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An Afferent Vagal Nerve Pathway Links Hepatic PPARα Activation to Glucocorticoid-Induced Insulin Resistance and Hypertension

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SUMMARY

Glucocorticoid excess causes insulin resistance and hypertension. Hepatic expression of PPARα (*Ppara*) is required for glucocorticoid-induced insulin resistance. Here we demonstrate that afferent fibers of the vagus nerve interface with hepatic *Ppara* expression to disrupt blood pressure and glucose homeostasis in response to glucocorticoids. Selective hepatic vagotomy decreased hyperglycemia, hyperinsulinemia, hepatic insulin resistance, *Ppara* expression, and phosphoenolpyruvate carboxykinase (PEPCK) enzyme activity in dexamethasone-treated *Ppara*$^{+/+}$ mice. Selective vagotomy also decreased blood pressure, adrenergic tone, renin activity, and urinary sodium retention in these mice. Hepatic reconstitution of *Ppara* in nondiabetic, normotensive dexamethasone-treated PPARα null mice increased glucose, insulin, hepatic PEPCK enzyme activity, blood pressure, and renin activity in sham-operated animals but not hepatic-vagotomized animals. Disruption of vagal afferent fibers by chemical or surgical means prevented glucocorticoid-induced metabolic derangements. We conclude that a dynamic interaction between hepatic *Ppara* expression and a vagal afferent pathway is essential for glucocorticoid induction of diabetes and hypertension.

INTRODUCTION

Insulin resistance, a potentially lethal metabolic disorder associated with diabetes, hypertension, and vascular disease, is poorly understood. Glucocorticoid excess, whether caused by exogenous administration, endogenous overproduction associated with Cushing's syndrome, or accelerated conversion of inactive to active glucocorticoids in tissues (Masuzaki et al., 2001; Paterson et al., 2004), alters lipid metabolism and produces insulin resistance. Given the phenotypic similarities between glucocorticoid excess and insulin resistance caused by diet-induced obesity, defining how glucocorticoids cause insulin resistance is likely to be relevant to common obesity-related diseases like diabetes and hypertension. In mice, the development of diabetes and hypertension following chronic glucocorticoid exposure requires expression of the lipid-activated transcription factor PPARα in liver (Bernal-Mizrachi et al., 2003).

A member of the nuclear receptor superfamily (Berger and Moller, 2002), PPARα orchestrates the adequate provision of substrates for critical tissues such as the central nervous system (CNS) in response to prolonged fasting. PPARα activation promotes fatty-acid oxidation (Reddy, 2001), ketogenesis (Rodriguez et al., 1994), and gluconeogenesis (Bernal-Mizrachi et al., 2003; Koo et al., 2004; Patsouris et al., 2004). Fasted mice that are deficient in PPARα develop hypoglycemia and fatty liver (Kersten et al., 1999). The same phenotype develops in PPARα wild-type mice when deprived of dietary and synthesized fat (Chakravarthy et al., 2005), highlighting the role of lipid in activation of the receptor. Physiological responses mediated by PPARα, like those occurring with fasting in the short-term, may become pathophysiological in the setting of long-term stressors such as obesity and glucocorticoid exposure. Consistent with this notion, two independent groups have reported that PPARα deficiency prevents glucose intolerance caused by diet-induced obesity (Guerre-Millo et al., 2001; Tordjman et al., 2001).

Maladaptive PPARα signaling is not limited to effects on glucose metabolism. PPARα deficiency also protects mice from hypertension induced by either high-fat feeding (Tordjman et al., 2001) or glucocorticoids (Bernal-Mizrachi et al., 2003), suggesting that chronic activation of PPARα in mice initiates a cascade of events resulting in several detrimental components of insulin resistance. Species differences between mice and humans in terms of PPARα biology could limit the relevance of these mouse studies to human disorders. However, recent data indicate that

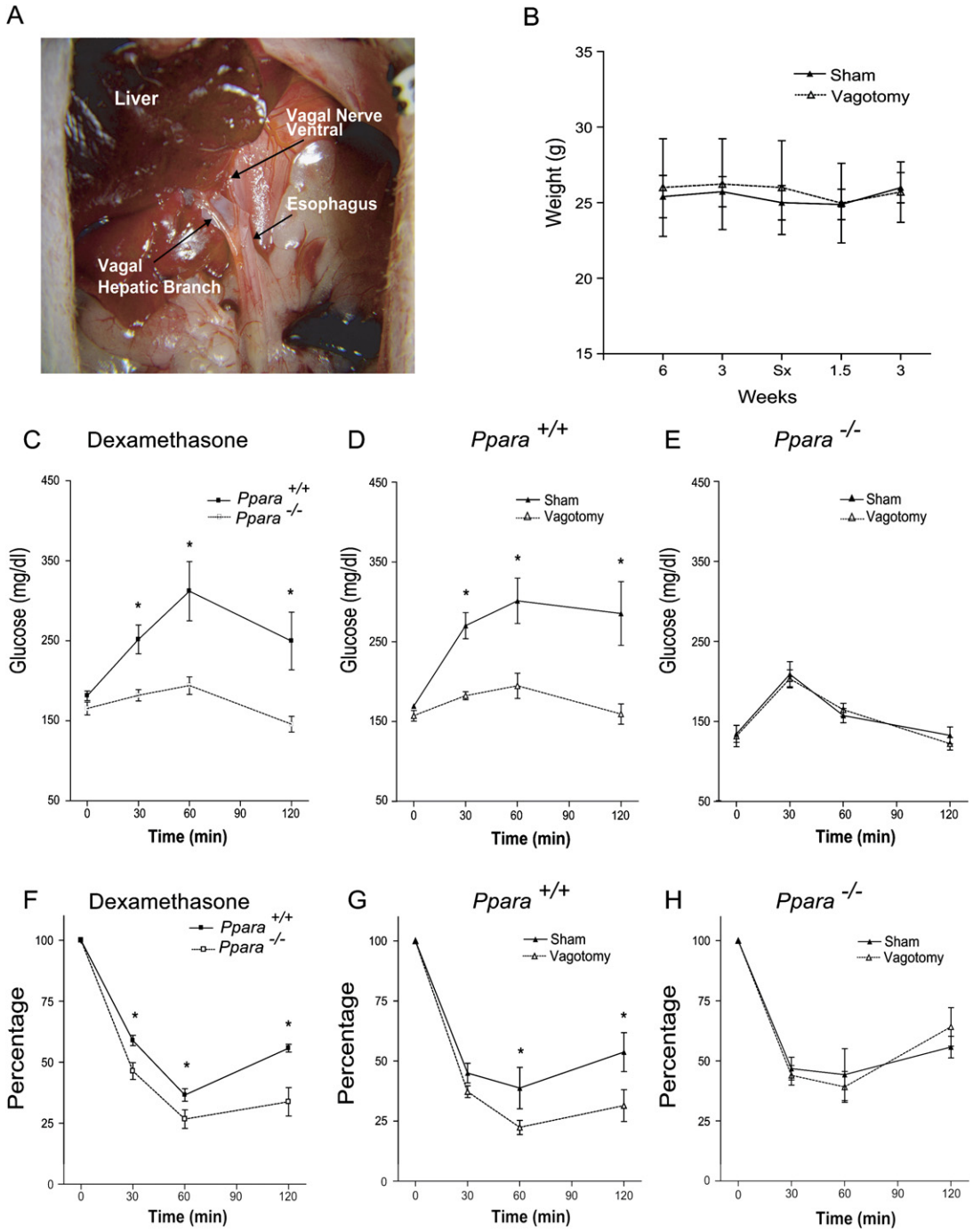

Figure 1. Selective Hepatic Vagotomy Reverses Glucocorticoid-Induced Insulin Resistance

(A) Operative field for selective nerve interruption. The liver is visible in the upper left quadrant. The esophagus, ventral trunk of the vagus nerve, and hepatic branch of the vagus are denoted by arrows.

(B) Body weight before and after selective hepatic vagotomy or sham surgery. *Ppara*$^{+/+}$ mice were treated with Dex for 5 months (1 mg/kg i.p. every other day) and then underwent either selective hepatic vagotomy or sham surgery. Weight data in grams are presented for ten mice in each group around the time of the surgery. Weeks on the horizontal axis are not to scale and represent time before and after surgery, which is indicated as "Sx."

(C) Glucose tolerance testing (i.p. administration of 1 g/kg D-glucose to fasted mice at time 0 followed by blood glucose measurements) in *Ppara*$^{+/+}$ (n = 13) and *Ppara*$^{-/-}$ (n = 13) mice after 5 months of treatment with Dex.

(D) Glucose tolerance testing in *Ppara*$^{+/+}$ mice treated with Dex as in Figure 1C and then randomized to either selective hepatic vagotomy (n = 7, open symbols) or sham surgery (n = 6, closed symbols).

(E) Glucose tolerance testing in *Ppara*$^{-/-}$ mice treated with Dex as in Figure 1C and then randomized to either selective hepatic vagotomy (n = 5, open symbols) or sham surgery (n = 5, closed symbols).

