Brain-liver connections: role of the preautonomic PVN neurons

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O’Hare JD, Zsombok A. Brain-liver connections: role of the preautonomic PVN neurons. Am J Physiol Endocrinol Metab 310: E183–E189, 2016. First published December 8, 2015; doi:10.1152/ajpendo.00302.2015.—Diabetes mellitus (DM) is the most common metabolic disorder. The National Diabetes Statistics Report from 2014 published by the Centers for Disease Control and Prevention (8a) estimated that 29.1 million people, which is 9.3% of the US population, suffer from diabetes mellitus. Even more alarming information is that the number of new diabetes cases showed an increase of 1.7 million within 2 yr, and in 2010 diabetes mellitus was listed as the seventh leading cause of death. The coexisting conditions and complications of diabetes mellitus are severe, including hypo- and hyperglycemic events, high blood pressure, obesity, high cholesterol levels, and many more. Undoubtedly, there is a huge demand for treatment and prevention of these conditions that justifies the search for new approaches and solutions for better management of whole body metabolism. Emerging evidence demonstrates that the autonomic nervous system is largely involved in the regulation of glucose homeostasis; however, the underlying mechanisms are still under investigation. Within the hypothalamus, the paraventricular nucleus (PVN) is in a unique position to integrate neural and hormonal signals to command both the autonomic and neuroendocrine outflow. This minireview will provide a brief overview on the role of preautonomic PVN neurons and the importance of the PVN-liver pathway in the regulation of glucose homeostasis.

paraventricular nucleus; liver-related neurons; glucose homeostasis

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thletic outflow are unaffected (4, 7, 33, 34). Studies conducted in rodents also support the differential control of sympathetic outflow, and a recent review summarized the findings in detail (34). Brown adipose tissue (BAT) is involved in thermoregulation and thus in energy homeostasis, and its function is controlled by the sympathetic nervous system. Selective activation of BAT sympathetic nerve activity by hypothermia, disinhibition of neurons in the rostral raphe pallidus, the differences in basal discharges, the characteristics of stimulated BAT vs. splanchnic sympathetic nerve activity, and the sensitivity of BAT and splanchnic sympathetic nerve activity to baroreceptor reflex all demonstrated the existence of a distinct subset of premotor neurons that regulate vasoconstriction and BAT thermogenesis (35). These findings revealed the existence of tissue-specific subsets of preautonomic neurons, which differentially control sympathetic outflow, in this case, the BAT. Moreover, it is highly likely that metabolically important organs (e.g., liver, pancreas) are also differentially controlled; however, the understanding of the neural circuits mediating the differential control of sympathetic and parasympathetic outflows remains an open area of investigation.

The imbalance of the autonomic nervous system is due to altered neuronal activity in autonomic centers of the brain (75), and selective modulation of brain circuits may represent a possible means of both controlling glycemic balance and preventing complications. Within the hypothalamus, the paraventricular nucleus (PVN) is an integrative autonomic center, and this minireview will provide a brief overview of the role of preautonomic PVN neurons and the importance of the PVN-liver pathway in the regulation of glucose homeostasis.

Control of the Liver by Preautonomic PVN Neurons: In Vivo Findings

The first evidence of the brain being able to regulate glucose metabolism originated from Claude Bernard in 1854 (1). Bernard, who established the concept of homeostasis and was the founder of modern experimental physiology, demonstrated that puncturing the floor of the fourth ventricle resulted in increased peripheral glucose levels mimicking diabetic conditions. These early studies did not establish precise underlying mechanisms (e.g., insulin levels) or site of action (e.g., nuclei) but clearly showed that manipulating brainstem circuits influences peripheral function, in this case glucose levels. Then, with the discovery of insulin, the major focus of research shifted to the establishment of insulin-dependent mechanisms, mainly in the periphery. On the other hand, over the past few decades, with the discovery of new and more precise experimental approaches and the discovery of hormones such as leptin, which exerts its main effect through brain circuits, the original idea of regulation of glucose homeostasis via the brain has risen again (50).

Glucose production of the liver and modulation of glucose clearance via the actions of hormones, including insulin, which promotes glucose uptake in the skeletal muscle, are the major mechanisms controlling plasma glucose levels. The liver plays a crucial role in the maintenance of glucose homeostasis, and evidence supports a governing role for the autonomic nerves in the control of hepatic functions (5, 44). Activation of hepatic sympathetic innervation increases endogenous glucose production and glycogenolysis, whereas activation of the parasympathetic innervation decreases glucose production and promotes glucose storage (36, 52, 55, 56, 64). Hormones and nutrients, including glucose, leptin, and fatty acids, are also using the brain-liver pathway to regulate glucose homeostasis (28, 37, 40, 41, 46), and detailed information about the metabolic sensing, the neural innervation of the liver, or the autonomic control of hepatic lipid metabolism can be found in previous reviews (5, 44, 69, 72).

