Central insulin-mediated regulation of hepatic glucose production

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Abstract. Insulin controls hepatic glucose production (HGP) and maintains glucose homeostasis through the direct action of hepatic insulin receptors, as well as the indirect action of insulin receptors in the central nervous system. Insulin acts on insulin receptors in the hypothalamic arcuate nucleus, activates ATP-sensitive potassium channels in a phosphoinositide 3-kinase (PI3K)-dependent manner, induces hyperpolarization of the hypothalamic neurons, and regulates HGP via the vagus nerve. In the liver, central insulin action augments IL-6 expression in Kupffer cells and activates STAT3 transcription factors in hepatocytes. Activated STAT3 suppresses the gene expression of gluconeogenic enzymes, thereby reducing HGP. It has become evident that nutrients such as glucose, fatty acids, and amino acids act upon the hypothalamus together with insulin, affecting HGP. On the other hand, HGP control by central insulin action is impeded in obesity and impeded by insulin resistance due to disturbance of PI3K signaling and inflammation in the hypothalamus or inhibition of STAT3 signaling in the liver. Although the mechanism of control of hepatic gluconeogenic gene expression by central insulin action is conserved across species, its importance in human glucose metabolism has not been made entirely clear and its elucidation is anticipated in the future.

Key words: Hypothalamus, Insulin, Liver, Vagus nerve, STAT3

The liver plays a central role in the maintenance of whole-body glucose homeostasis, by adjusting glucose production according to the energy balance. The master regulator of hepatic glucose production (HGP) is insulin, as the liver is exposed to portal blood, which displays high insulin concentrations of approximately three times that in venous blood [1]. Indeed, in diabetes mellitus, the increase of HGP is closely related to the rise of fasting blood level [2]. HGP is strongly controlled by direct actions of insulin on hepatocytes [3]. When bound to its receptor, insulin suppresses HGP via the activation of phosphoinositide 3-kinase (PI3K) signal transduction pathway consisting of insulin receptor substrate (IRS), PI3K, phosphoinositide-dependent kinase 1 (PDK1) and Akt [4]. Thus, liver-specific insulin receptor knockout mice or impaired PI3K signal transduction exhibit hyperinsulinemia and glucose intolerance with impaired insulin-dependent suppression of HGP [5-8]. Meanwhile, insulin is known to control HGP not only by direct mechanisms via hepatic insulin receptors, but also by indirect mechanisms mediated by insulin actions on other organs (Fig.1). It has been reported that insulin still suppresses HGP partially even in mice with PI3K signal transduction inhibition in the liver [8, 9]. Further, in an investigation of the changes in HGP after intravenous and intraportal insulin administration, it was shown that the differences in portal blood insulin level, that is, the direct insulin action on hepatocytes, do not necessarily correlate with the effect of insulin on HGP suppression [10].

Indirect suppression of HGP by insulin is accomplished through the reduction of the release of glycerol and free fatty acids from the adipose tissue, which are the substrates and energy source of gluconeogenesis, and the decrease in the expression of hepatic gluconeogenic genes, including phosphoenolpyruvate carboxykinase (PEPCK) and glucose-6-phosphatase (G6Pase) [11]. Actually, in an investigation using dogs, maintenance of blood free fatty acid at a constant level by the intravenous administration of fat emulsion and hepamin is reported to inhibit suppression of HGP during hyperinsulinemic-euglycemic clamp [12].
This review provides an overview of the mechanisms by which central insulin action exerts control over hepatic glucose metabolism via the vagus nerve.

**Central insulin action and glucose/energy metabolism**

Central insulin action is known to play important roles in regulating energy metabolism (e.g., via food intake and thermogenesis). Studies using monkeys and rats have revealed that intracerebroventricular (icv) insulin administration diminishes the amount of food intake and body weight [16-18]. This food intake-lowering effect of insulin is reported to be reversed by the icv administration of PI3K inhibitor [19]. In addition, brain/neuron-specific insulin receptor knockout mice (NIRKO mice) were reported to display significant increases in the amounts of food intake and adipose tis-
Brain-insulin and glucose metabolism

Brain-insulin and glucose metabolism of HGP in L1 mice is aggravated when insulin receptors are reintroduced into their POMC neurons [31]. However, it has been reported that under a leptin receptor-lacking condition, insulin action in POMC neurons is important to the suppression of HGP [32].

