Regulatory role of GABAergic neurons in the lateral hypothalamic area and zona incerta in glucose metabolism

LONG, Yu[1]

[1]Department of Physiological Sciences, School of Life Science, The Graduate University for Advanced Studies, SOKENDAI

Introduction

Glucose and energy homeostasis are vital for the maintenance of biological functions in animals. The lateral hypothalamic area (LHA) in the brain emerges as a key component involved in the regulation of food intake and peripheral metabolism. In the LHA, approximately 55% LHA neurons are GABAergic neurons encoding vesicular GABA transporter (Vgat), while approximately 45% LHA neurons are glutamatergic neurons encoding vesicular glutamate transporter 2 (Vglut2). Stimulation of LHA GABAergic neurons induced rapid binge-like eating, whereas the ablation of the neurons attenuated food consumption. In contrast, stimulation of LHA glutamatergic neurons suppressed food consumption.

The zona incerta (ZI), a brain region located in close proximity to the LHA, was described as a "zone of uncertainty". Recent investigations have shed light on certain shared characteristics between the LHA and ZI, including involvement in attentional processes, narcolepsy, and energy homeostasis. ZI neurons are predominantly GABAergic (~85%). Stimulation of LHA and ZI (LHA/ZI) GABAergic neurons induced rapid binge-like eating regardless of caloric content. These studies underscore the potential significance of LHA/ZI GABAergic neurons, along with LHA glutamatergic neurons, in the regulation of energy homeostasis. However, their involvement in glucose metabolism remains elusive. In the present study, I investigated the regulatory role of LHA/ZI GABAergic neurons, as well as LHA glutamatergic neurons in glucose metabolism in mice.

Materials and Methods

I used the chemogenetic method called designer receptors exclusively activated by designer drug (DREADD) to activate the neurons. Adeno-associated virus (AAV) expressing the excitatory DREADD hM3Dq was administered into the LHA/ZI region of vesicular GABA transporter (Vgat)-Cre or vesicular glutamate transporter 2 (Vglut2)-Cre knock-in mice (referred to as $Vgat^{LHA/ZI}$-hM3Dq or $Vglut2^{LHA}$-hM3Dq mice). DREADD ligand clozapine-N-oxide (CNO) or saline was intraperitoneally injected in $Vgat^{LHA/ZI}$-hM3Dq or $Vglut2^{LHA}$-hM3Dq mice.

Chemogenetic CNO administration into Vgat$^{LHA/ZI}$-hM3Dq and Vglut2^{LHA}-hM3Dq mice increased neuronal activation maker, c-Fos, expression in LHA/ZI GABAergic neurons and LHA glutamatergic neurons, respectively.

I conducted a series of physiological assessments on the subject mice after the neuronal activation, encompassing the administration of glucose tolerance tests (GTT), insulin tolerance tests (ITT), pyruvate tolerance tests (PTT) and basal blood glucose level. I also examined plasma insulin concentration during GTT before and after glucose administration. For the liver that plays a most important regulatory role in glucose metabolism, my subsequent investigation focused on whether activation of LHA/ZI GABAergic neurons changes glycogen and glucose 6-phosphate (G6P) contents and mRNA amount of glucose 6-phosphatase (G6Pase, *g6pc*) and phosphoenolpyruvate carboxykinase (PEPCK, *pck1*), or the enzymatic activity of fructose 1,6-bisphosphatase (F1,6BPase) in the liver. Finally, I examined 2DG (2-[^{14}C]deoxyglucose) uptake in the liver and other peripheral tissues during GTT.

Results

Activation of LHA/ZI GABAergic neurons increases glucose tolerance during GTT.

First, I examined whether activation of LHA/ZI GABAergic neurons affects glucose metabolism. After 16-h fasting, Vgat$^{LHA/ZI}$-hM3Dq mice received IP administration of saline or CNO, followed by IP injection of glucose 30 min later. Blood glucose concentration elevation after glucose administration was significantly reduced in CNO-injected group when compared with saline-injected group. These results suggest that activation of LHA/ZI GABAergic neurons increased glucose tolerance during GTT.

Activation of LHA glutamatergic neurons does not alter glucose tolerance during GTT.

To explore the role of glutamatergic neurons in the LHA on glucose metabolism, I activated LHA glutamatergic neurons and performed GTT. In contrast to Vgat$^{LHA/ZI}$-hM3Dq mice, change in blood glucose concentration during GTT was not different between CNO and saline injection in Vglut2^{LHA}-hM3Dq mice. These results suggest that activation of LHA glutamatergic neurons does not affect glucose metabolism during GTT.

Activation of LHA/ZI GABAergic neurons does not alter plasma insulin concentrations during GTT.

