Induction of Obesity and Hyperleptinemia by Central Glucocorticoid Infusion in the Rat

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It has been claimed that factors favoring the development or maintenance of animal or human obesity may include increases in glucocorticoid production or hyperresponsiveness of the hypothalamic-pituitary-adrenal axis. In normal rats, glucocorticoids have been shown to be necessary for chronic intracerebroventricular infusion of neuropeptide Y to produce obesity and related abnormalities. Conversely, glucocorticoids inhibited the body weight–lowering effect of leptin. Such dual action of glucocorticoids may occur within the central nervous system, since both neuropeptide Y and leptin act within the hypothalamus. The aim of this study was to determine the effects of glucocorticoids (dexamethasone) given intracerebroventricularly to normal rats on body weight homeostasis and hypothalamic levels of neuropeptide Y and corticotropin-releasing hormone. Continuous central glucocorticoid infusion for 3 days resulted in marked sustained increases in food intake and body weight relative to saline-infused controls. The infusion abolished endogenous corticosterone output and produced hyperinsulinemia, hypertriglyceridemia, and hyperleptinemia, three salient abnormalities of obesity syndromes. Central glucocorticoid infusion also produced a marked decrease in the expression of uncoupling protein (UCP)-1 and UCP-3 in brown adipose tissue and UCP-3 in muscle. Finally, chronic central glucocorticoid administration increased the hypothalamic levels of neuropeptide Y and decreased those of corticotropin-releasing hormone. When the same dose of glucocorticoids was administered peripherally, it resulted in decreases in food intake and body weight, in keeping with the decrease in hypothalamic neuropeptide Y levels. These results suggest that glucocorticoids induce an obesity syndrome in rodents by acting centrally and not peripherally. ***Diabetes*** **48:365–370, 1999**

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Received for publication 28 July 1998 and accepted in revised form 26 October 1998.

CRH, corticotropin-releasing hormone; HPA, hypothalamic-pituitary-adrenal; NPY, neuropeptide Y; RIA, radioimmunoassay; UCP, uncoupling protein.

Elevated circulating levels of glucocorticoids or hypersensitivity to these hormones has long been thought to play a role in the development and maintenance of obesity syndromes. In obese humans, particularly those with abdominal obesity, increased production of glucocorticoids, increased concentrations of tissue glucocorticoids and glucocorticoid receptors, and overresponsiveness of the hypothalamic-pituitary-adrenal (HPA) axis to different neuropeptides and stress tasks have been reported (1–6). In rodents, it has been shown that obesity is often accompanied by hypercorticism, overresponsiveness of the HPA axis, or increased 24-h urinary corticosterone output (7,8). It has also been observed that adrenalectomy normalized several defects of obese rodents, including increased food intake, increased body weight, high levels of plasma insulin and related increased fat storage, and low thermogenic activity of brown adipose tissue (9–15). These effects of adrenalectomy were reversed by glucocorticoid replacement (10,12,14,15). In addition, it has been suggested that the effects of glucocorticoids in inducing food intake or producing obesity are related to an action of these hormones within the central nervous system (16,17). This suggestion is in keeping with observations made in rodents and humans that glucocorticoids stimulate food intake (18,19), a complex process that is controlled by the central nervous system—the hypothalamus, in particular. With regard to the latter considerations, many syndromes of obesity in animals are characterized by an increase in hypothalamic neuropeptide Y (NPY) levels, one among several of the hypothalamic neuropeptide candidates implicated in the stimulation of food intake (20,21). When NPY was infused intracerebroventricularly in normal rats, it was found to produce an obesity syndrome with its salient facets, that is, hyperphagia, increased body weight, hyperinsulinemia, hypercorticosteronemia, muscle insulin resistance, and increased fat storage (22–24). Such robust hormonal-metabolic changes elicited by intracerebroventricular NPY failed to occur when the neuropeptide was similarly administered to adrenalectomized rats (25) and reappeared after intracerebroventricular NPY plus intracerebroventricular dexamethasone administration (K.E.Z., I.C., B.J., F.R.-J., unpublished observations). It was of further interest to discover that the intracerebroventricular effects of leptin in decreasing food intake and body weight were markedly amplified when intracerebroventricular leptin was administered in adrenalectomized rats, to be reduced

again by glucocorticoid replacement (26). Overall, these observations further suggested that glucocorticoids may exert a central effect in influencing body weight homeostasis.

