Glucocorticoid Signaling in the Arcuate Nucleus Modulates Hepatic Insulin Sensitivity

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Glucocorticoid receptors are highly expressed in the hypothalamic paraventricular nucleus (PVN) and arcuate nucleus (ARC). As glucocorticoids have pronounced effects on neuropeptide Y (NPY) expression and as NPY neurons projecting from the ARC to the PVN are pivotal for balancing feeding behavior and glucose metabolism, we investigated the effect of glucocorticoid signaling in these areas on endogenous glucose production (EGP) and insulin sensitivity by local retrodialysis of the glucocorticoid receptor agonist dexamethasone into the ARC or the PVN, in combination with isotope dilution and hyperinsulinemic–euglycemic clamp techniques. Retrodialysis of dexamethasone for 90 min into the ARC or the PVN did not have significant effects on basal plasma glucose concentration. During the hyperinsulinemic–euglycemic clamp, retrodialysis of dexamethasone into the ARC largely prevented the suppressive effect of hyperinsulinemia on EGP. Antagonizing the NPY1 receptors by intracerebroventricular infusion of its antagonist largely blocked the hepatic insulin resistance induced by dexamethasone in the ARC. The dexamethasone-ARC–induced inhibition of hepatic insulin sensitivity was also prevented by hepatic sympathetic denervation. These data suggest that glucocorticoid signaling specifically in the ARC neurons modulates hepatic insulin responsiveness via NPY and the sympathetic system, which may add to our understanding of the metabolic impact of clinical conditions associated with hypercortisolism.
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Clinical conditions with glucocorticoid excess such as Cushing syndrome are accompanied by deranged glucose metabolism and hepatic insulin resistance, which is reversible after treatment (1). Several hypothalamic nuclei including the arcuate nucleus (ARC) and the paraventricular nucleus (PVN) have recently been shown to coregulate hepatic glucose production and insulin sensitivity (2–6). Thus, in addition to their well-known direct peripheral effects, glucocorticoids may also affect glucose metabolism through receptor activation in defined circuits of the central nervous system (CNS).

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Glucocorticoid signaling involves two receptor systems, i.e., the mineralocorticoid receptor, which in the CNS is mainly restricted to the septum, hippocampus and amygdala, and the glucocorticoid receptor, which is more widely expressed in different brain regions including the hypothalamus (7–11). In the hypothalamus, glucocorticoid receptors are expressed abundantly in the PVN and in the ARC, as demonstrated by immunohistochemistry, in situ hybridization, and receptor autoradiography studies (7–11). Mineralocorticoid receptors show weak immunoreactivity in the PVN, and its mRNA expression is not detectable by in situ hybridization methods (12). The strong expression of glucocorticoid receptors in the PVN, especially in the parvocellular subdivision (8,10), is thought to be mainly involved in the negative feedback of corticosterone on the hypothalamus-pituitary-adrenal (HPA) axis. However, the PVN not only governs neuroendocrine pathways but also represents an important hypothalamic center for the control of the autonomic nervous system, as reflected by the abundance of preautonomic neurons. Accumulating evidence suggests that the autonomic nervous system plays an essential role in the regulation of hepatic glucose metabolism and insulin sensitivity (5,13). We therefore hypothesized that glucocorticoid action in the CNS might affect glucose metabolism through activation of receptors on preautonomic neurons in the PVN.

Interestingly, intracerebroventricular infusion of dexamethasone (a glucocorticoid receptor agonist) stimulates food intake and body weight gain (14), while it decreases peripheral glucose uptake (15). The specific neuronal targets for these effects of dexamethasone in the CNS have not yet been clearly identified. One obvious possibility is that this phenomenon involves glucocorticoid signaling in the ARC, thereby antagonizing the effects of insulin on neuropeptide Y (NPY)-containing ARC neurons (16,17). This is supported by the observations that NPY is one of the strongest known hypothalamic orexigenic signals (18) and that peripheral administration of dexamethasone or corticosterone increases NPY mRNA expression in the ARC (19,20).

In the current study, we investigated whether modulation of hypothalamic glucocorticoid signaling specifically in the ARC or the PVN would influence peripheral glucose metabolism. In all experiments, the retrodialysis technique was used to slowly and exclusively deliver dexamethasone locally into specific hypothalamic nuclei under stress-free experimental conditions. Endogenous glucose production (EGP) was measured by the stable isotope dilution method, and hepatic insulin sensitivity was determined using hyperinsulinemic–euglycemic clamps. In experiment 1, dexamethasone was delivered into the ARC or the PVN to see whether there is a nucleus-specific effect on EGP and hepatic insulin sensitivity. In experiment 2, dexamethasone was only delivered into ARC, but this treatment was combined with the intracerebroventricular infusion of an

NPY1 receptor antagonist to investigate the involvement of NPY signaling in the molecular underpinnings of dexamethasone-induced changes in hepatic insulin sensitivity. In experiment 3, dexamethasone was delivered into the ARC after hepatic sympathetic and parasympathetic denervation to test whether dexamethasone-ARC (Dex-ARC) effects on peripheral glucose metabolism were relayed via the autonomic output from the hypothalamus to the liver.

