More News About NUCB2/Nesfatin-1: A New Factor in the Hypothalamic Control of Glucose Homeostasis?

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Insulin resistance and declining insulin production define a spectrum of metabolic diseases afflicting a growing portion of the population (1). Current estimates suggest that 26 million Americans have diabetes, with the incidence of type 2 diabetes involving insulin resistance and β-cell decompensation far exceeding that of type 1 diabetes. Treatment of type 2 diabetes involves secretagogues that increase insulin secretion and insulin sensitizers to improve insulin receptor (INSR) action (and likely include other effects on glucose production) (1). These drugs exhibit a short duration of efficacy in many patients and often require a multidrug approach as the disease progresses and insulin injections when other treatment options fail. A clear need exists for continued research aimed at developing more effective strategies for maintaining glycemic control.

In this issue, Yang et al. (2) report that intracerebroventricular (ICV) infusion of nesfastin-1 improves glucose homeostasis in diet-induced obese rats by inhibiting hepatic glucose production. Improved insulin action is one mechanism for the reduction in hepatic glucose production. Increased phosphorylation of the INSR and insulin receptor substrate-1 was observed in lean and obese rats following ICV administration of nesfatin-1. Tyrosine phosphorylationof multiple residues in the insulin receptor substrate-1 coding sequence is an important early event following INSR activation. This leads to an intracellular signaling cascade that facilitates changes in energy metabolism by regulating gene transcription and enzymatic activity (3). The changes in phosphorylation of AKT, AMP-dependent protein kinase, mammalian target of rapamycin, and mammalian target of rapamycin complex 2 that were also observed were consistent with improved actions of these important downstream effectors in the INSR signaling cascade. Activity of phosphoenolpyruvate carboxykinase, the rate-limiting enzyme in gluconeogenesis, was also reduced in livers of lean and diet-induced obese rats administered nesfatin-1 ICV. Finally, a modest effect to stimulate whole-body glucose disposal and increased glucose uptake in skeletal muscle was also observed.

Nesfatin-1 is an 82–amino acid peptide derived from the posttranslational processing of nucleobindin-2 (NUCB2), and was originally reported by Oh-I et al. (4) in 2006 to function as a "satiety" factor (Fig. 1). Daily ICV injections of nesfatin-1 reduced food intake and attenuated weight gain in rats (4). Importantly, antibodies that inhibit nesfatin-1 action or antisense oligonucleotides that target *Nucb2* expression had the opposite effect: increasing food intake and weight gain. Other laboratories reported similar inhibitory effects of nesfatin-1 on food intake in rats and mice (5). Subsequent studies reported that the functions of nesfatin-1 are consistent with a role in metabolic homeostasis including inhibiting gastric motility, stimulating the adrenal axis and autonomic function, and stimulating of glucose-induced insulin secretion from β-cells (5). Oh-I et al. reported that *Nucb2* mRNA is expressed in the arcuate and paraventricular (PVN) nuclei of the hypothalamus and in the lateral hypothalamus (4). Subsequent analysis indicated that *Nucb2* mRNA is widely expressed in the central nervous system, where it exhibits associations with neuropeptides involved in regulating ingestive behaviors, gonadal function, and the stress reponse (5).

To appreciate the results presented in this issue of *Diabetes*, it is important to be aware that, at least in rodents, actions involving hypothalamic neurons are essential for glucose homeostasis. Most hypothalamic neuropeptides and neurotransmitters regulating appetite also affect peripheral glucose metabolism (6–8). Well-studied examples include the regulation of insulin sensitivity in peripheral tissues through modulation of autonomic activity by leptin and melanocortin receptors expressed by hypothalamic (and extrahypothalamic) neurons (9). Indeed, the effective control of glucose homeostasis by insulin requires the actions of INSRs expressed in the hypothalamus (7). The attenuated response of hypothalamic neurons to signals of metabolic status such as insulin, glucose and leptin has been hypothesized to be a contributing factor in deteriorating control of metabolic homeostasis in obesity (6,7).

