Intracerebroventricular Administration of Neuropeptide Y Induces Hepatic Insulin Resistance via Sympathetic Innervation

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OBJECTIVE—We recently showed that intracerebroventricular infusion of neuropeptide Y (NPY) hampers inhibition of endogenous glucose production (EGP) by insulin in mice. The downstream mechanisms responsible for these effects of NPY remain to be elucidated. Therefore, the aim of this study was to establish whether intracerebroventricular NPY administration modulates the suppressive action of insulin on EGP via hepatic sympathetic or parasympathetic innervation.

RESEARCH DESIGN AND METHODS—The effects of a continuous intracerebroventricular infusion of NPY on glucose turnover were determined in rats during a hyperinsulinemic-euglycemic clamp. Either rats were sham operated, or the liver was sympathetically (hepatic sympathectomy) or parasympathetically (hepatic parasympathectomy) denervated.

RESULTS—Sympathectomy or parasympathectomy did not affect the capacity of insulin to suppress EGP in intracerebroventricular vehicle–infused animals (50 ± 8 vs. 49 ± 6 vs. $55 \pm 6\%$, in hepatic sympathectomy vs. hepatic parasympathectomy vs. sham, respectively). Intracerebroventricular infusion of NPY significantly hampered the suppression of EGP by insulin in sham-denervated animals (29 ± 9 vs. $55 \pm 6\%$ for NPY/sham vs. vehicle/sham, respectively, $P = 0.038$). Selective sympathetic denervation of the liver completely blocked the effect of intracerebroventricular NPY administration on insulin action to suppress EGP (NPY/hepatic sympathectomy, $57 \pm 7\%$), whereas selective parasympathetic denervation had no effect (NPY/hepatic parasympathectomy, $29 \pm 7\%$).

CONCLUSIONS—Intracerebroventricular administration of NPY acutely induces insulin resistance of EGP via activation of sympathetic output to the liver. *Diabetes* **57:2304–2310, 2008**

Since Claude Bernard first observed in the 1850s that puncture of the floor of the fourth cerebral ventricle elevates blood glucose levels, the fundamental role of the brain in the control of glucose metabolism has been firmly established (1,2). Intracerebroventricular infusion of insulin inhibits endogenous glucose production (EGP) (3), and downregulation of hypothalamic insulin receptors by antisense oligonucleotides precludes suppression of EGP by (circulating) insulin to a considerable extent (4). Thus, hypothalamic insulin signaling appears to play a role in the control of EGP. Insulin inhibits neuropeptide Y (NPY)-producing neurons in the arcuate nucleus of the hypothalamus (5). Intracerebroventricular administration of NPY hampers the capacity of insulin to suppress EGP (6), suggesting that silencing of arcuate NPY neurons may contribute to the inhibitory effect of intracerebroventricular insulin administration on EGP.

The downstream mechanism responsible for the effects of hypothalamic NPY on hepatic fuel flux remains to be established. Arcuate NPY neurons project to the paraventricular nucleus (PVN) and various other hypothalamic nuclei (7). The hypothalamus is a major source of forebrain input into the sympathetic nervous system (8); it partakes in the control of cholinergic outflow to visceral organs (9,10), and it orchestrates the release of various pituitary hormones (11,12). Either of these neuroendocrine systems can impact on EGP (13–15).

Here, we test the hypothesis that intracerebroventricular administration of NPY hampers the ability of insulin to suppress EGP via autonomic nervous inputs to the liver. To this end, rats whose livers were selectively stripped of sympathetic or parasympathetic nerves received intracerebroventricular infusion of NPY or vehicle. The capacity of insulin to inhibit EGP was quantified by hyperinsulinemic-euglycemic clamp.

