Cardiovascular effects of leptin and orexins

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Shirasaka, Tetsuro, Mayumi Takasaki, and Hiroshi Kannan. Cardiovascular effects of leptin and orexins. Am J Physiol Regul Integr Comp Physiol 284: R639–R651, 2003; 10.1152/ajpregu.00359.2002.—Leptin, the product of the ob gene, is a satiety factor secreted mainly in adipose tissue and is part of a signaling mechanism regulating the content of body fat. It acts on leptin receptors, most of which are located in the hypothalamus, a region of the brain known to control body homeostasis. The fastest and strongest hypothalamic response to leptin in ob/ob mice occurs in the paraventricular nucleus, which is involved in neuroendocrine and autonomic functions. On the other hand, orexins (orexin-A and -B) or hypocretins (hypocretin-1 and -2) were recently discovered in the hypothalamus, in which a number of neuropeptides are known to stimulate or suppress food intake. These substances are considered important for the regulation of appetite and energy homeostasis. Orexins were initially thought to function in the hypothalamic regulation of feeding behavior, but orexin-containing fibers and their receptors are also distributed in parts of the brain closely associated with the regulation of cardiovascular and autonomic functions. Functional studies have shown that these peptides are involved in cardiovascular and sympathetic regulation. The objective of this article is to summarize evidence on the effects of leptin and orexins on cardiovascular function in vivo and in vitro and to discuss the pathophysiological relevance of these peptides and possible interactions.

mean arterial pressure; heart rate; sympathetic nerve activity; catecholamine; hypothalamic paraventricular nucleus; depolarization; sympathoexcitation

