

Regulation of corticotropin-releasing factor and its types 1 and 2 receptors by leptin in rats subjected to treadmill running-induced stress

Qingling Huang, Elena Timofeeva and Denis Richard

Laval Hospital Research Center and Merck-Frosst/IRSC Obesity Research Chair, 2725 chemin Sainte-Foy, Québec, Canada G1V 4G5

(Requests for offprints should be addressed to D Richard; Email: denis.richard@crhl.ulaval.ca)

Abstract

The present study was conducted to investigate the long-term effects of subchronic elevation of central leptin levels on the expression of corticotropin-releasing factor (CRF) and its types 1 and 2 receptors in the brain of rats subjected to treadmill running-induced stress. PBS or recombinant murine leptin was infused continuously for a period of 5 days into the third ventricle of rats with the aid of osmotic minipumps at a delivery rate of 2 µg/day. On the fifth day of infusion, rats were killed under resting conditions or after a session of treadmill running, which is known to induce a stress response in rats. Leptin treatment significantly decreased food intake, body weight, white adipose tissue weight, glucose and insulin plasma contents, and blunted the treadmill running-induced elevation in plasma levels of corticosterone. Leptin infusion prevented stress-induced *de novo* synthesis of CRF in

the paraventricular hypothalamic nucleus (PVN), which was measured using the intronic probe for CRF heteronuclear RNA. The induction of the type 1 CRF receptor (CRF₁R) in the PVN and supraoptic nucleus in running rats was also significantly blunted by leptin. In contrast, leptin treatment strongly increased the expression of type 2 CRF receptor (CRF₂R) in the ventromedial hypothalamic nucleus (VMH). The present results suggest that subchronic elevation of central levels of leptin blunts treadmill running-induced activation of the hypothalamic–pituitary–adrenal axis through the inhibition of activation of the CRFergic PVN neurons, and potentially enhances the anorectic CRF effects via the stimulation of expression of CRF₂R in the VMH.

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Introduction

Corticotropin-releasing factor (CRF) is a 41 amino acid peptide widely distributed throughout the brain, and particularly concentrated in the medial parvocellular division of the hypothalamic paraventricular nucleus (PVN; Vale *et al.* 1981, Sawchenko & Swanson 1985). The CRF neurons of the parvocellular PVN are predominantly involved in the control of the hypothalamic–pituitary–adrenal (HPA) axis. Activation of all components of the HPA axis, including the stimulation of PVN CRFergic neurons and the significant increase in adrenocorticotropin (ACTH) and corticosterone plasma levels, is seen in rats subjected to stressful conditions such as treadmill running (Richard *et al.* 1996, Timofeeva *et al.* 2003). After a session of treadmill running, about all PVN CRFergic neurons express the immediate early gene *c-fos*, a marker of persisting neuronal activity (Timofeeva *et al.* 2003). Besides its stress-related hypophysiotropic effects, CRF blunts energy balance through concomitantly reducing energy intake and augmenting energy expenditure (Richard *et al.* 2002). Central administration of CRF decreases food intake, as well as food hoarding, and increases thermogenesis (Gosnell *et al.* 1983, Rothwell 1990, Cabanac & Richard 1995, Buwalda *et al.* 1997).

The central effects of CRF are mediated through two distinct high-affinity membrane receptors, which are referred to as CRF types 1 (CRF₁R; Perrin *et al.* 1993) and 2 (CRF₂R; Lovenberg *et al.* 1995) receptors. The CRF₁R is widely distributed throughout the brain and the anterior and intermediate lobes of the pituitary. Within the PVN, CRF₁R mRNA is barely detectable under basal conditions, but can be readily induced by stressful stimuli (Rivest *et al.* 1995). The CRF₁R has been suggested to mediate the corticotropic effects of CRF and assure basal and stress-induced HPA axis activity (Desouza *et al.* 1991). The expression of the CRF₂R is restricted to the particular limbic regions and the ventromedial hypothalamic nucleus (VMH). The VMH is thought to mediate the anorectic effects of CRF through the CRF₂R (Vaughan *et al.* 1995), and lesions of VMH increase food intake and lead to obesity (Egawa *et al.* 1993, Dube *et al.* 1999). The expression of the CRF₂R in the VMH is significantly lower in genetically obese rats than in lean rats (Richard *et al.* 1996, Timofeeva & Richard 1997). There is evidence that the central effects of leptin may be mediated at least partially through the VMH, where the leptin receptors are expressed (Elmqvist *et al.* 1998a,b, Nishiyama *et al.* 1999).

The adipocyte-derived hormone leptin circulates in the blood in proportion to body adiposity (Zhang *et al.* 1994,

Campfield *et al.* 1995) and has a marked potential in reducing energy deposition by decreasing food intake and increasing energy expenditure (Halaas *et al.* 1995, Pellemounter *et al.* 1995, Levin *et al.* 1996, Huang *et al.* 1998). Leptin may affect the HPA axis at different levels. Plasma leptin is inversely correlated with diurnal variations in glucocorticoid levels (Korbonits *et al.* 1997, Licinio *et al.* 1997). Leptin administration inhibits adrenal glucocorticoid synthesis and release (Bornstein *et al.* 1997, Kruse *et al.* 1998), and may reduce the ACTH stress-induced secretion (Trottier *et al.* 1998, Oates *et al.* 2000, Wilson *et al.* 2005). The effects of leptin on the synthesis and secretion of CRF from the PVN remain largely controversial. *In vitro* studies have demonstrated a stimulatory (Costa *et al.* 1997, Raber *et al.* 1997) as well as an inhibitory (Heiman *et al.* 1997) effect of leptin on CRF release. Acute administration of leptin may induce the expression of *c-fos* (Van Dijk *et al.* 1996, Elmquist *et al.* 1997, 1998a, Badoer & Ryan 1999, Elias *et al.* 2000) and increases the CRF mRNA levels (Nishiyama *et al.* 1999) in the PVN. However, chronic administration of leptin reduces ACTH and glucocorticoids secretion and inhibits stress-induced Fos expression in the PVN neurons (Huang *et al.* 1998, Oates *et al.* 2000).

