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Roux-en-Y gastric bypass does not affect daily water intake or the drinking response to dipsogenic stimuli in rats

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Roux-en-Y gastric bypass does not affect daily water intake or the drinking response to dipsogenic stimuli in rats. Am J Physiol Regul Integr Comp Physiol 307: R114 –R120, 2014. First published June 4, 2014; doi:10.1152/ajpregu.00135.2014.—Bariatric surgery is currently the most effective treatment for severe obesity, and Roux-en-Y gastric bypass (RYGB) is the most common approach in the United States and worldwide. Many studies have documented the changes in body weight, food intake, and glycemic control associated with the procedure. Although dehydration is commonly listed as a postoperative complication, little focus has been directed to testing the response to dipsogenic treatments after RYGB. Accordingly, we used a rat model of RYGB to test for procedure-induced changes in daily water intake and in the response to three dipsogenic treatments: central administration of ANG II, peripheral injection of hypertonic saline, and overnight water deprivation. We did not find any systematic differences in daily water intake of sham-operated and RYGB rats, nor did we find any differences in the response to the dipsogenic treatments. The results of these experiments suggest that RYGB does not impair thirst responses and does not enhance any satiating effect of water intake. Furthermore, these data support the current view that feedback from the stomach is unnecessary for the termination of drinking behavior and are consistent with a role of orosensory or postgastric feedback.

Obesity rates have increased dramatically over the past several decades, and considerable research has been devoted to understanding the underlying causes, with the hope of developing better treatments. Bariatric surgery is widely considered the most effective treatment for severe obesity, and the number of procedures performed each year have risen in the United States and in other parts of the world (6, 7, 46). Several surgical approaches are used for bariatric surgery, but the most common over the past decade worldwide is the Roux-en-Y gastric bypass (RYGB) (6, 7). A rodent model of RYGB (8) has been developed and has provided important information about the various changes in behavior and the endocrine system that occur after surgical manipulation of the gut (13, 33). In spite of this increased attention and previous reports of dehydration after RYGB (10, 22, 24, 26), little research has focused on the effect of these manipulations on water intake and the ability to respond to dipsogenic treatments. This is a potentially important consideration because many patient populations undergoing gastric bypass surgery may be especially likely to develop dehydration because of some relevant comorbidity with obesity that is associated with dehydration (e.g., hyperglycemia).

Given the well-known role of stomach and intestinal feedback in the control of food intake, it may not be surprising that eliminating a portion of the gastrointestinal tract affects food intake, but this mechanical change may not be wholly responsible for the efficacy of the procedure. Indeed, there are many endocrine changes that follow bariatric surgery, and the possibility that these underlie the behavioral changes is a topic of rigorous investigation (1, 3, 11, 13, 23, 33, 37, 45, 47). Although the contributions of mechanoreceptors in the gut and hormonal signaling to the brain are well known to participate in the control of food intake, much less is known about the signals that affect fluid intake satiation. Some parallels seem to exist, however, and recent studies have shown that some of the same gut hormones that affect food intake also have effects on fluid intake (21, 27, 28, 30, 31, 36, 42). The role of feedback from the gut in the control of fluid intake, however, remains poorly understood. Accordingly, models of gastric bypass provide a means to test whether the same type of feedback from the gut that affects food intake also plays a role in the maintenance or termination of water intake.

To test the effect of gastric bypass surgery on water intake, we performed Roux-en-Y gastric bypass on laboratory rats, following a protocol developed previously (8). Daily food and water intakes were measured, and rats were each subjected to three separate dipsogenic treatments: central injection of ANG II, subcutaneous injection of hypertonic saline, and overnight water deprivation. Water intake during the stimulated water intake tests was further analyzed to test for changes in licking patterns that are associated with different orosensory or postingestive feedback.

