Adrenalectomy Prevents the Obesity Syndrome Produced by Chronic Central Neuropeptide Y Infusion in Normal Rats

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Neuropeptide Y (NPY) in the hypothalamus plays an important role in the regulation of food intake and body weight and seems to be implicated in the etiology of obesity. When intracerebroventricularly (ICV) infused for 6 days in normal rats, NPY resulted in hyperphagia, increased body weight gain, hyperinsulinemia, hypercorticosteronemia, and hypertriglyceridemia compared with vehicle-infused control rats. NPY infusion also resulted in an insulin-resistant state in muscles and in a state of insulin hyperresponsiveness in white adipose tissue, as assessed by the measurement of the in vivo glucose utilization index of these tissues during euglycemic-hyperinsulinemic clamps. All of these hormono-metabolic effects produced by chronic central NPY infusion were completely prevented when rats were adrenalectomized before NPY administration. Adrenalectomy per se had no effect on any of the parameters mentioned above. The levels of mRNA for the *obese* gene were increased in white adipose tissue after 6 days of ICV NPY infusion in normal rats, and white adipose tissue weight was also increased. These effects of ICV NPY infusion were markedly decreased by prior adrenalectomy, although NPY infusion was able to somewhat enhance the low white adipose tissue *obese* mRNA levels and tissue weight of adrenalectomized rats. In conclusion, intact adrenal glands, and probably circulating corticosterone in particular, are necessary for the establishment of most of the hormonal and metabolic effects induced by chronic ICV infusion of NPY in normal rats. *Diabetes* 46:209–214, 1997

The adrenal gland and glucocorticoids play a fundamental role in syndromes of obesity and insulin resistance. High plasma levels of glucocorticoids are common to genetically obese *fa/fa* rats, *db/db* and *ob/ob* mice, and obese humans (including those with Cushing's syndrome), all of which demonstrate obesity and insulin resistance (1–5). Furthermore, exogenous administration of glucocorticoids to normal animals and humans results in some of the metabolic characteristics of obesity, such as hyperinsulinemia, β-cell hyperplasia, and insulin resistance (6,7). Of special interest is the fact that much of the pathology of rodents with obesity of genetic (8–12), dietary (13), or hypothalamic (14–16) origin can be at least partially normalized or prevented by adrenalectomy. Moreover, administration of even trace (10,15,16) amounts of glucocorticoids to adrenalectomized, obese rodents results in reappearance of their obesity syndrome, highlighting the permissive role these steroids play in the development of obesity.

An endogenous substance implicated in the etiology of obesity is neuropeptide Y (NPY). This neurotransmitter is particularly abundant in the hypothalamus, where it is involved in the regulation of fuel homeostasis (17,18) as well as in the secretion of various hormones such as insulin and glucocorticoids, as recently reviewed (19,20). It is of note that the hypothalamic levels of NPY and/or its message are increased in many genetic models of rodent obesity such as *fa/fa* and *cp/cp* rats (21,22) as well as *ob/ob* and *db/db* mice (23). This increase has been detected early after weaning in *fa/fa* rats and may be an etiologic factor in their obesity that starts to become overt at this time (21). Such a viewpoint is strengthened by the observation that chronic administration of exogenous NPY to specific hypothalamic nuclei or into the cerebral ventricles of normal rats results in many of the defects of obesity, including a marked increase in food intake, accelerated body weight gain, hypercorticosteronemia, hyperinsulinemia, muscle insulin resistance, and increased triglyceride accumulation within white adipose tissue (24).

In light of these observations, it is possible that the effect of adrenalectomy in preventing the full expression of rodent obesity could be mediated by effects on hypothalamic NPY-ergic activity. For example, type II adrenal steroid (glucocorticoid) receptor agonists cause an increase in NPY mRNA and/or peptide levels in the hypothalamus after central or peripheral administration in vivo (25–30) or after direct application to hypothalamic cells in vitro (30), while adrenalectomy of normal rats has been reported to reduce hypothalamic NPY gene expression (25,26,28,29). The aim of this work was to assess the role of glucocorticoids in the obesity syndrome produced by chronic central NPY infusion in normal rats. This was done by comparing normal and adrenalectomized rats with respect to their hormonal and metabolic responses to 6-day intracerebroventricular (ICV) NPY infusion.