The present study was designed to investigate the long-term effects of chronically elevated central leptin levels on the expression of the primary CRF transcript and CRF types 1 and 2 receptors in the brain of rats stressed by treadmill running. The results of this study suggest that daily leptin infusion inhibits stress-induced activation of PVN CRFergic neurons by blunting *de novo* CRF synthesis as well as the induction of CRF₁R. In addition, the present results confirm the ability of centrally infused leptin to increase the expression of VMH CRF₂R, which could potentially enhance the anorectic effects of CRF.

Materials and Methods

Animals and treatments

Thirty-two adult male Wistar rats were purchased from the Canadian Breeding Laboratories (St Constant, Quebec, Canada). All rats were cared for and handled according to the Canadian Guide for the Care and Use of Laboratory Animals and the present protocol was approved by our institutional animal care committee. The rats were individually housed in wire-bottom cages that were suspended above absorbent paper, and a stock diet was available *ad libitum* (Charles River Rodent Animal Diet, distributed by Ralston Products, Woodstock, Ontario, Canada). They were subjected to a 12 h light:12 h darkness cycle (light on 0645 h) and kept under an ambient temperature of 23 ± 1 °C.

On the tenth day after arrival, all rats were subjected to brain surgery to implant a guide cannula into the third ventricle. Rats were first anesthetized intraperitoneally with 1.5 ml mixture containing 20 mg/ml ketamine and 2.5 mg/ml xylazine and placed in a stereotaxic apparatus

(David Kopf Instruments, Tujunga, CA, USA). Then, a 22 gauge guide cannula was aimed at the third ventricle using the following stereotaxic coordinates: 2.3 mm posterior to bregma at the midline and 8.4 mm ventral to the brain surface (Paxinos & Watson 1997). The guide cannula was secured with screws and dental cement (Dentsply International, York, PA, USA) and was directly connected to the Alzet osmotic minipump (Model 2001; ALZA Pharmaceuticals, Palo Alto, CA, USA; 1.0 µl/h), which contained PBS (pH 7.4) or recombinant murine leptin (2 µg/day per rat; high purity, endotoxin level < 0.1 ng/µg; US Biological, Swampscott, MA, USA). PBS or leptin was continuously infused into the third ventricle for 5 days. Thereafter, the rats were killed either (i) at rest (PBS-resting; leptin-resting), (ii) immediately following a treadmill running session lasting 60 min (PBS-running; leptin-running), or (iii) at rest following a recovery period of 120 min after 60-min running (PBS-post-running; leptin-post-running). The running session took place between 0730 and 0910 h. During running, the speed of the motor-driven treadmill was raised from 10 to 25 m/min by increase of 5 m/min every 15 min. The rats were forced to run continuously for 60 min. Each experimental group included five to six rats.

Body weight, food intake, and tissue weight

Measurements of body weight and food intake were performed every morning between 0700 and 0800 h. Rats weighed between 270 and 290 g at the time of surgery. Epididymal white adipose tissue (eWAT) was quickly dissected out during intracardial perfusion with saline.

Plasma determinations

An intracardial blood sample was taken from anesthetized rats immediately before the intracardial perfusion. Serum corticosterone was determined by a competitive protein-binding assay (sensitivity 0.058 nmol/l; inter-assay coefficient of variation 9.0%) using plasma from a dexamethasone-treated female rhesus monkey as the source of transcortin (Murphy 1967).

Brain preparation

Rats were anesthetized with 1.5 ml mixture containing 20 mg/ml ketamine and 2.5 mg/ml xylazine. Without delay, they were intracardially perfused with 50 ml ice-cold isotonic saline followed by 500 ml paraformaldehyde (4%) solution. Brains were removed and kept in paraformaldehyde for an additional period of 7 days. They were then transferred overnight to a solution containing paraformaldehyde and sucrose (10%) and sliced using a sliding microtome (HistoSlide 2000, Reichert-Jung, Heidelberg, Germany). Brain sections were taken from the olfactory bulb to the brain stem. Thirty micrometer thick sections of brains were collected and stored at -30 °C in a cold cryoprotecting solution containing

sodium phosphate buffer (50 mM), ethylene glycol (30%), and glycerol (20%).

