Title

Adropin and insulin resistance: Integration of endocrine, circadian, and stress signals regulating glucose metabolism

Journal

Obesity, 29(11)

ISSN

1930-7381

Authors

Butler, Andrew A

Havel, Peter J

Publication Date

2021-11-01

DOI

10.1002/oby.23249

Peer reviewed

Received: 13 May 2021 | Revised: 18 June 2021 | Accepted: 22 June 2021

DOI: 10.1002/oby.23249

Obesity A Research Journal | THE OBESITY SOCIETY | WILEY

PERSPECTIVE

Adropin and insulin resistance: Integration of endocrine, circadian, and stress signals regulating glucose metabolism

Andrew A. Butler[1] | **Peter J. Havel**[2]

[1]Department of Pharmacology & Physiology, Henry and Amelia Nasrallah Center for Neuroscience, Saint Louis University School of Medicine, Saint Louis, Missouri, USA

[2]Department of Molecular Biosciences, School of Veterinary Medicine and Department of Nutrition, University of California Davis, Davis, California, USA

Correspondence
Andrew A. Butler, Department of Pharmacology & Physiology, Saint Louis University School of Medicine, 1402 S. Grand Blvd, St. Louis, MO 63104, USA.
Email: andrew.butler@health.slu.edu

Funding information
The research in the authors' laboratories has been supported by grants from the National Institutes of Health (R21NS108138 to AAB and RO1 DK-095980, RO1 HL-121324 and U24 DK-092993 to PJH), American Diabetes Association (AAB) and Novo Nordisk (AAB).

Abstract

Dysregulation of hepatic glucose production (HGP) and glucose disposal leads to hyperglycemia and type 2 diabetes. Hyperglycemia results from the declining ability of insulin to reduce HGP and increase glucose disposal, as well as inadequate ß-cell compensation for insulin resistance. Hyperglucagonemia resulting from reduced suppression of glucagon secretion by insulin contributes to hyperglycemia by stimulating HGP. The actions of pancreatic hormones are normally complemented by peptides secreted by cells distributed throughout the body. This regulatory network has provided new therapeutics for obesity and type 2 diabetes (e.g., glucagon-like peptide 1). Other peptide hormones under investigation show promise in preclinical studies. Recent experiments using mice and nonhuman primates indicate the small secreted peptide hormone adropin regulates glucose metabolism. Here, recent expression profiling data indicating hepatic adropin expression increases with oxidative stress and declines with fasting or in the presence of hepatic insulin resistance and how adropin interacts with the pancreatic hormones, insulin, and glucagon to modulate glycemic control are discussed.

With recent advances in obesity and type 2 diabetes (T2D) therapeutics, some might conclude that all major hormonal pathways governing energy balance and glucose homeostasis have been identified and thoroughly investigated. However, mammalian genomes contain many highly conserved short open frame sequences (sORF) encoding a multitude of secreted peptides that may regulate metabolic homeostasis (1,2), some of which could provide further treatments for obesity and T2D.

The biological activities of the small secreted peptide adropin are indicative of a potentially important regulator of glucose metabolism. The sORF encoding adropin was discovered by Genentech's Secreted Protein Discovery Initiative (2). The first experiments connecting adropin to metabolism profiled hepatic gene expression in male C57BL/6J mice (3). Expression of the energy homeostasis associated (*ENHO*) transcript encoding adropin was suppressed with obesity and fasting and rapidly increased when refed after overnight fasting (3,4). These responses suggested involvement in metabolic adaptation to food intake.

Expression profiling using mouse models now suggests a more complex relationship. A variety of metabolic conditions suppress hepatic adropin expression: catabolic states induced by overnight fasting, increased hepatic lipogenesis (e.g., consumption of high-sugar diets or severe hyperphagic obesity syndromes) in hypercholesterolemia, and genetic disruption of the circadian-clock activator BMAL1 (aryl hydrocarbon receptor nuclear translocator-like protein 1) (3–6). Expression of adropin in nonhuman primate (NHP) and mouse liver exhibits a circadian profile with peaks coinciding with food intake (6,7). Control of transcription is complex and it appears to involve nuclear receptor components of the clock (ROR, REV-ERB) and epigenetic modification (4,6,7). The liver clock per se was shown to be insufficient for full expression, indicating circadian signaling from extrahepatic tissues is involved (4).