PPARα activation in normal humans increases blood pressure (Subramanian et al., 2006), results that complement mouse studies implicating PPARα in blood pressure control. Exactly how PPARα activation affects both blood pressure and glucose is unknown.

The CNS integrates signals from peripheral sites like the liver to modulate blood pressure and glucose. Infusion of long-chain fatty acids into the portal vein, a major conduit to the liver, has effects that suggest involvement of the CNS including increases in circulating levels of epinephrine and norepinephrine, elevated blood pressure, and accelerated hepatic glucose production (Benthem et al., 2000; Grekin et al., 1995, 1997). Hepatic signals are likely transmitted to the CNS by the vagus nerve since vagal activity is increased by portal or jejunal infusion of lipids (Randich et al., 2001). To test the hypothesis that the vagus nerve mediates PPARα-dependent metabolic dysfunction induced by glucocorticoids, we studied the effects of selective hepatic branch vagotomy in *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ mice as well as the effects of selective disruption of vagal afferents following chronic dexamethasone therapy.

RESULTS

Selective Hepatic Vagotomy Reverses Insulin Resistance

In a series of experiments, *Ppara*$^{+/+}$ (n = 37) and *Ppara*$^{-/-}$ (n = 37) mice were treated with dexamethasone (Dex) (1 mg/kg) by i.p. injection every other day for 5 months, followed by selective hepatic vagal nerve sectioning in Dex-treated *Ppara*$^{+/+}$ mice (n = 20) and Dex-treated *Ppara*$^{-/-}$ mice (n = 20). An intraoperative photograph demonstrating the isolation of the hepatic branch of the vagus nerve is shown in Figure 1A. As a control, sham surgery (isolation of the nerve without resection) was performed on Dex-treated *Ppara*$^{+/+}$mice (n = 17) and Dex-treated *Ppara*$^{-/-}$ mice (n = 17). As an additional control, separate cohorts of *Ppara*$^{+/+}$ (n = 26) and *Ppara*$^{-/-}$ (n = 20) animals were treated with normal saline instead of Dex. Saline-treated mice also underwent either selective hepatic vagotomy or the sham procedure. Interventions were performed in five sequential experiments, each yielding the same results. Consistent with previous results (Bernal-Mizrachi et al., 2003), there was no effect of chronic Dex treatment on body weight in either genotype (see Figures S1A and S1B in the Supplemental Data available with this article online). Metabolic studies were performed 3 weeks after surgery when animals recovered their body weight (Figure 1B) and food intake had returned to normal (Figure S1C). Serum levels of alanine aminotransferase, a marker of liver inflammation, were normal 3 weeks after surgery (data not shown).

As expected (Bernal-Mizrachi et al., 2003), fasting levels of glucose and insulin were higher in Dex-treated *Ppara*$^{+/+}$ mice as compared to Dex-treated *Ppara*$^{-/-}$ mice (Table S1). There were no differences between genotypes in cholesterol, NEFA, triglycerides, body weight, or body composition at baseline or with Dex (Table S1). Dex-treated *Ppara*$^{+/+}$ mice had higher glucose levels during glucose tolerance testing as compared to Dex-treated *Ppara*$^{-/-}$ mice (Figure 1C). This glucose intolerance in Dex-treated *Ppara*$^{+/+}$ mice was abolished by selective hepatic vagotomy, but not by sham surgery (Figure 1D). Glucose tolerance was unaffected by either procedure in Dex-treated *Ppara*$^{-/-}$ animals (Figure 1E). Fasting insulin levels were lower in *Ppara*$^{+/+}$ animals after vagotomy as compared to sham surgery, but there were no effects on serum chemistries, body composition, or glucagon levels (Table S2). Insulin levels at 30 min during glucose tolerance testing were also lower in vagotomized as compared to sham-operated Dex-treated *Ppara*$^{+/+}$ mice (0.33 ± 0.07 versus 0.70 ± 0.09 ng/ml; p = 0.04). Dex-treated *Ppara*$^{+/+}$ mice became less hypoglycemic than Dex-treated *Ppara*$^{-/-}$ animals during insulin tolerance testing, indicating insulin resistance (Figure 1F). Vagotomy resulted in greater insulin responsiveness during insulin tolerance testing in Dex-treated *Ppara*$^{+/+}$ mice as compared to sham surgery (Figure 1G). These effects of enhanced insulin sensitivity following vagotomy persisted for at least 3 months after surgery (data not shown). Vagotomy had no effect in *Ppara*$^{-/-}$ mice (Figure 1H). Chronic saline injections had no effect on fasting serum chemistries and glucose or insulin tolerance testing in either genotype (data not shown).

Vagotomy Effects on Glucose Production and *Ppara* Expression

In hyperinsulinemic-euglycemic clamp experiments performed 6 weeks after surgery, endogenous glucose production was suppressed to a greater degree by insulin in vagotomized as compared to sham-operated Dex-treated *Ppara*$^{+/+}$ mice (Figure 2C, left bars). There was no effect of vagotomy on saline-treated *Ppara*$^{+/+}$ mice (Figure 2C, right bars). There were no significant effects on peripheral insulin sensitivity as measured by the clamp-determined rate of glucose disposal (Figure 2B). Enzyme activity for PEPCK, a key enzyme in gluconeogenesis, was 42% lower in the livers of vagotomized Dex-treated mice as compared to sham-operated mice (Figure S2A). Taken together, these results suggest that interruption of vagal nerve signaling blocks Dex-induced glucose intolerance in part by decreasing gluconeogenesis.

(F) Insulin tolerance testing (injection of fasted mice with 0.75 U/kg insulin at time 0 followed by blood glucose measurements) for the animals in Figure 1C.

(G) Insulin tolerance testing for the animals in Figure 1D.

(H) Insulin tolerance testing for the animals in Figure 1E. In this and all other figures, results are presented as mean ± SEM. *p < 0.05 at the same time point by two-tailed, unpaired t test. Panels show results from representative experiments. Similar results were seen in at least three independent experiments with different cohorts of mice.

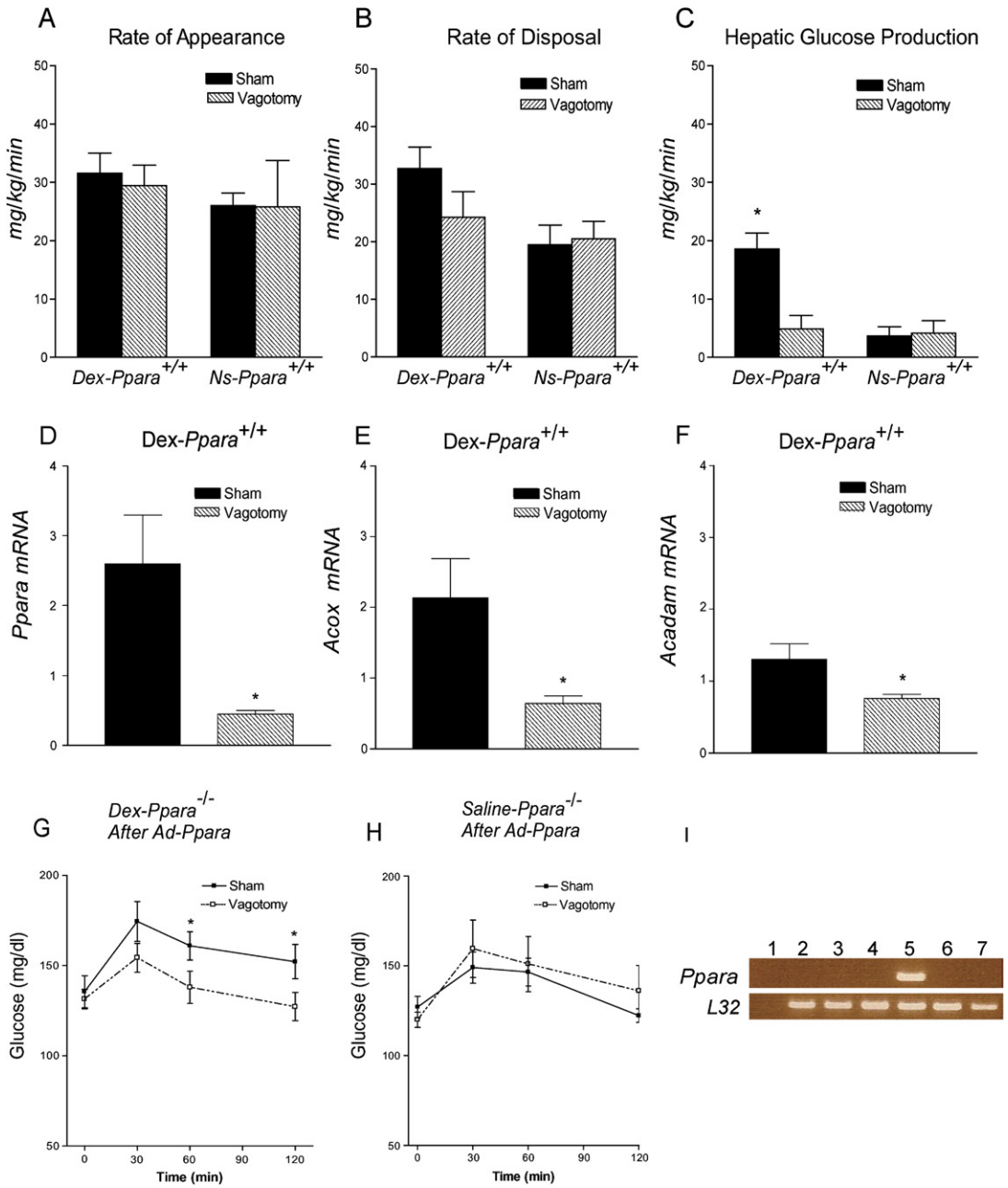

Figure 2. Effects of Vagotomy on Insulin Sensitivity, Hepatic Gene Expression, and Reconstitution of PPARα in *Ppara*$^{-/-}$ Mice

