
James P. Warne, Michelle T. Foster, Hart F. Horneman, Norman C. Pecoraro, Hanna K. de Jong, Abigail B. Ginsberg, Susan F. Akana, and Mary F. Dallman

Department of Physiology, University of California San Francisco, San Francisco, California

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Warne JP, Foster MT, Horneman HF, Pecoraro NC, de Jong HK, Ginsberg AB, Akana SF, Dallman MF. The gastroduodenal branch of the common hepatic vagus regulates voluntary lard intake, fat deposition, and plasma metabolites in streptozotocin-diabetic rats. Am J Physiol Endocrinol Metab 294: E190–E200, 2008. First published October 30, 2007; doi:10.1152/ajpendo.00336.2007.— The common hepatic branch of the vagus nerve negatively regulates lard intake in rats with streptozotocin (STZ)-induced, insulin-dependent diabetes. However, this branch consists of two subbranches: the hepatic branch proper, which serves the liver, and the gastroduodenal branch, which serves the distal stomach, pancreas, and duodenum. The aim of this study was to determine whether the gastroduodenal branch specifically regulates voluntary lard intake. We performed a gastroduodenal branch vagotomy (GV) on nondiabetic, STZ-diabetic, and STZ-diabetic insulin-treated groups of rats and compared them with sham-operated counterparts. All rats had high steady-state corticosterone levels to maximize lard intake. Five days after surgery, all rats were provided with the choice of chow or lard to eat for another 5 days. STZ-diabetics resulted in a reduction in lard intake that was partially rescued by either GV or insulin treatment. Patterns of white adipose tissue (WAT) deposition differed after GV- and insulin-induced lard intake, with subcutaneous WAT increasing exclusively after the former and mesenteric WAT increasing exclusively in the latter. GV also prevented the insulin-induced reduction in the STZ-elevated plasma glucagon, triglycerides, free fatty acids, and total ketone bodies but did not alter the effect of insulin-induced reduction of plasma glucose levels. These data suggest that the gastroduodenal branch of the vagus inhibits lard intake and regulates WAT deposition and plasma metabolite levels in STZ-diabetic rats.

Insulin; glucocorticoids; obesity; diabetes; food intake

The common hepatic branch of the vagus nerve consists of fibers directly serving the liver parenchyma, bile ducts, and portal vein, collectively called the hepatic branch proper, with those serving the duodenum, pancreas, pylorus, and distal gastric antrum, collectively known as the gastroduodenal branch (6). Signaling through the common hepatic branch is important in energy metabolism since the fibers are sensitive to a variety of hormonal and metabolic stimuli (4), including carbohydrates (33), fats (42), and amino acids (51).

When rats are presented with the choice of lard and chow to consume, the total number of calories ingested is influenced by prevailing corticosterone levels, whereas insulin influences the composition of calories ingested (26, 40). Specifically for insulin, streptozotocin (STZ)-induced insulin-dependent diabetes reduces lard intake to favor chow, a situation that is reversed by exogenous insulin treatment (26, 54, 56). STZ-diabetes also results in behavioral, autonomic, endocrine, and neuroendocrine characteristics of chronic stress in rodents (47). Hence, the levels of corticosterone and insulin, and the ratio of the two, profoundly influence food intake, notably under conditions of chronic stress (12).

Studies using common hepatic branch vagotomy (HV) surgery have shown the common hepatic branch of the vagus nerve to be important in the regulation of food intake. In STZ-diabetic high-corticosterone rats, HV mimics the actions of insulin in restoring voluntary lard intake to nondiabetic levels, suggesting that, independent of insulin, the hepatic vagus nerve ordinarily exerts an inhibitory influence on lard intake (54). Subsequent studies utilizing the afferent-specific neurotoxin capsaicin (53) have revealed that afferent signaling through the common hepatic branch is key to this phenomenon. HV also abolishes the stimulatory actions of mercaptoacetate, an inhibitor of fatty acid oxidation, on food intake (29, 49) and prevents the lard-induced inhibition of food intake and changes in neuropeptide expression arising from STZ-induced, insulin-dependent diabetes (27, 28). Collectively, these data show that the common branch of the hepatic vagus regulates lard intake and hepatic fatty acid content.