The conditions that increase adropin expression are not well characterized but are correlated with activation of stress responses. Although intake of high-fat/low-sugar diets rapidly increased adropin expression (3), expression also increased with caloric restriction

(CR) (4). In the latter study, adropin expression correlated with genes involved in cellular stress responses. Hepatic adropin expression was increased by compounds that induce oxidative stress (e.g., paraquat) (4). These responses could be adaptive, as adropin actions involving nuclear factor erythroid 2-related factor 2 were shown to protect the liver from oxidative stress (8). One implication is that hepatic adropin expression is induced in response to cellular stress. Presumably, normal regulation of adropin in response to oxidative stress, and any potential protective role, are compromised in situations of severe obesity and insulin resistance.

An unexpected observation is a positive correlation between adropin and phosphoenolpyruvate carboxykinase 1 (PCK1), a major control point in gluconeogenesis (4). This relationship was observed in the liver transcriptome in samples collected over 24 hours from mice fed ad libitum or subjected to CR. These data provide a window into the liver transcriptome under conditions ranging from short-term energy deficit between meals in mice subject to prolonged CR, milder energy deficits during the lights-on period when nocturnal mice rest, "normal" voluntary feeding in the dark phase, or recent binge-feeding (mice subject to CR consume 80% to 90% of daily kilocalorie intake within 1 hour of ad libitum food presentation). Overnight fasting of mice housed at room temperature induced a severe catabolic state (9). Although fasting suppresses hepatic adropin expression and increases hepatic glucose production (HGP), this relationship is not observed in mice subjected to a broader range of metabolic conditions involving coactivation of HGP and adropin expression.

Positive correlations with PCK1 and acute responses of liver adropin expression to fasting and refeeding suggest functions related to adapting metabolism to shifts in energy balance. Experiments using adropin knockout mice (AdrKO), mice treated with synthetic adropin, and in primary cultured hepatocytes all suggest adropin inhibits HGP (4,10–13). This effect is bidirectional as liver-specific AdrKO mice exhibited increased glucose excursions following intraperitoneal injections of pyruvate, suggesting increased HGP (4). Adropin appears to "modulate" glucagon receptor signal transduction. Activation of the putative adropin receptor has an inhibitory effect on the induction of cAMP-dependent protein kinase (PKA) signaling by glucagon. This could indicate an inverse relationship between liver adropin expression and the ability of glucagon to increase HGP. Alternatively, adropin has the opposite effect on insulin action, improving hepatic insulin action in conditions of obesity and insulin resistance (12,13).

Two candidate cell-surface receptors have been identified: an orphan G-protein coupled receptor (GPR19) (14) and a glycosylphosphatidylinositol-anchored neuronal membrane protein that functions as a cell adhesion molecule (NB3/CNTN6) (15). Both are predominantly expressed in the nervous system (16). GPR19 inhibited cAMP signaling in cultured cells (17), whereas Notch signaling was implicated in liver glucose metabolism (18). However, how adropin signaling from the cell surface of hepatocytes affects glucose metabolism needs to be clarified.

Experiments conducted in mouse models support a role for adropin in glucose homeostasis. AdrKO mice exhibited increased HGP assessed by hyperinsulinemic-euglycemic clamp (10). Activation of hepatic protein kinase B (AKT/PKB) signaling by insulin was, however, normal, suggesting a potentially novel and atypical mechanism (10). Whether changes in glucagon signaling observed in liver-specific AdrKO mice explain the phenotype requires further investigation (4). Administration of recombinant adropin to diet-induced obese mice enhances insulin signaling in skeletal muscle and liver (12,19). This effect is observed in perfused hearts *ex vivo*, suggesting a direct effect of adropin (4,12,20). Adropin treatment is also unique because it was shown to acutely enhance oxidative glucose disposal in diet-induced obese mice (19). Other laboratories have also observed direct effects of adropin in cultured cells and isolated perfused hearts to regulate fuel substrate selection to favor the oxidation of glucose over fat (20–22). Adropin could therefore potentially represent a unique therapy targeting T2D by improving insulin sensitivity while simultaneously enhancing oxidative glucose disposal.

