham-drug, CHB-saline, and CHB-drug). Body weights were not significantly different between sham (399 ± 4.0 g, n = 19), Gas (394 ± 6.7 g, n = 20), and Cel (398.8 ± 6.6 g, n = 22)-vagotomized animals before testing (P > 0.05). Three days of baseline measures were collected before testing with cisplatin or apomorphine, and 10 and 3 days of data were collected after injection with cisplatin and apomorphine, respectively.

Statistical Analysis

Food, water, and kaolin intake data are expressed as means ± SE. In initial comparisons no differences were noted between groups in each experiment, and data were pooled for subsequent analyses. Body weight (BW) data are presented as percent change from baseline before treatment by the following equation: % BW change = [daily BW after injection/(mean of BW 1 and 2 days before 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 with 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 Fig. 9, one-sample t-tests were used to determine whether 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 sham vagotomy condition)/(mean cisplatin effect in sham vagotomy condition) × 100.

RESULTS

Experiment 1: Effects of CHB Vagotomy

Cisplatin-induced pica. ANOVA results showed a significant vagotomy × injection × time interaction [F(12,420) = 5.97, P < 0.00001]. Figure 2 shows results from post hoc analyses revealing significant increases in kaolin intake follow-
Experiment 1: Effects of CHB Vagotomy on Cisplatin-Induced Pica

Cisplatin-induced pica. In Fig. 2, effects of CHB vagotomy on cisplatin-induced pica are shown. Cisplatin (6 mg/kg) or saline was injected (ip) after 3 days of baseline measurements. *P < 0.05, saline vs. cisplatin; †P < 0.05, sham-operated vs. CHB vagotomy cisplatin conditions.

Using cisplatin treatment compared with 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 (all P < 0.05). It was also observed that CHB-vagotomized cisplatin-treated rats exhibited significantly lower intakes of kaolin compared with cisplatin-treated sham-vagotomized rats on postinjection days 1 and 4 (both P < 0.05).

Cisplatin-induced reduction in body weight. We did not observe a significant three-way vagotomy × injection × time interaction on body weight change or a vagotomy × injection effect (both P > 0.05); however, significant vagotomy × time [F(10,350) = 2.87, P < 0.002] and injection × time [F(10,350) = 29.82, P < 0.0001] interactions were detected. Post hoc analyses showed significant body weight loss due to cisplatin compared with saline injection in sham-operated and CHB-vagotomized rats from days 1 to 10 after 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 to 10 (all P < 0.05; Fig. 3, top).

Cisplatin-induced reduction in food intake. A significant vagotomy × injection × 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 after cisplatin treatment compared with saline injection in both sham-operated and CHB-vagotomized rats from day 1 to day 10 (all P < 0.05); however, in CHB-vagotomized animals, this reduction was significantly less than in cisplatin-treated sham-operated animals from day 1 to day 6 (all P < 0.05; Fig. 3, middle).

Cisplatin-induced changes in water consumption. A significant vagotomy × injection × 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-operated rats treated with cisplatin compared with saline injection on day 2 (P < 0.05; Fig. 3, bottom). There was a significant increase in water intake after cisplatin treatment compared with 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-vagotomized rats compared with saline-injected controls from day 6 to day 10 after injection (all P < 0.05).

Apomorphine-induced pica, body weight change, food intake, and water intake. ANOVA assessments revealed no significant vagotomy × injection × time, vagotomy × injection, or vagotomy × time interactions (all P > 0.05). A significant injection × 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 Fig. 4 reveal kaolin intake to be increased similarly in both sham-operated and CHB-vagotomized rats on posttreatment day 1 (both P < 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 × injection × time, vagotomy × injection, or vagotomy × time interactions (all P > 0.05). A significant injection × day interaction was observed for cis-
Cel-vagotomized rats exhibited significantly greater suppression of food intake than cisplatin-treated sham-operated controls on days 2–4 and 6–10, while Gas-vagotomized cisplatin-treated rats showed significantly lower food intakes compared with sham-operated animals treated with cisplatin on days 3–8 (all \( P < 0.05 \)).

Cisplatin-induced changes in water consumption. ANOVA showed no significant vagotomy × injection × time, vagotomy × injection, or vagotomy × time interactions for water intake (all \( P > 0.05 \)). However, there was a significant injection × 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 Fig. 7 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 (all \( P < 0.05 \)), 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 after injection, while intake decreased in sham-operated rats only on day 2 (all \( P < 0.05 \)). Cel-vagotomized rats also displayed significantly decreased water intake on days 3, 9, and 10 relative to sham-operated animals treated with cisplatin, while Gas-vagotomized rats showed greater reductions in water intake compared with cisplatin-injected sham-operated rats on days 3 and 4 after injection (all \( P < 0.05 \)).

Apomorphine-induced pica, body weight change, food intake, and water intake. ANOVA showed no significant vagotomy × injection × time, vagotomy × injection, or vagotomy × time interactions for food intake (all \( P > 0.05 \)). Post hoc analyses revealed significant increases in pica on days 1 and 2 in all cisplatin-treated groups, regardless of vagotomy condition (all \( P < 0.05 \); Fig. 5). 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-vagotomized controls (\( P < 0.05 \)).

