

The Hepatic Vagus Mediates Fat-Induced Inhibition of Diabetic Hyperphagia

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Diabetic rats both overeat high-carbohydrate diet and have altered hypothalamic neuropeptide Y (NPY) and corticotropin-releasing factor (CRF). In contrast, a high-fat diet reduces caloric intake of diabetics to normal, reflected by normal hypothalamic NPY and CRF content. How the brain senses these changes in diet is unknown. To date, no hormonal changes explain these diet-induced changes in caloric intake. We tested whether the common branch of the hepatic vagus mediates the fat signal. We presented fat in two ways. First, diabetic and vehicle-treated rats were offered a cup of lard in addition to their normal high-carbohydrate diet. Second, we switched diabetic rats from high-carbohydrate diet to high-fat diet, without choice. In streptozotocin-treated rats, both methods resulted in fat-induced inhibition of caloric intake and normalization of hypothalamic neuropeptides to nondiabetic levels. Strikingly, common branch hepatic vagotomy (unlike gastroduodenal vagotomy) entirely blocked these fat-induced changes. Although a shift in hepatic energy status did not explain the lard-induced changes in diabetic rats, the data suggested that common hepatic branch vagotomy does not interfere with hepatic energy status. Furthermore, common branch hepatic vagotomy without diabetes induced indexes of obesity. Abnormal function of the hepatic vagus, as occurs in diabetic neuropathy, may contribute to diabetic obesity. *Diabetes* 52: 2321–2330, 2003

In insulin-dependent diabetes, the hormonal system of signals to the brain that ensures normal food intake is aberrant. Rats with uncontrolled streptozotocin (STZ)-induced diabetes overeat when fed a high-carbohydrate diet and display reduced body fat, high corticosterone concentrations (1), very low insulin and leptin concentrations (2–4), and impaired glucose utilization (5). Although the underlying mechanisms of diabetic hyperphagia are unknown, hypothalamic neuropeptide Y (NPY) and corticotropin-releasing factor (CRF) are hypothesized to participate in diabetic hyperphagia (6–9). In

rats with STZ diabetes, NPY expression in the hypothalamic arcuate nucleus (Arc), associated with stimulating food intake (10,11), is increased and CRF expression in the hypothalamic paraventricular nucleus (PVN), associated with inhibiting feeding (12,13), is decreased. In addition, changes in NPY and CRF are often associated with changes in the concentrations of circulating insulin, corticosterone, and leptin, hormones known to be involved in the regulation of energy intake and expenditure through their effects on hypothalamic and brainstem neuropeptide systems (8,14). Central administration of either insulin or leptin in STZ rats decreases NPYmRNA in the PVN and reduces food intake. Furthermore, corticosterone is elevated in uncontrolled diabetes, and corticosterone administration in diabetic rats increases both Arc NPYmRNA and food intake (15) and decreases CRFmRNA (16). Taken together, these observations support the hypothesis that the effects of insulin, corticosterone, and leptin on hypothalamic and downstream brainstem neuropeptide systems may activate diabetic hyperphagia. However, when switched from high-carbohydrate to high-fat diet, diabetic rats normalize food intake (17) and hypothalamic neuropeptide expression, without changes in these hormones (18). Thus, how the brain senses these changes in diet cannot be explained by invoking this set of hormonal feedback signals.

Although hormonal feedback regulation of diabetic hyperphagia has been studied extensively, neuronal feedback has not. Because the liver is an important site of fat oxidation, we hypothesized that hepatic vagal pathways from the liver to the brain mediate the fat-induced changes in hypothalamic neuropeptides and feeding that occur in diabetic rats. Neural feedback in fat-induced feeding signals is important, and liver energy status is potentially important in neural signals (19–22). Vagotomy and small lesions in the brainstem pathway from vagal input to hypothalamus inhibit both feeding and hypothalamic Fos (immediate early gene) responses to mercaptoacetate, which interferes with fat metabolism (19–21). The Fos response to 2,5-anhydro-D-mannitol, a fructose analog that also stimulates food intake and inhibits hepatic glycolysis, glycogenolysis, and gluconeogenesis (22), is also blocked by lesion of the hepatic vagus (20).

Earlier studies (18) used equicaloric high-carbohydrate and high-fat diets to test the effect of high-fat diet on food intake and hypothalamic neuropeptides in rats with STZ-induced diabetes. However, presenting a high-fat diet ensures intake and does not represent choices made daily by patients with type 1 diabetes. We therefore gave rats a choice to consume additional fat. In experiment 1, STZ- and vehicle-treated rats were offered lard in addition to

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AGRP, agouti-related peptide; Arc, arcuate nucleus; CRF, corticotropin-releasing factor; ME, median eminence; NPY, neuropeptide Y; POMC, proopiomelanocortin; PVN, paraventricular nucleus; STZ, streptozotocin.

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their normal high-carbohydrate diet. In the second experiment, we switched hyperphagic diabetic rats onto a high-fat diet to test whether the hepatic vagal control that we observed was due to high fat or to choice.

Dissection of the common hepatic vagal branch not only removes neural transmission from the liver to the brain but also interferes with signals from the proximal duodenum to the brain, and gastroduodenal vagotomy has been suggested as the proper control procedure for common hepatic branch vagotomy (23). Thus, in experiment 3, we offered lard to gastroduodenal vagotomized diabetic rats and measured caloric intake and hypothalamic NPY content.

It seems clear that hepatic energy status (state of liver oxidation, availability of phosphate and stores of ATP) is key for the increase in food intake after injection of 2,5-anhydro-D-mannitol (22,24,25). Therefore, the decrease in caloric intake observed in diabetic rats switched from a high-carbohydrate diet to a high-fat diet (18) might result from a shift in hepatic energy status. In the absence of insulin, diabetics produce and lose large quantities of glucose and do not synthesize fat stores, coinciding with low concentrations of hepatic ATP. Increased availability of fat for oxidation in diabetes is associated with higher hepatic ATP resulting in decreased food intake (26). Therefore, differences in hepatic ATP might occur in diabetic rats eating lard that, in turn, would lead to a signal mediated by the hepatic vagus to the brain. To test whether the fat-induced inhibition of food intake in STZ-induced diabetic rats involves a shift in hepatic energy status, we gave STZ rats, with and without an intact hepatic vagal nerve, the choice of lard in addition to their normal high-carbohydrate diet in experiment 4.

RESEARCH DESIGN AND METHODS

Male rats (Sprague-Dawley, B&K International, Gilroy, CA) weighing 250 ± 10 g were individually housed in hanging wire cages in a temperature- (21–23°C) and light-controlled (lights on at 0600–1800) room. Rats were allowed to adapt to their new environment for at least 4 days. All procedures were approved by the University of California San Francisco IACUC. All rats had ad libitum access to pelleted rat chow (Purina Chow #5008; Purina, St. Louis, MO) and tap water except where noted. Food and body weight were measured daily. The lard used in experiments 1, 3, and 4 was purchased locally (Armour lard; 9 kcal/g). The compositions of the equal caloric high-carbohydrate and high-fat diets in experiment 2 were similar to those described (18). High-fat diet contained 63% fat, 13% carbohydrate, and 24% protein, whereas high-carbohydrate diet contained 63% carbohydrate, 13% fat, and 24% protein (Labdiets, Richmond, IN).

