The hypothalamic paraventricular nucleus (PVN) integrates preautonomic and neuroendocrine control of energy homeostasis, fluid balance, and the stress response. We recently demonstrated that glucocorticoids act via a membrane receptor to rapidly cause endocannabinoid-mediated suppression of synaptic excitation in PVN neurosecretory neurons. Leptin, a major signal of nutritional state, suppresses CB1 cannabinoid receptor-dependent hyperphagia (increased appetite) in fasting animals by reducing hypothalamic levels of endocannabinoids. Here we show that glucocorticoids stimulate endocannabinoid biosynthesis and release via a Gαs–cAMP–protein kinase A-dependent mechanism and that leptin blocks glucocorticoid-induced endocannabinoid biosynthesis and suppression of excitation in the PVN via a phosphodiesterase-3B-mediated reduction in intracellular CAMP levels. We demonstrate this rapid hormonal interaction in both PVN magnocellular and parvocellular neurosecretory cells. Leptin blockade of the glucocorticoid-induced, endocannabinoid-mediated suppression of excitation was absent in leptin receptor-deficient obese Zucker rats. Our findings reveal a novel hormonal crosstalk that rapidly modulates synaptic excitation via endocannabinoid release in the hypothalamus and that provides a nutritional state-sensitive mechanism to integrate the neuroendocrine regulation of energy homeostasis, fluid balance, and the stress response.

**Key words:** leptin; glucocorticoids; endocannabinoids; neuroendocrine; stress; energy homeostasis

**Introduction**

We recently demonstrated that stress levels of glucocorticoids (GCs) cause fast, CB1 cannabinoid receptor-dependent retrograde inhibition of glutamatergic excitatory synaptic inputs to neurosecretory cells of the hypothalamic paraventricular nucleus (PVN) (Di et al., 2003, 2005a), which control the major endocrine systems involved in maintaining energy homeostasis and fluid balance and in the homeostatic adaptation to different kinds of stress. This effect, which we refer to here as GC-induced, endocannabinoid-mediated suppression of synaptic excitation (GSE), was demonstrated in different PVN parvocellular neuronal subpopulations that control the hypothalamic–pituitary–adrenal (HPA) axis and the hypothalamic–pituitary–thyroid (HPT) axis, as well as in the magnocellular neurons from the PVN and from the supraoptic nucleus (SON), which integrate the hypothalamic–neurohypophysial system. The HPA axis controls circulating levels of GCs, which are the main endocrine signal in the adaptation to stress and are also a major signal for the maintenance of energy homeostasis via control of metabolism and appetite (Castonguay, 1991; Sapolsky et al., 2000). Fasting is a metabolic stress that increases the circulating levels of GCs and decreases the circulating levels of leptin (Chowers et al., 1969; Ahima, 2000), the primary endocrine signal of the nutritional state. This hormonal profile sets the stage for vital behavioral and physiological adaptations that take place during caloric deficit to promote the reestablishment of energy balance. A major behavioral aspect of this homeostatic adaptation to fasting is the development of hyperphagia, which was shown to depend on a decrease in leptin levels, an increase in GC levels, and activation of central CB1 receptors (Castonguay, 1991; Ahima, 2000; Di Marzo et al., 2001). Thus, during fasting, circulating GCs and hypothalamic levels of endocannabinoids are increased and both are involved in hypothalamic stimulation of feeding (Tempel et al., 1993; Ahima, 2000; Di Marzo et al., 2001; Kirkham et al., 2002). Leptin, conversely, suppresses hyperphagia in fasting animals by reducing endocannabinoid levels in the hypothalamus, probably via blockade of endocannabinoid biosynthetic pathways (Di Marzo et al., 2001).
Together, these findings suggested to us that crosstalk between leptin and GCs may rapidly modulate hypothalamic neuronal activity via opposing regulation of endocannabinoid biosynthesis and release of the endocannabinoids andonoids, Newport, RI) was stored as a 2 mM solution in water and was freshly washed in ether to remove any residual free dexamethasone. AM251 [N-((piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide] (Tocris Cookson, Ballwin, MO) was stored as a 10 mM solution in DMSO. 2-AG (Tocris Cookson) was stored at 5 mg/ml in anhydrous ethanol. Murine leptin (Sigma) was reconstituted and stored as a 78 mM solution in 15 mM HCl plus 7.5 mM NaOH. Cilostamide (Cil) (Tocris Cookson) was stored as a 100 mM solution in DMSO. H-89 [N-(2-p-bromo-cinnamylamino)-ethyl]-5-isouquinoline-sulfonamide 2HCl (Calbiochem, La Jolla, CA) was stored as a 100 µM solution in DMSO. All bath-applied drugs were stored at −20°C and dissolved to their final concentrations in ACSF just before bath application.