The effect of intracerebroventricular leptin administration in decreasing food intake and body weight of normal rats has been reported to be caused by at least a decreased hypothalamic NPYergic activity (27–29), together with an increased hypothalamic corticotropin-releasing hormone (CRH) content (29–31). Thus it was hypothesized that glucocorticoids' effects on body weight homeostasis could be elicited centrally, implicating changes opposite to those of leptin on the neuropeptides just mentioned.

In the present studies, therefore, we examined the hypothesis that several days' intracerebroventricular administration of the synthetic glucocorticoid, dexamethasone, might change body weight homeostasis, and that it might do so by altering the relative levels of hypothalamic NPY and CRH. To ensure that the observed effects of glucocorticoids were central and not peripheral (from potential leakage into the periphery), dexamethasone was also infused peripherally, and some of the consequences on body weight homeostasis and the status of hypothalamic neuropeptides were determined.

RESEARCH DESIGN AND METHODS

Animals. Normal rats of the Zucker strain (*Fa*/*Fa*), bred in our animal quarter, weighing 200–220 g were housed individually under conditions of controlled temperature (23°C) and illumination (7:00 A.M. to 7:00 P.M.). They were allowed ad libitum access to a standard laboratory food (Provimi Lacta, Cossonay, Switzerland) and tap water.

Placement of intracerebroventricular cannulas. At 10–11 weeks of age, rats were anesthetized with intramuscular ketamin and xylazine at 45 and 9 mg/kg, respectively (Parke-Davis, Baar, and Bayer Leverkusen, Switzerland) for the placement of a cannula in the right lateral cerebral ventricle (32). They recovered for 1 week, during which time they were handled daily and habituated to the blood sampling procedure. Several days before the experiments, the drinking response to the intracerebroventricular injection of angiotensin II (25 ng in 5 µl phosphate-buffered saline) (Novabiochem, Laüfelfingen, Switzerland) was measured to confirm correct placement of the intracerebroventricular cannula (33). Only those rats that drank 8 ml or more water in the 30 min after injection were used for further studies (~90% of the animals).

Infusion of synthetic glucocorticoid. Because we are unable to include corticosterone in minipumps, dexamethasone was used instead; moreover, dexamethasone binds with a higher affinity than corticosterone to type II glucocorticoid receptors known to be implicated in ingestive behavior (34,35). Dexamethasone diluted in isotonic saline (5 µg/day) or isotonic saline alone (control animals) was infused intracerebroventricularly for 3 days via a subcutaneously placed osmotic minipump (model 2001; Alza, Palo Alto, CA) (32). In another group of rats, the same dose of dexamethasone (5 µg/day), or saline for controls, was infused intraperitoneally to discriminate between central and peripheral effects of the hormone.

Measurements and tissue sampling. During intracerebroventricular infusions, food intake and body weight were measured daily and 200-µl blood samples were collected from the tip of the tail into EDTA-coated tubes at 9:00 A.M. each day, 90 min after removal of food from cages. Plasma was stored at –20°C until used for measurements of leptin levels. At the end of the 3rd experimental day, rats were killed by decapitation, trunk blood was taken, and plasma was stored at –20°C until further analysis. Interscapular brown adipose tissue, inguinal white adipose tissue, and quadriceps muscle were removed, freeze-clamped in liquid nitrogen, and stored at –70°C for subsequent measurements. Brains were also quickly removed and frozen on a freezing table. For NPY quantification, serial sections were cut and hypothalamic nuclei microdissected as previously described (36). In another group of rats used for CRH measurements, mediobasal hypothalami were dissected from a frontal brain slice cut between the middle of the optic chiasm and the mammillary bodies. The block extended laterally to the perihypothalamic sulcus and superiorly to the anterior commissure. After tissue preparation, both NPY and CRH contents were measured by radioimmunoassay (RIA), as previously reported (36,37). Plasma insulin and corticosterone levels were measured by RIA (38,39). Plasma leptin levels were measured by RIA, using a commercial kit (Linco Research, St. Louis, MO). Plasma triglyceride and glucose concentrations were determined using kits from bioMérieux (Marcy-l'Etoile, France) and Boehringer Mannheim (Mannheim, Germany), respectively.