Common hepatic branch and gastroduodenal vagotomies. Rats were anesthetized with ketamine (75 mg/kg intramuscularly; Abbott Lab, Chicago, IL) and xylazine (10 mg/kg intramuscularly; Butler, Columbus, OH). A midline laparotomy was performed. The common hepatic vagal branch (hepvag) was transected by stretching the fascia containing the common hepatic vagal branch. A myelin-specific dye (Toluidine Blue; Sigma Chemicals, St. Louis, MO) revealed the common hepatic branch as it separates from the left vagal trunk. Stained neural tissue was transected between ventral vagal trunk and liver. Small, blue-stained branches running in the fascia between stomach and liver were also transected. For gastroduodenal vagotomy (duovag), the common hepatic vagus was stretched and followed down to the duodenum. The branches were visualized as above, and all branches on top of and above the duodenum were removed. Sham-operated (sham) rats underwent all procedures except transection of the neural tissue. During all procedures, the abdominal cavity was bathed with saline to prevent drying of the viscera. Ketoprofen (10 mg/kg subcutaneously; Abbott Lab) was given postoperatively. **STZ-induced diabetes.** Seven days later, rats were eating normally and showed normal weight gain, then hepvag, duovag, and sham rats were anesthetized with isoflurane and given either STZ (Sigma Chemicals, St. Louis,

MO; 65 mg/kg in citrate buffer pH 4.2, subcutaneously) or the citrate buffer (vehicle, subcutaneously).

Protocols

Experiment 1. Twelve of 20 hepvag rats and 16 of 26 sham rats received STZ; the rest received citrate vehicle (veh). All were fed pellet chow for 6 days until the diabetic rats were hyperphagic. Four rats did not become diabetic (one hepvag-STZ and three sham-STZ rats), and one rat in each diabetic group died of diabetic complications. Five sham-veh, six sham-STZ, four hepvag-veh, and six hepvag-STZ rats received lard in addition to the pellet chow; the remaining rats received pulverized pellets. After 3 days, all rats were decapitated between 0900 and 1000. Blood was collected from the trunk into chilled plastic tubes containing EDTA, kept on ice until centrifuged, and then aliquoted. Brains were immediately removed from the skull and were immersion-fixed in 4% paraformaldehyde in PBS (0.1 mol/l, pH 7.2).

Experiment 2. Nine hepvag rats and nine sham rats received STZ. All were given the high-carbohydrate diet for 6 days until all were hyperphagic. One hepvag-STZ and one sham-STZ rat died. Four rats of each group were then switched to the high-fat diet; the others remained on high-carbohydrate diet. After 3 days, rats were decapitated between 0900 and 1000; blood and brains were collected.

Experiment 3. Seven duovag rats and five hepvag rats were made diabetic and after 6 days of normal pellet chow were exposed to lard for 3 days. Four sham-veh rats were maintained on normal chow during the course of the experiment. Three of the seven duovag-STZ rats and one hepvag-STZ rat died before the end of the experiment. The remaining procedures were as described in experiment 1.

Experiment 4a. Fourteen hepvag rats and 14 sham rats received STZ, and 7 sham rats received vehicle. Two sham-STZ rats died of diabetic complications. All rats were fed normal chow for 5 days until diabetic rats were hyperphagic. Three days after STZ injection, a tail nick was performed (2 h after lights on) to withdraw 0.5 ml of blood for hormone measurements. One hour before lights off, seven hepvag-STZ and seven sham-STZ rats received lard in addition to the pelleted chow, whereas others received pulverized pellets. In the morning of the second day (41 h later), all rats were anesthetized as described in LIVER ENERGY STATUS.

Experiment 4b. Eighteen hepvag rats and 23 sham rats received STZ; 4 nonoperated rats received vehicle. One hepvag-STZ and two sham-STZ rats died. STZ rats were hyperphagic on normal chow after 5 days. Groups of hepvag-STZ and sham-STZ rats were anesthetized, and the livers were freeze-clamped at 6, 13, and 30 h after presentation of lard. Five sham-STZ rats and four veh rats received pulverized pellets and were anesthetized as described above.

Plasma. Glucose was measured using the glucose oxidase assay on a plate reader (Sigma Diagnostics, St. Louis, MO), and corticosterone, insulin, and leptin were determined with radioimmunoassays (ICN Biochemicals, Orangeburg, NY, and Linco Research, St. Charles, MO, respectively).

Immunocytochemistry. Brains were sliced at 30 μ m from the optic chiasm to the ventromedial nuclei, and 1:6 sections at the appropriate level were stained for CRF (anti-rabbit CRF [lot c-70], diluted 1:10,000, gift from Dr. W. Vale, Salk Institute, San Diego, CA), NPY, and galanin (anti-rabbit NPY or anti-rabbit galanin, diluted 1:6,000; gifts from Dr. R.M. Buijs, NIBR, the Netherlands). Immunostaining was performed as described previously (27). For ensuring that the exposure to DAB was similar between groups, sections were stained in 24-well plates; all sections were stained with the same solution. DAB exposure was limited so that we did not measure maximum DAB staining. Semiquantitative densitometric analysis was performed on relative levels of NPY-peptide (Arc [medial part]), CRF peptide (median eminence outer layer [bregma, -2.8 mm]), and the galanin peptide (anterior parvocellular PVN [bregma, -1.4 mm]). An illumination objective of 10 \times was used, and digital images were made with a digital camera connected to a computer with NIH Image software (ImageJ-1.29 \times). Optical density readings through any given cell group, corrected for background, were taken at regularly spaced (120 μ m) intervals. Mean group values were determined from averaged readings of multiple sections (at least three) from individual rats (four per group). The number of slices analyzed for each rat varied depending on the region and distribution of the peptide (three of four sections per rat) (28,29).

Liver energy status. Rats were anesthetized (ketamine/acepromazine [10:1; 1 ml/kg intramuscularly]), and a piece of the median lobe (~ 3 g) was freeze-clamped, homogenized, and stored at -70°C and analyzed later as described elsewhere (30). The phosphorylation potential was calculated as $[\text{ATP}]/([\text{ADP}] \times [\text{Pi}])$.

