Cardiovascular Responses Evoked by Leptin Acting on Neurons in the Ventromedial and Dorsomedial Hypothalamus

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Abstract—Leptin, a circulating hormone produced by adipose tissue, is believed to act on the hypothalamus to increase sympathetic vasomotor activity, in addition to its well-known effects on appetite and energy expenditure. In this study, we determined the cardiovascular effects of direct application of leptin to specific cell groups within the hypothalamus that are known to be activated by circulating leptin. In rats anesthetized with urethane, microinjections of leptin (16 ng in 20 nL solution) were made into the ventromedial hypothalamic nucleus, dorsomedial hypothalamic nucleus, and paraventricular nucleus. Compared with vehicle solution, microinjections of leptin into the ventromedial hypothalamic nucleus evoked significant increases in arterial pressure and renal sympathetic nerve activity, but not heart rate. In contrast, microinjections of leptin into the dorsomedial hypothalamic nucleus evoked significant increases in arterial pressure and heart rate but not renal sympathetic nerve activity, whereas microinjections of leptin into the paraventricular nucleus had no significant effect on any of the measured cardiovascular variables. These results indicate that the ventromedial and dorsomedial hypothalamic regions might be important sites at which leptin activation leads to increases in sympathetic vasomotor activity and heart rate, as occurs in obesity-related hypertension. (Hypertension. 2003;42:488-493.)

Key Words: hypothalamus • sympathetic nervous system • arterial pressure • heart rate • brain • hypertension, experimental • obesity

Intravenous administration of leptin increases the activity of sympathetic nerves that innervate brown adipose tissue. In addition to these effects, it has also been shown that intravenous administration of leptin increases the activity of sympathetic nerves that innervate the kidneys, hindlimb vasculature, and adrenal glands in anesthetized rats, whereas long-term intravenous or intracarotid infusions of leptin increase arterial pressure and heart rate (HR) in conscious rats. It has therefore been suggested that leptin-induced increases in the activity of sympathetic vasomotor and cardiac nerves might be an important component in obesity-related hypertension. The leptin-induced increases in sympathetic vasomotor activity, arterial pressure, and HR appear to be mediated via action on the brain, because short-term administration of leptin into the lateral ventricles increases arterial pressure in conscious rats. Similarly, long-term administration of leptin into the lateral ventricles of conscious rats also results in a sustained increase in arterial pressure.

Circulating leptin has been shown to access the brain via a saturable transport system, but the site(s) in the brain at which leptin acts to increase sympathetic vasomotor activity is unknown. Studies on the expression of Fos (the protein product of the immediate-early gene c-fos) as a marker of neuronal activation have found that neurons in several hypothalamic and brainstem nuclei contained significantly increased numbers of Fos-positive neurons after intravenous infusion of leptin. Furthermore, by combining Fos immunohistochemistry with in situ hybridization, Elias et al found that some leptin-activated neurons in the hypothalamus (but not in the brain stem) also contained mRNA for the long form of the leptin receptor, which is believed to mediate the physiologic effects of leptin in the hypothalamus. Such double-labeled neurons in the hypothalamus were found in the region surrounding the median eminence as well as in the retrochiasmatic area and the arcuate, ventromedial, dorsomedial, and ventral premammillary nuclei.

Of the aforementioned groups of nuclei, the ventromedial and dorsomedial hypothalamic nuclei (VMH and DMH, respectively) are of particular interest with regard to the possible site of action of leptin in inducing increases in...
sympathetic vasomotor activity. In the case of the VMH, previous studies have shown that microinjections of leptin into this nucleus evoke an increase in the levels of circulating catecholamines, as well as increased glucose uptake in peripheral tissues, which was dependent on an intact sympathetic innervation. These studies therefore indicate that leptin-sensitive neurons in the VMH can increase the activity of sympathetic nerves that regulate catecholamine secretion and glucose uptake but do not demonstrate whether there is also activation of sympathetic vasomotor nerves. It is well established that the DMH contains neurons that, when activated, evoke increases in sympathetic vasomotor activity, arterial pressure, and HR. Thus, it is also possible that activation of leptin-sensitive neurons in this nucleus might increase sympathetic vasomotor activity. In light of these previous observations, the aim of this study was to test the hypothesis that leptin-sensitive neurons in the VMH or DMH affect cardiovascular function. For this purpose, we determined the effects of microinjections of leptin into the VMH and DMH on arterial pressure, HR, and renal sympathetic nerve activity (RSNA). In addition, as a control, microinjections of leptin were also made into the paraven-tricular nucleus (PVN), which is also known to play an important role in the regulation of sympathetic outflow, but which has a low level of expression of leptin receptors.

