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Am J Physiol Endocrinol Metab 287:E1107-E1113, 2004. First published 10 August 2004;
doi:10.1152/ajpendo.00038.2004

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Physiological regulation of hypothalamic IL-1 β gene expression by leptin and glucocorticoids: implications for energy homeostasis

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Submitted 27 January 2004; accepted in final form 2 August 2004

Wisse, Brent E., Kayoko Ogimoto, Gregory J. Morton, Charles W. Wilkinson, R. Scott Frayo, David E. Cummings, and Michael W. Schwartz. Physiological regulation of hypothalamic IL-1 β gene expression by leptin and glucocorticoids: implications for energy homeostasis. *Am J Physiol Endocrinol Metab* 287: E1107–E1113, 2004. First published August 10, 2004; doi:10.1152/ajpendo.00038.2004.—Interleukin-1 β (IL-1 β) is synthesized in a variety of tissues, including the hypothalamus, where it is implicated in the control of food intake. The current studies were undertaken to investigate whether hypothalamic IL-1 β gene expression is subject to physiological regulation by leptin and glucocorticoids (GCs), key hormones involved in energy homeostasis. Adrenalectomy (ADX) increased hypothalamic IL-1 β mRNA levels twofold, measured by real-time PCR ($P < 0.05$ vs. sham-operated controls), and this effect was blocked by subcutaneous infusion of a physiological dose of corticosterone. Conversely, hypothalamic IL-1 β mRNA levels were reduced by 30% in *fa/fa* (Zucker) rats, a model of genetic obesity caused by leptin receptor mutation ($P = 0.01$ vs. lean littermates), and the effect of ADX to increase hypothalamic IL-1 β mRNA levels in *fa/fa* rats ($P = 0.02$) is similar to that seen in normal animals. Moreover, fasting for 48 h (which lowers leptin and raises corticosterone levels) reduced hypothalamic IL-1 β mRNA levels by 30% ($P = 0.02$), and this decrease was fully reversed by refeeding for 12 h. Thus leptin and GCs exert opposing effects on hypothalamic IL-1 β gene expression, and corticosterone plays a physiological role to limit expression of this cytokine in both the presence and absence of intact leptin signaling. Consistent with this hypothesis, systemic leptin administration to normal rats (2 mg/kg ip) increased hypothalamic IL-1 β mRNA levels twofold ($P < 0.05$ vs. vehicle), an effect similar to that of ADX. These data support a model in which expression of hypothalamic IL-1 β is subject to opposing physiological regulation by corticosterone and leptin.

interleukin-1 β ; cytokine; corticosterone; food intake; appetite; obesity; anorexia

THE CLINICAL FINDINGS OF ANOREXIA and weight loss in patients with adrenal insufficiency and of increased appetite and weight gain in conditions of glucocorticoid (GC) excess have long suggested a role for the adrenal gland in energy homeostasis. Moreover, most rodent models of obesity are dependent on the action of GCs, since they are prevented or reversed by adrenalectomy (ADX) (29). Furthermore, anorexia induced by the adipocyte hormone leptin is augmented by ADX via a mechanism that is blocked by GC administration (30), suggesting that GCs play a physiolog-

ical role to antagonize the central actions of leptin. The observation that the weight-reducing effects of ADX are especially potent in animals that lack a leptin signal, however, suggests that GCs regulate energy homeostasis via a mechanism that is, at least partly, leptin independent. Together, these observations raise the possibility that the opposing effects of GCs and leptin in energy homeostasis (1, 30) involve the reciprocal regulation of a common downstream central nervous system (CNS) pathway.

Several recent observations identify the proinflammatory cytokine interleukin-1 β (IL-1 β) as a potential target for the opposing effects of leptin and GCs on hypothalamic systems governing energy balance. Administration of IL-1 β , either centrally or peripherally, inhibits food intake potently (21), and both IL-1 β and IL-1 receptors are expressed by neurons in regions of the hypothalamus associated with energy homeostasis (14, 16, 20). At pharmacological doses, GCs inhibit hypothalamic IL-1 β mRNA expression and reduce inflammation-associated fever (2), whereas ADX increases the expression of interleukin-1 β -converting enzyme in the hypothalamus and sensitizes mice to febrile reactions (15). Similarly, GCs inhibit, and ADX enhances, the ability of IL-1 β administration to activate the hypothalamic-pituitary-adrenal (HPA) axis (12, 27). These findings raise the possibility that hypothalamic IL-1 β gene expression is subject to physiological regulation by GCs and that, as in the regulation of fever and the HPA axis, IL-1 β -producing cells in the hypothalamus important to the control of energy homeostasis may be sensitive to the inhibitory actions of GCs.