Statistics. Data were analyzed by two-way ANOVA using two factors: group (sham-veh, sham-diab, vag-veh, vag-diab) and treatment (no lard, lard or high-carbohydrate diet, high-fat diet). Significant interaction ($P < 0.05$) was

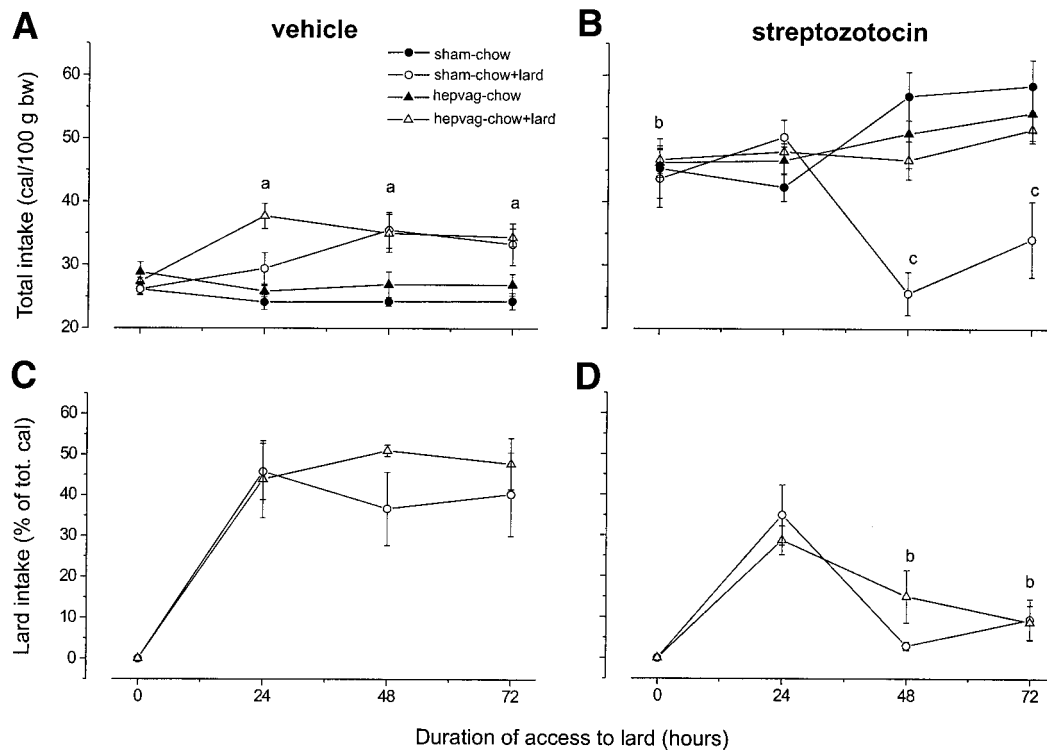


FIG. 1. Twenty-four-hour total caloric intake and lard intake (as % of total intake) for all veh-treated (left) and STZ-treated (right) rats. Intake shown on day 0 represents the intake over 24 h before the rats gained access to lard. Significant effects of hepatic vagotomy, STZ, and lard were measured using repeated measures ANOVA. **A:** When eating chow, hepvag-veh rats consume significantly more calories than sham-veh rats ($F[1,7] = 6.1$; $P < 0.05$). In both groups, lard+chow significantly increases caloric intake (a; $P < 0.05$); there are no significant differences in total caloric intake between hepvag-veh and sham-veh on chow+lard ($P = 0.12$). **B:** STZ significantly increases total caloric intake in both hepvag and sham rats (b; $P < 0.001$); lard decreases caloric intake in sham-STZ rats (c) but not in hepvag-STZ. **C:** Lard consumption as a percentage of total caloric intake in vehicle-treated rats \pm hepatic vagotomy is constant over the 3 days of lard+chow access. There is no significant difference between hepatic vagotomized and shams. **D:** In both STZ-diabetic groups, lard consumption (as % of total caloric intake) declines over the 3 days of access. There is no significant difference between the STZ groups (sham versus hepatic vagotomy). Lard intake (as % of total intake) is lower in STZ-treated rats at 48 and 72 h than in vehicle-treated rats (b).

followed by post hoc tests of individual group differences (Tukey-Kramer). In the text, tables, and figures, all data are presented as means \pm SE.

RESULTS

Experiment 1: common branch hepatic vagotomy in STZ-diabetic rats consuming lard. Caloric intake, body weight gain, and corticosterone concentrations were significantly increased after common branch hepatic vagotomy (Figs. 1 and 2A and B, Table 1), and there was a trend toward increased insulin concentrations ($P = 0.09$; Table 1). Both sham-veh and hepvag-veh rats increased total caloric intake when lard was present (effect of lard, $F[1,14] = 31$, $P < 0.001$), eating similar amounts of lard and chow over 3 days (effect of hepvag, $F[1,7] = 1$; $P = 0.35$). It is interesting that lard ingestion increased leptin and insulin significantly only in hepvag-veh rats (leptin:interaction effect, $F[3,32] = 5.2$; $P < 0.004$; insulin interaction, $F[3,32] = 3.0$; $P < 0.05$; Fig. 2B, Table 1).

Five days after STZ injection, hepvag-STZ and sham-STZ rats were hyperphagic (0 h; Fig. 1A and B; effect of STZ, $F[1,36] = 76$; $P < 0.001$). During the first 24 h of lard, sham-STZ and hepvag-STZ rats maintained total caloric intake that was similar to the preceding day, but they substituted 30–40% of the total calories with calories from lard. A similar percentage was seen in the vehicle-treated rats (24 h; Fig. 1C and D). During the subsequent 24 h, sham-STZ rats decreased caloric intake to nondiabetic levels, whereas hepvag-STZ rats did not decrease intake,

eating the same number of calories as on the previous day (48 h; Fig. 1B; interaction effect, $F[1,18] = 12.5$; $P < 0.003$). This pattern continued in the hepvag-STZ rats for the third 24-h period, whereas sham-STZ rats increased their total caloric intake slightly, predominantly from high carbohydrate chow (72 h; Fig. 1). Both sham-veh and hepvag-veh groups increased total caloric intake with 40% calories from lard over the total 72 h that lard was available (Fig. 1C), whereas the sham-STZ and hepvag-STZ rats decreased lard intake after the first 24 h (effect of lard, $F[2,34] = 7.5$; $P < 0.002$; Fig. 1D).

During lard access, hepvag-STZ rats (as with sham-STZ and hepvag-STZ groups without lard) did not gain body weight, despite the higher caloric intake; the sham-STZ group tended to lose weight, probably because of lower caloric intake (Fig. 2A and B). At the end of the experiment, STZ decreased insulin and leptin and increased glucose and corticosterone, independent of hepatic vagotomy (Table 1). Furthermore, lard ingestion in diabetic rats did not alter plasma concentrations of glucose, insulin, leptin, or corticosterone.