**Drugs applied via the patch pipette.** (R)-Adenosine, cyclic 3’,5’- (hydrogenophosphorothioate) triethylammonium (cAMP-Rp), triethylammonium salt (Tocris Cookson) was stored as a 40 mM solution in water and diluted to its final concentration with the recording pipette solution. Rabbit antisera against rat Gaα and Gaα (Chemicon, Temecula, CA) were directly diluted in the recording pipette solution to a final dilution of 1:2000. Pipette tips were filled with regular pipette solution, and the pipettes were backfilled with the solution containing the test drug.

**Quantitative analysis of endocannabinoids in acute slices.** Two contiguous 350 µm coronal hypothalamic slices containing the PVN were sectioned on a vibratome, bisected along the midline and trimmed around the PVN by cutting laterally at the medial edge of the fornix, dorsally at the vertex of the third ventricle, and ventrally at ~1.5 mm from the base of the brain. Horizontal cerebellar slices (350 µm) were used as controls. Trimmed hemi-slices were stored for 2–3 h in ACSF at room temperature and then preincubated for 20 min in ACSF at 34°C. The two contiguous hemi-slices from each side were pooled together into control and test groups. Test slices were transferred to modified ACSF (maCSF) (aCSF containing 1 µM TTX, 10 µM bicuculline, and 0.025% BSA) with the specific drug treatment, whereas the contralateral control slices were transferred to maCSF without treatment for the same incubation time at 34°C. Test groups were treated as follows: Dex, 10 min of 1 mM dexamethasone in maCSF; Dex + Lep, preincubation in maCSF with leptin (10 nM) for 5 min before transfer to maCSF with leptin (10 nM) plus Dex (1 µM); Dex + H-89, preincubation in maCSF with H-89 (1 mM) for 5 min before transfer to maCSF with H-89 (1 µM) plus Dex (1 µM). Immediately after treatment, slices were collected and homogenized in 1 ml of ice-cold methanol in preparation for LC/MS/MS analysis. One milliliter samples of incubating buffer also were collected from each assay to control for changes in the levels of endocannabinoids in solution. LC/MS/MS analysis of the endocannabinoids AEA and 2-AG was performed on chloroform methanol (2:1) lipid extracts, which were

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**Materials and Methods**

**Animals.** Male Sprague Dawley rats (3–4 weeks) and lean Zucker (Fa/?) or obese Zucker (fa/fa) rats (5–6 weeks) (Charles River Laboratories, Wilmington, MA) were used according to a protocol approved by the Tulane University Institutional Animal Care and Use Committee and in accordance with United States Public Health Service guidelines.

**Slice preparation.** Rats were decapitated under deep pentobarbital sodium anesthesia (50 mg/kg body weight). The brain was immediately transferred to ice-cold (0–1°C), oxygenated artificial CSF (aCSF), blocked, and sliced. The composition of the aCSF was as follows (in mM): 140 NaCl, 3 KCl, 1.3 MgSO4, 1.4 NaH2PO4, 2.4 CaCl2, 11 glucose, and 5 HEPES. pH adjusted to 7.2–7.3 with NaOH. Two contiguous coronal hypothalamic slices (350 µm) containing the PVN were sectioned on a vibratome, bisected along the midline (i.e., at the third ventricle), and incubated in oxygenated aCSF at room temperature for >1.5 h before being transferred to the recording chamber. Hemi-slices were transferred to the recording chamber and allowed to equilibrate at 32–34°C for at least 20 min before recordings.

