Celiac vagotomy reduces suppression of feeding by jejunal fatty acid infusions

James E. Cox, CA William J. Tyler, Alan Randich, Gary R. Kelm¹ and Stephen T. Meller¹

Department of Psychology, Campbell Hall, University of Alabama at Birmingham, Birmingham, AL 35294; ¹Procter and Gamble Co., OTC-HCTD, Health Care Research Center, 8700 Mason-Montgomery Rd., Mason, OH 45040, USA

^{CA}Corresponding Author

Received 18 October 2000; accepted 1 February 2001

We investigated the role of the celiac branch of the vagus nerve in suppression of food intake produced by jejunal fatty acids infusions. Following selective celiac vagotomy or sham surgery, adult, male Sprague–Dawley rats received 7 h infusions of linoleic acid or saline through indwelling jejunal catheters on four consecutive days. Although linoleic acid still produced

Key words: Linoleic acid; Rats; Satiety; Small intestine; Vagal afferents

significant suppression of intake in rats with celiac vagotomy, it was less effective in these animals than in controls. The temporal pattern of results suggested that celiac afferent fibers are involved in mediating both pre- and postabsorptive effects of infused fatty acids. *NeuroReport* 12:1093–1096 © 2001 Lippincott Williams & Wilkins.

INTRODUCTION

Subdiaphragmatic vagotomy has been found to abolish suppression of sham feeding produced by duodenal infusion of triglycerides and fatty acids [1,2]. In tests of normal feeding, Novin et al. [3] reported that duodenal triglyceride (peanut oil) infusions did not significantly reduce intake in rats with truncal vagotomy. These outcomes have been interpreted as suggesting a vagally mediated preabsorptive satiating action by lipids within the small intestine [1]. Consistent with anatomical studies showing that the celiac branches provide the predominant vagal innervation of all but the first few cm of the small intestine [4,5], Walls and colleagues [6] reported that celiac deafferentation eliminated suppression of normal feeding resulting from infusion of sodium oleate into the distal duodenum. An intriguing aspect of the latter report is that transection of celiac fibers appeared to eliminate not only the 30 min suppression produced by the infusion but also the observed decrease in cumulative intake measured 24h after lipid administration.

In all of the studies cited above, infusions were of short duration, ≤ 30 min. We have recently investigated suppression of food intake by intestinal infusions of linoleic or oleic acid using a substantially different protocol: slow (0.2 ml/h, 0.027 kcal/min) infusions of neat fatty acids were delivered into the jejunum over a 7h period on 4 consecutive days [7]. The importance of the celiac vagus for the observed suppression was suggested by the observation that multi-unit activity of celiac vagal afferents was increased by jejunal administration of these substances [8]. The aim of the present experiment was to examine the effect of selective celiac vagotomy (CVX) on suppression of short- and long-term intake by linoleic acid in our protocol and, in so doing, assess the generality of the conclusion drawn by Walls and colleagues [6] that celiac afferent fibers mediate the satiating action of lipids within the distal small intestine.

MATERIALS AND METHODS

Animals: Procedures were approved by the Institutional Animal Care and Use Committee at the University of Alabama at Birmingham. Adult, male Sprague–Dawley rats (Harlan) with a mean weight at surgery of 372 g were group housed prior to surgery and housed individually in wire-mesh cages thereafter. They were adapted for ~3 weeks to a nutritionally complete liquid diet (vanilla-flavored Boost, Mead-Johnson, 1 kcal/ml) from graduated sipper tubes in the test chambers, which were constructed from plexiglass cylinders 12 inches in diameter.

Surgery: Before surgery, rats received injections of atropine sulfate (0.15 mg, i.p.) and the antibiotic sulfamethoxazole/trimethoprim (0.2 ml, i.m.) and were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Following laparotomy rats first underwent selective celiac vagotomy or sham surgery. For vagotomy the accessory celiac branch was dissected free of the esophagus immediately after it exited the ventral vagal trunk and sectioned by cauterization. The celiac branch proper was exposed at its exit from the dorsal trunk. It typically consisted of multiple bundles running through the tissue in the angle between the esophagus and the left gastric artery and vein [9]. All presumed branches in that region were cauterized. After vagotomy, a polyurethane catheter (microrenathane 065, Braintree Scientific, Braintree, MA) was inserted into the jejunum 50 cm from the ileocecal junction. It was secured

J. E. COX ET AL.

with two purse-string sutures (6-O silk) around the point of entry. In addition, two sutures were glued to the catheter with cyanoacrylate cement and tied to the serosa. A piece of marlex mesh (Bard, Cranston, RI) was tied over the entry wound to facilitate healing. The other end of the catheter was threaded through an opening in the abdominal wall and then passed subcutaneously to an exit on the dorsal surface of the neck. It was secured to underlying muscles with silk sutures and marlex mesh and capped with monofilament fishing line.