Methods

General Procedures

Experiments were performed on Sprague-Dawley rats (320 to 500 g) anesthetized with urethane (1.4 g/kg IP). All experiments were carried out in accordance with the Guidelines for Animal Experimentation of the National Health and Medical Research Council of Australia. Body temperature was monitored and maintained in the range 37°C to 38°C. Catheters were placed in a femoral vein and artery, and the trachea was cannulated. The rat was artificially ventilated at a level that maintained end-tidal CO₂ in the range 4.0% to 4.5%. The head was placed in a stereotaxic frame with the tooth bar fixed 19 mm below the interaural line. A small craniotomy was made near the bregma to allow for later insertion of a micropipette into the VMH, DMH, or PVN. The renal sympathetic nerve was exposed, as described in detail previously. After completion of all surgical procedures, neuromuscular blockade was induced with alcuronium chloride (0.1 mg/kg IV every 1 to 2 hours). The procedures for ensuring maintenance of an adequate level of anesthesia with and without neuromuscular blockade were as described previously. The mean arterial pressure (MAP), HR, and RSNA were recorded continuously, as described previously.

Microinjections of Drugs

Microinjections of recombinant murine leptin (Sigma; 16 ng in 20 nL solution) were made by pressure into sites in the VMH, DMH, or PVN with use of a micropipette held in a micromanipulator. The coordinates for injections into the DMH were determined from preliminary experiments in which the DMH sympathoexcitatory region was identified by injections of bicuculline, which is known to evoke a powerful sympathoexcitatory response when injected into this region. The coordinates for injections into the VMH and PVN were then determined from those DMH coordinates by taking into account the relative positions of the VMH and PVN with respect to the DMH, with reference to the atlas of Paxinos and Watson.

In control experiments, the vehicle saline solution (20 nL) or a solution of lipopolysaccharide (LPS, 2 ng in 20 nL; Sigma) was injected instead of the leptin solution. LPS was injected because it is a possible contaminant of recombinant murine leptin. There was, however, no detectable difference between the effects of injection of saline or LPS solution, so the results for these 2 groups of experiments were pooled.

Experimental Procedures

After all surgical procedures were completed, there was a waiting period of 20 minutes to allow the measured cardiovascular variables to stabilize. The baseline MAP, HR, and RSNA were then recorded for an additional 15 to 30 minutes. A microinjection of leptin, the control vehicle, or LPS solution was then made into the VMH, DMH, or PVN on one side. In some experiments, another microinjection was made into another site on the other side, after a waiting period of ~45 minutes and when cardiovascular variables had stabilized.

Histology

After all experimental procedures were completed, microinjections of the vehicle solution (20 nL) containing Fast Green dye were made into most of the sites (17 of 31) in the VMH, DMH, or PVN by using the same coordinates as those used for injection of leptin or vehicle control solution. The animal was then euthanized with an overdose of sodium pentobarbitone, and the brain was removed and placed in fixative solution. Subsequently, 50-μm-thick coronal sections of the hypothalamus were examined under a microscope, and the labeled microinjection sites were mapped onto standard hypothalamic sections according to the atlas of Paxinos and Watson. The locations of the injection sites were also compared with the distribution of Fox-positive neurons in the hypothalamus, as mapped in a preliminary study, in which leptin solution was infused intravenously into conscious rats (A.J. Marsh et al, unpublished observations).