RESEARCH DESIGN AND METHODS

Male Wistar rats (Harlan Nederland, Horst, the Netherlands) were housed in a temperature-controlled room on a 12-h light-dark cycle with lights on at 7:00 A.M. Food and water were available ad libitum, except during experimental sessions, when only water was available. Animals weighed between 260 and 340 g at the time of the experiments. All animal experiments were performed in accordance with the regulations of Dutch law on animal welfare, and the institutional ethics committee for animal procedures approved the protocol. **Surgical procedures.** Rats were anesthetized with 0.08 ml/100 g body wt i.m. Hypnorm (Janssen Pharmaceutica, Beerse, Belgium) and 0.04 ml/100 g body wt s.c. Dormicum (Genthon, Nijmegen, the Netherlands). Postoperative care was provided with a subcutaneous injection of 30 μl/100 g body wt 1:10 Fynadine (Dopharma, Raamsdonksveer, the Netherlands). All animals re-

TABLE 1
Plasma parameters 1

	Glucose (mmol/l)		Insulin (ng/ml)		Glucagon (pmol/l)	
	Basal	Hyperinsulinemic	Basal	Hyperinsulinemic	Basal	Hyperinsulinemic
Vehicle/sham	7.3 ± 0.5	6.7 ± 0.3	2.2 ± 0.2	4.2 ± 0.5	53.4 ± 5.8	46.0 ± 7.7
Vehicle/hepatic sympathectomy	5.9 ± 0.3*	6.4 ± 0.4	3.1 ± 0.8	4.5 ± 0.4	68.2 ± 8.1	33.7 ± 4.8
Vehicle/hepatic parasympathectomy	7.7 ± 0.5	6.5 ± 0.3	2.9 ± 0.7	6.2 ± 1.0	48.3 ± 7.3	35.3 ± 6.1
NPY/sham	6.2 ± 0.3	7.0 ± 0.4	2.7 ± 0.8	6.4 ± 1.0	60.3 ± 11.5	43.1 ± 8.3
NPY/hepatic sympathectomy	6.5 ± 0.2	6.3 ± 0.3	2.9 ± 0.4	5.4 ± 0.7	60.8 ± 6.1	38.9 ± 8.2
NPY/hepatic parasympathectomy	7.6 ± 0.5	7.2 ± 0.1	3.0 ± 0.7	4.9 ± 0.6	63.0 ± 5.8	48.3 ± 5.1

Data are means ± SE for at least five rats per group. Plasma glucose, insulin, and glucagon concentrations under basal or hyperinsulinemic conditions in rats that received an intracerebroventricular infusion of NPY or vehicle and of which the liver was sympathetically (hepatic sympathectomy) denervated, parasympathetically (hepatic parasympathectomy) denervated, or sham operated. *$P < 0.05$ vs. vehicle sham.

ceived two permanent catheters for infusion and blood sampling and underwent stereotaxic surgery. The liver was selectively stripped of its sympathetic or parasympathetic nerves, or a sham operation was performed. After a recovery period of 1 week, hyperinsulinemic-euglycemic clamp experiments were performed.

Hepatic sympathectomy. Hepatic sympathectomy was performed as previously described (16,17). A left-to-right incision was performed in the midline. The liver lobes were gently pushed up, and ligaments around the liver lobes were severed to free the bile duct and portal vein complex, which were isolated from each other. At the level of the hepatic portal vein, the hepatic artery breaks up into the hepatic artery proper and the gastroduodenal artery. This division occurs on the ventral surface of the portal vein. At this point, the arteries were separated via blunt dissection from the portal vein. Nerve bundles running along the hepatic artery proper were removed using microsurgical instruments under an operating microscope (magnification ×25). Any connective tissue attachments between the hepatic artery and portal vein were also broken, eliminating any possible nerve crossings. This procedure does not impair the vagal input to the liver, as shown previously (16).

Hepatic parasympathectomy. Hepatic parasympathectomy was performed as previously described (16). In short, a left-to-right incision was performed in the midline. The fascia containing the hepatic branch was stretched by gently moving the stomach and the esophagus. With the aid of a binocular-operating microscope, the neural tissue was transected between the ventral vagus trunk and liver. Also, small branches running in the fascia between the stomach and the liver were transected. We also dissected the nerves running over the stomach to the left lobe of the liver as described by Magni and Carobi (18). Particular care was taken not to damage the dorsal and ventral trunks innervating the stomach and abdominal tissues or blood vessels that run along the hepatic vagus branches.