**Electrophysiological recording.** Whole-cell patch-clamp recordings were performed in putative magnocellular and parvocellular neurons at 32–34°C at a holding potential of ~65 mV in the presence of the voltage-gated sodium channel inhibitor tetrodotoxin (TTX) (1 µM) and the GABA<sub>a</sub> receptor blocker bicuculline (10 µM). Pipette tips were pulled from borosilicate glass (1.65 mm outer diameter, 1.2 mm inner diameter; KG33; Garner Glass, Claremont, CA) with a Flaming/Brown P-97 micropipette puller (Sutter Instruments, Novato, CA) to a resistance of 4–6 MΩ. The pipette solution contained the following (in mM): 120 K-gluconate, 10 KCl, 1 NaCl, 1 MgCl2, 1 EGTA, 2 Mg-ATP, 0.3 Na-GTP, and 10 HEPES, pH adjusted to 7.3 with KOH. The osmolality of the solution was adjusted to 300 mOsm with 20 mM D-sorbitol. Putative magnocellular and parvocellular cells were distinguished on the basis of their morphology, positions within the PVN, as well as the transient outward rectification seen in magnocellular and not in parvocellular neurons (Luther et al., 2000), and the parvocellular neuroendocrine cells were distinguished from the parvocellular preautonomic neurons by location and by their lack of low-threshold calcium spike, as described by Stern (2001) and Luther et al. (2002) (supplemental Fig. 1, available at www.jneurosci.org as supplemental material). After the whole-cell recording configuration was obtained, all cells were observed for 5–10 min for stability before a 3 min baseline control recording of miniature EPSCs (mEPSCs) was recorded. Drugs were applied immediately after baseline recordings.

**Drugs applied in the perfusion bath.** Water-soluble dexamethasone (Dex) (1 or 2.5 µM) and corticosterone (Cort) (1 µM) (Sigma, St. Louis, MO) were dissolved directly in aCSF to their final concentrations. Dexamethasone hemisuccinate–bovine serum albumin (Dex-BSA) (Ster...
analysis to detect parent/daughter ion pairs simultaneously. The selected mode to detect MS2 spectra and selected reaction mode for quantitative sheath gas was argon at 1.5 mTorr. The instrument was run on full scan running on negative ion detection mode. Electrospray voltage was 3 kV; triple quadrupole mass spectrometer (Thermo-Finnigan, San Jose, CA) ionization probe (Elkins-Sinn, Cherry Hill, NJ) on a TSQ Quantum solution A in 10 min. LC effluents were diverted to an electrospray-isocratic for 5 min, after which the system returned to 100% solvent 100% of solvent B (99.99:0.01 methanol/acetic acid) in 30 min, and run 45 min gradient protocol starting with solvent solution A (40:60:0.01 methanol/water/acetic acid, pH 4.5) at a flow rate of 300 l/min, reached 2.1 mm, 5 min) drug treatment as specified in the text. For biochemical analyses, one-way ANOVA was used for comparison between the mean endocannabinoid concentration in control slices and the mean endocannabinoid concentration in treated groups, and between treated groups. For this analysis, the values of control hemi-slices used in all experiments were pooled together. Probability values of <0.05 were considered significant.

**Results**

Leptin blocks glucocorticoid-induced biosynthesis of 2-AG and AEA in the PVN

Acute hypothalamic slices trimmed around the PVN were homogenized immediately after a 10 min treatment with the synthetic GC Dex (1 μM) and analyzed by LC/MS/MS. Dex caused a significant increase in the levels of the endocannabinoids AEA (control, 12.5 ± 2.1 pmol/mg protein; Dex, 40.5 ± 10.5 pmol/mg protein; n = 6; p < 0.0005) and 2-AG (control, 302.5 ± 83.9 pmol/mg protein; Dex, 685.5 ± 125.0 pmol/mg protein; n = 6; p < 0.001) (Fig. 1). The same treatment in cerebellum slices had no effect on levels of AEA (control, 4.1 ± 0.5 pmol/mg protein; Dex, 5.1 ± 0.8 pmol/mg protein; n = 6; p = 0.36) or 2-AG (control, 33.8 ± 10.7 pmol/mg protein; Dex, 52.3 ± 14.8 pmol/mg protein; n = 6; p = 0.34) (Fig. 1). A 5 min pretreatment with leptin (10 nM) blocked the Dex-induced increase in endocannabinoid levels and caused a rather significant reduction in 2-AG levels (155.4 ± 66.2 pmol/mg protein; n = 6; p = 0.0005) and a nonsignificant reduction in AEA levels (8.6 ± 2.4 pmol/mg protein; n = 6; p = 0.29) (Fig. 1). To control for possible elution of endocannabinoids into the incubation buffer, we also analyzed buffer samples, which revealed only negligible amounts of endocannabinoids in solution in both...
control and treated groups (data not shown). These results demonstrate a fast, GC-induced biosynthesis of AEA and 2-AG that is rapidly and completely blocked by leptin, thus revealing a non-genomic GC–leptin interaction that regulates endocannabinoid levels in the PVN.

