Effects of central and systemic administration of leptin on neurotransmitter concentrations in specific areas of the hypothalamus

Kimberly A. Clark,1 Sheba M. J. MohanKumar,1,2 Badrinarayanan S. Kasturi,2 and P. S. MohanKumar1,2

1Neuroscience Program, and 2Department of Pathobiology and Diagnostic Investigation, Neuroendocrine Research Laboratory, College of Veterinary Medicine, Michigan State University, East Lansing, Michigan

Submitted 17 May 2005; accepted in final form 3 October 2005

Clark, Kimberly A., Sheba M. J. MohanKumar, Badrinarayanan S. Kasturi, and P. S. MohanKumar. Effects of central and systemic administration of leptin on neurotransmitter concentrations in specific areas of the hypothalamus. Am J Physiol Regul Integr Comp Physiol 290: R306–R312, 2006. First published October 6, 2005; doi:10.1152/ajpregu.00350.2005.—Leptin, a hormone produced by adipocytes, has been shown to affect a number of central functions, such as regulation of the hypothalmo-pituitary-adrenal axis, feeding, and body weight regulation. Because hypothalamic monoamines are intricately involved in the regulation of these functions, we hypothesized that leptin may produce its effects by altering the activity of these neurotransmitters. To test this hypothesis, male rats received peripheral (0, 100, or 500 μg ip), or central (0 or 5 μg icv) injections of leptin. The animals were killed 5 h later, and their brains were removed, frozen, and sectioned. Serum was collected to measure leptin and corticosterone by RIA. The paraventricular nucleus (PVN), arcuate nucleus (AN), ventromedial hypothalamus (VMH), dorsomedial dorsal nucleus (DMD), median eminence (ME), and medial preoptic area (MPA) were obtained using Palkovits’ microdissection technique, and monoamine concentrations in these areas were determined using HPLC-EC. Intraperitoneal administration of leptin increased serum leptin concentrations in a dose-dependent manner (P < 0.05). Both intraperitoneal and intracerebroventricular administration of leptin decreased serum corticosterone significantly (P < 0.05). Norepinephrine (NE) concentration decreased significantly in the PVN, AN, and VMH after both intraperitoneal and intracerebroventricular administration of leptin (P < 0.05). NE concentrations decreased significantly in DMN after intracerebroventricular administration of leptin (P < 0.05). Leptin treatment (both ip and icv) decreased dopamine concentrations significantly in the PVN. Serotonin (5-HT) concentration decreased significantly in the PVN after both intraperitoneal and intracerebroventricular injections of leptin and decreased in the VMH only with intracerebroventricular treatment of leptin. Leptin did not affect any of the monoamines in the ME and MPA. These results indicate that both central and systemic administration of leptin can affect hypothalamic monoamines in a region-specific manner, which, in turn, could mediate many of leptin’s central and neuroendocrine effects.

Leptin, an adipocyte-derived hormone, plays a critical role in metabolic homeostasis by serving as a signaling molecule to the brain (10). It is believed that the primary role of leptin is to act as a signal of nutritional status to the hypothalamus, thereby modulating neurotransmitter systems that regulate food intake and energy expenditure (10, 40). In addition, leptin also regulates several central and neuroendocrine functions (39, 46). Leptin is known to affect the reproductive system (3, 13) and the stress axis (46), and it also plays an important role in the regulation of several hormones, such as thyroid hormones (21), growth hormone (11), and reproductive hormones, such as luteinizing hormone and prolactin (17).

The mechanisms by which leptin produces these central and neuroendocrine effects are not clear. There is evidence that indicates that leptin modulates various neuropeptides and neuromodulators. Neuropeptide Y (NPY) is one such substance that is involved in the regulation of certain neuroendocrine effects associated with leptin (23, 46). Apart from NPY, other brain neurotransmitters, specifically, the hypothalamic monoamines—norepinephrine (NE), dopamine (DA), and serotonin (5-HT)—could play a critical role in many of leptin’s central and neuroendocrine effects. Hypothalamic monoamines play a critical role in the regulation of feeding behavior, sympathetic outflow, and alterations in the hypothalmo-pituitary-adrenal (HPA) and hypothalmo-pituitary-gonadal (HPG) axes, as well as other neuroendocrine functions (26, 35), all of which are also affected by leptin.