HYPERPHAGIA (OVEREATING) is often associated with energy overstorage and obesity, which may lead to a myriad of serious health problems, including heart disease, hypertension, and type 2 diabetes. Thus understanding the complex pathological mechanisms underlying hyperphagia and obesity has important clinical significance. The concept of the hypothalamus playing a role in the regulation of feeding behavior and energy homeostasis was originally based on observations of brain lesions (69). Lesions of the ventromedial hypothalamus (VMH) produce hyperphagic obesity, whereas lesions of the lateral hypothalamus (LH) induce hypophagia and weight loss, suggesting that satiety and feeding centers existed in the VMH and LH, respectively (7). Recent advances have led to a greater understanding of the signaling pathways that regulate these centers, particularly those involving the satiety center in the VMH (7), which is dominated by the hormone leptin (11, 103). Leptin, the protein product of the ob/ob gene, is a 167-amino acid protein produced and secreted by adipocytes in direct proportion to adiposity in rats and humans (11, 32, 103). Leptin suppresses food intake by inhibiting neuropeptide Y (NPY) secretion from the arcuate nucleus (18, 67, 70, 88), by acting on the VMH through increasing the production of the melanocyte-stimulating hormone (MSH), or by decreasing the agouti-related peptide (AGRP), an antagonist of MSH at the MC4 receptor (25, 26). In addition, leptin receptor modifications and modifications in the ob/ob gene, which result in a lack of leptin production, also result in obesity, hyperinsulinemia,
and hypercorticosteronemia (72). In contrast, two novel hypothalamic peptides that stimulate food consumption when administered centrally were discovered in an intracellular calcium influx assay on multiple cells expressing individual orphan G protein-coupled receptors (76). Two research groups reported finding almost simultaneously (21, 76). These peptides are known as orexins (orexin-A and -B) (76) or hypocretins (hypocretin-1 and -2) (21). Orexin-A (hypocretin-1) consists of 33 amino acids and has an NH2 terminal pyroglutamyl residue and COOH terminal amide group (76). Orexin-B (hypocretin-2) consists of 28 amino acids and is 46% identical to orexin-A (76). Intracerebroventricular administration of orexin-A and -B stimulates food intake in a dose-dependent manner (76). In addition to the appetite-promoting activity of orexins, their mRNA levels were upregulated more than twofold after a 48-h fasting period (76). The mRNA is expressed abundantly and specifically in the lateral hypothalamic nucleus (LHA) and adjacent areas (76), a region implicated in the central regulation of feeding behavior and energy homeostasis (7). Orexin-A and -B neurons are restricted to the lateral and posterior hypothalamus, whereas both orexin-A and -B nerve fibers projected widely into the olfactory bulb, cerebral cortex, thalamus, hypothalamus, and brain stem (20, 71). In contrast, expression of orexin receptor mRNA (OX1R and OX2R) is widely distributed in the rat brain (96). The expression patterns for OX1R and OX2R are distinct. Within the hypothalamus, OX1R mRNA is most abundant in the ventromedial hypothalamic nucleus (VMH) (76, 96) and moderate levels are detected in the medial preoptic area, lateral anterior and dorsomedial hypothalamic nuclei (DMH), lateral mammillary nucleus, and posterior hypothalamic area. In contrast, OX2R mRNA is expressed predominantly in the hypothalamic paraventricular nucleus (PVN) and moderate levels are detected in the VMH and DMH and the posterior and lateral hypothalamic areas (96). The difference in expression patterns for OX1R and OX2R mRNA in the VMH and PVN is significant. Although it appears that both nuclei play key roles in the neuronal circuitry of feeding regulation, the hypothalamus is the main integrative center for a number of other neuroendocrine and autonomic nervous functions in addition to feeding behavior (7, 91). For example, the PVN of the hypothalamus, which is enriched with a vast number of neuroendocrine modulators (19, 36), has been implicated in the stress response, control of pituitary function, body fluid homeostasis, analgesia, and cardiovascular and gastrointestinal functions (4, 90, 91). Hypothalamic PVN is a heterogeneous structure comprised of neuronal populations that are grouped generally into magnocellular (type 1) and parvocellular (type 2) neurons (91, 94). Both magnocellular and parvocellular neurons can be further subdivided on the basis of peptide expression, projection targets, and/or location in the nucleus (91). Magnocellular neurons can be oxytocinergic neurons or vasopressinergic neurons, whereas parvocellular neurons can be either neuroendocrine or preautonomic neurons (89, 91). The preautonomic parvocellular neurons project to rostral ventrolateral medulla (RVLM) and to the intermediolateral cell column (IML) of the spinal cord, which are involved in the regulation of heart rate (HR) and arterial pressure (AP) (4, 80, 91). Electrical and chemical stimulation of the PVN increases AP and renal sympathetic nerve activity (RSNA) in conscious rats (47). Conversely, VMH is involved in the homeostatic regulation of body metabolism mediated via sympathetic nerves (84). Several peptides, which affect food intake, have an effect on cardiovascular response and sympathetic nerve activity (9, 22, 83). This review describes results that provide anatomical and physiological evidence for the cardiovascular and sympathetic effects of leptin and orexins and discusses the significance of each peptide and their possible interaction.

**LEPTIN AND CARDIOVASCULAR FUNCTION**

Leptin binding sites have been found in brain regions that are important in cardiovascular control (93). Leptin receptor mRNA is also found extensively in the central nervous system (CNS) (40, 79, 93), and leptin has been shown to activate neurons in many nuclei of the hypothalamus, including the PVN and VMH (23, 24, 102). The intracerebroventricular or intravenous administration of leptin produces marked changes in AP (17, 81), HR, sympathetic nerve activity (SNA) (34, 35, 60), and renal excretory function (45, 75) in rats and rabbits.