RESEARCH DESIGN AND METHODS

All the animal experiments were conducted under the approval of the animal care committee of the Royal Netherlands Academy of Arts and Sciences. Male Wistar rats weighing 300–350 g (Harlan Nederland, Horst, the Netherlands) were housed in individual cages, with a 12/12-h light-dark schedule (lights on at 0700 h, as Zeitgeber time [ZT]0). Food and water were available ad libitum unless otherwise indicated.

Surgery preparation. Animals underwent surgeries according to the different experimental designs under anesthesia with intramuscular 0.8 mL/kg Hypnorm (Janssen, Buckinghamshire, U.K.) and 0.4 mL/kg s.c. Dormicum (Roche, Almere, the Netherlands).

Silicon catheters were inserted into the right jugular vein and left carotid artery for infusions and blood sampling, respectively. With a standard Kopf stereotaxic apparatus, either bilateral microdialysis probes or intracerebroventricular probes or both (6) were placed into the ARC and PVN or lateral cerebral ventricle. Coordinates were adapted from the atlas of Paxinos and Watson (Table 1). All catheters and probes were fixed on top of the skull and secured with dental cement. Hepatic sympathetic, parasympathetic, and sham denervations were applied according to previously published methods (21). After recovery, animals were connected to a multichannel fluid infusion swivel (Instech Laboratories, Plymouth Meeting, PA) 1 day before the experiment for adaption. Food was restricted to 20 g on the night prior to experiments. The start of the experiment (i.e., $t = 0$) was at ZT3.

Glucose kinetics. To study glucose kinetics, in all groups, [6.6-$^2H_2$]glucose as a primed (8.0 μmol in 5 min) followed by a continuous (16.6 μmol/h) infusion was used as a tracer (>99% enriched; Cambridge Isotope Laboratories, Andover, MA). A first blood sample was taken at $t = -5$ min for measuring background enrichment of [6.6-$^2H_2$]glucose. The [6.6-$^2H_2$]glucose infusion started at $t = 0$ min. After an equilibration time of 90 min, blood samples were taken at $t = 90$, 95, and 100 min for determining glucose enrichment and plasma glucose and corticosterone concentrations. Thereafter, the [6.6-$^2H_2$] glucose infusion was continued for another 110 min, after which point blood samples were taken at $t = 200$, 205, and 210 min for determining glucose enrichment during the retrodialysis of dexamethasone and vehicle (Ringer's solution). Thereafter, the insulin infusion was started (prime 7.2 mU/kg · min for 4 min) followed by a continuous infusion (3 mU/kg · min) from $t = 210$ min until $t = 370$ min (vide infra). At $t = 210$ min, an additional and variable infusion of a 25% glucose solution (enriched 1% with [6,6-$^2H_2$]glucose) was started to maintain euglycemia (5.5 ± 0.2 mmol/L), as determined by 10-min carotid catheter blood sampling. At the end of the clamp, five blood samples were taken, with a 10-min interval from $t = 330$ to $t = 370$ min (6).

Retrodialysis and intracerebroventricular infusion. In experiment 1, retrodialysis probes were aimed to be placed into the PVN and ARC. We included animals in which the probes appeared to be misplaced upon histological examination as a separate post hoc misplacement control group; thus, animals were divided into six groups: vehicle-PVN, dexamethasone-PVN (Dex-PVN), vehicle-ARC, dexamethasone-ARC (Dex-ARC), vehicle–misplacement control (vehicle-MC), and dexamethasone–misplacement control (Dex-MC). From $t = 0$–100 min, all six groups received retrodialysis of vehicle (3 μL/min; Ringer's). After the blood samples for the calculation of basal EGP were taken, i.e., from $t = 100$ min onward, in the dexamethasone groups (Dex-PVN, Dex-ARC, and Dex-MC) the retrodialysis of Ringer's was replaced by dexamethasone retrodialysis (200 μmol/L, 3 μL/min). In the vehicle group, the retrodialysis of Ringer's was continued until the end of the study. At the end of the experiments, brains were removed for Nissl staining to validate the probe placements. In experiments 2 and 3, retrodialysis probes were only placed into ARC. In experiment 2, NPY1 receptor antagonist BIBP3226 (5 nmol/5 μL/h i.c.v.; Tocris Bioscience) was infused from $t = 100$ to the end of the study.

Effects of dexamethasone on NPY mRNA and protein expressions in rat hypothalamic cell line. For examination of whether there are direct effects of dexamethasone on NPY mRNA and protein expressions, the hypothalamic cell line CLU211 (CELLutions) was incubated with vehicle for 4 h or 10^{-7} mol/L dexamethasone for 4 h. For quantitative real-time PCR, total RNA was extracted from treated cells using RNeasy mini columns (Qiagen, Valencia, CA) following the manufacturer's instructions. Total RNA (1 μg) was used for cDNA synthesis. Quantitative real-time PCR was performed using Taqman master mixture (Applied Biosystems). A standard curve was used to obtain the relative concentration of *NPY* gene, and ribosomal protein 32 (*Mrpl32*) was used as the housekeeping gene.

