

Adrenalectomy Reverses Obese Phenotype and Restores Hypothalamic Melanocortin Tone in Leptin-Deficient *ob/ob* Mice

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In genetically obese leptin-deficient *ob/ob* mice, adrenalectomy reverses or attenuates the obese phenotype. Relative to lean controls, *ob/ob* mice also exhibit decreased hypothalamic proopiomelanocortin (POMC) mRNA and increased hypothalamic agouti-related peptide (AGRP) mRNA and neuropeptide Y (NPY) mRNA. It has been hypothesized that this profile of hypothalamic gene expression contributes to the obese phenotype caused by leptin deficiency. To assess if reversal of obese phenotype by adrenalectomy entails normalization of hypothalamic gene expression, male wild-type and *ob/ob* mice were adrenalectomized (with saline supplementation) or sham adrenalectomized at 2 months of age. Mice were sacrificed 2 weeks after adrenalectomy, during which time food intake and body weight were monitored daily. After sacrifice, hypothalamic gene expression was assessed by Northern blot analysis as well as in situ hybridization. In wild-type mice, adrenalectomy significantly decreased AGRP mRNA but did not significantly influence POMC or NPY mRNA. In *ob/ob* mice, adrenalectomy reduced the levels of plasma glucose, serum insulin and corticosterone, and food intake toward or below wild-type levels, and it restored hypothalamic POMC and AGRP mRNA but not NPY mRNA to wild-type levels. These studies suggest that adrenalectomy reverses or attenuates the obese phenotype in *ob/ob* mice, in part by restoring hypothalamic melanocortin tone toward wild-type levels. These studies also demonstrate that factors other than leptin may play a major role in regulating hypothalamic melanocortin function. *Diabetes* 49:1917–1923, 2000

It has long been appreciated that adrenalectomy prevents and may even reverse the glucose intolerance and obese phenotype of *ob/ob* mice (1–6) as well as the obese phenotype of many other forms of obesity (7,8). Because obesity in *ob/ob* mice is associated with greatly elevated glucocorticoid levels and obesity in adrenalectomized *ob/ob* mice is restored with glucocorticoid injections (7), adrenalectomy appears to exert its antiobesity

effect by reducing glucocorticoid levels, but the mechanism through which glucocorticoids are exerting this effect remains unknown. The obesity of *ob/ob* mice is also associated with a characteristic profile of hypothalamic gene expression, relative to wild-type controls, including elevated hypothalamic neuropeptide Y (NPY) mRNA (9–12), reduced hypothalamic proopiomelanocortin (POMC) mRNA (12–14), and elevated agouti-related peptide (AGRP) mRNA levels (15,16). This profile of hypothalamic gene expression is thought to play a key role in causing the obese phenotype of *ob/ob* mice, because a targeted mutation of the NPY gene reduces the obese phenotype of *ob/ob* mice (17), and accumulating evidence strongly suggests that the melanocortin system, enhanced by hypothalamic POMC and reduced by hypothalamic AGRP, plays a critical role in regulating body weight and metabolic status (18). These data suggest that adrenalectomy might reverse the obese phenotype of *ob/ob* mice by reversing the profile of hypothalamic gene expression in these mice or, alternatively, that adrenalectomy reverses obese phenotypes through a mechanism independent of hypothalamic gene expression. To test these hypotheses, the present study examined the effect of adrenalectomy on hypothalamic gene expression in *ob/ob* mice.

