TRANSLATIONAL PHYSIOLOGY

Food restriction, refeeding, and gastric fill fail to affect emesis in musk shrews

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Horn CC, Still L, Fitzgerald C, Friedman MI. Food restriction, refeeding, and gastric fill fail to affect emesis in musk shrews. Am J Physiol Gastrointest Liver Physiol 298: G25–G30, 2010. First published November 5, 2009; doi:10.1152/ajpgi.00366.2009.—Nausea and emesis are common side effects of gastrointestinal disease. Reports indicate that ghrelin and endocannabinoids, agents that stimulate appetite, also reduce emesis evoked by chemotherapy treatment, which suggests that stimulation of feeding inhibits the emetic system. In the following study we conducted a more direct test of this hypothesis by determining the impact of manipulating the motivation to eat on emesis, using food restriction and refeeding. Emesis was induced in musk shrews, a commonly used animal model for emesis research, using the cancer chemotherapy agent cisplatin (20 mg/kg ip), nicotine (2 mg/kg sc), or motion (1 Hz, horizontal, 4-cm displacement), because these treatments are known to target separate emetic pathways: gut vagal afferents, arcuate postrema, and vestibular pathways, respectively. Twenty-four hours of food restriction was sufficient to stimulate food intake, and 1 h of refeeding filled the stomach. The results indicate that food restriction, refeeding, and gastric fill had no significant effects on the amount of emesis produced by any of the emetic treatments tested here. This suggests that, although activation of the emetic system might have prominent effects on food intake, neural controls for feeding behavior do not significantly affect the neural pathways for emesis. These results may have implications for how we treat patients who experience a constellation of side effects, including nausea and emesis, since stimulating appetite may not necessarily inhibit emetic pathways.

nausea; emesis; vagus; cisplatin

TRANSLATIONAL HIGHLIGHTS Reports suggest that stimulating appetite using drugs might reduce vomiting evoked by cancer chemotherapy treatment. The present study shows that stimulating appetite, by use of food restriction, and refeeding in musk shrews does not affect emesis induced by a variety of stimuli, including chemotherapy. These results may have implications for how we treat patients who experience side effects, including nausea and emesis produced by chemotherapy.

The primary function of nausea and vomiting is to defend against the ingestion of toxins. Vomiting empties toxins from the gut, and, arguably, nausea can serve as a learned response that prevents the future ingestion of toxins (14). Many diseases and conditions, as well as their treatments, cause nausea and vomiting (emesis) (26). Severe and prolonged emesis can affect patients’ nutritional health, quality of life, and willingness to continue treatments that include nausea and vomiting as prominent side effects. It is more difficult to treat nausea than emesis, and our understanding of the biology of nausea is limited because it is difficult to assess nausea in animal models (5, 26).

Although of great benefit, antiemetic drugs are not always effective for controlling emesis (e.g., Ref. 1) and often are not adequate for reducing nausea (13). It would be beneficial to find other approaches or adjunctive methods to control nausea and vomiting. Appetite and nutritional factors are sometimes cited as variables that influence the degree of nausea and vomiting (e.g., Ref. 7). Clinical evaluations tend to link appetite with nausea and emesis; for example, the grading of nausea used by the National Cancer Institute (9) in clinical trials is sometimes related to the amount of reduced appetite. In a converse fashion, administration of appetite stimulants, such as cannabinoids and ghrelin (10, 36), inhibits emesis in ferrets and least shrews (22, 24, 33). This suggests that activation of neural systems controlling appetite inhibit emesis, perhaps by stimulating a competitive response (feeding) to vomiting.

As a first step toward evaluating the potential impact that feeding may have on emesis we studied musk shrews, a well-established model of emesis (16, 19, 32). To expand on the findings that cannabinoids and ghrelin inhibit emesis, we sought to determine whether fasting, a natural stimulus for feeding and subsequent refeeding (sufficient to fill the stomach) would modify emesis induced by activation of different neural pathways: gut vagal afferents (using the cancer chemotherapy agent cisplatin) (4, 19), the area postrema (using nicotine) (6, 32), and the vestibular system (using horizontal motion) (16). Our initial work used musk shrews originally derived from Guam. However, we discovered that this Guam strain was insensitive to motion-induced emesis, so we completed our studies using a Taiwan strain known to show a potent emetic response to motion (e.g., Refs. 2, 8, 16, 31).