Most parameters mentioned above were measured in intraperitoneal dexamethasone-infused rats and the respective controls at the end of a 3-day experimental period.

Uncoupling protein expression. In other groups of animals and at the end of a 3-day intracerebroventricular infusion of dexamethasone or vehicle, different tissues were removed and Northern blot analysis of uncoupling protein (UCP)-1, UCP-2, and UCP-3 was carried out as described previously, using probes defined elsewhere (40). Ratios of UCP to β-actin mRNA levels were used throughout.

Statistical analysis. For daily measurements of changes in food intake, body weight, and plasma leptin levels, we used one-way analysis of variance for repeated measures followed by multiple Bonferroni comparisons. For the rest of the results, we used the two-tailed Student's t test for unpaired data. Values of $P < 0.05$ were accepted as statistically significant.

RESULTS

The intracerebroventricular infusion of dexamethasone for 3 days in normal rats resulted in a marked and sustained increase in food intake relative to vehicle-infused control animals (Fig. 1), accompanied by about 15 g body weight gain at the end of the experimental period, compared with a value of about 5 g for control rats (Fig. 1).

We then measured plasma hormones and substrates in intracerebroventricular dexamethasone-treated rats and their respective controls. In the intracerebroventricular dexamethasone-infused group, plasma corticosterone levels were barely detectable, while those of insulin were three times higher than in controls (Fig. 2). In addition, in the intracerebroventricular dexamethasone-infused rats, plasma leptin levels started to rise from the 1st experimental day onward, while they remained low and stable in control animals (Fig. 3). At the end of the experimental period, no intergroup difference in blood glucose levels was detected (6.4 ± 0.2 and 6.5 ± 0.3 mmol/l for vehicle- and dexamethasone-infused rats, respectively), but triglyceridemia was much higher in the intracerebroventricular dexamethasone-infused than in vehicle-infused rats (Fig. 4).

Some of the tissues containing uncoupling proteins were investigated. In brown adipose tissue, it was observed that the expression of UCP-1 and UCP-3 was markedly decreased in

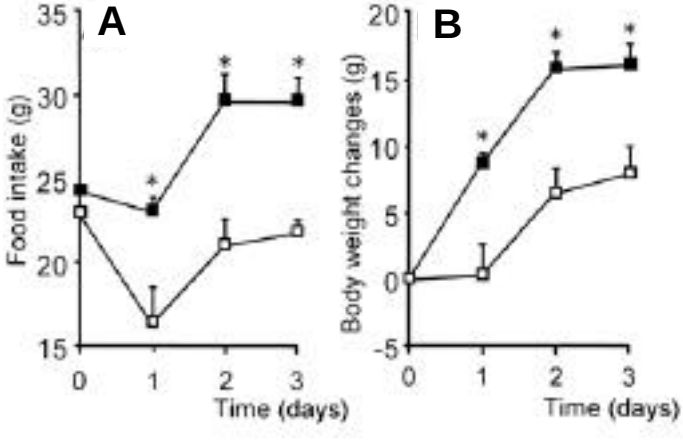

FIG. 1. Effect of a 3-day intracerebroventricular dexamethasone infusion on food intake (A) and change in body weight (B) in normal rats. Treated rats were infused with 5 µg/day dexamethasone (■) and controls with isotonic saline (□). Data are means ± SE of 10 animals per group. Statistical analysis was performed using one-way analysis of variance followed by multiple Bonferroni comparisons. *$P < 0.05$ vs. respective vehicle-infused controls.