For Western blotting, standard Western blot analysis was performed with the following procedure: the same amount of protein (250–400 μg) from whole cell lysates was placed in 1.5-mL tubes and brought up to equal volumes with lysis buffer. Lithium dodecyl sulfate sample buffer (4×) was added to each tube in a 1:4 ratio and incubated at 70°C for 10 min. After heating, the mixture was kept at room temperature for 20 min. The lysates were then centrifuged at 10,000 rpm, resolved by SDS-PAGE, and transferred to nitrocellulose membranes (45 μmol/L pore size; Hybond-ECL). After the transfer, the membranes were blocked in 2% blocking reagent provided in the ECL Advance Western Blotting kit (Amersham) for 1 h. Primary NPY and glyceraldehyde-3-phosphate dehydrogenase antibodies were diluted in Tris-buffered saline with Tween (TBST) (pH 7.4) and incubated with the membrane for 1 h at room temperature with gentle rocking. Subsequently, membranes were washed in TBST (three times for 20 min) and incubated with the secondary antibody for 1 h and washed again in TBST (three times for 20 min). Membranes were then developed using the Amersham ECL Advance Western kit.

Analytical methods. Plasma glucose concentration was measured by a handheld glucometer (Freestyle Flash; Abbott), and plasma corticosterone concentrations were measured using radioimmunoassay kits (LINCO Research, St. Charles, MO). Plasma [6,6-$^2H_2$]glucose enrichment was measured by gas chromatography–mass spectrometry.

Statistics and calculations. Results are expressed by averaging values from three plasma samples at the end of the equilibration state ($t = 90$–95–100 min), the end of the dexamethasone infusion state ($t = 200$–205–210 min), and five plasma samples at the end of the clamp experimental period ($t = 330$–340–350–360–370 min). ANOVA and post hoc Student t tests were performed to compare the group differences. EGP was calculated using the modified forms of the Steele equations.

RESULTS

Probe placements were examined in Nissl-stained sections. For the ARC and PVN groups, rats were included if bilateral probes were placed either inside the nuclei or within the direct vicinity of the nucleus, i.e., not beyond 200 μm distance from the borders of the nuclei. Rats with probes exceeding these ranges were categorized as misplacement control.

Experiment 1: specific administration of dexamethasone into the ARC induces hepatic insulin resistance. Before the retrodialysis of vehicle or dexamethasone into ARC, PVN, or misplacement areas, basal plasma glucose levels did not differ between the six experimental groups. During the retrodialysis of vehicle or dexamethasone,

TABLE 1
Stereotactic coordinates for placements of microdialysis probes

	Anteroposterior	Lateral	Ventral	Angle
PVN (mm)	−1.88	2.0	−7.9	10
ARC (mm)	−3.30	2.0	−9.7	8
Intracerebroventricular (mm)	−0.8	2.0	−3.4	0

Tooth bar was set as −3.2 mm. The ventral coordinates were standardized for 300 g body wt; for every additional 25 g body wt, the probes would be placed 0.1 mm deeper.

glucose concentration did not change within any of the groups; also, no difference in plasma glucose level was found between the six groups (Fig. 1*A*). Basal EGP did not show significant differences between any groups (Fig. 1*B*). Also, after 90 min of dexamethasone or vehicle treatment, EGP was not significantly different between the six treatment groups. In all, group mean EGP decreased (average 27%) during the retrodialysis of vehicle or dexamethasone. As we have previously demonstrated (6), this decline of EGP is caused by the removal of food from the rat home cage before the start of the experiment and the fasting along the experimental period. Only in the Dex-ARC and Dex-MC groups did this decrease reached significance (Fig. 1*B*).

During the hyperinsulinemic–euglycemic clamp, hyperinsulinemia significantly suppressed EGP in the three vehicle groups, as well as in the Dex-PVN and the Dex-MC groups (Fig. 1*B*). Mean suppression of EGP in those groups was 64% (Fig. 1*C*). However, in the Dex-ARC group, severe hepatic insulin resistance was found, with no significant suppression of EGP by hyperinsulinemia. ANOVA detected a significant effect of group [$F(5,28) = 5.66$, $P = 0.002$] during the clamp condition. Post hoc testing showed that EGP during hyperinsulinemia was significantly higher and suppression significantly less in the Dex-ARC group than in the other five groups ($P < 0.01$ for all groups).

R_d was not significantly different between the six groups. However, post hoc analysis on the separate groups revealed that R_d in the Dex-PVN group was significantly higher than in the vehicle-PVN or Dex-ARC group (Fig. 1*D*).

