Hypothalamic control of glucose and lipid metabolism in skeletal muscle

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Received: January 16, 2017 / Accepted: January 26, 2017

Abstract The hypothalamus controls glucose and lipid metabolism in peripheral tissues. Recent studies have revealed that the ventromedial hypothalamus (VMH) and arcuate nucleus (ARC) of the hypothalamus play an important role in the regulation of glucose and lipid metabolism in skeletal muscle and the liver. The fat-derived hormone leptin was thus shown to stimulate glucose uptake and fatty acid oxidation in red-type skeletal muscle by activating VMH neurons - likely mediated in part by augmentation of synaptic plasticity between leptin receptor and proopiomelanocortin (POMC)-expressing neurons in the ARC and melanocortin receptor (MCR)-expressing VMH neurons - and consequent activation of sympathetic nerves innervating the muscle tissue. The VMH - sympathetic nerve axis was also found to be activated by orexin-positive neurons in mediation of hedonic feeding-induced glucose uptake in red-type skeletal muscle. The effects of orexin and leptin on glucose metabolism in skeletal muscle are interconnected with those of insulin, with the action of VMH also being necessary for the beneficial effects of exercise on metabolism. Leptin ameliorates diabetic phenotypes in animals with uncontrolled insulin-deficient diabetes as well as in patients with or animal models of lipodystrophy through the central nervous system (CNS). Finally, a single injection of fibroblast growth factor 1 (FGF1) into the lateral ventricle was shown to induce sustained remission of hyperglycemia in several animal models of type 2 diabetes, at least in part by increasing glucose uptake in skeletal muscle. The CNS thus plays an important role in the control of glucose and lipid metabolism, with the VMH as well as POMC neurons being implicated as key regulators of such metabolism in skeletal muscle.

Keywords: glucose metabolism, fatty acid metabolism, skeletal muscle, ventromedial hypothalamus, proopiomelanocortin neuron, sympathetic nervous system

Introduction

The hypothalamus controls energy balance by regulating food intake and calorie expenditure\textsuperscript{1}, the latter of which encompasses glucose and lipid utilization in thermogenic tissues such as skeletal muscle and brown adipose tissue (BAT). We found that electrical stimulation of the ventromedial hypothalamus (VMH) in rats increases glucose production by the liver, but also increases glucose utilization in skeletal muscle, heart and BAT through activation of the sympathetic nervous system without a change in the plasma concentration of insulin\textsuperscript{2}. Glucose utilization by BAT in response to VMH stimulation was also shown to be related to thermogenesis induced by activation of sympathetic nerves innervating the tissue\textsuperscript{3}. In addition, glucose uptake in skeletal muscle was increased by VMH stimulation in both anesthetized rats and animals treated with a muscle relaxant, suggesting that muscle contraction is unlikely to serve as a primary regulator of increased glucose uptake in the tissues by VMH stimulation.

Electrical stimulation of the VMH activates multiple types of resident neurons as well as axonal projections that pass through this brain region. However, we and others found that the fat-derived hormone leptin, which selectively activates VMH neurons that express the transcription factor SF1 (steroidogenic factor 1)\textsuperscript{4}, increases whole-body glucose turnover and glucose uptake in red-type skeletal muscle, heart, and BAT via the sympathetic nervous system\textsuperscript{5-8}, similar to the effects of electrical stimulation of the VMH\textsuperscript{2}. Furthermore, under the hyperinsulinemic-euglycemic condition, leptin was found to enhance insulin-induced glucose uptake in red-type skeletal muscle, heart, and BAT as well as insulin-induced suppression of hepatic glucose production through the VMH\textsuperscript{9}. We also showed that leptin stimulates fatty acid oxidation in skeletal muscle via activation of AMP-activated protein kinase (AMPK) both directly through interaction with the leptin receptor in skeletal muscle as well as...
as indirectly through activation of sympathetic nerves innervating the tissue\(^\text{(10)}\). SF1 neurons in the VMH were recently found to be required for the beneficial effects of exercise on metabolism including a reduction in fat mass, improvement in glycemia, increase in energy expenditure, and induction of the transcriptional coactivator PGC1\(\alpha\) (peroxisome proliferator–activated receptor \(\gamma\) coactivator 1\(\alpha\)) in muscle\(^\text{(13)}\). Furthermore, the orexin system is activated by a nonhomeostatic regulatory mechanism associated with taste stimulation and increases glucose metabolism in skeletal muscle during feeding via the VMH and sympathetic nerves innervating the tissue\(^\text{(12)}\).

The importance of the CNS in the control of glucose metabolism has also been indicated by studies showing that leptin markedly ameliorates metabolic abnormalities and insulin resistance associated with diabetes in both individuals with lipodystrophy and animal models\(^\text{(13,14)}\). Administration of a pharmacological dose of leptin also ameliorated metabolic abnormalities in rodents with uncontrolled insulin-deficient diabetes even in the presence of extremely low plasma insulin levels\(^\text{(15)}\). Moreover, a single injection of FGF1 into the lateral ventricle was recently shown to induce sustained remission of diabetic hyperglycemia in type 2-diabetic model animals by increasing glucose utilization in skeletal muscle and the liver\(^\text{(16)}\). These various observations thus implicate the CNS as a key player in the regulation of glucose utilization in skeletal muscle.

In this review, recent studies that have examined the role of the CNS in the control of glucose and lipid metabolism in skeletal muscle and the liver will be discussed. In particular, the focus is on the regulatory role of the VMH in glucose and fatty acid utilization in skeletal muscle.