Despite the fact that the majority of fibers within the common hepatic branch originate from the gastroduodenal branch (5), few studies have compared the contribution of signaling through the gastroduodenal branch to that of the hepatic branch proper to the effects mediated by the common hepatic branch. One strategy to explore this is to compare the effects of HV with specific gastroduodenal branch vagotomy (GV) to infer the role of the hepatic branch proper. This has been done successfully in studies examining hormone-stimulated nerve activity (22), and comparison of HV with GV (27) revealed that lard-induced inhibition of diabetic hyperphagia is regulated through the hepatic branch proper.

We sought to assess the role of vagal signaling through the gastroduodenal branch in the regulation of lard intake. These findings could then be compared with those previously obtained after HV (54) to deduce the involvement of the gastroduodenal branch vs. the hepatic branch proper in lard intake behavior as well as the involvement of the branch in regulation of insulin-sensitive hormones (leptin, glucagon), metabolites [triglycerides, free fatty acids (FFA), glycerol, total ketone
bodies], and liver glycogen levels and white adipose tissue (WAT) depot weights.

MATERIALS AND METHODS

Male rats (Sprague-Dawley; Simonsen, Gilroy, CA) weighing 30 ± 2 g were housed individually in hanging wire cages in a temperature- (22°C) and light-controlled (lights on 0600–1800) room. Rats were allowed to adapt to their new environment for 4 days before experimentation. All experimental procedures were approved by the University of California San Francisco Institutional Animal Care and Use Committee. The rats had ad libitum access to pelleted rat chow (Purina Chow no. 5008, 3.31 kcal/g; Purina, St. Louis, MO) and water throughout the experiment.

Preliminary study. To illustrate the importance of maintaining equal corticosterone concentrations across all groups, a preliminary study was performed comparing rats with high steady-state plasma corticosterone levels that correspond to near-circadian maximum concentrations (1) to rats that had free running, unclamped corticosterone levels. The two groups were prepared as follows. For the high-steady-state corticosterone group (“high”; n = 6), the rats received a subcutaneous pellet of corticosterone, outlined below for the main experiment. These rats were allowed 5 days to recover after surgery (day 0) and then presented diet choices of chow, lard, and 32% sucrose solution (4 kcal/g dry wt; Safeway, Pleasanton, CA) for 5 days until they were killed by decapitation. This group was compared with a group of rats with unclamped, free-running corticosterone (“free”; n = 4) that had no surgical manipulations and were offered only chow to eat throughout the study. The treatment schedule was identical to that of the main experiment.

The results, presented in Fig. 1, show that high corticosterone is associated with increased (P < 0.01) insulin (Fig. 1A) and leptin (Fig. 1C) concentrations and mesenteric WAT (mWAT) weight (Fig. 1F) and decreased (P < 0.01) adrenal (Fig. 1D) and thymus (Fig. 1E) weights. Interestingly, plasma glucose (free vs. high, 103.9 ± 1.5 vs. 109.9 ± 5.0 mg/dl) and subcutaneous WAT (scWAT) weight (Fig. 1F) were identical. The corticosterone/insulin ratio between the two groups, however, was also identical (Fig. 1B). Similar results were obtained comparing citrate-injected, corticosterone-clamped rats with untreated, unclamped rats. Both were both presented with diet choices 5 days after surgery (day 0); however, in this instance the choice period was 7 days as opposed to 5 days (data not shown). A linear corticosterone-insulin relationship has similarly been demonstrated (13, 26) in adrenalectomized rats replaced with a range of steady-state corticosterone levels. Hence, to alter the corticosterone-insulin relationship requires manipulation of both corticosterone and insulin levels. This is achieved through the use of corticosterone pellets to produce steady-state levels, as above, and through the use of STZ to destroy pancreatic β-cells coupled with steady-state exogenous insulin treatment via osmotic minipumps. This strategy was used in the main experiment.