Data Analysis

The average values of MAP, HR, and RSNA were measured over successive 5-minute periods starting 15 minutes before microinjection of the leptin or control vehicle solution and ending 30 minutes after microinjection. The baseline levels of MAP, HR, and RSNA were measured as the average values of these variables over the 15-minute period immediately preceding microinjection. The maximum changes in MAP, HR, and RSNA during the 20-minute period after leptin microinjections into the VMH, DMH, or PVN were compared with those after vehicle microinjection into the same region by using an unpaired t test. A probability value <0.05 was taken to indicate a statistically significant difference. All values are presented as mean±SEM.

Results

The baseline levels of MAP and HR averaged over the 15-minute period before microinjection of leptin were 82±3 mm Hg and 349±9 beats per minute (bpm), respectively, and before microinjections of the vehicle control solution were 96±3 mm Hg and 362±8 bpm, respectively. Microinjections of leptin into the VMH (n=5) evoked a significant cardiovascular response compared with control microinjections (n=8; Figures 1 and 2). In particular, there were gradually developing increases in MAP, HR, and RSNA that began within ~5 minutes after the leptin microinjection and reached a peak after 15 to 20 minutes (Figures 1 and 2). The mean maximal increase in RSNA during the 20-minute period after leptin microinjection was 28±5%, which was much greater than that during the same period after the control microinjection (6±2%, P<0.001). The increase in MAP after leptin microinjection was small (11±2 mm Hg) but was also significantly greater than that after the control microinjection (5±1 mm Hg, P<0.05). On the other hand, the increase in HR evoked by leptin microinjection into the VMH was not significantly different from that evoked by the control microinjection (P>0.1).
Microinjections of leptin into the DMH (n = 5) also evoked a significant cardiovascular response compared with the control microinjections (n = 5; Figure 2). In particular, there was a small increase in MAP (8 ± 2 mm Hg), which was significantly greater than that evoked by the control microinjections (2 ± 1 mm Hg, P < 0.05). There was also an increase in HR, which was significantly greater than that evoked by the control microinjections (42 ± 11 vs 11 ± 4 bpm, P < 0.01). Similarly, leptin microinjections into the DMH also evoked an increase in RSNA (18 ± 4%), but in this case, the increase was not significantly different from that evoked by the control microinjections (9 ± 4%, P > 0.1).

In the case of the PVN, microinjections of leptin (n = 4) resulted in small and variable changes in MAP, HR, and RSNA (Figure 2). These were not significantly different (P > 0.25 in all cases) from the changes in these variables after control microinjections (n = 4). All of the injection sites whose locations were determined histologically were confirmed to be within the VMH, DMH, or PVN, as shown in Figure 3.

Discussion

The main finding of the present study was that significant cardiovascular responses were evoked by microinjections of leptin into the VMH and DMH but not into the PVN. In particular, a significant increase in RSNA was evoked by leptin microinjection into the VMH only. The observations are consistent with a previous finding that neurons in the hypothalamus that synthesize the long form of the leptin receptor (which is believed to mediate the physiologic effects of leptin in the hypothalamus) are located in the VMH and DMH but not in the PVN.

Before considering the implications of these findings, some limitations of the study need to be considered. First, the experiments were performed in urethane-anesthetized rats, which was necessary to permit microinjections of small volumes (20 nL) into specific brain regions. Nevertheless, it is known that urethane does affect the tonic and reflex control...
of HR and sympathetic activity to some extent. Thus, the magnitude of the leptin-evoked changes in HR and sympathetic activity is likely to have been affected by the urethane anesthesia. At the same time, all experiments were performed under the same experimental conditions, and so comparisons can be made with respect to the effects of leptin microinjections into the different hypothalamic nuclei. Second, only the short-term effects of leptin application to the VMH, DMH, and PVN were studied. Under normal conditions, any changes in the concentration of leptin within these nuclei would occur much more slowly and be maintained at elevated levels for much longer periods. It is therefore conceivable that the long-term effects of leptin on cardiovascular variables in these nuclei might be different from those observed in these short-term experiments. Therefore, although our experiments have demonstrated that activation of leptin-sensitive neurons in the VMH and DMH can alter cardiovascular function, the importance of these nuclei in mediating the long-term cardiovascular actions of leptin remains to be determined.

