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Adrenalectomy Alters the Sensitivity of the Central Nervous System Melanocortin System

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Removal of adrenal steroids by adrenalectomy (ADX) reduces food intake and body weight in rodents and prevents excessive weight gain in many genetic and dietary models of obesity. Thus, glucocorticoids appear to play a key role to promote positive energy balance in normal and pathological conditions. By comparison, central nervous system melanocortin signaling provides critical inhibitory tone to regulate energy balance. The present experiments sought to test whether glucocorticoids influence energy balance by altering the sensitivity to melanocortin receptor ligands. Because melanocortin-producing neurons are hypothesized to be downstream of leptin in a key weight-reducing circuit, we tested rats for their sensitivity to leptin and confirmed reports that the hypophagic response to third ventricular (i3vt) leptin is increased in ADX rats and is normalized by glucocorticoid replacement. Next we tested rats for their sensitivity to the melanocortin agonist melanotan II and found that, as for leptin, ADX enhanced the hypophagic response via a glucocorticoid-dependent mechanism. The central nervous system melanocortin system is unique in that it includes the endogenous melanocortin receptor antagonist, AgRP. The orexigenic effect of i3vt AgRP was absent in ADX rats and restored by glucocorticoid replacement. We conclude that the potent weight-reducing effects of ADX likely involve heightened responsiveness to melanocortin receptor stimulation. *Diabetes* 52:2928–2934, 2003

O besity is a major risk factor for many diseases, including diabetes, hypertension, cardiovascular disease, and some cancers. Among the myriad factors mediating the regulation of food intake and body weight is the glucocorticoid, cortisol (corticosterone in rats), which is secreted from the adrenal cortex. Glucocorticoids are lipophilic molecules that freely cross the blood-brain barrier and interact with receptors distributed throughout the central nervous system, including the hypothalamus (1). In rodents, most forms of genetic obesity are associated with increased glucocorticoid levels, whereas a lack of glucocorticoids is linked to hypophagia and reduced body weight. Parallels exist in humans as well. Individuals with Addison's disease exhibit anorexia and weight loss due to adrenal insufficiency, whereas patients with Cushing's disease and other glucocorticoid excess syndromes have increased food intake (2) and visceral adiposity. In rodents, adrenalectomy (ADX) reverses many forms of obesity, while glucocorticoid replacement restores the obese state (2), and glucocorticoid administration to adrenal-intact animals increases food intake and weight gain (2,3).

Whereas glucocorticoids stimulate food intake and weight gain, leptin is a hormone that provides negative feedback in the control of food intake and adiposity. Leptin is secreted from adipocytes in direct proportion to the amount of stored fat (4,5), and plasma leptin levels fall during caloric restriction and rise during overfeeding (4,6–8). Genetic leptin deficiency results in profound hyperphagia and obesity in mice and humans (9), a syndrome that can be reversed by exogenous leptin treatment (10–12). These effects of leptin are due to activation of the leptin receptor in brain regions important for body weight regulation (5), including the hypothalamic arcuate nucleus (ARC) and adjacent areas (13).

Several lines of evidence suggest an interaction between leptin and the hypothalamic-pituitary-adrenal axis (14). Genetic deficiency of leptin or its receptor is characterized by hypercorticosteronemia in rodents (15); in leptin-deficient ob/ob mice, leptin replacement decreases corticosterone levels independent of its weight-reducing effects, suggesting a role for leptin to inhibit the hypothalamic-pituitary-adrenal axis (16). When rats are adrenalectomized, the ability of intracerebroventricular administration of leptin to reduce food intake and body weight is enhanced. This increase in sensitivity can be reversed by glucocorticoid replacement, suggesting that glucocorticoids play a physiological role to attenuate the response to leptin (2).

One potential mechanism whereby glucocorticoids can influence leptin action is via effects on intracellular signal transduction pathways activated by leptin in the brain (17). For example, ADX increases hypothalamic expression of leptin receptor and signal transducer and activator of transcription (STAT)-3 while it decreases expression of suppressor of cytokine signaling (SOCS)-3, an endogenous inhibitor of leptin receptor signaling. Thus, glucocorticoid deficiency is proposed to enhance leptin activation of the

Janus kinase (JAK)-STAT signaling pathway in key hypothalamic neurons and thereby potentiate its feeding effects (17).