The current study used a freely moving, nonstressed animal model, as nicely illustrated by the fact that during the basal state (i.e., before dexamethasone administration) plasma corticosterone concentrations of all animals were in the normal daytime range, i.e., 0–25 ng/mL (Fig. 1*E*), without significant differences between any of the groups. After dexamethasone or vehicle administration in the basal state, no significant difference was found between the six groups. A clear rise in the plasma corticosterone concentration was observed in the three vehicle groups at the end

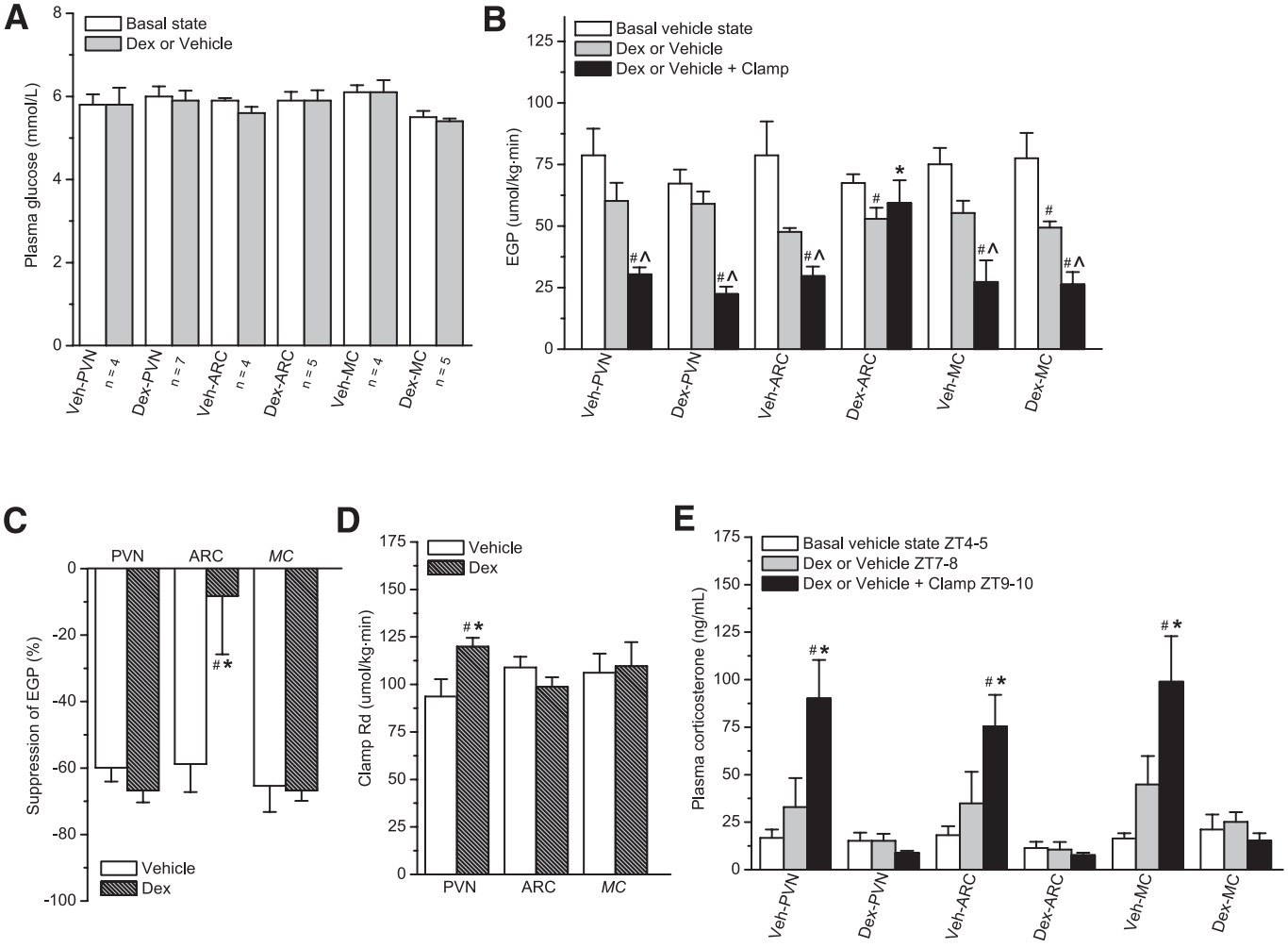

FIG. 1. Effects of dexamethasone (Dex) in different hypothalamic nuclei on various aspects of glucose metabolism. *A*: Plasma glucose concentrations at the end of the equilibration state (basal) and at the end of the 90-min dexamethasone or vehicle administration. *B*: EGP at the end of equilibration state (basal vehicle state), at the end of the 90-min dexamethasone or vehicle administration, and at the end of the hyperinsulinemic–euglycemic clamp. *C*: Percentage suppression of EGP by hyperinsulinemia at the end of the clamp. *D*: R_d at the end of the euglycemic–hyperinsulinemic clamp. *E*: Plasma corticosterone concentration at the end of equilibration state (basal vehicle state), at the end of the 90-min dexamethasone or vehicle administration, and at the end of the hyperinsulinemic–euglycemic clamp. *A–D*: #$P < 0.05$ vs. basal vehicle state; ^$P < 0.05$ vs. dexamethasone state; *$P < 0.05$ vs. other five groups during clamp. *E*: #$P < 0.05$ vs. basal vehicle state ZT4–5; *$P < 0.05$ vs. dexamethasone or vehicle ZT7–8. MC, misplacement control; Veh-ARC, vehicle-ARC; Veh-MC, vehicle-MC; Veh-PVN, vehicle-PVN.

