Physiological Regulation of Hypothalamic Interleukin-1beta (IL-1β) Gene Expression by Leptin and Glucocorticoids: Implications for Energy Homeostasis

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Running Head: Leptin and GCs control Hypothalamic IL-1β mRNA in Rats

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ABSTRACT
Interleukin-1β (IL-1β) 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-1β gene expression is subject to physiological regulation by leptin and glucocorticoids (GCs), key hormones involved in energy homeostasis. Adrenalectomy (ADX) increased hypothalamic IL-1β mRNA levels, measured by real-time PCR, by two fold (p<0.05 vs. sham-operated controls) and this effect was blocked by sc infusion of a physiological dose of corticosterone. Conversely, hypothalamic IL-1β mRNA levels were reduced by 30% in fa/fa (Zucker) rats, a model of genetic obesity due to leptin receptor mutation (p=0.01 vs. lean littermates), and the effect of ADX to increase hypothalamic IL-1β mRNA levels in fa/fa rats (p=0.02) is similar to that seen in normal animals. Moreover, fasting for 48h (which lowers leptin and raises corticosterone levels) reduced hypothalamic IL-1β mRNA levels by 30% (p=0.02) and this decrease was fully reversed by re-feeding for 12h. Thus, leptin and GCs exert opposing effects on hypothalamic IL-1β 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 (2mg/kg IP) increased hypothalamic IL-1β mRNA levels by two fold (p<0.05 vs vehicle), an effect similar to that of ADX. These data support a model in which expression of hypothalamic IL-1β is subject to opposing, physiological regulation by corticosterone and leptin.

Keywords: Cytokine, corticosterone, food intake, appetite, obesity, anorexia
Introduction
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) Moreover, 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 physiological 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 CNS pathway.

Several recent observations identify the pro-inflammatory cytokine interleukin-1β (IL-1β) as a potential target for the opposing effects of leptin and GCs on hypothalamic systems governing energy balance. Administration of IL-1β, either centrally or peripherally, inhibits food intake potently, (21) and both IL-1β 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-1β mRNA expression and reduce inflammation-associated fever (2), whereas ADX increases the expression of interleukin-1β converting enzyme (ICE) in the hypothalamus and sensitizes mice to febrile reactions. (15) Similarly, GCs inhibit, and ADX enhances, the ability of IL-1β administration to activate the hypothalamic-pituitary-adrenal (HPA) axis. (12, 27) These findings raise the possibility that hypothalamic IL-1β gene expression is subject to physiological regulation by GCs and that, as in the regulation of fever and the HPA axis, IL-1β-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-1β 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-1β signaling, since leptin-induced anorexia is attenuated in rodent models in which the action of IL-1β is blocked. (17) The finding that exogenous leptin administration increases hypothalamic IL-1β gene expression in mice via a mechanism that is suppressed by pharmacological GC administration (10, 11) suggests that hypothalamic IL-1β 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-1β and that this effect contributes to the potent actions of these hormones on energy balance.

In the current study, we sought to determine whether hypothalamic IL-1β is regulated by physiological input from leptin and GCs. Specifically, we sought to determine 1) if leptin or GC signaling is necessary for maintenance of normal hypothalamic levels of IL-1β mRNA; 2) if the opposing effects of these hormones are specific for hypothalamic IL-1β
expression relative to other anorexigenic cytokines; and 3) whether changes in hypothalamic IL-1β gene expression are correlated with effects of leptin and GCs on food intake. Our findings suggest that hypothalamic IL-1β 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 in lean (Fa/?) and obese (fa/fa) male Zucker rats, age 14-16 wk (Harlan, Indianapolis, IN) housed individually in a temperature-controlled room (23 ± 2°C) on a 12h 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 NIH 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 Inc., Richmond, IN) unless otherwise indicated.

**Surgical procedures**
Bilateral adrenalectomy (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) Post-operatively, 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 (100mg, 21d release (4.76mg/d), Innovative Research, Sarasota, FL) that normalizes plasma levels (4), yielding three study groups: 1) ADX + placebo pellet (ADX-P); 2) ADX + corticosterone pellet (ADX-C) and; 3) sham-operated + placebo pellet (Sham).