Main experiment. In the rat model utilized in the main experiment, plasma corticosterone was maintained at steady-state levels that correspond to near-circadian maximum concentrations (1), which stimulate lard intake (26). Voluntary lard intake was further manipulated by treatment with STZ, which reduces intake (26, 56). Thus, these manipulations produce a spectrum of lard intake that is insulin sensitive and is of a magnitude sufficient to reveal the effects of our experimental manipulations. Implantation of a corticosterone pellet...
also controls for any STZ-induced changes in corticosterone levels (46, 47) that would confound interpretation of the effects of insulin and GV on food intake. The superior mesenteric vein was chosen as the site of venous insulin treatment, as infusion from this location is similar to the ordinary path of insulin from the pancreas and has been shown (54, 56) to robustly promote lard intake. The dose of 3 U insulin/day was selected since it reliably promotes lard intake and can produce detectable changes in plasma insulin and liver glycogen levels in STZ-diabetic rats (54, 56). It was not necessary for this dose to control the diabetes but only to facilitate lard intake, since glucose has been indirectly precluded in our other studies (26, 56) as the insulin-sensitive signal that regulates lard intake.

**Surgical procedures and treatments.** The experimental design expanded upon a previously reported model (54, 56) with an identical treatment timetable but, in this instance, manipulation of the gastroduodenal branch of the common hepatic vagus. All procedures were performed in one surgery (day 0). All rats were anesthetized with ketamine (75 mg/kg im) and xylazine (10 mg/kg im). All rats received a 100 mg pellet of corticosterone (100%; Steraloids, Newport, RI) that was implanted subcutaneously through a small incision in the back (1). Rats were the divided into the following six groups (n = 6/group) for appropriate surgical manipulations: citrate injected (“citrate”), sham operated; citrate, GV operated; STZ-injected, saline infused (“STZ-saline”), sham operated; STZ-saline, GV operated; STZ injected, insulin infused (“STZ-insulin”), sham operated; and STZ-insulin, GV operated.

After all appropriate surgical manipulations were performed, all incisions were closed using silk suture, and ketoprofen (10 mg/kg sc) was administered as a postsurgical analgesic. The rats were then allowed 5 days to recover, during which incisions, body weight, and food and water intake were monitored daily at 1000. On day 5, all rats were also presented with an ad libitum supply of lard (9 kcal/g; Armour, Omaha, NE) at 1000 in a metal cup. Body weight and solid and liquid intakes were monitored daily (at 1000) for an additional 5 days. On day 10, all rats were killed by decapitation 4 h after lights on at 1000, and samples were quickly collected.

**Gastroduodenal vagotomy.** A midline incision was made, and the common hepatic vagal branch was visualized under a dissecting microscope (>10×; 20× magnification), as it separated from the left vagal trunk and followed down to the duodenum. All cephalad subbranches originating from this branch on top and above the duodenum, pylorus, and distal gastric antrum were transected in accordance with previous studies (27). Other tissues were held out of the field of view by saline-soaked sterile gauze, which also prevented drying of the viscera. The gastroduodenal branch runs alongside the gastroduodenal artery. The transaction procedure involved in a GV frequently involved damage to this artery. Although this is an unavoidable consequence of the procedure, studies (22) have shown that damage to this artery has little impact on blood flow to the duodenum and liver. The sham-operated groups underwent all procedures except for the transection of the neural tissue. The transected gastroduodenal branch was visualized under a dissecting microscope at death; in all cases the branch appeared cut. With the exception of mechanical probing of the duodenum and measurement of activity within the common branch of the hepatic vagus (22), which should be blocked by GV, there are no other documented means to verify the completeness of the surgery. This, therefore, represents a limitation for such a neural transaction procedure and thus a consideration when interpreting the results.

**STZ-induced diabetes and insulin replacement.** Insulin-dependent diabetes was induced by a subcutaneous injection of STZ (65 mg/kg in 2 ml/kg citrate buffer, pH 4.2; Sigma Chemical, St. Louis, MO). The rats in the nondiabetic groups were injected with 2 ml/kg citrate buffer. Insulin (3 U/day, Humulin R U500; Eli Lilly, Indianapolis, IN) or saline was infused into the superior mesenteric vein of STZ-treated rats via catheters (PE5 tubing, 1.5 cm, fused to PE60 tubing, 1.5 cm) attached to osmotic minipumps (Alzet, model 2002; Alza, Palo Alto, CA) as previously described (56). Briefly, the cecum was externalized onto saline-soaked gauze, and the superior mesenteric vein was identified and gently exposed. The catheter was then quickly inserted into the exposed vein and sealed into place using sterile glue (Vetabond; 3M Animal Care Products, St. Paul, MN). The cecum and osmotic minipump were then quickly internalized.