In vehicle-treated rats, common branch hepatic vagotomy and/or lard ingestion did not have clear effects on NPY and CRF peptide levels. Lard ingestion tended to decrease CRF peptide in hepvag-veh rats (Fig. 2C). STZ-induced diabetes decreased CRF peptide in the median eminence (ME; Fig. 2C) and increased NPY-icc in the Arc

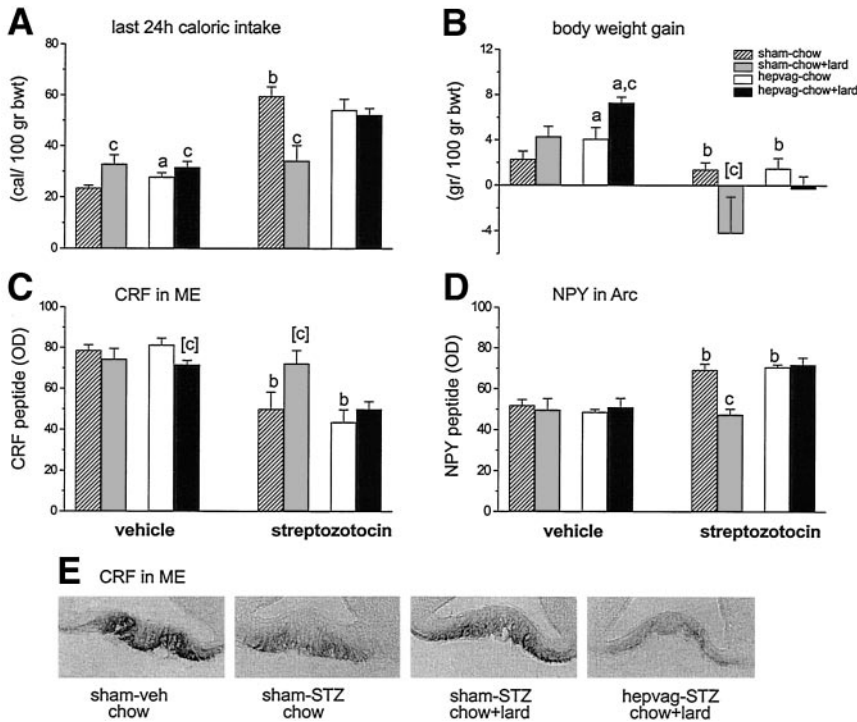


FIG. 2. Hepatic vagotomy in vehicle-treated rats increases food intake and body weight gain. Hepatic vagotomy in STZ-treated rats prevents the changes in lard-induced feeding and hypothalamic peptide. **A:** Hepvag-veh rats consume more calories than sham-veh (a), STZ-induced diabetes increases caloric intake in both sham- and hepvag-groups (b), access to chow+lard increases caloric intake in sham-veh and hepvag-veh groups, and decreases in sham-STZ (c). **B:** Body weight gain is higher in hepvag-veh rats (a), STZ-induced diabetes decreases weight gain (b), and lard ingestion increases weight gain even further in hepvag-vehicle and tends to decrease it in sham-STZ (c). **C:** CRF peptide expression in ME decreases with STZ-induced diabetes (b), lard ingestion tends to decrease CRF in hepvag-veh rats and to increase it in sham-STZ rats (c). **D:** NPY peptide expression in the Arc increases with STZ-induced diabetes (b), lard ingestion decreases NPY in sham-STZ rats (c). **E:** CRF peptide: decrease in CRF peptide in ME with STZ-induced diabetes, the increase in sham-STZ rats after lard ingestion, and the lack of an increase in hepvag-diab with lard (magnification $\times 10$). ^aSignificant effect of hepatic vagotomy $P < 0.05$; ^bsignificant effect of diabetes $P < 0.05$; ^csignificant effect of lard ingestion $P < 0.05$. Number between brackets is a trend $P < 0.1$.

(Fig. 2D). Lard ingestion decreased caloric intake in sham-STZ, decreased NPY to nondiabetic levels (interaction effect ($F[1,12] = 13$; $P < 0.004$), and tended to increase CRF (interaction effect, $F[1,12] = 4.9$; $P = 0.051$); in hepvag-STZ rats lard ingestion had no effect on CRF- and NPY peptide levels (Fig. 1C–E).

Experiment 2: common branch hepatic vagotomy in STZ-diabetic rats on a high-fat diet. Because diabetic rats in experiment 1 refused to eat lard after the first day of exposure, we used a forced high-fat diet to test whether the hepatic vagal control is general to high fat in the diet. Only hepvag-STZ and sham-STZ groups were used, and the rats were given a high-fat and high-carbohydrate diet (both 3.3 kcal/g) similar to that described before (18). Five days after induction of diabetes with a single injection of STZ,

rats were hyperphagic on the high-carbohydrate diet (hepvag-STZ from 33 ± 1.0 to 48 ± 2.2 and sham-STZ from 31 ± 0.5 to 51 ± 2.9 cal/100 g body wt/day). Again we saw a clear decrease in caloric intake to nondiabetic levels in sham-STZ rats eating fat (caloric intake in sham-STZ rats on the high-fat diet was not significantly different from that before diabetes was induced; $P = 0.2$). In contrast, caloric intake in the hepvag-STZ rats eating a high-fat diet was similar to that of hepvag-STZ rats eating the high-carbohydrate diet (Fig. 3A). Changes in body weight over the period of the experiment were not different among the four groups (data not shown), and there were no differences in plasma concentrations of glucose, insulin, or leptin (Table 2). Plasma corticosterone concentrations all were elevated for the time of the day (>10 $\mu\text{g}/\text{dl}$), although

TABLE 1

Effect of lard consumption, common branch hepatic vagotomy, and STZ-induced diabetes on circulating metabolic hormones and metabolites

	Sham-veh	Hepvag-veh	Sham-STZ	Hepvag-STZ
Body weight (g) before lard (n)				
No lard	328 \pm 6 (5)	351 \pm 13 (4)	309 \pm 10 (6)	299 \pm 6 (4)
Lard	330 \pm 8 (5)	351 \pm 14 (4)	302 \pm 10 (6)	306 \pm 9 (6)
Glucose (mg/dl)				
No lard	90 \pm 4	96 \pm 17	438 \pm 47	526 \pm 71
Lard	131 \pm 17*	118 \pm 13	518 \pm 24	480 \pm 40
Insulin (ng/ml)*				
No lard	4.6 \pm 0.7	5.8 \pm 0.6	0.9 \pm 0.3	0.5 \pm 0.2
Lard	6.2 \pm 1.4	9.7 \pm 2.0†	0.7 \pm 0.3	0.6 \pm 0.2
Leptin (ng/ml)				
No lard	3.8 \pm 0.5	4.0 \pm 0.7	0.5 \pm 0.1	0.2 \pm 0.2
Lard	7.3 \pm 1.9	10.4 \pm 1.8‡	0.6 \pm 0.1	0.6 \pm 0.2
Corticosterone ($\mu\text{g}/\text{dl}$)§				
No lard	2.6 \pm 0.8	4.4 \pm 0.5	9.0 \pm 3.3	12.8 \pm 5.0
Lard	1.5 \pm 0.5	3.4 \pm 0.6	7.8 \pm 2.5	12.9 \pm 2.5

Results are means \pm SE. † $P < 0.1$, ‡ $P < 0.05$ vs. no lard; § $P < 0.1$, * $P < 0.05$ vs. hepvag-veh (independent of lard). Body weight measures the day when lard was given.