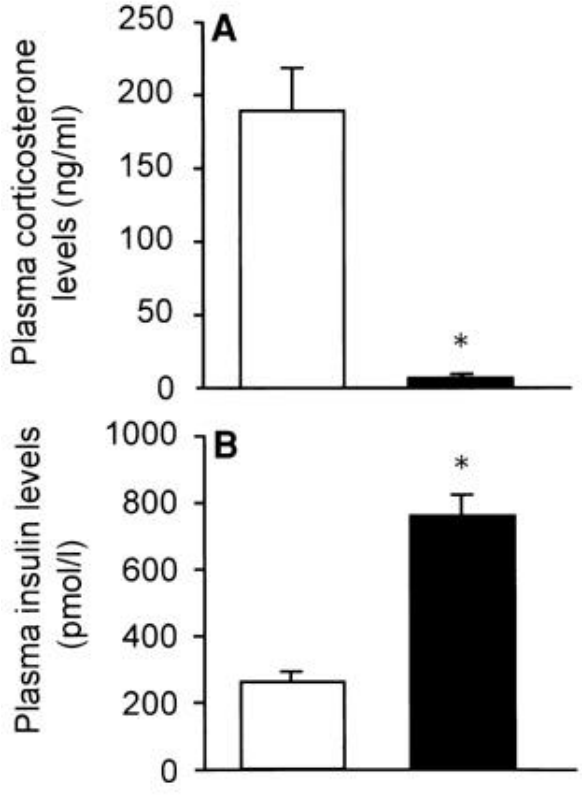

FIG. 2. Effect of a 3-day intracerebroventricular dexamethasone infusion on plasma corticosterone levels (*A*) and plasma insulin levels (*B*) in normal rats. Treated rats were infused with 5 µg/day dexamethasone (■) and controls with isotonic saline (□). Data are means ± SE of 10 animals per group. Statistical analysis was performed using two-tailed Student's *t* test for unpaired data. **P* < 0.05 vs. respective vehicle-infused controls.

the intracerebroventricular dexamethasone-infused animals compared with the vehicle-infused animals (Fig. 5), and that a similar decrease was observed for the expression of mus-

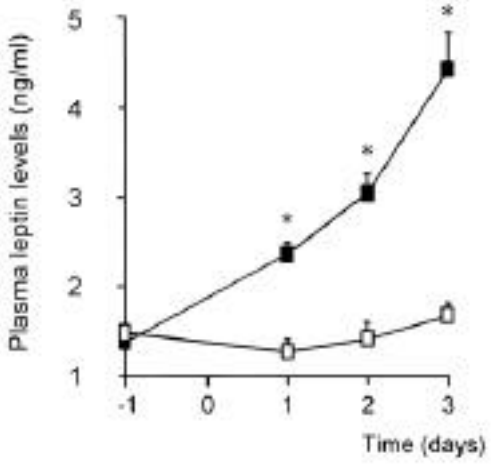

FIG. 3. Effect of a 3-day intracerebroventricular dexamethasone infusion on plasma leptin levels in normal rats. Treated rats were infused with 5 µg/day dexamethasone (■) and controls with isotonic saline (□). Data are means ± SE of 10 animals per group. Statistical analysis was performed using one-way analysis of variance followed by multiple Bonferroni comparisons. **P* < 0.05 vs. respective vehicle-infused controls.

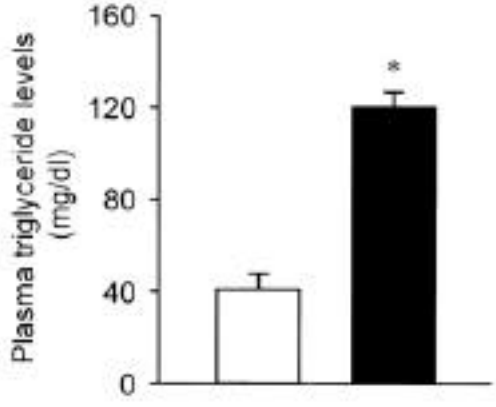

FIG. 4. Effect of a 3-day intracerebroventricular dexamethasone infusion on plasma triglyceride levels in normal rats. Treated rats were infused with 5 µg/day dexamethasone (■) and controls with isotonic saline (□). Data are means ± SE of 10 animals per group. Statistical analysis was performed using two-tailed Student's *t* test for unpaired data. **P* < 0.05 vs. respective vehicle-infused controls.

cle (red quadriceps) UCP-3 (Fig. 6), while no change in the expression of white adipose tissue UCP-2 was recorded (data not shown).