The autonomic nervous system consists of the sympathetic and parasympathetic branches and regulates the majority of organs in an opposite way; autonomic motor neurons for these systems are located in the spinal cord and brainstem, respectively, and convey information through the sympathetic and parasympathetic outflow. Neurons in higher brain areas projecting to these autonomic motor neurons, the so-called preautonomic neurons, are crucial for integration of brain signals, and establishing their cellular and molecular characteristics may help to understand how the central nervous system is able to control our peripheral organs and tissues (47, 48, 59, 61, 62). Remarkably, despite the fact that functions of the liver are governed by sympathetic and parasympathetic inputs, the exact location of the premotor inputs to the relevant sympathetic and parasympathetic motor neurons is not known.

Within the hypothalamus, the PVN is a crucial, integrative center that incorporates signals from numerous brain areas, including sites known for controlling energy and glucose homeostasis, and contributes largely to the regulation of the sympathetic and parasympathetic nervous system and thus modulates autonomic functions (69, 75). Studies using electrical stimulation of the PVN have demonstrated direct connections between the PVN and autonomic brainstem neurons or sympathetic neurons in the spinal cord and thus demonstrated the ability of PVN to relay information through both autonomic pathways (29, 45, 68). These studies also suggest that the PVN is likely to be one of the locations of the premotor inputs governing sympathetic and parasympathetic motor neurons. Unilateral norepinephrine injection into the PVN evoked an immediate hyperglycemia that reached the peak ~10 min following injection (15). The high glucose levels were associated with inhibition of insulin regardless of glucose levels (15), and the norepinephrine-induced hyperglycemia was largely reduced by a ganglionic blocker and was independent of corticosterone levels (15). These findings demonstrated that the hyperglycemia accompanied with inhibition of insulin is due to sympathetic activation (15). Furthermore, the revealed effects were similar to those observed following electrical stimulation of the splanchnic nerve, which caused hyperglycemia due to direct neural effects on the liver, secretion of glucagon, and catecholamine release (3, 13, 19, 20, 55).

In vivo administration of N-methyl-D-aspartate and the GABA_A antagonist bicuculline with bilateral microdialysis probes into the PVN increased plasma glucose levels significantly, which was accompanied with increased glucagon levels, without change in plasma insulin (18). Moreover, the glucose increase was absent in rats following sympathectomy, indicating that the increased plasma glucose levels were attained via the sympathetic nervous system (18). This study also suggested that GABAergic inhibition plays important role in the PVN-dependent regulation of glucose levels and raised the question about the origin of the GABAergic inputs. The suprachiasmatic nucleus (SCN) has been proposed as one of the
sources of GABAergic inputs that also could explain the daily rise in blood glucose levels with the withdrawal of the GABAergic inhibition of presym pathetic PVN neurons by the biological clock; however, further evidence is needed to prove this hypothesis (18).

Insulin through hypothalamic insulin receptors has been shown to inhibit hepatic glucose production (38), and one proposed mechanism is the inhibition of neuropeptide Y (NPY) neurons in the arcuate nucleus (49). NPY neurons send dense projections to the PVN, and it has been demonstrated that hepatic sympathectomy abolishes the NPY/insulin effect on liver glucose production, suggesting that the hypothalamic insulin effect may depend on modulation of presym pathetic PVN neurons (65). On the other hand, Pocai et al. (39) revealed that the parasympathetic outflow to the liver is involved in the inhibitory effect of hypothalamic insulin on hepatic glucose production. Similarly, the effect of leptin on insulin sensitivity of the liver was prevented by hepatic vagotomy (11), whereas orexin-dependent control of hepatic glucose production was blocked by sympathectomy of the liver (70). Taken together, these findings suggest that preautonomic PVN neurons are important integrating points in converging information to control hepatic glucose metabolism, as suggested before (17).

Certain pathophysiological conditions, including thyrotoxicosis, are linked to increased hepatic glucose production, hepatic insulin resistance, and hyperglycemia (9, 22). The hyperglycemia and increased hepatic glucose production was attenuated following hepatic sympathetic denervation (22). Bilateral infusion of triiodothyronine into the PVN elevated hepatic glucose production and plasma glucose levels, and hepatic sympathectomy prevented this effect. These findings demonstrated that the sympathetic outflow to the liver is mediated through the sympathetic nervous system without plasma glucoregulatory hormone concentrations being altered (21).