In situ hybridization histochemistry

In situ hybridization histochemistry was used to localize CRF heteronuclear (hn) RNA, CRF₁R mRNA, and CRF₂R mRNA on the brain sections taken from the entire brain. The protocol used was largely adapted from the technique described by Simmons *et al.* (1989). Briefly, one out of every five brain sections were mounted onto poly-L-lysine coated slides. The sections were then successively fixed in paraformaldehyde (4%), digested with proteinase K (10 µg/ml in 100 mM Tris-HCl containing 50 mM EDTA (pH 8.0)), acetylated with acetic anhydride (0.25% in 0.1 M triethanolamine (pH 8.0)), and dehydrated through graded concentrations (50, 70, 95, and 100%) of alcohol. Thereafter, 90 µl hybridization mixture, which contains an antisense ³⁵S-labeled cRNA probe (10⁷ c.p.m./ml), were spotted on each slide. The slides were sealed with the coverslips and incubated overnight at 58 °C in a slide warmer. The next day, the coverslips were removed and the slides were rinsed four times with SSC (0.6 M NaCl, 60 mM trisodium citrate buffer (pH 7.0)), digested with RNase A (20 µg/ml in 10 mM Tris-500 mM NaCl containing 1 mM EDTA), washed in descending concentrations of SSC (2×, 10 min; 1×, 5 min; 0.5×, 5 min; 0.1×, 30 min at 60 °C) and dehydrated through graded concentrations of alcohol. Slides were exposed to an X-ray film (Kodak). Once removed from the autoradiography cassettes, the slides were defatted in xylene, dipped in NTB2 nuclear emulsion (Eastman Kodak), and exposed from 7 to 21 days, before being developed in D 19 developer and fixed in rapid fixer (Kodak). Finally, tissues were rinsed in distilled water and counterstained with thionin (0.25%).

Antisense ³⁵S-labeled cRNA probes

The hn CRF cRNA probe was generated from 530 bp fragment of the CRF intron 1 (Dr S Watson, University of Michigan, Ann Arbor, MI, USA) subcloned into pGem-3 plasmid (Stratagene, La Jolla, CA, USA), and linearized with HindIII and EcoRI (Pharmacia Biotech, Inc.) for antisense and sense probes respectively. The CRF₁R cRNA was generated from a 1.3 kb PstI fragment of the rat CRF₁R cDNA (Dr W Vale, Peptide Biology Laboratory, The Salk Institute, La Jolla, CA, USA) subcloned into pBluescript-SKII plasmid (Stratagene), and linearized with BamHI and EcoRI (Pharmacia Biotech, Inc.) for antisense and sense probes respectively. The rat CRF₂R cRNA probe was prepared from a 275 bp fragment of the 5'-region cDNA of the CRF₂α receptor (Dr T W Lovenberg, Neurocrine Biosciences, Inc., San Diego, CA, USA) subcloned into a pBluescript SK+ vector (Stratagene), and linearized with EcoRI and BamHI for antisense and sense probes respectively. The radioactive riboprobes were synthesized, extracted, and precipitated as described elsewhere (Timofeeva *et al.* 2003, 2005). The

specificity of each probe was confirmed by the absence of positive signal in sections hybridized with sense probe.

Quantitative analysis of the hybridization signals

The hybridization signals revealed on NTB2 dipped nuclear emulsion slides were analyzed and quantified under a light microscope (Olympus, BX50) equipped with a black and white video camera (Sony, XC-77) coupled to a Macintosh computer (Power PC 7100/66) using Image software (version 1.55 non-FPU; Wayne Rasband, NIH, Bethesda, MD, USA). The optical density (OD) of the hybridization signal was measured under dark field illumination. Brain sections were matched for rostrocaudal levels as closely as possible according to the atlas of the rat brain (Paxinos & Watson 1997). The OD for each specific region was corrected for the average background signal, which was determined by sampling an unlabeled area outside the areas of interest. When no hybridization signal was visible under dark field illumination, the brain structures of interest were outlined under bright field illumination and then subjected to densitometric analysis under dark field illumination. Saturation of the hybridization signal was avoided by the creation of the calibration density profile plot for the strongest hybridization signals sampled for each region in every series. The luminosity of the system was then corrected, so that for the strongest hybridization signals, the reading OD did not exceed the half of the pixel value maximum, and this luminosity was conserved throughout the analysis of the entire series. The mean of OD for individual animals was used to determine the mean ± S.E.M. for each group.

Statistical analysis

A 2 × 3 ANOVA was used to examine the main and interaction effects of infusion (I; PBS, leptin) and activity (A; resting, running, and post-running) on the various dependent variables measured in this study. In the cases of significant I × A interactions, *a posteriori* comparisons were performed using the Bonferroni-Dunn multiple comparison procedure.

Results

Body weight, weight of white fat tissue, and food intake

Figure 1 and Table 1 demonstrate the effects of an intracerebroventricular infusion of leptin on body weight, food intake, and weight of eWAT. Infusion of leptin led to a substantial decrease in food intake and body weight. The effect of leptin on food intake and body weight was already significant on the first or the second day of infusion. Cumulative food intake measured upon five consecutive days of infusion was two times higher in PBS-treated rats than in leptin-infused animals (Table 1). The weight of the eWAT was also affected by leptin. Five days of leptin infusion produced a twofold reduction in eWAT weight compared