RESEARCH DESIGN AND METHODS

Animals and treatment. Male *ob/ob* mice (C57Bl/6J background) and their wild-type littermates were obtained at 2 months of age from the Jackson Laboratory (Bar Harbor, ME). Mice were individually housed with free access to food and water under a 12:12 h light-dark cycle (lights on at 0700). All studies had been approved by the appropriate institutional animal review board. One week after arrival, mice ($n = 136$) were either adrenalectomized or underwent sham adrenalectomy. Adrenalectomy was performed by bilateral flank incision under anesthesia (2,2,2-tribromoethanol, 0.015–0.017 ml/g body wt, i.p.). Sham adrenalectomy entailed the same procedure as adrenalectomy, except that the adrenal glands were grasped but not removed. At the time of adrenalectomy, mice were given dexamethasone (45 mg/kg body wt, i.p.), and drinking water was replaced with saline (0.9% NaCl). Body weight and food intake were measured daily for 2 weeks after surgery; mice were then killed in order following a balanced design. The mice were killed by decapitation toward the end of the light period (between 1700 and 1900 h) after a brief exposure to carbon dioxide; we have verified in pilot studies that this method of killing does not increase plasma corticosterone above levels observed without carbon dioxide exposure. Adrenalectomy was verified in each individual by visual inspection to ensure that no visible traces of adrenal glands were observed (see below). In two separate studies, hypothalamic mRNAs were assessed either by Northern blot analysis or by in situ hybridization. Brains for Northern blot analysis were quickly removed, and the hypothalamus was dissected out, frozen on dry ice, and stored at -70°C until use. Brains for in situ hybridization were similarly removed but blocked and frozen on slides with dry ice and stored at -70°C until use. The anterior lobe of the pituitary was also removed and frozen on dry ice and stored at -70°C until use. Epididymal white adipose tissue was also removed, frozen on dry ice, and stored at -70°C until use.

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AGRP, Agouti-related peptide; ANOVA, analysis of variance; NPY, neuropeptide Y; POMC, proopiomelanocortin.

Blood chemistry. Glucose was measured by a LifeScan One-Touch II glucose meter (Johnson & Johnson, Mountain View, CA); insulin and leptin were assayed by an enzyme-linked immunosorbent assay with commercial kits (Crystal Chemicals, Chicago) and corticosterone was assayed by a radioimmunoassay with a commercial kit (ICN Pharmaceuticals, Costa Mesa, CA).

Northern blot analysis. Northern blot analysis was carried out as previously described (12,16,19,20). Briefly, total RNA was extracted in TRIzol (Gibco, Gaithersburg, MD) and 3 μ g of total RNA from hypothalamus, estimated by spectrophotometer, was subjected to Northern blot analysis to measure POMC, NPY, and AGRP mRNA. Of total RNA, 5 μ g from adipose tissue or anterior pituitary were also subjected to Northern blot analysis to measure leptin or POMC mRNA levels, respectively. Northern blot analysis was performed by using single-stranded internally labeled DNA probes as described previously (12,16,19,20). To monitor RNA loading, membranes were reprobated and hybridized with 32 P-labeled probe encoding 18S ribosomal RNA. The total integrated densities of hybridization signals were determined by phosphorimager (Storm 860; Molecular Dynamics, Sunnyvale, CA).

In situ hybridization histochemistry. In situ hybridization was carried out as previously described (12,16). Coronal sections of 10- μ m thickness were cut through the mouse hypothalamus. The sections were fixed in 3% paraformaldehyde in 0.1 mmol/l phosphate buffer (pH = 7.0) containing 0.1% diethylpyrocarbonate (DEPC) that was dehydrated and stored at -20°C until use. Sections were sorted on the basis of histology to ensure that the anterior-posterior levels were matched between different mouse brains. Prehybridization and hybridization with the single-stranded internally labeled DNA were carried out as previously described (12,20). The total integrated densities of hybridization signals were determined by computerized densitometric scanning (MCID system; St. Catherine's, Ontario, Canada).

Statistical analysis. Statistical analysis entailed a two-way (wild-type-*ob/ob* \times adrenalectomy-sham-operated) analysis of variance (ANOVA) followed, when indicated by appropriate *P* values ($P < 0.05$), by Tukey-Kramer post-hoc test using the JMP statistical package implemented on the Macintosh operating system. $P < 0.05$ was considered significant.

RESULTS

The present studies involved two separate sets of mice treated identically (wild-type-*ob/ob* \times adrenalectomy-sham operation, killed 2 weeks after surgery); one set of mice was used to measure hypothalamic mRNA by Northern blot analysis, and one set was used to measure hypothalamic mRNA by in situ hybridization. The effects of genotype and adrenalectomy were essentially identical in each study, so the results for body weight, food intake, and blood chemistry are reported only for the first study.