Effects of leptin on food intake and body weight appear to be mediated in part by the hypothalamic melanocortin (MC) system (18). MCs (including α-melanocyte–stimulating hormone [α-MSH]) are cleavage products of proopiomelanocortin (POMC), which is synthesized by neurons in the ARC. These neurons express the long form of the leptin receptor (13), are activated by leptin, and project to other nuclei within the hypothalamus, including the paraventricular nucleus (PVN) (19), where MC3 and MC4 receptors are located (20–22). Central administration of α-MSH (23,24), or its synthetic analog, melanotan II (MTII), dose dependently reduces food intake in rodents (25,26), and endogenous melanocortin signaling is required for normal body weight regulation (18,27,28)

The effects of α-MSH are opposed by agouti-related protein (AgRP), an endogenous antagonist of MC3 and MC4 receptors (29) synthesized in ARC neurons that also synthesize neuropeptide Y. Glucocorticoids are implicated as negative regulators of melanocortin signaling because ADX reduces AgRP expression in the hypothalamus of normal mice and normalizes hypothalamic levels of POMC mRNA and AgRP mRNA in *ob/ob* mice (30). Thus, glucocorticoids may influence energy homeostasis via effects on neural systems downstream of the leptin receptor.

To confirm and extend the finding that central leptin sensitivity is heightened by the absence of glucocorticoids, we first performed a full dose-response curve for third ventricular (i3vt) leptin in ADX rats and sham-operated controls. To determine whether the absence of glucocorticoids also increases the hypophagic effects of a melanocortin agonist, we next performed a dose-response analysis of i3vt MTII on food intake in ADX rats and controls. In a separate group of rats, we also determined whether endogenous glucocorticoids promote the orexigenic actions of central AgRP. In a final set of studies, we examined the influence of glucocorticoids on the expression of MC4R mRNA. Because ADX causes a deficiency of circulating hormones in addition to glucocorticoids, in each of these experiments we also tested the ability of glucocorticoid replacement to reverse the effects of ADX.

RESEARCH DESIGN AND METHODS

Male Long-Evans rats weighing between 348 and 471 g were obtained from the breeding colony maintained by the Department of Psychology at the University of Washington or from Harlan (Indianapolis, IN) and housed at the University of Cincinnati. Rats were housed individually in clear, plastic cages with ad libitum access to pelleted rat diet (unless otherwise specified) and water in a temperature-controlled vivarium on a 12:12-h light:dark schedule. After ADX surgery, all animals had access to a 0.9% NaCl solution in addition to their water. The research with animals for all studies presented here was conducted in Association for Assessment and Accreditation of Laboratory Animal Care–approved facilities, conformed to federal National Institutes of Health and U.S. Department of Agriculture regulations, and was conducted with approval of our local Animal Care and Use Committee.

Procedures

i3vt cannulation. Animals were surgically implanted with a 22-gauge (Plastics One, Roanoke, VA) cannula with its tip in the third cerebral ventricle as previously described (31). Rats were anesthetized with ketamine (86 mg/kg i.p.)/xylazine (12.9 mg/kg). Verification of cannula placement was accomplished by i3vt injection of 10 ng of angiotensin II in 1 μl 0.9% saline. Animals that did not drink 5 ml of water within 60 min after this treatment were not included in the experiments.

ADX. A short incision was made in the skin rostral to each kidney. The perirenal fat and prominent adrenal veins were located, and both adrenals were isolated from the surrounding fat capsule and removed. Animals were allowed to recover for at least 10 days before experiments began. Because ADX also removes the mineralocorticoids that are necessary for sodium retention, all ADX animals had access to isotonic (0.9%) saline in addition to tap water. To maintain a balanced design, sham animals also had access to both of these fluids. ADX was verified by measuring plasma corticosterone levels by radioimmunoassay (RIA). Tail blood was sampled after the completion of behavioral testing at 1 h before lights off (100 μl from the tip of the tail) for determination of plasma corticosterone levels. Animals with plasma levels >1 μg/dl were removed from the study.

Corticosterone RIA. Plasma corticosterone levels were determined by RIA using rabbit antiserum raised against corticosterone-21-hemisuccinate (B3-163; Endocrine Sciences, Tarzana, CA). Assay sensitivity was 0.5 μg/dl, and the intra-assay coefficient of variation was between 2 and 5% for all experiments.

Plasma leptin, insulin, and glucose. Glucose was measured using a glucose oxidase method. Insulin was determined by a previously described RIA (32). Leptin values were determined using a commercially available RIA kit (Linco Research, St. Charles, MO).

Experiment 1: Effect of ADX on the feeding response to leptin

Behavioral procedures. Rats (sham, $n = 14$; ADX, $n = 12$) were adapted to a schedule in which food was removed from the cages, and the animals were weighed 3 h before the end of the light cycle. On experimental days, 45 min after food was removed, each animal was injected i3vt with 2 μl of recombinant human leptin (Calbiochem, San Diego, CA) dissolved in artificial cerebrospinal fluid or artificial cerebrospinal fluid alone via a Hamilton syringe. Food was returned 45 min before lights out, and the food hopper was weighed after 24 h. Each animal received four treatments (0, 0.35, 1, and 3.5 μg of leptin), with the order counterbalanced across subjects using a Latin-square procedure. There were at least 3 days with no injections between experimental days.