**Sample collection.** After the rats were killed, trunk blood was collected into chilled tubes containing 0.1 liters of EDTA (65 mg/ml). Tubes were centrifuged, and plasma was collected and stored at −80°C. Liver biopsies (100–200 mg) were quickly collected from the left lateral lobe, snap-frozen, and stored at −80°C. Position of each catheter and osmotic minipump was verified upon dissection. In all cases, one end of the catheter was attached to the minipump and the other end securely inserted into the superior mesenteric vein.

The WAT depots [scWAT, mWAT, epididymal WAT (eWAT), and perirenal WAT (pWAT)], stomach (with contents), thymus, adrenals, and spleen were subsequently removed, cleaned, and weighed. The WAT depots were defined anatomically as follows. The scWAT depot consisted of all WAT under the skin of the right side of the carcass from anterior to posterior midlines, including the inguinal fat pad, from the neck to the base of the tail in rostrocaudal extent. The eWAT depot comprised all of the discrete WAT that is attached to the cranial pole of the testis, surrounding the caput epididymidis and spermatic cord. The mWAT depot comprised all of the WAT attached to the digestive tract from the lower esophagus to the rectum. The pWAT depot consists of all of the WAT extending from the diaphragm, surrounding the kidney and adrenal, and extending caudally to the floor of the pelvis.

**Plasma and liver assays.** Plasma corticosterone, insulin, glucagon, and leptin concentrations were measured using commercially available radioimmunoassays (MP Biomedicals, Orangeburg, NY; and Linco Research, St. Charles, MO). Plasma glucose, triglycerides, glycerol, total ketone bodies, and FFA were measured colorimetrically using commercially available kits (Mega Diagnostics, Los Angeles, CA; Sigma-Aldrich, St. Louis, MO; and Wako Chemicals, Neuss, Germany). Plasma glucose-dependent insulintropic peptide (GIP; also known as gastric inhibitory peptide) was measured using a commercially available enzyme-linked immunosorbant assay kit (Linco Research). Liver glycogen was extracted and measured using a colorimetric assay outlined previously (31), and values were standardized to milligrams wet liver weight. All assays were as previously described (26, 56).

**Statistical analyses.** All data are presented as means ± SE. Data from the preliminary experiment were analyzed with the unpaired Student’s t-test. Data from the main experiment were analyzed by two-way ANOVA (factors being diabetic status and GV vs. sham). Significant (P < 0.05) effects were followed by post hoc tests of individual group differences (Tukey’s test). Where appropriate, repeated-measures ANOVA was employed to monitor changes with time.

**RESULTS**

Plasma insulin and corticosterone concentrations are presented in Fig. 2, A and B, respectively. The results clearly show that STZ significantly (P < 0.01) reduces plasma insulin levels. Insulin treatment to STZ-treated rats raises plasma insulin levels (P < 0.05), although not to nondiabetic levels (Fig. 2A). GV surgery did not modify circulating insulin levels. Plasma corticosterone concentrations (Fig. 2B) were not significantly different among any of the experimental groups. Fig. 2C shows the corticosterone/insulin ratio, which was greatest (P < 0.05) in the STZ-saline groups, followed by the STZ-insulin groups. The citrate groups had significantly (P < 0.05)
Table 1) were small and unaffected by any experimental manipulation. Thymus weights (Table 1) were also low but were further decreased by STZ treatment, as revealed by two-way ANOVA ($P < 0.05$). Total stomach and content weight (Table 1), which is elevated by subdiaphragmatic trunkal vagotomy (25, 43, 48), was unchanged with any experimental manipulation. This confirms the specificity of our neural transactions around the stomach and emphasizes that cutting the gastroduodenal branch of the vagus does not cause food retention, which might affect intake, in our experimental conditions.