Feeding tests: After surgery, rats were maintained on the liquid diet. Their light:dark cycle was shifted so that lights were out from 10.00 h to 22.00 h. Rats underwent ~3 weeks of additional adaptation in the test chambers, which included jejunal infusions of normal saline (0.2 ml/h for 7 h) from a syringe pump. Swivels in the delivery system allowed rats freedom of movement within the chambers.

Each rat underwent two 4-day sets of feeding tests on alternate weeks, receiving infusions of 60% linoleic acid (Sigma, St. Louis) during one set and normal saline during the other. Order of presentation of infusates was randomized. On each test-day, infusions began at 09.00 h and continued at 0.2 ml/h until 16.00 h for a total load of 1.4 ml (11.5 kcal). Liquid diet was presented at 10.00 h and was continuously available in the test chambers and later in home cages until 09.00 h the next day. Cumulative intake was measured each day 1, 3, 6, and 23 h after its initial presentation.

Verification of celiac vagotomy: Following completion of feeding tests, each rat received an injection of the retrograde tracer True Blue (1 mg, i.p.). After 72 h, it received an overdose of sodium pentobarbital and was perfused transcardially with normal saline followed by 10% phosphatebuffered formalin. The brain was removed and stored overnight in the dark in cold 30% sucrose-formalin. The following day, 40 µm serial coronal sections were taken through the medulla at the level of the area postrema (AP). Sections were subsequently examined using a fluorescence microscope. As expected, the dorsal motor nuclei of rats with celiac vagotomy exhibited a truncated appearance because of lack of label in the lateral-most aspect of the nucleus, which contains neurons giving rise to celiac-branch axons [10]. The following procedure was followed to provide a quantitative index of loss of label in laterally placed cells. In each rat, six coronal sections were selected: the two most rostral sections containing the AP, the two most caudal sections containing the AP, and the two sections midway between the rostral and caudal pairs. On the right and left sides of each section, we counted labeled neurons lateral to the medial border of the solitary tract (Fig. 1 in [5]). Mean numbers of cells/section were calculated for each side of the brain.

Data analysis: Means were compared with mixed between- and within-subjects analysis of variance (ANOVA) or independent-groups *t*-tests. Holm's test was used as a multiple-comparison procedure [11,12] (test statistic = t').

RESULTS

Verification of celiac vagotomy: There was an unambiguous between-group difference in numbers of labeled dorsal

motor nucleus neurons lateral to the medial boundary of the solitary tract. For both sides of the brain, the distributions of numbers of labeled cells lateral to the solitary tract did not overlap. In controls, the cluster of labeled cells was typically continuous to a point beyond this landmark, resulting in relatively high cell counts (means \pm s.e.m. = 3.60 ± 0.08 and 3.08 ± 0.18 for left and right, respectively). In rats with celiac vagotomy, labeled cells lateral to this boundary were either absent or were outliers, clearly separated from the medial cluster (0.60 ± 0.18 and 0.29 ± 0.11 for left and right, respectively). The decrease in the CVX group was significant (t(15) = 15.85, p < 0.0001).

Rats in the CVX group also showed evidence of damage to the dorsal gastric branch of the vagus. The right-side gastric column of the dorsal motor nucleus exhibited varying degrees of reduced labeling compared with the left. To assess the importance of this damage, the degree of depletion was categorized as none-to-moderate *vs* severe and point-biserial correlations were calculated between depletion and mean linoleic acid-induced suppression of intake at 1, 3 and 6 h as well as total suppression across the 4-day experiment. None of these correlations was significant (all p > 0.05, and all correlations were in the direction of predicting greater suppression from greater depletion). Parenthetically, when correlations were computed between depletion and baseline intakes at the various time points, none approached significance (all p > 0.50).