of the hyperinsulinemic clamp (ZT9–10) compared with the basal state, i.e., in line with the daily rhythm in plasma corticosterone concentrations that show a pronounced circadian peak before the onset of the dark period (i.e., ZT12). This rise in corticosterone at the end of the hyperinsulinemic period was completely absent in all three dexamethasone-treated groups. ANOVA detected a significant effect of group [$F(5,28) = 12.31$, $P < 0.001$), ($P < 0.01$ for each vehicle group vs. dexamethasone group). No differences were found among the three vehicle groups or among the three dexamethasone-treated groups.

Experiment 2: hepatic insulin resistance induced by dexamethasone administration into the ARC can be blocked by NPY1R antagonist. Intracerebroventricular infusion of the NPY1R antagonist BIBP3226 or vehicle together with retrodialysis of dexamethasone or vehicle into the ARC at $t = 100$–210 min had no effects on basal plasma glucose or EGP levels (Fig. 2*A* and *B*). All groups showed a trend of decreasing EGP during the intracerebroventricular and retrodialysis coadministration, but no significance was found compared with the basal level.

During the hyperinsulinemic–euglycemic clamp, hyperinsulinemia significantly suppressed EGP in the two vehicle-ARC retrodialysis groups with intracerebroventricular infusion of either vehicle or BIBP3226 (Fig. 2*B*). In the two Dex-ARC groups, hyperinsulinemia suppression of EGP was blocked by dexamethasone retrodialysis in the intracerebroventricular vehicle infusion animals, i.e., as seen in experiment 1, but not in the intracerebroventricular BIBP3226 infusion animals. No significant differences were found between this dexamethasone plus BIBP group and the two vehicle retrodialysis groups (Fig. 2*C*). R_d was not significantly different between any of the groups (Fig. 2*D*).

Other in vitro studies in cultured hypothalamic cells have shown that dexamethasone significantly increases the intracellular NPY levels, and in the current study, using a hypothalamic cell line, we confirmed that 4-h treatment with dexamethasone increases the NPY mRNA expression