RESEARCH DESIGN AND METHODS

Animals. Female Sprague Dawley rats, bilaterally adrenalectomized or sham-operated at 7 weeks of age, were purchased from IFFA CREDO (L'Arbresle, France) and housed individually under conditions of controlled temperature

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CRF, corticotropin-releasing factor; ICV, intracerebroventricular; NPY, neuropeptide Y.

(23°C) and illumination (6:00 A.M.–6:00 P.M.). They were allowed ad libitum access to standard laboratory meal (Provimi Lacta, Cossonay, Switzerland) and tap water (supplemented with 9 g/l NaCl for adrenalectomized rats).

Placement of chronic ICV cannulas. At 10 weeks of age rats were anesthetized with intramuscular ketamin/xylazine at 45 mg/kg and 9 mg/kg, respectively (Parke-Davis and Bayer AG, Leverkusen, Switzerland), for the placement of a cannula in the right lateral cerebral ventricle (24). Adrenalectomized rats were injected with corticosterone (3.0 mg in ethanol subcutaneously.) 1 h prior to anesthesia, as well as 2–5 h later. An intramuscular injection of 50 mg/kg amoxicillin (Smith Kline Beecham, Thörishaus, Switzerland) was given after surgery, and animals were left to recover for 2 weeks, during which time they were handled daily and habituated to the blood-sampling procedure. Several days before experimentation, the drinking response to the ICV injection of angiotensin II (25 ng in 5 µl phosphate-buffered saline; Novabiochem, Läufelfingen, Switzerland) was measured to confirm correct placement of the ICV cannula. Only those rats that drank ~8 ml or more water (or saline for adrenalectomized rats) in the 30 min after injection (~90% of the animals) were used for further studies.

Infusion of NPY. At 12 weeks of age, rats were designated to one of four experimental groups: *1*) normal (sham-operated and ICV infused with vehicle only); *2*) normal + ICV NPY (sham-operated and ICV infused with NPY); *3*) ADX (adrenalectomized and ICV infused with vehicle only); and *4*) ADX + ICV NPY (adrenalectomized and ICV infused with NPY). Porcine NPY (15 µg/day; Bachem, Bubendorf, Switzerland) or its vehicle (0.04 mol/l phosphate-buffered saline, pH 7.4, with 0.2% bovine serum albumin and 0.01% ascorbic acid) was ICV infused for 6 days via a subcutaneously placed osmotic minipump (model 2001, Alza Corporation, Palo Alto, CA) (24). During ICV infusion, ad libitum food intake and body weight were measured daily, and a 450-µl blood sample was collected from the tip of the tail into EDTA-coated tubes at 9:30 A.M. each day, 1.5–2.5 h after removal of food from cages. Plasma was stored at –20°C until further analysis.

Measurement of in vivo glucose utilization during euglycemic-hyperinsulinemic clamps. After 6 days of ICV infusion, 13-h fasted animals were anesthetized with sodium pentobarbital (55 mg/kg intraperitoneally) and prepared for euglycemic-hyperinsulinemic clamps (2) associated with the labeled 2-deoxyglucose technique to measure in vivo insulin-stimulated glucose utilization index in individual tissues as previously described and validated (31,32). Human insulin (Actrapid, Novo Nordisk Pharma, Zurich, Switzerland) was infused intravenously such that insulinemia was increased to an average steady-state level of 6,180 ± 1,125 pmol/l in all groups of rats (n = 20). Such values of insulinemia are known to maximally stimulate in vivo glucose metabolism in rats (2), allowing the study of maximum insulin responsiveness of tissue glucose utilization. Unlabeled glucose solution (200 g/l) was intravenously infused (Precidor Infors pump, Bottmingen, Switzerland) at a varying rate to maintain glycemia at an average level of 6.1 ± 0.1 mmol/l in all rats (n = 20) during the clamp, a value not significantly different from basal glucose concentrations (6.4 ± 0.2 mmol/l, n = 20). When a steady rate of glucose infusion was reached, 30 µCi of 2-deoxy-D-[1-^{3}H]glucose (11.2 Ci/mmol, Amersham, Aylesbury, U.K.) was injected intravenously to measure the maximum insulin-stimulated glucose utilization index of different types of muscle (quadriceps and gastrocnemius, separated into red and white components, extensor digitorum longus, tibialis) and of inguinal white adipose tissue.