**Integrative Action of Leptin**

*Effects induced by intracerebroventricular administration.* The effects of leptin on cardiovascular function have been studied in animals by determining changes in HR and AP induced by intracerebroventricular and intravenous administration of leptin. The administration of leptin intracerebroventricularly activates specific nuclear groups in the hypothalamus and brain stem known to regulate cardiovascular responses (24, 99). On the basis of this knowledge, it was hypothesized that leptin may affect cardiovascular function via a CNS site of action. To explore this possibility, cardiovascular responses induced by intracerebroventricular administration of leptin were investigated (17, 22, 60). Intracerebroventricular administration of leptin (5–50 μg) was found to elicit a dose-related increase in MAP and RSNA in conscious rabbits, with peak values obtained after 10 and 20 min, respectively, while producing no consistent, significant increases in HR (60). Correia et al. (17) reported that a chronic (2–4 wk) high dose (1,000 ng/h) of leptin increased AP and HR in conscious Sprague-Dawley (SD) rats. Acutely injected leptin did not induce tachycardia, possibly due to the prevention of a direct cardiac sympathoexcitatory effect as a result of baroreceptor reflex activation by elevated AP levels. Intracerebroventricular administration of leptin in a chloralose-urethane-anesthetized Wistar rat increased MAP, lumbar, and renal SNA with a reduction in blood flow in the iliac and superior...
mesenteric arteries, but not in the renal artery (22). Sympathoactivation induced by intracerebroventricular leptin administration was observed in the kidneys, adrenal glands, hindlimbs, and brown adipose tissue (BAT) (34, 35). Haynes et al. (33) demonstrated that an increase in RSNA induced by intracerebroventricular application of leptin was due to activation of hypothalamic melanocortin receptors; in contrast, sympathoactivation of thermogenic BAT by leptin was found to be independent of the melanocortin system. These data suggest that sympatoactivation caused by leptin is controlled by heterogeneous neural mechanisms, which only partly involve the melanocortin system. Leptin-induced sympatoactivation was apparent after transection of sympathetic nerves distal to the recording site (35), implying the involvement of efferent rather than afferent nerves. This was confirmed by the prevention of sympathoexcitatory and pressor effects of central leptin after the intravenous administration of a ganglion-blocking agent (27, 60). In contrast to administration of leptin intracerebroventricularly, intravenous administration at the same dose did not increase AP or RSNA in conscious rats (17) and rabbits (60). The pressor effect of leptin was proportional to the level of leptin in the cerebrospinal fluid (CSF) (17). These results suggest that the pressor and sympathoexcitatory effects of leptin are due to a central neural action. Leptin did not cause sympathoactivation in obese Zucker rats (35), which are known to possess a mutation in the gene for the leptin receptor (72). This implies that the sympathoexcitatory action of leptin requires the presence of an intact leptin receptor. Taken together these results suggest that intracerebroventricularly administered leptin produces pressor and sympathoexcitatory effects mediated via a central leptin receptor.