Hyperpolarization of neurons due to PI3K-dependent $K_{ATP}$ channel activation is an important mechanism of hypothalamic insulin action-mediated suppression of HGP. Intracerebroventricular administration of $K_{ATP}$ channel blocker glibenclamide results in the impediment of insulin-mediated suppression of HGP and reduction of blood glucose levels in rats, while icv administration of $K_{ATP}$ channel activator diazoxide results in suppressed HGP and reduced blood glucose levels [27, 28].

Central insulin action and the vagus nerve

Central insulin action controls HGP via the vagus nerve [27]. The efferent vagal fiber begins at the dorsal motor nucleus of the vagus nerve (DMV) in the medullary oblongata, descending alongside the esophagus and branching to the liver just below the diaphragm. When the hepatic branch of the vagus nerve is excised, icv insulin administration-induced suppression of HGP is attenuated [27]. The vagus nerve is known to maintain constant pacemaker-like activity [33], and it has been reported in an electrophysiological study of the rat under pentobarbital anesthesia that the nerve activity is enhanced by a rise in blood glucose, and inactivated by an increase in plasma insulin concentration [34]. However, the question of how the central insulin action actually controls the vagus nerve activity via hypothalamic neurons and suppresses HGP remains unanswered. HGP is also suppressed by icv administration of $\alpha$-MSH [35], and according to a patch clamp study, the agonist of melanocortin receptor, which is the receptor of $\alpha$-MSH, causes hyperpolarization of DMV neurons [36]. In addition, it has been revealed that diazoxide, which activates $K_{ATP}$ channel and induce hyperpolarization of neurons, suppresses HGP when administered to DMV [37]. From these findings, a possibility emerges that suppression of HGP is induced by the inactivation of the vagus nerve.

Efferent vagal nerve fibers synapse with postganglionic neurons in the hepatic portal region parasympathetic ganglia, and distribute themselves in the portal area where active gluconeogenesis takes place within the hepatic lobules [38, 39]. Besides regulating HGP, the vagus nerve is known to be closely involved in the regulation of food intake, body weight, and even displayed insulin resistance [20]. Insulin acts on the hypothalamic arcuate nucleus, containing the agouti-related peptide (AgRP) neurons which secrete food intake-promoting factors neuropeptide Y (NPY) and AgRP, and proopiomelanocortin (POMC) neurons which secrete food intake-suppressing factors such as $\alpha$-melanocyte stimulating hormone (\(\alpha\)-MSH) [21]. The administration of icv insulin attenuates NPY expression, while it increases the expression of POMC, the precursor of $\alpha$-MSH [22-24]. It is also known that insulin hyperpolarizes and inactivates both AgRP and POMC neurons by opening the ATP-sensitive potassium channel ($K_{ATP}$ channel) in a PI3K-dependent manner [25, 26].

Prompted by findings that NIRKO mice display not only increases in the amount of food intake and body weight but also insulin resistance [20], it was elucidated that central insulin action regulates glucose metabolism [15]. Indeed, the administration of insulin to the hypothalamus is reported to attenuate HGP and lower blood glucose value, without any changes in peripheral glucose uptake [27]. Also, the suppression of HGP following insulin administration is disturbed in NIRKO mice and hypothalamic insulin receptor knockdown rats [9, 28]. Intracerebroventricular administration of PI3K inhibitor was found to attenuate the insulin-mediated effects of lowering blood glucose levels and HGP in rats [28, 29]. This suggests that central insulin action controls HGP through a PI3K-dependent mechanism, affecting whole body glucose metabolism.