(A–C) Hyperinsulinemic-euglycemic clamp data from *Ppara*$^{+/+}$ mice after chronic treatment with Dex followed by selective hepatic vagotomy (n = 5) or sham surgery (n = 5) (left side of each panel) and after chronic treatment with saline followed by hepatic vagotomy (n = 4) or sham surgery (n = 4) (right side of each panel).

(D–F) Expression of *Ppara* and the known PPARα target genes *Acox* and *Acadam* in liver. RNA was analyzed by semiquantitative RT-PCR. Samples were isolated from *Ppara*$^{+/+}$ mice treated chronically with Dex and then subjected to selective hepatic vagotomy (n = 4) or sham surgery (n = 4).

(G) Glucose tolerance testing in Dex-treated mice. *Ppara*$^{-/-}$ mice were treated chronically with Dex followed by selective hepatic vagotomy (n = 7) or sham surgery (n = 7). After recovery from surgery, *Ppara*$^{+/+}$ expression was reconstituted in liver (as documented by RT-PCR; see below), and glucose tolerance testing was performed.

(H) Glucose tolerance testing in saline-treated mice. *Ppara*$^{-/-}$ mice were treated chronically with saline followed by selective hepatic vagotomy (n = 5) or sham surgery (n = 4). After recovery from surgery, *Ppara*$^{+/+}$ expression was reconstituted in liver, and glucose tolerance testing was performed. Results are presented as mean ± SEM. *$p < 0.05$ by two-tailed, unpaired t test except for in Figure 2C, where ANOVA was used.

(I) Tissue survey of gene expression by RT-PCR following treatment with the PPARα adenovirus. Lane 1, water control; lane 2, whole brain; lane 3, brainstem; lane 4, hypothalamus; lane 5, liver; lane 6, heart; lane 7, lung.

Selective hepatic vagotomy decreased *Ppara* expression in the liver, providing a potential mechanism for observed effects on glucose metabolism. Hepatic *Ppara* expression was ~6-fold lower in vagotomized Dex-treated animals as compared to sham-operated *Ppara*$^{+/+}$ animals (Figure 2D). Messenger RNA levels for acyl-CoA oxidase (*Acox*) and medium-chain acyl-CoA dehydrogenase (*Acadam*), known PPARα target genes, were decreased in vagotomized as compared to sham-operated mice (Figures 2E and 2F), but there was no effect on the expression of the coactivator PGC-1α (*Ppargc1*), another modulator of gluconeogenesis (data not shown).

Reconstitution of Hepatic *Ppara* Expression

When *Ppara* expression was reconstituted in the livers of normoglycemic Dex-treated *Ppara*$^{-/-}$ mice subjected to either vagotomy or sham surgery (see metabolic characteristics of these mice in Figures 1E and 1H), sham-operated animals had greater glucose excursions than vagotomized mice during glucose tolerance tests (Figure 2G). At 30 min after glucose injection, insulin levels were 3-fold higher in sham-operated as compared to vagotomized animals (0.9 ± 0.2 versus 0.3 ± 0.06 ng/ml; $p < 0.04$). Enzyme activity for PEPCK was ~75% lower in vagotomized Dex-treated *Ppara*$^{-/-}$ mice reconstituted with AdPPARα as compared to sham-operated animals treated with the same virus (Figure S2B). There was no effect on the glycemic response (Figure 2H) or insulin levels (data not shown) when *Ppara* expression was reconstituted in the livers of saline-treated mice. Treatment with control virus has no effect in this model (Bernal-Mizrachi et al., 2003). PPARα protein has been detected in the CNS (Moreno et al., 2004), raising the possibility that central activation of this receptor affects responses in our model. However, AdPPARα treatment of Dex-treated PPARα null mice produces glucose intolerance associated with *Ppara* expression in liver but not whole brain, brainstem, or hypothalamus (or other tissues; Figure 2I). These data suggest that hepatic expression of *Ppara* (but not CNS *Ppara* expression) and an intact hepatic vagus nerve are required for the development of glucocorticoid-induced insulin resistance.

Intact Vagus Nerve Is Required for Dex-Induced Hypertension

With Dex treatment, both systolic and diastolic blood pressure was ~20 mm Hg higher in *Ppara*$^{+/+}$ as compared to *Ppara*$^{-/-}$ mice (Figure 3A, left panel). Selective hepatic vagotomy in Dex-treated *Ppara*$^{+/+}$ mice decreased systolic blood pressure by 10 mm Hg ($p < 0.001$) and diastolic pressure by 9 mm Hg ($p < 0.01$) (Figure 3A, middle panel). These differences detected by tail-cuff determinations were confirmed invasively in anesthetized mice (Figure S3A). There was no effect of vagotomy on blood pressure in Dex-treated *Ppara*$^{-/-}$ mice (Figure 3A, right panel) or in saline-treated mice of either genotype (Figure 3B). Blood pressure measurements were made 3 weeks after surgery when there was no difference between vagotomized and sham-operated mice in sodium intake, water intake, or urine output (Figures S1D, S1F, and S1G). Plasma renin activity and urinary catecholamine levels, critical determinants of renal sodium handling and blood pressure, decreased by ~50% in Dex-treated *Ppara*$^{+/+}$ mice after vagotomy compared with sham-operated animals (Table 1). Urinary sodium excretion increased 3-fold after vagotomy in Dex-treated *Ppara*$^{+/+}$ mice (Table 1), consistent with less activity of the sympathetic nervous system and renin-angiotensin-aldosterone axis after selective hepatic vagotomy. There were no effects on serum leptin.

Reconstitution of Hepatic *Ppara* Expression and Blood Pressure

Reconstitution of *Ppara* expression in the livers of normotensive Dex-treated *Ppara*$^{-/-}$ mice subjected to either vagotomy or sham surgery by infusion of a PPARα adenovirus increased systolic blood pressure by 25 mm Hg ($p = 0.001$) and diastolic blood pressure by 16 mm Hg ($p = 0.04$) in sham-operated animals compared to vagotomized animals (Figure 3C). Blood pressure effects were confirmed invasively (Figure S3B). There was no effect on blood pressure in saline-treated mice (Figure 3D). Hepatic reconstitution of *Ppara* in sham-operated animals nearly doubled plasma renin activity ($p = 0.04$) and decreased urinary sodium ($p = 0.04$) as compared to vagotomized mice treated with the PPARα adenovirus (Table S3). These data suggest that hepatic *Ppara* activation and the presence of an intact vagus nerve promote glucocorticoid-induced hypertension through a renin-dependent mechanism.