Experiment 2: Effect of ADX on the response to leptin in corticosterone-replaced rats

Procedures. ADX animals had their saline drinking fluid replaced with a solution of 0.9% saline, 2.7 mg/ml corticosterone, and 0.5% alcohol to keep the corticosterone in solution. Providing ADX rats with this corticosterone solution results in plasma corticosterone levels comparable with those of sham-operated controls (33). Rats had access to this solution for 5 days before any behavioral testing. Food intake was subsequently determined after 24 h for both groups of rats (sham, $n = 12$; ADX, $n = 11$) following i3vt administration of either 3.5 μg of leptin or artificial cerebrospinal fluid, with order of treatments counterbalanced across subjects. Tail blood was sampled after the completion of behavioral testing at 1 h before lights off (100 μl from the tip of the tail) for determination of plasma corticosterone levels.

Experiment 3: Effect of ADX on the feeding response to a melanocortin agonist

Procedures. Surgical procedures and verifications were performed as described for experiment 1. All ADX animals maintained plasma corticosterone levels <1 μg/dl.

Behavioral procedures. Rats (sham, $n = 8$; ADX, $n = 9$) were adapted to a schedule in which food was removed from the cages 4 h before the end of the light cycle. On experimental days, 3 h after food was removed, all animals received an i3vt infusion of 2 μl of either MTII (Phoenix Pharmaceuticals, Mountain View, CA) dissolved in 0.9% saline or saline alone via a Hamilton syringe. Food was returned at the time of lights out, and the food hopper was weighed after 1, 4, and 24 h. Each animal received four doses of MTII (0, 0.1, 0.3, and 1.0 nmol), with the order counterbalanced across subjects using a Latin-square procedure. Animals were allowed at least 3 days without injections between experimental days.

Experiment 4: Effect of ADX on the feeding response to a melanocortin agonist in corticosterone-replaced rats

Procedures. ADX animals had their saline drinking fluid replaced with a corticosterone solution as described in experiment 2. Rats had access to this solution for 5 days before any behavioral testing. On the day before the beginning of behavioral testing, all rats had a 100-μl tail blood sample 1 h before lights off for determination of plasma corticosterone by RIA. Both groups of rats (sham, $n = 8$; ADX, $n = 7$) were then observed for their food intake response to 0.3 nmol of MTII and saline, with order of the conditions counterbalanced across subjects.

Experiment 5: Effect of ADX on the feeding response to AgRP

Procedures. Surgical procedures and verifications were performed as described for experiment 1. All ADX animals maintained plasma corticosterone levels <1 μg/dl.

Behavioral procedures. Rats (sham, $n = 15$; ADX, $n = 16$) were adapted to a schedule in which food was removed from the cages 5 h before the end of

TABLE 1
Food intake (24 h) in rats treated i3vt with leptin

Leptin dose (µg)	Sham	ADX
Vehicle	26.0 ± 1.20	19.2 ± 2.20*
0.35	22.1 ± 1.10	12.3 ± 1.68*
1.0	18.8 ± 1.41	8.7 ± 1.20*
3.5	16.8 ± 1.32	5.32 ± 0.80*

Data are means ± SE. *Significantly different from sham at the same time point and leptin dose.

the light cycle. On experimental days, 1 h after food was removed, all animals received an i3vt infusion of 2 µl of either AgRP (Phoenix Pharmaceuticals) dissolved in 0.9% saline or saline alone via a Hamilton syringe. Food was returned immediately after injection, and the food hopper was weighed after 4, 24, and 48 h. Each animal received three doses of AgRP (0, 0.1, and 1.0 nmol), with the order counterbalanced across subjects using a Latin-square procedure. Animals were allowed at least 3 days without injections between experimental days.

Experiment 6: Effect of ADX on the response to AgRP in corticosterone-replaced rats
Procedures. ADX animals had their normal saline drinking fluid replaced with a corticosterone solution as described in experiment 2. Rats had access to this solution for 5 days before any behavioral testing. The day before the beginning of behavioral testing, all rats had a 100-µl tail blood sample taken 1 h before lights off for determination of plasma corticosterone by RIA. Both groups of rats (sham, $n = 12$; ADX, $n = 14$) were then observed for their food intake response to 0.1 nmol of AgRP and saline, with order of the conditions counterbalanced across subjects.

Experiment 7: Effect of ADX and corticosterone replacement on hypothalamic MC4 receptor expression
Procedures. Half of a group of ADX animals had their corticosterone solution replaced with a 0.9% saline solution, so that there were three groups of rats for PCR analysis: sham ($n = 12$), ADX ($n = 7$), and ADX + CORT ($n = 7$). All animals were killed 1 h before lights out by brief exposure to CO_2 followed by rapid decapitation and removal of fresh brain tissue. Blood samples were also collected for the later determination of plasma leptin, insulin, and glucose.