The importance of the ANS and in particular the PVN in the regulation of hepatic glucose production was further demonstrated with intracerebroventricular and PVN infusions of pituitary adenyl cyclase-activating polypeptide (PACAP) (71). PACAP administration into the PVN resulted in hyperglycemia and increased hepatic glucose production, and this effect was largely but not fully mediated via the sympathetic innervation of the liver. The combination of brain injections, retrograde tracing from the thoracic spinal cord, hepatic denervation, and immunostaining showed that PACAP via sympathetic-related preautonomic PVN neurons largely controls hepatic glucose production and thus plasma glucose levels (71).

The in vivo findings reviewed in this section contain complex experimental approaches, including delivery of drugs into specific brain areas, in this case to the PVN; therefore, certain methodological concerns have to be pointed out. In general, the correct placement of the microdialysis probe or the injection cannula into the targeted brain area always has to be verified, and results should be analyzed and interpreted following verification of the correct site. Negative responses following placement outside of the target area also give important information and could be used as negative control sites. In addition to the verification of the correct site, the spread of the administered drug is always a concern. The distribution of the drug could be assessed by coadministering a colored dye; however, despite the overlap between the coadministered drug and dye, the distribution is most likely not equal. Furthermore, the spread of the drug also depends on its concentration. Based on these methodological issues, we cannot entirely exclude that the administered drugs affected only the PVN or that the neighboring nuclei were unaffected. On the other hand, Kalsbeek et al. (18) estimated the spread of the administered drugs to be able to address this concern, and they demonstrated that the PVN is the key area. In their study, plasma glucose and corticosterone levels were analyzed following infusion of drugs into four different brain areas. Comparing the effects of drugs in the PVN with the effects in the neighboring brain nuclei (dorsomedial, ventromedial, and suprachiasmatic nuclei) was used to calculate the effective radius of the drug infusion, and the study found that the hyperglycemic effect of the drug was delayed when the drug was applied at the border of PVN compared with injection within the PVN. Furthermore, administration outside of the PVN had no effect on plasma glucose levels. Based on these data, these authors suggested that the PVN is responsible for the observed effect on glucose levels, and in their study, preautonomic PVN neurons are key structures (18). Because of experimental limitations, not every study uses or can use this type of evaluation; therefore, there is a chance that the neighboring nuclei could contribute to the demonstrated effects; however, Kalsbeek et al. (18) addressed this issue convincingly in one of their studies.

Together, the above-mentioned in vivo findings support the existence of the brain-dependent regulation of glucose metabolism and demonstrate the importance of the PVN in the brain-liver pathway; however, more detailed studies are necessary to understand the underlying mechanisms, including the neural circuits, molecular mechanisms, neurotransmitters, neuromodulators, and receptors regulating preautonomic neurons and thereby modulating glucose production of the liver.

**Control of the Liver By Preautonomic PVN Neurons: Anatomic and Cellular Studies**

Anatomic studies using anterograde and retrograde tracers in combination with immunohistochemistry were used to investigate the connections between the brain and liver. However, for a long time, there has been limited amount of information about the location, phenotype, and connectivity of preautonomic neurons. The development of neural tract tracing methods, including the use of transsynaptic retrograde viral tracers, allowed the identification of specific brain nuclei and a more accurate distribution of organ-related neurons and led to the description of detailed neural pathways connecting the hypothalamus and visceral organs, including the brain-liver pathways (6, 10, 18).

It has been well accepted that many hypothalamic nuclei, including the ventromedial hypothalamus (VMH), dorsomedial hypothalamus (DMH), lateral hypothalamus (LH), and PVN, contribute to the autonomic outflow and modulate metabolic activity in a variety of tissues (17, 64). The PVN as a critical command component of the autonomic pathway integrates signals from a variety of brain areas, including the above-mentioned hypothalamic nuclei, and it is in a unique position to integrate neuronal and humoral signals to organize the autonomic and neuroendocrine outflow, as mentioned above (48, 59, 61, 69, 75).
Early studies by Shimazu (53, 54) postulated that the LH directly relays information through the parasympathetic nuclei, which builds the vagal hepatic branch of the liver, whereas the VMH is involved in the control of the sympathetic pathway to the liver. Then, studies with retrograde transneuronal viral tracers revealed more detailed and refined pathways demonstrating that the PVN projects to the liver through both sympathetic and parasympathetic pathways (6, 27). Labeling with pseudorabies virus in rats resulted in a reproducible infection pattern and revealed polysynaptic neural connection between the liver and brain (26). Short-time (3 days) survival demonstrated pseudorabies labeling in the intermediolateral column of the spinal cord without labeling in parasympathetic cell groups. Intermediate survival time (4–5 days) revealed viral labeling in parasympathetic and sympathetic cell groups of the brainstem and also in the PVN (26). Longer survival time revealed labeling in hypothalamic areas connected to the PVN, including the medial preoptic area, DMH, arcuate nucleus, LH, circumventricular organs, and SCN (26). This study demonstrated the presence of polysynaptic autonomic pathways to the liver and also demonstrated that the PVN is able to control both sympathetic and parasympathetic pathways and integrate information from other nuclei (6, 26, 64).