We studied aspects of the hypothalamic neuropeptide status at the end of the experimental period by measuring the amounts of CRH and NPY in the whole hypothalamus and the arcuate nucleus, respectively. As seen in Fig. 7, the amount of CRH was decreased while that of NPY was increased in the intracerebroventricular dexamethasone-infused group relative to controls. As all these dexamethasone-induced changes could conceivably be related to leakage of the hormone into the circulating blood, additional control experiments were carried out. Assuming a complete leakage of dexamethasone out of the cerebral ventricles, the same amount of the hormone previously infused intracerebroventricularly was administered intraperitoneally for the same 3-day period,

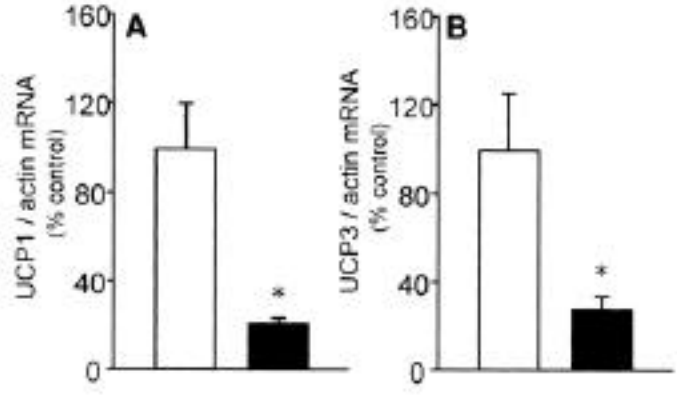

FIG. 5. Effect of a 3-day intracerebroventricular dexamethasone infusion on UCP-1 (*A*) and UCP-3 (*B*) in brown adipose tissue of normal rats. Treated rats were infused with 5 µg/day dexamethasone (■) and controls with isotonic saline (□) (for experimental details, see METHODS). Data are means ± SE of 10 animals per group. Statistical analysis was performed using two-tailed Student's *t* test for unpaired data. **P* < 0.05 vs. respective vehicle-infused controls.

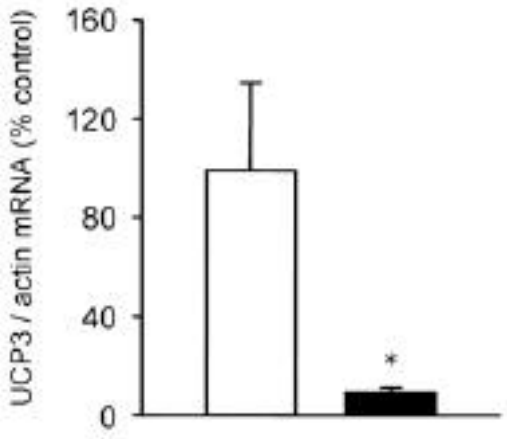

FIG. 6. Effect of a 3-day intracerebroventricular dexamethasone infusion on UCP-3 in muscle of normal rats. Treated rats were infused with 5 µg/day dexamethasone (■) and controls with isotonic saline (□) (for experimental details, see METHODS). Data are means ± SE of 10 animals per group. Statistical analysis was performed using two-tailed Student's *t* test for unpaired data. *P < 0.05 vs. respective vehicle-infused controls.