RNA isolation and cDNA synthesis. Brains were rapidly removed after decapitation and placed in RNA Later (Ambion, Austin, TX). Hypothalami were dissected, and total RNA was extracted using TRI Reagent (Molecular Research Center, Cincinnati, OH). The Superscript first-strand synthesis system (Invitrogen, Carlsbad, CA) was used to synthesize cDNA from 5 µg of total RNA.

Semiquantitative expression of MC4R mRNA. A primer and probe set was designed to amplify a portion of the rat MC4R gene (accession U67863) using MGB Eclipse Design Software 3.0 from Epoch Biosciences (available at www.epochbio.com). Primer sequences from 5′ to 3′ were CAGTGTTTCTT TCCATGTTAT and CTGTGGGGTGATGCAAT. A FAM-labeled probe composed of the sequence (5′ to 3′) TTGTTCTTTCTGTGGT was used for detection of the PCR product. The probe also included a MGB and the Eclipse dark quencher. A standard curve ($R^2 = 0.99$) was calculated using a dilution series spanning four orders of magnitude and the efficiency (E = 95%) of the reaction calculated from the slope of the plot threshold cycle (C_T) versus log starting concentration. Samples were run in triplicate and normalized to constitutively expressed ribosomal protein L32.

Statistics. Food intake data for experiment 1 were analyzed using a repeated measures two-way ANOVA, and food intake data for experiments 3 and 5 were analyzed using three-way (surgery × drug × time point) repeated-measures ANOVA. Experiments 2 and 7 were analyzed using one-way ANOVAs, and experiments 4, 6, and 7 were analyzed using two-way ANOVAs. Plasma values in experiment 7 were analyzed using one-way ANOVA. All pairwise comparisons of mean differences were conducted using Tukey honestly significant difference post hoc comparisons. Differences between group means were considered statistically significant if $P < 0.05$.

RESULTS

Experiment 1. There was a significant main effect of leptin dose on food intake at 24 h in both ADX and sham-operated rats ($P < 0.05$ in both cases). Leptin administration reduced food intake to a greater extent in ADX animals than sham animals at all doses tested ($P < 0.05$ in each case) at 24 h (Table 1). When assessed as the
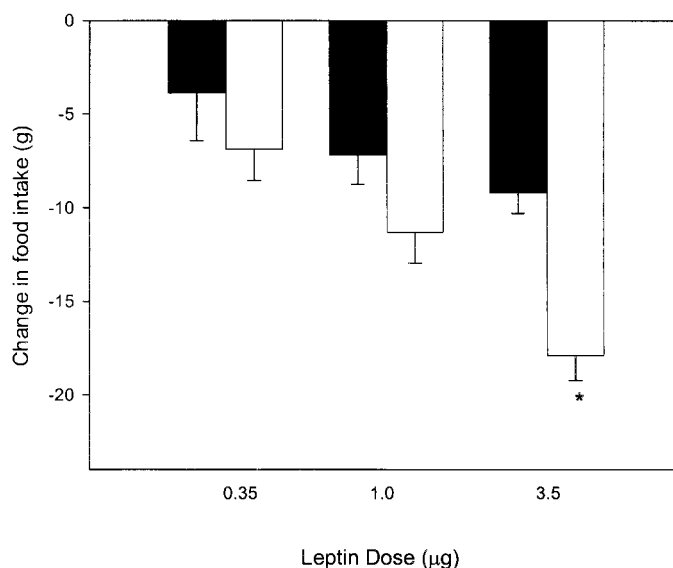

FIG. 1. Mean ± SE 24-h food intake in rats that received either bilateral ADX or sham surgery and were treated i3vt with leptin or vehicle. Data are represented as absolute change from when animals were injected with vehicle. ■, sham; □, ADX. *Significantly different from paired sham ($P < 0.05$).

absolute change in food intake from when the animals were injected with vehicle, leptin only reduced food intake to a significantly greater extent in ADX compared with sham animals at the 3.5-µg dose ($P < 0.05$) (Fig. 1).

Experiment 2. Plasma corticosterone concentrations were significantly reduced (to undetectable levels) in ADX compared with sham animals (6.4 ± 1.5 µg/dl) when assessed 1 h before lights out ($P < 0.05$). ADX animals that had corticosterone replaced in their drinking water (ADX + CORT) had plasma corticosterone concentrations similar to sham animals (5.8 ± 1.1 µg/dl) ($P > 0.05$). Expressed as a percentage of each animal's own vehicle intake, administration of 3.5 µg leptin significantly reduced food intake in both sham and ADX + CORT animals ($P < 0.05$ in both cases). Leptin reduced food intake to a similar extent in sham and ADX + CORT animals ($P > 0.05$). Relative to vehicle-treated controls, i3vt leptin reduced intake to a greater extent in ADX animals compared with both sham and ADX + CORT animals ($P < 0.05$ in both cases) (Fig. 2).