As shown in Fig. 3, all rats initially lost weight. The STZ treatment further exacerbated the corticosterone-induced weight loss with no effect of insulin treatment, as previously documented (56), or GV surgery. All rats gained weight upon provision of the lard. Collectively, the findings presented in Figs. 2 and 3 and Table 1 show the long-term efficacy of STZ treatment, insulin treatment, and steady-state corticosterone levels shown by body and tissue weights and exemplified by plasma insulin and corticosterone measurements at the end of the experiment.

Food intake is presented in Fig. 4. Chow intake (Fig. 4A) rose after surgery, and intake of the STZ-treated groups exceeded that of the citrate injected from day 4 onward. Provision of lard caused a reduction in chow intake in the citrate-treated groups and a transient drop in chow intake in the STZ-treated groups. Total chow intake for the last 5 days of the experiment (Fig. 4B) shows, in the sham-operated groups, that the elevation in chow intake was partially reduced by insulin treatment in the STZ-treated rats. GV surgery did not affect chow intake in any of the groups; however, the normally inhibitory effect of insulin on chow intake was negated when comparing the STZ-diabetic saline and insulin-infused, GV-operated groups.

Lard intake is shown in Fig. 4C. On the first day of presentation, all groups consumed the lard. Thereafter, as exemplified by the total, 5-day, lard intake (Fig. 4D), STZ treatment resulted in a drastic reduction in intake that was significantly ($P < 0.05$) attenuated by GV or insulin treatment. The GV surgery did not modify lard intake of the citrate-injected, nondiabetic group nor did it affect insulin treatment-induced lard intake. Total caloric intake (Fig. 4C), however, was significantly ($P < 0.001$) increased by STZ treatment but unaffected by insulin treatment or GV surgery.

STZ treatment, insulin treatment, and GV surgery modified WAT weights (Fig. 5). Compared with the citrate-injected (nondiabetic) groups, STZ treatment significantly reduced the weight of all fat pads examined. Both GV- and insulin-induced lard intake resulted in significant ($P < 0.05$) increases in eWAT (Fig. 5A) and pWAT (Fig. 5B) weight. However, only insulin infusion elevated mWAT ($P < 0.05$; Fig. 5B) weight, and GV surgery of the STZ-saline group exclusively elevated scWAT weight ($P < 0.05$; Fig. 5D). These changes in WAT weight also impacted plasma leptin, a WAT-derived adipokine. Specifically, STZ treatment resulted in a significant reduction ($P < 0.001$) in plasma leptin levels, paralleling the reduction in WAT weight. This reduction in plasma leptin levels was significantly ($P < 0.05$) attenuated by either GV or insulin infusion, reflecting the increases in WAT weight under these conditions.

Plasma glucagon, GIP, glucose, and liver glycogen are presented in Fig. 6. STZ treatment resulted in significantly ($P < 0.05$) elevated plasma glucagon (Fig. 6A) and glucose
Fig. 6D) and reduced liver glycogen content (Fig. 6C). Insulin treatment served to fully restore liver glycogen content and plasma glucagon levels and partially restored the levels of glucose to nondiabetic levels. Although GV did not alter the effects of STZ (saline infused), the surgery did significantly (P < 0.05) prevent the actions of insulin on liver glycogen content and plasma glucagon without affecting plasma glucose levels. Plasma GIP levels were unaffected by any experimental manipulation (Fig. 6B).

Figure 7 shows the plasma fatty acid levels. STZ treatment (saline infusion) resulted in significantly (P < 0.05) elevated total ketone bodies (Fig. 7A), triglycerides (Fig. 7B), and FFA (Fig. 7C) concentrations, which were unaffected by GV surgery. Insulin treatment served to fully restore plasma triglycerides, and FFA and partially restored the levels of total ketone bodies to nondiabetic levels, effects that were prevented by GV surgery. Two-way ANOVA revealed a significant effect of insulin treatment on glycerol levels (P < 0.05; Fig. 7D) that were otherwise unaffected by GV surgery. Specifically, post hoc analysis revealed that the STZ-insulin-infused, sham-operated group was significantly (P < 0.05) lower than the STZ-saline-infused groups.

**DISCUSSION**

Collectively, these results show that, in STZ-diabetic rats, GV partially restores lard intake to nondiabetic levels without affecting total caloric intake, similar to the effects of insulin treatment. However, lard intake-associated increases in WAT depot weights differed with the various experimental manipulations.