Selective Disruption of Vagal Afferent Fibers

Since both afferent (Uno et al., 2006) and efferent (Lam et al., 2005) fibers of the vagus have been implicated in systemic metabolic effects, vagal afferents were selectively disrupted using two independent techniques in animals treated with Dex. Hepatic vagal branch application of capsaicin, which is toxic to unmyelinated afferent fibers, disrupted vagal afferent fibers (Figures 4C and 4D, arrows) but did not affect myelinated fibers (Figures 4C and 4D, arrowheads) as demonstrated by electron microscopy 10 days following the procedure. Unmyelinated fibers from vehicle-treated vagus nerves were unaffected (Figure 4A, arrows), as were fibers in the posterior vagal trunk (which was untreated) of animals subjected to capsaicin treatment of the hepatic vagal branch (Figure 4B). There was no difference in food intake between capsaicin- and control-treated mice 10 days after the procedure (Figure 4E) and no difference in weight (Figure 4F). Capsaicin-induced disruption of vagal afferent fibers reversed glucose intolerance (Figure 4G). To confirm this observation, vagal afferents were surgically interrupted at the brainstem. Representative views of the surgical approach are shown in Figures 5A–5C, with the area denoted by the rectangle in Figure 5A expanded in Figure 5B and the area denoted by the rectangle in Figure 5B expanded in Figure 5C. Body weights following the central afferent vagotomy and the sham procedure (in which the dura was opened and the field visualized without surgical resection) are

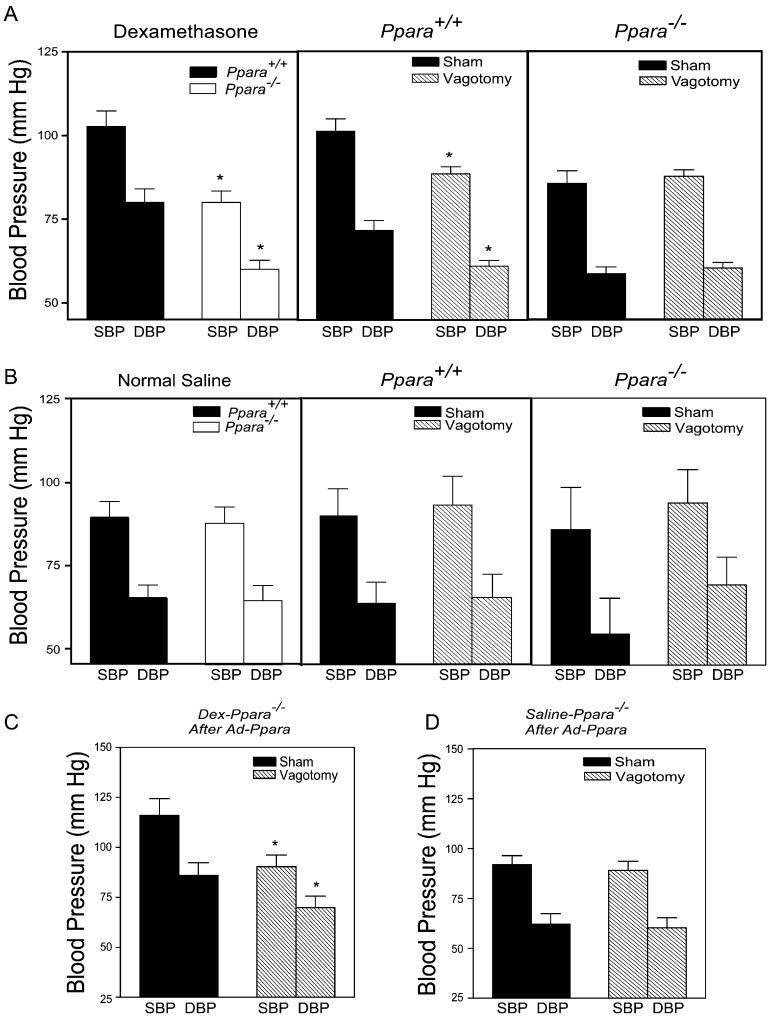

Figure 3. Effects of Vagotomy on Blood Pressure

(A) *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ mice were treated with Dex for 5 months (1 mg/kg i.p. every other day), and systolic (SBP) and diastolic (DBP) blood pressure was determined by tail cuff (left panel; n = 13 for each genotype). Mice then underwent either selective hepatic vagotomy or sham surgery, and blood pressure measurements were repeated following recovery in *Ppara*$^{+/+}$ (middle panel; n = 14 for vagotomy, n = 10 for sham surgery) and *Ppara*$^{-/-}$ (right panel; n = 14 for vagotomy, n = 8 for sham surgery) mice. The blood pressure effects in *Ppara*$^{+/+}$ mice were confirmed invasively (see Figure S3).

(B) *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$mice were treated with normal saline injections for 5 months, and systolic (SBP) and diastolic (DBP) blood pressure was determined by tail cuff (left panel; n = 10 for each genotype). These control mice then underwent either selective hepatic vagotomy or sham surgery, and blood pressure measurements were repeated following recovery in *Ppara*$^{+/+}$ (middle panel; n = 5 for vagotomy, n = 5 for sham surgery) and *Ppara*$^{-/-}$ (right panel; n = 5 for vagotomy, n = 5 for sham surgery) mice.

(C) *Ppara*$^{-/-}$ mice were treated chronically with Dex followed by selective hepatic vagotomy (n = 7) or sham surgery (n = 7). After recovery from surgery, *Ppara*$^{+/+}$ expression was reconstituted in liver (as documented by RT-PCR), and blood pressure was measured by tail cuff (shown here) and confirmed invasively (shown in Figure S3).

(D) *Ppara*$^{-/-}$ mice were treated chronically with saline followed by selective hepatic vagotomy (n = 5) or sham surgery (n = 5). After recovery from surgery, *Ppara*$^{+/+}$ expression was reconstituted in liver, and blood pressure was measured. Results are presented as mean ± SEM. *$p < 0.05$ by two-tailed, unpaired t test.

shown in Figure 5D. Three weeks after the surgeries, central afferent vagotomy lowered blood pressure (Figure 5E) and improved glucose tolerance (Figure 5F) in Dex-treated mice.

DISCUSSION

Despite important anti-inflammatory properties, glucocorticoids are potentially dangerous due to side effects that include insulin resistance, diabetes, and hypertension. Here we show that glucocorticoid-induced glucose intolerance and elevated blood pressure depend on an afferent vagal nerve pathway initiated by expression of the lipid-activated transcription factor PPARα in the liver.

Several lines of evidence implicate the autonomic nervous system in the regulation of hepatic metabolism, although results vary based on the experimental model. In dogs, chronic denervation of the liver has little effect on glucose metabolism (Wada et al., 1995), while acute disruption of the vagus decreases hepatic glucose production by inhibiting glycogenolysis (Cardin et al., 2002). In rats, both vagotomy (Xie and Lautt, 1996; Matsuhisa et al., 2000) and electrical stimulation of the vagus (Peitl et al., 2005) have been reported to induce insulin resistance. Also in rats, the hypothalamic infusion of fatty acids suppresses hepatic glucose production through mechanisms involving descending vagal input (Lam et al., 2005). Adenoviral expression of PPARγ2 in liver decreases peripheral adiposity in a process that depends on the afferent vagus (Uno et al., 2006). By extending selective hepatic vagotomy and disruption of the afferent vagus to mice with genetic manipulations of PPARα, there are three conclusions we can make about the role of the vagus nerve in metabolism.

First, an intact hepatic branch of the vagus in the presence of hepatic *Ppara* expression is required for the development of glucocorticoid-induced diabetes. Vagotomy reversed hyperglycemia in *Ppara*$^{+/+}$ mice (Figure 1D), and reconstitution of *Ppara* expression in the livers of Dex-treated *Ppara*$^{-/-}$ mice induced hyperglycemia after

Table 1. Effects of Selective Hepatic Vagotomy versus Sham Surgery in Dex-Treated *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ Mice

	Ppara$^{+/+}$			*Ppara*$^{-/-}$		
	Sham	Vagotomy	p	Sham	Vagotomy	p
Renin (ng/ml/hr)	22.5 ± 3.4 (n = 8)	14 ± 2.2 (n = 8)	0.01	23 ± 4.0 (n = 8)	18.2 ± 1.2 (n = 8)	0.3
Urinary norepinephrine (ng/day)	530 ± 84 (n = 8)	272 ± 71 (n = 8)	0.03	267 ± 48 (n = 8)	186 ± 50 (n = 8)	0.4
Urinary sodium (nmol/mg Cr/g/day)	0.07 ± 0.01 (n = 7)	0.11 ± 0.01 (n = 9)	0.01	0.07 ± 0.01 (n = 7)	0.09 ± 0.01 (n = 12)	0.3
Leptin (pg/ml)	4.08 ± 1.3 (n = 5)	5.1 ± 1.9 (n = 5)	0.6	6.2 ± 0.5 (n = 5)	5.4 ± 1.2 (n = 5)	0.5

Ppara$^{+/+}$ and *Ppara*$^{-/-}$ mice were treated with Dex for 5 months (1 mg/kg i.p. every other day) and then subjected to either selective hepatic vagotomy or sham surgery. Dex-induced elevated blood pressure was corrected by vagotomy in *Ppara*$^{+/+}$ mice as shown in Figure 3. At the time of blood pressure measurement, plasma renin activity as well as serum leptin was measured, and 24 hr urine collections were obtained and assayed for norepinephrine and sodium. Results are presented as mean ± SEM.

sham surgery but not after vagotomy (Figure 2G). PPARα was not a passive participant in this process. Glucocorticoids are known to induce hepatic *Ppara* gene expression (Lemberger et al., 1994, 1996), and *Ppara* is an important mediator of gluconeogenesis (Bernal-Mizrachi et al., 2003; Koo et al., 2004). Surprisingly, selective hepatic vagotomy decreased hepatic *Ppara* expression and the expression of two PPARα target genes, *Acox* and *Acadam* (Figures 2D–2F), without affecting expression of the coactivator *Ppargc1*. These findings, occurring in weight-stable mice with normal food intake and no evidence of hepatic dysfunction, suggest that vagal input is critical for the maintenance of glucocorticoid-induced transcriptional responses in the liver.