Verification of liver denervation. From previous studies, we knew that the success rate of sympathetic denervation of the liver may be <100%, whereas hepatic parasympathectectomy is easier and always accurate (17). Therefore, we checked the completeness of the sympathectomy by comparing hepatic norepinephrine concentrations in both sympathectomized groups with those in the sham-operated group receiving vehicle. After the experimental procedures, liver aliquots were processed and analyzed using a commercially available norepinephrine radioimmunoassay (RIA) (RE29325; IBL-Hamburg, Hamburg, Germany). Tissue samples were homogenized in ice-cold 0.1 N HCL and 1 mmol EDTA solution (1 ml/50 mg wet tissue) and centrifuged at 13,000 rpm for 15 min at 4°C. Samples of 200 μl supernatant were transferred to the extraction plate of the RIA kit, and bidest was added to a final volume of 1 ml. A RIA was then performed according to the manufacturer's protocol.

Norepinephrine levels were profoundly decreased in all but one of the sympathectomized animals (hepatic norepinephrine levels, 0.53 ± 0.06 vs. 0.14 ± 0.08 vs. 0.03 ± 0.02 ng/mg protein in vehicle/sham, vehicle/hepatic sympathectomy, and NPY/hepatic sympathectomy, respectively). The results obtained in one animal (which received vehicle) were not included in our analysis, because the norepinephrine concentration in its liver did not show a clear reduction compared with the sham-operated animals, indicating incomplete sympathectomy.

Placement of catheters. Rats were provided with two permanent catheters of sterile silicon. One was inserted into the right jugular vein for infusion of fluids and one into the left carotid artery for blood sampling. Both catheters were attached to bent steel tubings that were fixed on top of the rat skulls by means of four stainless-steel screws and dental cement.

Stereotaxic surgery. A cannula was implanted into the left lateral ventricle using the following coordinates from Bregma: 0.8 mm posterior, 2.0 mm lateral, and 3.2 mm ventral. Dental cement and four screws were used to fix the cannula onto the skull.

Experimental procedures. Animals were divided in six different groups: intracerebroventricular vehicle-infused sham-operated animals ($n = 7$), vehicle-infused hepatic sympathectomized animal ($n = 5$), vehicle-infused hepatic parasympathectomized animals ($n = 6$), NPY-infused sham-operated animals ($n = 7$), NPY-infused hepatic sympathectomized animals ($n = 7$), and NPY-infused hepatic parasympathectomized animals ($n = 7$). During the experiments, the input port of the intracerebroventricular cannula and the jugular vein catheter were connected to remote syringes via a double-fluid swivel interconnected with polyethylene tubing. This allowed all manipulations to be performed outside the cages without touching the animals. The rats were connected 1 day before the actual experiment to familiarize them with the experimental setup. The night preceding the experiment, animals were fed normally until 8:00 A.M., when food was removed and the output port of the carotid artery catheter was connected to polyethylene tubing. From 8:30 to 11:00 A.M., the intracerebroventricular infusion was started to empty the dead space in the tubing between the fluid swivel and intracerebroventricular cannula. As of 11:00 A.M., hyperinsulinemic-euglycemic clamps were performed as described previously (19). At 11:00 A.M., 10 μg/h intracerebroventricular NPY (dose based on prior experiments documenting effects on EGP in mice; Bachem, Bubendorf, Switzerland) (6) and vehicle infusions were started at a rate of 5 μl/h until the end of the experiments. First, a primed (1-μCi) continuous (7-μCi/h) infusion of [³H]glucose (Amersham, Little Chalfont, U.K.) was given for 75 min for determination of basal rates of glucose turnover. Subsequently, insulin was administered in a primed (10-mU) continuous (62.5-mU/h) intravenous infusion for 2 h to attain steady-state circulating insulin levels of ~5.3 ng/ml. A variable infusion of 25% D-glucose was used to maintain euglycemia (measured at 10-min intervals) (Freestyle; TheraSense, Disetronic Medical Systems, Vianen, the Netherlands). Blood samples (200 μl) were taken during the basal period (at 60 and 75 min after the start of NPY or vehicle infusion) and during the clamp period (at 90, 105, and 120 min after the start of insulin infusion) for determination of plasma glucose, insulin, glucagon, corticosterone, and [³H]glucose specific activities. At the end of the experiments, animals were killed, and tissue samples were taken and frozen in liquid nitrogen for subsequent analysis.