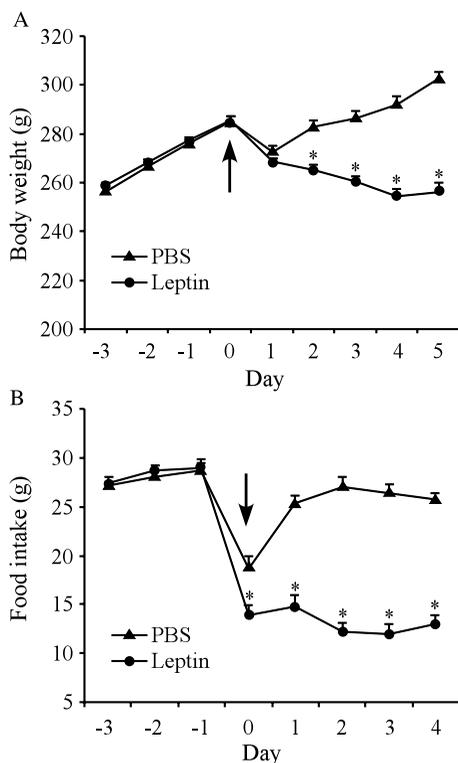


Figure 1 Daily measurements of (A) body weight and (B) food intake in rats infused intracerebroventricularly with PBS or recombinant murine leptin (2 µg/day per rat). Values are means \pm S.E.M. *Significantly different from PBS-infused rats. Arrow indicates start of infusion.

with PBS-treated animals (Table 1). Acute treadmill running did not noticeably affect body weight or eWAT weight.

Plasma glucose, insulin, and corticosterone

Table 1 presents the plasma levels of glucose, insulin, and corticosterone in PBS- and leptin-infused rats in resting, running, or post-running conditions. Plasma glucose and insulin levels were significantly lower in leptin-treated rats compared with PBS controls. Plasma corticosterone levels were not apparently different between resting leptin- and PBS-treated rats and noticeably increased in running rats in both the PBS- and leptin-infused animals. It is noteworthy that the increase in plasma corticosterone levels induced by running was apparently blunted by leptin infusion; increases in corticosterone response to running were 22.9 vs 14.5 times above basal levels in PBS and leptin rats respectively.

Expression of the CRF hnRNA in the PVN

The effects of leptin treatment and running stress on the expression of the CRF gene were evaluated by measuring the expression of CRF hnRNA using an intronic probe complementary to the intron 1 of the rat CRF gene. Intronic

Table 1 Final body weight (BW), cumulative food intake, epididymal white adipose tissue (eWAT), and plasma variables of the PBS- and leptin-infused Wistar rats before (resting), during (60-min; running), and after (120-min; post-running) treadmill running session. Values are presented as means \pm S.E.M.

	Rest		Running		Post-running		ANOVA (P)	
	PBS (n=5)	Leptin (n=5)	PBS (n=5)	Leptin (n=6)	PBS (n=5)	Leptin (n=6)	Infusion (I)	Activity (A)
Final BW (g)	305.2 \pm 6.4	252.8 \pm 5.8	293.6 \pm 2.9	252.6 \pm 6.9	290.9 \pm 4.4	249.5 \pm 7.9	<0.0001	0.3700
Cumulative food intake (g)	126.3 \pm 4.8	64.9 \pm 4.1	123.7 \pm 3.5	63.3 \pm 7.5	119.586 \pm 6.6	68.9 \pm 7.6	<0.0001	0.9445
eWAT (g)	1.122 \pm 0.057	0.550 \pm 0.103	1.144 \pm 0.102	0.822 \pm 0.098	1.111 \pm 0.110	0.673 \pm 0.079	<0.0001	0.3017
Plasma								
Glucose (mmol/l)	8.758 \pm 0.303	7.770 \pm 0.646	10.823 \pm 0.266	8.371 \pm 0.441	8.569 \pm 0.274	7.705 \pm 0.264	0.0001	0.0013
Insulin (nmol/l)	0.458 \pm 0.071	0.091 \pm 0.018*	0.356 \pm 0.050	0.269 \pm 0.052 [†]	0.210 \pm 0.023	0.083 \pm 0.013*	<0.0001	0.0016
Corticosterone (µmol/l)	0.065 \pm 0.055	0.073 \pm 0.041	1.491 \pm 0.116	1.058 \pm 0.064	0.501 \pm 0.166	0.339 \pm 0.181	0.0587	<0.0001
Interaction (I/A)								0.6049
								0.6410
								0.4285
								0.0935
								0.0071
								0.2079

Values were compared by 2 \times 3 ANOVA. Significant I/A interactions were further analyzed using Bonferroni-Dunn multiple comparison procedure. *Significantly different from the PBS-infused rats within the same activity. [†]Significantly different from the resting rats within the same infusion, $P < 0.05$.

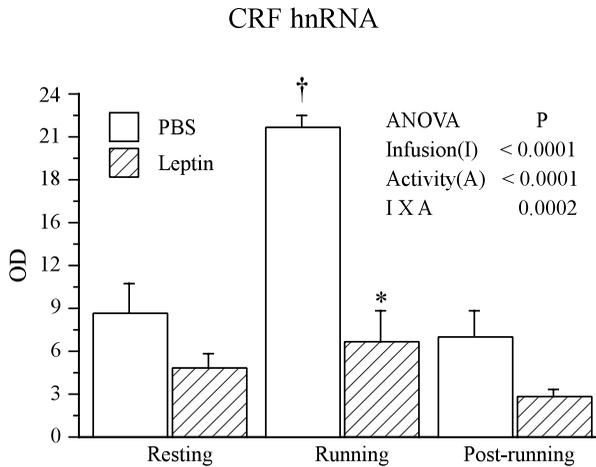


Figure 2 The optical densities (ODs) of the hybridization signals of the CRF heteronuclear (hn) RNA in the paraventricular hypothalamic nucleus (PVN) in resting, running, and post-running rats injected with PBS or leptin. The main and interaction effects of infusion (I; PBS, leptin) and activity (A; resting, running, or post-running) were determined using 2 × 3 ANOVA, followed by a *posteriori* Bonferroni–Dunn multiple comparison procedure. *Significantly different from the PBS-infused rats within the same activity; †significantly different from the resting rats within the same infusion, $P < 0.05$.

probe allows the rapid detection of the primary transcript of CRF (Rivest *et al.* 1995, Lacroix *et al.* 1996). The expression of CRF hnRNA increased during treadmill running in PBS, but not in leptin-treated rats (Figs 2 and 3). The levels of hnCRF transcript in PBS-treated rats returned to the basal values 2 h after running (Figs 2 and 3).