Body weight and food intake. A transient decrease followed by complete recovery of body weight 2 weeks after surgery was observed in both sham-operated (net change in body weight 2 weeks after surgery -0.1 ± 0.5 g; $n = 8$) and adrenalectomized (-0.3 ± 0.3 g; $n = 14$) wild-type mice (Fig. 1A). The effect of adrenalectomy on body weight change was not significant in wild-type mice ($P > 0.05$, Tukey-Kramer). On the other hand, sham-operated *ob/ob* mice exhibited a transient decrease in body weight but had recovered their body weight by 1 week after surgery. By 2 weeks after surgery, they had gained significant weight compared with their preoperation weights (2.5 ± 0.6 g; $n = 10$) $P < 0.05$, Tukey-Kramer) (Fig. 1A). In contrast, adrenalectomized *ob/ob* mice did not recover body weight lost after surgery, and 2 weeks after surgery, they had lost significant weight compared with preoperation weights (-4.3 ± 1.8 g; $n = 9$; $P < 0.05$, Tukey-Kramer) (Fig. 1A).

Hyperphagia in *ob/ob* mice, as indicated by average daily food intake 1 week before killing, was completely reversed by adrenalectomy (Fig. 1B). Thus, whereas sham-operated *ob/ob* mice consumed more food (6.9 ± 0.6 g/day) than sham-operated (4.9 ± 0.2 g/day) or adrenalectomized (5.3 ± 0.2 g/day) wild-type mice ($P < 0.05$, Tukey-Kramer), adrenalectomized *ob/ob* mice consumed significantly less food (4.1 ± 0.5 g/day) than these groups ($P < 0.05$, Tukey-Kramer). The effect of adrenalectomy on food intake was not significant in wild-type mice ($P > 0.05$, Tukey-Kramer).

Blood chemistry. As with hyperphagia, the hyperglycemia of *ob/ob* mice was completely corrected by adrenalectomy (Fig. 2A). Thus, blood glucose was significantly elevated in sham-operated *ob/ob* mice (14.8 ± 1.7 mmol/l) compared with either sham-operated (8.4 ± 0.7 mmol/l) or adrenalectomized (8.3 ± 0.3 mmol/l) wild-type mice ($P < 0.05$, Tukey-Kramer). Similar to the effect on food intake, adrenalectomy reduced blood glucose (6.0 ± 0.4 mmol/l) in *ob/ob* mice to below levels observed in wild-type mice, thereby leading to an over-correction of the hyperglycemia that usually characterizes *ob/ob* mice. There was no effect of adrenalectomy on blood glucose levels in wild-type mice ($P > 0.05$, Tukey-Kramer).

Adrenalectomy largely, but not completely, corrected the hyperinsulinemia characteristic of *ob/ob* mice (Fig. 2B). Thus, serum insulin was extremely elevated in sham-operated *ob/ob* mice ($20,388 \pm 3,134$ pmol/l) compared with sham-operated (469 ± 134 pmol/l) or adrenalectomized (205 ± 36 pmol/l) wild-type mice ($P < 0.05$, Tukey-Kramer). Adrenalectomy significantly reduced serum insulin in *ob/ob* mice ($1,756 \pm 140$ pmol/l), but in contrast to other variables, the correction of serum insulin by adrenalectomy was not complete, and serum insulin remained elevated compared with the wild-type mice (Tukey-Kramer) (Fig. 2B). Furthermore, in contrast with