Analytical procedures. Plasma levels of glucose, insulin, glucagon, corticosterone, T3, T4, and testosterone were measured by a commercially available kit, an insulin ELISA, glucagon RIA, corticosterone ELISA, T3 and T4 ELISA, and testosterone ELISA (Instruchemie, Delfzijl, the Netherlands; Mercodia, Uppsala, Sweden; Euro-diagnostica, Malmö, Sweden; Lucron Bioproduct, De Pinte, Belgium; IDS, Boldon, U.K.; Endocrine Technologies, Newark, NJ [T3, T4, and testosterone], respectively). Total plasma [³H]glucose was determined in 7.5 μl plasma and in supernatants after trichloroacetic acid (20%) precipitation and water evaporation to eliminate tritiated water.

Calculations. Turnover rates of glucose (μmol · min⁻¹ · kg⁻¹) were calculated during the basal period and in steady-state clamp conditions as the rate of tracer infusion (dpm/min) divided by the plasma specific activity of [³H]glucose (dpm/μmol). The ratio was corrected for body weight. EGP was calculated as the difference between the tracer-derived rate of glucose appearance and the glucose infusion rate.

Statistical analysis. Differences between groups were first determined by Kruskal-Wallis nonparametric tests for multiple independent samples. Subsequently, differences between each group in comparison with the vehicle sham group were determined by Mann-Whitney nonparametric tests for independent samples. A P value <0.05 was considered statistically significant. All values shown represent means ± SE.

RESULTS

Plasma parameters. Plasma glucose, insulin, glucagon, corticosterone, T3, T4, and testosterone concentrations in basal and hyperinsulinemic conditions are shown in Tables 1 and 2. Plasma glucose and insulin levels were similar in all groups, both in basal and hyperinsulinemic conditions, except for basal glucose concentrations, which were significantly ($P = 0.048$) lower in sympathectomized animals receiving vehicle. Insulin infusion reduced plasma glucagon, T3, and testosterone levels, but the circulating concentrations of none of these hormones were different between groups within basal or hyperinsulinemic conditions.

Basal glucose turnover. In basal conditions, glucose turnover (reflecting EGP) was not affected by NPY administration. Parasympathetic denervation of the liver significantly increased basal glucose turnover, whereas sympathetic denervation had no effect (vehicle/sham, 83 ± 6 μmol · min^{-1} · kg^{-1}; vehicle/hepatic sympathectomy, 81 ± 7 μmol · min^{-1} · kg^{-1}; vehicle/hepatic parasympathectomy, 101 ± 5 μmol · min^{-1} · kg^{-1} [$P = 0.017$ vs. vehicle/sham]; NPY/sham, 91 ± 12 μmol · min^{-1} · kg^{-1}; NPY/hepatic sympathectomy, 75 ± 7 μmol · min^{-1} · kg^{-1}; and NPY/hepatic parasympathectomy, 116 ± 10 μmol · min^{-1} · kg^{-1} [$P = 0.008$ vs. vehicle/sham]; Fig. 1).

Insulin-mediated glucose metabolism. The rate of glucose infusion necessary to maintain euglycemia during insulin infusion is shown in Fig. 2. Neither sympathectomy nor parasympathectomy affected the glucose infusion rate in animals receiving vehicle (hepatic sympathectomy, 72 ± 6 μmol · min^{-1} · kg^{-1}; hepatic parasympathectomy, 73 ± 4 μmol · min^{-1} · kg^{-1}; and sham, 71 ± 5 μmol · min^{-1} · kg^{-1}). Intracerebroventricular NPY infusion clearly reduced the glucose infusion rate required to maintain euglycemia in sham-denervated animals, reflecting the induction of insulin resistance by NPY (NPY/sham, 49 ± 5 μmol · min^{-1} · kg^{-1}; $P = 0.007$ vs. vehicle/sham). Hepatic sympathectomy completely abolished this effect of NPY on the glucose infusion rate (NPY/hepatic sympathectomy, 65 ± 5 μmol · min^{-1} · kg^{-1}; NS vs. vehicle/sham), whereas the inhibitory impact of NPY on glucose infusion remained unabated in parasympathectomized animals (NPY/hepatic parasympathectomy, 52 ± 6 μmol · min^{-1} · kg^{-1}; $P = 0.007$ vs. vehicle/sham).