**Table 1. Spleen, thymus, adrenal, and stomach and content weights**

<table>
<thead>
<tr>
<th></th>
<th>Citrate</th>
<th>STZ-Saline</th>
<th>STZ-Insulin</th>
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<tr>
<td></td>
<td>Sham</td>
<td>GV</td>
<td>Sham</td>
</tr>
<tr>
<td>Spleen</td>
<td>1.88±0.04a</td>
<td>1.95±0.06a</td>
<td>2.27±0.11b</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.44±0.06a</td>
<td>0.46±0.05a</td>
<td>0.35±0.05ab</td>
</tr>
<tr>
<td>Left adrenal</td>
<td>0.054±0.002</td>
<td>0.053±0.001</td>
<td>0.063±0.004</td>
</tr>
<tr>
<td>Right adrenal</td>
<td>0.053±0.002</td>
<td>0.053±0.001</td>
<td>0.063±0.004</td>
</tr>
<tr>
<td>Stomach and contents</td>
<td>12.64±1.07</td>
<td>15.23±3.05</td>
<td>17.25±2.67</td>
</tr>
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</table>

Values are means ± SE and in mg/g body STZ, streptozotocin; GV, gastroduodenal branch vagotomy. Efficacy of induction of STZ-diabetes and insulin infusion is shown in the spleen and thymus weights. Steady-state corticosterone infusion is shown by the terminal plasma levels and the low weight of the adrenals and thymi. Stomach and content weight is sensitive to subdiaphragmatic trunkal vagotomy; however, GV does not alter this parameter. Different letters indicate statistically significant (P < 0.05) differences (a is different from b, but neither are different from ab, etc.).
lations. Intake after GV resulted in increased scWAT weight, whereas intake during insulin treatment resulted in increased mWAT weight, and both increased pWAT and eWAT weight. GV also prevented the actions of insulin treatment to STZ-diabetic rats on plasma glucagon, total ketone bodies, triglycerides, and FFA and liver glycogen content, interestingly, without affecting the actions of insulin on plasma glucose levels. Comparison of these data with those after HV in an otherwise identical model (53, 54) revealed both similarities and differences between the outcomes of these two neural

Fig. 4. Intake of chow (A and B) and lard (C and D) and total calories (E and F). Data are presented as daily changes in intake (A, C, and E) and caloric intake (B, D, and F) during the last 5 days of the study (choice period), calculated by the sum of the daily caloric (in kcal) intake/100 g body wt. Insulin infusion served to partially reduce the STZ-induced increase in chow intake, an effect that was negated by GV. Lard intake was reduced by STZ treatment, which was prevented by either GV or insulin treatment. Total caloric intake was elevated by STZ treatment but unaffected by either GV or insulin treatment. Different letters indicate significant (P < 0.05) differences among treatment groups.
transection procedures (diagrammatically illustrated in Fig. 8) and outlined for all variables in Table 2.

We have no independent verification of the completeness of our GV other than the effects of the surgery that are reported here as well as the observation that the cut nerve ends were still separated at the end of the experiment. However, the differences in the results from GV surgery compared both with sham-operated groups and with the others we have performed in this series of experiments (53–56) suggests that the gastroduodenal branch of the vagus was, indeed, severed.

For all variables examined, nondiabetic rats showed no effect of GV. Key to revealing the role of the gastroduodenal branch of the vagus could be the metabolically important corticosterone/insulin ratio (50). The nondiabetic rats with high steady-state corticosterone levels had elevated circulating insulin levels compared with levels expected for intact, naïve rats with freely secreted corticosterone. Manipulating insulin levels with STZ independent of changes in corticosterone tips this otherwise self-adjusting balance (exemplified in Fig. 1), which reveals the importance of the gastroduodenal branch of the vagus was, indeed, severed.