In contrast to the effect of GCs, pharmacological administration of leptin increases IL-1 β protein concentration and mRNA content in the hypothalamus (10, 17). Moreover, the action of leptin in the brain appears to depend, at least in part, on functional IL-1 β signaling, since leptin-induced anorexia is attenuated in rodent models in which the action of IL-1 β is blocked (17). The finding that exogenous leptin administration increases hypothalamic IL-1 β gene expression in mice via a mechanism that is suppressed by pharmacological GC administration (10, 11) suggests that hypothalamic IL-1 β is a target for the opposing effects of leptin and GCs. These findings raise the as-yet-untested possibility that leptin and GCs exert opposing physiological actions to regulate hypothalamic signaling by IL-1 β and that this effect contributes to the potent actions of these hormones on energy balance.

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In the present study, we sought to determine whether hypothalamic IL-1 β is regulated by physiological input from leptin and GCs. Specifically, we sought to determine 1) whether leptin or GC signaling is necessary for maintenance of normal hypothalamic levels of IL-1 β mRNA, 2) whether the opposing effects of these hormones are specific for hypothalamic IL-1 β expression relative to other anorexigenic cytokines, and 3) whether changes in hypothalamic IL-1 β gene expression are correlated with effects of leptin and GCs on food intake. Our findings suggest that hypothalamic IL-1 β expression is regulated in a reciprocal manner by physiological input from GCs and leptin, consistent with a model in which this cytokine functions as a “downstream” mediator of opposing hypothalamic actions of these two hormones on energy homeostasis.

MATERIALS AND METHODS

Animals. Studies were conducted using male Wistar rats, age 8 wk (Charles River Laboratories, Wilmington, MA), and lean (*Fa/?*) and obese (*fa/fa*) male Zucker rats, age 14–16 wk (Harlan, Indianapolis, IN), housed individually in a temperature-controlled room (23 \pm 2°C) on a 12:12-h light-dark cycle. All protocols were approved by the Institutional Animal Care and Use Committee of the University of Washington, Seattle, WA, and were performed in accordance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. All animals were provided with ad libitum access to water and pelleted rodent chow (Test Diet 5012; LabDiet, Richmond, IN) unless otherwise indicated.

Surgical procedures. Bilateral ADX or a sham operation (identification of adrenal glands bilaterally without removal of tissue) was performed on weight-matched Wistar rats and obese *fa/fa* rats under general anesthesia (inhaled isoflurane), using a midline approach modified from standardized methods (19, 28). Postoperatively, all animals were provided continuous access to both tap water and normal saline (0.9%), and food intake and body weight were measured daily at the onset of the light cycle. For ADX studies in Wistar rats, each animal received a subcutaneously (sc) implanted pellet at the time of surgery containing either placebo or corticosterone at a dose [100 mg, 21-day release (4.76 mg/day); Innovative Research, Sarasota, FL] that normalizes plasma levels (4), yielding three study groups: 1) ADX plus placebo pellet (ADX-P), 2) ADX plus corticosterone pellet (ADX-C), and 3) sham-operated plus placebo pellet (Sham).

Effects of ADX and leptin on hypothalamic IL-1 β content in Wistar rats. One week following ADX or sham surgery, Wistar rats in each of the three groups (ADX-P, ADX-C, and Sham, $n = 8$ –12/group) received a single intraperitoneal injection of either saline ($n = 4$ –6) or leptin (2 mg/kg) in 0.3 ml of saline ($n = 4$ –6) 1 h before the onset of the dark cycle. Food intake was measured until 2 h after dark cycle onset, and animals were subsequently euthanized by decapitation following brief exposure to inhaled CO₂. Brains were removed at the time of death, and trunk blood was collected for determination of plasma corticosterone and leptin levels.

Effect of ADX on hypothalamic IL-1 β content in Zucker rats. After ADX or sham surgery, animals were divided into three groups: 1) ADX animals with access to chow provided ad libitum (ADX, $n = 8$), 2) sham-operated animals with ad libitum access to chow (Sham-AL, $n = 7$), and 3) sham-operated animals individually pair fed to a weight-matched ADX animal (Sham-PF, $n = 6$). The pair-feeding regimen was initiated 3 days after the surgery by providing each Sham-PF animal with a quantity of food at dark cycle onset equal to the amount consumed in the previous 24 h by a partner in the ADX group. All animals were killed during the light cycle 10 days postoperatively as described above. Age-matched lean (*Fa/?*) Zucker rats that had not been subjected to a surgical procedure were killed in the same manner.

Effect of fasting and refeeding on hypothalamic IL-1 β mRNA content in Wistar rats. Animals were divided into three groups: 1) ad libitum fed, 2) 48-h fast, and 3) 48-h fast followed by a 12-h refeeding period. Timing of the feeding paradigms was designed so that the time of death for each group was the same 2 h after the onset of the light cycle.