Rossetti and colleagues recently provided evidence for links between the CNS and hepatic glucose production (Obici et al., 2002) involving the hepatic branch of the vagus (Lam et al., 2005). Our data show that selective hepatic vagotomy has no effect on glucose production in saline-treated wild-type mice (Figure 2). This procedure also had no effect on glucose production in saline-treated rats (see Supplementary Figure 3 in Lam et al., 2005). Lam et al. showed that an Intralipid/heparin infusion decreases glucose production through vagal efferents. The current study shows that glucocorticoid treatment increases glucose production through vagal afferents. Collectively, the results indicate that the vagus can mediate increased or decreased hepatic glucose production through different fibers depending on the specific stimulus.

The second conclusion we can make is that an intact hepatic branch of the vagus in the presence of hepatic *Ppara* expression is required for the development of glucocorticoid-induced hypertension. Vagotomy reversed established blood pressure elevations in *Ppara*$^{+/+}$ mice (Figure 3A) in concert with lower catecholamines, decreased renin activity, and less sodium retention (Table 1). Reconstitution of *Ppara* expression in the livers of Dex-treated *Ppara*$^{-/-}$ mice elevated blood pressure after sham surgery, but not after vagotomy (Figure 3C). Consistent with the current results, glucocorticoid-induced hypertension is thought to be caused by increased sodium retention (Whitworth et al., 1979). The pathophysiology may also involve enhanced vascular reactivity to pressors (Walker et al., 1994) and altered vascular nitric oxide production (Wallerath et al., 1999). Our data point to a critical role of an intact vagus nerve responding to hepatic *Ppara* expression in mediating the blood pressure response to chronic glucocorticoid exposure.

Our third conclusion is that intact vagal afferent fibers are required for glucocorticoid-induced glucose intolerance and hypertension. In animals treated with Dex, disruption of the afferent vagus using topical application of the selective neurotoxin capsaicin (Figure 4) reversed glucose tolerance (Figure 4G). Surgical interruption of vagal afferents at the brainstem (Figure 5) reversed glucose intolerance (Figure 5F) and lowered blood pressure (Figure 5E). These surgical results should be interpreted with the caveat that, although physiological effects resembled those with capsaicin, neuroanatomical verification as presented in the rat model (Walls et al., 1995) was not performed in these mice. Hepatic expression of a different nuclear receptor, PPARγ2, also appears to require intact vagal afferents to produce systemic effects, but these effects are strikingly different from those induced by PPARα: induction of hepatic expression of PPARγ2 increases insulin sensitivity (Uno et al., 2006), while Dex induction of hepatic expression of PPARα in the current study decreases insulin sensitivity. The fact that afferent vagal fibers are involved in both suggests that metabolic signals sent by each nuclear receptor in the liver are distinct. While neuroanatomical data for the vagus in mice are limited, rat studies indicate that sensory vagal fibers do not appear to provide substantial direct innervation of hepatocytes, instead tracking with portal triads in proximity to branches of the hepatic artery, portal vein, and bile ducts (Berthoud and Neuhuber, 2000). It is plausible that metabolic responses involve a regulatory compartment of hepatocytes located near portal triads. Since there may be little direct innervation of hepatocytes by the vagus, induction of PPARα-dependent genes in these cells could increase extracellular metabolites in the portal triad, resulting in vagal afferent signals ultimately translated by the brain into peripheral effects that include glucose intolerance and hypertension. Clarifying the nature of these

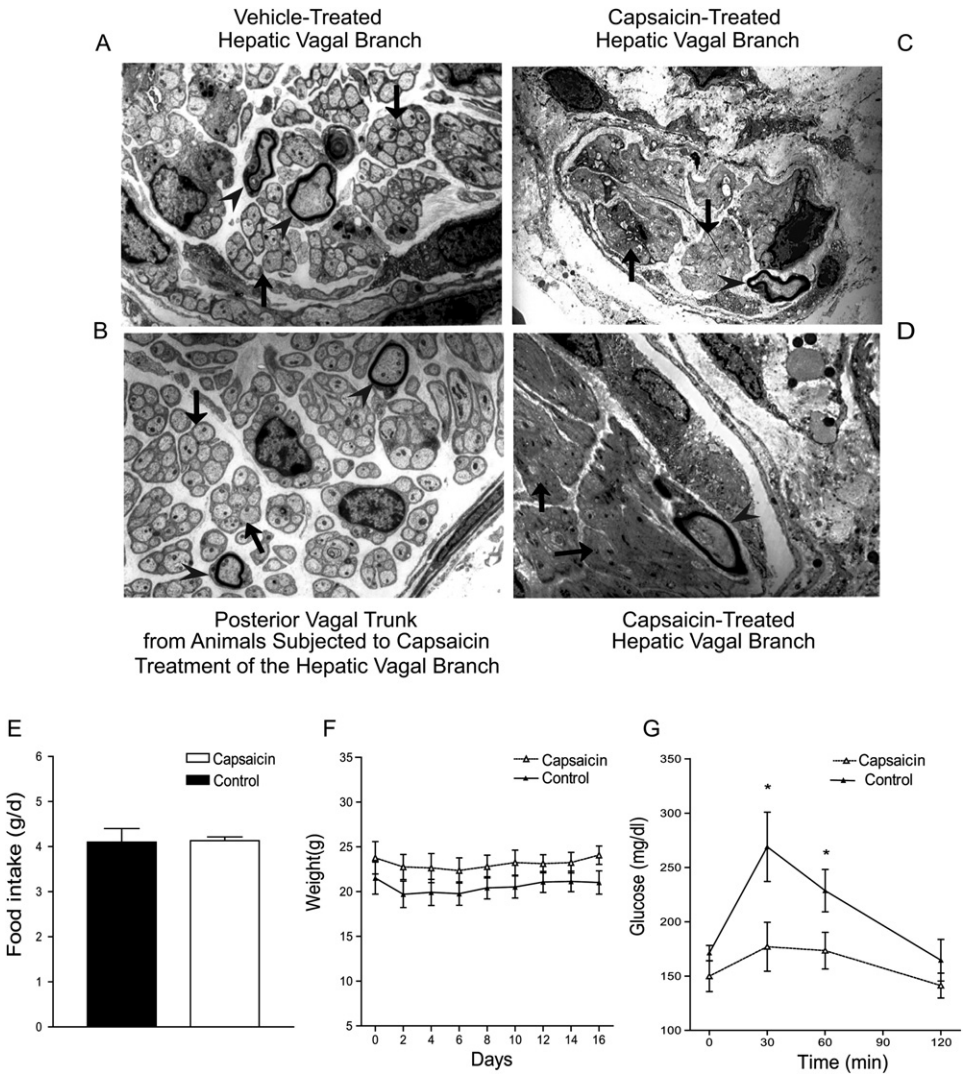

Figure 4. Selective Peripheral Afferent Vagal Denervation Reverses Dex-Induced Insulin Resistance

Dex-treated *Ppara*$^{+/+}$ mice underwent either capsaicin or vehicle application to the hepatic vagal branch. Electron microscopy (EM) of hepatic vagal nerve sections (A–D) and physiological studies (E–G) were performed 10 days later. In (A)–(D), arrows represent unmyelinated fibers and arrowheads represent myelinated fibers.

(A) EM assessment of the vagus in vehicle-treated mice.

(B) EM evaluation from the posterior vagal trunk of an animal subjected to capsaicin treatment of the hepatic vagal branch.

(C and D) EM of hepatic vagal nerves from two different capsaicin-treated animals.

(E) Food intake in grams per day from four animals in each group.

(F) Body weight after capsaicin or vehicle treatment. Weight data in grams are presented for five mice in each group. The horizontal axis represents days after the surgical procedure.

(G) Glucose tolerance testing in capsaicin-treated (n = 5) and vehicle-treated (n = 5) mice. Results are presented as mean ± SEM. *$p < 0.05$ at the same time point by two-tailed, unpaired t test. Panels show results from representative experiments. Similar results were seen in two independent experiments with different cohorts of mice.

PPARα-dependent metabolites and the characteristics of the vagal afferent signals they generate could yield new approaches for treating insulin resistance.

The vagus nerve derives its name from the Latin word for "wanderer," a reference to the meandering anatomic distribution of the nerve. Given recent discoveries that this nerve suppresses inflammation (Borovikova et al., 2000), inhibits seizure activity (Uthman et al., 2004) and depression (Nahas et al., 2005), modulates CNS responses to fatty acids (Lam et al., 2005), and establishes a circuit between the liver and CNS leading to either enhanced insulin sensitivity (Uno et al., 2006) or diabetes and hypertension (the current work), it appears that the full extent of the nomadic journey of the vagus is still unknown.

