Role of Corticotrophin-Releasing Factor in Effects of Leptin on Sympathetic Nerve Activity and Arterial Pressure


Abstract—Leptin and corticotrophin-releasing factor increase sympathetic nervous activity to interscapular brown adipose tissue, kidneys, and adrenal glands. Leptin is known to increase hypothalamic corticotrophin-releasing factor. In this study, we tested the hypothesis that leptin-dependent increases in sympathetic nervous activity are mediated through increases in central nervous system corticotrophin-releasing factor activity. We examined the effects of intracerebroventricular administration of corticotrophin-releasing factor and intravenous leptin on sympathetic nervous activity to interscapular brown adipose tissue through multifiber neurography in anesthetized Sprague-Dawley rats pretreated with intracerebroventricular α-helical corticotrophin-releasing factor (α-CRF) or vehicle. Centrally administered corticotrophin-releasing factor substantially increased interscapular brown adipose tissue sympathetic nervous activity. The responses to corticotrophin-releasing factor were substantially attenuated in animals pretreated with α-helical corticotrophin-releasing factor (α-CRF). Leptin-dependent increases in interscapular brown adipose tissue sympathetic nervous activity were significantly inhibited by pretreatment with α-helical corticotrophin-releasing factor (α-CRF). Interestingly, leptin also significantly increased arterial pressure over 6 hours, but this pressor action was not attenuated by the corticotrophin-releasing factor receptor antagonist. These results suggest that corticotrophin-releasing factor may mediate the sympathoexcitatory effect of leptin on thermogenic tissue without altering its cardiovascular actions. (Hypertension. 2001;38:384-388.)

Key Words: arteries ■ blood pressure ■ leptin ■ sympathetic nervous system ■ metabolism

Leptin is a 16-kDa peptide mostly secreted by white adipose tissue that signals to the hypothalamus the state of fat and energy storage.\(^1\) Leptin inhibits appetite\(^2\) and increases sympathetic activity to thermogenic interscapular brown adipose tissue (IBAT) in rodents,\(^3\) favoring weight loss.\(^2\) Increases of sympathetic output to the kidneys, adrenal glands, and hindlimbs are also caused by intravenous or intracerebroventricular (ICV) administration of leptin.\(^3\) Leptin-deficient obese mice (ob/ob mice) have low arterial pressure, supporting a physiological role for endogenous leptin in the maintenance of arterial pressure.\(^4\) In addition, it has been shown that leptin deficiency is associated with orthostatic hypotension and an impaired cold pressor response in humans, suggesting that leptin contributes physiologically to the sympathetic regulation of arterial pressure.\(^5\)

Whether leptin acts direct or indirectly to cause sympathoexcitation in the hypothalamus is still unclear. Leptin alters hypothalamic expression of several peptide neurotransmitters that act on sympathetic tone. Leptin is known to inhibit central nervous system expression of neuropeptide Y,\(^6\) which has sympathoinhibitory properties.\(^7\) On the other hand, leptin increases the expression of corticotrophin-releasing factor (CRF)\(^8\) and pro-opiomelanocortin, the precursor of the melanocortin receptor agonist α-melanocyte–stimulating hormone.\(^9\) We have demonstrated that sympathetic activity to IBAT and other organs is increased by the melanocortin type 4 receptor agonist MT-II.\(^10\)

It has been shown that ICV administration of CRF suppresses appetite and that the anorectic action of leptin may depend partially on activation of hypothalamic CRF.\(^11\) Therefore, it appears that CRF-dependent mechanisms play a role in the regulation of appetite. Additionally, CRF increases sympathetic activity to IBAT, kidneys, and adrenal glands,\(^7,12\) a response similar to that observed after ICV administration of leptin.\(^3\) It is possible that leptin-induced sympathetic activation might be at least partly mediated by CRF-dependent mechanisms. Therefore, we tested the hypothesis that leptin increases sympathetic outflow through activation of CRF-containing central nervous system pathways. To examine this issue, we studied the sympathetic effects of third cerebral ventricle administration of a CRF receptor antagonist (α-helical CRF\(_{9-41}\)) on leptin-induced sympathetic activation to IBAT in the anesthetized lean Sprague-Dawley rat.

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Methods
All procedures were approved by the University of Iowa Animal Research Committee. Experiments were performed in Sprague-Dawley male rats (300 to 310 g) from Harlan Sprague Dawley Inc (Indianapolis, Ind).

Procedures
Rats were anesthetized with pentobarbital (50 mg/kg IP) and secured in a Kopf 900 stereotactic instrument. Briefly, a 23-gauge stainless-steel guide cannula (16 mm in length) was lowered 10° from vertical into the third ventricle according to standard stereotactic procedures. The coordinates with respect to bregma were −1.0 mm anteroposterior, 1.5 mm lateral from midline, and −9.0 mm doroventral from the dura. ICV injections were made in a volume of 5 to 10 μL over 10 minutes. After experiments, methylene blue was injected through the ICV cannula to confirm its proper location. Stained brains were removed for microtome slicing. Arterial pressure and direct multifer recordings of sympathetic nerve activity (SNA) to brown adipose tissue were measured as described previously.3

Design
Studies were performed at least 7 days after placement of the third ventricular catheter. Baseline measurements of arterial pressure, heart rate, and SNA were continuously recorded for 30 minutes. After administration of experimental agents, hemodynamic and SNA measurements were recorded continuously for 360 minutes. Terminally, rats were euthanized with barbiturate overdose. Two protocols were performed.