Blood collection and tissue processing. After removal, brains were immediately frozen under crushed dry ice. Mediobasal hypothalamus (defined caudally by the mammillary bodies, rostrally by the optic chiasm, laterally by the optic tract, and superiorly by the apex of the hypothalamic third ventricle) was dissected and stored at -80°C before RNA extraction. Trunk blood was collected into chilled, heparinized tubes and centrifuged at 1,500 rpm for 30 min, and plasma was stored at -20°C . Plasma corticosterone (26) and leptin (Linco, St. Louis, MO) concentrations were determined by radioimmunoassay.

Real-time PCR. Hypothalamic RNA was extracted using RNeasy Lysis Buffer according to the manufacturer's instructions (Tel-Test, Friendswood, TX) for RT-PCR analysis. RNA was quantified by spectrophotometry at 260 nm, and 1.5 μg of RNA were reverse-transcribed at 42°C for 1 h with 10 U of AMV reverse transcriptase (Promega, Madison, WI). PCR was performed on a LightCycler (Roche Molecular Biochemicals, Indianapolis, IN) using a 50-ng sample of hypothalamic cDNA added to the commercially available LightCycler PCR master mix (FastStart DNA Master SYBR Green I, Roche Molecular Biochemicals). Primers were designed to span a single sequence derived from two exons (i.e., separated by an intron in genomic DNA and primary RNA transcripts to minimize amplification from non-mRNA-derived templates) and were optimized for *IL-1 β* , *GAPDH*, *NPY*, *POMC*, *Agrp*, *SOCS-3*, and *TNF- α* . Primer sequences are listed as follows: *IL-1 β* : forward 5'-tacaaggagacaagcaacgaca-3', reverse 5'-gatccacctctccagctgca-3'; *GAPDH*: forward 5'-aacgacccttcattgac-3', reverse 5'-tccacgacatactcagcac-3'; *NPY*: forward 5'-accaggcagagatatggcaaga-3', reverse 5'-ggacatttctgtccttctctatta-3'; *POMC*: forward 5'-cgctcctactctatggagcactt-3', reverse 5'-tcacctaccagctccctcttg-3'; *Agrp*: forward 5'-agggcatcagaaggcctgaccagg-3', reverse 5'-cattgaagaagcggcagtagcacgt-3'; *SOCS-3*: forward 5'-gagtaccccccaagagagcttacta-3', reverse 5'-ctctttaaagtggagcatcactg-3'; *TNF- α* : forward 5'-catcttctcaaaactcgatgacaa-3', reverse 5'-tgggagtagataaggtacagccc-3'. All mRNA expression levels were normalized to *GAPDH* mRNA content, and nontemplate controls were incorporated into each PCR run. Correct amplification of IL-1 β mRNA by PCR was verified by sequencing of the PCR product (data not shown).

Statistical methods. Comparisons between group mean values were performed using an unpaired Student's *t*-test for two-group comparisons. For comparisons involving three or more groups, one-way ANOVA was performed using the least significant difference post hoc test for multiple comparisons. Interactions between surgical (i.e., ADX vs. Sham) and drug (i.e., leptin vs. vehicle) treatments were analyzed using a two-way ANOVA. Statistical analyses were performed using Statistical Package for the Social Sciences (SPSS, version 10.1; SPSS, Fullerton, CA). The null hypothesis of no difference between groups was rejected at $P < 0.05$. All values are presented as means \pm SE.

RESULTS

Effect of ADX on hypothalamic IL-1 β mRNA content, body weight, food intake, and plasma leptin and corticosterone concentrations in Wistar rats. Relative to sham-operated animals, ADX caused a twofold increase in hypothalamic IL-1 β content ($P = 0.02$), and this effect was fully reversed by low-dose subcutaneous corticosterone administration (Fig. 1A). By comparison, hypothalamic levels of mRNA encoding TNF- α , another major inflammatory cytokine, were not signif-