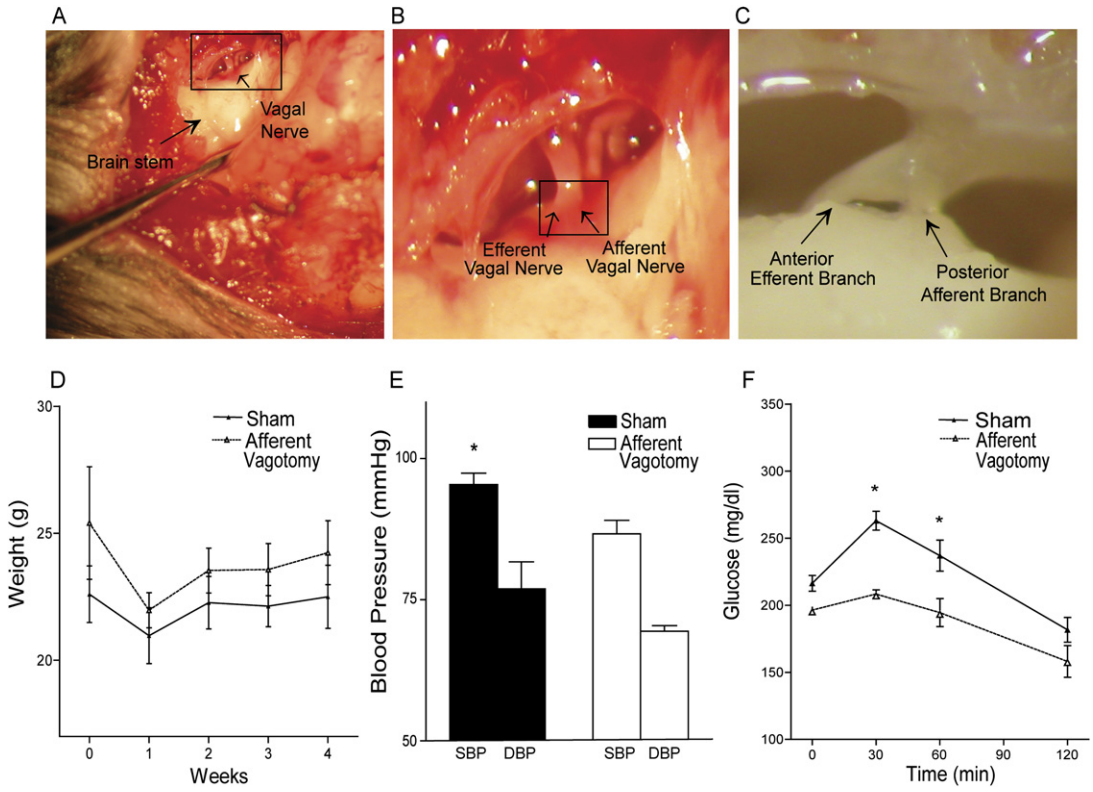

Figure 5. Central Afferent Vagal Nerve Sectioning Reverses Dex-Induced Hypertension and Insulin Resistance

Dex-treated *Ppara*$^{+/+}$ mice were randomly assigned to either selective section of the vagal afferent fibers in the brainstem or a sham operation.

(A–C) Views of the operative field, with the area enclosed by the rectangle in (A) expanded in (B) and the rectangle in (B) expanded in (C).

(D) Body weight in grams is presented for three mice in each group. The horizontal axis represents weeks after the surgical procedure.

(E) Tail-cuff systolic (SBP) and diastolic (DBP) blood pressure was obtained 3 weeks after the procedure (n = 3 for each group).

(F) Glucose tolerance testing 3 weeks after central afferent vagal sectioning (n = 3) and the sham operation (n = 3). Results are presented as mean ± SEM. *$p < 0.05$ at the same time point by two-tailed, unpaired t test.

EXPERIMENTAL PROCEDURES

Animals

The *Ppara*$^{+/+}$ and *Ppara*$^{-/-}$ mice used for these studies were produced by crossing *Ppara*$^{-/-}$ animals (a kind gift from Dr. Frank Gonzalez and developed at the NIH as described by Lee et al., 1995) with inbred C57BL/6 animals to the N6 generation. Littermate mice of both sexes were treated with either Dex (1 mg/kg i.p.) or an equal volume of normal saline every other day beginning at the age of 8 weeks. The same effects of interventions were seen in both sexes. Animals were housed in a specific-pathogen-free barrier facility with free access to water and standard mouse chow (providing 6% of calories as fat). The Washington University Animal Studies Committee approved these experiments, and all procedures were performed as recommended by the NIH Guide for the Care and Use of Laboratory Animals and the Laboratory Animal Welfare Act.

Selective Hepatic Vagotomy

Vagotomy was performed by modifying a procedure previously described in rats (Bellinger and Williams, 1981). Mice were anesthetized with pentobarbital (60 mg/kg). The abdominal wall was incised at midline, the stomach was pulled down, and the ligaments attaching the liver to the stomach were severed. The anterior ligament and the membranous connection of the left lobe of the liver to the diaphragm were cut to give better visibility of the esophageal-hepatic attachments. This region contains a neurovascular bundle including the hepatic branch of the vagus nerve. This branch was selectively ligated by silk sutures and cauterized (Smith and Jerome, 1983). The tissue at the ligation site was resected and examined microscopically to ensure that the manipulated structure represented nerve. After experiments, animals were sacrificed and the adequacy of the procedure was assessed by demonstrating the absence of vagal nerve tissue between the silk sutures and by tracing the nerve remnant to its origin in the neck by dissection.

Selective Disruption of Vagal Afferents

Two independent techniques, topical application of capsaicin and surgical interruption of fibers at the brainstem, were used to disrupt vagal afferents.

Capsaicin (10 mg) was sonicated with 0.1 ml Tween 80 for 10 min and brought to 1 ml with olive oil. Following induction of pentobarbital anesthesia, the hepatic vagal nerve was exposed as noted above. Gauze soaked in capsaicin, isolated from surrounding tissues by paraffin paper, was wrapped around the nerve for 30 min, with additional agent applied twice during that period to yield a total dose of 0.5 mg per mouse. Tween 80 in olive oil was applied to the nerve in control animals. Physiological evaluations were conducted 10 days after surgery.

Surgical interruption of the afferent fibers at the left side of the brainstem was performed using a dorsolateral approach. Unilateral sectioning was pursued due to the technically demanding nature of

the procedure and because previous data in rats indicate that the hepatic vagus projects predominantly to the left side of the brainstem (Magni and Carobi, 1983; Berthoud et al., 1992). Following induction of anesthesia with ketamine (75 mg/kg) and medetomidine (1 mg/kg, i.p.), the head and dorsal surface of the upper thorax were shaved and swabbed with Betadine, the eyes were protected with artificial tears, and the animal was secured into a stereotaxic device. Temperature was maintained using a heating pad and a heat lamp attached to a temperature probe. Muscles attached to the occipital portion of the skull were resected to expose the foramen magnum. Fine-tipped forceps were used to cut through the ventrolateral aspect of the skull rostrally to expose the brainstem. Care was necessary to avoid breaching the venous sinus near the rostral end of the brainstem. The brainstem was gently retracted to allow visualization of the vagus nerve between where it attaches to the brainstem and where it exits the skull through the posterior lacerated foramen. The dura was opened using iridectomy scissors to visualize the posterior cranial nerves. When the rootlets of the vagus were identified, the dorsal rootlets were avulsed from the brainstem using a dura hook inserted between the dorsal (afferent) and ventral (efferent) rootlets. After deafferentation, artificial dura (Duragen) was applied to the exposed area. Muscles were sutured back onto their insertion points on the skull, and the skin was closed. The animals were removed from the stereotaxic device and placed on a separate heating pad, and the anesthetic reversal agent atipamezole (1 mg/kg, s.c.), warm saline (2 cc, i.p.), enrofloxacin (2.5 mg/kg, s.c.) and buprenorphine (0.1 mg/kg, s.c.) were administered. Animals were closely monitored during recovery and, when fully awake, were placed in clean cages with water and nutritional supplements (Nutri-Cal and Froot Loops) available on or near the cage floor so that access would require minimal neck movement. Saline, antibiotics, and analgesia were continued for 3 days. Physiological evaluations were conducted 3 weeks after surgery.

Analytical Procedures

Glucose, insulin, cholesterol, triglycerides, nonesterified fatty acids, and leptin were assayed as described (Bernal-Mizrachi et al., 2002). Glucose and insulin tolerance tests were performed as described in procedures separated by at least 1 week (Marshall et al., 1999). Body composition was determined by dual-energy X-ray absorptiometry. Twenty-four hour urine collections for urinary sodium and catecholamines were as described (Bernal-Mizrachi et al., 2003). Renin activity was measured in 500 μl aliquots of pooled plasma from ten animals per condition by radioimmunoassay of in vitro-generated angiotensin I using a kit from DiaSorin. Electron microscopy was performed by the Washington University Cell Biology & Physiology Core with tissue prepared as described (Han et al., 2004).