METHODS

Subjects. Studies were performed on adult (>40 days of age) male musk shrews (Suncus murinus). Shrew colonies were created from...
Food intake. To determine the length of fasting required to significantly stimulate appetite, we fasted animals for 3 and 24 h. Two separate food intake studies (using within-subjects designs), one with Guam-derived shrews (n = 4) and another with Taiwan-derived shrews (n = 8), were conducted. Initially, half the animals were fed ad libitum and half were fasted for 3 h. For the 3-h fasted condition, food was removed at ~0900 and returned at ~1200. Food was weighed at 0, 1, 2, and 3 h starting at ~1200. At least 2 days later, the group assignment was reversed and the experiment was repeated. At least 3 days after the second feeding test, the same animals were retested under ad libitum feeding or 24-h fasting conditions (food removed at ~1200 the prior day and tested at ~1200), and treatments were reversed at least 3 days later.

Gastric emptying and body weight. To determine the impact of refeeding on stomach fill we fasted animals for 24 h and refed for 1 h and weighed the stomach contents. We also included ad libitum-fed, 3-h fasted, and 24-h fasted conditions for comparison. Separate gastric emptying studies using Guam-derived and Taiwan-derived shrews were conducted. Musk shrews were housed under four conditions: ad libitum, fasted for 3 h, fasted for 24 h, or fasted for 24 h and refed for 1 h. Data represent the mean ± SE of wet and dry contents of the stomach, intestine (10 cm starting at the pyloric sphincter), or extended (ext.) intestine (remaining intestinal segment, ~30–33 cm) of variable length (25–36 cm).

Emesis. Three stimuli known to reliably produce emesis in musk shrews (e.g., Refs. 16, 19, 27) were used: cisplatin (20 mg/kg ip; Sigma-Aldrich, cis-diamineplatinum dichloride, no. P4394), nicotine [2 mg/kg sc; Sigma-Aldrich, (−)-nicotine, no. 36733], and motion (1 Hz, 4 cm horizontal, 10 min with reciprocal shaker; see below). Cisplatin and nicotine were prepared in physiological saline (0.9% NaCl). Cisplatin was injected intraperitoneally in a volume of 10 ml/kg body wt, and nicotine was administered subcutaneously in a volume of 2 ml/kg body wt. Immediately after injection, animals were placed into a clear plastic container (28 × 17 × 12 cm) without food or water, and a trained observer recorded the timing and number of emetic responses for 120 min for cisplatin tests and for 30 min for nicotine tests.

For motion experiments, animals were placed individually in a clear plastic container (28 × 17 × 12 cm) on a reciprocal shaker (Taitec, Double Shaker R-30, Taiyo Scientific Industrial). Animals were allowed 3 min to acclimate before the shaker was turned on. Horizontal motion continued for 10 min with 4-cm displacement (2 cm left, 2 cm right) at a frequency of 1 Hz. Observation was continued

Fig. 1. Food intake 3 h after 3-h fast (top) or 24-h fast (bottom) in Guam- and Taiwan-derived musk shrews, compared with ad libitum food intake. *P < 0.05, planned comparison, ad libitum vs. fasted condition at a specific time period. Data are means ± SE.

stock obtained from Dr. Emilie Rissman at the University of Virginia (descendants from animals originally collected in Guam) (e.g., Ref. 35) and Dr. John Rudd at the Chinese University of Hong Kong (descendants from Taiwan) (23, 34). Sixty-two Guam-derived shrews (mean = 37.9 ± 0.6 g, range = 29.4–49.6 g) and 104 Taiwan-derived shrews (mean = 64.5 ± 0.8 g, range = 38.7–88.9 g) were used. Shrews were housed individually in a temperature-controlled vivarium with a 12:12-h light-dark cycle (0700–1900 light period) and ad libitum access to tap water and food [mixture of 75% Purina Cat Chow Complete Formula and 25% Complete Gro-Fur mink food pellets (Milk Specialty, New Holstein, WI), except as indicated. This protocol was approved by the Monell Chemical Senses Center Institutional Animal Care and Use Committee.