### The VMH is a target of leptin and controls glucose metabolism in skeletal muscle

The hypothalamus plays a key role in the control of energy homeostasis and glucose and lipid metabolism. Neurons in the arcuate nucleus (ARC) of the hypothalamus that express proopiomelanocortin (POMC) or both neuropeptide Y (NPY) and agouti-related peptide (AgRP) are thought to be the principal mediators of the regulation of energy homeostasis and peripheral metabolism by leptin\(^\text{(17)}\) (Fig. 1). \(\alpha\)-Melanocyte-stimulating hormone (\(\alpha\)-MSH) released from POMC neurons and AgRP released from NPY/AgRP neurons reciprocally regulate energy metabolism - in particular, food intake and energy expenditure - by acting at the melanocortin 4 receptor (MC4R)\(^\text{(17)}\). Activation of AgRP neurons in the ARC stimulates feeding behavior through antagonism of MC4R in the paraventricular nucleus of the hypothalamus (PVH)\(^\text{(18)}\). Moreover,
direct activation of MC4R-expressing neurons in the PVH inhibits feeding, whereas their inhibition stimulates it\(^8\). However, the finding that MC4R-expressing PVH neurons do not affect whole-body energy expenditure\(^9\) suggested that energy expenditure and substrate utilization in thermogenic tissues are regulated by MC4R-expressing neurons in other brain regions.

Subsets of VMH neurons express either the leptin receptor (Ob-Rb) or MC4R. Most of these Ob-Rb-positive neurons also express SF1, which is also known as adrenal 4-binding protein (AD4BP)\(^{10,20}\). Ablation of the leptin receptor gene in SF1-expressing neurons was found to induce obesity and insulin resistance in mice\(^{21}\). The VMH has long been known to increase glucose production by the liver through activation of the sympathetic nervous system as well as stimulation of the secretion of glucocorticoid hormones such as glucagon and glucocorticoids. Electrical stimulation of the VMH was thus shown to increase blood glucose levels by stimulating hepatic glucose production via activation of the sympathetic nervous system\(^{22}\). More recently, genetic disruption of glutamate release from SF1 neurons in mice was found to attenuate recovery from insulin-induced hypoglycemia\(^{21}\). Another recent study showed that activation of the TRPV1 (transient receptor potential vanilloid 1) ion channel, by electromagnetic manipulation in glucokinase-expressing neurons of the VMH, elicited a hyperglycemic response\(^{24}\).

In contrast to such elicitation of a hyperglycemic response, the VMH also increases glucose utilization and insulin sensitivity in certain peripheral tissues without a change in plasma insulin concentration (Fig. 1). We thus found that electrical stimulation of the VMH increased glucose utilization in interscapular BAT, heart, and skeletal muscle, but not in white adipose tissue (WAT), in rats with increased glucose production and hyperglycemia\(^2\). This effect of VMH stimulation was not due to an increase in the plasma insulin level and was associated with the thermogenic response of BAT induced by activation of the sympathetic nervous system\(^{25}\). We also found that injection of leptin into the VMH or peripherally, but not into the lateral hypothalamus (LH), stimulated glucose uptake in red-type skeletal muscle, heart, and BAT in mice and rats through activation of sympathetic nerves\(^{6-9}\), similar to the effect of electrical stimulation of the VMH in rats\(^{25}\). The enhancement of glucose uptake in these peripheral tissues - in particular, that of skeletal muscle - was induced >6 h after leptin injection peripherally or into the VMH\(^{8,9}\), suggesting that leptin activates the VMH peripheral tissue network by affecting neuronal plasticity in the brain.

Leptin was shown to increase sympathetic nerve activity in peripheral tissues such as BAT and hind limbs\(^{20}\). Leptin-induced glucose uptake in peripheral tissues was accompanied by a slow increase in sympathetic nerve activity in these tissues, with the activity peaking at ~6 h after leptin injection\(^{26}\). Surgical denervation of sympathetic nerves innervating BAT prevented leptin-induced glucose uptake in the tissue\(^{25}\). In addition, guanethidine, which blocks sympathetic nerve activity, and the β-adrenergic receptor (β-AR) antagonist, propranolol, were each found to abolish leptin-induced glucose uptake in peripheral tissues, whereas adrenal demodulation did not\(^7\). These observations suggested that leptin-induced glucose uptake in skeletal muscle as well as in heart and BAT is mediated by sympathetic nerves that innervate these tissues.

Both Ob-Rb and MC4R are abundant in the ARC, VMH, PVH, and dorsomedial nucleus of the hypothalamus (DMH). To explore the role of these hypothalamic nuclei in leptin-induced glucose uptake in peripheral tissues, we examined the effects of direct injection of leptin or the melanocortin receptor (MCR) agonist MT-II into each nucleus in mice\(^8\). Injection of leptin or MT-II into the VMH increased glucose uptake in red-type skeletal muscle, heart, and BAT, similar to the effect of peripheral injection of leptin. Glucose uptake in peripheral tissues, induced by leptin injection into the VMH, was abolished by intracerebroventricular (icv) injection of the MCR antagonist SHU9119. In contrast, injection of leptin into the ARC or injection of MT-II into the PVH induced only a small increase in glucose uptake in BAT, with no effect on that in skeletal muscle or heart\(^6\). Leptin injection into the PVH or DMH, or MT-II injection into the ARC or DMH, did not increase glucose uptake in any peripheral tissue. Together, these results suggest that Ob-Rb and MCR in VMH neurons each play an important role in leptin-induced glucose uptake in peripheral tissues (Fig. 1).