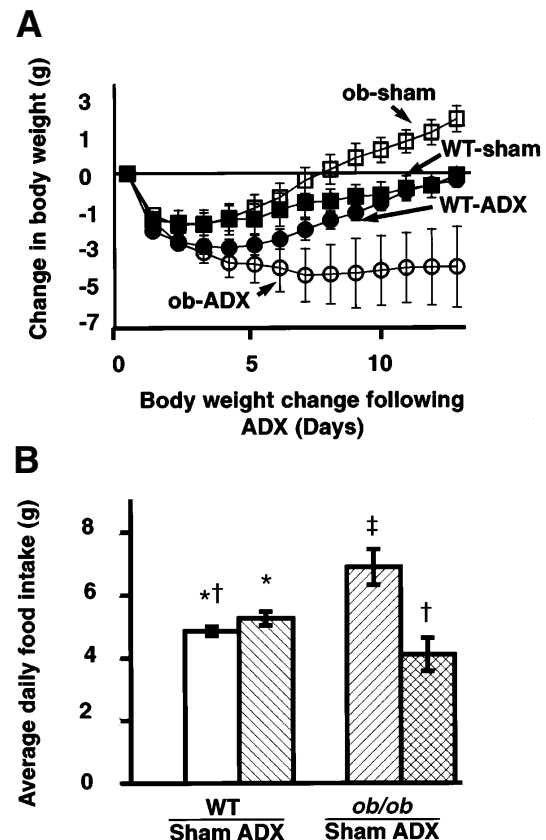


FIG. 1. Effect of adrenalectomy (ADX) or sham-adrenalectomy (sham) on body weight (A) and food intake (B) in wild-type (WT) or *ob/ob* (*ob*) mice. Data are expressed as means \pm SE. Groups with different symbols are statistically different ($P < 0.05$) by ANOVA followed by Tukey-Kramer post hoc comparisons.

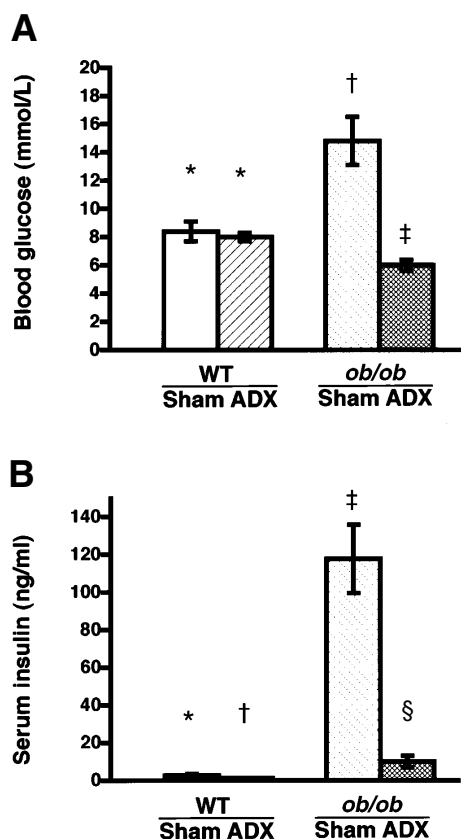


FIG. 2. Effect of adrenalectomy (ADX) or sham-adrenalectomy (sham) on blood glucose (A) and serum insulin (B) in wild-type (WT) or *ob/ob* mice. Data are expressed as means \pm SE. Groups with different symbols are statistically different ($P < 0.05$) by ANOVA followed by Tukey-Kramer post hoc comparisons.

blood glucose, adrenalectomy also significantly reduced serum insulin in wild-type mice ($P < 0.05$, Tukey-Kramer).

Adrenalectomy completely reversed the elevated plasma corticosterone characteristic of *ob/ob* mice. Thus, plasma corticosterone levels were higher in sham-operated *ob/ob* mice ($2.1 \pm 0.3 \mu\text{g/dl}$) versus sham-operated ($0.7 \pm 0.2 \mu\text{g/dl}$) or adrenalectomized ($0.3 \pm 0.1 \text{ ng/ml}$) wild-type mice. However, adrenalectomy reduced plasma corticosterone in *ob/ob* mice ($0.2 \pm 0.05 \mu\text{g/dl}$) to levels statistically indistinguishable from levels exhibited by adrenalectomized wild-type mice ($P > 0.05$, Tukey-Kramer).

Because adrenalectomy reduced serum corticosterone levels by only $\sim 50\%$ in wild-type mice, which is consistent with previous studies (21), it was of interest to ensure that the adrenalectomy procedure resulted in biologically significant effects. Because adrenalectomy in rats (22) and mice (23) leads to elevated expression of POMC mRNA in the anterior pituitary, we determined levels of POMC mRNA from the anterior pituitary of mice in the present study. Adrenalectomy resulted in a fivefold elevation of pituitary POMC mRNA in wild-type mice (expressed as a percentage of wild-type sham-operated mice: $518 \pm 100\%$ in adrenalectomized vs. $100 \pm 38\%$ in sham-operated wild-type mice, $P < 0.05$, Tukey-Kramer) and a threefold elevation of pituitary POMC mRNA in *ob/ob* mice ($772 \pm 105\%$ in adrenalectomized vs. $212 \pm 86\%$ sham-operated *ob/ob* mice, $P < 0.05$, Tukey-Kramer). Although there was a consistent trend toward an elevation of pituitary

POMC mRNA in *ob/ob* compared with wild-type mice, which was significant by Student's *t* test in both sham-operated and adrenalectomized mice, these differences did not achieve significance by the more appropriate (and stringent) Tukey-Kramer post-hoc test ($P > 0.05$).