Role of CRF Receptors in Regulation of IBAT Sympathetic Nerve Traffic
Rats received ICV injection of vehicle (0.9% NaCl, n=10) or CRF (Phoenix Pharmaceuticals Inc) in doses of 1 μg (n=15) and 5 μg (n=7). Other animals received the CRF-1 and CRF-2 receptor antagonist α-helical CRF₉₋₄₁ (Phoenix Pharmaceuticals Inc), either alone (30 μg, n=7) or followed 10 minutes later by CRF (1 μg, n=8). The CRF doses were based on published studies showing sympathoactivation to ICV administration of CRF in doses between 1 and 5 μg.7,13 The dose of α-helical CRF₉₋₄₁ was based on previous studies demonstrating blockade of sympathoexcitatory and pressor responses at doses of 10 to 35 μg.13,14

Role of CRF Receptors in Leptin-Dependent IBAT Sympathoexcitation
Rats received ICV injection of vehicle (n=12) or α-helical CRF₉₋₄₁ (30 μg, n=11). All rats then received murine leptin (1 mg/kg IV, Amgen Inc) as a 500 μg/kg loading dose over 10 minutes, followed by an infusion at 167 μg/kg per hour for 3 hours. This regimen results in a rapid and sustained increase in plasma leptin concentration to >100 ng/mL3 and has been shown to consistently increase sympathetic outflow to the kidneys and IBAT.3

Data Analyses
Results are expressed as mean±SEM. Sympathetic nerve firing rate was corrected for background noise by subtracting postmortem measurements from the measurement obtained at each time point during the experiment. Values from 3 separate baseline measurements did not differ significantly; therefore, they were averaged for each animal. After baseline, each continuously recorded variable was averaged every 5 minutes for 6 hours. Because there is significant interindividual variation in baseline SNA, these data are also expressed as percentage change from baseline. Differences between active treated and control rats were assessed by use of the Student’s t test or repeated-measures ANOVA, with statistical testing by the Scheffé F test. Statistical analysis was performed by use of StatView software for Macintosh (version 4, Abacus Concepts Inc). A value of P<0.05 was considered to be statistically significant.

Results

CRF Receptor-Dependent Sympathetic Effects on IBAT
Third cerebroventricular administration of CRF produced a substantial dose-dependent increase in IBAT SNA (P<0.001 versus vehicle, Figure 1A and Table). ICV injection of α-helical-CRF₉₋₄₁ significantly antagonized the CRF-induced sympathetic response to IBAT (P<0.001 versus CRF alone, Figure 1B and Table). ICV administration of α-helical CRF₉₋₄₁ alone did not have sympathetic effects (Table).

Role of CRF Receptors in Leptin-Dependent Sympathoactivation to IBAT
Intravenous leptin caused a marked slow-onset increase in the SNA response to IBAT (P<0.01 versus vehicle, Table and Figure 2) However, SNA response to IBAT was substantially inhibited by pretreatment with α-helical CRF₉₋₄₁ (P<0.05 versus leptin alone, Figure 2). Interestingly, leptin significantly increased arterial pressure over 6 hours (Table). In contrast to IBAT sympathoexcitation, the pressor effect of leptin was not attenuated by α-helical CRF₉₋₄₁.

Discussion
The present study explored the role of CRF in the sympathoexcitatory effect of leptin to IBAT. First, we have confirmed that ICV administration of CRF dose-dependently increases SNA to IBAT. Second, we have shown that leptin-dependent
sympathetic activation to thermogenic IBAT depends substantially on CRF receptor activation. Third, we have demonstrated that an acute pressor effect of intravenous leptin is not blocked by the CRF receptor antagonist.

Several reports have indicated that CRF might have an important role in appetite control, particularly under stress conditions. Stress paradigms, such as repeated restraint, cause temporary appetite inhibition and sustained reduction in body weight, which is totally reversed by ICV administration of a CRF receptor antagonist. Energy metabolism is also regulated by CRF. ICV injections of CRF have been shown to increase sympathetic activity and GDP binding to mitochondria in IBAT. These results indicate that ICV CRF increases thermogenic metabolism in IBAT.