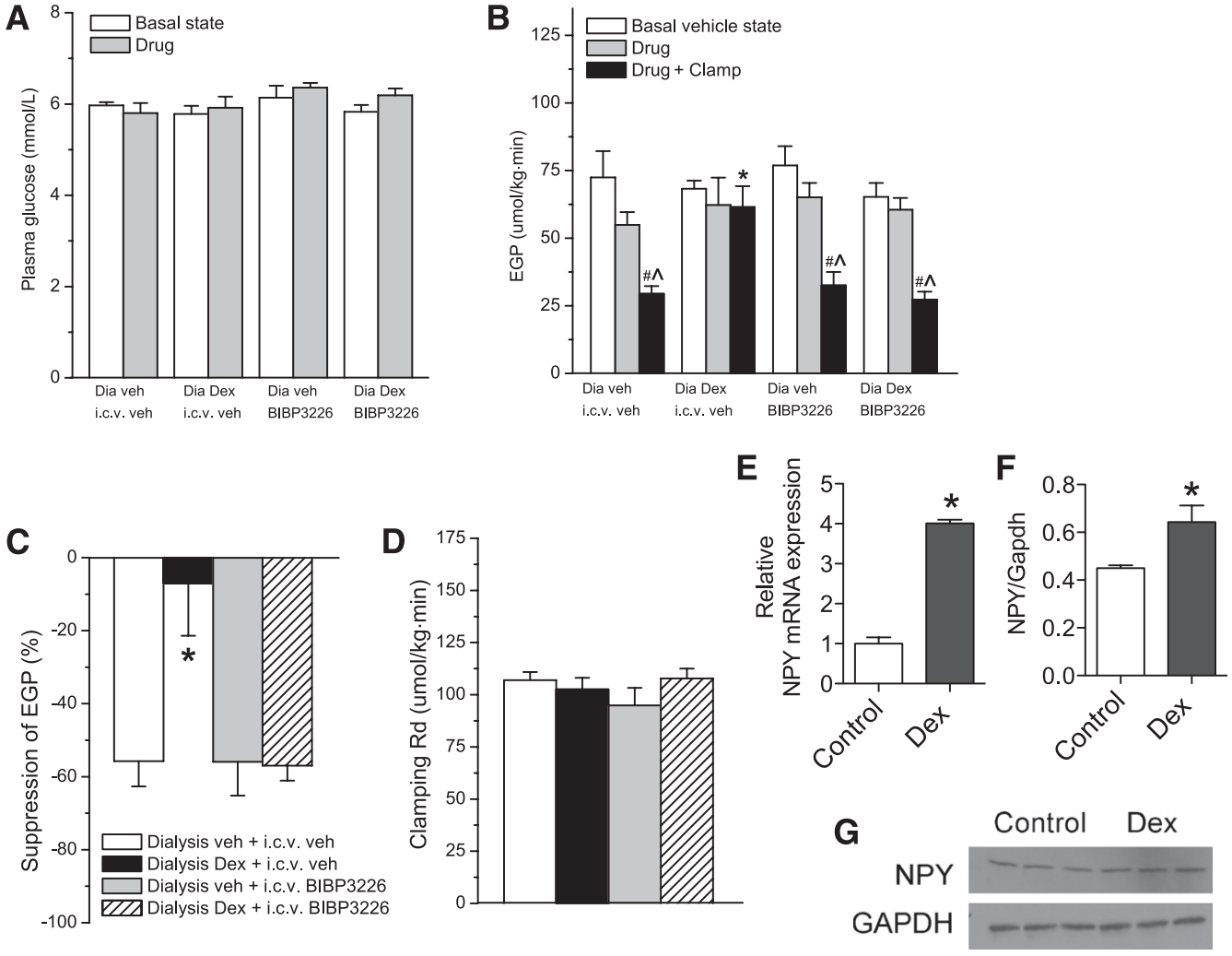

FIG. 2. Intracerebroventricular infusion of the NPY1R antagonist BIBP3226 blocks Dex-ARC–induced hepatic insulin resistance. *A*: Plasma glucose concentrations at the end of the basal state and at the end of the 90-min Dex-ARC or vehicle (veh) in combination with intracerebroventricular BIBP3226 or vehicle infusion. *B*: EGP at the end of basal vehicle state, at the end of the 90-min Dex-ARC/BIBP state, and at the end of the hyperinsulinemic–euglycemic clamp. *C*: Percentage suppression of EGP by hyperinsulinemia at the end of the clamp. *D*: R_d at the end of the euglycemic–hyperinsulinemic clamp. *E*: NPY mRNA expression in hypothalamic cells after dexamethasone (Dex) treatment. *F*: NPY protein expression level in hypothalamic cells after dexamethasone treatment. *G*: Western blotting for NPY in comparison with housekeeping protein glyceraldehyde-3-phosphate dehydrogenase (GAPDH). #$P < 0.05$ vs. basal vehicle state; ^$P < 0.05$ vs. dexamethasone or vehicle state; *$P < 0.05$ vs. the other three groups in *B* and *C*; *$P < 0.05$ vs. control in *E* and *F*. Dia, dialysis.

4.0-fold (Fig. 2*E*) and NPY protein expression 1.4-fold (Fig. 2*F* and *G*).

Experiment 3: hepatic insulin resistance induced by dexamethasone administration into the ARC can be blocked by hepatic sympathetic denervation. Hepatic sympathetic, parasympathetic, or sham denervation per se had no effects on basal plasma glucose or EGP (Fig. 3*A* and *B*). After 90-min dexamethasone retrodialysis into the ARC, all three groups showed a clear trend of EGP decline, as seen in experiments 1 and 2, and this decrease reached significance in the sham denervation group (Fig. 3*B*). During the hyperinsulinemic–euglycemic clamp, Dex-ARC blocked the hyperinsulinemic suppression of EGP in the parasympathetic and sham denervated groups. However, this blocking effect was eliminated by hepatic sympathetic denervation, as in these animals EGP during the clamp significantly decreased (Fig. 3*B*) and the hyperinsulinemic suppression of EGP was stronger than in the parasympathetic and sham denervated groups (Fig. 3*C*). No significant differences were observed between the three groups with respect to their R_d (Fig. 3*D*).

DISCUSSION

Selective administration of dexamethasone within the hypothalamic nuclei PVN and ARC had no effect on either basal plasma glucose concentrations or basal EGP. However, local administration of dexamethasone into the ARC, but not into the PVN, during a hyperinsulinemic–euglycemic clamp induced severe hepatic insulin resistance. Dex-ARC–induced hepatic insulin resistance was completely prevented by either intracerebroventricular coadministration of the NPY1 receptor antagonist BIBP3226 or by hepatic sympathetic denervation. Our data suggest that specific activation of glucocorticoid receptors in the ARC stimulates NPY release, which in turn activates sympathetic preautonomic output from hypothalamus to liver (22,23). The counteractive effect of dexamethasone on the insulin-induced inhibition of hepatic glucose production thus seems to be mediated by a very specific subset of hypothalamic neurons. On the other hand, the inhibitory effect of dexamethasone on the HPA axis did not differ between ARC and PVN or the area surrounding these two nuclei, as shown by an equal inhibition of the circadian rise in circulating plasma corticosterone levels in all dexamethasone-treated groups. Thus, the feedback action of dexamethasone on the HPA axis seems to be based on a more widespread phenomenon mediated via several hypothalamic circuits. Although all three dexamethasone-treated groups showed a similar inhibitory pattern with regard to the daily rise in plasma corticosterone, the dexamethasone effects on hepatic insulin sensitivity clearly differed between groups. Together, these data indicate that the inhibitory effect of dexamethasone on hepatic insulin sensitivity probably does not involve a change in glucocorticoid signaling within the liver. It is well known that glucocorticoid- and corticotropin-releasing factor receptors in the ventromedial hypothalamus are important for the counterregulatory response to hypoglycemia by stimulating hepatic glucose production (24,25). While the present data show that the effect of glucocorticoids on NPY expression in the ARC is more related to inhibition of hepatic insulin sensitivity, the data on the counterregulatory response clearly indicate that the HPA axis may control different aspects of peripheral glucose metabolism via distinctive mechanisms.