Expression of the CRF₁R mRNA in the PVN and supraoptic nucleus (SON)

The CRF₁R transcript was barely detectable in resting rats in both the PVN and SON. However, CRF₁R mRNA was significantly induced in these structures in PBS-treated rats after treadmill running. The increase in the CRF₁R mRNA expression in both the PVN and SON in PBS-infused animals was not observed immediately after running, but during the post-running period, as shown on the histogram illustrating the ODs of the hybridization signal of the CRF₁R mRNA in both structures (Fig. 4), and on the photomicrographs taken at the level of the PVN (Fig. 5) and SON (Fig. 6). The chronic leptin infusion significantly blunted the induction of expression of the CRF₁R mRNA during the post-running period in the PVN and SON (Figs 4–6). In both structures, the increase in CRF₁R mRNA expression after running was significantly low in leptin-infused rats compared with PBS-treated animals.

Expression of the CRF₂R mRNA in the VMH

The levels of expression of the CRF₂R mRNA in the VMH were significantly higher in leptin-infused rats compared with the PBS-treated animals. The analysis of the ODs of the hybridization signal of CRF₂R mRNA is shown in Fig. 7. The photomicrographs in Fig. 8 depict the positive signal for CRF₂R transcript in the VMH of resting rats infused with PBS or leptin. Resting, running, and post-running leptin-infused rats demonstrated higher levels of CRF₂R mRNA

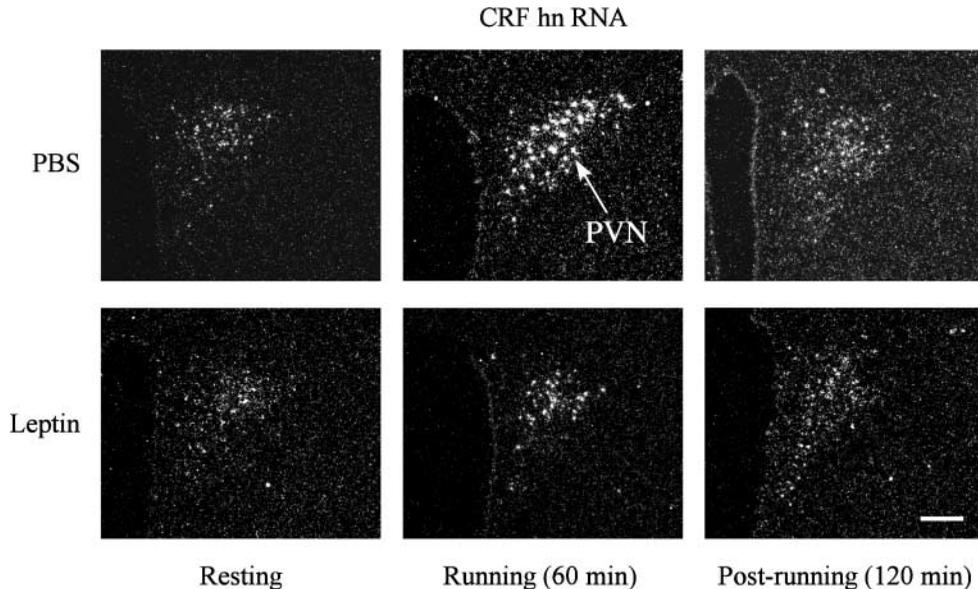


Figure 3 Dark field photomicrographs of coronal brain sections taken from the hypothalamic paraventricular nucleus (PVN), illustrating positive hybridization signal of the CRF heteronuclear (hn) RNA. The brain sections (30 μm thick) were obtained from PBS (top panels) or leptin (bottom panels) infused rats before (resting), during (running), or after (120-min post-running) treadmill running. The scale bar corresponds to 200 μm.