Our studies expand upon those (53, 54) that have shown that, under conditions of STZ-diabetes and high corticosterone, the common branch of the hepatic vagus normally inhibits lard intake without affecting total caloric intake. Our study shows that it is the gastroduodenal branch, not the hepatic branch proper, that regulates this phenomenon. In contrast, lard-induced inhibition of food intake in STZ-diabetic (but not corticosterone-clamped) rats is attenuated by HV but not GV (27, 28). Hence, the two component branches of the common branch of the hepatic vagus clearly act differently, with the gastroduodenal branch regulating lard intake and the hepatic branch proper responding to lard intake.

Approximately 30% of the fibers in the common hepatic branch are of nonvagal origin (41), which might be sympathetic postganglionic or dorsal root afferent fibers. It is likely that the gastroduodenal branch has a similar composition of fibers; hence, the effects of GV (as well as HV) could be due to effects on the sympathetic nervous system. However, capsaicin treatment of the common hepatic branch in similarly prepared STZ-diabetic rats selectively ablates vagal, but not sympathetic, fibers and, similar to HV and GV, promotes lard intake (53, 54). An afferent hepatic vagal pathway has also been reported (3) to be essential in the development of glucocorticoid-induced insulin resistance and hypertension. Studies mapping c-fos activation after insulin-induced lard ingestion in STZ-diabetic, high-corticosterone-clamped rats (55) have revealed the importance of the nucleus of the tractus solitarius, which is the site of the first synapse for vagal afferents (6), as well as a plethora of sites throughout the brain explicably linked to food motivation and reward (24). Hence, we propose that the putative inhibitory lard intake signal is afferent in nature, projecting from the gastroduodenal branch to brain regions that are known to influence palatable food intake.

There are several other possibilities for how GV might increase lard intake. Jejunal infusions of lipids can increase the activity of hepatic vagal afferents (42), which can decrease food intake in an HV-sensitive fashion (11). Dietary lipids could thus directly signal through the gastroduodenal branch to regulate fat intake. Mechanical probing of the serosal surface

Fig. 5. White adipose tissue (WAT) weights of the epididymal WAT (eWAT; A), perirenal WAT (pWAT; B), mWAT (C), and scWAT (D) depots, normalized to g body wt, together with plasma leptin levels (E), at the end of the study. Insulin treatment of diabetic rats generally increased weight of all fat pads except for scWAT, whereas GV surgery in STZ-saline-treated rats increased all but mWAT. GV did not modify any effects of insulin. The increase in WAT weight was consequently accompanied by increases in circulating leptin levels. Different letters indicate significant ($P < 0.05$) differences among treatment groups.
of the duodenum or stomach results in nerve activity within the common hepatic branch of the vagus that can be prevented by GV (22). STZ-diabetes results in slower gastric motility (30) and gastric retention (32), a trend evident in our sham-operated groups. Accordingly, insulin-induced gastric motility (44) and acid secretion (18) are abolished by HV.

Similar to GV, insulin treatment also increased lard intake in STZ-diabetic rats. Although direct association between these two findings has not been directly shown, it is possible that insulin acts to overcome the inhibitory lard intake signal that arises from the gastroduodenal branch of the vagus. This action does not occur at the gastroduodenal branch, since insulin can still promote lard intake even when the nerve has been transected. It is unclear where insulin is acting in this regard.

One possible site of insulin action is the brain, since insulin receptors are present throughout (21). Certainly, in our rodent model, insulin and lard intake result in distinct and generally nonoverlapping site-specific patterns of c-fos immunoreactivity throughout the brain (55). Central insulin action, in conjunction with leptin, is likely responsible for the reduction in chow intake observed, in keeping with the actions of these anorexigenic hormones within the arcuate nucleus (37).

WAT weight was clearly affected by both prevailing insulin levels and GV in a depot-specific manner. STZ treatment causes a profound loss of WAT weight. Insulin treatment increased the weight of all WAT depots except scWAT; however, GV surgery in the STZ-treated, saline-infused group increased all but mWAT weight. Insulin clearly has the predominant effect over WAT deposition, as GV surgery did not modify the effects of insulin. The mechanisms behind these depot-specific effects have yet to be explored. Other studies (52) have also shown the common branch of the hepatic vagus to be important in regulating the pattern of fat deposition to the WAT depots. Comparison with the results of HV (Table 2) suggests that signals from the hepatic branch proper might negate those from the gastroduodenal branch since HV does not similarly change WAT weight (54).