Insulin-mediated glucose disposal was similar in all groups (vehicle/sham, 110 ± 7 μmol · min^{-1} · kg^{-1}; vehicle/hepatic sympathectomy, 111 ± 6 μmol · min^{-1} · kg^{-1}; vehicle/hepatic parasympathectomy, 125 ± 6 μmol · min^{-1} · kg^{-1}; NPY/sham, 119 ± 11 μmol · min^{-1} · kg^{-1}; NPY/hepatic sympathectomy, 98 ± 5 μmol · min^{-1} · kg^{-1}; and NPY/hepatic parasympathectomy, 134 ± 11 μmol · min^{-1} · kg^{-1}; Fig. 3A).

The suppression of EGP by insulin is shown in Fig. 3B. Hepatic sympathectomy or hepatic parasympathectomy did not impact on the capacity of insulin to suppress EGP in animals receiving vehicle (vehicle/sham, 55 ± 6%; vehicle/hepatic sympathectomy, 52 ± 9%; and vehicle/hepatic parasympathectomy, 49 ± 6%). Intracerebroventricular NPY infusion significantly hampered the inhibitory action of insulin on EGP in sham-denervated animals (NPY/sham, 29 ± 9%; $P = 0.038$ vs. vehicle/sham). Hepatic sympathectomy completely abolished the effect of NPY on EGP (NPY/hepatic sympathectomy, 57 ± 7%; NS vs. vehicle/sham), whereas the inhibitory impact of NPY on the suppression of EGP remained unabated in parasympathectomized animals (NPY/hepatic parasympathectomy, 29 ± 7%; $P = 0.017$ vs. vehicle/sham).

TABLE 2
Plasma parameters 2

	Corticosterone (ng/ml)		T3 (ng/ml)		T4 (ng/ml)		Testosterone (ng/ml)	
	Basal	Hyperinsulinemic	Basal	Hyperinsulinemic	Basal	Hyperinsulinemic	Basal	Hyperinsulinemic
Vehicle/sham	25.7 ± 7.6	23.0 ± 5.5	1.8 ± 0.3	1.1 ± 0.2	43.5 ± 3.9	49.6 ± 9.5	0.41 ± 0.09	0.24 ± 0.08
Vehicle/hepatic sympathectomy	25.3 ± 12.3	16.8 ± 5.8	2.0 ± 0.4	1.4 ± 0.2	52.5 ± 8.4	41.4 ± 3.5	0.65 ± 0.16	0.20 ± 0.10
Vehicle/hepatic parasympathectomy	44.2 ± 2.6	25.9 ± 5.1	1.3 ± 0.2	1.3 ± 0.3	63.4 ± 4.4	55.3 ± 6.4	0.66 ± 0.20*	0.32 ± 0.10*
NPY/sham	28.2 ± 5.9	31.5 ± 5.1	1.8 ± 0.2	1.1 ± 0.2	57.0 ± 6.8	51.5 ± 5.9	0.66 ± 0.15	0.15 ± 0.05
NPY/hepatic sympathectomy	29.7 ± 4.6	26.9 ± 7.8	1.9 ± 0.4	1.0 ± 0.3	49.6 ± 4.1	52.8 ± 4.1	0.71 ± 0.22	0.37 ± 0.15
NPY/hepatic parasympathectomy	39.9 ± 2.3	32.3 ± 2.1	1.3 ± 0.3	1.4 ± 0.4	46.7 ± 2.9	68.6 ± 14.0	0.39 ± 0.09	0.18 ± 0.02