The hypothalamus receives monoaminergic innervation from different parts of the brain and brain stem (32). The paraventricular nucleus (PVN), which is rich in corticotropin-releasing hormone (CRH) neurons that are crucial for the functioning of the HPA axis, receives innervation from organized monoaminergic nuclei (35). Deafferentation of the hypothalamus that severs connection from these nuclei can disrupt the normal functioning of the HPA axis (45). Also, chemicals that are known to either decrease or augment monoaminergic activity can affect CRH and luteinizing hormone-releasing hormone secretion (35, 38). Taken together, these studies indicate that monoaminergic activity in the hypothalamus is critical for the regulation of the HPA and HPG axes. Hypothalamic monoamines are also involved in the regulation of food intake and secretion of other hormones, such as thyroid hormone and growth hormone (35). Therefore, it is clear that monoamines could play an important role in mediating many of leptin’s central and neuroendocrine effects. However, detailed studies examining the effects of exogenous leptin on hypothalamic monoamines in vivo have not been done so far.

The aim of this study, therefore, was to investigate the effects of peripheral and central administration of leptin on hypothalamic areas that are involved in the central and neuroendocrine effects associated with leptin in an in vivo setting. This would enable us to map the changes in monoamines in...
LEPTIN AND HYPOTHALAMIC MONOAMINES

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (3- to 4-mo-old), weighing between 300 and 350 g were obtained from Harlan Sprague-Dawley (Indianapolis, IN). They were housed in light-controlled (lights on from 0700 to 1900), air-conditioned (23 ± 2°C) animal quarters and were fed rat chow and water ad libitum. Animals used in the experiments were in accordance with the National Institutes of Health’s Guide for the Care and Use of Laboratory Animals and were approved by the institutional animal care and use committee.

Implantation of intracerebroventricular cannula into the lateral ventricle. Two groups of rats were anesthetized with pentobarbital sodium (50 mg/kg body wt ip). They were implanted with a stainless steel cannula (22-gauge) in the right lateral ventricle stereotaxically, before they were used in the treatment protocol. The animals were given at least 1 wk of rest recovering from surgery, the animals were given at least 1 wk of rest before they were used in the treatment protocol.

Treatment. The animals without cannulas were randomly divided into three groups of seven rats per group. On the day of the experiment, animals were brought into the laboratory at least 2 h before treatment. They were given intraperitoneal injections of 0 (control), 100, or 500 μg of rat recombinant leptin (n = 7; R&D Systems, Minneapolis, MN) in 250 μl of saline. Animals with cannulas in the lateral ventricle received an intracerebroventricular infusion (n = 4).

At the time of treatment, the stylet was replaced with a 30-gauge inner cannula that was connected to a Hamilton syringe. The tip of the inner cannula extended 0.5 mm beyond the tip of the guide cannula implanted into the lateral ventricle. Leptin (5 μg in 5 μl) or the vehicle (saline, 5 μl) was infused slowly into the lateral ventricle over 5 min. Animals were killed 5 h later, and their brains were removed quickly and frozen using dry ice. Trunk blood was collected, and the serum was separated and stored at −20°C until RIA analysis.

Palkovits’s microdissection. Palkovits’s microdissection procedure was used to isolate discrete areas of the hypothalamus, as described before (33). Briefly, the brain was sectioned serially in 300-μm increments from the bregma to lambda using a cryostat (Slee Mainz, London, UK). The sections were transferred to microscope coverslips and placed on a cold stage maintained at −10°C. Six hypothalamic areas namely, the PVN, arcuate nucleus (AN), medial preoptic area (MPA), dorsomedial dorsal nucleus (DMD), ventromedial hypothalamus (VMH), and median eminence (ME) were microdissected with the help of a stereotoxic atlas (37). The areas were removed using a 500-μm punch. Care was taken to include all subdivisions of individual nuclei from multiple serial sections, depending on the location of the nuclei. The microdissected tissue was stored in 0.1 M HClO4 at −70°C until analyzed for neurotransmitter concentrations using HPLC-EC.