**Leptin regulates insulin sensitivity in skeletal muscle and the liver through distinct signaling pathways**

Leptin increases glucose utilization in peripheral tissues without a change in plasma glucose and insulin levels, suggesting that leptin maintains the plasma glucose concentration by increasing glucose production as well as glucose utilization in peripheral tissues in an insulin-independent manner. We therefore examined whether leptin increases whole-body glucose turnover - that is, both glucose production and glucose utilization - in mice under both basal and hyperinsulinemic-euglycemic conditions\(^8\). Under the basal insulin condition, leptin maintained the plasma glucose level but increased the rate of whole-body glucose turnover by increasing both hepatic glucose production as well as glucose utilization in peripheral tissues including red-type skeletal muscle. Furthermore, during a hyperinsulinemic-euglycemic clamp, leptin increased whole-body insulin sensitivity including that in skeletal muscle. Leptin acted synergistically with insulin infusion to increase glucose uptake in red-type skeletal muscle. Interestingly, under the hyperinsulinemic-euglycemic
condition, leptin was found to inhibit glucose production by increasing insulin sensitivity in the liver. The leptin-induced suppression of glucose production in the liver thus occurred only under the hyperinsulinemic condition, not under the basal insulin condition.

We next explored the leptin signaling pathways in the VMH responsible for regulation of glucose metabolism in skeletal muscle and the liver under basal insulin and hyperinsulinemic-euglycemic conditions. Peripheral injection of leptin as well as leptin injection into the VMH increased whole-body glucose utilization and glucose uptake in red-type skeletal muscle under basal insulin and hyperinsulinemic-euglycemic conditions via activation of the signaling pathway mediated by extracellular signal-regulated kinase (ERK) and its upstream kinase MEK in the VMH. Under the basal insulin condition, leptin-induced glucose utilization and glucose production were equal and the plasma glucose level was maintained. The increased hepatic glucose production induced by leptin at basal insulin levels thus appears to be reflected by the increase in glucose utilization in peripheral tissues including skeletal muscle. Consistent with this notion, the leptin-induced increase in hepatic glucose production under the basal insulin condition was also found to be mediated by MEK-ERK signaling in the VMH.

In contrast, the leptin-induced suppression of hepatic glucose production during a hyperinsulinemic-euglycemic clamp was mediated by signal transducer and activator of transcription 3 (STAT3) signaling in the VMH. These results suggest that VMH neurons regulate hepatic glucose production and muscle glucose uptake under the hyperinsulinemic-euglycemic condition by distinct signaling pathways in the VMH: MEK-ERK signaling in the VMH stimulates glucose utilization in skeletal muscle, whereas STAT3 signaling in the VMH suppresses glucose production in the liver. However, the increase in insulin sensitivity in the liver induced by leptin might also be mediated by other brain regions such as the ARC and brain stem. The enhancement of the insulin-induced suppression of hepatic glucose production elicited by peripheral injection of leptin was thus only partially attenuated by injection of a STAT3 inhibitor into the VMH, whereas the effect of leptin on glucose uptake in skeletal muscle was abolished by injection of a MEK inhibitor into the VMH. This finding was consistent with the previous observation that restoration of Ob-Rb signaling in the ARC of leptin receptor-deficient Koletsky rats improved glycogenolysis in the liver under normal insulin level. The reciprocal effect of leptin on glucose metabolism in the liver may account for the fact that leptin administration does not induce hypoglycemia in humans or experimental models. At low insulin levels, leptin stimulates hepatic glucose production and maintains the blood glucose concentration. In contrast, under physiological conditions with high insulin levels, which are usually accompanied by high blood glucose concentrations, leptin enhances the insulin-induced suppression of glucose production and accumulation of glycogen in the liver. The reciprocal effect of leptin on glucose metabolism in the liver is also supported by a previous study showing that an iv injection of leptin regulates gluconeogenesis and glycogenolysis in the liver through distinct mechanisms in the brain. Leptin has been shown to inhibit expression of gluconeogenic genes in the liver, while it stimulates glycogenolysis in the tissue under a normal insulin level.

Of note, injection of a MEK inhibitor into the VMH did not suppress the MT-II-induced increases in glucose uptake and insulin sensitivity in skeletal muscle, whereas the effects of leptin were abolished by such injection of the inhibitor. These results suggest that MEK-ERK signaling regulates presynaptic POMC neurons in the VMH. Given that MEK-ERK signaling increases synaptic activity, leptin may stimulate synaptic plasticity of POMC neurons and MCR-expressing neurons in the VMH through activation of such signaling in VMH neurons. Indeed, we found that leptin increased the phosphorylation level of synapsin in the VMH in a manner dependent on MEK-ERK signaling. Furthermore, leptin was shown to induce the expression of brain-derived neurotrophic factor (BDNF) in the VMH through MC4R signaling, and this effect strengthened synaptic activity between POMC neurons and MC4R-expressing neurons in the VMH.
possible that Ob-Rb–expressing VMH neurons enhance synaptic activity of POMC neurons and MCR-expressing VMH neurons either at the point of their synaptic connection or directly through activation of POMC neurons in the ARC\textsuperscript{30}. This notion is consistent with the slow increases in both sympathetic nerve activity in the hind limb and glucose uptake in skeletal muscle induced by leptin injection.

Leptin-induced activation of MEK-ERK signaling in the VMH thus increases insulin sensitivity and glucose utilization in red-type skeletal muscle through activation of MCR in the VMH (Fig. 2). Ob-Rb-expressing VMH neurons likely activate POMC neurons either in the ARC itself or at their synaptic connections with MCR-expressing VMH neurons in a manner dependent on the MEK-ERK pathway. The MEK-ERK pathway thereby stimulates synaptic plasticity at POMC neurons and MCR-expressing neurons in the VMH. Whereas other brain sites may contribute to the leptin-induced enhancement of the suppressive effect of insulin on hepatic glucose production, leptin-activated STAT3 signaling in the VMH mediates this enhancement in the liver.