METHODS

Animals. A total of 18 adult male Sprague-Dawley rats were used in the analysis. Rats were obtained from Harlan Laboratories (Indianapolis, IN). Rats were individually housed in stainless-steel wire mesh cages in a temperature- and humidity-controlled room on a
12:12-h light-dark cycle, and all experiments were performed early in the lights-on phase of the cycle. All experimental protocols were approved by the Institutional Animal Care and Use Committee of the State University of New York at Buffalo, and the handling and care of animals were in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Surgery. Rats underwent RYGB (n = 7) or sham (n = 11) surgery procedures. All rats were food-deprived overnight and then anesthetized by inhaled isoflurane. The RYGB procedure was similar to that described previously (8). Briefly, after preoperative injection of 0.4 ml/kg enrofloxacin and 5 mg/kg ip carprofen, a midline incision was used to access the abdominal cavity. For the RYGB procedure, the small bowel was transected ~20 cm distal to the pylorus of the stomach, creating a proximal and distal end of the small bowel. The proximal end, being still continuous with the remaining portion of the stomach, constituted the biliopancreatic limb and was anastomosed to the ileum ~25–30 cm from the cecum, creating the common channel. For formation of the gastric pouch, the stomach was transected ~5 mm below the gastroesophageal junction, creating a gastric pouch of a size of no more than 2%-3% of original stomach size. The Roux-en-Y reconstruction was completed by connecting the distal end of the small bowel to the gastric pouch, leading to the formation of the alimentary limb. Rats in the sham group received the same median laparotomy, and the stomach and intestine were moved and manipulated, without incision, to approximate portions of the treatment of the rats in the RYGB group. No transections, incisions, or anastomosis were performed on the gut of the rats in the sham group.

Immediately after the RYGB or sham surgical procedures, a subset of rats (RYGB, n = 5; sham, n = 9) were implanted with chronic indwelling cannulas aimed at the lateral ventricle. To this end, rats were anesthetized using a combination of ketamine (70 mg/kg im; Fort Dodge Animal Health, Fort Dodge, IA) and xylazine (5 mg/kg im; Spectrum Chemical, Gardena, CA) before being secured in a stereotaxic apparatus. A small burr hole was drilled, and guide cannulas (26 gauge; Plastics One, Roanoke, VA) were implanted using the following coordinates: 0.9 mm posterior to bregma, 1.4 mm lateral to midline, and 1.8 mm ventral to dura. Cannulas were affixed to the skull with bone screws and dental cement. All rats were maintained on a liquid diet (Ensure) for 3 days, after which standard chow was returned. At least 5 days after surgery, accurate cannula placement was verified by the response to injection of 10 ng ANG II. Rats that drank at least 5 ml of water in 30 min after ANG II were included in subsequent testing.

Intake and body weight measures. Rats were weighed on all week days. Food and water intakes were measured on all week days, and an average daily intake on weekends was calculated by dividing the total weekend intake by two. Total water intake during each measurement period was calculated by the difference in pretest and posttest water bottle weight. Food hoppers were weighed before and after specified intervals, and spillage was collected on a plastic transparency under each cage and was included in the measures as uneaten food.

Stimulated water intake tests. The rats that were not implanted with intracerebroventricular cannula were used for daily intake and body weight measures only, and two rats from the sham group were excluded from a subset of the stimulated water intake tests because of issues with cannula patency.

The other rats were tested for responses to dipsogenic treatments in the following order: intracerebroventricular injection of 10 ng ANG II, subcutaneous injection of hypertonic saline, and overnight water deprivation. These tests began 2–4 wk postsurgery, and each test was separated by 5–7 days.

Injections of ANG II were performed using a Hamilton syringe attached to water-filled PE-50 tubing and were made through a 33-gauge injection cannula fabricated to extend beyond the guide cannula into the lateral ventricle. The ANG II (10 ng) was delivered in 1 ml of TBS, and the injection cannula was left in place for ~30 s after each injection. Rats were given immediate access to water after the injection was complete. For the response to hypertonic saline, a 2-ml subcutaneous bolus of 2 M NaCl was injected immediately after a single injection of 0.1 ml lidocaine (2%) at the same site. Water was removed for 30 min before the intake test began.

Each stimulated water intake test lasted 30 min. Volume was calculated by weighing the bottles before and after each test, and the distribution of intake was determined by counting licks using a contact lickometer (designed and constructed by the Psychology Electronics Shop, University of Pennsylvania, Philadelphia, PA). The lickometer interfaced with a computer using an integrated USB digital I/O device (National Instruments, Austin, TX) and was processed in a MatLab (MathWorks, Natick, MA) software environment before being ported to Excel for final analysis. All measures were collected in the home cage in which water spouts were behind an electrically isolated metal plate with a 3.175 mm-wide opening through which the rat needed to lick to reach the spout, minimizing the possibility of nontongue contact with the spout.