HPLC-EC. The HPLC-EC system and the details of the mobile phase have been described previously (5, 33). Briefly, the system consisted of a Shimadzu LC-10 AT VP pump, a phase II, 5-μm ODS reverse-phase, C-18 column (Phenomenex, Torrance, CA), a glassy carbon electrode placed inside a Shimadzu CTO-10 AT/VP column oven, and a LC-4C amperometric detector (Bioanalytical Systems, West Lafayette, IN) connected to a computer with the class VP chromatopac software (Shimadzu, Columbia, MD). The composition of the mobile phase was as follows: monochloroacetic acid (14.14 g/l), sodium hydroxide (4.675 g/l), octanesulfonic acid disodium salt (0.3 g/l), EDTA (0.25 g/l), acetonitrile (35 ml/l), and tetrahydrofuran (14 ml/l). The mobile phase was made with pyrogen-free water, filtered, and degassed through a Milli-Q purification system (Millipore, Bedford, MA). The final pH of the mobile phase was adjusted to 3.1 using NaOH. The mobile phase was pumped at a flow rate of 1.8 ml/min. The range of the detector was 1.0 nA full scale, and the potential of the working electrode was 0.65 V. The column oven was set at 37°C. At the time of HPLC analysis, tissue samples were thawed and homogenized in 150 μl of 0.1 M HClO4 using a microultrasonomic cell disruptor (Kontes, Vineland, NJ) and centrifuged at 10,000 g for 10 min. Fifty microliters of the supernatant along with 25 μl of the internal standard (0.05 M dihydroxybenzylamine) were injected into the HPLC system. The sensitivity of the system was <1 pg.

Protein assay. A 20-μl aliquot of the tissue homogenate was used in duplicate in the protein assay. Protein levels were determined using a microplate bicinechonic acid assay (Pierce, Rockford, IL). Absorbance at 562 nm was obtained using an ELX 800 microplate reader (Biotek Instruments, Winooski, VT). Neurotransmitter concentrations were expressed as picogram per microgram protein.

Radioimmunoassay. Radioimmunoassay kits from Linco Research (St. Charles, MO), and the Coat-A-Count kit from Diagnostic Products (Los Angeles, CA) were used to measure leptin and corticosterone levels, respectively, as described before (5).

Statistical analysis. Changes in the concentrations of neurotransmitters and hormones after intraperitoneal administration of leptin were analyzed by one-way ANOVA followed by post hoc Fisher’s least significant difference test. Changes in neurotransmitters and hormones after intracerebroventricular administration were analyzed using unpaired Student’s t-test.

RESULTS

Serum leptin. Serum leptin levels (means ± SE; ng/ml) in control animals and those treated with intraperitoneal or intracerebroventricular injections of leptin are shown in Fig. 1A. In control animals that were given intraperitoneal injections of the vehicle, leptin levels were 4.7 ± 1.0. Intraperitoneal administration of 100 μg of leptin increased serum leptin concentrations to 8.45 ± 1.3 (P < 0.05). A 500-μg injection of leptin given intraperitoneally increased this further to 30.1 ± 5.8 (P < 0.05). In contrast, intracerebroventricular administration of leptin did not affect serum leptin levels.

Serum corticosterone. Serum corticosterone levels (means ± SE; ng/ml; Fig. 1B) in control animals that were treated with the vehicle (ip) were 118.8 ± 17.1. Treatment with 100 or 500 μg of leptin (ip) decreased corticosterone concentrations to 75.9 ± 8.68 and 55.6 ± 12.3, respectively (P < 0.01). Similarly, in animals that were given 5.0 μg of leptin intracerebroventricularly, serum corticosterone levels decreased by 75% (39.4 ± 10.6) compared with the respective control group (125.3 ± 27.6; P < 0.05).