The VMH contains a heterogeneous population of neurons. Principle neurons in the VMH to regulate peripheral tissue metabolism remains unclear. Recent study showed that activation of a subset of SF1 neurons in the VMH, which appear to be glucose-excitatory neurons, regulates insulin sensitivity in skeletal muscle and the liver via uncoupling protein 2 (UCP2) and mitochondrial fission\textsuperscript{31}. Further studies are necessary to explore the principle neurons in the VMH in the control of muscle glucose metabolism.

Leptin increases fatty acid oxidation in skeletal muscle through activation of AMPK mediated by muscle leptin receptors or the medial hypothalamus-sympathetic nerve axis

In addition to its effects on glucose metabolism, leptin stimulates lipid oxidation, as revealed by a decrease in the respiratory quotient. The protein kinase AMPK regulates contraction-induced glucose uptake and fatty acid oxidation in muscle. We therefore examined AMPK activity in skeletal muscle after injection of leptin into the medial hypothalamus or intravenously in mice\textsuperscript{10} (Fig. 3). Intravenous injection of leptin induced a biphasic effect on the activity of AMPK containing the α2 catalytic subunit (α2 AMPK) in red-type skeletal muscle such as the soleus, with a twofold increase being apparent at ~15 min, a return to baseline by 60 min, and a second twofold increase at 6 h. Injection of leptin into the medial hypothalamus including the VMH and ARC also increased the activity of α2 AMPK in red-type skeletal muscle, with this effect peaking at 1 h and persisting for up to 6 h. The activity of α1 AMPK in soleus muscle was not affected.

Fig. 2  Distinct signaling pathways in the VMH underlie leptin-induced glucose uptake in skeletal muscle and suppression of hepatic glucose production. The leptin receptor (Ob-Rb) in the VMH plays a key role in the regulation by leptin of glucose metabolism and insulin sensitivity in skeletal muscle and the liver. Leptin activates the MEK-ERK signaling pathway in the VMH and thereby increases insulin sensitivity and glucose utilization in red-type skeletal muscle through activation of POMC neurons (either in the ARC itself or at their synaptic connections with VMH neurons) and consequent activation of MCR in the VMH. The MEK-ERK pathway is thus thought to promote plasticity at synapses formed by POMC neurons and MCR-expressing neurons in the VMH. Whereas other brain sites may contribute to the enhancement of the suppressive effect of insulin on hepatic glucose production by leptin, leptin-induced activation of STAT3 signaling in the VMH mediates this effect of leptin in the liver. (modified from Ref. 9)
by intravenous or intrahypothalamic injection of leptin. Mutant (db/db) mice that lack the long form of the leptin receptor Ob-Rb did not manifest an increase in α2 AMPK activity in the soleus in response to leptin injection. These observations thus showed that peripheral injection of leptin induced a biphasic activation of α2 AMPK in red-type skeletal muscle, whereas hypothalamic injection of the hormone induced sustained α2 AMPK activation in muscle with a time course similar to that of the late phase of the activation induced by peripheral leptin injection10).

We explored the role of the sympathetic nervous system in the leptin-induced activation of AMPK in muscle10). Unilateral denervation of the sciatic nerve, which disrupts motor innervation, resulted in a 62% decrease in the catecholamine content of the soleus compared with that of the contralateral, intact muscle. Denervation of the sciatic, femoral, and obturator nerves, which removes both sympathetic and motor innervation, reduced the catecholamine content of the soleus to 6% of the control level. Denervation of all three nerves prevented the activation of AMPK in soleus muscle induced by hypothalamic injection of leptin as well as the late phase, but not the early phase, of AMPK activation elicited by intravenous leptin injection. In contrast, denervation of the sciatic nerve alone did not affect leptin-induced activation of AMPK in soleus muscle, despite the complete loss of motor function. The effect of leptin on AMPK activity is thus independent of motor activity. Together, these findings suggest that the late phase of AMPK activation in red-type skeletal muscle induced by peripheral injection of leptin as well as the AMPK activation in this tissue induced by hypothalamic injection of leptin are mediated by the sympathetic nerves innervating the muscle. Consistent with this notion, as mentioned above, peripheral injection of leptin increases the activity of sympathetic nerves that innervate the hind limb with a time course similar to that of the late phase of AMPK activation in muscle induced by peripheral leptin injection. In contrast, the early phase of AMPK activation elicited by intravenous leptin injection may reflect a direct effect of the hormone. Indeed, leptin increased α2 AMPK activity in soleus muscle ex vivo10) (Fig. 3).

What is the mechanism by which sympathetic nerves activate AMPK in soleus muscle? AMPK is activated by receptors such as α1-AR that are coupled to the G protein Gαs. Such receptors stimulate intracellular Ca2+ signaling and thereby activate the AMPK kinase CaMKK (Ca2+/calmodulin-dependent protein kinase kinase). The adipokine adiponectin activates AMPK in skeletal muscle via CaMKK by increasing the cytosolic Ca2+ concentration25). Furthermore, we found that α1-AR, but not β-AR, contributes to the activation of AMPK in the soleus mediated by sympathetic nerves8). The activation of AMPK in red-type skeletal muscle by leptin and the sympathetic nervous system thus appears to be mediated by an α1-adrenergic mechanism, whereas the stimulation of glucose uptake is mediated by β-AR. We also found that MCR in the brain participates in the hypothalamic effect of leptin on α2 AMPK activity in soleus muscle33). An icv injection of the MCR agonist MT-II thus activated AMPK in muscle, whereas the MCR antagonist SHU9119 blunted the effect of leptin. Injection of MT-II into the VMH was also recently shown to increase energy expenditure, the temperature of muscle and BAT, as well as sympathetic nerve and AMPK activity in red-type skeletal muscle of mice34).