Serum leptin was not significantly influenced by adrenalectomy in wild-type mice in the first study ($0.9 \pm 0.2 \text{ ng/ml}$ in sham-operated mice vs. $0.6 \pm 0.2 \text{ ng/ml}$ in adrenalectomized mice). Because the trend to reduce leptin in this study was not significant, we also examined serum leptin used for in situ hybridization analysis in the second study. In this study also, the effect of adrenalectomy on serum leptin was not significant, but in this study the trend was in the opposite direction ($0.6 \pm 0.2 \text{ ng/ml}$ in sham-operated mice vs. $1.4 \pm 0.6 \text{ ng/ml}$ in adrenalectomized mice). Serum leptin in *ob/ob* mice was below the level of detectability in this assay.

Hypothalamic gene expression levels by Northern blot analysis. By Northern blot analysis, adrenalectomy completely corrected the low hypothalamic POMC mRNA characteristic of *ob/ob* mice (Fig. 3A). Therefore, hypothalamic POMC mRNA in sham-operated *ob/ob* mice was only $37 \pm 12\%$ of the level observed in sham-operated wild-type mice ($100 \pm 27\%$, $P < 0.05$, Tukey-Kramer), whereas adrenalectomy led to a fivefold elevation of hypothalamic POMC mRNA in *ob/ob* mice to $164 \pm 66\%$ the level observed in sham-operated wild-type mice ($P < 0.05$, Tukey-Kramer). Adrenalectomy did not significantly influence hypothalamic POMC mRNA in wild-type mice ($P > 0.05$, Tukey-Kramer).

As with food intake, blood glucose, and other parameters, adrenalectomy actually overcorrected the elevated hypothalamic AGRP mRNA characteristic of *ob/ob* mice (Fig. 3B). Thus, hypothalamic AGRP mRNA in sham-operated *ob/ob* mice was $295 \pm 109\%$ of the level observed in sham-operated wild-type mice ($100 \pm 42\%$, $P < 0.05$), whereas adrenalectomy of *ob/ob* mice led to a reduction of AGRP mRNA to significantly below sham-operated wild-type levels ($49 \pm 15\%$, $P < 0.05$). Interestingly, adrenalectomy also significantly reduced hypothalamic AGRP mRNA in wild-type mice to significantly below the levels observed in sham-operated wild-type mice ($18 \pm 6\%$, $P < 0.05$, Tukey-Kramer).

As expected, genotype and adrenalectomy influenced hypothalamic NPY mRNA similarly to AGRP (Fig. 3C). Consequently, hypothalamic NPY mRNA in sham-operated *ob/ob* mice was significantly higher ($305 \pm 86\%$, $P < 0.05$, Tukey-Kramer) than both the levels observed in sham-operated wild-type mice ($100 \pm 28\%$), and after adrenalectomy, the observed levels of hypothalamic NPY mRNA in *ob/ob* mice were no longer significantly higher ($183 \pm 48\%$) than levels observed in sham-operated wild-type mice ($P > 0.05$, Tukey-Kramer). On the other hand, levels of hypothalamic NPY mRNA in adrenalectomized *ob/ob* mice were not significantly lower than levels observed in sham-operated *ob/ob* mice. Moreover, these NPY mRNA levels were significantly higher than levels observed in adrenalectomized wild-type mice (Fig. 3C).

Hypothalamic gene expression levels by in situ hybridization. A second set of mice was treated identically to those described above, and brains were taken to assess hypothalamic gene expression by in situ hybridization. The correction by adrenalectomy of body weight, food intake, and blood chemistry were essentially identical to the effects observed in the first study (data not shown). Similarly, the