The effect of leptin on monoamines in the paraventricular nucleus. Changes in monoamine concentrations (means ± SE; pg/μg protein) in the PVN after intraperitoneal or intracerebroventricular administration of leptin are shown in Fig. 2. NE concentrations in the PVN of animals that were given the vehicle (ip) were 61.6 ± 10.2. Although administration of the lower dose of leptin (ip) did not have any effect on NE concentration, treatment with 500 μg of leptin (ip) decreased it by 50% (30.5 ± 4.0; P < 0.05). Similarly, intraperitoneal injections of the higher dose of leptin also decreased DA and 5-HT concentrations significantly in the PVN (P < 0.05). In contrast, intracerebroventricular administration of leptin decreased the levels of NE from 43.2 ± 6.2 in the control group to 22.1 ± 5.3 in the leptin-treated group. A similar decrease was observed in the levels of DA (3.8 ± 1.7) and 5-HT (4.4 ± 2.3), compared with the control group (22.1 ± 5.3 and 13.1 ± 3.1, respectively; P < 0.05).

Downloaded from http://ajpregu.physiology.org/ by 10.22033.237.32 on October 5, 2016
The effect of leptin on monoamines in the arcuate nucleus.

Fig. 3. shows changes in monoamine levels (means ± SE; pg/μg protein) in the AN after intraperitoneal or intracerebroventricular administration of leptin. NE levels in control animals were 45 ± 3.2 and decreased significantly with both 100 and 500 μg of leptin (ip) treatment (29.2 ± 1.9 and 32.5 ± 3.5, respectively; P < 0.05). A similar decrease was observed with intracerebroventricular administration of leptin (23.5 ± 1.4) compared with the control group (44.2 ± 3.8; P < 0.05). However, leptin treatment (both ip and icv) did not affect DA or 5-HT levels in the AN.

Effect of leptin on monoamines in the ventromedial hypothalamus. Similar to what was observed in the PVN, only the highest dose of leptin (500 μg ip) was effective in suppressing NE levels in the VMH (5.2 ± 0.9) compared with the control group (12.6 ± 1.2; P < 0.05). A similar decrease was observed after intracerebroventricular administration of leptin (5.4 ± 1.5) compared with the control group (11.1 ± 1.4; P < 0.05). Intraperitoneal treatment of leptin did not affect DA concentrations in the VMH. However, intracerebroventricular administration of leptin decreased 5-HT levels in this nucleus (5.1 ± 0.9 compared with 13.5 ± 2.1 in the control; Fig. 4).
LEPTIN AND HYPOTHALAMIC MONOAMINES

Administration of leptin decreased NE significantly in the DMN concentrations in the DMN. In contrast, intracerebroventricular administration of vehicle for leptin or 5 μg of leptin did not produce any effect on monoamine concentrations in the MPA and ME.

**DISCUSSION**

Leptin plays a significant role in energy homeostasis by acting as a signaling molecule to the brain, and by doing so, it also affects several central and neuroendocrine functions (10, 46). Some of the neuroendocrine effects of leptin are regulation of the stress axis and reproductive axis, inhibition of thyroid hormone secretion, and influence on growth hormone production (3, 10, 11, 13, 21, 46). However, the mechanisms by which leptin produces its neuroendocrine effects are not clear. Results from the present study indicate that leptin can cause regionspecific changes in monoamine levels in the hypothalamus when given systemically or centrally, and it can also reduce serum corticosterone levels significantly. Because hypothalamic monoamines are known to be involved in the regulation of several releasing hormones (35), leptin-induced changes in hypothalamic monoamines that were observed in this study could play an important role in bringing about leptin’s various neuroendocrine effects.

The effect of exogenous leptin on hypothalamic monoamines has not been studied extensively. It is important to note that the present study is the first systemic study, as opposed to previous work, which was done in vitro. The in vitro studies involving leptin’s effects on hypothalamic monoamines have had contradictory results. A recent report by Hastings et al. (18) showed that leptin had no significant effect on hypothalamic NE or 5-HT overflow. In contrast to this, another in vitro study found that leptin inhibits depolarization-induced NE and DA release from rat hypothalamic neuronal endings without modifying basal levels (9). Our recent in vitro studies provide evidence that leptin does, indeed, decrease basal NE release from the hypothalamus in a dose-dependant manner and that this effect may be mediated through GABA (16). Although this study indicated that leptin was capable of decreasing NE from 40.2 ± 4.3 in the control group to 27.6 ± 4.1 in the leptin-treated group (P < 0.05). Neither intraperitoneal nor intracerebroventricular treatment produced any change in monoamine concentrations in the MPA and ME.