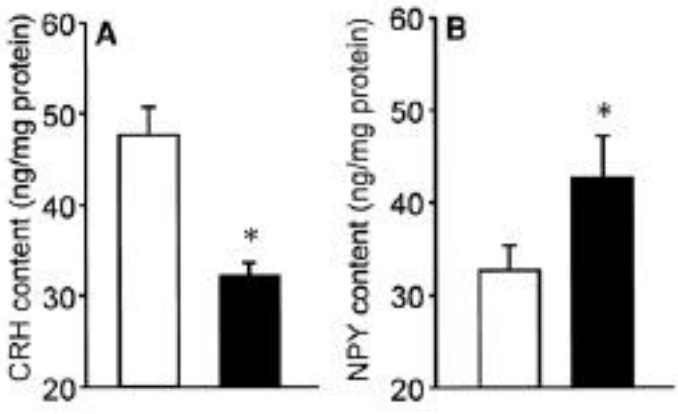

FIG. 7. Effect of a 3-day intracerebroventricular dexamethasone infusion on hypothalamic neuropeptides in normal rats: CRH (*A*) and NPY (*B*). Treated rats were infused with 5 µg/day dexamethasone (■) and controls with isotonic saline (□). Mediobasal hypothalamuses were used for CRH measurements, and arcuate hypothalamic nuclei were used for quantification of NPY content (for further experimental details, see METHODS). Data are means ± SE of 10 animals per group. Statistical analysis was performed using two-tailed Student's *t* test for unpaired data. *P < 0.05 vs. respective vehicle-infused controls.

and most of the parameters mentioned above were measured (Table 1). It was observed that intraperitoneal dexamethasone infusion resulted in definite body weight loss, slight but significant decrease in food intake, and increases in plasma insulin and leptin levels that were less marked than those measured when the intracerebroventricular route of dexamethasone infusion was used. Plasma corticosterone levels were similarly inhibited by both intraperitoneal and intracerebroventricular routes of administration. In keeping with this similarity was the observation that the hypothalamic CRH content was decreased by intraperitoneal as well as intracerebroventricular infusion. In marked contrast to intracerebroventricular dexamethasone administration, intraperitoneal infusion of the hormone resulted in a significant decrease in arcuate nucleus NPY levels.

DISCUSSION

These data show that the continuous (3-day) intracerebroventricular infusion of the synthetic glucocorticoid, dexamethasone, to normal rats stimulated their food intake and rate of body weight gain relative to vehicle-infused controls. Such intracerebroventricular infusion of glucocorticoids was accompanied by marked hyperinsulinemia, hypertriglyceridemia, and hyperleptinemia, three salient abnormalities encountered in most obesity syndromes, at least in rodents (41,42).

TABLE 1
Effect of intraperitoneal dexamethasone infusion on food intake, body weight, neuropeptides, and hormones

	Control	Intraperitoneal dexamethasone
Food intake (g)	20.4 ± 0.5	18.2 ± 0.6*
Change in body weight (g)	5.4 ± 1.8	–6.4 ± 2.3*
Arcuate nucleus NPY (ng/mg protein)	46.7 ± 5.0	33.8 ± 2.5*
Hypothalamic CRH (ng/mg protein)	30.3 ± 4.3	18.7 ± 2.2*
Corticosterone (ng/ml)	81.0 ± 20.9	3.0 ± 3.0*
Insulin (pmol/l)	133.4 ± 18.0	257.7 ± 30.3*
Leptin (ng/ml)	1.2 ± 0.1	2.8 ± 0.4*

Data are means ± SE of five to six rats per group. Statistical analysis was performed by one-way analysis of variance with repeated measures followed by multiple Tukey's comparisons. *P < 0.05 for intergroup differences.

The observed increase in plasma leptin levels could be partly due to intracerebroventricular glucocorticoid-elicited hyperinsulinemia, since that hormone is a well-documented trigger of adipose tissue *ob* gene expression and leptin secretion (43–45). Continuous intracerebroventricular glucocorticoid infusion abolished the peripheral corticosterone output, in agreement with its well-substantiated negative feedback effect on the HPA axis.

The stimulatory effect of intracerebroventricular glucocorticoids on food intake and body weight is fitting with the observation that intracerebroventricular glucocorticoid infusion resulted in an actual increase in the amount of the potent orexigenic peptide, NPY, in the site of its synthesis, the arcuate nucleus. This increase is in keeping with previous observations showing that a continuous intracerebroventricular infusion of NPY resulted in increased food intake, body weight, insulin output, and fat storage at the expense of substrate utilization by the muscle mass (23,46). When NPY-infused rats were prevented from overeating, similar but less marked hormonal-metabolic alterations were observed (23). Thus, it is postulated that in the absence of hyperphagia (pair-feeding), intracerebroventricular dexamethasone effects might be qualitatively, but not quantitatively, similar to those described in the present study.