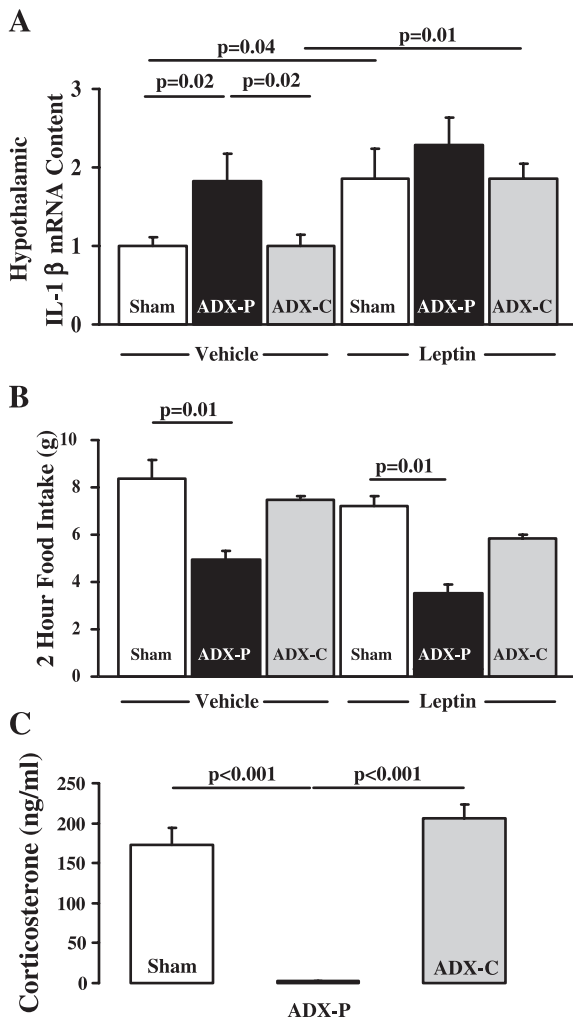


Fig. 1. A: relative hypothalamic IL-1 β mRNA content in sham-operated with placebo pellet (Sham, open bar), adrenalectomized (ADX) with placebo pellet (ADX-P, filled bar), and ADX with corticosterone (100 mg) pellet (ADX-C, gray bar) animals. All animals received either vehicle (data on left) or 2 mg/kg of leptin ip (data on right) 30–60 min before dark cycle onset; $n = 4$ –6/ surgical group. Time of death was 2 h into the dark cycle. Quantification of hypothalamic mRNA content was by RT-PCR expressed relative to GAPDH mRNA content for each animal. B: 2-h postinjection food intake (g) on day of experimental protocol. C: plasma corticosterone values (ng/ml) from trunk blood at time of death, as measured by specific RIA. Data are means \pm SE, with statistical analysis by 1-way ANOVA.

icantly altered by ADX ($P = 0.8$), nor were levels of mRNA encoding proopiomelanocortin (POMC, $P = 0.7$), neuropeptide Y (NPY, $P = 0.4$), or agouti-related protein (AGRP, $P = 0.4$; Table 1). Daily food intake was significantly decreased by ADX (ADX-P 19 ± 1 vs. Sham 26 ± 1 g/day, $P < 0.001$), and this effect was partially corrected by corticosterone (ADX-C 23 ± 1 g/day, $P = 0.002$ and $P = 0.02$, vs. ADX-P and Sham groups, respectively). This reduction of 24-h food intake was paralleled by decreases of intake measured during the first 2 h of the dark cycle, a time when the rate of food consumption is maximal (Fig. 1B). Analysis of plasma corticosterone levels at the end of the study confirmed the success of the ADX procedure (corticosterone 2 ± 1 ng/ml in ADX-P animals, $P < 0.001$, vs. Sham and ADX-C), whereas corticosterone pellet implantation resulted in circulating levels that were slightly

Table 1. Relative hypothalamic mRNA content for the 3 surgical groups by treatment

| | TNF- α | POMC | NPY | AGRP |
|---------|---------------|---------------|---------------|---------------|
| Sham-P | | | | |
| Vehicle | 1.0 \pm 0.2 | 1.0 \pm 0.1 | 1.0 \pm 0.4 | 1.0 \pm 0.4 |
| Leptin | 1.1 \pm 0.4 | 1.0 \pm 0.1 | 0.8 \pm 0.2 | 0.7 \pm 0.2 |
| ADX-P | | | | |
| Vehicle | 1.0 \pm 0.1 | 0.9 \pm 0.1 | 0.9 \pm 0.3 | 0.6 \pm 0.2 |
| Leptin | 1.5 \pm 0.2 | 1.1 \pm 0.2 | 0.7 \pm 0.3 | 0.6 \pm 0.2 |
| ADX-C | | | | |
| Vehicle | 1.0 \pm 0.2 | 1.0 \pm 0.1 | 0.4 \pm 0.1 | 0.5 \pm 0.1 |
| Leptin | 1.2 \pm 0.2 | 1.4 \pm 0.1 | 0.8 \pm 0.2 | 0.9 \pm 0.2 |

Data are means \pm SE. Treatments were vehicle or leptin (2 mg/kg ip) before onset of dark cycle. POMC, proopiomelanocortin; NPY, neuropeptide Y; AGRP, agouti-related protein; Sham-P, sham adrenalectomy (ADX) + placebo pellet; ADX-P, ADX + placebo pellet; ADX-C, ADX + corticosterone pellet. Time of death was 2 h into the dark cycle. Quantification of hypothalamic mRNA content was by RT-PCR expressed relative to GAPDH mRNA content for each animal.