**Effect of leptin on monoamines in other areas of the hypothalamus.** Monoamine concentrations in the DMD, MPA, and ME after intraperitoneal and intracerebroventricular leptin treatment are shown in Table 1. Intraperitoneal treatment with leptin did not produce any effect on monoamine concentrations in the DMN. In contrast, intracerebroventricular administration of leptin decreased NE significantly in the DMN

**Table 1. Changes in NE, DA, and 5-HT in the DMD, MPA, and ME after intraperitoneal and intracerebroventricular administration of vehicle (control) and leptin**

<table>
<thead>
<tr>
<th>Area</th>
<th>Treatment</th>
<th>NE, pg/μg</th>
<th>DA, pg/μg</th>
<th>5-HT, pg/μg</th>
</tr>
</thead>
<tbody>
<tr>
<td>DMD</td>
<td>intraperitoneal</td>
<td>Control</td>
<td>40.1±4.3</td>
<td>6.3±1.8</td>
</tr>
<tr>
<td></td>
<td>100 μg leptin</td>
<td>45.5±8.1</td>
<td>6.8±1.2</td>
<td>19.2±2.6</td>
</tr>
<tr>
<td></td>
<td>500 μg leptin</td>
<td>43.2±3.1</td>
<td>4.9±0.6</td>
<td>12.1±1.4</td>
</tr>
<tr>
<td></td>
<td>intracerebroventricular</td>
<td>Control</td>
<td>40.2±4.3</td>
<td>7.1±2.4</td>
</tr>
<tr>
<td></td>
<td>5 μg leptin</td>
<td>27.6±4.1*</td>
<td>5.7±1.3</td>
<td>15.9±3.8</td>
</tr>
<tr>
<td>MPA</td>
<td>intraperitoneal</td>
<td>Control</td>
<td>49.1±9.2</td>
<td>11.1±1.7</td>
</tr>
<tr>
<td></td>
<td>100 μg leptin</td>
<td>41.0±9.8</td>
<td>8.0±1.1</td>
<td>10.9±3.8</td>
</tr>
<tr>
<td></td>
<td>500 μg leptin</td>
<td>40.6±5.4</td>
<td>11.4±4.0</td>
<td>23.6±7.7</td>
</tr>
<tr>
<td></td>
<td>intracerebroventricular</td>
<td>Control</td>
<td>43.4±12.8</td>
<td>12.0±0.8</td>
</tr>
<tr>
<td></td>
<td>5 μg leptin</td>
<td>40.5±3.8</td>
<td>14.4±1.2</td>
<td>12.7±1.4</td>
</tr>
<tr>
<td>ME</td>
<td>intraperitoneal</td>
<td>Control</td>
<td>45.7±6.3</td>
<td>107.9±25</td>
</tr>
<tr>
<td></td>
<td>100 μg leptin</td>
<td>35.4±5.4</td>
<td>53.1±10</td>
<td>18.2±1.6</td>
</tr>
<tr>
<td></td>
<td>500 μg leptin</td>
<td>41.7±5.8</td>
<td>102.3±30</td>
<td>25.9±7.9</td>
</tr>
<tr>
<td></td>
<td>intracerebroventricular</td>
<td>Control</td>
<td>46.2±6.3</td>
<td>84.9±6.8</td>
</tr>
<tr>
<td></td>
<td>5 μg leptin</td>
<td>30.7±3.4</td>
<td>65.7±20</td>
<td>13.7±6.8</td>
</tr>
</tbody>
</table>

Values are presented as means ± SE; *P < 0.05 compared to the respective control group. NE, norepinephrine; DA, dopamine; DMD, dorsomedial nucleus; MPA, medial preoptic area; and ME, median eminence.