The increases in MAP evoked by leptin microinjections into the VMH and DMH would have increased the activity of arterial baroreceptors, which might therefore have buffered the associated increases in HR and RSNA. Although such buffering might have occurred, this would not explain the fact that the RSNA increase evoked from the VMH was greater than that evoked from the DMH, because the small pressor response (and thus, degree of baroreceptor activation) evoked from the VMH (increase in MAP of 11 ± 2 mm Hg) was, if anything, slightly greater than that evoked from the DMH (increase in MAP of 8 ± 2 mm Hg).

Finally, the possibility should be considered that the observed leptin-induced effects could have been caused by diffusion of leptin to nuclei surrounding the DMH or VMH. This seems unlikely in the case of the DMH, because there are few neurons in the region immediately surrounding the DMH either that are activated by leptin (as indicated by Fos expression) or that contain the long form of the leptin receptor. In the case of the VMH, however, some of the injection sites at which leptin evoked a sympathoexcitatory response were centered in the ventral part of that nucleus, which does not contain a high density of leptin-sensitive neurons, as indicated by either Fos expression or the presence of the long form of the leptin receptor. On the other hand, such leptin-sensitive neurons are located within the dorsomedial subdivision of the VMH, as well as in the arcuate nucleus and retrochiasmatic area. The latter 2 regions are both close to the ventral border of the VMH and thus, to the centers of the injection sites in the ventral part of the VMH. It therefore follows that the renal sympathoexcitatory effects evoked from leptin microinjections into the ventral part of the VMH in our experiments could have been due to diffusion of the leptin to leptin-sensitive neurons into either the dorsomedial subdivision of the VMH or the nearby arcuate nucleus and retrochiasmatic area. In previous studies in which leptin microinjections were made into the VMH, the possibility that the arcuate nucleus or retrochiasmatic area was involved in mediating the evoked effects was even more likely, because in those studies, the injection volumes were much larger (500 or 1000 nL, compared with 20 nL in the present study). In the following discussion, therefore, we will refer to the VMH region (ie, the region encompassing the VMH together with the nearby portions of the arcuate nucleus and retrochiasmatic area).

It has been shown previously that microinjections of leptin into the VMH region of the rat evoke an increase in the levels of circulating epinephrine and norepinephrine, as well as increased glucose uptake in peripheral tissues, which was dependent on an intact sympathetic innervation. Although these studies indicated that leptin can activate neurons in the VMH region that regulate sympathetic nerves affecting energy balance, no measurements were made of cardiovascular variables. The results of the present study show for the first time that activation of leptin-sensitive neurons in the VMH region can increase sympathetic vasmotor activity. Thus, in addition to regulating food intake and energy expenditure, the VMH region might play a central role in regulating sympathetic vasmotor activity in response to increased levels of leptin.

Leptin microinjections into the DMH also resulted in a significant cardiovascular response, but the pattern of the response appeared to be different from that evoked in the VMH region. In particular, in contrast to the VMH region, leptin microinjections into the DMH evoked a significant increase in HR. Compared with the vehicle control, microinjection of leptin into the DMH also evoked an increase in RSNA, although this failed to reach statistical significance.