Analytical procedures relative to clamp studies. 2-deoxy-D-[1-^{3}H]glucose-specific activity in blood samples was determined as previously published (24). Uptake and phosphorylation of 2-deoxy-D-[1-^{3}H]glucose by various tissues during the clamp was estimated as described (31,32) and expressed as a glucose utilization index (ng/min × mg tissue). Since white adipose tissue weight varied between the four groups of rats (Table 1), the glucose utilization index of this tissue was expressed in ng/min × µg protein. Protein content of white adipose tissue was measured by Bio-Rad microassay (Bio-Rad Laboratories GmbH, Munich, Germany) after protein extraction (33).

Plasma hormone and metabolite measurements. Plasma insulin concentrations were measured by radioimmunoassay (24) using guinea pig anti-rat insulin serum (Linco Research Inc., St. Louis, MO) for basal samples. Guinea pig anti-porcine insulin serum having 100% cross-reactivity with rat and human insulin (Sorin Biomedica, Saluggia, Italy) was used to detect endogenous as well as the exogenously infused human insulin used during euglycemic-hyperinsulinemic clamps. Corticosteronemia was measured by radioimmunoassay with a limit of detection ~30 pmol/l (34), and a kit from CIS Bio International (Gif-Sur-Yvette, France) was used for measurement of plasma ACTH. Plasma triglyceride and glucose concentrations were determined using kits from BioMérieux (Marcy L'Etoile, France) and Boehringer (Mannheim, Germany), respectively.

Northern blot analysis. Ratios of *ob*/β-actin mRNA levels in inguinal white adipose tissue (expressed as a percentage of control values) were determined as described in a previous study (35).

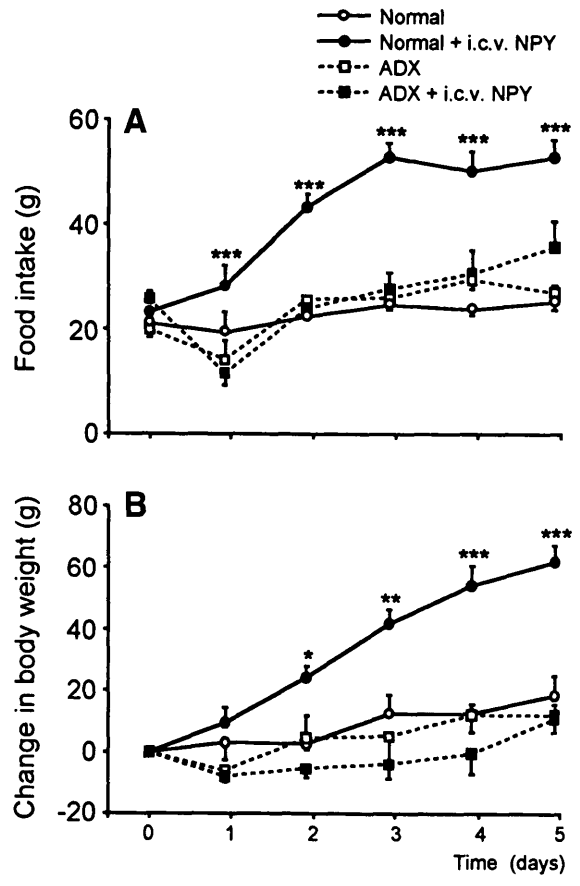

FIG. 1. Daily food intake (*A*) and cumulative change in body weight (*B*) during ICV infusion of NPY (15 µg/day) in normal or adrenalectomized (ADX) rats compared with respective vehicle-infused controls. Mean body weight at the start of ICV infusion was 252 ± 4 g, with no difference between the four groups. Data are means ± SE of 5–7 rats per group. *P < 0.05; **P < 0.01; ***P < 0.001 vs. corresponding value of vehicle-infused normal rats.

Statistical analysis. For the daily measurements of food intake, change in body weight, plasma insulin, triglyceride, glucose, and corticosterone concentrations, differences between the four groups of rats were assessed by one-way analysis of variance with repeated measures followed by post-hoc Duncan's range test. When two groups were found to be significantly different, multiple Bonferroni comparisons were made to locate differences at each time point. Differences between the four groups of rats with respect to glucose utilization index of various tissues as well as *ob* mRNA levels and weight of white adipose tissue depots were determined by one-way analysis of variance followed by Student-Newman-Keul's multiple comparisons tests. For all analyses, values of P < 0.05 were accepted as being statistically significant.