Fig. 4. Changes in monoamine concentrations in the ventromedial hypothalamic nucleus after intraperitoneal administration of vehicle or leptin (100 or 500 μg) (A) and after intracerebroventricular administration of vehicle for leptin or 5 μg of leptin (B). Animals (n = 4–8) were killed 5 h after administration of vehicle or leptin. Brains were removed quickly and frozen immediately. Monoamine concentrations were measured as described in MATERIALS AND METHODS. *P < 0.05 compared with respective control groups.
release from the whole hypothalamus, it was not clear whether leptin would decrease NE levels in different hypothalamic nuclei. This is important to study because NE levels stimulate both the HPA and HPG axes, yet leptin is known to suppress HPA activity (4) but stimulate HPG function (13). It is, therefore, critical to see whether leptin is capable of differentially affecting the nuclei that help regulate these two axes.

Therefore, in the present study, we wanted to map the effects of leptin on hypothalamic monoamines simultaneously in multiple nuclei. Palkovits’s microdissection in combination with HPLC is a useful tool to accomplish this. Although some may consider neurotransmitter release that is measured using microdialysis/push-pull perfusion to be a more realistic assessment of neurotransmitter activity, steady-state monoamine concentration also proves to be a useful index of neurotransmitter activity. We have compared both release and concentration in other studies and have found that both are valuable tools. Although release provides an excellent profile of neurotransmitter activity over a period of time, concentration provides information on neurotransmitter activity in multiple areas at any given time point. In this study, we measured monoamine concentrations in several nuclei because there were no comparable in vivo studies examining the effects of leptin on monoamine levels in vivo. This would help us understand how leptin affects monoaminergic terminals differentially to modulate its many effects on body functions.

Studies examining the effects of leptin on the HPA axis have been controversial. Earlier studies indicated that leptin is capable of increasing CRH mRNA levels in the PVN (34, 36). This was attributed to the anorexigenic actions of CRH and its ability to increase energy expenditure (25). Intracerebroventricular administration of leptin has been shown to increase plasma corticosterone levels in rats (34, 47). Systemic administration of leptin also increases corticosterone levels 60 and 120 min postinjection (31). More recently, however, leptin has been shown to decrease HPA axis activity. Leptin was found to decrease the preparturient rise in adrenocorticotropic hormone and cortisol in the sheep fetus (50). It is conceivable that leptin can decrease serum corticosterone because in conditions such as diabetes, in which there is a well-documented decrease in leptin levels, as there is a concurrent increase in HPA activity (5, 32). Moreover, rats with defective leptin receptors have constantly elevated HPA activity (7). All of these observations indicate that a reduction in leptin levels or the inability to sense leptin causes activation of the HPA axis, whereas an increase in leptin levels may suppress HPA axis activity. In fact, we have previously shown that treatment of diabetic rats with leptin can normalize serum corticosterone levels. This was accomplished by a reduction in NE levels in the PVN (5). Taken together, these studies support our present findings that leptin treatment can, indeed, decrease serum corticosterone levels.

The decrease observed in serum corticosterone levels in the present study after both central and systemic administration of leptin can be associated with the reduction in NE levels in the PVN. The PVN has many CRH cell bodies and receives rich noradrenergic innervation from the brain stem (38). Direct administration of NE into the PVN is known to stimulate CRH transcription (22). Moreover, lesioning of the ventral noradrenergic bundle that carries the majority of the noradrenergic innervation to the hypothalamus results in a dramatic suppression of the stress axis (45). Thus it is evident that NE levels in the PVN play a stimulatory role in CRH secretion. Because leptin treatment decreased NE levels in the PVN, it is possible that this could be responsible for the decrease in plasma corticosterone seen in the present study.

Besides being a crucial part of the stress axis, the PVN is also involved in feeding behavior. NE levels in the PVN are known to stimulate feeding behavior. NE injections into the PVN (27) and treatment with α-2 adrenergic agonists, such as clonidine (49), are known to stimulate feeding. Moreover, lesioning of noradrenergic fibers that innervate the PVN have suppressed feeding behavior, suggesting that NE levels in the PVN are critical for feeding (44). Because leptin supresses feeding, the reduction in NE levels observed in the PVN that was observed in this study correlates well with this behavior. Besides the PVN, the VMH is also known to be important in feeding behavior (24). NE levels in the VMH are known to stimulate feed intake (43). In the present study, leptin treatment produced a dose-dependent decrease in NE levels in the VMH, indicating that this could be another possible target for leptin to decrease feeding.