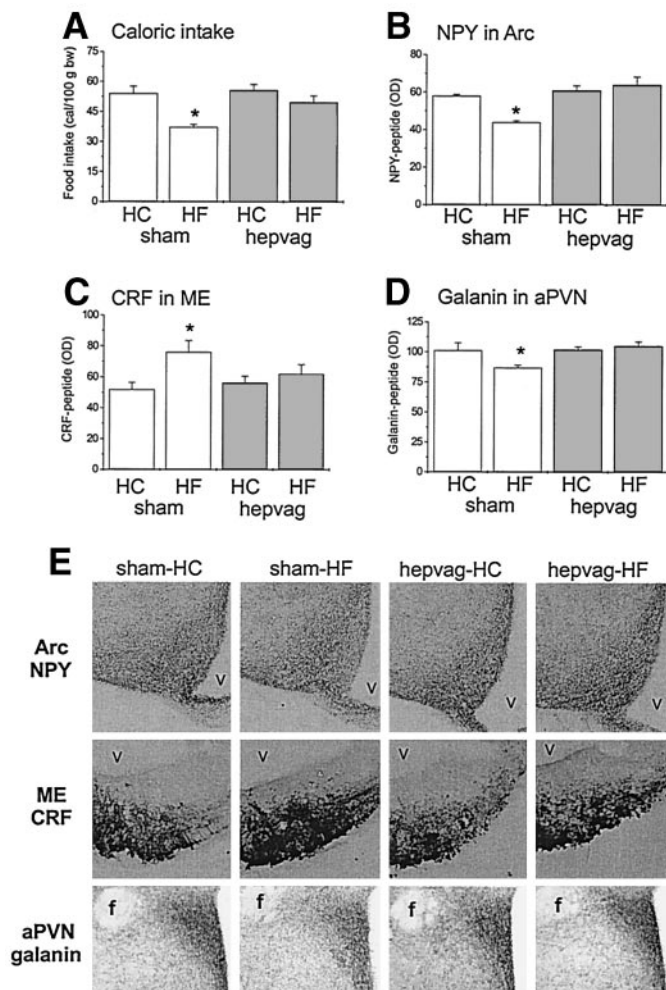


FIG. 3. Hepatic vagotomy prevents the changes in fat-induced feeding and peptide in STZ-induced diabetic rats. **A:** Caloric intake corrected for body weight on the fourth day equal caloric density feeding of high-carbohydrate or high-fat diet feeding (interaction effect $F[1,12] = 6.1$; $P < 0.03$). **B:** NPY (interaction effect $F[1,8] = 21$; $P < 0.002$). **C:** CRF (interaction effect $F[1,8] = 6.8$; $P < 0.04$). **D:** Galanin peptide expression (interaction effect $F[1,8] = 6.1$; $P < 0.039$). Bars represent mean \pm SE ($n = 3-4$). **E:** Immunocytochemistry for NPY in the Arc, CRF in the ME, and galanin in the aPVN (magnification $\times 10$) in sham-STZ and hepvag-STZ groups eating either a high-carbohydrate or a high-fat diet. v, third ventricle; f, fornix. HC, high carbohydrate; HF, high fat. * $P < 0.05$ vs. sham-HC, hepvag-HC, and hepvag-HF.

they decreased significantly in sham-STZ rats that were switched to a high-fat diet (Table 2).

The decrease in caloric intake in sham-STZ rats that were switched to a high-fat diet was accompanied by significant decreases in NPY peptide in Arc and increases

in CRF peptide in ME. Hepatic vagotomy completely blocked these fat-induced changes (Fig. 3B, C, and E). Like NPY peptide, galanin peptide was decreased in sham-STZ rats that were switched to a high-fat diet, and this was blocked by hepatic vagotomy (Fig. 3D and E).

Experiment 3: gastroduodenal-vagotomy in STZ-diabetic rats consuming lard. Food intake and body weight gain were not different between duovag and hepvag rats. Six days after the administration of STZ, both groups were hyperphagic compared with the vehicle group (Fig. 4A, 0 h). During the first 24 h of lard presence, both STZ groups maintained total caloric intake similar to that of the preceding day (Fig. 1A, 24 h), but they substituted 30–40% of the total with calories from lard. During the subsequent 24 h, duovag-STZ rats decreased caloric intake to non-diabetic levels, whereas hepvag-STZ rats did not decrease intake, eating the same amount of calories as the previous day (48 h; Fig. 4A and B). This feeding pattern continued in the hepvag-STZ rats for the third 24-h period, whereas caloric intake in duovag-STZ rats remained decreased (72 h; Fig. 4A and B). Body weight loss after STZ was comparable between both groups. Lard ingestion and subsequent inhibition of food intake in duovag-STZ rats decreased body weight further, whereas hepvag-STZ rats gained weight, although still significantly less than sham-veh rats (Fig. 4C). As in experiment 1, Arc NPY peptide levels in hepvag-STZ rats with lard were significantly higher than in sham rats, whereas duovag-STZ rats with lard had similar NPY peptide levels as sham-veh rats that were not given lard (Fig. 4D). STZ in both hepvag and duovag rats increased plasma concentrations of glucose and corticosterone and decreased plasma concentrations of insulin and leptin. There were no differences between these values in the STZ groups (Table 3).

Experiment 4a: hepatic energy status in STZ-diabetic rats consuming lard. Five days after STZ, hepvag-STZ and sham-STZ rats were hyperphagic compared with the sham-veh group (47.5 ± 1.0 , 44.5 ± 1.7 , and 29.1 ± 0.8 respectively; effect of STZ, $F[2,29] = 44$; $P < 0.001$). Caloric intake between the two STZ groups was not different ($P = 0.2$). After lard, total caloric intake in sham-STZ and hepvag-STZ rats showed the usual patterns (Fig. 5A, left). Sham-STZ rats decreased caloric intake after 24 h of lard, whereas hepvag-STZ rats remained hyperphagic. Without lard, STZ decreased hepatic energy status in both hepvag-STZ and sham-STZ rats: hepatic ATP, the ATP/ADP ratio, and the phosphorylation potential all were decreased. There were no significant differences in hepatic energy status between the STZ groups,

TABLE 2

Effect of a high-fat diet and common branch hepatic vagotomy in STZ-induced diabetes on circulating metabolic hormones and metabolites

	Sham-HC STZ	Sham-HF STZ	hepvag-HC STZ	hepvag-HF STZ
Body weight before diet switch (g)	279 \pm 14	296 \pm 14	286 \pm 10	287 \pm 10
Glucose (mg/dl)	531 \pm 78	516 \pm 32	524 \pm 54	671 \pm 65
Insulin (ng/ml)	0.3 \pm 0.2	0.6 \pm 0.3	0.3 \pm 0.2	0.5 \pm 0.1
Leptin (ng/ml)	0.8 \pm 0.2	0.7 \pm 0.3	1.2 \pm 0.3	1.2 \pm 0.6
Corticosterone (μ g/dl)	49.5 \pm 10.6	10.8 \pm 3.7*	22.4 \pm 10.6	29.4 \pm 16.3

Plasma concentrations at the end of the experiment. * $P < 0.001$ vs. sham-HC ($n = 4$ for every group). Body weight measures the day when lard was given.