Experiment 3. There was a significant effect of MTII dose on food intake at 1, 4, and 24 h in both sham and ADX animals ($P < 0.05$ in all cases). MTII reduced food intake to a greater extent in ADX animals compared with sham animals at all doses of MTII at 4 h ($P < 0.05$ in all cases), at 0.1 and 0.3 nmol at 1 h, and at 1.0 nmol at 24 h ($P < 0.05$ in all cases), although there were no significant interactions (Table 2). When assessed as the absolute change in food intake from when the animals were injected with vehicle, the 4-h intake was significantly reduced in ADX compared with sham animals at the 0.1 and 0.3 nmol doses ($P < 0.05$) but not at the 1.0 nmol dose ($P > 0.05$) (Fig. 3). MTII significantly reduced body weight at all doses compared with vehicle injections in both sham and ADX animals ($P < 0.05$ in all cases), but there were no differences in this reduction between surgery groups ($P > 0.05$) (e.g., at the 0.3-nmol MTII dose, 24-h body weight change
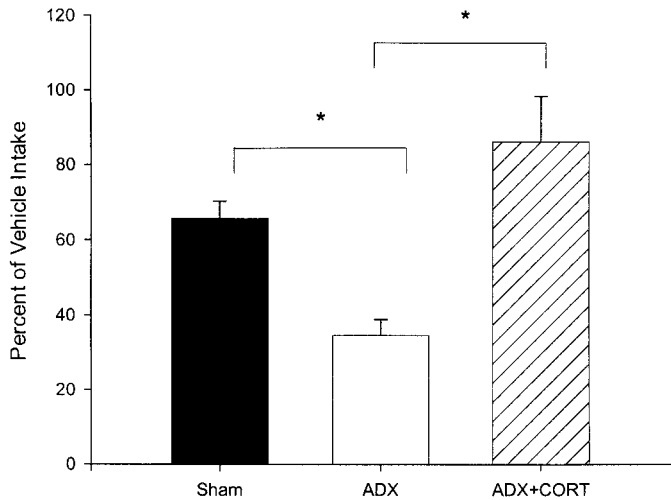
FIG. 2. Mean ± SE 24-h food intake in rats that received either ADX or sham surgery or that received ADX surgery and 5 consecutive days of corticosterone in the drinking water (2.7 mg/ml) and were treated i3vt with 3.5 µg leptin or vehicle. Data are represented as the percentage of each group's mean vehicle intake. ■, sham; □, ADX; ▨, ADX + CORT. *Significantly different from ADX ($P < 0.05$).
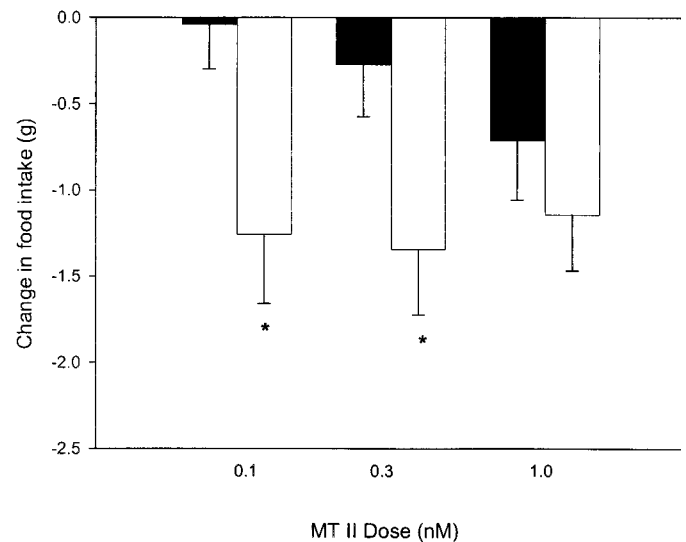

FIG. 3. Mean ± SE 4-h food intake in rats that received either ADX or sham surgery and were treated i3vt with MTII or vehicle. Data are represented as absolute change from when animals were injected with vehicle. ■, sham; □, ADX. *Significantly different from paired sham ($P < 0.05$).

in sham was -25.69 ± 2.34 g and in ADX was -21.49 ± 2.47).

Experiment 4. Plasma corticosterone concentrations were significantly reduced (to undetectable levels) in ADX compared with sham animals (6.46 ± 0.79 µg/dl) when assessed at 1 h before lights out ($P < 0.05$). ADX + CORT animals had plasma corticosterone concentrations similar to sham animals (7.6 ± 0.85 µg/dl) ($P > 0.05$). Administration of 0.3 nmol MTII reduced food intake comparably in both sham and ADX + CORT animals ($P < 0.05$ in both cases). MTII significantly reduced food intake in both groups ($P < 0.05$) and to a similar extent in sham and ADX + CORT animals ($P > 0.05$) (Fig. 4).