Effects induced by intravenous administration. Intravenous infusion of leptin increases Fos production in spinally projecting neurons in the hypothalamic PVN, and this directly influences sympathetic nerve activity (5). Chronic intravenous infusion of leptin in conscious SD rats at 1 μg·kg⁻¹·min⁻¹ (1,152–3,456 μg) significantly increases MAP and HR after 3 and 4 days, respectively (81). This dose of leptin also increases plasma levels of leptin, explaining the slow onset of increase in AP and HR levels induced by increasing levels of circulating leptin. All variables returned to control levels when leptin infusion is stopped. A low dose of leptin (0.1 μg·kg⁻¹·min⁻¹; 345.6 μg) did not affect these variables (81). Some studies reported that AP and HR levels were unaffected by acute leptin infusion (33, 34, 35). One possible explanation is that AP and HR levels were measured while the animal was under anesthesia (33–35). Another explanation is that the acute administration of leptin, either by a single bolus injection or by short-term infusion, induced plasma leptin levels that were too low to influence the level of AP or HR. Leptin increases the norepinephrine turnover in interscapular BAT (15) and acute intravenous infusion of leptin in anesthetized SD rats increases SNA in the adrenals, BAT, and the kidneys (34, 35). Since the leptin-induced elevation of AP and HR is abolished by α₁ and β-adrenergic receptor blockage, the mechanism controlling these events may be mediated by activation of the sympathetic nervous system (12). How the large (M₀, 16,000) leptin protein crosses the blood-brain barrier to activate the OB-RB receptor in the CNS is not known. It is possible that the hypothalamic leptin receptor is in a region where there is a weak or non-existent blood-brain barrier. The functional leptin receptor OB-RB is expressed in endothelial cells (87). This is significant because the vascular endothelium is known to play a critical role in AP homeostasis, in part by its ability to produce potent vasoactive factors, principal among these being the vasodilator nitric oxide (NO) (66). Intravenous infusion of leptin (10–1,000 μg/kg) increases serum NO concentrations in a dose-dependent manner in anesthetized Wistar rats (27) and enhances the increase in AP and HR levels under NO synthesis inhibition (53). This suggests that leptin originating from the peripheral tissue increases the sympathetic outflow through the CNS and may tonically modulate the cardiovascular function mediated via local NO. However, Mitchell et al. (63) demonstrated that acute intravenous infusion of leptin had no significant effect on hemodynamics in the presence of an NO synthase inhibitor, despite a significant increase in lumbar SNA in conscious rats. Chronic intravenous infusion of leptin, under impaired NO synthesis, moderately enhances the hypertensive effects of leptin and severely amplifies the tachycardia caused by hyperleptinemia in conscious rats (53). Acute injection of leptin does not induce hypertension and may reflect the brief duration of leptin administration. It is also possible that the depressor action of NO may prevent the pressor responses induced by sympathetic activation. A further possibility is that changes in baroreceptor reflex activity induced by leptin may modulate the direct cardiovascular response, as well as NPY, which attenuates baroreflex sensitivities by intracerebroventricular injection (61). These findings suggest that stimulation of endothelium-derived NO has a depressor effect and opposes the pressor effect mediated by the direct sympathoexcitatory effects of leptin. It is likely that intravenously administered leptin modulates cardiovascular function mediated by the central sympathetic nervous system and/or the peripheral NO system.

Cellular Action of Leptin

The leptin receptor (Ob-Rb) gene has at least six splice variants (103). The observations that the Ob-Rb variant is highly expressed in the hypothalamus and that the obese diabetic db/db mouse mutation is found in the Ob-Rb variant strongly suggest that leptin normally exerts its effects on this hypothalamic receptor. Systemic administration of leptin (1 mg/kg) in the ob/ob mouse activates Fos protein expression in the PVN (102), and intracerebroventricular administration of leptin (3.5 μg) induces c-Fos-like expression, in addition to enhancing levels of CRH mRNA in the PVN.
in Long-Evans rats (99). Furthermore, intracerebroventricular and intraperitoneal administration of leptin (1 mg/kg) induces Fos expression in the dorsal, ventral, and lateral parvocellular subdivisions of the PVN (24). These subnuclei are a major source of descending axons to autonomic preganglionic neurons within the medulla and spinal cord. These results suggest that circulating leptin activates PVN neurons and regulates physiological functions. To examine this possibility, Powis et al. (73) examined the direct membrane effects of leptin on the PVN neuron of rat brain slices using whole cell patch-clamp recording techniques. Bath applications of leptin (1–100 nM) produced dose-related depolarizations in 82% of the type 1 (magnocellular) neurons tested and 67% of the type 2 (parvocellular) neurons tested. Similar depolarizations were observed in response to bath application of leptin during synaptic transmission blockage using the sodium channel blocker TTX, indicating that leptin has a postsynaptic site of action in PVN neurons. A voltage-clamp study revealed that leptin-induced currents have a reversal potential between −25 and −30 mV, indicating that a nonspecific mix of cations carries this current. These results suggest that leptin has an excitatory effect on CNS neurons, in particular PVN neurons.