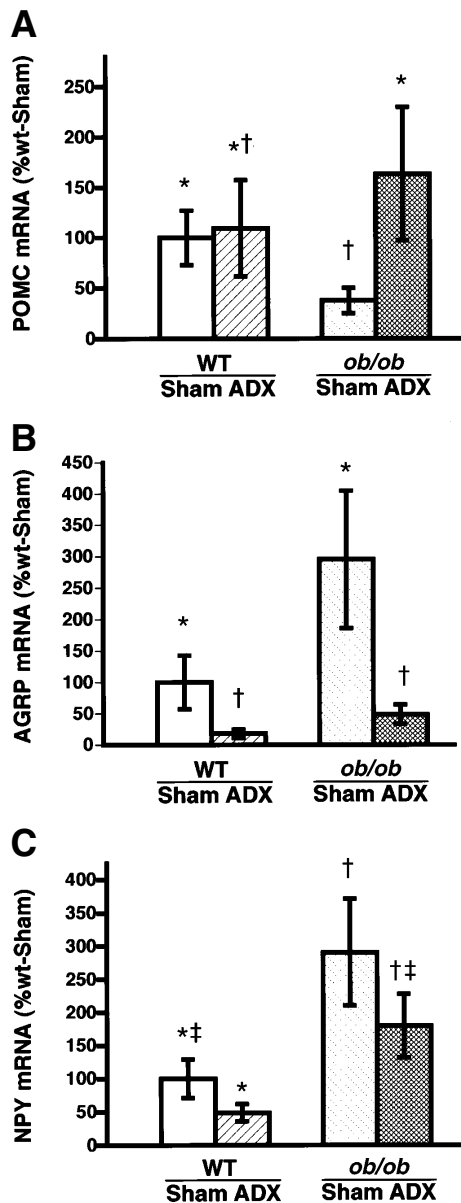


FIG. 3. Effect of adrenalectomy (ADX) or sham-adrenalectomy (sham) on hypothalamic POMC mRNA (A), AGRP mRNA (B), and NPY mRNA (C) in wild-type (WT) or *ob/ob* mice. Data are expressed as a mean percentage \pm SE of wild-type sham-adrenalectomized levels. Groups with different symbols are statistically different ($P < 0.05$) by ANOVA followed by Tukey-Kramer post hoc comparisons.

results of the in situ hybridization closely reflected the results of the Northern blot analysis. Thus, in situ hybridization adrenalectomy of *ob/ob* mice decreased AGRP mRNA by 80% (and completely corrected the expression of this mRNA to wild-type levels) (Table 1), increased POMC mRNA by 50%, and decreased NPY mRNA by 30%, although these latter effects did not achieve statistical significance by in situ hybridization (Table 1).

DISCUSSION

Although the hyperphagia, insulin resistance, and glucose intolerance of *ob/ob* mice are due to the absence of the peptide leptin (24,25), it is remarkable that these obese pheno-

TABLE 1

Effects of adrenalectomy and leptin deficiency on hypothalamic gene expression as assessed by in situ hybridization

	AGRP (%)	POMC (%)	NPY (%)
Wild-type/sham-operated	100 \pm 45*	100 \pm 21*	100 \pm 40*
Wild-type/adrenalectomized	43 \pm 9†§	97 \pm 18*	32 \pm 11†
<i>ob/ob</i> -sham-operated	436 \pm 136‡	26 \pm 4†	288 \pm 105*
<i>ob/ob</i> -adrenalectomized	56 \pm 29*§	41 \pm 6†	187 \pm 43*

Data are expressed as means \pm SE ($n = 6$ per group). Groups with different symbols are statistically different ($P < 0.05$) by ANOVA followed by Tukey-Kramer post hoc comparisons.

types in *ob/ob* mice can be largely or completely corrected by adrenalectomy (1–6). Many effects of leptin, and thus leptin deficiency, are thought to be due to the reduced hypothalamic melanocortin tone and the elevated hypothalamic NPY mRNA levels (18,26) that characterize *ob/ob* mice. If adrenalectomy acts through the same pathway as leptin, this would predict that adrenalectomy of *ob/ob* mice would increase hypothalamic melanocortin tone (thereby increasing hypothalamic POMC mRNA and decreasing hypothalamic AGRP mRNA levels) and decrease hypothalamic NPY mRNA. Conversely, if adrenalectomy were to reduce the obese phenotype through a leptin-independent pathway, then adrenalectomy would accentuate, rather than attenuate, the obesity-associated hypothalamic profile of gene expression, as is observed when *ob/ob* and *db/db* mice lose weight during fasting (12,27). The results of the present study generally support the hypothesis that adrenalectomy reverses the obese phenotype of *ob/ob* mice at least in part through the same pathway through which leptin acts. In a preliminary report, Arvaniti and Richard (28) also reported that adrenalectomy reduced hypothalamic AGRP mRNA in both wild-type and *ob/ob* mice. It should be noted, however, that the present studies address only mRNA levels, not peptide secretion, so it remains to be demonstrated if adrenalectomy actually influences the actual release of peptide in parallel with its effects on mRNA levels.