Data are means ± SE for at least five rats per group. Plasma corticosterone, T3, T4, and testosterone concentrations under basal or hyperinsulinemic conditions in rats that received an intracerebroventricular infusion of NPY or vehicle and of which the liver was sympathetically (hepatic sympathectomy) denervated, parasympathetically (hepatic parasympathectomy) denervated, or sham operated. *These data are based on four (basal) and three (hyperinsulinemic) rats only and therefore must be considered with caution.
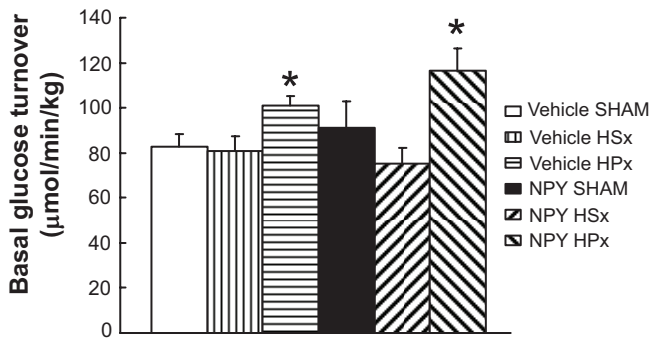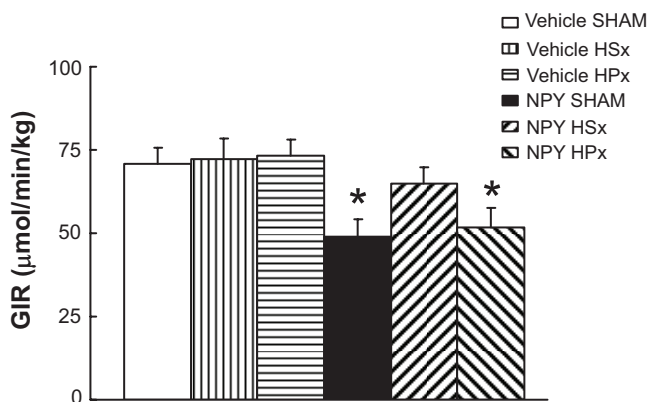
FIG. 1. Basal glucose turnover. Basal glucose turnover in rats that were sham operated or in which the liver was sympathetically (HSx) or parasympathetically (HPx) denervated and that received an intracerebroventricular infusion of NPY or vehicle. Values are means ± SE for at least five animals per group. *P < 0.05 vs. vehicle sham group.

DISCUSSION

Here, we report that selective hepatic sympathectomy completely reverses the deleterious effect of intracerebroventricular NPY administration on the capacity of insulin to inhibit EGP in Wistar rats. In contrast, selective destruction of parasympathetic nerves innervating the liver does not modify the impact of NPY on hepatic insulin sensitivity. These data corroborate previous findings in rats and mice, demonstrating that intracerebroventricular administration of NPY hampers insulin action to inhibit glucose and VLDL production (6,20). The present results suggest that NPY neurons modulate this inhibitory effect of insulin on glucose production via efferent sympathetic nerves innervating the liver.

The role of the autonomic nervous system in the control of glucose metabolism is well established. The liver is densely innervated by sympathetic and parasympathetic nerves that can be traced back to the PVN (16,21). Activation of sympathetic nerves stimulates glycogen phosphorylase and inhibits glycogen synthase, thereby increasing glycogen breakdown and glucose production. In contrast, parasympathetic activation enhances glucose uptake by the liver (13). Neurons in control of sympathetic and parasympathetic nerves innervating the liver were identified in various hypothalamic nuclei by viral tracing technology (16). The hypothalamus is critically involved in the control of autonomic outflow to various tissues (8–10), it contains many neurons expressing NPY receptors in

FIG. 2. Glucose infusion rate. Glucose infusion rate (GIR) in rats that were sham operated or in which the liver was sympathetically (HSx) or parasympathetically (HPx) denervated and that received an intracerebroventricular infusion of NPY or vehicle during a hyperinsulinemic-euglycemic clamp. Values are means ± SE for at least five animals per group. *P < 0.05 vs. vehicle sham group.
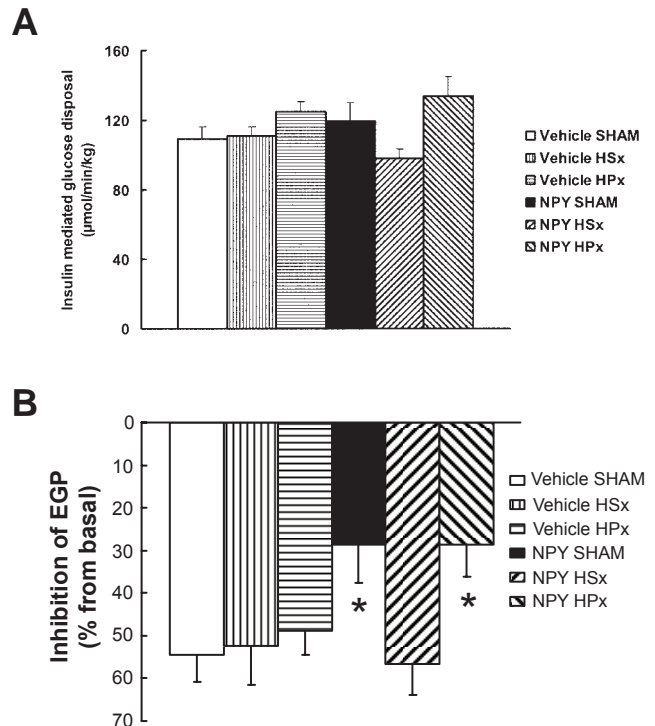
FIG. 3. Insulin-mediated glucose disposal and inhibition of EGP by insulin. Insulin-mediated glucose disposal (A) and inhibition of EGP by insulin (B) in rats that were sham operated or in which the liver was sympathetically (HSx) or parasympathetically (HPx) denervated and that received an intracerebroventricular infusion of NPY or vehicle during a hyperinsulinemic-euglycemic clamp. Values are means ± SE for at least five animals per group. *P < 0.05 vs. vehicle sham group.