Experiment 5. There was a significant effect of AgRP at both 0.1 and 1.0 nmol on food intake at 24 and 48 h in sham animals ($P < 0.05$ in all cases) but not at 4 h ($P > 0.05$). In ADX animals, however, AgRP was ineffective in increasing food intake at 0.1 and 1.0 nmol at all time points measured ($P > 0.05$) (Fig. 5 shows 24-h data, and Table 3 shows the other time points). AgRP significantly increased body weight at both doses in sham animals ($P < 0.05$ in both cases) but not in ADX animals ($P > 0.05$ in both cases) (e.g., at the 0.1-nmol AgRP dose, 24-h body weight change in sham was 20.40 ± 4.7 g and in ADX was 1.76 ± 4.7 g).

Experiment 6. Plasma corticosterone concentrations were significantly reduced (to undetectable levels) in ADX compared with sham animals (10.7 ± 1.7 µg/dl) when assessed at 1h before lights out ($P < 0.05$). ADX + CORT animals had plasma corticosterone concentrations similar

to sham animals (6.3 ± 2.8 µg/dl) ($P > 0.05$). Administration of 0.1 nmol AgRP significantly increased food intake in both sham and ADX + CORT animals ($P < 0.05$ in both cases). AgRP increased food intake to a similar extent in sham and ADX + CORT animals ($P > 0.05$) (Fig. 6).

Experiment 7. Mean ± SE expression of MC4 receptor relative to L32 control was similar across sham (52.43 ± 5.79), ADX (65.42 ± 10.90), and ADX + CORT (44.17 ± 3.35) groups ($P > 0.05$). ADX animals had significantly lower plasma leptin, insulin, and glucose compared with sham animals (leptin: 8.87 ± 1.14 vs. 1.98 ± 0.36 ng/ml; insulin: 428.30 ± 44.71 vs. 174.67 ± 21.05 pmol/l; glucose: 10.19 ± 0.42 vs. 8.23 ± 0.31 mmol/l; for sham and ADX, respectively) ($P < 0.05$ in all cases).

DISCUSSION

ADX has long been known to have antiobesity effects in rodents, and more recently, these effects have been attributed to a sensitization of leptin action (2). Consistent with previous findings, we report that ADX reduced spontaneous food intake and enhanced the ability of i3vt leptin to reduce food intake and that replacement of glucocorticoids reversed this effect (17). The removal of glucocorticoids had a similar effect to augment the anorectic action of i3vt MTII, and this effect was reversed by glucocorticoid replacement. Conversely, the orexigenic action of i3vt AgRP was completely absent in ADX animals compared with sham animals, and this effect was again reversed by

TABLE 2
Food intake (1, 4, and 24 h) in rats treated i3vt with MTII

MTII dose (nmol)	Sham (1 h)	ADX (1 h)	Sham (4 h)	ADX (4 h)	Sham (24 h)	ADX (24 h)
Vehicle	3.04 ± 0.33	2.36 ± 0.31	5.88 ± 0.46	4.98 ± 0.44	24.00 ± 1.49	22.41 ± 1.41
0.1	1.84 ± 0.36	$0.33 \pm 0.38*$	3.09 ± 0.51	$0.56 \pm 0.52*$	15.88 ± 1.63	8.91 ± 1.68
0.3	1.94 ± 0.34	$0.39 \pm 0.38*$	2.81 ± 0.51	$0.48 \pm 0.52*$	9.54 ± 1.61	5.49 ± 1.70
1.0	0.54 ± 0.33	0.66 ± 0.35	2.33 ± 0.47	1.21 ± 0.44	11.4 ± 1.45	$4.89 \pm 1.41*$

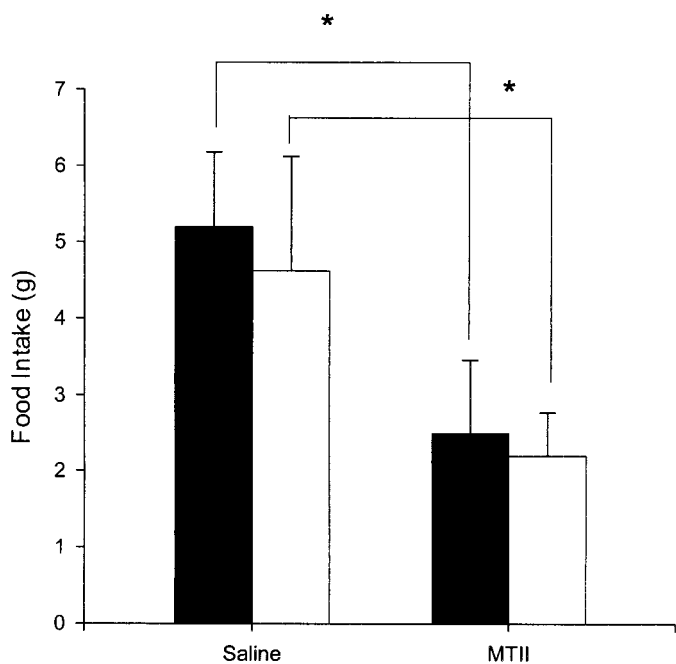Data are means ± SE. *Significantly different from sham at the same time point and MTII dose.

