Published in final edited form as:

Physiol Behav. 2016 August 1; 162: 147–150. doi:10.1016/j.physbeh.2016.04.012.

Hypoglycemia Unawareness Prevention: Targeting Glucagon Production

Willis K. Samson¹, Lauren M. Stein¹, Mollisa Elrick¹, Alison Salvatori¹, Grant Kolar², John A. Corbett³, and Gina L.C. Yosten¹

¹Department of Pharmacology and Physiology, Saint Louis University School of Medicine St. Louis, MO 63104

²Department of Pathology, Saint Louis University School of Medicine St. Louis, MO 63104

³Department of Biochemistry Medical College of Wisconsin Milwaukee, WI

Abstract

Insulin-dependent individuals with diabetes are at risk for a severe hypoglycemic event that may predispose them to several repeat episodes during which the normal counter regulatory mechanisms that protect against hypoglycemia fail to be activated. This state of hypoglycemia unawareness is characterized by a failure of glucagon release, preventing mobilization of endogenous glucose stores from the liver. We describe the discovery of a novel hormone, produced in pancreatic delta cells, which stimulates glucagon production and release, particularly under low glucose conditions. We hypothesize that this hormone, called neuronostatin, may be effective as a co-therapy with insulin to prevent repeated, potentially fatal episodes of recurrent hypoglycemia.

Keywords

hypoglycemia; glucagon; neuronostatin; insulin

1.1 Introduction

Regardless of level of intensity of therapy, all insulin-dependent, diabetic individuals are at significant risk (11) of experiencing a severe episode of hypoglycemia, defined as blood glucose levels less than 50 mg/dl. Large patient-based studies have demonstrated that severe hypoglycemia (13, 27) increases risk for diseases of the cardiovascular, respiratory and digestive systems. While normal physiologic responses to this event may or may not correct low blood glucose levels during the initial episode, these individuals are at risk during the ensuing days and weeks for a additional hypoglycemic events during which those compensatory mechanisms fail to be activated (3, 5, 12). This apparent unawareness of the

Corresponding Author: Willis K. Samson, PhD, DSc, Department of Pharmacology and Physiology, Saint Louis University, 1402 South Grand Boulevard, St. Louis, MO 63104, Tel: (314) 977-8677, Fax: (314) 977-8670, samsonwk@slu.edu.

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final citable form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

hypoglycemic state can result in mild consequences (nausea, dizziness) or even more serious, life threatening unconsciousness, coma and death. Although the exact mechanisms underlying hypoglycemic unawareness are not entirely understood, it is clear that the normal compensatory mechanisms, including glucagon secretion and epinephrine release, are not recruited and intervention is required to bring blood glucose levels into the normal range. These interventions include immediate ingestion, if the individual is conscious, of sugary fluids or in other cases injection of glucagon itself to recruit endogenous stores of glucose from primarily the liver. Because the compensatory responses of pancreatic alpha cells are compromised in hypoglycemic unawareness (16), this cell type represents a novel target for the development of therapeutics designed to protect against the lack of a normal compensatory response. This identifies the alpha cell as a target for novel therapeutics designed to protect against the failure of the normal compensatory response.

2.1 The Alpha Cell as a Target

Glucagon release from the alpha cell is primarily regulated by ambient levels of insulin. When blood glucose levels fall below 80 mg/dl, the stimulus for insulin secretion from the pancreatic beta cell is withdrawn (3, 22) and the combination of decreased blood glucose and insulin levels removes the brake on the alpha cell leading to glucagon secretion and the recruitment of endogenous fuel stores. Are there other, endogenous factors that control glucagon production and secretion and is the reason for the failure of the alpha cell to respond to hypoglycemia due to lack of recruitment of those unknown factors? Even more importantly, if endogenous factors trophic to the alpha cell exist, can they be developed as therapeutic agents to protect against recurrent episodes of debilitating hypoglycemia? This review presents the developing evidence for one of those potential approaches, based upon the discovery of a novel hormone, produced primarily in the delta cell of the pancreas, that has been shown to stimulate glucagon production and release (4, 19, 20). The delta cell is the site of somatostatin production in the pancreatic islet. While somatostatin exerts inhibitory effects on insulin and glucagon secretion in vitro, its physiological role in intra-islet communication control of insulin secretion is yet to be clearly shown. However, a paracrine action of somatostatin to inhibit glucagon secretion has been demonstrated under pharmacologic conditions (9, 10).