A key element in the design of the present study was the time after surgery, 2 weeks, at which the mice were sacrificed. This time point was chosen to allow lean wild-type and adrenalectomized mice to return to their original body weights. Reports on the effect of adrenalectomy on hypothalamic POMC and NPY mRNA in lean rats have been contradictory. Some reports indicate that adrenalectomy reduces hypothalamic POMC mRNA in lean rats (29), some reports indicate no effect of adrenalectomy on hypothalamic POMC mRNA (30), and some reports indicate that adrenalectomy increases hypothalamic POMC mRNA (31). Similarly, some studies reported that adrenalectomy can reduce NPY mRNA in lean rats (32–34) and that very high glucocorticoid levels can induce NPY mRNA in adrenalectomized ad libitum fed rats (34). However, other studies have failed to observe an effect of adrenalectomy on hypothalamic NPY mRNA in lean rats (35). We have observed in pilot studies that adrenalectomy reduced hypothalamic POMC mRNA in lean rats, as previously reported (29), but in such rats, body weight and serum leptin levels were reduced (T.M.M., unpublished observations). Because hypothalamic gene expression is regulated by factors such as leptin, which are reduced when

body weight is decreased, we hypothesized that the variable results of adrenalectomy on hypothalamic gene expression might be due to variable body weights (and, therefore, leptin) after surgery. Although it has been observed that adrenalectomy produces a greater and more prolonged decrease in body weight and food intake than sham surgery during the first week after surgery (36), we had observed that in our hands, by 2 weeks after surgery, these parameters were fully recovered in both sham-operated and adrenalectomized wild-type mice.

Therefore, to facilitate the most informative assessment of effects of adrenalectomy in *ob/ob* mice, mice were killed 2 weeks after surgery, at which time body weights of all wild-type groups had similarly recovered to presurgery levels (Fig. 1A). Under these circumstances in wild-type mice, adrenalectomy did not significantly influence food intake (Fig. 1B), plasma glucose (Fig. 2A), hypothalamic POMC mRNA (Fig. 3A) or hypothalamic NPY mRNA (Fig. 3C). On the other hand, although body weight and food intakes were restored to sham-operated levels, hypothalamic AGRP mRNA (Fig. 3B), serum insulin (Fig. 2B), and corticosterone levels were reduced in adrenalectomized wild-type mice.

The effects of adrenalectomy on obese phenotypes in the present study were essentially identical to those reported previously (1–6), including the observation that blood glucose and food intake were actually lower in adrenalectomized *ob/ob* mice than adrenalectomized wild-type mice (1) (Fig. 2A). Similarly, plasma insulin was largely, but not completely, corrected to wild-type levels (Fig. 2B). The key novel observations in the present study were that adrenalectomy stimulated hypothalamic POMC mRNA to wild-type levels (Fig. 2A) and that it reduced hypothalamic AGRP mRNA to wild-type levels (Fig. 2B); thus, hypothalamic melanocortin tone was restored in adrenalectomized *ob/ob* mice. If effects of adrenalectomy on obese phenotypes were due to mechanisms independent of melanocortin tone, then the subsequent reduction in circulating nutrients (including insulin and glucose) would reduce melanocortin tone to the same degree as the reduction in fasted *ob/ob* and *db/db* mice (12,27). Rather, because melanocortin tone was increased by adrenalectomy and because extensive evidence has suggested that impaired melanocortin tone can cause obesity (18), these results are consistent with the hypothesis that the effects of adrenalectomy to reverse obese phenotypes are due, at least in part, to the restoration of hypothalamic melanocortin tone.

It should be noted that the pattern of NPY expression was similar to the pattern of AGRP (increased in *ob/ob* mice and decreased by adrenalectomy). However, the effects of adrenalectomy on NPY mRNA in *ob/ob* mice were not statistically significant. We interpret these results to be consistent with our own previous conclusions (37) and those of others (38): AGRP and NPY are regulated similarly, but AGRP is generally regulated more robustly. Thus, especially because peptide secretion was not measured, our data do not directly address whether NPY may mediate some effects of adrenalectomy.