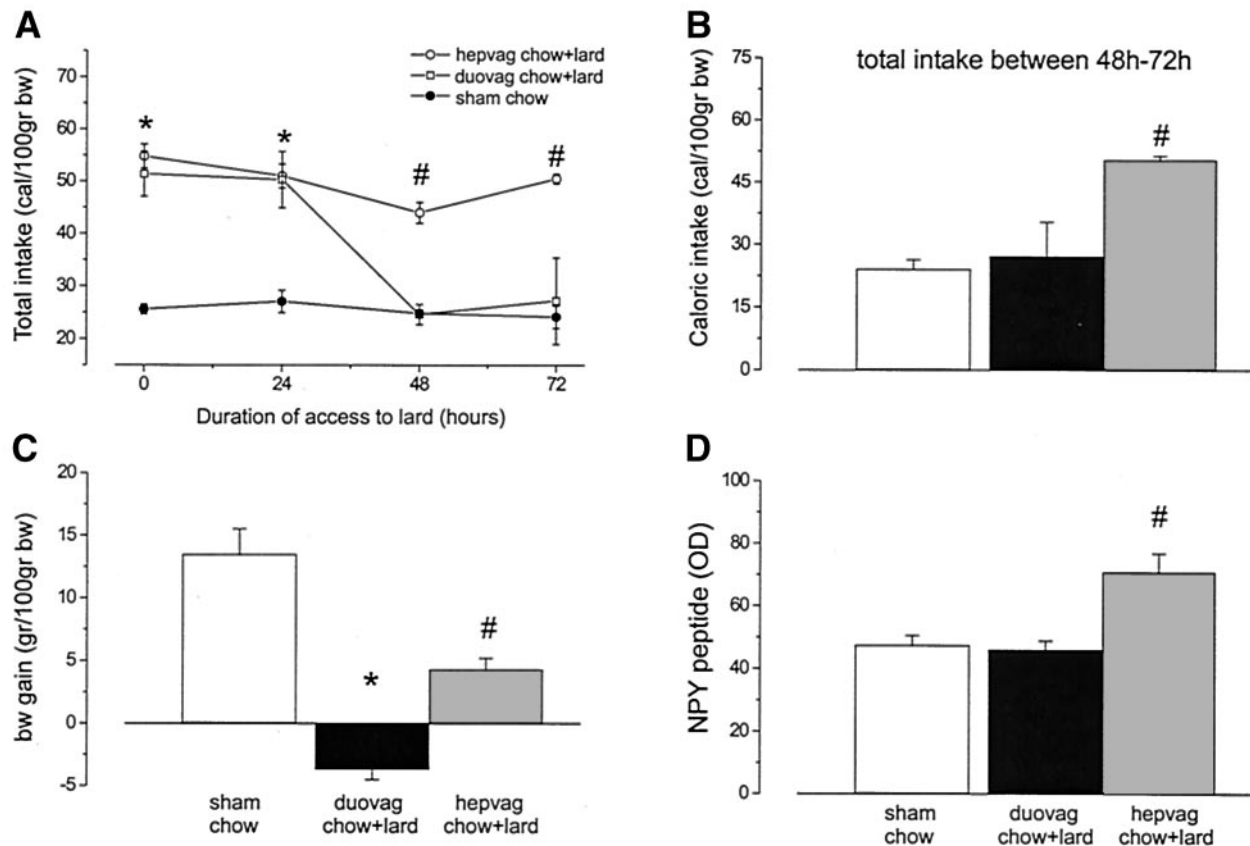


FIG. 4. *A* and *B*: Total caloric intake in sham rats on chow and in gastroduodenal and hepatic vagotomized rats treated with STZ with access to lard and chow. Access to lard and chow (starting on day 0) decreases total caloric intake in duovag-STZ rats to normophagic levels (sham-chow levels), unlike hep vag-STZ rats. *C*: Body weight gain over 4 days of access to chow+lard. *D*: NPY peptide expression in the Arc (*significantly different from duovag-STZ and sham-chow rats; $P < 0.05$; #significant differences between hep vag-STZ and duovag-STZ and hep vag-STZ and sham-veh rats, Student's *t*: $P < 0.05$).

and lard ingestion did not change hepatic energy status (Fig. 5*B–D*, left).

Experiment 4b. We next measured hepatic energy status from initiation of lard inhibition in caloric intake occurred. Hep vag-STZ and sham-STZ rats again ate similar calories on the day lard was first given and significantly more calories than sham-veh rats (Fig. 5*E*, right). STZ again decreased hepatic energy status (Fig. 5*F–H*). After 5 h of lard (lard was given at lights off), hep vag-STZ and sham-STZ rats consumed similar amounts of calories (± 30 cal/100 g body wt; Fig. 5*E*), with lard representing $57.3\% \pm 3.3$ and $58.7\% \pm 3.8$ of total intake, respectively. Between 5 and 13 h after lard access, hep-STZ and sham-STZ rats

ate only chow and no lard, resulting in a cumulative intake at 13 h of ± 40 cal/100 g body wt (Fig. 5*E*). Thus, over the first 24-h period of lard access, rats consumed lard during the first 5 h of the dark period. Between 13 and 30 h of lard access, inhibition of caloric intake occurred in sham-STZ rats; hep-STZ rats maintained caloric intake, although they did not eat more lard (Fig. 5*E*).

Hepatic energy status in hep vag-STZ and sham-STZ rats during 30 h of lard access did show significant differences (effect of time, ATP $F[2,24] = 6.4$, $P < 0.006$; ATP/ADP ratio $F[2,24] = 3.5$, $P < 0.05$); phosphorylation ratio $F[2,24] = 4.6$; $P < 0.03$). No significant differences emerged between hep vag-STZ and sham-STZ rats in any measures of hepatic energy status. All measures in hep vag-STZ and sham-STZ rats at all times were significantly lower than in sham-veh rats ($F[2,34] = 3.5$; $P < 0.05$), and post hoc analysis revealed significant differences for sham-veh versus hep vag-STZ and sham-veh versus sham-STZ at all times ($P < 0.05$).