Our group identified a second biologically active peptide derived from the somatostatin pre-prohormone and verified that this peptide, called neuronostatin, was indeed produced in pancreatic delta cells (20). Importantly, neuronostatin has been demonstrated to act directly on the alpha cell to stimulate glucagon production and release, and via an indirect action to inhibit glucose stimulated insulin secretion (19).

2.2 The Discovery of Neuronostatin

Using a bioinformatic approach, Aaron Hsueh (Stanford University) recognized the presence of a second, potentially bioactive peptide in the somatostatin pre-prohormone. Upstream from somatostatin in the prohormone are located multiple potential dibasic cleavage sites suggesting that post-translational processing might result in liberation of a peptide sequence with a C-terminal glycine, predicting amidation, a common feature of many biologically

active peptides. In collaboration with the Hsueh lab and Jaw Kang Chang at Phoenix Pharmaceuticals, we purified the peptide from porcine pancreas and spleen and demonstrated his prediction to be correct. As a collaborative team, we went on to raise antibodies to the peptide and establish both a radioimmunoassay and an ELISA, with which we characterized the tissue distribution of neuronostatin-like immunoreactivity in the rat. As predicted neuronostatin colocalized with somatostatin in all tissues examined. Most relevant to this review, the delta cell was the sole cell type in pancreas in which neuronostatin-like immunoreactivity was detected. This established the potential for the peptide to act within the islet in a paracrine fashion to control hormone release.

Plasma levels of neuronostatin were observed to fluctuate dependent upon fed state, rising during an overnight fast and returning quickly to baseline after refeeding (4). This led us to interrogate the possible role of neuronostatin in the regulation of glucose homeostasis using two distinct approaches. First, we showed that neuronostatin significantly blunted glucose stimulated insulin secretion from isolated rat islets (19). This effect was absent in beta cell cultures suggesting that neuronostatin's effect in whole islets was indirect requiring recruitment of some additional trophic agent, perhaps even glucagon itself or somatostatin release from the delta cells (7, 9, 10, 21). These results were mirrored in the second approach when neuronostatin was infused into conscious male rats prior to and during a glucose challenge (19). Neuronostatin treatment delayed glucose clearance and significantly dampened the in vivo insulin response. This led us to hypothesize that at least one action of the peptide was to circumscribe insulin secretion protecting ambient levels of plasma glucose. We then turned to the alpha cell as a potential site of action.

2.3 Neuronostatin Acts Directly on the Pancreatic Alpha Cell

Neuronostatin was demonstrated to significantly increase proglucagon mRNA levels in cultured alpha cells and in a concentration dependent fashion enhance glucagon release under low glucose conditions (19). These effects were replicated in isolated islets. This lead us to hypothesize that neuronostatin contributes to physiologic regulation of glucose homeostasis via an action on the alpha cell. Since the administration of large doses of neuronostatin increased c-Jun levels in vivo (20), an effect also observed in alpha cell cultures (4), it suggested that this hormone required some form of receptor to transmit the action of the peptide. Proof would require the identification a unique neuronostatin receptor and demonstration that it localized on the alpha cell itself.

Using a deductive reasoning strategy developed by Dr. Yosten (25), we identified several transformed cell lines that responded to neuronostatin with an increase in cellular activity. Then, because a prior collaborative effort with Dr. Jun Ren (University of Wyoming) had revealed that the cardiotropic effects of neuronostatin were dependent upon signaling via activation of protein kinase A (6), Dr. Yosten hypothesized that the neuronostatin receptor would be one of the approximately 120 "orphan G protein-coupled receptors" of the Type A or Type B classes as cataloged by IUPHAR (15). Dr. Yosten screened neuronostatin-responsive cell lines for common expression of these orphans and identified several candidates. Compromise of the expression of only one of those candidates, GPR107,

resulted in the loss of the action of neuronostatin in a gastric tumor cell line, KATOIII cells (25). Thus we had our candidate receptor and we returned to our alpha cell cultures.

Compromise of GPR107 expression in cultured alpha cells similarly resulted in the loss of the action of neuronostatin to increase proglucagon mRNA levels (4). Remarkably this siRNA-mediated approach also worked in isolated rat islets providing strong evidence for the handshake between ligand (neuronostatin) and receptor (GPR107). Additional studies employed siRNA to examine the effects of receptor compromise on neuronostatin-induced signaling in cultured alpha cells. We had identified the signaling cascade activated in alpha cells to be, like the case in isolated cardiomyocytes, dependent upon PKA phosphorylation and activation of NF-kappaB. As was the case when examining proglucagon mRNA levels, phosphorylation of PKA by neuronostatin was blocked by compromise of GPR107 expression in cultured alpha cells (4).