Central insulin action regulates HGP

Insulin action in the AgRP neurons plays an important role in the central insulin action-mediated suppression of HGP [30]. Increased HGP is displayed by the AgRP neuron-specific insulin receptor knockout mice [30]. It has also been reported that by restoring the insulin receptor expression specifically in the AgRP neurons of the mice with hypothalamic insulin receptor deficiency (L1 mice) which have impeded insulin-dependent suppression of HGP, the insulin-mediated suppression of HGP also recovers [31]. On the other hand, the role of POMC neurons in the central insulin action-mediated regulation of HGP has not been entirely elucidated. Insulin-induced suppression of HGP is not disturbed in POMC neuron-specific insulin receptor knockout mice [30]. It has been also shown that the impediment of insulin-mediated suppression of HGP in L1 mice is aggravated when insulin receptors are reintroduced into their POMC neurons [31]. However, it has been reported that under a leptin receptor-lacking condition, insulin action in POMC neurons is important to the suppression of HGP [32].
in hepatic glucose metabolism. Evidence includes insulin resistance in rats after vagotomy [40, 41], enhanced hepatic glycogen synthesis activity due to vagal nerve stimulation in rabbits [42], and enhanced glucose utilization in perfused rat liver caused by vagal nerve stimulation in the presence of insulin [43]. On the other hand, the mechanism by which the vagus nerve regulates glucose metabolism in hepatocytes remains unknown. It has been reported that vagotomy induces insulin resistance in rats, and this resistance was alleviated by intraportal acetycholine administration [41]. However, although hepatocytes express M3 muscarinic receptors (M3mAChR), hepatocyte-specific M3mAChR knockout and overexpression mice do not display phenotypes with a clear metabolic abnormality, including alteration in hepatic gluconeogenic enzyme expression [44]. This shows that central insulin action-mediated HGP control cannot be explained by the direct action of acetycholine released from the vagus nerve on hepatocytes.

Central insulin action effector molecules in the liver

It is known that icv insulin-mediated suppression of HGP is caused by the attenuation of gene expression of gluconeogenic enzymes such as PEPCK and G6Pase [28]. We found that the transcription factor signal transducer and activator of transcription 3 (STAT3) plays an important role as an effector molecule of central insulin action-mediated gluconeogenic enzyme gene expression control [9]. STAT3 is a ligand-activated transcription factor, and the interleukin-6 (IL-6) family of cytokines is known to be its main ligand [45]. In primary cultured hepatocyte, gene expressions of PEPCK and G6Pase diminish following STAT3 activation [46]. Also, overexpression of constitutively active STAT3 in the liver of leptin receptor-deficient db/db mice displaying obesity and diabetes leads to reduced gene expression of PEPCK and G6Pase, and lowering of blood glucose levels [46]. Activated STAT3 is reported to bind directly to the G6Pase promoter region to suppress G6Pase gene expression [47]. Moreover, liver-specific STAT3-deficient mice display insulin resistance due to the increase in gluconeogenic enzyme expression and HGP, which suggests the importance of hepatic STAT3 in the maintenance of glucose homeostasis [9]. Hepatic STAT3 and PI3K-mediated direct insulin action on the liver have an additive effect in suppressing HGP. Indeed, liver-specific PDK1/STAT3 double knockout mice have increased gluconeogenic enzyme expression and HGP compared with liver-specific PDK1-deficient mice [9].

As seen with food intake, glucose tolerance test and hyperinsulenic-euglycemic clamp, STAT3 is activated in the liver following icv insulin administration and diazoxide administration [9, 48, 49]. On the other hand, STAT3 activation in the liver following glucose tolerance test is attenuated in NIRKO mice [9]. These results show that central insulin action induces hepatic STAT3 activation. We have also found that Kupffer cells play an important role in the central insulin action-mediated hepatic STAT3 activation. In a liposome encapsulated clodronate-mediated Kupffer cell depletion mouse model, icv insulin-induced hepatic STAT3 activation does not take place [50]. A mechanism has been elucidated, in which central insulin action augments IL-6 secretion by Kupffer cells, which acts on hepatocytes in a paracrine manner to activate STAT3 (Fig. 1). Indeed, IL-6-deficient mice, which displays maturity-onset obesity and insulin resistance [51], show impeded icv insulin-mediated suppression of HGP as did liver-specific STAT3-deficient mice [9].