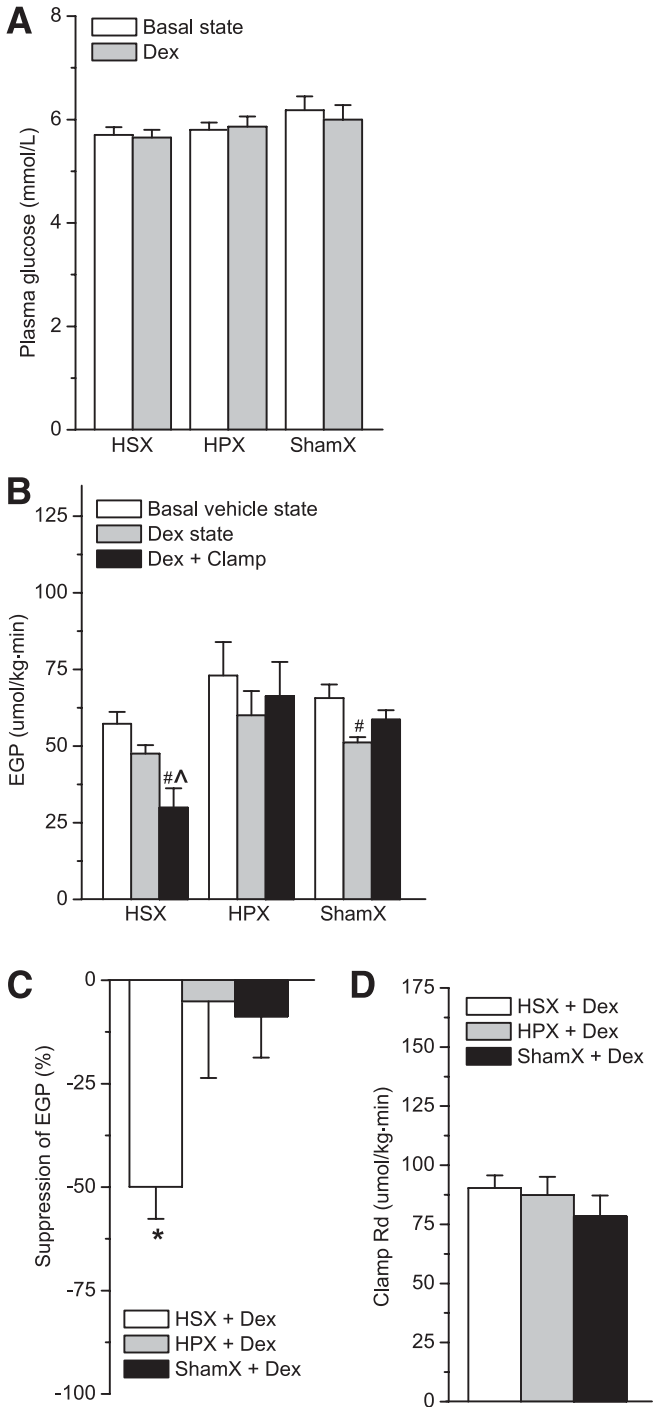

FIG. 3. Hepatic sympathetic denervation eliminates hepatic insulin resistance induced by Dex-ARC. *A*: Plasma glucose concentrations at the end of the basal state and at the end of the 90-min Dex-ARC within the different denervation groups. *B*: EGP at the end of basal vehicle state, at the end of the 90-min Dex-ARC state, and at the end of the hyperinsulinemic–euglycemic clamp. *C*: Percentage suppression of EGP by hyperinsulinemia at the end of the clamp. *D*: R_d at the end of the euglycemic–hyperinsulinemic clamp. HSX, hepatic sympathetic denervation; HPX, hepatic parasympathetic denervation; ShamX, sham denervation. #P < 0.05 vs. basal vehicle state; ^P < 0.05 vs. dexamethasone (Dex) state; *P < 0.05 vs. HPX and ShamX.