If the mouse data are translatable to humans, low adropin activity should correlate with hyperglycemia. In an NHP model, low plasma adropin concentrations correlate with fasting hyperglycemia and with plasma biomarkers suggesting dysregulation of hepatic lipoprotein metabolism and increased adiposity (7,23). Transcriptomic data in the liver of NHPs suggest adropin expression peaks around mealtime and that it is coregulated with genes involved in glucose metabolism and second messenger signal transduction pathways involving G proteins and receptor tyrosine kinases. In primates, peak hepatic adropin expression may thus anticipate and/or coincide with the activation of signal transduction pathways mediating the responses of hepatocytes to endocrine and/or metabolite signals of increased nutrient flux.

Our analysis of relationships between circulating levels of adropin and circulating indices of glucose and lipid metabolism in humans is less clear. Low plasma adropin concentrations correlate with evidence of impaired insulin action in studies of subjects using narrowly defined age, weight, and health criteria for inclusion (5,24). On the other hand, in a larger study with fewer restrictions for inclusion, we reported an inverse relationship with non-high-density lipoprotein cholesterol that could be explained by an inhibitory effect of cholesterol on *ENHO* expression (6). Fructose consumption rapidly induces insulin resistance and dyslipidemia in humans and rapidly increases plasma adropin concentrations, and this effect was most pronounced in subjects with existing dyslipidemia at baseline prior to sugar consumption (25). As with the early studies of other secreted peptides, issues of assay specificity and validation are significant caveats in the interpretation of the data on plasma adropin concentrations.

To conclude, we have discussed several mechanisms by which adropin acts to regulate glucose homeostasis. Adropin signaling in the liver through an as-yet unidentified receptor interacts with insulin and glucagon to lower HGP. Suppression of adropin expression may contribute to increased HGP in obesity. Data showing enhancement of insulin action and glucose oxidation in cardiac and skeletal muscle also suggest a role in lower glucose (19,20). Clearly,

further assessment of the clinical relevance of the current findings on regulation of adropin and its physiological actions is warranted. The identity of the cell-surface receptors for adropin and the signal transduction pathways need to be fully explored. Finally, models more relevant to humans (e.g., NHPs) should be used to explore the relationship between low hepatic adropin expression and hyperglycemia.

ACKNOWLEDGMENTS

The authors apologize that because of space limitations we were unable to cite a number of other important studies on the biology adropin.

CONFLICT OF INTEREST

The authors declared no conflict of interest.