higher than those of sham-operated controls but well within the physiological range (corticosterone 206 ± 18 and 173 ± 21 ng/ml for ADX-C and Sham groups, respectively, $P = 0.02$; Fig. 1C). Plasma leptin levels were significantly lower among ADX-P animals (1.2 ± 0.2 ng/ml) compared with either Sham (4.4 ± 0.6 ng/ml) or ADX-C (9.9 ± 2.0 ng/ml) ($P < 0.001$ for ADX-P vs. Sham and ADX-C). To summarize, ADX decreased food intake and increased hypothalamic IL-1 β mRNA content while markedly lowering plasma leptin levels, and each of these responses was prevented by low-dose corticosterone administration.

Hypothalamic IL-1 β mRNA expression in lean and obese Zucker rats. As expected, body weight (640 ± 11 vs. 380 ± 4 g, $P < 0.001$) and daily food intake (36.8 ± 1.5 vs. 22 ± 0.3 g, $P < 0.001$) were elevated in obese Zucker (*fa/fa*) rats relative to lean Zucker (*Fa/?*) littermates. By comparison, hypothalamic IL-1 β mRNA levels were reduced in *fa/fa* rats by 30% compared with lean animals ($P = 0.01$; Fig. 2), whereas neither plasma corticosterone levels (132 ± 20 vs. 125 ± 14 ng/ml, $P = 0.8$) nor hypothalamic levels of TNF- α mRNA ($P = 0.9$) differed significantly between groups. As previously reported (13), POMC mRNA content in the hypothalamus of *fa/fa* animals was only 30% of values measured in lean animals ($P < 0.001$).

Effect of ADX on hypothalamic IL-1 β mRNA expression in obese Zucker rats. Although both ADX and sham surgery were associated with acute decreases of food intake and body weight

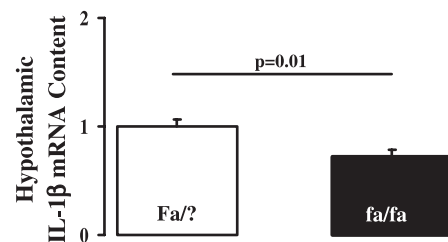


Fig. 2. Relative hypothalamic IL-1 β content in lean (*Fa/?*, open bar) and obese (*fa/fa*, filled bar) Zucker rats; $n = 6$ /group. Animals were killed during the middle of the light cycle. Quantification of hypothalamic mRNA content was by RT-PCR expressed relative to GAPDH mRNA content for each animal. Statistical analysis was by unpaired, 2-tailed Student's *t*-test. Data are means \pm SE.

in *fa/fa* rats, weight loss was transient among sham-operated animals fed ad libitum (Sham-AL), whereas it was sustained both in the ADX group and in sham-operated rats that were pair fed to the intake of the ADX group (Sham-PF) (-33 ± 10 and -68 ± 5 g, respectively; $P = 0.004$ and $P < 0.001$ for both ADX and Sham-PF, respectively, vs. Sham-AL). Weight loss in the ADX group was associated with a 35% decrease in daily food intake relative to the Sham-AL group (ADX 20 ± 2 vs. Sham-AL 31 ± 1 g, $P < 0.001$), whereas by design, average food intake in Sham-PF animals was identical to that of the ADX group. Plasma corticosterone levels confirmed successful induction of GC deficiency by ADX (1 ± 1 vs. 132 ± 20 ng/ml for ADX vs. Sham-AL, $P < 0.001$), whereas plasma corticosterone levels in the Sham-PF group were increased (283 ± 27 ng/ml, $P < 0.001$), consistent with the well-documented effect of chronic energy restriction to activate the HPA axis (25).

As in Wistar rats, induction of GC deficiency in *fa/fa* rats by ADX induced a nearly twofold increase of hypothalamic IL-1 β mRNA expression relative to both the Sham-AL and Sham-PF groups (Fig. 3). By comparison, ADX had no significant effect on hypothalamic levels of either TNF- α or POMC mRNA in *fa/fa* rats compared with either Sham-operated control group (Fig. 3). Because the effects of ADX on IL-1 β gene expression were not detected in sham-operated *fa/fa* controls pair fed to the intake of the ADX group, they cannot be attributed to the effect of ADX to reduce food intake or body weight of these animals.