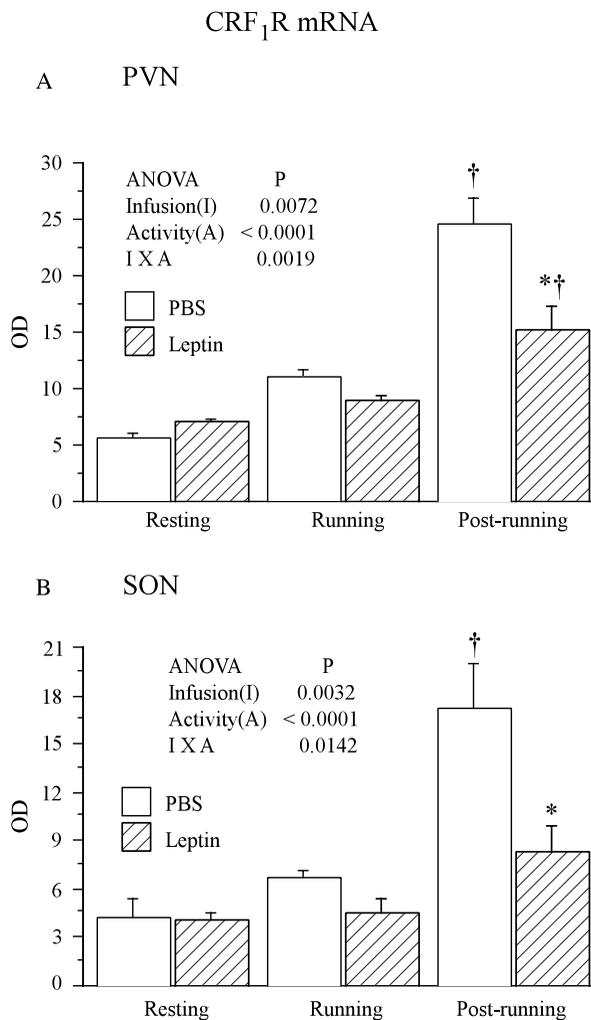


Figure 4 The optical densities (ODs) of the hybridization signals of the CRF₁R mRNA in the paraventricular hypothalamic nucleus (PVN; A) and the supraoptic nucleus (SON; B) in resting, running, and post-running rats injected with PBS or leptin. The main and interaction effects of infusion (I; PBS, leptin) and activity (A; resting, running, or post-running) were determined using 2 × 3 ANOVA, followed by a *posteriori* Bonferroni–Dunn multiple comparison procedure. *Significantly different from the PBS-infused rats within the same activity; †significantly different from the resting rats within the same infusion, $P < 0.05$.

than PBS-treated animals, but within the leptin- or PBS-treated groups, the expression of CRF₂R mRNA did not change during or after a session of treadmill running (Fig. 7).

Discussion

The present study demonstrates that the subchronic brain infusion of leptin alters the stress response induced by treadmill running in rats (Richard *et al.* 1996, Timofeeva

et al. 2003). Leptin lessens corticosterone secretion as well as the expression of CRF and the CRF₁R mRNA in rats subjected to 1 h treadmill running. Our results also confirm the stimulatory effects of leptin on the expression of the CRF₂R mRNA in the VMH (Nishiyama *et al.* 1999).

Although, daily intracerebroventricular leptin infusion did not affect plasma levels of corticosterone in resting animals, it considerably blunted running-induced hypercorticism. This finding is consonant with previous results that demonstrated the ability of leptin to reduce the HPA axis activity in response to stress through actions exerted on the various components of the pituitary–adrenal system. Leptin has been reported to inhibit both the synthesis and release of adrenal glucocorticoids (Bornstein *et al.* 1997, Kruse *et al.* 1998), and reduce the magnitude and duration of stress-induced ACTH secretion (Trottier *et al.* 1998, Oates *et al.* 2000). In addition, it has been demonstrated that leptin may prevent the response of the pituitary–adrenal axis to insulin administration in rat (Giovambattista *et al.* 2000) and attenuate the elevation in plasma cortisol and ACTH following an unpredictable stress in rhesus monkeys (Wilson *et al.* 2005).

The present results also suggest that leptin may reduce the hypothalamic drive exerted by the hypophysiotropic CRF neurons on the HPA axis. Leptin infusion suppressed the expression of CRF in the PVN neurons during stress. In fact, while treadmill-induced expression of CRF hnRNA was readily detected in the parvocellular PVN of PBS control rats, it was barely apparent in rats chronically infused with leptin. The use of the intronic probe allows for the detection of the CRF primary transcript, whose brain constitutive expression is low but rapidly increases in the PVN in response to stressful conditions (Rivest *et al.* 1995, Lacroix *et al.* 1996). Whether the leptin effect on stress-induced activation of the CRF gene is due to a direct leptin action on the PVN neurons or involves the inhibition of larger assemblies of stress-related circuits has yet to be untangled. Leptin might directly affect the CRF-producing parvocellular PVN neurons as these cells moderately express the leptin receptor (Elmqvist *et al.* 1998b). Leptin could increase the inhibitory tone on the hypothalamic neurons (Jo *et al.* 2005).

The PVN induction of CRF hnRNA after treadmill running was accompanied by the induction of the CRF₁R gene, which was not only seen in the PVN but also in the SON. Several lines of evidence emerging from studies using CRF₁R transgenic/knockout mice, CRF₁R antisense oligonucleotide, and CRF₁R agonists/antagonists have suggested that CRF₁R mediates the endocrine, autonomic, and behavioral effects of CRF during stress (for review, see Behan *et al.* 1996, Takahashi 2001, Bale & Vale 2004). In resting conditions, the CRF₁R transcript is barely detectable in the PVN, but it is readily induced by stress (Rivest *et al.* 1995). The induction of CRF₁R in the PVN has indeed been shown in several stress paradigms (Desouza *et al.* 1991, Rivest *et al.* 1995, Timofeeva & Richard 1997, Timofeeva *et al.* 2003, Wang *et al.* 2004) and has to be seen as a valid marker of stress. The present study reports for