Short-term intake: In Fig. 1 data are represented as mean suppression calculated from difference scores between tests with saline and linoleic acid administration. Suppression of intake at 1, 3, and 6 h is shown for individual days, as is mean suppression pooling across the four test days. Linoleic acid suppressed intake in both groups, but the degree of suppression was generally less in rats with celiac vagotomy. At 1 h, intake was significantly reduced in controls on 2 of the 4 days of testing (p < 0.05) but was not significantly affected on any individual day in the CVX group (all p > 0.05). At both 3 and 6 h intake was significantly reduced by fatty acid infusions on every day in controls vs 3 out of 4 days in vagotomized rats. On the other hand, when data were pooled across days, linoleic acid significantly suppressed intake at all three times in both groups (all p < 0.05). Comparisons of degree of suppression in the two groups indicates consistently lower mean values in the CVX group, but the difference in pooled mean suppression was significant only at 6 h (t'(16) = 3.96, p < 0.005). However, it should be noted that for the 3h point, ANOVA revealed significant variation in the between-group difference across days (F(3,48) = 2.90,p < 0.05), entailing comparisons on individual days; rats with celiac vagotomy exhibited significantly less suppression than controls on Day 2 (t'(16) = 3.07, p < 0.02) but not on the other 3 days (p > 0.05).

Long-term intake: As shown in Fig. 2, celiac vagotomy reduced suppression of cumulative intake produced by jejunal infusions of linoleic acid on 4 consecutive days. ANOVA revealed a significant group × infusate × time interaction (F(15,240) = 4.06, p < 0.0001). A subsequent test of differential linear trends indicated that the difference in slopes between saline and linoleic acid tests was smaller in



Fig. 1. Mean differences in cumulative intake between linoleic acid and saline tests for rats in the control (open bars) and celiac vagotomy (CVX; cross hatching) groups after 1, 3 and 6 h access to liquid diet. Values are shown for individual days and pooling across days (Mean). Error bars indicate s.e. Baseline intakes did not differ significantly between groups at any time point (all p > 0.20). * p < 0.05, and ** p < 0.01 for comparisons between saline and linoleic acid.



Fig. 2. Cumulative intake across four days in the control and celiac vagotomy groups on tests with daily 7h infusions of saline (open circles) or linoleic acid (closed circles). Baseline intakes did not differ significantly between groups (p > 0.25).

the celiac vagotomy group (82.4 and 74.7 ml/day, respectively) than in controls (87.9 and 69.3 ml/day, respectively; F(1,16) = 5.30, p < 0.05); this contrast accounted for 92.4% of the interaction sum of squares. Total cumulative suppression was 57% less in rats with vagotomy (32.0 ± 12.7 kcal) than in controls (74.3 ± 16.0 kcal; t(16) = 2.07, p < 0.05). None the less, significant suppression was still present after the celiac branches were severed (t(10) = 2.52, p < 0.05).

DISCUSSION

Our results are consistent with a role of fibers in the celiac branches of the vagus in the satiating action of intestinal lipid infusions. Selective celiac vagotomy significantly attenuated both short- and long-term suppression of intake produced by linoleic acid. However, significant suppression remained, indicating that these branches are not the sole mediators of this suppression.

With regard to short-term intake (i.e. intake during infusions), the importance of celiac fibers was most evident in the 6h observations. At that time, the amount by which infusions reduced intake was significantly attenuated by celiac vagotomy; mean suppression in the CVX group (8.9 kcal) was only 39% of that seen in controls (22.2 kcal). Observed effects of vagotomy were less compelling for the shorter intervals, but the overall pattern of results suggested reduced effectiveness of linoleic acid in the absence of celiac fibers. In conjunction with our previous report of activation of celiac afferents by jejunal fatty acid infusions [8], these outcomes provide qualified support for the hypothesis that these fibers mediate the preabsorptive satiating action of lipids within the intestine [1,6]. It is noteworthy that our infusion protocol addressed two criticisms that have been directed at previous studies. First, the infusate we used was a free fatty acid and not a sodium salt and was, therefore, unlikely to produce intestinal damage [13]. Second, the infusion rate we used (0.027 kcal/ min) can be considered physiological based on comparison with published estimates of the rate of gastric emptying of lipids [14-16]. These procedures increase the likelihood that our results are relevant to satiation normally engaged by ingestion of lipids.

Our observation that celiac vagotomy attenuated suppression of cumulative intake across the 4-day test period is in agreement with the observation by Walls and colleagues [6] that celiac fibers play a role in the long-term (in their case 24 h) effect of fatty acid infusions. However, interpretation of these outcomes in terms of the celiac innervation of the intestine appears problematic because current conceptions of the role of vagal innervation of the gastrointestinal tract in satiation propose that vagal afferents mediate short-latency effects of intraluminal substances [1]. In this view, longer-term effects of infused or ingested nutrients involve postabsorptive actions. Thus, we speculate that our results indicate that, in addition to preabsorptive effects within the intestine, celiac afferents mediate the satiating action of lipids on the liver. Friedman and colleagues have argued that inhibitory effects of lipid ingestion involve alterations in hepatic metabolism and develop slowly, over a period of several hours [14,15]. Furthermore, and although the major vagal innervation of the liver is provided by the hepatic branch, anatomical and electrophysiological studies have suggested that some afferent fibers from the liver travel in the celiac branch [17-19]. In addition, one study found that inhibition of feeding resulting from i.p. epinephrine, presumed to result from action on the liver, was attenuated by celiac vagotomy [20].