FIG. 4. Mean ± SE 4-h food intake in rats that received either sham surgery or that received ADX surgery and 5 consecutive days of corticosterone in drinking water (2.7 mg/ml) and were treated i3vt with 0.3 nmol MTII or vehicle. ■, sham; □, ADX + CORT. *Significantly different from saline-treated rats ($P < 0.05$).

glucocorticoid replacement. Finally, there was a nonsignificant trend for MC4R mRNA to be upregulated in ADX compared with sham animals. These data support other studies, suggesting an interaction between glucocorticoids and the melanocortin system. For example, some (but not all) studies (30,34,35) have reported that ADX decreases hypothalamic POMC and AgRP mRNA. Collectively, these data suggest that the opposing actions of glucocorticoids on leptin action in the brain are likely to involve effects mediated, at least in part, downstream of the leptin receptor and via interactions with the melanocortin sys-
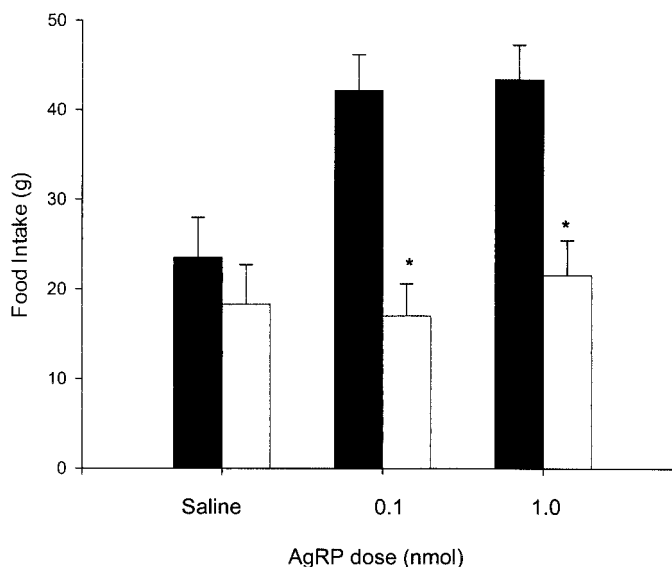
TABLE 3
Food intake (4 and 48 h) in rats treated i3vt with AgRP

AgRP dose (nmol)	Sham (4 h)	ADX (4 h)	Sham (48 h)	ADX (48 h)
Vehicle	2.85 ± 1.25	2.78 ± 1.28	58.05 ± 6.91	42.45 ± 4.78
0.1	8.78 ± 1.12	3.80 ± 1.02*	77.89 ± 6.14	38.47 ± 5.32*
1.0	8.65 ± 1.44	3.68 ± 1.52*	81.53 ± 7.99	50.83 ± 6.92*

Data are means ± SE. *Significantly different from sham at the same time point and AgRP dose.

tem and its targets to influence food intake and body weight.

Consistent with previous work (2), the present data suggest that endogenous glucocorticoids render an individual less sensitive to the anorectic effects of leptin. One proposed explanation for this effect is that glucocorticoids inhibit leptin action by altering expression of leptin receptor and/or downstream signaling molecules such as STAT-3 and SOCS-3 (17). The increase in leptin receptor expression could be due in part to the decrease in plasma leptin observed in ADX rats. Recent data also indicate an important role for phosphatidylinositol 3-kinase in leptin's ability to reduce food intake, opening up another intracellular signaling pathway that glucocorticoids may act upon to alter the response to leptin (36).

A key finding of the current study is that regardless of the effects that glucocorticoids may have on the cellular response to leptin receptor activation, they also may reduce leptin sensitivity by acting downstream at the level of the central nervous system melanocortin system. One possible mechanism to explain these effects is to propose that glucocorticoids reduce either the abundance or responsiveness of MC4 receptors, which are concentrated in key hypothalamic areas such as the PVN (37). The present data suggest that MC4 receptor mRNA may be upregulated