Data analyses. Licking behavior was analyzed to test for differences in the total number of licks and lick patterns. For the latter, analysis of licking bursts, defined as at least two licks with a maximum interlick interval of 1 s, included tests for changes in the number of bursts (burst number) and the average number of licks per burst (burst size). Group differences in the interlick interval were also evaluated. Food and water intake measures were calculated as raw intake and were normalized to body weight. Hypothetical testing was performed using Statistica (StatSoft, Tulsa OK). Body weight and daily intake measures were analyzed by mixed-design two-way ANOVAs testing for within-subject effects of time and between-subjects effects of surgery. Student-Newman-Keuls pairwise comparisons were used to probe any significant main or interaction effects. Student’s t-tests were used for group comparisons in the acute tests. Statistical significance was set at P < 0.05 for all tests.

RESULTS

Body weight. As illustrated in Fig. 1, control rats steadily gained weight after the sham procedure at a rate of 2–5% per week. In contrast, rats in the RYGB group lost ~11% of their presurgery weight in the first week after surgery, after which body weight remained relatively stable for the remainder of the

Fig. 1. Average weekly body weight in sham and RYGB rats. Values are presented as means ± SE body weight of 11 sham and 7 RYGB presurgery and during the weeks after surgery. *P < 0.05.

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A mixed-design, two-way ANOVA testing for a within-subjects effect of time and a between-subjects effect of surgery detected main effects of time ($F_{7,112} = 2.69, P < 0.05$) and surgery ($F_{1,16} = 34.81, P < 0.01$), and a significant time $\times$ surgery interaction ($F_{7,112} = 20.79, P < 0.01$). Student-Newman-Keuls pairwise comparisons found that the group differences were only significant during the first week, when the RYGB group ate significantly less than controls.

**Daily water intake.** Similar to the measures of food intake, we measured daily water intake and calculated average daily intake per week (Fig. 3). The pattern of intake appeared similar to that for food intake, but the statistical analysis revealed no significant main or interaction effects. Analysis of intake data after normalizing to body weight found no significant main effects, but did find a significant time $\times$ surgery interaction ($F_{6,90} = 3.20, P < 0.001$). Student-Newman-Keuls pairwise comparisons, however, did not detect any group differences at any particular time.

**Stimulated water intake tests.** To evaluate the ability of rats with RYGB to respond to dipsogenic treatments, we performed three separate tests on each rat: central injection of 10 ng ANG...
II, subcutaneous injection of 2 ml hypertonic saline (2 M NaCl), and 24-h water deprivation. As shown in Fig. 4, measures of total intake and measures of intake normalized to body weight did not find any differences between RYGB and sham rats.

To further probe the fluid intake by RYGB and sham rats, we analyzed drinking microstructure during the stimulated water intake tests. The results of these analyses are shown in Fig. 5. Consistent with the similar volume consumed by rats in both groups, there were no group differences in the number of licks in any of the tests. Although it seemed reasonable to predict that the surgical manipulation of the gastrointestinal system would affect postingestive feedback, which has been shown to affect the number of discrete bursts of licking (4, 14), our analysis found no group differences in burst number during any of the drinking tests. Likewise, we found no group differences in the number of licks per burst (burst size) or within-burst interlick interval during any of the drinking tests.

**DISCUSSION**

The number of bariatric surgical procedures performed worldwide increased from ~150,000 in 2003 to nearly 350,000 in 2008. According to data from 2011, the total number of procedures appeared to reach a plateau with Roux-en-Y gastric bypass (RYGB) remaining the most common approach, representing 46.6% of all procedures performed in 2011 (7). Many studies on the efficacy and safety of this procedure have been conducted, but to the best of our knowledge, none have specifically focused on fluid intake. One previous study found greater diuresis accompanied by natriuresis and delayed water intake in RYGB rats (9), but the timing of the intake difference in this study suggests that it was secondary to the difference in sodium handling, rather than due to any differences in dipsogenic potency or intake satiation. Our studies used the same rat model and found that RYGB did not prevent normal responsiveness to dipsogenic treatments. Intake by rats in the RYGB group was similar to those by controls. Likewise, we found no striking differences in daily water intake or in the licking patterns during the acute stimulated intake tests. We were able to detect differences in food intake like those shown previously in rats (e.g., Ref. 8) and found that the difference in intake was only present in the first postsurgical week when intake was normalized by body weight.