AMPK phosphorylates acetyl-CoA carboxylase (ACC) and thereby increases fatty acid oxidation in skeletal muscle (Fig. 3). Phosphorylation of ACC inhibits its activity and thereby reduces the cellular content of malonyl-CoA, which relieves the inhibition of carnitine palmitoyltransferase 1 (CPT1) and increases fatty acid oxidation. Peripheral or hypothalamic injection of leptin was found to suppress ACC activity in soleus muscle through phosphorylation of the enzyme10). Similar to its effects on α2 AMPK activity in soleus muscle, intravenous or hypothalamic injection of leptin induced biphasic or sustained effects, respectively, on both ACC phosphorylation and fatty acid oxidation in this tissue10) (and unpublished data). The increase in the level of ACC phosphorylation apparent at 6 h after intravenous or hypothalamic injection of leptin was abolished by surgical denervation of the sciatic, femoral, and obturator nerves innervating the hind limb10). Furthermore, leptin was shown to induce phosphorylation of ACC in cultured muscle cells expressing Ob-Rb, and this effect was attenuated by forced expression of a dominant negative mutant of AMPK10).

The down-regulation of ACC activity in soleus muscle by leptin indicates that leptin stimulates fatty acid oxidation in this tissue via disinhibition of CPT1 (Fig. 3). We found that peripheral or hypothalamic administration of leptin increased fatty acid oxidation in red-type skeletal muscle such as the soleus33) as well as in the heart and BAT (unpublished data) of mice injected with radioisotope-labeled 2-bromopalmitic and palmitic acids. The effect of hypothalamic injection of leptin on fatty acid oxidation in soleus muscle as well as the late phase (6 h) of the increase in fatty acid oxidation induced by peripheral leptin injection were abolished by surgical denervation of all three nerves innervating the hind limb. In contrast, the early phase (15 min) of the increase in fatty acid oxidation induced by peripheral injection of leptin was not blocked by surgical denervation, as was the case for the effects of such injection on ACC phosphorylation and α2 AMPK activity. Given that leptin stimulates lipolysis in WAT35), its stimulation of fatty acid oxidation in red-type skeletal muscle likely contributes to the suppression of lipotoxicity in muscle. Consistent with this notion, SF1 neurons in the VMH were shown to be necessary for the beneficial effects of exercise on metabolism. Deletion of SF1 in these neurons thus attenuated metabolic responses to exercise including the reduction in fat mass, improvement in glycemia, increase in energy expenditure, and induction of PGC1α expression in skeletal muscle10).
Orexin increases glucose uptake and insulin sensitivity in skeletal muscle via the VMH-sympathetic nerve system

Orexin-A and orexin-B are neuropeptides that are expressed in specific populations of neurons in the lateral (LH) and perifornical areas of the hypothalamus. These orexin neurons are activated during motivated behaviors and active waking, and they regulate sympathetic nerve activity, energy expenditure, and the blood glucose level as well as feeding, wakefulness, and reward seeking. The actions of orexins are mediated by orexin receptor type 1 (OX-R1) and orexin receptor type 2 (OX-R2) in brain regions including hypothalamic nuclei such as the VMH. Narcolepsy, which is accompanied by a specific loss of orexin neurons, is associated with an increased risk for type 2 diabetes and obesity. Deficiency of orexin neurons in mice results in obesity associated with hypophagia. Furthermore, both insulin sensitivity in peripheral tissues and whole-body glucose metabolism are increased in orexin transgenic mice.

Orexin neurons innervate VMH neurons, and OX-R1 is abundant in the VMH. We therefore examined whether hypothalamic orexins stimulate muscle glucose metabolism via the sympathetic nervous system. Injection of orexin-A into the VMH indeed stimulated glucose uptake in skeletal muscle via activation of sympathetic nerves innervating the tissue. Furthermore, we found that the orexin system is activated by a nonhomeostatic regulatory mechanism associated with taste stimulation and that it increases glucose metabolism in skeletal muscle during feeding.

We first examined the effects of orexin injection into the VMH on glucose turnover and glucose uptake in skeletal muscle in rats and mice. Injection of orexin-A increased whole-body glucose turnover and glucose uptake in skeletal muscle. The effect on glucose uptake was pronounced in red-type skeletal muscle such as the soleus and was maximal at 2 h after orexin-A injection. Orexin-B also increased glucose uptake in soleus muscle, albeit to a lesser extent than did orexin-A. Orexins activate NPY neurons in the ARC. However, icv injection of NPY or AgRP did not induce a significant increase in glucose uptake in skeletal muscle, suggesting that VMH neurons, but not NPY/AgRP neurons, mediate this effect of orexins. Indeed, the mouse VMH contains many orexin-positive processes, some of which are located in close proximity to SF1 neurons, and orexin increased the firing rate of these neurons.

We next examined sympathetic nerve activity by measuring norepinephrine turnover in skeletal muscle. Injection of orexin-A into the VMH increased norepinephrine turnover in red-type skeletal muscle. Furthermore, peripheral administration of guanethidine (a blocker of sympathetic nerve activity), but not adrenal demodulation, blunted the effect of orexin-A on glucose uptake in skeletal muscle. These results suggest that orexin stimulates

**Fig. 3** Stimulatory effect of leptin on fatty acid oxidation in red-type skeletal muscle. Leptin activates AMPK in red-type skeletal muscle through 2 distinct mechanisms: a direct effect of leptin and the hypothalamic-sympathetic nervous system. Activation of AMPK phosphorylates and inhibits acetyl-CoA carboxylase (ACC) activity, which, in turn, inhibits malonyl-CoA synthesis, activates carnitine palmitoyltransferase I (CPT1) and thereby increases mitochondria import and fatty acid oxidation in muscle. (modified from Ref. 58)
glucose uptake in red-type skeletal muscle via orexin receptor–expressing VMH neurons and sympathetic nerves innervating the tissue.