RESULTS

As shown in Fig. 1, food intake and cumulative change in body weight were markedly increased during 6-day ICV NPY infusion in normal rats relative to their vehicle-infused controls. In contrast, ICV NPY infusion had no effect on food intake nor on body weight gain in adrenalectomized rats, which had values similar to those of vehicle-infused normal rats (Fig. 1). Parallel results were obtained for plasma insulin and triglyceride concentrations (Fig. 2), which were four- to eightfold increased by ICV NPY infusion in normal rats but were unaltered by NPY infusion in adrenalectomized animals. There was no difference in glycemia between the four groups of rats at any time point during the experiment, average morning values being 5.6 ± 0.2 mmol/l, 5.3 ± 0.2 mmol/l, 6.0 ± 0.2 mmol/l, and 5.4 ± 0.2 mmol/l in

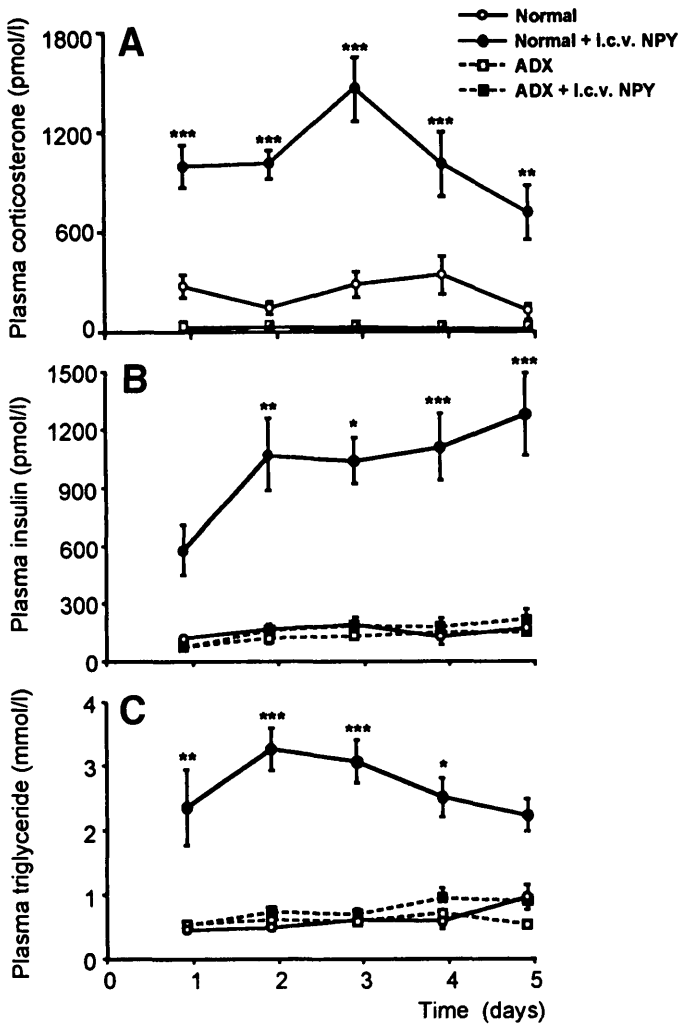

FIG. 2. Plasma corticosterone (*A*), insulin (*B*), and triglyceride (*C*) concentrations during ICV infusion of NPY (15 µg/day) in normal or adrenalectomized (ADX) rats compared with respective vehicle-infused controls. Data are means ± SE of 5–7 rats per group. $*P < 0.05$; $**P < 0.01$; $***P < 0.001$ vs. corresponding value of vehicle-infused normal rats.

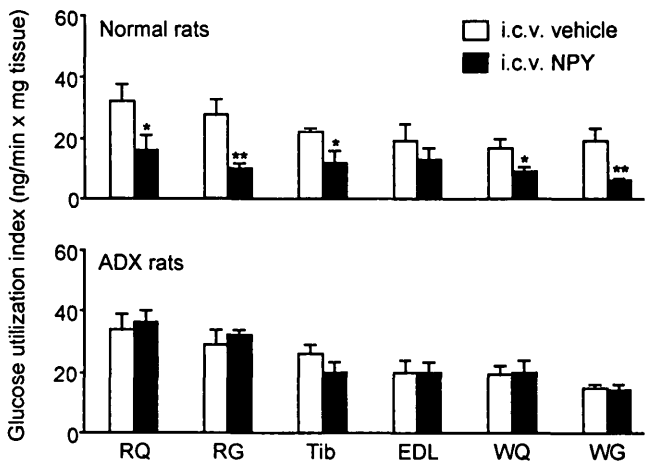

FIG. 3. Glucose utilization index of various muscles (RQ, red quadriceps; RG, red gastrocnemius; Tib, tibialis; EDL, extensor digitorum longus; WQ, white quadriceps; WG, white gastrocnemius) measured during euglycemic-hyperinsulinemic clamps after 6 days of ICV NPY infusion (15 µg/day) in normal or adrenalectomized (ADX) rats, compared with corresponding vehicle-infused controls. Data are means ± SE of 3–6 rats per group. $*P < 0.05$; $**P < 0.01$ vs. vehicle-infused normal rats.