NE levels in the AN are associated with stimulation of growth hormone (GH) secretion (35). Because there is an inverse relationship between leptin and GH, the reduction in NE levels in the AN could contribute to the decrease in GH (25). Moreover, the AN is part of a complex neuronal network that interacts with a variety of orexigenic and anorexigenic peptides to modulate food intake and GH release (12).

Besides NE, DA is also important in neuroendocrine functions associated with leptin. In the present study, DA levels in the PVN decreased after leptin treatment but were not affected in any other hypothalamic area. DA is believed to be inhibitory to thyrotropin hormone secretion (35). The decrease in DA levels observed in the PVN could, therefore, be related to the stimulatory effect that leptin has on thyrotropin-releasing hormone neurons (42).

Serotonin, on the other hand, increases with feeding (35) just like leptin. It is believed to be a potent inhibitor of feeding behavior (1, 15). Depletion of serotonin increases food intake and promotes obesity (8), indicating that serotonin could be a mediator of leptin’s actions. Moreover, serotonergic neurons that project to the hypothalamus express leptin receptor mRNA (4, 19). All of these studies suggest that leptin treatment could possibly increase serotonin levels in the brain. However, in the present study, we observed a reduction in serotonin levels after leptin treatment. This could be due to the fact that our observations were made 5 h after leptin treatment, and we could have missed any increase in serotonin levels that could have occurred earlier. More studies are needed to examine the effect of leptin administration on serotonin levels to determine the role of serotonin in leptin-induced inhibition of feeding.

Besides the PVN, VMH, and AN, the DMD was another area that was affected by leptin treatment. The DMD plays an important role in relaying information to neural pathways that mediate the neuroendocrine and behavioral responses to stress. It is rich in NPY and other neurons and may participate in CRH secretion by the PVN (6). Stressful situations are known to increase NE levels in the DMD (30). The decrease in NE levels observed in the DMD could be related to the suppression of stress axis activity after leptin treatment.
The mechanism by which leptin affects the hypothalamus is still unclear. Leptin has been shown to cross the blood-brain barrier via a saturable transport mechanism (2) and its receptors have been identified in several parts of the brain, including the hypothalamus (19, 20). Functional leptin receptors have been found in the brain stem (20), where leptin may act to modulate the neuroendocrine control of food intake and energy expenditure. Hosoi et al. (20) have shown that noradrenergic fibers arising from the brain stem (A1, A2, and A6) are rich in leptin receptors, and it is known that these regions project to the PVN and VMH in the hypothalamus (35). The reduction in NE and DA observed in this study could be attributed to an inhibitory effect on tyrosine hydroxylase (TH) synthesis in brain stem neurons, as TH is common for the synthesis of both NE and DA. Leptin may also act directly at the level of the hypothalamus, as leptin receptors are found in abundance in hypothalamic nuclei, particularly the PVN and VMH (46). Besides monoamines, leptin may affect a wide range of neurotransmitters and neupeptides, such as GABA, histamine, and other anorexigenic peptides, the complex interaction of which could mediate many of its central and neuroendocrine effects (10, 13, 16, 28, 46).

In summary, the results from this study indicate that leptin modulates monoaminergic concentrations in specific hypothalamic areas that regulate a host of central and neuroendocrine functions affected by leptin. This could be a possible mechanism of leptin’s actions. Further study is required to understand the possible routes, sites, and cellular mechanisms involved in this process.

ACKNOWLEDGMENTS

This study was partially supported by National Institutes of Health Grant AG 05980 to P. S. MohanKumar and National Science Foundation Grant IBN 0236385 to S. M. J. MohanKumar and P. S. MohanKumar.

REFERENCES

33. MohanKumar SM, MohanKumar PS, and Quadri SK. Specificity of interleukin-1beta-induced changes in monoamine concentrations in hypotha-