To explore further the role of sympathetic nerves in this system, we examined the effect of orexin-A injection into the VMH on glucose uptake in mice lacking β1-, β2-, and β3-ARs (β-less mice)21. Orexin-A did not increase muscle glucose uptake in these mice. Furthermore, injection of orexin-A into the VMH of wild-type mice did not affect plasma glucose, insulin, or epinephrine concentrations, food intake, locomotor activity, the electromyogram of back muscle, or the activity of α2 AMPK in muscle. These results indicate that orexin-induced glucose uptake in red-type skeletal muscle is not mediated by muscle contraction, insulin secretion, or AMPK activity.

We examined the effects of orexin-A injection into the VMH on the insulin signaling pathway in red-type skeletal muscle21. Orexin-A increased the phosphorylation of insulin receptor substrate–1 (IRS-1), Akt, and AS160, but it did not affect that of the β subunit of the insulin receptor (IR), in muscle of wild-type mice. These effects of orexin-A were not observed in β-less mice. Furthermore, orexin-A increased the amount of phosphatidylinositol (PI) 3-kinase activity associated with IRS-1 in wild-type mice but not in β-less mice. These results suggested that orexin-induced glucose uptake in red-type skeletal muscle is mediated by activation of the insulin signaling pathway (but not that of IR) via sympathetic nerves and β-AR in the muscle cells. Red-type skeletal muscle of mice preferentially expresses the β2 isoform of β-AR22, and peripheral injection of a β2-adrenergic antagonist, but not that of a β1-adrenergic antagonist, inhibited the orexin-A–induced increase in glucose uptake by red-type skeletal muscle in these animals22.

To examine further the role of β2-AR in skeletal muscle, we restored expression of the receptor in the soleus of β-less mice expressing β2-AR under the control of the CAG promoter, not in that of those expressing the receptor under the control of the HSA gene promoter12. Unexpectedly, orexin-A injection into the VMH markedly enhanced the insulin-induced increases in muscle glucose uptake and glycogen synthesis. Orexin-A also enhanced the insulin-induced tyrosine-phosphorylation of IR in red-type skeletal muscle, whereas orexin-A injection alone had no effect on IR phosphorylation. Insulin signaling events such as phosphorylation of the protein kinase Akt and of glycogen synthase kinase 3β (GSK3β) as well as the activity of glycogen synthase a in red-type skeletal muscle were also induced to a greater extent by the combination of insulin and orexin-A than by either agent alone.

We next examined the effects of orexin and insulin on glycogen synthesis in red-type skeletal muscle of β-less mice in which the expression of β2-AR had been restored in the right soleus under the control of either the muscle-specific HSA or the universal CAG promoter22. We confirmed that electroproporation of the soleus, with a vector encoding enhanced green fluorescent protein (EGFP) under the control of the CAG promoter, conferred expression of EGFP not only in myocytes but also in nonmyocyte cells including blood vessel cells. In contrast, the HSA gene promoter conferred EGFP expression only in myocytes. Unexpectedly, orexin-A injection into the VMH increased glycogen synthesis and glycogen content only in the soleus of β-less mice expressing β2-AR under the control of the CAG promoter, but not in that of those expressing the receptor under the control of the HSA gene promoter. These results suggested that the enhancement of insulin-induced glycogen synthesis in red-type muscle by orexin requires β2-AR in nonmyocyte cells of the muscle (Fig. 4).

Orexin neurons are thought to contribute to highly rewarded and motivated behaviors such as hedonic feeding36. The calorie-free sweetener saccharin and glucose are often used as conditioned reinforcers in animal experiments. Saccharin or glucose ingestion increases the abundance of orexin mRNA in the hypothalamus of rats43. Carbohydrate feeding also activates sympathetic outflow40. We therefore examined whether orexin neurons stimulate glucose metabolism in skeletal muscle after hedonic feeding motivated and conditioned with saccharin21. In mice trained to drink saccharin solution spontaneously within 10 min of its presentation, saccharin ingestion induced a significant increase in the expression of the transcription factor c-Fos in orexin neurons, without affecting that in melanin-concentrating hormone (MCH) neurons in the LH. The orexin knockout mice ingested only a small volume of saccharin solution during the initial 10-min period, consistent with the previous observation that orexin knockout mice are not able to adapt to a restricted feeding schedule45. Spontaneous saccharin drinking after training significantly enhanced insulin-induced glucose uptake and glycogen synthesis in red-type skeletal muscle, but not those in WAT. The plasma insulin level before or after insulin injection did not differ
between animals drinking saccharin or water, probably as a result of the anticipatory response induced by training. However, injection of an orexin receptor antagonist into the VMH or peripheral injection of a β2-AR antagonist blunted the effects of saccharin on insulin-induced glucose uptake and glycogen synthesis in red-type skeletal muscle without affecting saccharin drinking behavior and without a change in the plasma insulin concentration. The saccharin-induced enhancement of insulin-induced muscle glucose metabolism required the training of mice with saccharin for several days. These results thus suggested that conditioned taste stimulation with saccharin activates the orexin–VMH–sympathetic nerve axis, and thereby enhances insulin-induced glucose uptake and glycogen synthesis in red-type skeletal muscle (Fig. 4).