FIG. 5. Mean ± SE 24-h food intake in rats that received either ADX or sham surgery and were treated i3vt with AgRP or vehicle. ■, sham; □, ADX. *Significantly different from paired sham ($P < 0.05$).
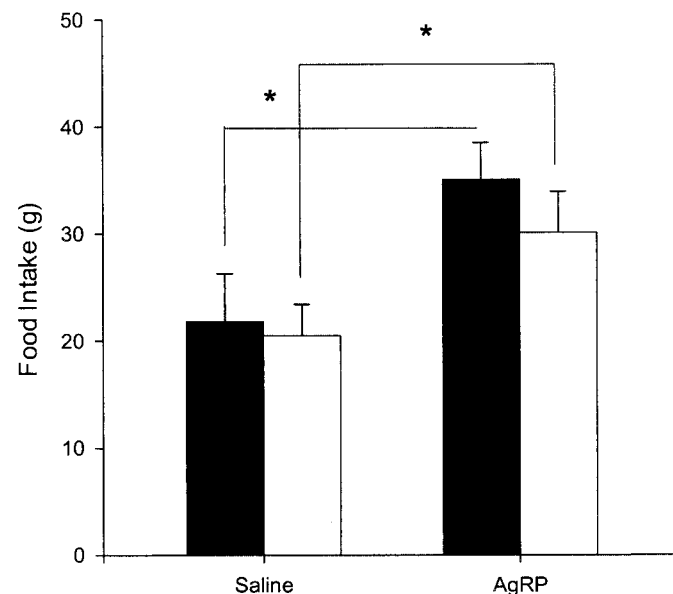

FIG. 6. Mean ± SE 24-h food intake in rats that received either sham surgery or that received ADX surgery and 5 consecutive days of corticosterone in drinking water (2.7 mg/ml) and were treated i3vt with 0.1 nmol AgRP or vehicle. ■, sham; □, ADX + CORT. *Significantly different from saline-treated rats ($P < 0.05$).

in ADX animals compared with sham animals, which would account for the increased sensitivity to MTII in ADX animals. MC4 receptor activity could also be upregulated without significant changes in gene expression. Alternatively, glucocorticoids could alter the central nervous system melanocortin system via action on ARC neurons to alter the release of either α-MSH or AgRP. The levels of endogenous ligands for the MC4 receptor can influence the ability of exogenous compounds to bind and alter the activity of MC4 receptor signaling. Although the finding is controversial, ADX has been demonstrated to increase POMC mRNA (34), suggesting that glucocorticoids normally inhibit POMC tone. Others, however, have reported (38) that POMC mRNA in the medial basal hypothalamus is decreased after ADX, which would be consistent with a compensatory upregulation of MC4 receptor activity. Consistent with ADX increasing melanocortin tone, ADX reduces hypothalamic AgRP mRNA without affecting serum leptin concentrations (30), suggesting that glucocorticoids might increase the activity of AgRP neurons. Thus after ADX, less AgRP would be available to occupy MC4 receptors, making exogenous MTII more capable of binding and activating MC4 receptors.

Interestingly, glucocorticoid deficiency had potent effects on the ability of both MTII to reduce food intake and AgRP to increase food intake. Teleologically, such results make sense because both results are likely to bias the animal to consume less food and gain less weight, such as occurs after ADX. However, these results are not easily explained by a single effect of glucocorticoids directly on MC4 receptor expression or activity. Moreover, such results also cannot be the result of simply changing levels of α-MSH or AgRP in the synapse. We would like to offer a potential mechanism for glucocorticoid action that involves influencing the actions of syndecan-3 in the PVN. The syndecans are a family of highly abundant cell-surface heparin sulfate proteoglycans that are able to bind many extracellular peptides (39). Evidence suggests that syndecan-3 acts as a coreceptor for AgRP, normally facilitating the action of AgRP at melanocortin receptors. During fasting, syndecan-3 is predominately in the active membrane-bound form where it can facilitate the actions of AgRP to reduce melanocortin signaling (40). If membrane-bound syndecan-3 is increased by glucocorticoids, its absence could be predicted to increase the actions of MC4 receptor agonists while simultaneously reducing the efficacy of AgRP. At this point, this possibility remains speculation, and future research is needed to address whether glucocorticoids regulate both the expression and membrane status of syndecan-3 within the PVN.

It is not necessarily inconsistent, however, that ADX animals were both more sensitive to MTII and insensitive to AgRP. MC4R knockout mice do not respond to MTII, suggesting that MTII is acting primarily on MC4 receptors rather than on MC3 receptors (41). On the other hand, AgRP is effective in these knockouts, indicating that AgRP does not require the MC4 receptor, and may be acting at the MC3 receptor (41) or via an unidentified mechanism independent of competitive antagonism at the MC4 receptor (42).

Glucocorticoids exert myriad effects on behavior and physiology, and glucocorticoid receptors are widespread throughout the central nervous system and periphery (43). Glucocorticoid receptors are present in regions of the hypothalamus important for the regulation of food intake and body weight, including the ARC, PVN, and lateral hypothalamus (43). Therefore, it is likely that glucocorticoids influence the activity or responsiveness to numerous neuropeptide systems in addition to the leptin-melanocortin system, and as previously reported, insulin and neuropeptide Y (44,45). The current data suggest that the important effects that glucocorticoids exert to alter food intake and energy balance involve significant actions on the melanocortin system. Future studies will need to investigate the role of specific glucocorticoid receptor populations in these effects on food intake and body weight to elucidate the complicated circuitry by which glucocorticoids influence energy balance.

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