**Leptin blocks the glucocorticoid-induced, endocannabinoid-mediated suppression of excitation in PVN magnocellular and parvocellular neuroendocrine cells**

Our LC/MS/MS analysis of PVN slices containing both parvocellular and magnocellular subdivisions showed a complete blockade of GC-induced endocannabinoid release by leptin, suggesting that leptin suppresses endocannabinoid release from each of the magnocellular and parvocellular populations of the PVN; we showed previously that multiple cell types from both the magnocellular and parvocellular neuroendocrine cell groups undergo GSE (Di et al., 2003, 2005a). To determine the neurophysiologic significance and the intracellular mechanisms of the GC–leptin interactions that regulate endocannabinoid biosynthesis and release, whole-cell patch-clamp recordings were performed in putative PVN magnocellular and parvocellular neuroendocrine cells to test for leptin blockade of GSE. In the magnocellular neurons, a 10 min bath application of Cort (1 μM) or Dex (2.5 μM) caused a rapid (~1 min onset) reduction in the frequency of mEPSCs (Cort, −32.6 ± 3.6%; n = 6; p < 0.05) and was blocked by the CB1 antagonist AM251 (1 μM) (+30.1 ± 18.0%; n = 7; p = 0.17) (Fig. 2B, D). A 5 min preapplication of leptin (10 nM), although without effect alone on mEPSCs, completely abolished GSE in all of the PVN magnocellular neurons tested (Lep + 2.5 μM Dex, +13.3 ± 7.0%; n = 6, p = 0.08; Lep + 1 μM Cort, +9.9 ± 23.1%; n = 6, p = 0.32) (Fig. 2C,D).

In putative parvocellular neuroendocrine cells, as reported previously (Di et al., 2003), a 10 min bath application of Dex (2.5 μM) also caused a rapid reduction (~1 min onset) in the frequency of mEPSCs (Dex, −36.6 ± 3%; n = 6; p < 0.01) without affecting mEPSC amplitude (Fig. 3A, C). Like in the magnocellular neurons, a 5 min preapplication of leptin (10 nM), although without effect alone on mEPSCs, also completely abolished GSE in all of the parvocellular neurons tested (Lep + 2.5 μM Dex, +9.6 ± 4%; n = 5; p = 0.28) (Fig. 3B, C). This indicated that leptin blockade of GSE is a common feature among the different PVN neurosecretory subpopulations.

**Leptin blockade of GSE is mediated by Ob-Rb-dependent activation of PDE-3B**

Genetically obese Zucker rats (fa/fa) are homozygous for a defective leptin receptor gene, which prevents leptin receptor signaling in these animals, whereas lean Zucker rats (FA/?) express at least one normal allele for the leptin receptor and present a normal response to leptin. Obese Zucker rats chronically show a physiological, hormonal, and behavioral profile similar to those of fasted rats, which includes hyperphagia, elevated circulating levels of GCs, and increased hypothalamic levels of endocannabinoids (Freedman et al., 1985; Ahima, 2000; Di Marzo et al., 2001). Leptin failed to block GSE in PVN magnocellular neurons in slices from obese Zucker rats (−32.9 ± 10.1%; n = 7; p < 0.01) but had an inhibitory effect on GSE in slices from lean Zucker rats (+3.0 ± 16.8%; n = 6; p = 0.39) (Fig. 4A–C) similar to that seen in slices from normal Sprague Dawley rats.