To examine whether the decrease of glucose concentration after activation of LHA/ZI GABAergic neurons both in the presence and absence of glucose administration is attributable to the alterations

in plasma insulin concentration, I measured plasma insulin concentration before (0 min) and 15 min after glucose administration during GTT in $Vgat^{LHA/ZI}$-hM3Dq mice with saline or CNO administration. The insulin concentration was not different between saline- and CNO-injected groups before and 15 min after glucose administration. These results suggest that activation of LHA/ZI GABAergic neurons does not affect plasma insulin concentration with and without glucose administration.

Activation of LHA/ZI GABAergic neurons does not alter the insulin action on blood glucose concentrations during ITT.

To examine whether activation of LHA/ZI GABAergic neurons affects the insulin action on glucose metabolism, I conducted ITT. $Vgat^{LHA/ZI}$-hM3Dq mice were deprived of food for 2 h prior to receiving IP administration of saline or CNO, and injected with insulin 30 min after saline or CNO administration. Interestingly, blood glucose concentration was significantly decreased in CNO-injected group at 30 min after CNO administration (before insulin administration) when compared to saline injected group. Following insulin administration, however, both saline and CNO-injected groups showed similar decrease of blood glucose concentration. These results suggest that activation of LHA/ZI GABAergic neurons does not alter the effect of insulin on blood glucose concentration. These results, taken together, suggest that the enhancement of glucose tolerance after activation of LHA/ZI GABAergic neurons is independent of changes in plasma insulin concentration or insulin action on glucose metabolism.

Activation of LHA/ZI GABAergic neurons decreases basal blood glucose concentration at early time point.

Blood glucose concentration significantly decreased before insulin injection during ITT. I next examined whether activation of LHA/ZI GABAergic neurons exerts an effect on basal blood glucose concentration in the absence of glucose administration. $Vgat^{LHA/ZI}$-hM3Dq mice were deprived of food for 2 h, followed by IP injection of saline or CNO. Notably, blood glucose concentration was significantly decreased in CNO-injected group at 30 min after the administration compared with saline-injected group. There was no statistical difference at other tested time points. These results indicate that activation of LHA/ZI GABAergic neurons temporally decreases basal blood glucose concentration at early time point of the neural stimulation.

Activation of LHA and ZI GABAergic neurons increases pyruvate tolerance during PTT.

I next conducted PTT to examine whether activation of LHA/ZI GABAergic neurons changes gluconeogenesis. Vgat$^{LHA/ZI}$-hM3Dq mice were fasted for 16 h, and received IP administration of saline or CNO, followed by IP injection of pyruvate 30 min later. Blood glucose concentration significantly reduced in CNO-injected group at 30, 60 and 90 min after pyruvate administration. These results indicate that activation of LHA/ZI GABAergic neurons decreases pyruvate-derived glucose production, suggesting that activation of LHA/ZI GABAergic neurons suppresses gluconeogenesis.

Activation of LHA/ZI GABAergic neurons decreases glycogen and G6P contents in the liver.

The results of PTT suggest that glucose production in the liver could be affected by activation of LHA/ZI GABAergic neurons. To test the possibility, I examined whether activation of LHA/ZI GABAergic neurons affects key enzymes or glucose metabolites involved in gluconeogenesis in the liver. Vgat$^{LHA/ZI}$-hM3Dq mice were fasted for 16 h, and injected with CNO or saline, and liver samples was collected 30 min after CNO or saline injection. I first investigated whether activation of LHA/ZI GABAergic neurons affect the rate-limiting enzymes for gluconeogenesis in the liver. The mRNA amount of G6Pase and PEPCK, or the enzymatic activity of F1,6BPase was not changed in CNO-injected group. In contrast, activation of LHA/ZI GABAergic neurons significantly decreased the amounts of glycogen and G6P in the liver in the CNO-injected group. Given that the activation of GABAergic neuron in the LHA/ZI suppresses gluconeogenesis and decreases blood glucose concentration at early time point of the neural stimulation, the decrease of glycogen content in the liver is due to the enhancement of glycogen breakdown in the tissue to maintain blood glucose concentration.

Activation of LHA and ZI GABAergic neurons does not affect glucose uptake in peripheral tissues after glucose administration.

Finally, I examined 2DG uptake in the liver and other peripheral tissues during GTT. Vgat$^{LHA/ZI}$-hM3Dq mice were subjected to GTT, together with the injection of ^{14}C-labeled 2DG. Activation of LHA/ZI GABAergic neurons tended to increase 2DG uptake in the liver at 120 min after glucose administration, although it was not statistically significant. 2DG uptake in the liver at 30 min was not different between the CNO-injected and saline-injected groups after glucose administration. 2DG uptake in other tissues at 120 min after glucose administration was also not different.