The residual suppression of intake that we observed in rats with celiac vagotomy was probably mediated, a least in part, by afferent fibers of the hepatic vagal branch. Although its intestinal innervation is concentrated in the proximal duodenum, hepatic-branch fibers apparently supply the entire small intestine [21]. These fibers provide a likely substrate for preabsorptive action of infused fatty acids. By the same token, a number of studies have suggested that hepatic branch afferents are involved in inhibition of feeding resulting from post-prandial changes in hepatic metabolism [22–25].

CONCLUSION

Food intake is suppressed by infusion of lipids into the small intestine. Previous studies, primarily employing short-duration (e.g. 30 min) infusions, have established the importance of the vagus nerve for this effect. Results of one study [6] pointed in particular to a role for afferent fibers of the celiac branches of the vagus. Our results extend this last finding by demonstrating that selective celiac vagotomy attenuated the effectiveness of long-duration (7h) infusions of linoleic acid given on 4 consecutive days. We interpret this result as suggesting a role for celiac afferents in both the preabsorptive and postabsorptive actions of this infusate by virtue of its intestinal and hepatic innervation, respectively.

REFERENCES

- 1. Greenberg D and Smith GP. Psychosom Med 58, 559-569 (1996).
- 2. Yox DP, Stokesberry H and Ritter RC. Am J Physiol 260, R503–R508 (1991).
- 3. Novin D, Sanderson J and Gonzalez M. Physiol Behav 22, 107–113 (1979).
- Berthoud HR, Carlson NR and Powley TL. Am J Physiol 260, R200–R207 (1991).
- 5. Phillips RJ and Powley TL. Am J Physiol 274, R1626-R1638 (1998).
- Walls EK, Phillips RJ, Wang FB, Holst MC and Powley TL. Am J Physiol 269, R1410–R1419 (1995).
- 7. Cox J, Tyler WJ, Randich A et al. Am J Physiol 278, R604-R610 (2000).
- 8. Randich A, Tyler WJ, Cox JE et al. Am J Physiol 278, R34-R43 (2000).
- 9. Prechtl JC and Powley TL. J Comp Neurol 235, 182-195 (1985).
- 10. Powley TL, Fox EA and Berthoud HR. *Am J Physiol* **253**, R361–R370 (1987).
- 11. Howell DC. Statistical Methods for Psychology, 4th edn. Belmont, CA: Duxbury; 1997.
- 12. Seaman MA, Levin JR and Serlin RC. Psychol Bull 110, 577-586 (1991).
- Ramirez I, Tordoff MG and Friedman MI. Am J Physiol 273, R1779–R1785 (1997).
- 14. Friedman, MI, Ramirez I and Tordoff MG. Am J Physiol 270, R688–R692 (1996).
- 15. Horn CC, Tordoff MG and Friedman MI. *Am J Physiol* 270, R761–R765 (1996).
- Maerz LL, Sankaran H, Scharpf SJ and Deveney CW. Am J Physiol 267, R1163–R1167 (1994).
- 17. Berthoud HR, Kressl M and Neuhuber WL. Anat Embryol 186, 431–442 (1992).
- 18. Carobi C, Della Torre G and Magni F. Neurosci Lett 62, 255-260 (1985)
- Rogers RC, Kahrilas PJ and Hermann GE. J Autonom Nerv Syst 11, 223–225 (1984).
- 20. Tordoff MG and Novin D. Physiol Behav 29, 605-613 (1982).
- Phillips RJ, Baronowsky EA and Powley TL. J Comp Neurol 384, 248–270 (1997).
- 22. Boutellier S, Lutz TA, Volkert M and Scharrer E. Am J Physiol 277, R301–R305 (1999).
- 23. Friedman MI. Am J Clin Nutr 67, 513S-518S (1998).
- 24. Langhans W and Scharrer E. J Autonom Nerv Syst 18, 13-18 (1987).
- 25. Lutz TA, Diener M and Scharrer E. Am J Physiol 273, R442-R445 (1997).

Acknowledgments: This work was supported by an award from The Procter and Gamble Co. to J.C.