Retrograde viral labeling following hepatic parasympathectomy revealed presympathetic liver-related neurons in the PVN and in the areas that project to the PVN, including medial preoptic area (MPO), anterior hypothalamic area, DMH, arcuate nucleus, VMH, circumventricular organs, and the SCN (18). Hepatic sympathectomy in combination with the retrograde viral labeling identified preparasympathetic liver-related neurons in the PVN and in the connected areas, including DMH, MPO, VMH, and SCN (18). It has to be mentioned that the studies revealing presympathetic liver-related neurons in the hypothalamus also identified neurons in the rostral ventrolateral, ventromedial medulla, raphe pallidus, and A5 region. These brain areas are known to project to sympathetic vasomotor preganglionic neurons that innervate the entire body, and thus it is likely that they contribute to the vasomotor innervation of the liver. This does not exclude the possibility that the labeled presympathetic neurons contribute to the non-

vasomotor sympathetic innervation of the liver, but with the retrograde transsynaptic viral labeling, the neurons contributing to vasomotor and nonvasomotor sympathetic innervation of the liver cannot be distinguished. On the other hand, studies identifying preparasypathetic inputs to the liver are likely to identify the premotor parasympathetic neurons because of the lack of parasympathetic innervation of the blood vessels.

Labeling with this pseudorabies virus approach was also used to investigate segregation between sympathetic- and parasympathetic-projecting liver-related neurons (6). The analysis demonstrated a complete separation between presympathetic and preparasypathetic liver-related neurons in the hypothalamus (6) and suggested that preautonomic PVN neurons project through either the sympathetic or parasympathetic pathway. Interestingly, both presympathetic and preparasypathetic neurons were immunopositive for oxytocin, which suggest that both groups of preautonomic neurons can contain the same neuropeptide (Fig. 1) (6). Stanley et al. (57) revealed time-dependent expression of preautonomic neurons in hypothalamic nuclei following pseudorabies inoculation of the liver in mice. Then, using immunostaining, their study determined that a subset of liver-related neurons colocalized with neurons expressing oxytocin and corticotropin-releasing hormone but not vasopressin. These studies are consistent with previous findings indicating that the hypothalamic PVN is important in hepatic autonomic innervation and in the control of hepatic glucose metabolism (18, 64). Furthermore, demonstrating the distribution of liver-related PVN neurons laid down the anatomic background for physiological:functional studies.

Interestingly, “command” preautonomic PVN neurons were identified following simultaneous injections of the liver and epididymal white adipose tissue (57). Identification of these dual-labeled central command neurons supports the theory of coordinated response to more than one organ, which was suggested for sympathetic activation of the heart and adrenal gland (16). The hypothesis that one neuron can control more than one organ was also supported by observations from Buijs et al. (6) demonstrating that many preautonomic neurons project to the liver and adrenal gland; however, the above-discussed issue regarding vasomotor and nonvasomotor sym-

Fig. 1. Overview of brain-liver connections. Liver-related preautonomic neurons in the para-ventricular nucleus (PVN) of the hypothalamus receive inputs from a variety of hypothalamic nuclei and via the sympathetic (SNS) and parasympathetic nervous systems (PNS) modulate glucose levels. Some of the liver-related neurons express corticotropin-releasing hormone (CRH) and oxytocin, whereas the phenotype of the majority of liver-related PVN neurons is unknown. In vivo administration of neurotransmitters and modulators influences glucose levels, whereas the exact underlying mechanisms (e.g., receptors) are unclear. POA, preoptic area; AH, anterior hypothalamus; DMH, dorsomedial hypothalamus; VMH, ventromedial hypothalamus; SCN, suprachiasmatic nucleus; CVO, circumventricular organs; LTS, low-threshold spikes; TRPV1, transient receptor potential vanilloid type 1; HGP, hepatic glucose production.
pathetic innervation of the liver has to be kept in mind. On the contrary, complete segregation of preautonomic neurons projecting to the intra-abdominal fat and subcutaneous fat tissue was shown by Kreier et al. (25), whereas command neurons were projecting to intra-abdominal organs, suggesting an organization based on body compartments. Interaction between neurons of the sympathetic and parasympathetic pathways also has to be considered, and connections have been proposed at the level of presympathetic PVN neurons, which can have collaterals projecting to parasympathetic-related neurons (6, 59, 66).