Cisplatin-induced reduction in body weight. ANOVA showed no significant vagotomy × injection × time, vagotomy × injection, or vagotomy × time interactions for alterations in body weight (all \( P > 0.05 \)). There was a significant injection × time interaction for 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 Fig. 6 depict body weight loss in all cisplatin-treated groups from day 1 to day 10 (all \( P < 0.05 \)). Cel-vagotomized rats exhibited significantly greater body weight loss than cisplatin-treated sham-vagotomized controls on days 7–10, while Gas-vagotomized cisplatin-treated rats showed significantly increased weight loss compared with sham-operated animals treated with cisplatin on days 5–10 (\( P < 0.05 \); Fig. 6, top).

Cisplatin-induced reduction in food intake. ANOVA showed no significant vagotomy × injection × time, vagotomy × injection, or vagotomy × time interactions for food intake (all \( P > 0.05 \)). There was a significant injection × 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 day 1 to day 10 (all \( P < 0.05 \); Fig. 6, bottom). Cel-vagotomized rats exhibited significantly greater suppression of food intake than cisplatin-treated sham-operated controls on days 2–4 and 6–10, while Gas-vagotomized cisplatin-treated rats showed significantly lower food intakes compared with sham-operated animals treated with cisplatin on days 3–8 (all \( P < 0.05 \)).
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 neural mechanisms parallel 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.

DISCUSSION

The present 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 after injection; Fig. 9), as well as decreased reductions in food intake and body weight, compared with control animals after 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.

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 3 days of baseline measurements. aP < 0.05, saline vs. cisplatin, all surgical conditions; bP < 0.05, saline vs. cisplatin, all surgical conditions, and sham-operated vs. Gas vagotomy cisplatin conditions; cP < 0.05, saline vs. cisplatin, all surgical conditions, and sham-operated vs. Cel vagotomy cisplatin conditions; dP < 0.05, saline vs. cisplatin, all surgical conditions, sham-operated vs. Gas vagotomy cisplatin conditions, and sham-operated vs. 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 3 days of baseline measurements. *P < 0.05, saline vs. cisplatin; †P < 0.05, sham-operated vs. Gas or Cel vagotomy cisplatin conditions.

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 Refs. 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 subbranch of the CHB blocks the electrophysiological response of the CHB to cisplatin treatment (27).

The present data support an important role for CHB vagal afferents in the generation of cisplatin-induced pica. It should be noted 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 after injection and a 55% reduction for days 2–10 (Fig. 9). The sum of all past and present 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-HT3 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).

Because 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 midjejunum 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 show that cisplatin-induced emesis is greatly reduced after 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 both in the blood (9, 18, 38) and in isolated ileal preparations (11) after cisplatin treatment, suggesting the possibility that the central nervous system, 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 be the first step in assessing the role of the vagus in cisplatin-induced pica in the rat. However, because of the well-known sequelae of reduced gastrointestinal motility, basal food intake, as well as the usually required use of a liquid maintenance diet after 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 Refs. 7, 25).

In contrast to the results of CHB vagotomy, Cel-vagotomized rats showed a trend indicative of increased cisplatin-induced pica relative to sham-operated controls, 57% over days 2–10, while Gas-vagotomized animals showed little difference from sham-operated 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-operated rats on day 1 after 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-operated animals treated with cisplatin.

Previous electrophysiological work showed that midjejunal mesenteric fibers, likely of vagal origin, are sensitive to cis-
platin (19). Anatomic data indicate that jejunal vagal fibers are mostly derived from the celiac branches of the vagus (6, 63), and the present 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 after 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 before injections, which is consistent with previous data (59). Food intake and body weight in Gas and Cel rats tended to be lower than in control rats. 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 present 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 present study, e.g., the average negative correlation (Pearson’s R) in experiment 1 between food and kaolin intake was $-0.28 \pm 0.11$ for cisplatin-treated sham-vagotomized rats and $-0.24 \pm 0.14$ for cisplatin-treated CHB-vagotomized animals.

Water intake in sham-vagotomized rats (Figs. 3 and 7) after cisplatin treatment was significantly decreased at 2 days after injection, which could be associated with the decrease in food intake. By 4–6 days after injection with cisplatin water consumption was significantly greater than in 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 1–4; Fig. 7) immediately after cisplatin treatment were not seen in sham-vagotomized 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). Antiemetic treatments may differ depending on efficacy for the acute (up to 1 day after treatment) and delayed (1–5 days after 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 present data show increased pica in intact rats most markedly within the first and second days after injection, as well as a reappearance of significant kaolin intake on the fourth day after cisplatin treatment, but also on days 6–10 (Figs. 2 and 5). A few recent reports have assessed pica beyond 72 h after 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 not only be an applicable model for 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 h after cisplatin treatment.

Although cancer itself plays an important role in anorexia and cachexia (muscle wasting), chemotherapy also seems to 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 antiemetic (see, e.g., Refs. 51, 56). Our findings of attenuated food intake suppression in cisplatin-treated CHB-vagotomized animals are intriguing, because it may be an indicator of both reduced malaise but also increased appetite or disrupted signaling of feedback cues involved in the control of food intake.

*Perspectives and Significance*

The present 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 NTS and amygdala (22). Together, the present 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 the mouse, which are nonvomiting species. In humans, chemotherapy-induced anorexia often accompany symptoms of nausea and vomiting, and current treatment directions are beginning to consider both antiemetic treatments as well as appetite-stimulating compounds to improve patient health. De-
linement of the physiology of pica should greatly aid in the separation of neural systems responsible for the control of feeding behavior from those producing malaise and how they might interact.

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