TABLE 3

Effect of an HF diet and gastroduodenal vagotomy or common branch hepatic vagotomy in STZ-induced diabetes on circulating metabolic hormones and metabolites

	Sham-veh	Duovag-STZ	Hep vag-STZ
Body weight before lard (g)	325 ± 7	282 ± 10	300 ± 15
Insulin (ng/ml)	$3.2 \pm 0.9^*$	0.2 ± 0.1	0.4 ± 0.2
Leptin (ng/ml)	$6.0 \pm 2.5^*$	1.6 ± 0.9	0.3 ± 0.2
Corticosterone (μ g/dl)	$0.7 \pm 0.5^*$	21.4 ± 9.2	24.1 ± 13.9

Plasma concentrations at the end of the experiment. * $P < 0.001$ veh vs. STZ ($n = 4$ for every group). Body weight measures the day when lard was given.

DISCUSSION

Consistent with several other studies, we show that a high-fat diet normalizes food intake in STZ-diabetic rats (17,18). Furthermore, hypothalamic NPY peptide decreases and CRF peptide increases, supporting the data of Chavez et al. (18), who found that the mRNAs of these peptides were normalized in diabetic rats consuming a high-fat diet. Because presenting a high-fat diet forces

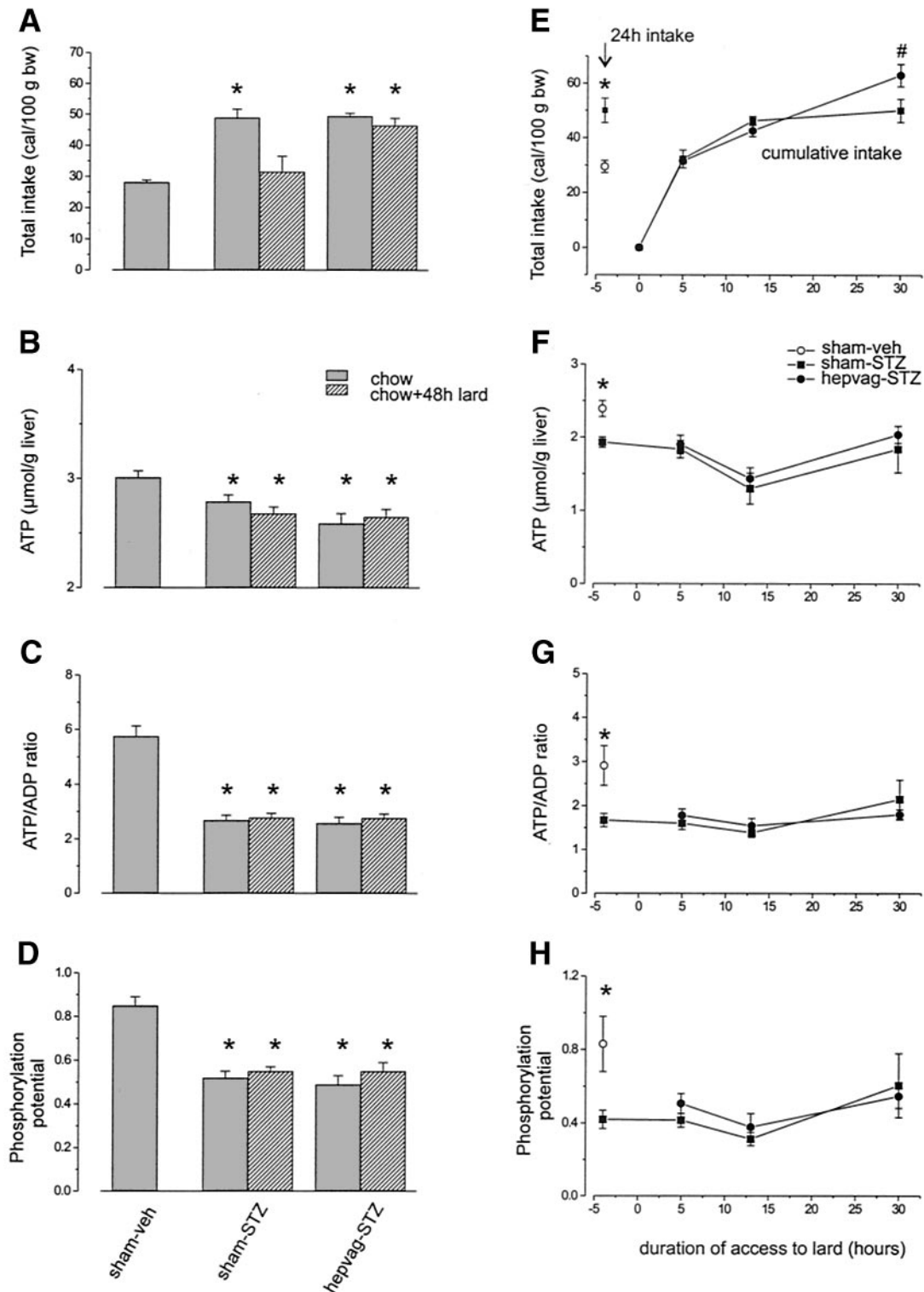


FIG. 5. Hepatic ATP content, ATP/ADP ratio, phosphorylation potential, and caloric intake in sham-STZ and hepvag-STZ rats with 48 h of access to chow or chow+lard (left, *A-D*) or with access to chow (-4 h), and 5 h or 13 h or 30 h of access to chow+lard (right, *E-H*) compared with sham-veh rats with access only to chow. STZ treatment significantly lowers ATP, ATP/ADP ratio, and phosphorylation potential ($[ATP]/([ADP] + [Pi])$) in both sham-STZ and hepvag-STZ rats. There are no significant differences between hepvag-STZ and sham-STZ rats. An effect of time was found in all parameters shown in the right panel (repeated measures ANOVA, $P < 0.05$). Caloric intake decreased with lard consumption in sham-STZ rats and not in hepvag-STZ rats (*significant effect of lard, $P < 0.05$).

intake and does not represent daily life, we also studied rats with the choice of consuming fat. Fat consumed by choice also results in fat-induced inhibition of caloric intake and normalization of CRF and NPY peptide-ir to

nondiabetic levels. Furthermore, lard ingestion by diabetic rats either by choice or forced with a high-fat diet does not change plasma concentrations of glucose, insulin, leptin, or corticosterone, consistent with findings of others (18).

Striking, common branch hepatic vagotomy completely blocks both the fat-induced decrease in caloric intake in diabetic rats and normalization of hypothalamic peptides. Moreover, unlike common branch hepatic vagotomy, gastroduodenal vagotomy does not prevent the fat-induced inhibition of caloric intake and normalization of NPY peptide levels in STZ-induced diabetes. Our findings, therefore, strongly suggest that in diabetic rats, the ingested fat results in a signal to the CNS that is mediated by the hepatic vagal nerve innervating the liver and not the proximal gastroduodenal region.