There is much less information available about the cellular properties of preautonomic liver-related PVN neurons. PVN consists of magnocellular neurons, parvocellular neuroendocrine cells, and parvocellular preautonomic neurons (31, 32, 60). These neurons can be distinguished based on their electrophysiological (e.g., pre- and postsynaptic) properties (63). Preautonomic PVN neurons express low-threshold spikes (LTS) and strong inward rectification, which are common features (32, 58). However, besides the common characteristics like LTS, the characterization of the electrophysiological and morphological properties of the preautonomic PVN neurons revealed a heterogeneous neuronal population (58). This heterogeneity could be associated with their target of innervation and/or differential modulation of sympathetic and parasympathetic outflow or related to the neurochemical phenotype of the preautonomic neurons, as suggested by Stern (58). The segregation of the presympathetic and parasymmpathetic PVN neurons innervating organs related to glucose metabolism is also demonstrated by retrograde tracing studies that revealed no overlap between spinally projecting neurons and neurons projecting to the dorsal motor nucleus of the vagus (42, 43).

Our laboratory used retrograde viral labeling to identify liver-related PVN neurons (10, 74). The excitatory neurotransmission of preautonomic liver-related PVN neurons was investigated in a control and a type 1 diabetic mouse model (10). In this study, we did not find a significant difference between the frequencies of spontaneous or miniature excitatory postsynaptic currents among the groups. On the other hand, the transient receptor potential vanilloid type 1 (TRPV1)-dependent regulation of liver-related PVN neurons (10, 74). The excitatory neurotransmission of preautonomic liver-related PVN neurons was diminished in type 1 diabetic mice (10). We revealed that in vitro and in vivo insulin application restored TRPV1 activity in a phosphatidylinositol 3-kinase/PKC-dependent manner and also stimulated TRPV1 trafficking to the plasma membrane (10). These data suggested that TRPV1 plays an important role in the regulation of liver-related PVN neurons and that the diabetic condition alters the TRPV1-driven excitatory neurotransmitter release. We have to note that in these experiments liver-related PVN neurons were identified and recordings were conducted from these neurons, but our study does not differentiate the presym pathetic or parasymmpathetic nature of the preautonomic neurons. Despite these data, there is still limited information on the cellular characteristics of liver-related PVN neurons.

Future Perspectives

During the past few decades, significant effort has been made to further delineate brain-dependent regulation of glucose metabolism; however, despite this effort, more detailed studies are necessary to further understand the underlying mechanisms. In general, the information available about the tissue-specific subset of presympathetic and preparasym- pathetic neurons in the hypothalamus is limited. The retrograde viral labeling technique, used in combination with sympathetic and/or parasympathetic, has identified specific brain areas, but due to the above-mentioned technical limitations, many questions are still not answered. The phenotype of the majority of liver-related preautonomic neurons is not known. It would also be intriguing to know the neuromodulators, neurotransmitters, nutrients, hormones, and receptors that are able to regulate the activity of the liver-related neurons (Fig. 1). Determining these regulators in a control condition would be important to identify their potential involvement in the development of pathophysiological conditions or ways of restoring normal neuronal functions. Since it does appear that the diabetic state alters autonomic function, both sympathetic and parasympathetic, once the fundamental regulatory players involved in the control of liver-related neurons and outflow are identified, it will be possible to further investigate how those transmitters/regulators are altered in a diabetic state or other disease conditions affecting the ANS. Then, with a greater understanding of how the ANS may go awry in a diabetic state, it may be possible to intervene on some of those processes. Furthermore, despite the importance of organ-related circuits, including the brain-liver pathway, our understanding of the mechanisms controlling these circuits, the origin of inputs to liver-related PVN neurons, and factors modulating these inputs is limited. The recent thriving of optogenetic and pharmacogenetic approaches in combination with transgenic animals has been useful and successful in revealing brain circuits for homeostatic control such as feeding (2, 23, 24) and opens new avenues to discover the elements of brain circuits regulating glucose homeostasis.

Together, delineating the brain-visceral organ circuits, including the brain-liver circuitry, may have potential therapeutic value via opening new strategies for better control of glucose homeostasis via the ANS.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

J.D.O. and A.Z. prepared figures; J.D.O. and A.Z. drafted manuscript; J.D.O. and A.Z. edited and revised manuscript; J.D.O. and A.Z. approved final version of manuscript.

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