The lack of a significant cardiovascular response to microinjections of leptin into the PVN, even though this nucleus is known to play a major role in cardiovascular regulation, is entirely consistent with the fact that this nucleus has a low level of expression of leptin receptors. Furthermore, this finding supports the view that the cardiovascular responses...
evoked by leptin into the VMH region or DMH are due to a specific effect on leptin receptors. The observations suggest that leptin-evoked activation of renal sympathetic outflow, but not increases in HR, is evoked more readily from neurons in the VMH region than in the DMH. It is interesting to note, therefore, that recent evidence indicates that different central pathways mediate the leptin-induced activation of sympathetic outflows to the kidney and brown adipose tissue. In particular, it has been shown that leptin-induced increases in sympathetic outflows to the kidney and brown adipose tissue are dependent on different central neurotransmitter systems (the melanocortin and corticotrophin-releasing factor systems, respectively).26,27 Furthermore, a recent study has shown that leptin-induced activation of these 2 sympathetic outflows are also differentially modulated by the baroreceptor reflex.28 Thus, it is conceivable that leptin-sensitive neurons in the VMH region and DMH exert differential control over the sympathetic outflows to brown adipose tissue and the kidney. Further studies are needed to test this possibility.

The time course of sympathoactivation in response to leptin microinjection into the VMH region was slow, reaching a peak after 15 to 20 minutes. Similarly, injections of a much larger dose of leptin (5 μg, ~300 times greater) into the lateral ventricle in the conscious rat also resulted in a gradual increase in RSNA.7 The intracellular mechanisms that are activated by leptin in hypothalamic neurons are not fully understood, although they are known to involve the enzyme activated by leptin in hypothalamic neurons are not fully understood, although they are known to involve the enzyme phosphoinositol-3 kinase29 as well as the transcription factor, signal transducer and activator of transcription 3 (STAT3).30 Thus, the slow time course of action of leptin on RSNA presumably reflects the time course of activation of these or other components of the intracellular signaling pathway.

Only limited information is available in regard to the descending pathways from the DMH and VMH region that might mediate the leptin-evoked increases in RSNA and HR. It has been shown that the increase in HR evoked by disinhibition of DMH neurons is dependent on synaptic connections with neurons in the medullary raphe,31 which contains premotor neurons that project to the spinal sympathetic outflow.32 Thus, the leptin-induced increase in HR evoked from the DMH might be mediated via this pathway. In the case of the VMH region, previous anatomic studies have not identified spinally projecting neurons within the VMH itself, with the possible exception of some neurons on the ventral border of the nucleus.33–35 There are, however, many spinally projecting neurons in the nearby retrochiasmatic area and lateral arcuate nucleus that project to the spinal cord.33–35 Furthermore, Elias et al35 have shown that many of the spinally projecting neurons in the retrochiasmatic area and lateral arcuate nucleus are activated by leptin and also contain the peptide cocaine-and-amphetamine-regulated transcript (CART). In addition, they showed that there is a high density of CART immunoreactivity in fibers within the intermediolateral column of the spinal cord,35 suggesting that the CART-containing, leptin-sensitive neurons in the retrochiasmatic area and lateral arcuate nucleus within the VMH region might directly innervate sympathetic preganglionic neurons. It is therefore possible that these neurons might mediate, at least in part, the cardiovascular response evoked by leptin in the VMH region.

Leptin-sensitive neurons within the VMH might also regulate sympathetic outflow via indirect connections with sympathetic preganglionic nuclei. It has been shown that the VMH projects to many nuclei in the forebrain and brain stem, including several nuclei that are known to have connections with sympathetic outflow, such as the DMH, midbrain periaqueductal gray, parabrachial nucleus in the pons, and the nucleus of the solitary tract in the medulla.36,37 Thus, in summary, the anatomic evidence is consistent with the hypothesis that leptin-sensitive neurons within the VMH region can regulate sympathetic vasomotor activity via direct and/or indirect descending pathway(s) to sympathetic preganglionic neurons.

Perspectives
The discovery that an increase in circulating leptin can lead to an increase in sympathetic vasomotor activity in addition to its effects on appetite and energy expenditure has led to the suggestion that leptin might play a role in obesity-associated hypertension.5 Although obesity is often associated with leptin resistance, it has been shown recently that this is selective, such that the renal sympathoexcitatory effects of leptin are preserved while the metabolic actions are blocked.38,39 This in turn implies that there are different central pathways that subserve these different actions in leptin. The present study suggests that the VMH region might be an important site of action of leptin in increasing RSNA and thus, could be a key component of the central pathways involved in obesity-associated hypertension.

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