normal, normal + ICV NPY, ADX, and ADX + ICV NPY-infused rats, respectively (n = 5–7 rats per group). Plasma corticosterone levels of adrenalectomized rats were consistently below the limit of detection of the radioimmunoassay, ~30 pmol/l (Fig. 2). In contrast, vehicle-infused normal rats exhibited basal values of corticosteronemia of 150–350 pmol/l, and this was increased by severalfold during the 6 days of ICV NPY infusion (Fig. 2). Plasma ACTH concentrations were also significantly elevated due to central NPY infusion in normal rats (7.9 ± 1.3 and 57.5 ± 15.0 pmol/l in normal and normal + ICV NPY-infused rats, respectively, n = 5–7, $P < 0.01$). Adrenalectomized rats had plasma ACTH concentrations approximately 100-fold greater than those of normal vehicle-infused rats, and NPY infusion did not affect these levels (data not shown).

The insulin-stimulated glucose utilization index, measured during euglycemic-hyperinsulinemic clamps using maximally effective plasma insulin concentrations (2), was decreased in most muscles of normal rats after 6 days of ICV NPY infusion (Fig. 3). Thus this decrease, indicating muscular insulin resistance to maximal plasma insulin concentrations, was present in all six muscles shown in Fig. 3 and reached statistical significance in five of these six muscles. In contrast, muscles of adrenalectomized rats did not become insulin resistant following 6 days of ICV NPY infusion (Fig. 3), and their glucose utilization indexes were similar to those of normal vehicle-infused control rats.

Figure 4 shows the insulin-stimulated glucose utilization index of inguinal white adipose tissue, expressed in ng/min × µg protein as the weight of this tissue varied between the four groups of animals (see Table 1). Compared with vehicle-infused normal rats, 6-day ICV NPY infusion in these rats resulted in a significant fourfold increase in the inguinal white adipose tissue glucose utilization index. However, no such effect of central NPY administration was observed in adrenalectomized rats, which were no different from vehicle-infused normal rats with respect to the inguinal white adipose tissue glucose utilization index.

Table 1 shows the weight of the inguinal, mesenteric, and retroperitoneal white adipose tissue depots in the four experimental conditions. NPY significantly increased the weight of each of these three white adipose tissue depots when centrally infused in normal rats. Adrenalectomized vehicle-infused rats had white adipose tissue weights 30–60% smaller than those of normal controls, and central NPY infusion in adrenalectomized rats increased these tissue weights to values approaching those of normal vehicle-infused rats.

Finally, the relative abundance of *ob* mRNA was significantly increased in the inguinal white adipose tissue of NPY-infused normal rats compared with their vehicle-infused controls (Fig. 5). Vehicle-infused adrenalectomized rats showed fivefold lower levels of *ob* mRNA than did vehicle-infused normal rats (Fig. 5, open columns), and this decrease was reconfirmed and reached statistical significance in a separate experiment using different groups of animals (data not shown). Central NPY infusion in adrenalectomized rats tended to increase their low levels of *ob* expression to values similar to those of vehicle-infused normal rats.

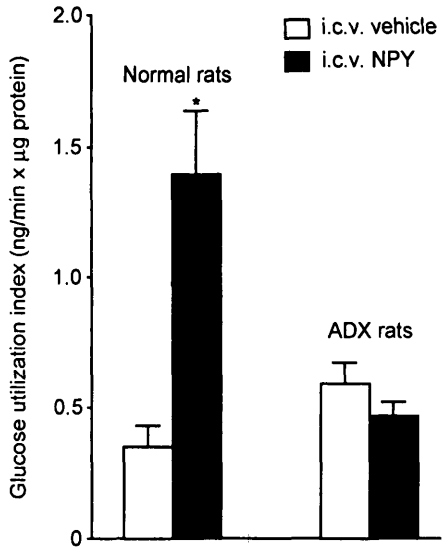

FIG. 4. Glucose utilization index of inguinal white adipose tissue measured during euglycemic-hyperinsulinemic clamps after 6 days of ICV NPY infusion (15 µg/day) in normal or adrenalectomized (ADX) rats compared with corresponding vehicle-infused controls. Data are means ± SE of 3–6 rats per group. *P < 0.05 vs. vehicle-infused normal rats.