These results, taken together, suggest that activation of LHA/ZI GABAergic neurons increases glucose tolerance during GTT by suppressing gluconeogenesis in the liver.

Discussion

The LHA and ZI play an important role in feeding. LHA/ZI GABAergic neurons and LHA glutamatergic neurons have been shown to regulate feeding and peripheral metabolism. Previous studies also showed that electrical stimulation of the LHA decreased blood glucose levels and suppressed gluconeogenesis in the liver. However, the actual subtypes of LHA/ZI neurons controlling glucose metabolism remain unknown. My results demonstrate that activation of GABAergic$^{LHA/ZI}$, but not glutamatergic^{LHA} neurons, increase glucose tolerance during GTT. I found that activation of LHA/ZI GABAergic neurons decreased the pyruvate-dependent glucose production. Furthermore, I found that activation of the neurons decreased the basal blood glucose concentration and glycogen and G6P contents in the liver at early time point of the neural stimulation. These results suggest that LHA/ZI GABAergic neurons inhibit gluconeogenesis, most likely in the liver, thereby increasing glucose tolerance after glucose administration. Decrease of glycogen content in the liver is probably due to the enhancement of glycogen breakdown in the tissue to maintain the basal blood glucose concentration. The concept of functional zonation in the liver is well established. Periportal region in the liver expresses abundantly genes involved in gluconeogenesis, fatty acid degradation, and amino acid degradation. In contrast, periportal region expresses genes involved in glycolysis, cholesterol production, and xenobiotic metabolism. Thus, gluconeogenesis and glycogen breakdown could be regulated in the liver independently.

To explore whether activation of LHA/ZI GABAergic neurons regulates glucose uptake in peripheral tissues including the liver, I examined 2DG uptake into tissues under the condition of GTT. My results indicate that activation of LHA/ZI GABAergic neurons tended to increase 2DG uptake in the liver at 120 min after glucose administration. Glucose uptake in other peripheral tissues (kidney, heart, soleus, red and white portion of gastrocnemius, BAT, inguinal WAT and epididymal WAT) at 120 min did not change. Thus, it might be possible that activation of LHA/ZI GABAergic neurons increases glucose uptake in the liver during GTT, responding to the reduction of tissue G6P and glycogen contents after suppression of gluconeogenesis in the liver. Further examination is required to conclude whether LHA/ZI GABAergic neurons affect glucose uptake in the liver. Measurement of the whole body glucose production and utilization by the constant infusion of [^{3}H]glucose into blood circulation under the steady state condition with and without insulin infusion may be useful to quantify the rate of whole body glucose production and utilization after activation of LHA/ZI GABAergic neurons.

The mechanism by which LHA/ZI GABAergic neurons suppress glucose production from the liver remains unclear. I found that activation of LAH/ZI GABAergic neurons had little effect on plasma insulin concentrations before and after glucose administration. Furthermore, it did not show any effect on blood glucose reduction during ITT. These results suggest that LHA/ZI GABAergic neurons increases glucose tolerance during GTT independently of plasma insulin concentration or insulin action on glucose metabolism.

The mRNA abundance of *g6pc* (G6Pase) and *pck1* (PEPCK) or the enzymatic activity of F1,6BPase was not affected by activation of LHA/ZI GABAergic neurons. Thus, activation of LHA/ZI GABAergic neurons quickly inhibits glucose production without change in the gene expressions. In addition, the activity of F1,6BPase is regulated by F2,6BP. Further examination is necessary to determine whether activation of LHA/ZI GABAergic neurons alters the activity of G6Pase, F1,6BPase or PEPCK in the liver.

Previous studies using trans-synaptic viral tracers revealed that LHA neurons have neural efferent connection to the liver, suggesting that LHA/ZI GABAergic neurons control glucose metabolism in the liver via the autonomic nervous system. It has been reported that stimulation of sympathetic nerves innervating the liver increases gluconeogenesis and glycogen breakdown in the tissue, while stimulation of parasympathetic nerves innervating the liver suppresses the gluconeogenesis. Recent study revealed that central action of insulin decreases glucose production from the liver by suppressing vagal activation in the liver. Therefore, LHA/ZI GABAergic neurons may inhibit glucose production from the liver through the autonomic nerves innervating the liver.

In summary, I demonstrate that activation of LHA/ZI GABAergic neurons increases glucose tolerance after glucose administration probably through the suppression of glucose production in the liver. These findings underscore the significance of LHA/ZI GABAergic neurons in regulating glucose homeostasis. Further investigations are warranted to elucidate the precise neural pathways and physiological role of LHA/ZI GABAergic neurons in the regulation of liver glucose metabolism.