Experimental data suggesting a link between nesfatin-1 and the melanocortin system provide further rationale for the experiments described by Yang et al. and suggest future research. For example, it is not clear whether melanocortin neurons in the PVN implicated in the effects of nesfatin-1 on feeding are involved in mediating these actions, whereas actions of nesfatin-1 in other regions of the brain that regulate autonomic functions may also be involved (Fig. 1). *Nucb2* expression in the PVN is stimulated by central injection of α-melanocyte–stimulating hormone whereas the anorectic response to nesfatin-1 is inhibited by SHU9119, an antagonist for the melanocortin-4 receptor (10). Melanocortin-4 receptors regulate satiety and also modulate autonomic outputs that affect insulin action in the liver (11–13). Although Yang et al. report increased Fos immunoreactivity in the arcuate and PVN with nesfatin-1 treatment (2), they did not investigate the identity of neurons involved. Future studies could therefore investigate these neural pathway(s) and whether central antagonism of melanocortin signaling inhibits the effects of nesfatin-1 on glucose homeostasis (4). Sophisticated genetic tools have been developed that could be used to
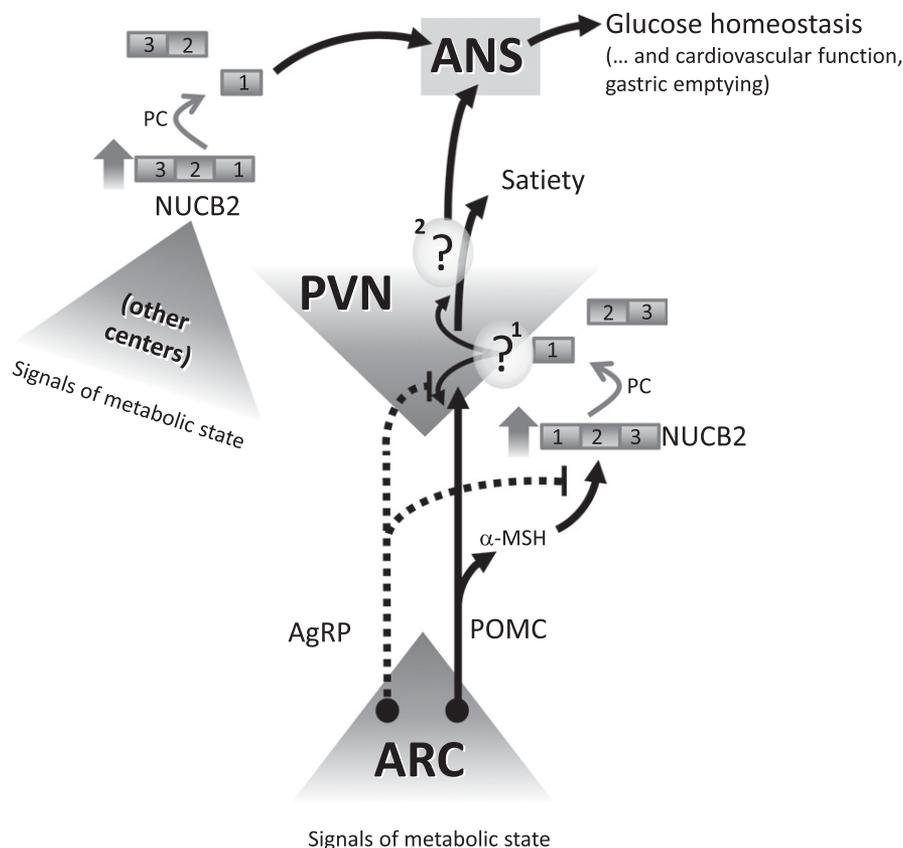
FIG. 1. Yang et al. (2) report that nesfatin-1 modulates autonomic outputs governing glucose homeostasis. However where and how this occurs is not known. This schematic highlights the interaction reported between nesfatin-1 and the melanocortin system based on data suggesting the interaction is important in regulating satiety (4). Proopiomelanocortin (POMC) neurons regulated by signals of metabolic state (e.g., insulin, leptin, serotonin) release α-melanocyte–stimulating hormone (α−MSH); ICV administration of α-melanocyte–stimulating hormone stimulates *Nucb2* expression and presumably posttranslational modification of the full-length NUCB2 protein containing nesfatin-1, -2, and -3 in the PVN. NUCB is presumably posttranslationally modified by a prohormone convertase (PC), releasing nesfatin-1. The actions of nesfatin-1 on satiety are inhibited by SHU9119, a nonselective antagonist at the melanocortin-3 and -4 receptors expressed in this region of the brain (10). However, although the blockade of function by SHU9119 could involve inhibiting nesfatin-1 synthesis/release (i.e., nesfatin-1 acts downstream to mediate the actions of melanocortins on satiety), other currently unknown mechanisms (as indicated by ?[1]) are involved. Melanocortin-4 receptors expressed in the PVN regulate satiety; however, studies where the Cre-Lox system was used to target expression of the *Mc4r* gene in mice suggest they may not be involved or are insufficient for regulating autonomic pathways governing metabolic homeostasis (14). The regulation of glucose homeostasis by nesfatin-1 may therefore involve as yet to be identified neurons in the PVN ([2]?), or nesfatin-1 expressed in numerous other regions of the brain linked to the control of the autonomic nervous system (ANS) (5). It should also be noted that nesfatin-1 is coexpressed in other PVN neurons implicated in regulating ingestive behaviors, including corticotrophin-releasing factor and oxytocin (5). ARC, arcuate.

further investigate the interaction between nesfatin-1 and melanocortins (11,14–16).

Another important question is whether attenuated nesfatin-1 activity in the hypothalamus contributes to insulin resistance in obesity. A conditional targeting strategy to target nesfatin-1 production in various brain regions might be informative. This would be a technically challenging experiment as nesfatin-2 and -3 produced from the processing of NUCB2 may also have important functions. One approach would be to define and then target critical residues needed for nesfatin-1 function. The problem with this strategy is structure-activity relationship studies require knowledge about the identity of the receptor(s) involved. Lack of knowledge about the receptor limits our ability to determine where the peptide is working in the brain and periphery. Identification of this receptor(s) and the intracellular signaling pathways involved are critical end points in future studies investigating mechanism of action.

Finally, while the result reported by Yang et al. is important progress, it is also important to acknowledge some of the limitations to their work. Although nesfatin-1 is present in

rat cerebrospinal fluid and human plasma samples, an important question is whether (and where) full length NUCB2 protein is processed into nesfatin-1 in the brain (4,17). Whether the hypothalamus regulates peripheral glucose metabolism in larger mammals such as humans is also unclear (18). Finally, history has also shown that the link between neuropeptides controlling appetite and the autonomic control of insulin action can be problematic when compounds targeting these systems reach the clinic (19). Answers to these questions can only come from further research that investigates whether this poorly understood protein will have potential for developing new therapies to improve glycemic control in type 2 diabetes.

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