Central insulin action-mediated HGP control and type 2 diabetes mellitus

The importance and mechanisms of central insulin action-mediated hepatic gluconeogenesis regulation have been elucidated from rodent-based studies. However, it has been reported that while icv insulin activates hepatic STAT3 and suppresses gluconeogenic enzyme gene expression in dogs, their HGP is not reduced [49]. These findings indicate that while central insulin action-mediated mechanism that controls hepatic gluconeogenic enzyme expression is preserved across the species, its importance in whole body glucose metabolism differs greatly among the species.

In humans, it is possible to administer peptide hormones to the central nervous system intranasally. Indeed, it is possible to increase the cerebrospinal fluid insulin values without altering the peripheral blood insulin values by administrating 40 IU intranasal insulin to healthy individuals [52]. Intranasal administration of 40 IU insulin diminishes HGP under the condition of pancreatic clamp, which maintain a steady level of plasma insulin and glucagon by somatostatin administration [53]. Further, it has been reported that intra-
nasal insulin administration affects parasympathetic output, which is detected by the measurement of heart rate variability in the electrocardiogram [54]. Also, in a human pancreatic clamp study of the effect of oral diazoxide (which passes the blood brain barrier and induces central-mediated HGP suppression in rodents), HGP is suppressed by diazoxide administration [48].

It has been reported that intranasal administration of a high dosage of insulin (160 IU) enhances insulin sensitivity [54], and clinical application of central insulin action in treating type 2 diabetes mellitus is anticipated. However, all the HGP-diminishing effects of intranasal insulin and oral diazoxide administration were in fact observed in healthy individuals [48, 53, 54], and they may not necessarily be reproducible in type 2 diabetes mellitus accompanied by obesity. The disturbance of central insulin action-mediated suppression of HGP has been reported in a rodent study using a high fat diet-induced obesity model [55], and dysfunction of central insulin action following high fat diet-induced obesity is reported to be due to inflammation of the hypothalamus and PI3K signal transduction failure caused by S6 kinase activation [55-57]. Indeed, dysfunction of insulin-dependent suppression of HGP is reported to be ameliorated by administrating neutralizing antibodies for pro-inflammatory cytokines in the cerebral ventricle of obese and insulin-resistant mice [56]. In addition, in obesity and hepatic steatosis, impaired STAT3 activation in the liver could also be a possible factor contributing to the disturbance of central insulin action-mediated suppression of HGP. Indeed, in obesity and hepatic steatosis, the acetylation of STAT3, which is necessary for the activation of STAT3, is diminished, resulting in impaired activation [58-60].

Concluding remark

Insulin controls HGP directly and indirectly through the hepatocyte insulin receptor and central insulin action, respectively. Central insulin action activates IL-6/STAT3 signaling in the liver via the vagus nerve and hepatic Kupffer cells, and suppresses hepatic gluconeogenic enzyme gene expression. The importance of such central nervous system regulation of HGP in whole body glucose metabolism is not fully elucidated, especially in humans. However, several papers have recently reported that nutrients such as glucose, long-chain fatty acids and amino acids (e.g., leucine, proline and histidine, etc.) act on the central nervous system to subsequently regulate HGP in addition to central insulin action [50, 61-64]. These findings lead to the proposal that the importance of the sum of central actions that regulate HGP, including humoral factors like insulin and nutrients on the central nervous system, should be considered in whole body glucose metabolism. The appeal of central actions as therapeutic targets for type 2 diabetes mellitus is lost by the fact that central nervous system-mediated regulation of HGP is impeded in obesity and insulin resistance. To overcome this problem, a deeper and more detailed elucidation of the mechanism of central nervous system-mediated hepatic glucose metabolism regulation and its impediment is necessary.

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Brain-insulin and glucose metabolism