Because the anorexigenic effects of central leptin injection are mediated by leptin receptor-dependent activation of PDE-3B (Zhao et al., 2002), we tested whether the leptin blockade of GSE is PDE-3B dependent in magnocellular and parvocellular neuroendocrine cells from normal Sprague Dawley rats. Bath application of the PDE-3B inhibitor cilostamide (1 μM) prevented the leptin-mediated blockade of GSE in both putative magnocellular (−29.9 ± 5.9%; n = 6; p < 0.05) and parvocellular neuroendocrine (−31.4 ± 2%; n = 5; p < 0.05) cells (Fig. 5A–C). These results together indicate that the leptin blockade of GSE is mediated by a receptor encoded for by the Ob gene. RT-PCR analysis of PVN and supraoptic nucleus tissue punches confirmed the presence of mRNA encoding for Ob-Rb, the long splice variant of the leptin receptor encoded for by the Ob gene, in these hypothalamic nuclei (Fig. 6).
Glucocorticoid-induced synthesis of endocannabinoids is mediated by a Glucocorticoid receptor (G)–cyclic AMP (cAMP)–Protein Kinase A (PKA) pathway

Because PDE-3B activation causes a reduction of cellular cAMP levels by converting it to 5'AMP (Zhao et al., 2002; Sahu, 2004), our data indicate that the crosstalk between leptin and GC signaling pathways regulates endocannabinoid biosynthesis in PVN neuroendocrine cells by controlling cellular cAMP levels. Therefore, we tested in putative magnocellular neurons whether GCs are coupled to endocannabinoid biosynthesis via G-protein-coupled receptor activation of a cAMP-dependent pathway. Intracellular application (via the patch pipette) of an antiserum against the Glucocorticoid receptor subunit completely blocked GSE (−2.4 ± 8.2%; n = 6; p = 0.41), whereas a Glucocorticoid receptor subunit had no effect on GSE (Fig. 7A, C). Intracellular application of the AMPA/PKA antagonist cAMPS-Rp (50 μM) also blocked GSE (Fig. 7B, C). These findings suggest that GSE depends on the postsynaptic activation of a glucocorticoid receptor coupled to a stimulatory G-protein and cAMP. Additional LC/MS/MS provided direct evidence that PKA activation is necessary for the GC-induced endocannabinoid biosynthesis and release in the PVN. Thus, bath application of the PKA inhibitor H-89 (1 μM) blocked the Dex-induced increase in endocannabinoid levels in trimmed PVN slices, causing instead a significant decrease in 2-AG levels (AEA, 12.7 ± 4.9 pmol/mg protein, p = 0.99; 2AG, 105.4 ± 48.0 pmol/mg protein, n = 5, p < 0.05) (Fig. 7D). Consistent with GC actions at a putative membrane GC receptor, as shown in our previous studies (Di et al., 2003, 2005a), GSE in PVN magnocellular neurons was also seen with the membrane-impermeant Dex-BSA conjugate (2.5 μM) (−42.6 ± 5.2%; n = 9; p < 0.01) (Fig. 7C).

Discussion

Here we demonstrate a crosstalk between GC- and leptin-activated signaling pathways that rapidly controls endocannabinoid biosynthesis and release, thereby modulating CB1-dependent inhibition of excitatory synaptic inputs to PVN neurosecretory cells involved in the regulation of energy homeostasis, fluid balance, and the adaptation to stress. The rapid onset of GSE (−1 min) and of the leptin suppression of GSE (−5 min) indicates a nongenomic interaction between these hormones. Our data show that GCs stimulate endocannabinoid synthesis by activation of a membrane receptor coupled to a Glucocorticoid–cAMP–PKA pathway, which is consistent with studies showing that GCs activate a nongenomic, PKA-dependent signaling pathway in mouse neuroblastoma cells (Han et al., 2005) and that cAMP and PKA are required for GC-induced apoptosis in leukemic cells (Tiwari et al., 2005). Furthermore, Cadas et al.

Figure 4. Leptin fails to suppress GSE in PVN neurons from leptin receptor-deficient obese Zucker rats. A, Representative recording of mEPSCs in a PVN magnocellular neuron from an obese Zucker rat before (control) and after a 9-min application of Dex (2.5 μM) in the presence of 10 nM leptin (Dex + Lep). B, Time course of mean normalized mEPSC frequency recorded in PVN magnocellular neurons from lean (n = 6) and obese (n = 7) Zucker rats showing that 5 min leptin (10 nM) preapplication prevents GSE in lean but not in genetically obese Zucker rats. C, Summary histogram showing the mean normalized mEPSC frequency recorded over the last 3 min of a 10 min application of Dex or Lep + Dex in PVN magnocellular neurons from lean (n = 7 and 6, respectively) and obese (n = 5 and 7) Zucker rats. Means ± SE. *p < 0.05; **p < 0.01.