As a result of a less complete blood brain barrier in the proximity of one of the circumventricular organs, the median eminence, the ARC represents a population of neurons in the brain that is more accessible for many

blood-borne signals such as insulin, leptin, thyroid hormone, ghrelin, or glucocorticoids. Moreover, the receptors for all of these hormones are abundantly expressed in this nucleus (9,26–29). Despite the fact that it has been suggested that dexamethasone poorly penetrates the brain (30), radioautographic studies in adrenalectomy (ADX) rats have consistently shown that "small amounts" of peripherally administrated dexamethasone do selectively reach the ventral part of the ARC (31). The clearest evidence for glucocorticoids directly acting on ARC neurons is the changes induced by glucocorticoids in NPY expression and release (19,20) in conjunction with the presence of glucocorticoid-binding elements in the *NPY* gene (32). In addition, the effects of ghrelin, which is released by the stomach and also acts within the ARC to alter food intake, depend on glucocorticoids because none of these actions of ghrelin can be induced after ADX (33). Together, these data indicate that glucocorticoids capably interact with ARC signaling and that this cross-talk may represent a key mechanism by which ARC neurons control glucose metabolism.

Previously, we showed that intracerebroventricular administration of NPY could prevent the inhibitory effect of systemic hyperinsulinemia on hepatic glucose production. In addition, we showed that the NPY-induced insulin resistance was mediated via sympathetic innervation of the liver (5). However, owing to the generalized CNS effect inherent to the intracerebroventricular administration protocols, we were not able to pinpoint the effect of insulin to any specific subpopulation of NPY receptor–containing neurons. In the current study, we uncover a role for the NPY-containing arcuate neurons with the help of the more anatomically refined microdialysis technique. The antagonistic effect of the intracerebroventricular administration of NPY1R receptor antagonist clearly indicates that the main action of dexamethasone in the ARC was to increase NPY activity and release. Our studies suggest that the increased release of NPY by the ARC in turn induces hepatic insulin resistance. The results of our observations in rodent models of the hepatic denervations in our previous study (5) and the current study clearly implicate the sympathetic branch of the autonomic nervous system in the stimulatory effect of NPY on hepatic glucose production. In view of the intracerebroventricular administration route of the NPY1R antagonist, however, it is not clear yet which preautonomic neurons in the hypothalamus are responsible for the increased sympathetic input to the liver.

Chronic intracerebroventricular infusion of dexamethasone (for 2 days) increases food intake and decreases muscle tissue glucose uptake, and both effects require an intact subdiaphragmatic vagus nerve (15). Interestingly, some of the metabolic effects of chronic intracerebroventricular infusion of NPY depend on the presence of circulating corticosterone, since bilateral adrenalectomy prevents the effects on muscle glucose uptake and insulin sensitivity of adipose tissue. Moreover, these effects also depend on an intact subdiaphragmatic vagus nerve (34). In summary, these data support synergisms in metabolic control on multiple levels between hypothalamic NPY signaling and corticosteroid action in the CNS as well as in the periphery.

Intriguingly, some of these earlier experiments implicate the parasympathetic branch of the autonomic nervous system in the joint metabolic effects of dexamethasone and NPY, whereas in the current experiments we did not find any evidence of the involvement of hepatic parasympathetic innervation. Differences in surgical denervation protocols and acute versus chronic study design may account for some of the discordance between studies. Collectively, all available data indicate that depending on the metabolic (im)balance, NPY projections orchestrate the balance between sympathetic and parasympathetic preautonomic neurons to efficiently adjust hepatic glucose metabolism. Although a number of studies in rodents have shown that intracerebroventricular administration of insulin can inhibit EGP (2,35,36), recent studies in dogs indicated that brain insulin only modestly suppresses net hepatic glucose output by enhancing hepatic glucose uptake and glycogen synthesis. But despite its suppression of the expression level of the gluconeogenic genes *PEPCK* and *G6pase*, it did not suppress hepatic glucose production or gluconeogenesis (37). Therefore, regarding the modulation of insulin sensitivity by Dex-ARC, we have to consider two possibilities: either *1*) dexamethasone in the ARC inhibits the central action of insulin via interactions within the hypothalamus at the level of NPY neurons in the ARC or *2*) dexamethasone inhibits liver insulin sensitivity at the level of liver via its stimulation of the NPY–hepatic sympathetic autonomic nerves. However, with both mechanisms, ultimately hypercortisolism will result in an inhibition of hepatic insulin sensitivity.

In conclusion, the NPY-containing neurons in the arcuate nucleus represent an important conduit for numerous afferent signals to sense, process, and convey feedback messages to higher brain centers and back to the periphery. As the current study used an acute hypercortisolism in specific hypothalamic nuclei to study its effects on hepatic insulin sensitivity, the mechanistic insight reported here only represents a first step toward a better understanding of the metabolic side effects of hypercortisolism. Therefore, whether the currently presented mechanism can fully explain the pathological changes during long-term hypercortisolism (such as Cushing syndrome) or therapeutic dexamethasone requires further investigation including complex combinations of cell-specific genetic loss- and gain-of-function studies.

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C.-X.Y. performed experiments and wrote the manuscript. E.Fo., W.A., Y.G., and A.A. performed experiments. S.E.l.F., M.J.S., E.Fl., R.M.B., and M.H.T. contributed to discussion and reviewed and edited the manuscript. A.K. contributed to the discussion and wrote, reviewed, and edited the manuscript.

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