DISCUSSION

This work shows that most of the hormonal and metabolic effects produced by the chronic ICV infusion of NPY in normal rats were prevented by prior adrenalectomy. Such NPY infusion in normal rats resulted in hyperphagia, increased body weight gain, hyperinsulinemia, hypercorticosteronemia, and hypertriglyceridemia. It also resulted in an insulin resistance of the in vivo glucose utilization index in muscles and an insulin hyperresponsiveness of such indexes in white adipose tissue, in keeping with previous work (24). These NPY-induced effects were not observed during NPY's central infusion in adrenalectomized rats. This finding extends previous reports to show that not only are genetic (8–12), dietary (13), and hypothalamic (14–16) obesities in rodents prevented by adrenalectomy, but NPY-induced hormonometabolic changes leading to obesity also depend on intact adrenal glands, probably on circulating corticosterone in particular. In agreement are previous reports that showed that the feeding response to central NPY injection was attenuated by adrenalectomy (36–38) but restored by corticosterone replacement (36). In the latter study (36), hypophysectomy was likewise effective in attenuating NPY-induced feeding in rats, this effect being prevented by peripheral corticosterone replacement. These findings provide further evidence that the lack of circulating corticosterone in adrenalectomized rats, not the increase in ACTH secretion, results from removal of glucocorticoid inhibition on the hypophysis (39), which reduces or prevents the effects of central NPY administration. The observation that NPY-induced feeding in rats is abolished by the central administration of selective type II (glucocorticoid) adrenal steroid receptor antagonists (40) suggests that the action of corticosterone within the central nervous system most likely is necessary for the obesity-like effects of chronic ICV NPY infusion. This is in keeping with the observation that several aspects of the obesity syndrome of *ob/ob* and gold thioglucose-treated mice can be restored, after adrenalectomy, by ICV injection of glucocorticoids, including the preferential type II receptor agonist dexamethasone (10,16,41). Additional studies are required for precise identification of the adrenal hormone, its site(s) of action, and the receptor(s) required for the effects of central NPY on body energy balance as shown in this study.

It is known that adrenalectomy in rats causes increases in expression and release of corticotropin-releasing factor (CRF) in the hypothalamus due to the absence of feedback inhibition by corticosterone (39). Chronic administration of exogenous CRF to the hypothalamus has been shown to decrease food intake, body weight gain, and insulinemia of normal lean or genetically obese *fa/fa* rats (42), effects which are opposite to those of chronic central NPY administration to normal rats (24). In the present investigation, however, ICV vehicle-infused adrenalectomized rats were similar to vehicle-infused normal rats with respect to food intake, body weight gain, and insulinemia, despite the elevated hypothalamic CRF expression (indicated by high plasma ACTH concentrations) in the former group. It therefore seems unlikely that an increase in hypothalamic CRF-ergic activity could explain the lack of effect of chronic ICV NPY infusion on feeding and other parameters in adrenalectomized rats.

The finding that adrenalectomized rats fail to respond to chronic ICV NPY infusion with the anabolic changes observed after NPY infusion in intact rats may partially explain why adrenalectomy diminishes the expression of genetic obesity in rodents. Thus, adrenalectomy of these rats could act by blocking the effects of their elevated hypothalamic NPY-ergic content (21–23). Another possibility is that adrenalectomy attenuates genetic obesity syndromes in rodents by reducing the elevated hypothalamic NPY expression observed in these animals. This would be in keeping with the observations that glucocorticoids, acting most probably at type II adrenal steroid receptors in the central nervous system, stimulate NPY gene expression in the hypothalamus and that adrenalectomy, at least in some studies, results in reduced hypothalamic NPY gene expression (25–30).