Figure 5. Leptin blocks GSE in both magnocellular and parvocellular PVN neurons by activating PDE-3B. A, Time course of normalized mean mEPSC frequency recorded from PVN magnocellular and parvocellular neurons showing that, in the presence of cilostamide (1 μM), 5 min preapplication of leptin (10 nM) failed to prevent GSE during 10 min application of 2.5 μM Dex. B, Summary histogram showing mean normalized mEPSC frequency recorded over the last 3 min of a 10 min application of Dex (n = 6), Lep + Dex (n = 5), or Cil + Lep + Dex (n = 5) in magnocellular neurons and of Dex (n = 6), Lep + Dex (n = 5), or Cil + Lep + Dex (n = 5) in parvocellular neurons. Means ± SE. *p < 0.05; **p < 0.01. In the summary histogram (B), the data from the groups Dex and Lep + Dex for both magnocellular and parvocellular neurons are repeated here for comparison.

Figure 6. RT-PCR analysis of the Ob-Rb leptin receptor isoform expression in rat PVN and SON. Using Ob-Rb-specific primers that yielded a 92 bp amplimer, the expression of Ob-Rb was detected in punches of PVN and SON from three individual rats (1, 2, 3) but not in rat liver. Although the same primers could amplify rat brain genomic DNA, no PCR product was detected in any of the PVN or SON samples in which reverse transcriptase was omitted (−RT), indicating no genomic DNA contamination. M, Marker; L, liver; G, genomic DNA.
Glucocorticoid-induced biosynthesis of endocannabinoids and the subsequent CB1-dependent suppression of excitatory inputs to PVN neurons were blocked completely by the PDE-3B-dependent actions of leptin, indicating that leptin prevents PKA-dependent endocannabinoid biosynthesis by reducing cAMP levels. The dependence on PDE-3B and the absence of effect in genetically obese Zucker rats indicate that leptin blockade of GSE is likely to be mediated by Ob-Rb, which is thought to be the only leptin receptor capable of activating PDE-3B in the brain (Sahu, 2004). Our RT-PCR data and previous immunohistochemical studies (Hakansson et al., 1998; Yarnell et al., 1998; Matsuda et al. 1999) indicate that Ob-Rb is expressed in the PVN. Because leptin suppresses activity-dependent release of endocannabinoids in the lateral hypothalamus (Jo et al., 2005), it will be interesting to test whether leptin can also block the oxytocin (OT)-, α-melanocyte-stimulating hormone- and activity-dependent release of endocannabinoids that has been demonstrated in different hypothalamic nuclei (Hirasawa et al., 2004; Di et al., 2005b; Jo et al., 2005; Sabatier and Leng, 2006).

Our LC/MS/MS analysis of PVN slices along with the data from our electrophysiological experiments indicate that leptin-mediated blockade of GSE is a common feature among PVN parvocellular and magnocellular neurosecretory cells. Such a broad regulatory influence over PVN neuroendocrine output is consistent with the well established nutritional state-dependent regulation by leptin, GCs, and endocannabinoids of different fundamental aspects of homeostasis. For instance, in fasting animals, circulating levels of leptin decrease whereas both circulat-

**Figure 7.** Signaling mechanism mediating glucocorticoid-induced retrograde endocannabinoid release. **A-C.** Left, Representative recordings before (control) and after −9 min of drug application; right, time course of mean normalized mEPSC frequency. **A.** The dexamethasone effect was inhibited by intracellular application of a Goαs antiserum (n = 6) but not a Goαq11 antiserum (n = 4). **B.** Intracellular application of a PKA antagonist, cAMPS-Rp (50 μM), inhibited the dexamethasone effect (n = 6). **C.** Summary histogram showing mean normalized mEPSC frequency recorded over the last 3 min of a 10 min application of membrane-impermeant Dex-BSA (n = 9) and of dexamethasone in the presence of the Goαs antiserum (n = 6), the Goαq11 antiserum (n = 4), and cAMPS-Rp (n = 8); means ± SE. *p < 0.05; **p < 0.01. **D.** LC/MS/MS analysis showing that the PKA antagonist H-89 (1 μM) blocked the Dex-induced rise in PVN levels of AEA and 2-AG; means ± SE. *p < 0.05 versus control group; **p < 0.001 versus control group; ***p < 0.0005 versus control group; #p < 0.05; ##p < 0.005 versus PVN (Dex) group. The PVN (control) (n = 16) and PVN (Dex) (n = 5) groups are the same presented in the Figure 1, which were used here for statistical comparison and for clarity.
dependent hyperphagia develops whenever leptin signaling is re-
duced or impaired.