To examine the physiological relevance of these effects of orexin, we trained mice to lick and taste a small volume of glucose solution on each of three consecutive days, and then injected an orexin receptor antagonist or saline into the VMH immediately before the next scheduled oral glucose ingestion. The blood glucose level after glucose ingestion was significantly increased by injection of the orexin receptor antagonist into the VMH, whereas the plasma insulin level did not differ between the groups of animals. These results suggest that orexin receptors in the VMH regulate the blood glucose level after oral glucose ingestion in a manner independent of plasma insulin level. The effect of the orexin receptor antagonist required the training of mice with glucose for several days. In contrast, when glucose was intraperitoneally administered, injection of the orexin receptor antagonist into the VMH had no effect on either plasma glucose or insulin levels, even after training of the mice by glucose tasting. Furthermore, oral glucose ingestion resulted in a higher blood glucose concentration in orexin knockout mice than in wild-type mice, even though the plasma insulin level was significantly higher in the former animals.

Together, these various observations suggest that the orexin–VMH–sympathetic nerve system is activated by a nonhomeostatic regulatory mechanism associated with taste stimulation and that it regulates muscle glucose metabolism in response to feeding (Fig. 4). Delivery of insulin to skeletal muscle cells via blood vessels plays a key role in the regulation of whole-body glucose utilization. Both β2-AR in blood vessels and insulin induce vascular relaxation. Our finding that orexin injection into the VMH enhanced the insulin-induced tyrosine-phosphorylation of IR in skeletal muscle suggests that orexin might promote insulin delivery to muscle cells by inducing the activation of β2-AR in muscle cells. An orexin–VMH–sympathetic nerve–β2-AR pathway might thus regulate glucose metabolism in red-type skeletal muscle through...
two distinct mechanisms: (i) orexin in the VMH triggers activation of PI 3-kinase, Akt, and other downstream insulin signaling molecules (but not of IR) in muscle, at least in part through activation of β2-AR in myocytes, and (ii) when the plasma insulin level is high, orexin promotes insulin delivery to myocytes through activation of β2-AR in blood vessels and thereby enhances the stimulatory effect of insulin on glucose metabolism in muscle (Fig. 4). A similar mechanism likely operates with regard to leptin-induced glucose uptake in red-type skeletal muscle. We have thus found that such leptin-induced glucose uptake is abolished in β-less mice, but is restored by forced expression of β2-AR in both nonmyocyte cells and myocytes (unpublished data).

Orexin neurons are activated by fasting, and this activation is associated with food-seeking behavior and enhancement of arousal level. The orexin–VMH–sympathetic nerve–β2-AR system may thus also regulate glucose metabolism in red-type skeletal muscle during fasting. Although the activity of sympathetic nerves in certain tissues such as BAT is markedly suppressed during fasting, that in skeletal muscle is maintained constant. The action of orexin in the VMH might therefore support the metabolic demands of fasting-related behavior such as food-seeking by maintaining glucose utilization in skeletal muscle.

The CNS improves glucose metabolism in insulin-deficient and type 2 diabetes

Severe insulin resistance and diabetes are associated with so-called “lipodystrophy”, which is characterized by reduced leptin production as a result of a greatly reduced adipose tissue mass. The inability to store fat in adipocytes leads to the accumulation of fat in nonadipose tissues including the liver and skeletal muscle among others and thereby gives rise to metabolic impairments such as diabetes.

Several lines of evidence from both humans and animal models suggest that leptin deficiency contributes to the insulin resistance and diabetes that accompany lipodystrophy. Transplantation of WAT from wild-type mice thus reduced insulin resistance in a mouse model of lipodystrophy, whereas transplantation of WAT from ob/ob mice, which harbor a mutant version of the leptin gene, had no such effect. Furthermore, leptin administration ameliorated the diabetic phenotype of a lipodystrophic mouse model in a manner at least in part independent of the associated reduced food intake and body weight. In addition, normalization of the plasma leptin level by leptin administration markedly attenuated the diabetic phenotype of lipodystrophic patients. Leptin treatment is thus the most effective medical therapy for severe insulin resistance and diabetes in such patients. Although POMC neurons and VMH neurons may contribute to the effects of leptin in lipodystrophy, it has remained unknown how leptin might stimulate glucose and fatty acid utilization in skeletal muscle and suppress hepatic glucose production in the liver of lipodystrophic patients and animal models.

Another model of leptin deficiency is uncontrolled insulin-deficient diabetes such as that induced in mice by treatment with streptozotocin (STZ), which destroys pancreatic β-cells. In addition to a marked decline in circulating insulin levels, such uncontrolled insulin-deficient diabetes is characterized by both hyperglycemia and hyperphagia as well as a progressive loss of adipose tissue mass and a consequent pronounced decline in the plasma leptin concentration. The important role of leptin deficiency in insulin-deficient diabetes was indicated by the finding that systemic administration of leptin normalized both the hyperglycemia and hyperphagia in affected animals. Furthermore, icv injection of a low dose of leptin normalized hyperglycemia in mice with STZ-induced diabetes by suppressing hepatic glucose production and increasing glucose utilization in peripheral tissues such as skeletal muscle. More importantly, such leptin injection did not induce hypoglycemia, suggesting that leptin maintains the ability to increase hepatic glucose production when blood glucose levels decline. The effect of leptin on glycemia was not attributable to recovery of pancreatic β-cells, increased urinary excretion of glucose, or reduced food intake.

Systemic leptin administration was also shown to normalize the increased plasma glucagon and corticosterone levels observed in STZ-induced diabetic mice, with these effects thus accounting at least in part for the effect of leptin on hyperglycemia in these animals. However, whereas glucagon levels were returned to normal by physiological leptin replacement, hyperglycemia was only slightly ameliorated, suggesting that other factors may contribute to the antidiabetic effect of pharmacological doses of leptin. Given that normalization of the plasma leptin level results in a sufficient amelioration of diabetes in lipodystrophic patients and animal models, the absence of insulin may increase the dose of leptin required to correct metabolic abnormalities in insulin-deficient diabetes.