OREXINS AND CARDIOVASCULAR FUNCTION

The orexins were initially characterized as potent stimulants of food intake (76); however, mRNA mapping of the orexin receptors (OX1R and OX2R) (76, 96) and orexin nerve fibers (20, 97, 98) suggests that they have a role in other physiological functions, such as the regulation of blood pressure, the neuroendocrine system (98), and the sleep-waking cycle (13, 50, 56). For example, the administration of orexin-A or -B induces marked changes in AP, HR, RSNA, and plasma catecholamine (CA) in anesthetized (3, 14) and conscious (62, 77, 78, 86) animals.

Integrative Action of Orexins

Effects induced by intracerebroventricular administration. Intracerebroventricular injection of orexins induces c-Fos expression in the locus ceruleus, arcuate nucleus, central gray, raphe nuclei, NTS, supraoptic nucleus (SON), and PVN in Wistar rats (20, 54), indicating that central administration of orexin activates specific nuclear groups in the hypothalamus and brain stem known to regulate autonomic and neuroendocrine functions (91). We hypothesized that orexin might affect cardiovascular function mediated via a CNS site of action. To examine this possibility, the cardiovascular and sympathetic responses produced by the central administration of orexin-A and -B were studied in conscious, unrestrained Wistar rats (86), because anesthesia is well known to have a profound effect on the cardiovascular and autonomic nervous systems (101). Intracerebroventricular administration of orexin-A provoked a dose-related increase in MAP, HR, and RSNA in conscious rats (Fig. 1). The MAP and HR increased rapidly and reached peak values 10–15 min after orexin-A administration. Pressor effects induced by intracerebroventricularly administered orexin-A and -B were also observed in conscious SD rats (77) and rabbits (62). In urethane-anesthetized rats, intracisternal (14) or intrathecal (3) injections of orexin-A or -B increased MAP and HR in a dose-dependent manner. Intravenous injection of the same dose of orexin-A or -B used in the intracerebroventricular injection experiment failed to cause any cardiovascular and SNA change (62, 86). These results suggest that the pressor effects induced by orexin-A and -B were mediated via a CNS site of action. A high dose of orexin-A produced a

Fig. 1. Time course of changes in mean arterial pressure (MAP), heart rate (HR), and renal sympathetic nerve activity (RSNA) during the 60 min after intracerebroventricular administration of orexin-A (0.3 and 3.0 nmol) or vehicle (saline) in conscious rats. Vertical dotted line indicates the time, 0 min; bpm, beats/min. All data are means ± SE; n is the number of animals. *P < 0.05 vs. vehicle. †P < 0.05 vs. orexin-A (0.3 nmol). [Borrowed with permission from Shirasaka et al. (86).]
significant increase in RSNA 10 min after injection, which persisted for ~15 min (Fig. 1). RSNA also increased transiently at a low dose (0.3 nmol) of orexin-A. There was a statistically significant correlation coefficient ($r$) between RSNA and MAP ($r = 0.69$ and $r = 0.83$, respectively; both $P$ values $< 0.001$) or HR ($r = 0.76$ and $r = 0.89$, respectively; both $P$ values $< 0.001$) at 0.3- and 3.0-nmol doses in the orexin-A injected group (Fig. 2). Intracerebroventricularly administered orexin-B (3.0 nmol) also produced a significant increase in MAP, this response pattern being similar to that observed for orexin-A administration (Fig. 3). HR also rapidly increased and returned to control levels within 30 min of orexin-B (3.0 nmol) administration. In contrast to the results with orexin-A, RSNA did not increase significantly at a dose of 0.3 or 3.0 nmol. However, a 30-nmol dose of orexin-B resulted in an ~40% ($P < 0.001$) increase in RSNA 20 min after injection (unpublished data). For each dose, the maximum changes from control values for orexin-A and -B during the recording time (60 min) were compared (Fig. 4A). Central orexin-A induced an increase in MAP ~1.5-fold greater than orexin-B for both doses, but no significant differences were observed in HR. These results are consistent with findings by Chen et al. (14). The increase in RSNA produced by intracerebroventricular administration of orexin-A was greater than that produced by orexin-B at 3.0 nmol. To provide a description of the duration and magnitude of cardiovascular and sympathetic responses, the area under the curve (AUC) (2) was calculated for the 60-min period immediately after peptide injection for each animal within a group (Fig. 4B). The AUC in MAP and HR was significantly larger for orexin-A than for orexin-B at only 3.0
nmol. The AUC in RSNA was larger for orexin-A than for orexin-B at each dose. In almost all rats subjected to intracerebroventricular administration of orexin-A and -B (0.3 and 3.0 nmol, respectively) in conscious rats. All data are means ± SE; n is the number of animals. *P < 0.05 vs. orexin-B for each dose. [Borrowed with permission from Shirasaka et al. (86).]