Effect of fasting and refeeding on hypothalamic IL-1 β mRNA content in Wistar rats. In response to a 48-h fast, which potently decreases plasma leptin (7) and increases plasma corticosterone in rats (3), hypothalamic IL-1 β mRNA expression was reduced by 30% ($P = 0.02$), whereas rats that were allowed to refeed for 12 h demonstrated hypothalamic IL-1 β mRNA levels no different from those of ad libitum-fed controls (Fig. 4). The time of death was the same in all three groups of animals to prevent any confounding effect from diurnal variation in hypothalamic IL-1 β mRNA expression.

Leptin regulation of hypothalamic IL-1 β mRNA content in Wistar rats in the presence or absence of ADX. Two hours after leptin administration (2 mg/kg ip) to sham-operated rats, hypothalamic IL-1 β mRNA content was increased twofold relative to vehicle-injected, sham-operated animals ($P = 0.04$; Fig.

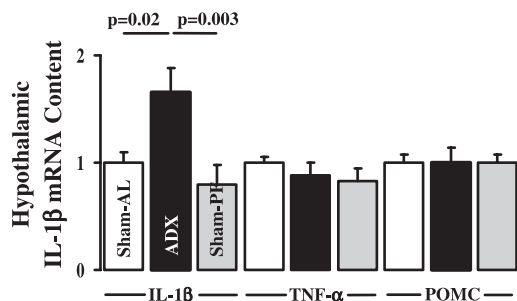


Fig. 3. Relative hypothalamic IL-1 β , TNF- α , and proopiomelanocortin (POMC) mRNA content in the 3 groups of obese *fa/fa* Zucker rats: 1) sham-ADX ad libitum fed (Sham-AL, open bar), 2) ADX (filled bar), and 3) sham-ADX pair fed (Sham-PF, gray bar); $n = 6-8$ /group. Quantification of hypothalamic mRNA content was by RT-PCR expressed relative to GAPDH mRNA content for each animal. Data are means \pm SE, with statistical analysis by 1-way ANOVA.

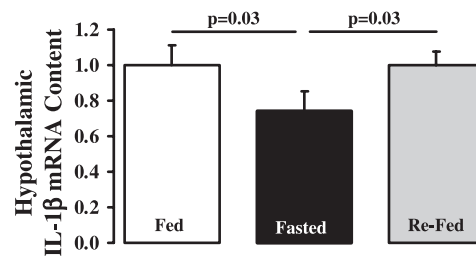


Fig. 4. Relative hypothalamic IL-1 β mRNA expression in 3 groups of Wistar rats: 1) ad libitum fed (Fed, open bar), 2) 48-h fasted (Fasted, filled bar), and 3) 12-h refeeding period following a 48-h fast (Re-Fed, gray bar); $n = 6-8$ /group. Onset of food restriction paradigm in groups 2 and 3 was designed so that time of death was the same. Quantification of hypothalamic mRNA content was by RT-PCR expressed relative to GAPDH mRNA content for each animal. Data are means \pm SE, with statistical analysis by 1-way ANOVA.

1A), in accord with previous studies showing increased hypothalamic IL-1 β protein following pharmacological leptin administration (17). This leptin effect was not associated with significant changes of either plasma corticosterone concentration (150 ± 28 vs. 195 ± 33 ng/ml, $P = 0.33$) or levels of TNF- α mRNA in the mediobasal hypothalamus ($P = 0.75$; Table 1). Among ADX rats that received a placebo pellet (ADX-P), leptin administration tended to increase hypothalamic IL-1 β mRNA, but this effect did not achieve statistical significance ($P = 0.3$), whereas leptin administration to corticosterone-replaced (ADX-C) rats caused an increase in hypothalamic IL-1 β mRNA levels ($P = 0.01$) equivalent to the effect seen in sham-operated animals.

Relative to vehicle treatment, leptin administration caused a significant decrease in 2-h food intake ($P = 0.05$ for leptin vs. vehicle by two-way ANOVA; Fig. 1B), although post hoc comparison of mean values from individual treatment groups failed to detect statistically significant differences for any of the three surgical groups at this early time point ($P = 0.2$, $P = 0.3$, and $P = 0.2$, respectively, for ADX-P, Sham, and ADX-C). To investigate whether 2-h food intake varied inversely with hypothalamic IL-1 β mRNA levels, we compared mean values of both parameters for all three groups (Sham, ADX-P, and ADX-C) under both treatment conditions (vehicle and leptin). As predicted, we found that 2-h food intake and IL-1 β mRNA levels (Fig. 5) were strongly and inversely correlated ($R^2 = 0.73$, $P = 0.02$).