It is noteworthy that the fasting-induced drop in leptin levels leads to the suppression of both the norepinephrine-dependent satiety response to cholecystokinin (CCK) and CCK-induced c-fos expression in both PVN parvocellular and magnocellular neurons (McMinn et al., 2000). Conversely, feeding, gastric dis-
tension, and systemic administration of CCK activate both par-
vocellular and magnocellular OT-secreting neurons of the PVN, causing a transient rise in circulating OT and central OT receptor-mediated anorexia (Verbalis et al., 1986; Renaud et al., 1987; Olson et al., 1991). Preautonomic parvocellular OT neu-
rons are thought to mediate CCK-induced short-term satiety (Olson et al., 1991, 1992; Ueta et al., 1993; Blevins et al., 2003), whereas peripheral OT secreted by magnocellular neurons has been shown to promote insulin-like anabolic adaptations appro-
piate to the sated state (Pittman et al., 1961). In adipose tissue, for instance, OT stimulates in vitro synthesis of protein and glyc-
cogen and facilitates lipogenesis but suppresses lipolysis (Mirsy and Periutti, 1961; Krahl, 1964; Muchmore et al., 1981). Fur-
thermore, peripheral OT may favor satiety by stimulating CCK release (Wu et al., 2002) and by potentiating glucose-induced insulin secretion in vitro and in vivo (Altszuler and Hampshire, 1981; Knudtzon, 1983; Gao et al., 1993; VanderWeele, 1994). It is likely, therefore, that the combination of high GC levels with concomitant low levels of leptin during fasting favors the release of endocannabinoids in the PVN, reducing the excitation of both parvocellular and magnocellular OT neurons in response to in-
puts triggered by satiety factors, such as CCK. This would con-
tribute to hyperphagia by causing a delay in satiation until leptin and GCs return to basal levels after nutritional recovery. This hypothesis is consistent with the findings of Blevins et al. (2004) showing that leptin potentiates the CCK-induced activation of PVN parvocellular OT neurons, which in turn activate brainstem neurons that control gastric motility and cause meal termination. However, it remains to be determined whether the GSE–leptin crosstalk also applies to these OT-secreting preautonomic neu-
rons of the PVN.

Another important aspect of the adaptation to fasting that has been shown to be under modulation by GCs, leptin, and endo-
cannabinoids is the decline in metabolic rate and energy expen-
diture resulting from the downregulation of the PVN TRH-
secreting neurons and reduced HPT axis outflow (Harris et al., 1978). Whereas GCs (Brabant et al., 1987) and exogenous canna-
binoids (Hillard et al., 1984) were shown to suppress the HPT axis, leptin prevents the fasting-induced suppression of the HPT axis (Ahima, 2000). We previously demonstrated GSE in PVN TRH neurons (Di et al., 2003), and our current results suggest that the drop in leptin levels in combination with the increase in GC levels during fasting is likely to favor GSE in PVN TRH-releasing neurons, contributing to the fasting-induced inhibition of the HPT axis.

Our findings are also consistent with abnormal HPA axis tone giving rise to increased appetite, obesity, and metabolic dysfunc-
tion (Freedman et al., 1985; Castonguay et al., 1986; Dallman and Stern, 1986; Argiles, 1989). For instance, in obese Zucker rats, which show characteristic eating and metabolic disorders, an in-
creased sensitivity to the central inhibitory GC feedback causes abnormally low basal secretion of both corticotrophin releasing hormone and vasopressin by parvocellular PVN neurons that control HPA axis at the posterior pituitary level (Plotzky et al., 1992). Our findings, along with the increased basal levels of hy-
opthalmal endocannabinoids observed in obese Zucker rats (Di

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