Like CRF, leptin promotes negative energy balance. Through complex and as-yet-unclear mechanisms involving melanocortins, neuropeptide Y, and CRF and/or CRF-related neuropeptides, leptin inhibits food intake and increases sympathetic activity to IBAT. There is substantial evidence indicating that at least part of these leptin-dependent actions are mediated by CRF. Leptin receptor–like immunoreactivity is expressed in CRF-containing neurons in the paraventricular nucleus (PVN) of the hypothalamus. The PVN seems to be an important structure in controlling body weight and feeding behavior, because PVN lesions cause hyperphagia and obesity. Also, ICV injection of leptin increases PVN CRF mRNA by ≈40% but does not alter CRF message in the PVN of leptin-resistant Zucker rats. This result indicates that leptin-induced changes in CRF mRNA in the PVN are specific and depend on leptin receptor integrity. In addition, functional studies on feeding behavior have demonstrated a close relationship between leptin and CRF central nervous system pathways. Administration of leptin into the third cerebral ventricle increases hypothalamic CRF concentrations and inhibits refeeding in fasted Wistar rats. Furthermore, simultaneous administration of α-helical CRF9-41 (CRF-1 and -2 receptor antagonist) inhibits the appetite-suppressant effect of leptin. Thus, food intake suppression caused by leptin appears to be partially mediated by central nervous system CRF-dependent mechanisms. The present study extends these previous data to suggest a role for central nervous system CRF in leptin-dependent sympathoactivation of IBAT.

The interactions between leptin and CRF receptors to control sympathetic-mediated thermogenic metabolism and feeding behavior indicate that CRF might have a role in the regulation of body weight in rodents. However, CRF-deficient knockout mice are phenotypically normal and do not exhibit obesity. CRF belongs to a family of CRF-like peptides that bind with lesser or greater affinity to CRF-1 and CRF-2 receptors. It is possible that altered expression of CRF-like peptides, such as urocortin, in the hypothalamus prevents major abnormalities in CRF-dependent mechanisms of weight control in CRF-knockout mice. It would be of interest to study the IBAT SNA responses to leptin in the CRF-knockout mouse. However, IBAT nerve recordings in mice pose a substantial technical challenge at present.

We found substantial but incomplete suppression of leptin-induced brown adipose tissue SNA by CRF receptor antagonism. This may reflect incomplete CRF receptor blockade. It is also likely that other neurotransmitters may interact with leptin to control sympathetic thermogenic metabolism. Melanocortins do not appear to be significant contributors to leptin-induced thermogenic sympathoactivation, given that this response is unaltered by SHU-9119, a melanocortin-4 receptor antagonist.

In contrast with our previous observations, we observed that intravenous leptin significantly increased arterial pressure by ≈20 mm Hg (Table). The difference from our previous reports may reflect the 6-hour observation period.
compared with the 3-hour period used previously. Most interestingly, leptin-induced increases in arterial pressure were not attenuated by CRF receptor blockade (Table). Although not a prespecified hypothesis of the present study, our data suggest that the effects of leptin on arterial pressure are not mediated by CRF-induced sympathoactivation. Other studies have supported a pressor effect of leptin, which is likely mediated by sympathoactivation. Our results are consistent with the hypothesis that leptin has divergent central neural mechanisms mediating its effects on metabolic as opposed to cardiovascular functions. Indeed, melanocortin-4 receptor blockade inhibits renal sympathoactivation to leptin but does not block thermogenic sympathoactivation.

There are potential limitations to the present study. First, anesthesia may alter the sympathetic responses to neuropeptides. However, we have shown previously that pentobarbital/chloralose anesthesia does not change renal or lumbar sympathetic activity in response to baroreflex stimulation or hemorrhage. Second, it is possible that α-helical CRF₉₄₁ did not specifically block the CRF receptor and may have acted at receptors for other neuropeptides mediating sympathoactivation. However, α-helical CRF₉₄₁ appears to be quite specific for CRF receptors, in that it does not inhibit growth hormone-releasing factor–stimulated secretion of growth hormone, gonadotrophin-releasing factor–stimulated secretion of leuteinizing and follicle-stimulating hormones, thyrotropin-releasing factor–stimulated secretion of thyrotropin or prolactin, or the secretion of adrenocorticotropic hormone induced by a different secretagogue, phorbol myristate acetate.

Third, the doses of agonists (leptin and CRF) are probably within the pharmacological range. Therefore, the sympathoexcitatory and pressor actions of leptin might be a pharmacological rather than physiological response. However, we have previously demonstrated that leptin-deficient ob/ob mice have lower arterial pressure than do their control littersmates, suggesting that leptin does contribute to physiological maintenance of arterial pressure. Studies in humans with leptin deficiency support a role for leptin in the sympathetic control of arterial pressure. Fourth, acute responses may not fully reflect the interaction between leptin and CRF-dependent sympathetic systems under chronic conditions. Fifth, the effect of CRF on leptin action may reflect a wider role for CRF in the regulation of sympathetic activation rather than a leptin-specific role. Finally, the exact CRF receptor responsible for sympathoactivation could not be identified, given that α-helical CRF₉₄₁ antagonizes CRF-1 and -2 receptors.

In summary, we have shown that CRF receptor activation substantially contributes to leptin-mediated sympathoactivation to IBAT. Therefore, CRF or CRF-related peptides appear to partially regulate the effects of leptin on sympathetically mediated thermogenic metabolism. In contrast, the pressor effect of leptin is likely not mediated by changes in activity of the CRF system.

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