The effect of central dexamethasone infusion on overall body weight homeostasis could have been attributed to leakage of the hormone into the peripheral blood. To examine this point, control experiments were carried out in which the same amount of dexamethasone was infused intraperitoneally for the same 3-day period. When comparing the effects of intracerebroventricular and intraperitoneal dexamethasone infusion, specific and important differences

were noted. First, intraperitoneal dexamethasone resulted in definite, though still moderate, decreases in food intake and body weight, whereas the opposite was observed when dexamethasone was given intracerebroventricularly, as mentioned above. Both intraperitoneal and intracerebroventricular dexamethasone infusion inhibited the HPA axis, as evidenced by marked decreases in plasma corticosterone levels relative to respective controls. Both intraperitoneal and intracerebroventricular dexamethasone stimulated insulin output, but to a lesser extent in the intraperitoneally (about twofold) than in the intracerebroventricularly (about threefold) infused group. As a likely consequence of both the decrease in body weight and the lesser insulin response, the intraperitoneal infusion of dexamethasone resulted in plasma leptin levels that were only twofold greater than controls, while they were threefold higher than controls in the intracerebroventricularly infused group.

The most striking difference between intracerebroventricular and intraperitoneal dexamethasone administration was found at the level of the regulation of hypothalamic NPY levels. Intracerebroventricular dexamethasone resulted in increased NPY content in the arcuate nucleus in the face of decreases in hypothalamic CRH levels. In contrast, intraperitoneal dexamethasone produced decreases in the levels of both NPY and CRH. We give the following interpretation for such divergent effects of intracerebroventricular and intraperitoneal dexamethasone on hypothalamic neuropeptide homeostasis. With the intracerebroventricular route of dexamethasone administration, the continuous presence of the steroid within the central nervous system appears to stimulate hypothalamic NPY synthesis and, by its mere concentration, to inhibit the effect of leptin on this process. This conclusion is in keeping with previous data indicating that glucocorticoids are inhibitory to leptin action (26). With the intraperitoneal route of dexamethasone administration, it is postulated that the peripheral levels of the steroid favor insulin output and, in combination with the latter, stimulate leptin secretion. It is further postulated that, given the dilution of intraperitoneally administered dexamethasone in the peripheral blood, its central concentration may not be sufficient to prevent the inhibitory effect of leptin on NPY or to favor its own stimulatory action on NPY synthesis. Since hypothalamic CRH levels are similarly decreased by either intracerebroventricular or intraperitoneal dexamethasone administration, it has to be postulated that the HPA axis is more sensitive to the steroid than the pathways implicated in the regulation of NPY homeostasis.

Central NPY has been shown previously to exert part of its peripheral effects by favoring the activity of the efferent vagus nerve (47). CRH reportedly exerts part of its effects via stimulating the activity of sympathetic efferents (32,48). It may thus be proposed that intracerebroventricular glucocorticoids, since they result in increased hypothalamic NPY levels and decreased hypothalamic CRH content, would shift the autonomic nervous system balance toward parasympathetic and away from sympathetic activity. This proposal would be in keeping with the present observation of a markedly decreased expression of UCP-1 and -3 at the level of brown adipose tissue and UCP-3 in muscle, and it would also be in agreement with previous reports showing a role of intracerebroventricular glucocorticoid infusion in stimulating the vagal drive to the endocrine pancreas (49,50).

In conclusion, the data of the present study indicate that intracerebroventricular glucocorticoid infusion in normal rats profoundly alters body weight and hormonal and metabolic homeostasis, favoring storage and overweight. These changes appear to be mediated mainly via an increase in NPY levels produced by central glucocorticoid infusion, substantiated by the observation that intraperitoneal administration of the same dose of dexamethasone produces body weight loss, in line with the decrease in NPY levels.

ACKNOWLEDGMENTS

This research has been supported by grant no. 31-53719.8 of the Swiss National Science Foundation (Bern, Switzerland) and by a grant-in-aid from Novartis Pharma A.G. (Basel, Switzerland), which also supplied the laboratories with recombinant mouse leptin.

We also would like to thank F. Pralong for valuable help with the CRH radioimmunoassay. We are indebted to P. Arboit, P. German, and A. Volery (Laboratoires de Recherches Métaboliques) and to G. Monnet (Novartis) for their skillful technical assistance.

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