Fig. 4. Bar graph showing maximal changes from control values (A) and the area under the curve (AUC; B) for MAP, HR, and RSNA during the 60 min after intracerebroventricular administration of orexin-A and -B (0.3 and 3.0 nmol, respectively) in conscious rats. All data are means ± SE; n is the number of animals. *P < 0.05 vs. orexin-B for each dose. [Borrowed with permission from Shirasaka et al. (86).]

Regional differences in sympathetic outflow are known to exist (100). To examine systemic sympathetic outflow induced by central orexins, plasma CA was measured under similar conditions to record nerve activity (Fig. 5). High doses of orexin-A and -B increase plasma norepinephrine (NE), the effect being larger and longer lasting with orexin-A. Therefore, it is likely that the orexin-induced increase in sympathetic nerve outflow leads to the increase in plasma NE, which in turn induces cardiovascular responses. Intracerebroventricularly administered orexin-A also significantly increases plasma epinephrine (Epi) levels 10 min after injection. Al-Barazanji et al. (1) demonstrated that intracerebroventricular injection of orexin-A results in a rapid and significant increase in plasma levels of ACTH and corticosterone and mRNA levels of CRF and AVP in the parvocellular neurons of the PVN. These results suggest that orexin-A acts centrally to activate the hypothalamic-pituitary-adrenal (HPA) axis and involves the stimulation of both CRF and AVP expression. Central orexin-A also increases plasma Epi, glucose, and AVP levels in conscious rabbits (62). The
elevated circulating level of Epi in addition to NE, after injections of a high dose of orexin-A, suggests that the sympathoadrenomedullary system (SA system) is activated. In contrast to orexin-A, central orexin-B did not produce an increase in plasma Epi. The large pressor response induced by central orexin-A, compared with that induced by orexin-B, may be due to activation of the SA system in addition to sympathetic outflow. This suggests that intracerebroventricular administration of orexin-A and -B induces cardiovascular responses via different central mechanisms.

Effects induced by intravenous administration. Orexins were initially thought to be synthesized exclusively in the brain in cell bodies in the lateral hypothalamic/perifornical area (76). It is known that prepro-orexin and orexin receptor mRNAs are also expressed in peripheral tissues such as kidney, adrenal, thyroid, testis, ovaries, and jejunum (46, 51). Although peripherally administered orexin-A enters the brain (49), reports of positive effects of intravenously administered orexin-A or -B are not as common. Thryotropin-releasing hormone (TRH) release from the rat hypothalamus in vitro was inhibited significantly in a dose-related manner with the intravenous injection of orexin-A (64). A high dose of intravenously administered orexin-A (3 mg/kg) induces analgesia in Wistar rats in the hotplate test; in addition, thermal hyperalgesia is induced by carrageenan (8). The level of intravenously administered orexins may have been too low to evoke other physiological responses. Alternatively, the action of leptin in peripheral tissue may be via an autocrine/paracrine mechanism.