*Effects of ADX and leptin on hypothalamic IL-1β content in Wistar rats.*
One week following ADX or sham surgery, Wistar rats in each of the 3 groups (ADX-P, ADX-C and Sham, n=8-12/group) received a single ip injection of either saline (n=4-6) or leptin (2mg/kg) in 0.3ml of saline, n=4-6) 1h prior to 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 sacrifice and trunk blood was collected for determination of plasma corticosterone and leptin levels.

*Effect of ADX on hypothalamic IL-1β content in Zucker rats*
Following ADX or sham surgery, animals were divided into three groups: 1) ADX with access to chow provided *ad libitum* (ADX, n=8); 2) sham-operated, also 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 3d
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 24h by a partner in the ADX group. All animals were sacrificed during the light cycle 10d post-operatively as described above. Age-matched lean (Fa/) Zucker rats that were not subjected to a surgical procedure were sacrificed in the same manner.

**Effect of fasting and re-feeding on hypothalamic IL-1β mRNA content in Wistar rats**

Animals were divided into three groups: 1) *ad libitum*-fed; 2) 48h fast; and 3) 48h fast followed by a 12h re-feeding period. Timing of the feeding paradigms was designed so that the time of sacrifice for each group was the same 2h 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 mamillary 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 prior to RNA extraction. Trunk blood was collected into chilled, heparinized tubes, 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 RNAzol B according to manufacturers’ instructions (Tel-Test Inc., TX) for RT-PCR analysis. RNA was quantified by spectrophotometry at 260 nm and 1.5 μg of RNA was reverse-transcribed at 42 ºC for 1 hour with 10 U of AMV reverse transcriptase (Promega, Madison, WI). PCR was performed on a LightCycler (Roche Molecular Biochemicals, 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-1β*, *GAPDH*, *NPY*, *POMC*, *Agrp*, *SOCS-3* and *TNF-α*. Primer sequences are listed for: *IL-1β*: fwd 5’ tacaaggagacacaagcaca 3’; *IL-1β* rev 5’ gatccacactctccagctgca 3’; *GAPDH*: fwd 5’ aacgaccccccttcattgac 3’; *GAPDH* rev 5’ tcaagacatctcagcagc 3’; *Npy* forward, 5’-acciagcagatagcgaagcaga-3’; *Npy* reverse, 5’-ggacatttctgtgtcctcactatta-3’; *POMC* forward, 5’-ctctttaaagtggagcatcatactg-3’; *POMC* reverse, 5’-tcacctaccagctccctttg-3’; *Agrp* forward 5’-aggcatcagaaggctgaccg-3’; *Agrp* reverse 5’-catcagaagacggctgaccct-3’; *SOCS-3* forward, 5’-gagttacccaaagagacggt-3’; *SOCS-3* reverse, 5’-ctctttaaagtggacacacttca-3’; *TNF-α* forward, 5’-catccttactcactcactctcact-3’; *TNF-α* reverse, 5’-tggagagtagtacactacgcc-3’. All mRNA expression levels were normalized to *Gapdh* mRNA content and non-template controls were incorporated into each PCR run. Correct amplification of IL-1β 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 3 or more groups, one-way ANOVA was performed using the LSD 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 Inc., Fullerton, CA, USA). The null hypothesis of no difference between groups was rejected at p <0.05. All values are presented as the mean ± SEM.