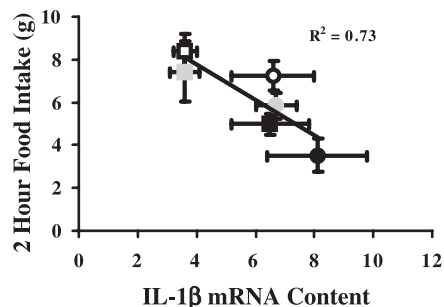


Fig. 5. Linear model regression analysis of 2-h food intake vs. relative hypothalamic IL-1 β mRNA in sham-ADX (open symbols), ADX with placebo pellet (filled symbols), and ADX with corticosterone (100 mg) pellet (gray symbols) animals ($n = 4-6$ /group) that were treated with either vehicle (squares) or 2 mg/kg leptin ip (circles) 30-60 min before dark cycle onset. Time of death was 2 h into the dark cycle. Data are means \pm SE.

DISCUSSION

The present studies were undertaken to determine whether hypothalamic IL-1 β mRNA expression is subject to physiological regulation by endogenous corticosterone and to determine whether corticosterone and leptin exert opposing effects on hypothalamic expression of this cytokine. Our results implicate corticosterone as a physiological inhibitor of hypothalamic IL-1 β gene expression, since ADX increased expression of IL-1 β mRNA via a mechanism dependent on corticosterone deficiency. Conversely, deficient leptin signaling in obese *fa/fa* rats was associated with reduced hypothalamic IL-1 β mRNA content, suggesting a physiological role for leptin signaling to increase IL-1 β biosynthesis in hypothalamus. These observations suggest that interventions that decrease leptin and increase corticosterone levels in circulation should lower hypothalamic IL-1 β gene expression. Consistent with this prediction, we found that a 48-h fast reduced hypothalamic IL-1 β mRNA content and that this effect was reversed after 12 h of refeeding. Our data further suggest that the effect of corticosterone deficiency to increase hypothalamic IL-1 β expression is not mediated via increased leptin signal transduction, since the stimulatory effect of ADX on hypothalamic IL-1 β mRNA content was similar between genetically normal rats and *fa/fa* rats and because the effect of ADX to increase hypothalamic IL-1 β mRNA expression in normal rats occurred despite a marked decrease of plasma leptin levels. These results collectively suggest that hypothalamic IL-1 β gene expression is subject to reciprocal physiological regulation by leptin and corticosterone and thereby identify a potential role for this cytokine in physiological actions mediated by hypothalamic actions of these hormones.

Although best known as a cytokine secreted by inflammatory cells, IL-1 β is also produced within neurons (23), and IL-1 β signaling has been documented in a variety of brain areas, including the hypothalamus, where this cytokine has effects on fever and HPA axis activation (2, 14, 20). Like leptin, IL-1 β potentially decreases food intake and reduces body weight when administered either peripherally or directly into brain ventricles (21). The hypothesis that hypothalamic IL-1 β signaling is tonically increased by leptin and decreased by GCs, therefore, provides a plausible mechanism to explain the opposing actions of these hormones in the control of energy homeostasis and other neuroendocrine responses (e.g., HPA activation).

Research spanning more than 40 years (8) has established a critical physiological role for adrenal hormones in the control of energy homeostasis, yet the underlying mechanisms mediating their effects on food intake remain incompletely defined. The effects of ADX on energy balance are profound, reducing food intake and body weight while increasing body temperature in many forms of rodent obesity (29). Although some evidence implicates enhanced leptin signaling as a mediator of ADX-induced negative energy balance (18), the fact that GC deficiency potentially attenuates obesity in animals with defective leptin signaling (*ob/ob* and *db/db* mice and *fa/fa* rats) suggests that other mechanisms may be involved as well. Our finding that ADX increases hypothalamic IL-1 β mRNA content, juxtaposed with the fact that IL-1 β administration causes anorexia, weight loss, and fever, raises the possibility that increased hypothalamic signaling via IL-1 β may contribute to

several pathological consequences induced by ADX. Because IL-1 β also potently activates hypothalamic corticotropin-releasing hormone (CRH)-producing neurons that exert primary control over the HPA axis, the hypothesis that ADX activates these neurons via a mechanism that is dependent, at least in part, on induction of this cytokine can be considered.

Our data suggest that corticosterone, the primary GC in rodents, is the critical adrenal hormone mediating tonic inhibition of hypothalamic IL-1 β expression, because the stimulatory effect of ADX on IL-1 β mRNA was reversed by administration of this hormone at a physiological dose. The fact that food intake and body weight were lower in the corticosterone-replaced ADX group relative to the sham-operated animals despite equivalent hypothalamic IL-1 β content suggests that negative energy balance per se was unlikely to have mediated the effect of ADX to increase hypothalamic IL-1 β mRNA expression. This conclusion is strengthened by our observation that fasting lowers expression of this cytokine.