It has been assumed that the actions of insulin and/or leptin in the brain are important for the increased food intake that occurs in diabetes (6,31). Our data show that without changes in plasma concentrations of insulin, leptin, or corticosterone, the hepatic vagus mediates changes in feeding and hypothalamic neuropeptides in diabetic rats. In rats on the forced high-fat diet, all plasma corticosterone concentrations are elevated for the time of the day (>10 mg/dl) but decrease significantly in sham-operated diabetic rats that were switched to the high-fat diet compared with those maintained on the high-carbohydrate diet. Because corticosterone concentrations in sham-STZ rats on high-fat diet are not significantly different from those in hepvag-STZ groups on high-carbohydrate and high-fat diets, it is unlikely that corticosterone mediates the fat-induced inhibition of caloric intake. Furthermore, this corticosterone concentration in diabetic rats is sufficient to promote hyperphagia (15). Thus, the decrease in corticosterone concentrations observed in sham-STZ rats on high-fat diet are not likely to mediate the decrease in food intake seen after high-fat diet. Changes in NPY and CRF peptides are associated with diabetic hyperphagia (6–9). In addition, mRNAs for other (an)orexogenic peptides in the hypothalamus, proopiomelanocortin (POMC), agouti-related peptide (AGRP), and galanin are altered by diabetes (14,32). It is interesting that the changes in POMC mRNA, AGRP mRNA, and body weight gain induced by diabetes were partially reversed after systemic insulin treatment, whereas NPY mRNA and CRF mRNA and food intake were totally reversed (14,33). It could well be that these peptides are more related to changes in energy expenditure or body weight than to food intake. Thus, although NPY and AGRP are synthesized in the same arcuate neurons, their expression may change separately. This suggests that (an)orexogenic neuropeptides may serve different roles in feeding and body weight regulation in rats with uncontrolled diabetes. Further experiments are necessary to determine whether POMC mRNA and AGRP mRNA are also associated with fat-induced changes in diabetes. Another peptide associated with food intake and fat preference in general is galanin. Because both hyperphagia in diabetes (32) and fat preference (34) are correlated with increases in the hypothalamic peptide galanin, we measured galanin peptide in the anterior portion of the PVN in both sham-STZ and hepvag-STZ rats on high-carbohydrate and high-fat diets. As with NPY, galanin was decreased significantly in sham-STZ rats that were switched to a high-fat diet, and this was blocked by common branch hepatic vagotomy. Whether the fat-induced decrease in caloric intake in sham-STZ rats correlates with galanin and fat preference or with food

ingestion in general is not clear but will be the subject of further study.

Although several studies suggest that fat ingestion in diabetic rats is not aversive (35–37), we cannot rule out the possibility that diabetic rats, eating lard or a high-fat diet, inhibit their food intake because lard and fat consumed during the first day cause malaise. However, the fat-induced inhibition of food intake is almost certainly not a consequence only of malaise. The absolute quantity of lard eaten by the diabetic rats was similar to that eaten by vehicle-treated rats, and vehicle-treated rats did not decrease food intake after eating lard, and diabetic rats did not increase their total caloric intake in the first 24 h that lard was available. Furthermore, adrenalectomized-diabetic rats, which show moderate diabetic signs with normal food intake and no body weight loss (38,39), show a similar inhibition in food intake after lard consumption while eating 75% less lard than diabetic rats with intact adrenals (S.E. la Fleur and M.F. Dallman, unpublished data). Further studies are necessary to determine whether malaise plays a role in the fat-induced inhibition in food intake.

Experiments on neural feedback in the fat-induced feeding signal and hepatic energy status (19–22,24,25) led us to hypothesize that hepatic energy status might signal fat-induced changes observed in STA-diabetic rats. Consistent with earlier reports, hepatic energy status was decreased in STZ-diabetic rats (26). Fat ingestion by choice, however, did not change hepatic energy status at any of the times studied. The effects of lard on caloric intake in diabetic rats occurred between 6 and 30 h, and during these times, we still observed significantly decreased hepatic energy status in both sham-STZ and hepvag-STZ rats, whereas at the 30-h time point, the sham-STZ rats clearly started to inhibit their feeding (Fig. 5). This suggests that a shift in hepatic energy status does not underlie the fat-induced changes in caloric intake and hypothalamic peptide expression when rats have the choice of eating lard. However, because hepatic vagotomy in STZ-induced diabetes did not alter hepatic energy status compared with nonvagotomized diabetic rats, this suggests that the surgical procedure does not interfere with hepatic energy balance. This supports our hypothesis that a neural signal afferent to the CNS is involved in the fat-induced inhibition of food intake rather than a denervation-induced change in the liver, *per se*. Because afferent fibers of the hepatic vagus are intermingled with efferent fibers, the influence of hepatic vagal efferent regulation on feeding regulation in diabetes cannot be excluded.

In addition to the role of the hepatic vagus in diabetic hyperphagia, the changes after common hepatic branch vagotomy in nondiabetic rats were interesting. Common hepatic branch vagotomy results in a small increase in food intake, body weight gain, and corticosterone concentrations and tends to increase insulin concentrations. Increased food intake, weight gain, and insulin after hepatic vagotomy have been reported by some (40,41); others have not found this (42–44). Lack of hepatic vagotomy effects may well be a consequence of a postsurgical loss of body weight (40). Our experience shows that preventing weight loss (through prophylactic use of ketoprofen at surgery [45]) is essential for eliciting the effects of hepatic vagotomy on features of obesity. It is interesting

that short-term lard consumption in hepvag-veh rats increases body weight and leptin significantly and tends to increase insulin and decrease CRF peptide levels in the median eminence. These changes are not observed in sham-veh rats, which consume similar amounts of calories with a similar percentage of calories from lard. To our knowledge, only one report has studied the first days of fat consumption in a mouse strain that is very sensitive to diet-induced obesity. Increased body weight occurred after 1 week, although increased leptin was already observed on days 1 and 2 (46). Other studies in rats show changes in body weight and leptin levels after 2 weeks of eating a high-fat diet (47,48). The metabolic changes that occur after 72 h of lard intake in hepvag-veh rats are similar to those observed after 72 h of central infusion of dexamethasone, which also leads to increased body weight gain and food intake (49). Taken together, we speculate that features of obesity are accelerated by removing the common hepatic vagal branch and that the common hepatic vagal branch plays a role in the onset of diet-induced obesity. A series of long-term studies with high-fat diet, however, will be necessary to clarify the role of the hepatic vagus in (diet-induced) obesity.

We have revealed a hepatic vagal afferent pathway to the brain that is important in feeding and regulating neuropeptide responses in type 1 diabetes; delineation of this pathway and its controls may provide new insights into a potentially important diabetic neuropathy. With time, diabetic neuropathy may blunt signals that normally ensure maintenance of energy balance.

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