**Results**

*Effect of ADX on hypothalamic IL-1β mRNA content, body weight, food intake and plasma leptin and corticosterone concentrations in Wistar rats*

Relative to sham-operated animals, ADX caused a two-fold increase in hypothalamic IL-1β content (p=0.02), and this effect was fully reversed by low-dose sc corticosterone administration (Fig 1A). By comparison, hypothalamic levels of mRNA encoding TNF-α, another major inflammatory cytokine, were not significantly altered by ADX (p=0.8), nor were levels of mRNA encoding POMC (p=0.7), NPY (p=0.4) or Agrp (p=0.4) (Table 1). Daily food intake was significantly decreased by ADX (ADX-P 19±1 vs. Sham 26±1g/day; p<0.001) and this effect was partially corrected by corticosterone (ADX-C 23±1g/day; p=0.002 and p=0.02 vs. ADX-P and Sham groups, respectively). This reduction of 24h food intake was paralleled by decreases of intake measured during the first 2h 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±1ng/ml in ADX-P animals, p<0.001 vs. Sham and ADX-C), while corticosterone pellet implantation resulted in circulating levels that were slightly higher than those of sham-operated controls but well within the physiological range (corticosterone: 206±18ng/ml and 173±21ng/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±0.2ng/ml) compared to either Sham (4.4±0.6ng/ml) or ADX-C (9.9±2.0ng/ml) (p<0.001 for ADX-P vs. Sham and ADX-C). To summarize, ADX decreased food intake and increased hypothalamic IL-1β mRNA content while markedly lowering plasma leptin levels, and each of these responses was prevented by low-dose corticosterone administration.

*Hypothalamic IL-1β mRNA expression in lean and obese Zucker rats*

As expected, body weight (640±11g vs. 380±4, p<0.001) and daily food intake (36.8±1.5g vs. 22±0.3, p<0.001) were elevated in obese Zucker (fa/fa) rats relative to lean Zucker (Fa/?) littermates. By comparison, hypothalamic IL-1β mRNA levels were reduced in fa/fa rats by 30% compared to lean animals (Fig 2, p=0.01), while 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-1β mRNA expression in obese Zucker rats

While both ADX and sham surgery were associated with acute decreases of food intake and body weight in fa/fa rats, weight loss was transient among sham-operated animals fed ad libitum (Sham-AL), while it was sustained in both the ADX group and in sham-operated rats that were pair-fed to the ADX group (Sham-PF) (-33±10g and -68±5g respectively; p=0.004, and p<0.001, for both ADX and Sham-PF 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±2g vs. Sham-AL 31±1g; p<0.001) while by design, average food intake in Sham-PF animals was identical to the ADX group. Plasma corticosterone levels confirmed successful induction of GC deficiency by ADX (1±1ng/ml vs. 132±20ng/ml for ADX vs Sham-AL, p<0.001) while plasma corticosterone levels in the Sham-PF group were increased (283±27ng/ml; p<0.001), consistent with the well-documented effect of chronic energy restriction to activate the hypothalamic-pituitary-adrenal axis. (25)

As in Wistar rats, induction of GC deficiency in fa/fa rats by ADX induced a nearly two-fold increase of hypothalamic IL-1β 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 to either Sham-operated control group (Fig 3). Since the effects of ADX on IL-1β 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 re-feeding on hypothalamic IL-1β mRNA content in Wistar rats

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

Leptin regulation of hypothalamic IL-1β mRNA content in Wistar rats in the presence or absence of ADX

Two hours following leptin administration (2mg/kg IP) to sham-operated rats, hypothalamic IL-1β mRNA content was increased by 2-fold relative to vehicle-injected, sham-operated animals (p=0.04, Fig 1A), in accordance with previous studies showing increased hypothalamic IL-1β protein following pharmacologic 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 adrenalectomized rats that received a placebo pellet (ADX-P), leptin administration tended to increase hypothalamic IL-1β 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-1β 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 2h-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, p=0.2, respectively; for ADX-P, Sham and ADX-C). To investigate whether 2h-food intake varied inversely with hypothalamic IL-1β 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 2h-food intake and IL-1β mRNA levels (Fig 5) were strongly and inversely correlated (R²=0.73, p=0.02).