Insulin treatment to STZ-diabetic rats served to restore, partially or completely, the levels of plasma FFA, total ketone bodies, triglycerides, and liver glycogen to nondiabetic levels as expected (2, 8, 16, 56). These actions of insulin were prevented by GV. This could be linked to the changes in plasma glucagon levels, which show a similar pattern to that of the metabolites. Glucagon serves to reduce liver glycogen content (19) and stimulates both adipocyte lipolysis (45) and hepatic ketogenesis (7, 35). FFA are additionally key determinants of total ketone body production, since they are the substrates for synthesis (34). Neural innervation of the pancreas can regulate α-cell glucagon output (20); hence, signaling through the gastroduodenal branch could regulate glucagon release that would consequently impact circulating metabolite levels. It is unclear why GV did not induce similar changes in the nondiabetic or STZ-diabetic, saline-infused groups. The corticosterone/insulin ratio is important for these metabolic parameters since corticosterone opposes many of the metabolic actions of insulin (40, 50). It is possible that, in combination with an increased corticosterone/insulin ratio, the effects of GV are evident only when the circulating concentration of insulin is high enough to exact some detectable metabolic change over that of the opposing corticosterone actions.

Fig. 6. Plasma glucagon (A), plasma glucose-dependent insulinogetic peptide (GIP; B), liver glycogen (C), and plasma glucose concentrations (D). STZ treatment reduced liver glycogen content and elevated circulating glucagon and glucose, effects that were prevented by insulin treatment. GV prevented all of the actions of insulin, with the exception of the effects on plasma glucose levels. GIP was unaffected by any experimental manipulation. Different letters indicate significant (P < 0.05) differences between treatment groups.
Despite attenuating the actions of insulin on liver glycogen and plasma glucagon, the actions of insulin on plasma glucose levels were unaffected by GV. This suggests that the hepatic branch proper is essential for the effects of insulin on hepatic glucose output, since HV and capsaicin treatment of the common hepatic branch also attenuate this action of insulin (53, 54). The importance of intact insulin signaling in the liver on hepatic glucose production has been shown in dogs (14) and in studies utilizing the liver-specific insulin receptor knockout mouse (15). However, controversy exists as to the importance

Table 2. Comparison between the effects of GV and HV on food intake, WAT weights, hormones, and metabolites in STZ-treated, high-corticosterone rats infused with either saline or insulin

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<tr>
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<td>GV</td>
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<td>Lard</td>
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<td>Glycerol</td>
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HV, hepatic branch vagotomy; WAT, white adipose tissue; eWAT, epididymal WAT; pWAT, perirenal WAT; mWAT, mesenteric WAT; scWAT, subcutaneous WAT; FFA, free fatty acids. *HV data summarize the findings from Warne et al. (54). †Treatment-induced increase; ‡ treatment-induced decrease; ↔ no change. INS signifies that treatment attenuated the effects of insulin; parantheses depict nonsignificant trend. @ No insulin effect was evident in that study, yet it was present in the other.
of liver vs. brain regulation of hepatic glucose output (17), with a number of convincing studies (23, 38, 39) showing the dominance of brain insulin action under certain experimental conditions. Our study clearly shows that insulin signaling through the hepatic branch proper is important for regulation of hepatic glucose output, suggesting that both liver insulin action and consequent central nervous system activity are important.

Plasma GIP, an incretin hormone that is released from the K cells of the duodenum (9) in response to glucose or lipid uptake to stimulate insulin secretion (10), was unchanged by any experimental manipulation. Since GV transects vagal fibers serving the duodenum, this suggests that GIP release is not under direct vagal control, which complements findings (36) showing that the common hepatic branch is also unresponsive to GIP. This also supports a role for hepatic, as opposed to duodenal, signals in the regulation of plasma glucose levels.

In conclusion, these data suggest that signaling through the gastroduodenal branch of the vagus inhibits fat, but not total caloric, intake in STZ-diabetic high-corticosterone-clamped rats. The gastroduodenal branch of the vagus also regulates the actions of insulin on plasma metabolite, but not glucose, levels under these experimental conditions, possibly via changes in glucagon release (Fig. 8).

GRANTS

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REFERENCES