Fig. 2. Gastric and intestinal contents after fasting and refeeding in Guam- and Taiwan-derived musk shrews. Animals were fed ad libitum, fasted for 3 h, fasted for 24 h, or fasted for 24 h and refed for 1 h. Data represent the mean ± SE of wet and dry contents of the stomach, intestine (10 cm starting at the pyloric sphincter), or extended (ext.) intestine (remaining intestinal segment, ~30 cm). *P < 0.05, planned comparison, a specific group vs. the ad libitum-fed group.

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for 5 min after the shaker was turned off. These parameters for motion exposure were determined by past studies to be optimal for inducing emesis in musk shrews (16).

Emetic responses were defined as a short burst of retches (3) that occurred with or without expulsion of material from the mouth (vomiting). Each animal was used only once in each emesis test. Tests were run with an equal number of control animals; for example, when two animals fasted for 24 h were tested for emesis after nicotine injection, two additional animals fed ad libitum were tested at the same time. The numbers of animals used in each condition is noted in the figures (5–6 per condition). Animals were tested with cisplatin and nicotine at 1200 and for motion exposure at 1500.

Initial studies used Guam-derived or Taiwan-derived shrews tested with ad libitum feeding or 24-h fasting. A final experiment was conducted with Taiwan-derived shrews to compare ad libitum feeding with 24-h fasting and 1-h refed group compared with ad libitum control animals (one-

**RESULTS**

**Food intake.** Twenty-four hours, but not 3 h, of food restriction stimulated food intake in Guam-derived and Taiwan-derived musk shrews [no significant effects for the 3-h studies; main effect of food restriction in Guam-derived shrews with 24-h food restriction, \( F(1,23) = 13.5, P < 0.05 \); interaction effect of food restriction and time in Taiwan-derived shrews with 24-h food restriction, \( F(2,47) = 14.2, P < 0.001 \); Fig. 1]. The increase in food intake after 24-h food restriction was seen at 0–1 h and 2–3 h in both Guam- and Taiwan-derived shrews compared with ad libitum-fed animals (Fig. 1; planned comparisons, \( P \) values < 0.05).

**Gastric emptying and body weight changes after fasting.** Compared with ad libitum-fed controls, both types of shrew fasted for 24 h and refed for 1 h had significantly more wet and dry stomach contents [one-way ANOVAs, \( F(2 \text{ or } 3,23) \geq 7.0, P < 0.005 \); planned comparisons, \( P \) values < 0.05; Fig. 2]. There were no statistically significant differences in the contents of the first 10 cm of the intestine (ANOVA or planned comparisons; Fig. 2). However, the Taiwan-derived shrews showed a significant increase in weight of contents of the extended intestine, both wet and dry, in the 24-h fast plus 1-h refed group compared with ad libitum control animals [one-

![Fig. 3. Emetic responses to cisplatin treatment after 24 h of fasting in Guam- and Taiwan-derived musk shrews. Top: number of emetic episodes: vomits (emetic episodes with expulsion) and retches (emetic episodes without expulsion). Numbers above each bar are \( n \) responders/\( n \) animals tested. Data are means ± SE. Bottom: latency to the first emetic response (the maximum time for the observation period was used if an animal did not respond). Data are median latency and a scatterplot of each value.](image)