Injection of leptin into the VMH was also found to ameliorate STZ-induced diabetes. Unexpectedly, however, examination of mice in which the leptin receptor gene was deleted specifically in SFI neurons revealed that leptin signaling in these VMH neurons was not required for the normalization of diabetic hyperglycemia by central leptin infusion. The role of other hypothalamic neurons in the leptin-induced normalization of hyperglycemia in insulin-deficient diabetes was further studied in mice in which Ob-Rb expression was genetically manipulated in such neurons. Leptin signaling in γ-aminobutyric acid (GABA)–expressing neurons and consequent suppression of their activity were found to be necessary for the leptin-induced correction of diabetic hyperglycemia in mice with STZ-induced diabetes. Restriction of Ob-Rb expression specifically to GABAergic neurons was suf-
cient to increase the survival rate, to partially ameliorate hyperglycemia, and to fully reverse hyperglucagonemia in insulin-deficient mice treated by icv injection of leptin. Leptin receptor expression in both GABAergic neurons and POMC neurons had additive metabolic effects and was sufficient to mediate the lifesaving and antidiabetic effects of leptin in insulin-deficient mice. Leptin receptor expression in these neurons thus restored leptin-induced glucose uptake in BAT and skeletal muscle as well as suppression of hepatic glucose production. These results suggest that leptin signaling in GABAergic neurons, as well as POMC neurons, is necessary to correct the metabolic abnormalities of insulin-deficient diabetes, whereas the role of the VMH remains elusive. Leptin may act on presynaptic GABAergic neurons that project to the VMH and POMC neurons or to other brain regions and thereby relieve the GABA-induced inhibition of postsynaptic neuronal activity. GABAergic neurons that express Ob-Rb are present in the ARC, DMH, and LH. In the ARC, NPY/AgRP neurons also release GABA, which potently inhibits POMC neuronal activity. Leptin suppresses the activity of these NPY/AgRP/GABAergic neurons and thereby activates POMC neurons15,17).

In contrast to the leptin deficiency associated with lipo-dystrophy and STZ-induced diabetes, the plasma leptin level is increased in type 2 diabetes with obesity. Leptin administration therefore does not ameliorate hyperglycemia in type 2 diabetes. However, icv injection of FGF1 was recently found to correct diabetic hyperglycemia in several mouse or rat models of type 2 diabetes16). A single injection of recombinant mouse FGF1 into the lateral ventricle thus induced a sustained reduction (persisting for at least 18 weeks) in blood glucose levels in obese animals such as ob/ob mice, mice with diet-induced obesity rendered diabetic by administration of a low dose of STZ, and Zucker fatty rats. This effect was not secondary to reduced caloric intake or weight loss and did not result in hypoglycemia, similar to the effect of leptin treatment in STZ-induced diabetes. Of note, both skeletal muscle and the liver appeared to be key targets for the central action of FGF1 under both basal and hyperinsulinemic-euglycemic conditions. Although the mechanism of this action of FGF1 remains unknown, FGF1 was found to activate tanyocytes lining the third ventricle of the brain. Tanyocytes are neuronal stem cells that line the lateral and third ventricles and which project to the hypothalamus. FGF1-induced neurogenesis may thus counteract a dysregulation of brain circuitry that contributes to the hyperglycemia associated with type 2 diabetes. These observations further support the notion that the CNS plays an important role in the control of glucose metabolism in skeletal muscle and possibly in the etiology of type 2 diabetes.

Concluding remarks

Claude Bernard first demonstrated that the brain can affect the blood sugar level in 185416). In a series of Takashi Shimazu’s studies of the 1960s, he showed that electrical stimulation of the VMH increased hepatic glucose production through activation of the sympathetic nervous system and stimulation of the secretion of gluconeogenic hormones17). We subsequently showed that the VMH regulates glucose and fatty acid utilization in skeletal muscle12,16). Both VMH neurons and POMC neurons in the ARC play an important role in the control of glucose utilization in skeletal muscle by leptin and orexin8,12). These effects on skeletal muscle are mediated by sympathetic nerves innervating the tissue. Leptin has recently been shown to correct hyperglycemia associated with lipodystrophy or insulin-deficient diabetes15), with hypothalamic GABAergic neurons appearing to be the target for this action of leptin15). More recently, a single injection of FGF1 into the lateral ventricle was shown to induce a sustained reduction in blood glucose level associated with increased glucose uptake in skeletal muscle and the liver in several rodent models of type 2 diabetes16). F1S neurons in the VMH have also been shown to be necessary for the beneficial effects of exercise on metabolism11). These and many other studies have thus revealed the important role of the hypothalamus in the control of glucose and lipid metabolism in skeletal muscle. Further investigations should provide a better understanding of the mechanisms by which the CNS controls muscle metabolism under physiological and pathophysiological conditions.

Conflict of Interests

The author declares that there is no conflict of interests regarding the publication of this article.

Acknowledgments

The author thanks past and present collaborators - especially Shiki Okamoto, Chitoku Toda, Tetsuya Shiuchi, Shigefumi Yokota, Eulalia A. Coutinho, Megumi Hayashi, and Kumiko Saito - who contributed to the studies described in this article. The author also thanks Takashi Shimazu for the supervision of our early studies. The work in the author’s laboratory is supported by a Grant-in-Aid for Scientific Research (B) (24390058) and a Grant-in-Aid for Exploratory Research from the Japan Society for the Promotion of Science; a Grant-in-Aid for Scientific Research on Innovative Areas from the Ministry of Education, Culture, Sports, Science, and Technology of Japan; by the Core Research for Evolutional Science and Technology (CREST) Program of the Japan Science and Technology Agency; and by the Japan Agency for Medical Research and Development.

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