Discussion

The current studies were undertaken to determine if hypothalamic IL-1β mRNA expression is subject to physiological regulation by endogenous corticosterone, and to determine if corticosterone and leptin exert opposing effects on hypothalamic expression of this cytokine. Our results implicate corticosterone as a physiological inhibitor of hypothalamic IL-1β gene expression, since ADX increased expression of IL-1β mRNA via a mechanism dependent on corticosterone deficiency. Conversely, deficient leptin signaling in obese fa/fa rats was associated with reduced hypothalamic IL-1β mRNA content suggesting a physiological role for leptin signaling to increase IL-1β biosynthesis in hypothalamus. These observations suggest that interventions that decrease leptin and increase corticosterone levels in circulation should lower hypothalamic IL-1β gene expression. Consistent with this prediction, we found that a 48 h fast reduced hypothalamic IL-1β 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-1β expression is not mediated via increased leptin signal transduction, since the stimulatory effect of ADX on hypothalamic IL-1β mRNA content was similar between genetically normal rats and fa/fa rats, and because the effect of ADX to increase hypothalamic IL-1β mRNA expression in normal rats occurred despite a marked decrease of plasma leptin levels. These results collectively suggest that hypothalamic IL-1β gene expression is subject to reciprocal, physiological regulation by leptin and corticosterone, and thereby identifies 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-1β is also produced within neurons, (23) and IL-1β 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-1β potently decreases food intake and reduces body weight when administered either peripherally or directly into brain ventricles.(21) The hypothesis that hypothalamic IL-1β 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
Research spanning more than forty 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 potently attenuates obesity in animals with defective leptin signaling (ob/ob and db/db mice, and fa/fa rats) suggests other mechanisms may be involved as well. Our finding that ADX increases hypothalamic IL-1β mRNA content, juxtaposed with the fact that IL-1β administration causes anorexia, weight loss and fever, raise the possibility that increased hypothalamic signaling via IL-1β may contribute to several pathological consequences induced by ADX. Since IL-1β also potently activates hypothalamic 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-1β expression, since the stimulatory effect of ADX on IL-1β 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-1β content suggests that negative energy balance per se was unlikely to have mediated the effect of ADX to increase hypothalamic IL-β mRNA expression. This conclusion is strengthened by our observation that fasting lowers expression of this cytokine.

That ADX increases hypothalamic IL-1β production is consistent with the previously reported effect of GC deficiency to increase production of pro-inflammatory cytokines in a variety of other tissues, including cerebral cortex. (6) In addition, we found that ADX increases hypothalamic IL-1β 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-1β signaling on energy homeostasis, we determined whether ADX-induced increases of hypothalamic IL-1β 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-1β 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 and colleagues suggest that functional IL-1β signaling is required for the anorexic response to leptin. (17) Because leptin-induced anorexia also depends on
activation of CRH (5), and since IL-1β is a potent activator of CRH-producing neurons (12), the possibility can be considered that IL-1β serves to link leptin signaling to activation of CRH neurons.

The concept that IL-1β serves as a ‘downstream’ leptin-dependent signal is strengthened by recent studies from Hosoi et al. demonstrating that pharmacological leptin administration stimulates the production of IL-1β in mouse hypothalamus (9-11), 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, upon signaling via IL-1β. Further, albeit indirect, evidence suggesting a physiological role for IL-1β in the hypothalamic control of energy balance stems from diurnal changes in the expression of this cytokine. Thus, IL-1β 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-1β.

While our finding of reduced IL-1β mRNA content in the hypothalamus of fa/fa rats implicates leptin signaling as a physiological determinant of hypothalamic IL-1β gene expression, it is also possible that IL-1β expression is reduced in these animals as a consequence of their obesity. This interpretation seems unlikely, however, since hypothalamic content of IL-1β 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-1β 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-1β synthesis. This conclusion in turn raises the possibility that deficient hypothalamic IL-1β 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-1β gene expression, add to a growing data base supporting the hypothesis that IL-1β 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. While neurons and glial cells are clearly capable of synthesizing IL-1β (23), the hypothalamic cell type(s) that express this cytokine in a leptin- and GC-sensitive manner have 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-1β 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-1β 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.