Hyperinsulinemic-Euglycemic Clamps

Clamp experiments were performed essentially as described (Marshall et al., 1999). [3-^{3}H]glucose was infused to steady state, regular human insulin was infused at 10 mU/kg/min, and 25% D-glucose was infused to maintain blood glucose at 120 mg/dl for at least 90 min. The [3-^{3}H]glucose infusion was continued during the clamp, with labeled glucose included in the 25% D-glucose infusion to match blood-specific activity at steady state. The rate of appearance of glucose (R_a), equal to the rate of glucose utilization (R_d) at steady state, was determined by dividing the infusion rate of labeled glucose by the specific activity. Endogenous glucose production was calculated by subtracting the cold glucose infusion rate from the clamp R_d.

Adenovirus Treatment

AdPPARα (containing the mouse *Ppara* cDNA) was generated and administered as described (Tordjman et al., 2002; Bernal-Mizrachi et al., 2003). Viruses were infused at a dose of 6×10^9 pfu in a total volume of 200 μl. Expression in tissues, almost exclusively limited to liver, was determined in a subset of animals sacrificed 3 days following infusion by RT-PCR using RNA from aorta, liver, kidney, heart, skeletal muscle, lung, and gonadal fat pad. The primers used were as follows: *Ppara* forward, 5′-TCGGCCTGGCCTTCTAAACA-3′, *Ppara* reverse, 5′-GTTACACCCTCCAGAACAGT-3′; *L32* forward, 5′-TAAGCGAAACTGGCGGAAAC-3′, *L32* reverse, 5′-TCATTTTCTTCGCTGCGTAGC-3′.

Blood Pressure

Noninvasive determinations of systolic and diastolic blood pressure were made over several days in conscious mice using a Kent Scientific tail-cuff system as described (Bernal-Mizrachi et al., 2002). For mice treated with adenoviruses, measurements began 4 days following the injection. Noninvasive results were confirmed using a PowerLab/8SP invasive monitoring instrument (AD Instruments).

Semiquantitative RT-PCR-Based Gene Expression

Analyses were done in a GeneAmp 7700 Sequence Detector (Applied Biosystems) using the following oligonucleotides: *Ppara* forward, 5′-ACTACGGAGTTCACGCATGTG-3′, *Ppara* reverse, 5′-TTGTCGTACACCAGCTTCAGC-3′, probe, 5′-AGGCTGTAAGGGCTTCTTTCGGCG-3′; *Ppargc1* forward, 5′-CGGAAATCATATCCAACCAG-3′, *Ppargc1* reverse, 5′-TGAGGACCGCTAGCAAGTTTG-3′, probe, 5′-TCATTCTCTTCATCTACTTTCTCAAATATGTTC-3′; *Aco* forward, 5′-GGATGGTAGTCCGGAGAACA-3′, *Aco* reverse, 5′-AGTCTGGATCGTTCAGAATCAAG-3′, probe, 5′-TCTCGATTTCTCGACGGCGCCG-3′; *Mcad* forward, 5′GGAAATGATCAACAAAAAAAGTATTT-3′, *Mcad* reverse, 5′-ATGGCCGCCACATCAGA-3′, probe, 5′-TGTCACACAGTAAGCACACATCATTGGCTG-3′; *L32* forward, 5′-AAGCGAAACTGGCGGAAAC-3′, *L32* reverse, 5′-GATCTGGCCCTTGAACCTTCT-3′, probe, 5′-CAGAGGCATTGACAACAGGGTGCG-3′. Results were normalized to the housekeeping gene *L32*, whose expression was unaffected by genotype or Dex treatment.

PEPCK Enzyme Activity

10% (w/v) liver homogenates were prepared at 0°C in 250 mmol/l sucrose, 5 mmol/l HEPES (pH 7.4) buffer and centrifuged at 3600 × g for 10 min. Supernatant fractions were further centrifuged at 130,000 × g for 30 min, and PEPCK enzyme activity was assayed by following the consumption of NADH as previously described (Petrescu et al., 1979; Jamieson et al., 1999).

Supplemental Data

Supplemental Data include three tables and three figures and can be found with this article online at http://www.cellmetabolism.org/cgi/content/full/5/2/91/DC1/.

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REFERENCES

Bellinger, L.L., and Williams, F.E. (1981). The effects of liver denervation on food and water intake in the rat. Physiol. Behav. *26*, 663–671.

Benthem, L., Keizer, K., Wiegman, C.H., de Boer, S.F., Strubbe, J.H., Steffens, A.B., Kuipers, F., and Scheurink, A.J. (2000). Excess portal venous long-chain fatty acids induce syndrome X via HPA axis and sympathetic activation. Am. J. Physiol. Endocrinol. Metab. *279*, E1286–E1293.

Berger, J., and Moller, D.E. (2002). The mechanisms of action of PPARs. Annu. Rev. Med. *53*, 409–435.

Bernal-Mizrachi, C., Weng, S., Li, B., Nolte, L.A., Feng, C., Coleman, T., Holloszy, J.O., and Semenkovich, C.F. (2002). Respiratory uncoupling lowers blood pressure through a leptin-dependent mechanism in genetically obese mice. Arterioscler. Thromb. Vasc. Biol. *22*, 961–968.

Bernal-Mizrachi, C., Weng, S., Feng, C., Finck, B.N., Knutsen, R.H., Leone, T.C., Coleman, T., Mecham, R.P., Kelly, D.P., and Semenkovich, C.F. (2003). Dexamethasone induction of hypertension and diabetes is PPAR-alpha dependent in LDL receptor-null mice. Nat. Med. *9*, 1069–1075.

Berthoud, H.-R., and Neuhuber, W.L. (2000). Functional and chemical anatomy of the afferent vagal system. Auton. Neurosci. *85*, 1–17.

Berthoud, H.-R., Kressel, M., and Neuhuber, W.L. (1992). An anterograde tracing study of the vagal innervation of rat liver, portal vein and biliary system. Anat. Embryol. (Berl.) *186*, 431–442.

Borovikova, L.V., Ivanova, S., Zhang, M., Yang, H., Botchkina, G.I., Watkins, L.R., Wang, H., Abumrad, N., Eaton, J.W., and Tracey, K.J. (2000). Vagus nerve stimulation attenuates the systemic inflammatory response to endotoxin. Nature *405*, 458–462.

Cardin, S., Walmsley, K., Neal, D.W., Williams, P.E., and Cherrington, A.D. (2002). Involvement of the vagus nerves in the regulation of basal hepatic glucose production in conscious dogs. Am. J. Physiol. Endocrinol. Metab. *283*, E958–E964.

Chakravarthy, M., Pan, Z., Zhu, Y., Tordjman, K., Schneider, J., Coleman, T., Turk, J., and Semenkovich, C.F. (2005). "New" hepatic fat activates PPARα to maintain glucose, lipid and cholesterol homeostasis. Cell Metab. *1*, 309–322.

Grekin, R.J., Vollmer, A.P., and Sider, R.S. (1995). Pressor effects of portal venous oleate infusion. A proposed mechanism for obesity hypertension. Hypertension *26*, 193–198.

Grekin, R.J., Dumont, C.J., Vollmer, A.P., Watts, S.W., and Webb, R.C. (1997). Mechanisms in the pressor effects of hepatic portal venous fatty acid infusion. Am. J. Physiol. Regul. Integr. Comp. Physiol. *273*, R324–R330.

Guerre-Millo, M., Rouault, C., Poulain, P., Andre, J., Poitout, V., Peters, J.M., Gonzalez, F.J., Fruchart, J.C., Reach, G., and Staels, B. (2001). PPAR-alpha-null mice are protected from high-fat diet-induced insulin resistance. Diabetes *50*, 2809–2814.

Han, D.-H., Nolte, L.A., Ju, J.-S., Coleman, T., Holloszy, J.O., and Semenkovich, C.F. (2004). UCP-mediated energy depletion in skeletal muscle increases glucose transport despite lipid accumulation and mitochondrial dysfunction. Am. J. Physiol. Endocrinol. Metab. *286*, E347–E353.

Jamieson, P.M., Nyirenda, M.J., Walker, B.R., Chapman, K.E., and Seckl, J.R. (1999). Interactions between oestradiol and glucocorticoid regulatory effects on liver-specific glucocorticoid-inducible genes: possible evidence for a role of hepatic 11beta-hydroxysteroid dehydrogenase type 1. J. Endocrinol. *160*, 103–109.

Kersten, S., Seydoux, J., Peters, J.M., Gonzalez, F.J., Desvergne, B., and Wahli, W. (1999). Peroxisome proliferator-activated receptor alpha mediates the adaptive response to fasting. J. Clin. Invest. *103*, 1489–1498.

Koo, S.H., Satoh, H., Herzig, S., Lee, C.H., Hedrick, S., Kulkarni, R., Evans, R.M., Olefsky, J., and Montminy, M. (2004). PGC-1 promotes insulin resistance in liver through PPAR-alpha-dependent induction of TRB-3. Nat. Med. *10*, 530–534.