![Fig. 4. Emetic responses to nicotine treatment after 24 h of fasting in Guam- and Taiwan-derived musk shrews. Top: number of emetic episodes: vomits (emetic episodes with expulsion) and retches (emetic episodes without expulsion). Numbers above each bar are \( n \) responders/\( n \) animals tested. Data are means ± SE. Bottom: latency to the first emetic response (the maximum time for the observation period was used if an animal did not respond). Data are median latency and a scatterplot of each value.](image)
Emesis after fasting and refeeding. Taiwan-derived shrews showed no effects of refeeding for 1 h after a 24-h fast on emetic responses produced by cisplatin, nicotine, or motion compared with ad libitum-fed animals (t-tests for number of emetic episodes or Mann-Whitney U-tests for emetic response latency; Fig. 6). During refeeding, food-restricted animals ate $1.7 \pm 0.7, 1.1 \pm 0.2, \text{and } 1.6 \pm 0.3 \text{ g of food in the cisplatin, nicotine, and motion studies, respectively.}$

**DISCUSSION**

The present results show that in musk shrews neither food restriction, food intake, nor gastric fill have significant effects on emesis produced by a variety of emetic stimuli. Our initial hypothesis was that fasting would reduce emesis because an increase in appetite for food can be viewed as a competing response to emesis. Endocannabinoids and ghrelin, which are well-known stimulants of food intake (10, 36), also reduce cisplatin-induced emesis in the ferret and least shrew (22, 24, 33). Similar to effects observed in rodents, ghrelin is present in the gastrointestinal tract of the musk shrew and endocannabinoids have been measured in the brain of the least shrew (11, 15). In rodents, fasting increases plasma levels of ghrelin and brain levels of endocannabinoids (12, 28), which suggests that similar changes might occur in musk shrews. However, we found no significant effects of food restriction and feeding on emesis produced by cisplatin or other emetic treatments. This is evidence that, although activation of emesis decreases ap-
petite and eating (e.g., patients with bouts of vomiting and sustained nausea are unlikely to eat), the reverse does not appear to be true: a change in appetite does not directly affect emesis. From this perspective, it also appears that the anti-emetic effect of cannabinoids or ghrelin is unrelated to their actions on feeding behavior or that the mechanisms that trigger eating behavior after administration of these agents are different from those involved in feeding induced by fasting. The present study also shows that the amount and latency for emesis is not affected by gastric fill. A 24-h fast emptied the stomach and refeeding filled the stomach, but neither condition affected emesis produced by any of the emetic stimuli.

Motion exposure produced no emesis in these Guam-derived shrews. In fact, we are unaware of any reports of motion-induced emesis in this strain of musk shrew. However, this response is not completely absent in Guam shrews since we have observed a few animals with motion-induced emesis in our colony (C. C. Horn, unpublished data). On the other hand, not all Taiwan-derived shrews display motion-induced emesis. In the present study, only ~58% of Taiwan-derived shrews showed motion-induced emesis, and not all shrews of this strain showed emesis to motion exposure in studies reported in the literature (e.g., Refs. 20, 23). It is possible that some shrews might be sensitive to motion-induced emesis under different parameters (e.g., a higher frequency of shaking) than those used here and in other studies (16).

Our results indicate that neither fasting nor refeeding has a significant effect on emesis. Five to six musk shrews were used in each of the present experiments, comparable to group sizes used in other studies on the pharmacology of emesis in musk shrews (e.g., Refs. 23, 25). Although the use of larger sample sizes might show statistically significant effects, the present data suggest that any effects that may be uncovered would be modest and not equivalent to the magnitude of effects produced by known antiepileptic treatments, including 5-HT3 and NK1 receptor antagonists (e.g., Refs. 23, 25). This information might be useful when considering how to reduce emesis in patients who receive treatments like cancer chemotherapy that produce strong effects of emesis and nausea (17). Some reports have suggested that food restriction might protect against the side effects of chemotherapy because food restriction reduces oxidative stress (21). In line with this idea are data pointing to a role for increased oxidative stress as a mediator of chemotherapy-induced emesis (e.g., Refs. 18, 29, 30). However, the present data indicate that the amount of emesis is not significantly affected by food intake or nutritional status before chemotherapy treatment, which therefore will likely prove to be an insignificant factor for the control of chemotherapy-induced emesis. Whether fasting prior to chemotherapy is a useful adjunctive treatment for emesis and nausea remains to be seen.

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GRANTS

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DISCLOSURES

No conflicts of interest are declared by the author(s).

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