Cellular Action of Orexins

The two known orexin receptors (OX1R and OX2R) belong to the G protein-coupled receptor superfamily with a proposed seven-transmembrane topology (76). OX1R and OX2R mRNAs are located exclusively in the rat brain. Orexin-A or -B evokes NE release from rat cerebrocortical slices (37). These findings are consistent with the idea that orexins are regulatory peptides that function within the CNS. The mRNA of OX1R and OX2R is differentially distributed, with OX1R mRNA most abundant in the VMH and OX2R mRNA predominantly expressed in the PVN. The preautonomic parvocellular neurons of the PVN send long descending projections to several areas within the CNS that are known to be important in cardiovascular function (4, 89, 91). These regions include the NTS, where baroreceptor and chemoreceptor afferents terminate, the dorsal vagal complex, which is present in the dorsomedial medulla and contains vagal preganglionic neurons, the RVLM, which is probably a major site for the generation of sympathetic tone for the vasculature, and the IML cell column of the thoracolumbar spinal cord, which is the site of sympathetic preganglionic motor neurons involved in the regulation of HR and BP (4, 90). Taken together, these data suggest that orexins are likely to affect PVN neurons and play a broad role in cardiovascular regulation.
regulatory role in the CNS. To examine whether orexins affect PVN neurons, we measured the changes in membrane potential induced by an application of orexins on the PVN neurons of a rat hypothalamic slice using the whole cell patch-clamp recording technique (85), according to cell classification (94). Bath applications of orexin-B (0.01–1.0 μM) depolarized 80.8% of type 1 and 79.2% of type 2 neurons in a dose-dependent manner in normal artificial CSF (aCSF) (Figs. 6 and 7). Orexin-A (1.0 μM) also induced depolarization in type 1 (magnocellular) and type 2 (parvocellular) neurons. These responses were accompanied by an increase in action potential firing (Fig. 6A). A similar reversible depolarization was observed in the presence of TTX (Fig. 6B), indicating that the depolarizing action is mediated by a postsynaptic orexin receptor. The direct postsynaptic excitatory action of orexins (hypocretins) was demonstrated in the locus ceruleus (30, 39, 44), arcuate nucleus (74), dorsal motor nucleus of the vagus (DMNV) (41), IML (3), and laterodorsal tegmental nucleus (LDT) (10). In further experiments involving the addition of Cd²⁺ in the aCSF-containing TTX (Fig. 6C), the increases in membrane potential induced by orexin-B significantly decreased in type 2 neurons (10.1 ± 0.64 to 7.84 ± 0.51 mV; n = 7, P < 0.05). This suggests that the orexin-evoked depolarization was produced in part by Cd²⁺-sensitive Ca²⁺ channels, which contribute to the release of glutamate from presynaptic nerve terminals, and that orexin-B also excites type 2 neurons, at least in part, by glutamatergic transmission. Therefore, it may be possible that orexin-B depolarizes type 2 neurons via both postsynaptic and presynaptic action. Monitoring membrane resistance during the response does not reveal a clear conductance change. Orexin-B has been reported to decrease or affect potassium conductance (44) or reduce afterhyperpolarization (39). Hwang et al. (41) demonstrated that orexins affect more than one conductance, which may include a nonselective cation conductance and a potassium conductance in DMNV neurons. It is possible that orexins excite type 1 and type 2 neurons of the PVN by increasing depolarizing conductance and decreasing hyperpolarizing conductance, leading to no change in conductance. Intracerebroventricularly administered orexins induce c-Fos expression in the PVN (20, 54). These studies suggest that
endogenous orexin-A and -B depolarize PVN neurons and increase the firing rate via postsynaptic receptors, leading to modulation of various physiological functions, including cardiovascular responses.

THE POSSIBLE CENTRAL INTERACTION BETWEEN OREXINS AND LEPTIN

Orexins were first characterized as stimulators of appetite and food consumption (76, 84); on the other hand, leptin was suspected to reduce food intake, mainly acting on neurons in the arcuate nucleus of the hypothalamus (103), and increase energy expenditure (18, 35). Intracerebroventricular or intraperitoneal administration of leptin inhibits a fasting-induced increase in prepro-orexin mRNA and orexin receptor (OX1R) mRNA levels (57) or reduces the orexin-A concentration in the rat hypothalamus (7). Horvath et al. (38) observed orexin fibers making direct contact with NPY cells and leptin receptors coexpressing in neurons in the arcuate nucleus. This suggests that orexins may act as a relay for leptin-induced actions in the CNS. Administration of leptin inhibits the electrical activity of orexin-sensitive neurons in the arcuate nucleus of the hypothalamus (74). The opposing actions of orexin and leptin on neuronal excitability are consistent with their opposing effects on food intake. These data sup-