various nuclei (22), and these receptors are involved in the control of autonomic activity. NPY injection into the third cerebral ventricle primarily impacts adjacent hypothalamic nuclei, including the PVN and the supraoptic nuclei (23), which exert differential effects on sympathetic outflow on NPY administration (24). Precise identification of the specific neurons and nuclei involved in the effects of NPY administration observed here requires further study.

Hypothalamic NPY neurons are involved in the control of pituitary hormone release, but our findings argue against the possibility that the acute effects of NPY on insulin action in the liver are mediated by endocrine cues. For example, NPY inhibits growth hormone secretion (25) and stimulates the activity of the pituitary-adrenal ensemble (26). Corticosteroids clearly promote glucose production (27,28), and adrenalectomy was shown to prevent or reduce at least some of the metabolic effects of subchronic intracerebroventricular NPY administration, i.e., hyperphagia, weight gain, and hyperinsulinemia (26,29,30). Intracerebroventricular NPY infusion for 5–7 days in rats was shown to decrease plasma IGF-1 and testosterone levels, endocrine cues well known for their effect on insulin action (31). However, NPY did not affect circulating levels of corticosterone, testosterone, glucagon, or thyroid hormones (T3 and T4) in the current experimental context. Moreover, the impact of NPY on insulin action was completely abolished by selective hepatic sympathectomy. In aggregate, the data suggest that the acute metabolic effects of central NPY administration are primarily caused by activation of sympathetic nerves innervating the liver, whereas the pituitary-adrenal ensemble and other endocrine cues may be engaged by more prolonged administration of NPY.

Selective hepatic parasympathectomy significantly increased EGP in the basal condition, whereas selective sympathectomy had no effect. This corroborates a previous report, documenting an increase of basal glucose production after parasympathetic denervation of the liver in rats (32). Conversely, parasympathetic activation by electrical stimulation of the hepatic nerve inhibits glucose production in cats (33). Thus, an increase of EGP in parasympathectomized animals may be explained by a predominance of sympathetic inputs to the liver in the absence of parasympathetic activity. Pocai et al. (34) have shown that intracerebroventricular infusion of insulin inhibits hepatic glucose production via hepatic vagal efferents. In particular, their data suggest that hepatic vagotomy negates the inhibitory impact of central insulin infusion on glucose production and halves the effect of systemic administration. In apparent contrast, hepatic parasympathectomy did not affect the capacity of insulin to suppress EGP in the current experimental context. The present finding is in agreement with another recent study of our group (35). As discussed in that paper, the contrast between the study of Pocai et al. and ours may relate to the lower level of peripheral hyperinsulinemia attained in our studies. For the effect of hepatic vagotomy to become apparent, insulin may have to be administered centrally, or peripheral insulin levels should be increased to such an extend that hepatic glucose production is inhibited >80%.

We show that NPY hampers insulin action to inhibit EGP via activation of sympathetic outflow to the liver. In fasting conditions, low circulating insulin levels allow expression of NPY in the hypothalamus, activating sympathetic outflow to the liver, which in its turn induces hepatic insulin resistance to maximize EGP. In the fed state, when high plasma insulin levels inhibit hypothalamic NPY expression, parasympathetic outflow to the liver prevails to inhibit glucose production. The fact that parasympathectomy did not impair the capacity of insulin to suppress EGP in intracerebroventricular vehicle–infused animals in the present study is in apparent contrast with this scenario. However, although parasympathectomy did not significantly affect the relative decline of EGP during hyperinsulinemia (Fig. 3B), it did increase the absolute rate of glucose production in hyperinsulinemic condition. In this respect, it is important to point out that parasympathectomy enhanced basal glucose production in the current experimental context, which is also counterintuitive in light of the fact that NPY expression and sympathetic activity are supposed to be elevated in fasting animals. However, our subjects fasted for only 4 h before the studies, because we wanted NPY levels to be low in vehicle-treated rats to maximize the contrast with NPY treated animals. Thus, parasympathetic tone may have dominated in basal conditions in our studies, explaining the fact that hepatic parasympathectomy increased EGP.