In addition to testing the ability to respond to dipsogenic treatments after RYGB, the experiments here provide some insight into the signals that terminate fluid intake. Indeed, the mechanisms underlying thirst termination are poorly understood. Interestingly, a perturbation in fluid homeostasis, such as increased plasma osmolality, that triggers drinking behavior and vasopressin secretion, is not restored until after the drinking behavior and vasopressin secretion terminates, arguing that the onset signal is separable from the mechanism controlling satiety. The act of drinking, however, more rapidly suppresses both vasopressin secretion and subsequent drinking. This has been shown in a variety of species, including rats (39), sheep (5), dogs (44), nonhuman primates (2), and humans (19, 35). These rapid effects of drinking on behavior and physiology are similarly shown in studies of sweating in humans (41) and panting in sheep (29). These findings point to something, perhaps outside of the CNS, that detects the restoration of fluid, suggesting that there is some feedback generated by the act of drinking itself. On the other hand, sham drinking, in which a gastric fistula allows ingested fluid to drain from the stomach before causing gastric fill, increases drinking behavior in dehydrated rats (25, 39). This could be seen to support the hypothesis that gastric fill is required for drinking termination, but confining water to the stomach, thereby exaggerating distention, does not decrease behavior (15, 16, 20). This has led to the suggestion that postgastric detection of the ingested water is at least a part of the mechanism responsible for drinking termination.

Postgastric feedback mechanisms play a role in food intake (33) and whether the enhancement of these signals is important
for the reduced intake, glycemic control, and loss of body weight after RYGB remains a controversial and an active area of research (1, 3, 11, 13, 23, 33, 37, 45, 47). Nevertheless, if enhanced gut-brain signaling caused by RYGB is responsible for any observed behavioral changes, it seems reasonable to predict that any enhancement of postgastric signaling related to food intake might be accompanied by enhancement of postgastric signaling related to water intake. The present studies found no support for this prediction, however, because RYGB had no effect on fluid intake after dipsogenic treatments.

In addition to suggesting a lack of enhancement of water-related signals, the present studies provide some clues regarding the anatomical location of any postigestive signals that give rise to fluid intake satiation. Specifically, given the rearrangement of the GI tract by the surgery, the present studies suggest that any postgastric feedback related to fluid intake satiation occurs by interactions with portions of the gut that are distal to the jejunum. How these signals are relayed to the brain to terminate drinking behavior remains an open question.

The experiments here are limited to the effect of RYGB on water intake, but may inform studies on the effect of RYGB on other types of fluid intake. Regulation of saline intake, for instance, appears to use a different satiating mechanism than that employed for water intake, and testing the effect of RYGB on saline intake remains an important area for future study. Indeed, the mechanism underlying saline intake satiety remains controversial, with some studies suggesting a minor, if any, role of postigestive signaling in sodium intake termination (18, 34) and others suggesting that the signaling is primarily postigestive (25, 38, 40). The knowledge that the water component of any saline intake measured is unaffected by the RYGB procedure could help with the interpretation of future studies. The present studies also shed light on a previous study using oral salt loading in RYGB rats (9). As mentioned above, this study found differences in fluid intake 8 h after an oral salt load. Given that our results show normal intake responses to a hypertonic challenge that was not delivered to the bypassed GI tract and given the normal response to the present dipsogenic treatments, we conclude that the differences in intake observed in this previous study are a more direct result of the increased diuresis observed, but not the result of any differences in dipsogenic sensitivity or reduced satiating potency of the consumed water. Recent studies also show that RYGB is associated with increased ethanol intake in rats (17, 43) and humans (12). One of the rat studies, however, found that RYGB was associated with increased water intake (43). We
did not replicate this effect in our studies and, therefore, conclude that the previously observed water intake was either a function of the ethanol provided or the high-fat diet provided to the experimental groups. Nevertheless, the present results suggest that these observed effects on ethanol intake are not due to a more generalized effect on fluid intake.

Perspectives and Significance

RYGB remains a common form of bariatric surgery for the treatment of severe obesity. To the best of our knowledge, the present studies provide the first test of the response to dipso-genic treatments in rats after RYGB. Our findings suggest intact responsiveness to these stimuli and provide some refinement to previous studies implicating that inhibition of drinking relies on postigestive signals.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

Author contributions: A.M. and J.S. performed experiments; A.M., J.S., and C.C. contributed to data analysis; A.M., J.S., C.C., T.A.L., and D.D. edited and revised the manuscript; A.M., J.S., C.C., T.A.L., and D.D. approved final version of manuscript; C.C., T.A.L., and D.D. interpreted results of experiments; J.S. and D.D. prepared figures; C.C., T.A.L., and D.D. conceived and designed research; D.D. drafted manuscript.

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