ORCID

Andrew A. Butler https://orcid.org/0000-0001-7196-0170

REFERENCES

1. Makarewich CA, Olson EN. Mining for micropeptides. *Trends Cell Biol*. 2017;27:685-696.
2. Clark HF, Gurney AL, Abaya E, et al. The secreted protein discovery initiative (SPDI), a large-scale effort to identify novel human secreted and transmembrane proteins: a bioinformatics assessment. *Genome Res*. 2003;13:2265-2270.
3. Kumar KG, Trevaskis JL, Lam DD, et al. Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism. *Cell Metab*. 2008;8:468-481.
4. Banerjee S, Ghoshal S, Stevens JR, et al. Hepatocyte expression of the micropeptide adropin regulates the liver fasting response and is enhanced by caloric restriction. *J Biol Chem*. 2020;295:13753-13768.
5. Stevens JR, Kearney ML, St-Onge M-P, et al. Inverse association between carbohydrate consumption and plasma adropin concentrations in humans. *Obesity (Silver Spring)*. 2016;24:1731-1740.
6. Ghoshal S, Stevens JR, Billon C, et al. Adropin: an endocrine link between the biological clock and cholesterol homeostasis. *Mol Metab*. 2018;8:51-64.
7. Butler AA, Zhang J, Price CA, et al. Low plasma adropin concentrations increase risks of weight gain and metabolic dysregulation in response to a high-sugar diet in male nonhuman primates. *J Biol Chem*. 2019;294:9706-9719.
8. Chen XU, Xue H, Fang W, et al. Adropin protects against liver injury in nonalcoholic steatohepatitis via the Nrf2 mediated antioxidant capacity. *Redox Biol*. 2019;21:101068. doi:10.1016/j.redox.2018.101068
9. Carper D, Coue M, Laurens C, Langin D, Moro C. Reappraisal of the optimal fasting time for insulin tolerance tests in mice. *Mol Metab*. 2020;42:101058. doi:10.1016/j.molmet.2020.101058
10. Ganesh-Kumar K, Zhang J, Gao SU, et al. Adropin deficiency is associated with increased adiposity and insulin resistance. *Obesity (Silver Spring)*. 2012;20:1394-1402.
11. Thapa D, Xie B, Manning JR, et al. Adropin reduces blood glucose levels in mice by limiting hepatic glucose production. *Physiol Rep*. 2019;7:e14043. doi:10.14814/phy2.14043
12. Gao SU, Ghoshal S, Zhang L, et al. The peptide hormone adropin regulates signal transduction pathways controlling hepatic glucose metabolism in a mouse model of diet-induced obesity. *J Biol Chem*. 2019;294:13366-13377.
13. Chen XU, Chen S, Shen T, et al. Adropin regulates hepatic glucose production via PP2A/AMPK pathway in insulin-resistant hepatocytes. *FASEB J*. 2020;34:10056-10072.
14. Stein LM, Yosten GL, Samson WK. Adropin acts in brain to inhibit water drinking: potential interaction with the orphan G protein-coupled receptor, GPR19. *Am J Physiol Regul Integr Comp Physiol*. 2016;310:R476-R480.
15. Wong C-M, Wang Y, Lee JTH, et al. Adropin is a brain membrane-bound protein regulating physical activity via the NB-3/Notch signaling pathway in mice. *J Biol Chem*. 2014;289:25976-25986.
16. Lonsdale J, Thomas J, Salvatore M, et al. The Genotype-Tissue Expression (GTEx) project. *Nat Genet*. 2013;45:580-585.
17. Rao A, Herr DR. G protein-coupled receptor GPR19 regulates E-cadherin expression and invasion of breast cancer cells. *Biochim Biophys Acta Mol Cell Res*. 2017;1864:1318-1327.
18. Pajvani UB, Accili D. The new biology of diabetes. *Diabetologia*. 2015;58:2459-2468.
19. Gao S, McMillan RP, Zhu Q, Lopaschuk GD, Hulver MW, Butler AA. Therapeutic effects of adropin on glucose tolerance and substrate utilization in diet-induced obese mice with insulin resistance. *Mol Metab*. 2015;4:310-324.
20. Altamimi TR, Gao SU, Karwi QG, et al. Adropin regulates cardiac energy metabolism and improves cardiac function and efficiency. *Metabolism*. 2019;98:37-48.
21. Thapa D, Stoner MW, Zhang M, et al. Adropin regulates pyruvate dehydrogenase in cardiac cells via a novel GPCR-MAPK-PDK4 signaling pathway. *Redox Biol*. 2018;18:25-32.
22. Thapa D, Xie B, Zhang M, et al. Adropin treatment restores cardiac glucose oxidation in pre-diabetic obese mice. *J Mol Cell Cardiol*. 2019;129:174-178.
23. Butler AA, Graham JL, Stanhope KL, et al. Role of angiopoietin-like protein 3 in sugar-induced dyslipidemia in rhesus macaques: suppression by fish oil or RNAi. *J Lipid Res*. 2020;61:376-386.
24. Butler AA, Tam CS, Stanhope KL, et al. Low circulating adropin concentrations with obesity and aging correlate with risk factors for metabolic disease and increase after gastric bypass surgery in humans. *J Clin Endocrinol Metab*. 2012;97:3783-3791.
25. Butler AA, St-Onge MP, Siebert EA, Medici V, Stanhope KL, Havel PJ. Differential responses of plasma adropin concentrations to dietary glucose or fructose consumption in humans. *Sci Rep*. 2015;5:14691. doi:10.1038/srep14691

How to cite this article: Butler AA, Havel PJ. Adropin and insulin resistance: Integration of endocrine, circadian, and stress signals regulating glucose metabolism. *Obesity (Silver Spring)*. 2021;29:1799–1801. https://doi.org/10.1002/oby.23249