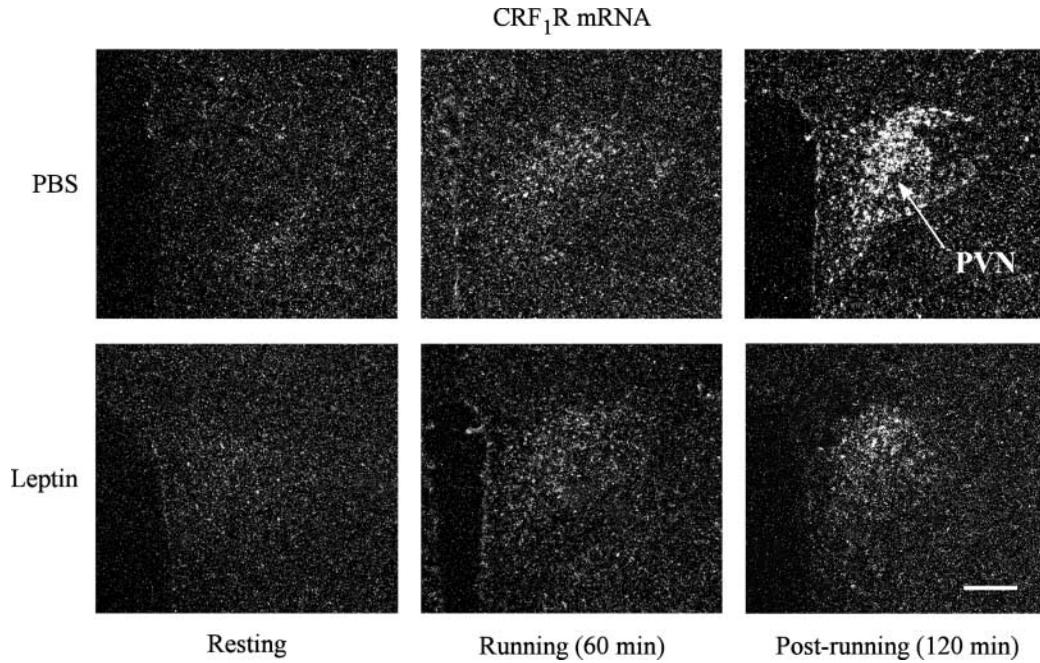


Figure 5 Dark field photomicrographs of coronal brain sections taken from the paraventricular hypothalamic nucleus (PVN) illustrating effects of leptin on CRF₁R mRNA before (resting), during (running), or after (120-min post-running) treadmill running. The scale bar corresponds to 200 μm.

the first time that leptin may blunt the stress-induced expression of CRF₁R in both the PVN and SON. The mechanism underlying this action remains to be delineated. The observation that the PVN expression of CRF₁R

followed that of CRF hnRNA nonetheless suggests that the reduced expression of CRF₁R following leptin might be a consequence of a leptin-induced reduction in the CRF tone, all the more since CRF has been reported to trigger the

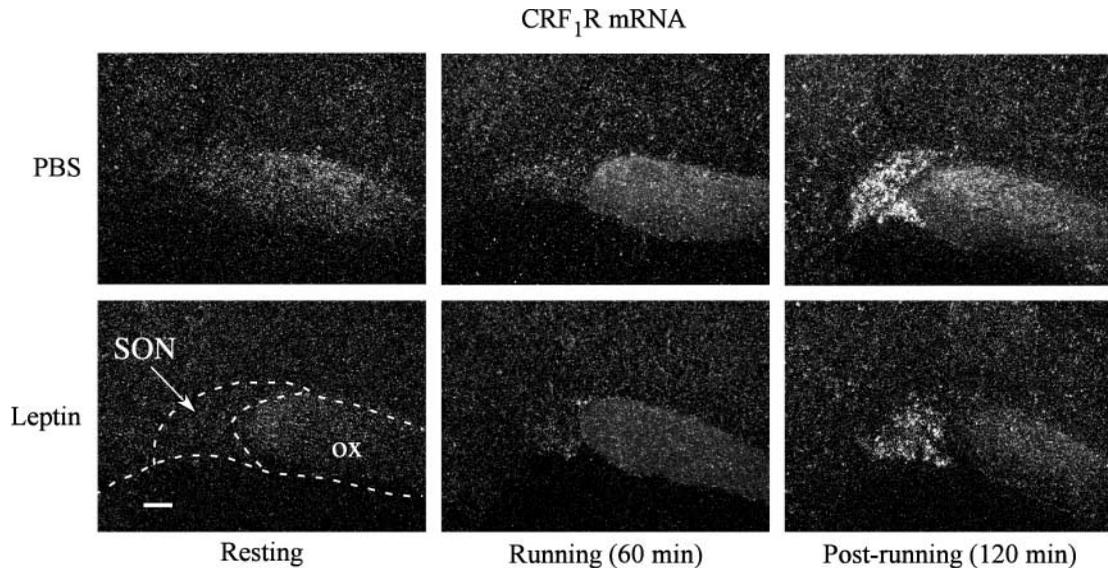


Figure 6 Dark field photomicrographs of coronal brain sections taken from the supraoptic nucleus (SON) illustrating effects of leptin on CRF₁R mRNA before (resting), during (running), or after (120-min post-running) treadmill running. The scale bar corresponds to 200 μm. OX, optic chiasm.