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Figure Legends

Fig 1. A. Relative hypothalamic IL-1β mRNA content in sham-adrenalectomized with placebo pellet (Sham, white bar), adrenalectomized with placebo pellet (ADX-P, black bar) and adrenalectomized with corticosterone (100mg) pellet (ADX-C, gray bar) animals. All animals received either vehicle (n=4-6/per surgical group), data shown on left side of panel, or 2mg/kg leptin (n=4-6/per surgical group), data shown on right side of panel, IP 30-60 min prior to dark cycle onset. Time of sacrifice was 2h into the dark cycle. Quantification of hypothalamic mRNA content was by RT-PCR expressed relative to GAPDH mRNA content for each animal. B. Two hour post-injection food intake (g) on day of experimental protocol C. Plasma corticosterone values (ng/ml) from trunk blood at time of sacrifice as measured by specific RIA. Data are means ± SEM, statistical analysis by One-way ANOVA.

Fig 2. Relative hypothalamic IL-1β content in lean (Fa/?) (white bar) and obese (fafa) (black bar) Zucker rats (n=6/group). Animals were sacrificed 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 by un-paired, two-tailed student t-test. Data are means ± SEM.

Fig 3. Relative hypothalamic IL-1β, TNF-α and POMC mRNA content in the 3 groups of obese fafa Zucker rats; 1) sham-adrenalectomized ad-libitum-fed (Sham-AL, white bar); 2) adrenalectomized (ADX, black bar) and; 3) sham-adrenalectomized 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 ± SEM, statistical analysis by One-way ANOVA

Fig 4. Relative hypothalamic IL-1β mRNA expression in 3 groups of Wistar rats; 1) ad-libitum-fed (Fed, white bar); 2) 48h fasted (Fasted, black bar) and; 3) 12h re-feeding period following a 48h 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 sacrifice was the same. Quantification of hypothalamic mRNA content was by RT-PCR expressed relative to GAPDH mRNA content for each animal. Data are means ± SEM, statistical analysis by One-way ANOVA

Fig 5. Linear model regression analysis of 2h food intake vs. relative hypothalamic IL-1β mRNA in; sham-adrenalectomized (white), adrenalectomized with placebo pellet (black) and adrenalectomized with corticosterone (100mg) pellet (gray) animals (n=4-6/group) that were treated with either vehicle (squares) or 2mg/kg leptin (circles) IP 30-60 min prior to dark cycle onset. Time of sacrifice was 2h into the dark cycle. Data are means ± SEM.

Table 1. Relative hypothalamic mRNA content for the three surgical groups by treatment (vehicle or leptin 2mg/kg IP) prior to the onset of the dark cycle. Time of sacrifice was 2h into the dark cycle. Quantification of hypothalamic mRNA content was
by RT-PCR expressed relative to GAPDH mRNA content for each animal. Data are means ± SEM.

Table 1

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<td>ADX-P</td>
<td>Vehicle</td>
<td>1.0±0.1</td>
<td>0.9±0.1</td>
<td>0.9±0.3</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>1.5±0.2</td>
<td>1.1±0.2</td>
<td>0.7±0.3</td>
</tr>
<tr>
<td>ADX-C</td>
<td>Vehicle</td>
<td>1.0±0.2</td>
<td>1.0±0.1</td>
<td>0.4±0.1</td>
</tr>
<tr>
<td></td>
<td>Leptin</td>
<td>1.2±0.2</td>
<td>1.4±0.1</td>
<td>0.8±0.2</td>
</tr>
</tbody>
</table>
Fig 1A

Hypothalamic IL-1β mRNA Content

- Sham
- ADX-P
- ADX-C
- Sham
- ADX-P
- ADX-C

Vehicle
Leptin

p = 0.04
p = 0.01
p = 0.02
p = 0.02
Fig 1B

2 Hour Food Intake (g)

- Sham
- ADX-P
- ADX-C
- Vehicle
- Leptin

p = 0.01

p = 0.01
Fig 3
Re-Fed

Fasted

Fed

p = 0.03

p = 0.03

1.2 1.0 0.8 0.6 0.4 0.2 0.0

IL-1β mRNA Content

Hypothalamic

Fig. 1