Fig. 8. Schematic diagram of possible mechanisms for the action of central leptin and orexins in cardiovascular, neuroendocrine, and sympathetic outflows. Leptin and orexins bind to their receptors of the magnocellular or parvocellular neurons of the hypothalamic paraventricular nucleus or arcuate nucleus neurons, causing their depolarization. Excitation of magnocellular neurons induces secretion of arginine vasopressin (AVP) from the posterior pituitary, antidiuresis, and vasoconstriction. Conversely, parvocellular neurons activate autonomic centers in the brain stem and spinal cord, increasing the HR and blood pressure or causing the release of CRF. Secretion of ACTH from the anterior pituitary is controlled by CRF and AVP, synthesized by the parvocellular neuron. Right lower vessel, norepinephrine released from the sympathetic nerve ending induces vasoconstriction. Activation of the renal sympathetic nerve induces antidiuresis and secretion of renin. Solid lines, a neural or humoral pathway. Dotted lines, functional influence. ARC, arcuate nucleus; CVOs, cerebroventricular organs; DVC, dorsal vagal complex; IML, intermediolateral cell column; LHA, lateral hypothalamus; PVN, paraventricular nucleus; Ma, magnocellular neuron; Pa, parvocellular neuron; RVLM, rostral ventrolateral medulla.
port the hypothesis that the arcuate nucleus is a site of integration for stimulatory and inhibitory drives on food intake, the former being mediated by the neuropeptide orexin from the LHA and the latter by leptin circulating in the blood. The hypothesis that control of feeding and energy metabolism involves leptin and orexins is supported by observations of morphological (28, 31, 68) and functional (6, 67, 84, 92) interactions between these peptides. With respect to cardiovascular function, the pressor, tachycardic, and sympathoexcitatory effects of orexins (14, 62, 77, 86) are similar to the effects of leptin (12, 17, 22, 34, 60). Neurons in the arcuate nucleus of the hypothalamus are known to establish functional synaptic contacts with the PVN neurons (55). Stressful stimuli significantly increase Fos protein levels in orexin neurons (104), and the orexin system is involved in the stress reaction mediated via a CRF (43). Leptin is an acute phase reactant with hematopoietic, immunomodulatory, and hepatocyte stimulating activity during the infectious and noninfectious stress responses (58). In critically ill patients, leptin levels increase significantly in response to stress-related cytokines (tumor necrosis factor, interleukin-1) (65) and may contribute to the anorexia and cachexia of infection. Considering the similarity in cardiovascular and sympathetic action of these peptides, it is possible that the orexins and leptin interact in the hypothalamus, most likely in the arcuate nucleus-PVN areas. Both peptides may be activated under stress conditions and cause an increase in AP, HR, and SNA as an adaptive response. The orexins and leptin have a direct excitatory postsynaptic effect on PVN neurons (73, 78, 85), leading to diverse pathophysiological consequences, including autonomic and cardiovascular functions associated with the stress reaction (Fig. 8). The majority of obese human subjects have high circulating concentrations of leptin (16). Leptin-induced sympathoexcitation may increase thermogenesis but may also contribute to the sympathetically mediated renal sodium reabsorption and hypertension of obesity. Although the pathophysiological role of the sympathoexcitatory effects of leptin and orexins is not clear, the close relationship between obesity, hypertension, and altered cardiovascular responses has been documented in a number of studies (48). Therefore, leptin and orexins may be the chemical mediators in the brain responsible for the generation and maintenance of hypertension that is associated with conditions of energy imbalance, such as obesity.

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