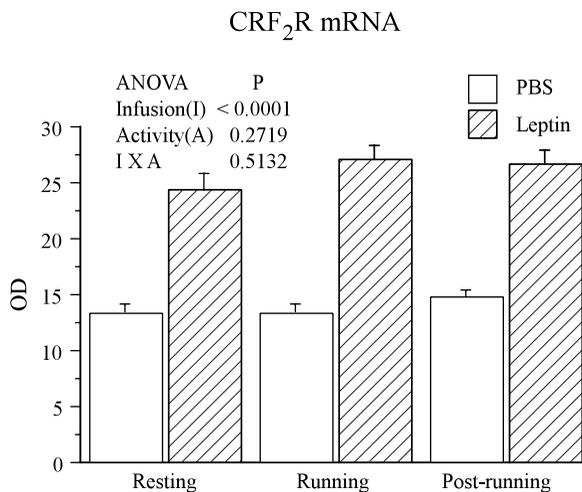


Figure 7 The optical densities (ODs) of the hybridization signals of the CRF₂R mRNA in the ventromedial hypothalamic nucleus (VMH) in resting, running, and post-running rats infused with PBS or leptin. The main and interaction effects of infusion (I; PBS, leptin) and activity (A; resting, running, or post-running) were determined using 2 × 3 ANOVA.

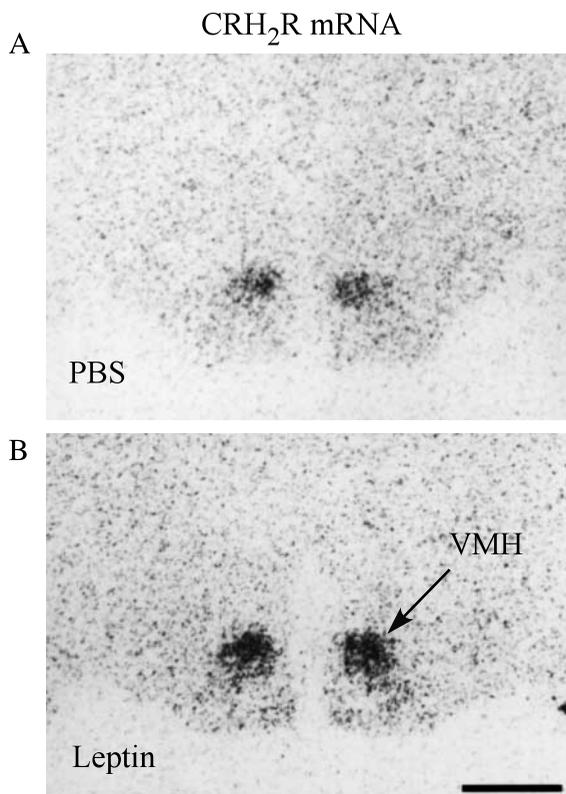


Figure 8 Film autoradiograms of coronal brain sections demonstrating the effects of leptin on CRF₂R transcript at the level of the ventromedial hypothalamic nucleus (VMH). The brain sections (30 μm thick) were obtained from resting rats infused with PBS (A) or leptin (B). The scale bar corresponds to 1 mm.

expression of its own type 1 receptor (Mansi *et al.* 1996). The CRF₁R seemingly favors further activation of CRF PVN neurons via an ultrashort positive feedback loop (Imaki *et al.* 2001). It is noteworthy that the repressing effect of leptin on CRF₁R mRNA was also seen in the SON, which is not recognized to markedly express CRF (Kawata *et al.* 1983, Merchenthaler 1984). Furthermore, the possibility for a direct effect of leptin on SON CRF₁R expression is weak due to the reported absence of the leptin receptors in this hypothalamic structure (Elmquist *et al.* 1998b).

In addition to binding to the CRF₁R, CRF also binds to the CRF₂R, which could be involved in the anorectic effects of CRF (Vaughan *et al.* 1995, Richard *et al.* 2000, 2002). The present study demonstrates an increased expression of CRF₂R mRNA in the VMH after leptin infusion into the brain. The levels of the CRF₂R transcript were independent of the running activity, but were about two times higher in leptin-infused rats compared with PBS-treated animals. These data are in agreement with the reported increase in the VMH CRF₂R expression after systemic administration of leptin (Nishiyama *et al.* 1999) and the positive correlation between the levels of VMH CRF₂R expression and plasma leptin concentrations (Makino *et al.* 1998). We have observed a decrease in the expression of CRF₂R in the VMH of food-deprived rats and in rats with uncontrolled diabetes, which exhibit decreases in plasma leptin levels (Timofeeva & Richard 1997, Huang *et al.* 2006). Leptin signaling deficiency due to the lack of leptin as in *ob/ob* mice, or to the dysfunctional leptin receptor as in *fa/fa* Zucker rats, also leads to a decrease in CRF₂R expression in the VMH (Richard *et al.* 1996, Timofeeva & Richard 1997). Leptin may directly affect the VMH neurons, which express the leptin receptor and exhibit leptin-induced Fos expression (Elmquist *et al.* 1997, 1998b, Elias *et al.* 2000). Through the expression of CRF₂R in the VMH, leptin may potentiate the anorectic effects of CRF.

In conclusion, the results of the present study provide evidence for the inhibitory effect of continuous central leptin infusion on the treadmill running-induced activation of the CRF hypophysiotropic system. Chronic leptin infusion prevented stress-induced *de novo* synthesis of CRF, and significantly decreased the induction of expression of CRF₁R in the PVN. In addition, leptin infusion increased the expression of the CRF₂R mRNA in the VMH. These results provide evidence that chronic increase in central leptin signaling may blunt the HPA axis response to stress and increase the anorectic effects of CRF.

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