TABLE 1
Weight of adipose tissue depots after 6 days of ICV NPY infusion (15 µg/day) in normal or adrenalectomized rats compared with respective vehicle-infused controls

	Normal	Normal + ICV NPY	ADX	ADX + ICV NPY
Inguinal (g)	1.56 ± 0.32	3.17 ± 0.41‡	1.14 ± 0.13	1.32 ± 0.07
Mesenteric (g)	1.17 ± 0.50	2.33 ± 0.34*	0.44 ± 0.01	0.75 ± 0.20
Retroperitoneal (g)	2.14 ± 0.16	4.35 ± 0.24‡	0.79 ± 0.03†	1.31 ± 0.09†§

Data are means ± SE of 3–6 rats per group. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. vehicle-infused normal rats; §P < 0.05 vs. vehicle-infused adrenalectomized rats. ADX, adrenalectomized.

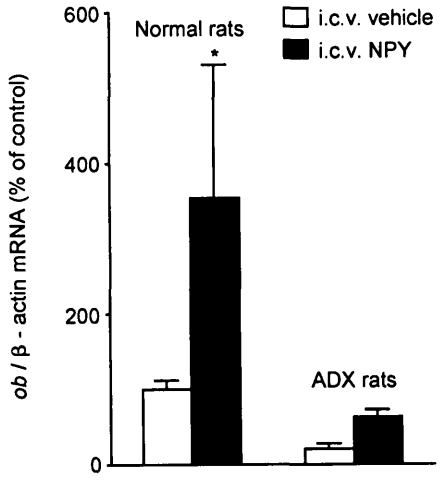

FIG. 5. Levels of *ob* mRNA in inguinal white adipose tissue after 6 days of ICV NPY infusion (15 μg/day) in normal or adrenalectomized (ADX) rats compared with corresponding vehicle-infused controls. Levels of *ob* mRNA are standardized against those of β-actin and are expressed as a percentage of normal vehicle-infused control rats (set at 100%). Data are means ± SE of 3–4 rats per group. *$P < 0.05$ vs. vehicle-infused normal rats.

Although the latter hypothesis awaits direct experimental evidence, the present data give further support for the role of hypothalamic NPY in the development of obesity syndromes.

In addition to the above-mentioned effects of adrenalectomy on the hormono-metabolic effects of ICV NPY infusion, this study shows the effects of adrenalectomy per se on the in vivo insulin response using the euglycemic-hyperinsulinemic clamp technique combined with measurements of tissue 2-deoxyglucose uptake in particular. It was found that adrenalectomy per se had no effect on the glucose utilization index of muscles or white adipose tissue under euglycemic-hyperinsulinemic conditions, in keeping with other observations (4,43,44). When performed in insulin-resistant genetically obese rats or mice, adrenalectomy resulted in clear increases in insulin-stimulated total body glucose disposal (43) and normalization of their depressed 2-deoxyglucose uptake by hindlimb muscles after insulin infusion (4). Likewise, the muscle insulin resistance induced by 6-day ICV NPY infusion in normal rats was prevented by prior adrenalectomy. These results provide further evidence that adrenal glucocorticoids, possibly in combination with hyperinsulinemia (6,45), contribute to the muscle insulin resistance of genetically obese and ICV NPY-infused rodents, although they apparently have no effect on insulin sensitivity and/or responsiveness in normal animals.

Finally, this report confirms our previous findings (35) that chronic central NPY infusion in normal rats markedly increased the expression of the *obese* (*ob*) gene in white adipose tissue, the gene product of which is centrally implicated in the control of feeding and body energy balance (23,46). A novel finding was that adrenalectomy per se reduced *ob* mRNA levels in white adipose tissue by fivefold, possibly due to the lack of circulating glucocorticoids, which have been shown to enhance *ob* expression both in vivo and in vitro (47,48). Adrenalectomy markedly diminished the increase in *ob* expression seen after ICV NPY infusion, although NPY did tend to increase the low *ob* mRNA levels of adrenalectomized rats toward normal control values. Parallel results were observed for the weight of white adipose tissue depots, showing that although ICV NPY influences *ob* expression and hypertrophy of white adipose tissue via a mechanism mainly dependent on intact adrenal glands, other as-yet-unknown mechanisms that are independent of changes in food intake, plasma corticosterone, or insulin concentrations could also play a role.

In conclusion, the obesity syndrome induced by chronic ICV NPY infusion in normal rats is largely prevented by adrenalectomy, although a small residual effect of NPY on white adipose tissue weight and *ob* expression persists even in the absence of intact adrenal glands. Further research is needed in order to determine the mechanism of this dependence on adrenal glucocorticoids for the anabolic effects of central NPY.

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