An important issue that remains to be resolved is the mechanism by which adrenalectomy increases hypothalamic melanocortin tone. Although leptin can increase hypothalamic melanocortin tone by increasing hypothalamic POMC mRNA (12,14) and reducing AGRP mRNA (16), clearly the

mechanism by which adrenalectomy has a similar effect in *ob/ob* mice is independent of leptin. Even in wild-type mice, the effect of adrenalectomy to reduce hypothalamic AGRP mRNA appears to be independent of leptin because the effects of adrenalectomy on serum leptin in wild-type mice are not significant or reliable. The most obvious potential mechanism is that adrenalectomy increases sensitivity to the central effects of insulin and/or glucose, as previously reported (39); this effect is consistent with the effects of adrenalectomy to dramatically reduce plasma insulin and glucose in *ob/ob* mice. Thus, by analogy with the regulation of hypothalamic NPY mRNA by insulin (40,41), the enhanced insulin and/or glucose tone after adrenalectomy may lead to an enhanced melanocortin tone. A possible role for glucose is supported by the presence of neurons in the hypothalamus, which sense physiological changes in glucose (42). We have observed that fasting stimulates hypothalamic AGRP mRNA and reduces hypothalamic POMC mRNA in diabetic wild-type mice in which fasting has no effect on either leptin or insulin (20). Furthermore, the toxin gold-thioglucose produces an insulin-dependent hypothalamic lesion that is facilitated by adrenalectomy (43) and blocked by glucose transport blockers (44), leading to dramatic reduction of hypothalamic POMC mRNA associated with obesity (45). We have also observed that leptin and glucose stimulate a largely overlapping set of neurons in the hypothalamus (X.-Y. Yang, L.M. Kow, C.V.M., unpublished observations). Nevertheless, it remains possible that glucocorticoids directly influence hypothalamic gene expression independent of effects of other nutritional factors.

The remarkable interrelationships among glucocorticoids, insulin, and obesity have long been appreciated and have stimulated much analysis (46–48). A particularly useful construct emphasizes the antagonism between insulin and glucocorticoids at both peripheral and central loci (48). Even though glucocorticoids are catabolic and insulin is anabolic in the periphery, the central effects of these hormones are precisely inverted (48). Thus, for example, insulin decreases hypothalamic NPY mRNA (40), whereas glucocorticoids are reported to increase hypothalamic NPY (34), which is consistent with the opposing effects of these hormones on body weight. A dramatic illustration of the antagonism between glucocorticoids and insulin in the present study is the remarkable reduction in both plasma insulin and plasma glucose by adrenalectomy in *ob/ob* mice (Fig. 2B). From this perspective, the present results may be viewed as a consequence of removing the glucocorticoid antagonism of the effects of insulin on the neural networks that regulate energy balance (48). Thus the natural history of obesity in leptin-deficient mice may be viewed as follows. First, as a direct consequence of leptin deficiency, glucocorticoids are elevated and feeding increases; the rise in glucocorticoids may be viewed as an appropriate compensation to mobilize peripheral nutrients and block satiety effects of peripheral signals in the perceived state of nutritional deficiency. As obesity develops, peripheral satiety signals, including insulin, glucose, and possibly fatty acids, increase dramatically, but their effects are blocked by the highly elevated glucocorticoids. Normalizing glucocorticoids abruptly by adrenalectomy permits the highly elevated insulin and other factors to produce robust satiety effects through insulin/glucose-sensitive neural networks in the

obese mice; because lean mice exhibit much lower levels of insulin, the effect of adrenalectomy is much less robust. The melanocortin system appears to constitute a key component of these insulin/glucose-sensitive neural networks, because yellow Agouti mice that are obese due to blockade of the melanocortin system are characterized by hyperinsulinemia, insulin resistance, and glucose (49). Taken together with the present study, these data support the hypothesis that adrenalectomy reverses obese phenotypes at least in part by removing the glucocorticoid blockade of insulin and/or glucose effects, thereby allowing insulin and/or glucose to stimulate the melanocortin system leading to satiety and other catabolic effects, independent of leptin.

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