Lam, T.K., Pocai, A., Gutierrez-Juarez, R., Obici, S., Bryan, J., Aguilar-Bryan, L., Schwartz, G.J., and Rossetti, L. (2005). Hypothalamic sensing of circulating fatty acids is required for glucose homeostasis. Nat. Med. *11*, 320–327.

Lee, S.S., Pineau, T., Drago, J., Lee, E.J., Owens, J.W., Kroetz, D.L., Fernandez-Salguero, P.M., Westphal, H., and Gonzalez, F.J. (1995). Targeted disruption of the alpha isoform of the peroxisome proliferator-activated receptor gene in mice results in abolishment of the pleiotropic effects of peroxisome proliferators. Mol. Cell. Biol. *15*, 3012–3022.

Lemberger, T., Staels, B., Saladin, R., Desvergne, B., Auwerx, J., and Wahli, W. (1994). Regulation of the peroxisome proliferator-activated receptor alpha gene by glucocorticoids. J. Biol. Chem. *269*, 24527–24530.

Lemberger, T., Saladin, R., Vazquez, M., Assimacopoulos, F., Staels, B., Desvergne, B., Wahli, W., and Auwerx, J. (1996). Expression of the peroxisome proliferator-activated receptor alpha gene is stimulated by stress and follows a diurnal rhythm. J. Biol. Chem. *271*, 1764–1769.

Magni, F., and Carobi, C. (1983). The afferent and preganglionic parasympathetic innervation of the rat liver, demonstrated by the retrograde transport of horseradish peroxidase. J. Auton. Nerv. Syst. *8*, 237–260.

Marshall, B.A., Tordjman, K., Host, H.H., Ensor, N.J., Kwon, G., Marshall, C.A., Coleman, T., McDaniel, M.L., and Semenkovich, C.F. (1999). Relative hypoglycemia and hyperinsulinemia in mice with heterozygous lipoprotein lipase (LPL) deficiency. Islet LPL regulates insulin secretion. J. Biol. Chem. *274*, 27426–27432.

Masuzaki, H., Paterson, J., Shinyama, H., Morton, N.M., Mullins, J.J., Seckl, J.R., and Flier, J.S. (2001). A transgenic model of visceral obesity and the metabolic syndrome. Science *294*, 2166–2170.

Matsuhisa, M., Yamasaki, Y., Shiba, Y., Nakahara, I., Kuroda, A., Tomita, T., Iida, M., Ikeda, M., Kajimoto, Y., Kubota, M., and Hori, M. (2000). Important role of the hepatic vagus nerve in glucose uptake and production by the liver. Metabolism *49*, 11–16.

Moreno, S., Farioli-Vecchioli, S., and Ceru, M.P. (2004). Immunolocalization of peroxisome proliferator-activated receptors and retinoid X receptors in the adult rat CNS. Neuroscience *123*, 131–145.

Nahas, Z., Marangell, L.B., Husain, M.M., Rush, A.J., Sackeim, H.A., Lisanby, S.H., Martinez, J.M., and George, M.S. (2005). Two-year outcome of vagus nerve stimulation (VNS) for treatment of major depressive episodes. J. Clin. Psychiatry *66*, 1097–1104.

Obici, S., Zhang, B.B., Karkanias, G., and Rossetti, L. (2002). Hypothalamic insulin signaling is required for inhibition of glucose production. Nat. Med. *12*, 1376–1382.

Paterson, J.M., Morton, N.M., Fievet, C., Kenyon, C.J., Holmes, M.C., Staels, B., Seckl, J.R., and Mullins, J.J. (2004). Metabolic syndrome without obesity: Hepatic overexpression of 11beta-hydroxysteroid dehydrogenase type 1 in transgenic mice. Proc. Natl. Acad. Sci. USA *101*, 7088–7093.

Patsouris, D., Mandard, S., Voshol, P.J., Escher, P., Tan, N.S., Havekes, L.M., Koenig, W., Marz, W., Tafuri, S., Wahli, W., et al. (2004). PPARalpha governs glycerol metabolism. J. Clin. Invest. *114*, 94–103.

Peitl, B., Dobronte, R., Nemeth, J., Mezey, G., Kovacs, P., Paragh, G., and Szilvassy, Z. (2005). The prandial insulin sensitivity-modifying effect of vagal stimulation in rats. Metabolism *54*, 579–583.

Petrescu, I., Bojan, O., Saied, M., Barzu, O., Schmidt, F., and Kuhnle, H.F. (1979). Determination of phosphoenolpyruvate carboxykinase activity with deoxyguanosine 5′-diphosphate as nucleotide substrate. Anal. Biochem. *96*, 279–281.

Randich, A., Spraggins, D.S., Cox, J.E., Meller, S.T., and Kelm, G.R. (2001). Jejunal or portal vein infusions of lipids increase hepatic vagal afferent activity. Neuroreport *12*, 3101–3105.

Reddy, J.K. (2001). Nonalcoholic steatosis and steatohepatitis. III. Peroxisomal beta-oxidation, PPAR alpha, and steatohepatitis. Am. J. Physiol. Gastrointest. Liver Physiol. *281*, G1333–G1339.

Rodriguez, J.C., Gil-Gomez, G., Hegardt, F.G., and Haro, D. (1994). Peroxisome proliferator-activated receptor mediates induction of the mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase gene by fatty acids. J. Biol. Chem. *269*, 18767–18772.

Smith, G.P., and Jerome, C. (1983). Effects of total and selective abdominal vagotomies on water intake in rats. J. Auton. Nerv. Syst. *9*, 259–271.

Subramanian, S., DeRosa, M.A., Bernal-Mizrachi, C., Laffely, N., Cade, W.T., Yarasheski, K.E., Cryer, P.E., and Semenkovich, C.F. (2006). PPARα activation elevates blood pressure and does not correct glucocorticoid-induced insulin resistance in humans. Am. J. Physiol. Endocrinol. Metab. *291*, E1365–E1371.

Tordjman, K., Bernal-Mizrachi, C., Zemany, L., Weng, S., Feng, C., Zhang, F., Leone, T.C., Coleman, T., Kelly, D.P., and Semenkovich, C.F. (2001). PPARα deficiency reduces insulin resistance and atherosclerosis in apoE-null mice. J. Clin. Invest. *107*, 1025–1034.

Tordjman, K., Standley, K., Bernal-Mizrachi, C., Leone, T., Coleman, T., Kelly, D.P., and Semenkovich, C.F. (2002). PPARα suppresses insulin secretion and induces UCP2 in insulinoma cells. J. Lipid Res. *43*, 936–943.

Uno, K., Katagiri, H., Yamada, T., Ishigaki, Y., Ogihara, T., Imai, J., Hasegawa, Y., Gao, J., Kaneko, K., Iwasaki, H., et al. (2006). Neuronal pathway from the liver modulates energy expenditure and systemic insulin sensitivity. Science *312*, 1656–1659.

Uthman, B.M., Reichl, A.M., Dean, J.C., Eisenschenk, S., Gilmore, R., Reid, S., Roper, S.N., and Wilder, B.J. (2004). Effectiveness of vagus nerve stimulation in epilepsy patients: a 12-year observation. Neurology *63*, 1124–1126.

Wada, M., Connolly, C.C., Tarumi, C., Neal, D.W., and Cherrington, A.D. (1995). Hepatic denervation does not significantly change the response of the liver to glucagon in conscious dogs. Am. J. Physiol. *268*, E194–E203.

Walker, B.R., Sang, K.S., Williams, B.C., and Edwards, C.R. (1994). Direct and indirect effects of carbenoxolone on responses to glucocorticoids and noradrenaline in rat aorta. J. Hypertens. *12*, 33–39.

Wallerath, T., Witte, K., Schafer, S.C., Schwarz, P.M., Prellwitz, W., Wohlfart, P., Kleinert, H., Lehr, H.A., Lemmer, B., and Forstermann, U. (1999). Down-regulation of the expression of endothelial NO synthase is likely to contribute to glucocorticoid-mediated hypertension. Proc. Natl. Acad. Sci. USA *96*, 13357–13362.

Walls, E.K., Wang, F.B., Holst, M.C., Phillips, R.J., Voreis, J.S., Perkins, A.R., Pollard, L.E., and Powley, T.L. (1995). Selective vagal rhizotomies: a new dorsal surgical approach used for intestinal deafferentations. Am. J. Physiol. Regul. Integr. Comp. Physiol. *269*, 1279–1288.

Whitworth, J.A., Coghlan, J.P., Denton, D.A., Graham, W.F., Humphery, T.J., and Scoggins, B.A. (1979). Comparison of the effects of 'glucocorticoid' and 'mineralocortocoid' infusions on blood pressure in sheep. Clin. Exp. Hypertens. *1*, 649–663.

Xie, H., and Lautt, W.W. (1996). Insulin resistance caused by hepatic cholinergic interruption and reversed by acetylcholine administration. Am. J. Physiol. *271*, E587–E592.