That ADX increases hypothalamic IL-1 β production is consistent with the previously reported effect of GC deficiency to increase production of proinflammatory cytokines in a variety of other tissues, including cerebral cortex (6). In addition, we found that ADX increases hypothalamic IL-1 β even in *fa/fa* rats with defective leptin signaling, demonstrating that this effect of GC deficiency is leptin independent. To further investigate potential effects of altered hypothalamic IL-1 β signaling on energy homeostasis, we determined whether ADX-induced increases of hypothalamic IL-1 β mRNA levels parallel its inhibitory effect on food intake. Our finding of a strong inverse relationship between these parameters across study groups identifies this cytokine as a potential mediator of anorexia induced by GC deficiency.

This conclusion is compatible with several published observations suggesting that hypothalamic IL-1 β signaling may participate in the physiological control of energy balance, in addition to its potential role in pathological weight loss (22). For example, data from Luheshi et al. (17) suggest that functional IL-1 β signaling is required for the anorexic response to leptin. Because leptin-induced anorexia also depends on activation of CRH (5) and because IL-1 β is a potent activator of CRH-producing neurons (12), the possibility can be considered that IL-1 β serves to link leptin signaling to activation of CRH neurons.

The concept that IL-1 β serves as a downstream leptin-dependent signal is strengthened by recent studies from Hosoi and colleagues (9–11) demonstrating that pharmacological leptin administration stimulates the production of IL-1 β in mouse hypothalamus, an observation extended by our current findings in rats. Moreover, the effect of leptin administration to increase body temperature is also prevented by pharmacological blockade of IL-1 receptors (17). Taken together, these findings identify a variety of actions of leptin in the CNS that may depend, at least in part, on signaling via IL-1 β . Further, albeit indirect, evidence suggesting a physiological role for IL-1 β in the hypothalamic control of energy balance stems from diurnal changes in the expression of this cytokine. Thus IL-1 β mRNA levels in rat hypothalamus are relatively reduced during the dark cycle (when plasma levels of corticosterone are high) and increased during the light cycle (when corticosterone is low) (24), a pattern accompanied by variations in food intake compatible with those induced by IL-1 β .

Although our finding of reduced IL-1 β mRNA content in the hypothalamus of *fal/fa* rats implicates leptin signaling as a physiological determinant of hypothalamic IL-1 β gene expression, it is also possible that IL-1 β expression is reduced in these animals as a consequence of their obesity. This interpretation seems unlikely, however, because hypothalamic content of IL-1 β mRNA was decreased, rather than increased, by fasting in normal rats (which lowers leptin levels). Moreover, our finding of similar plasma corticosterone levels in lean and obese Zucker animals eliminates differences in GC levels as a potential confounder. Combined with our finding that, despite comparable weight loss, ADX increased hypothalamic IL-1 β gene expression, whereas energy restriction (induced by pair feeding of sham-operated Zucker rats to the intake of those receiving ADX) did not, our data collectively suggest that endogenous leptin signaling is required for normal hypothalamic IL-1 β synthesis. This conclusion in turn raises the possibility that deficient hypothalamic IL-1 β signaling contributes to the development or maintenance of obesity in animals with impaired leptin signaling.

Our findings that GC deficiency raises, whereas deficient leptin signaling lowers, hypothalamic IL-1 β gene expression add to a growing database supporting the hypothesis that IL-1 β synthesis in the hypothalamus may require intact leptin signaling while being constrained by physiological input from GCs and that hypothalamic expression of this cytokine may be important in the regulation of energy homeostasis. Although neurons and glial cells are clearly capable of synthesizing IL-1 β (23), the hypothalamic cell type(s) that expresses this cytokine in a leptin- and GC-sensitive manner has yet to be identified. Accomplishing this goal is an important priority for future studies.

Our current work emphasizes the importance of ongoing efforts to determine the contribution made by hypothalamic IL-1 β signaling to the opposing actions of leptin and GCs on energy homeostasis and to clarify the mechanisms underlying these effects. An important additional priority for future studies is to evaluate the therapeutic potential of interventions that increase hypothalamic IL-1 β signaling in the treatment of obesity and of those that inhibit this signaling in the treatment of inflammatory anorexia and other forms of wasting illness.

ACKNOWLEDGMENTS

We are grateful to H. Nguyen, T. Huon, and G. Alkire for technical assistance.

GRANTS

This work was supported by grants from the National Institutes of Health to M. W. Schwartz (DK-12829, DK-52989, NS-32273), D. E. Cummings (DK-61516), and B. E. Wisse (DK-61384), Pilot & Feasibility Awards from the Clinical Nutrition and Research Unit (P30-DK-035816) and the Diabetes Endocrinology Research Center (P30-DK-17047) at the University of Washington (B. E. Wisse), and a grant from the Endocrine Fellows Foundation (B. E. Wisse)

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