The present study shows that administration of NPY into the lateral ventricle modulates the inhibitory effect of insulin on glucose production via activation of sympathetic output to the liver. Intracerebroventricular NPY injection also enhances sympathetic tone in vascular beds of heart, splanchnic tissues, and skeletal muscle (36). In contrast, in brown adipose tissue, it appears to inhibit sympathetic tone, and it activates parasympathetic outflow to the pancreas (24,37). NPY therefore seems to exert differential effects on autonomic output to various tissues, analogous to what is known about other peptides like leptin and cholecystokinin (38–40).

What are the potential (patho)physiological implications of these findings? NPY neuronal activity in the arcuate nucleus is governed by a variety of hormones and metabolites reflecting energy availability. Cues reflecting a state of plenty (i.e., leptin, insulin, gut-peptides, and glucose) inhibit NPY neurons (41–43). Circulating levels of these cues are low in the fasting state, which unleashes arcuate NPY release. Activation of sympathetic outflow to the liver by NPY subsequently induces insulin resistance of glucose production, facilitating glucose output, which is required for fostering the brain in the absence of food. In the context of chronic overfeeding, NPY gene expression in the arcuate nucleus is paradoxically elevated, probably as a result of insulin and leptin resistance of arcuate neurons (44,45). There is also substantial evidence of leptin resistance in obese humans (46), and myriad data support the contention that the sympathetic nervous system is exceedingly active in subjects with the metabolic syndrome (47–49 and references therein). Several of the components of the metabolic syndrome, like hypertension, obesity, and insulin resistance, are associated with adrenergic overdrive. In contrast, it has also been reported that the response of the sympathetic nervous system to various stimuli is diminished in obese humans (47–49), However, most investigators agree that obesity is marked by enhanced sympathetic tone in most tissues, at least in the basal state (47–49 and references therein). Our data suggest that elevation of hypothalamic NPY levels as a corollary of leptin resistance may contribute to sympathetic overdrive, hepatic insulin resistance, and increased glucose production in high-fat–fed rodents and man. If so, it is conceivable that selective (ant)agonism of specific NPY receptors can ameliorate hyperglycemia in obese individuals.

NPY affects a wide variety of physiological processes via distinct NPY receptors. The Y1, Y2, Y4, and Y5 receptor are involved in regulating food intake and/or energy expenditure. Blocking the Y1 or Y5 receptor and stimulation of the Y2 or Y4 receptor inhibits food intake. Hence, Y1 and Y5 antagonists and Y2 and Y4 agonists have been tested in experimental studies for the treatment of obesity and related diseases (rev. in 50,51). Intracerebroventricular and oral administration of the Y1 antagonist J-115814 suppresses feeding and induces sustained body weight loss in obese Zucker fatty rats. Y5 antagonists and Y2 or Y4 agonists have similar effects (rev. in 50). It is currently unknown which NPY receptor subtype(s) is involved in the control of metabolic processes. However, a recent study by Singhal et al. (52) indicates that the Y1 receptor might be important in this respect. Because it makes sense to assume that the mechanisms regulating food intake and metabolism are tightly coupled, it is reasonable to propose that any one of the receptors involved in the control of food intake simultaneously modulates EGP. However, this issue requires further study, particularly because identification of NPY receptor subtypes involved in metabolic control may pave the way for pharmacotherapy with NPY receptor (ant)agonists in patients with type 2 diabetes.

In conclusion, the present study shows that intracerebroventricular administration of NPY in rats acutely hampers the capacity of insulin to suppress EGP via activation of sympathetic nerves innervating the liver. Our data may provide an explanation for the sympathetic overdrive and hepatic insulin resistance that typify obese subjects with the metabolic syndrome. NPY receptor (ant)agonists and/or drugs that act to reduce sympathetic inputs to the

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