The physical association of neuronostatin with GPR107 then was demonstrated by confocal analyses, showing significant co-localization of the ligand and receptor on the membranes of cultured alpha cells. Likewise, neuronostatin and GPR107 co-localized on the cell membranes of glucagon immunoreactive cells in slices of human cadaveric pancreas (4). While this does not definitively demonstrate physical interaction of the ligand and receptor, it is a step in that direction.

2.4 Extrapancreatic Sites of Action of Neuronostatin

Neuronostatin immunoreactivity is present in a variety of tissues, as is GPR107 expression, including brain, particularly hypothalamus and medulla, and heart (4, 20, 25). In collaboration with Dr. Jun Ren, we have demonstrated negative chronotropic and inotropic effects of neuronostatin in vitro and in vivo (6, 26), suggesting that the changes in circulating hormone levels observed during fasting (4) may, in addition to effects on glucagon secretion, alter cardiovascular function (6, 26). The central nervous system actions of neuronostatin appear more related to autonomic regulation. Indeed, central administration of the neuronostatin in conscious rats resulted in an increase in mean arterial pressure that was prevented by pretreatment with the alpha-adrenergic blocker, phentolamine (24). This is in all likelihood a physiologically relevant action of neuronostatin, since compromise of central GPR107 expression prevents the pressor effect of neuronostatin given into the brain and alters baroreflex sensitivity (25).

3.1 Neuronostatin as a Potential Co-Therapy for the Prevention of Hypoglycemia Following a Severe Hypoglycemic Event

One of the most important mechanisms protecting against hypoglycemia is the dis-inhibition of alpha cell glucagon release. This compensatory mechanism, however, fails to be activated in the syndrome of hypoglycemia unawareness, either because the cause of the hypoglycemia is iatrogenic (excessive insulin exposure) (17), or because the cell itself fails to respond (is refractory) to the low glucose conditions (1, 16). Would it be possible to design a treatment regimen for insulin-dependent diabetic patients taking into consideration that failure of glucagon release, especially after a recent, severe hypoglycemic event? While

there is current interest in dual hormone treatment with insulin and glucagon (18), significant delivery problems have slowed development, none the least of which is the relative insolubility of glucagon. The technology requires constant glucose surveillance and the appropriate sensors and servomechanisms have yet to be developed. While early successes in the short term have been reported (23) other attempts have failed (2).

Perhaps the problem lies with the approach of using exogenous glucagon to override the hypoglycemic event. What if endogenous release could be raised such that an appropriate, slightly elevated baseline of glucagon was always available to provide the stimulus for hepatic gluconeogenesis and glycogenolysis? The discovery of an endogenous factor that up-regulates glucagon production and stimulates its release may have opened a new avenue for prevention of the devastating consequences of hypoglycemia unawareness, particularly if neuronostatin can be shown to stimulate glucagon secretion in individuals with type 1 diabetes. In other words, can neuronostatin activate glucagon production and release from alpha cells already compromised by antecedent hypoglycemia? Future studies should examine this potential protective approach of neuronostatin co-infusion following a severe hypoglycemic episode now that animal models of hypoglycemia unawareness have been developed (8, 14).

Acknowledgments

Personnel and experiments were supported by NIH HL 06623 and 121456 (WKS, GLCY), DK 052194 (JC), T32-GM008306 (ME, AS, LS), and Presidential Fellowships from Saint Louis University (ME, AS). The authors acknowledge the continued support of Aaron J. Hsueh (Stanford University), as well as Crystal Chang, Eng Tau and Jaw Kang Chang (Phoenix Pharmaceuticals).

References

1. Baker D, Evans M, Cryer P, Sherwin R. Hypoglycaemia and glucose sensing. *Diabetologia*. 1997; 40(Suppl 3):B83–88. [PubMed: 9345653]
2. Castle JR, Engle JM, El Youssef J, Massoud RG, Ward WK. Factors influencing the effectiveness of glucagon for preventing hypoglycemia. *Journal of diabetes science and technology*. 2010; 4:1305–1310. [PubMed: 21129324]
3. Cryer PE. Hypoglycemia, functional brain failure, and brain death. *The Journal of clinical investigation*. 2007; 117:868–870. [PubMed: 17404614]
4. Elrick MM, Samson WK, Corbett JA, Salvatori AS, Stein LM, Kolar GR, Naatz A, Yosten GL. Neuronostatin acts via GPR107 to increase cAMP-independent PKA phosphorylation and proglucagon mRNA accumulation in pancreatic alpha-cells. *American journal of physiology Regulatory, integrative and comparative physiology*. 2016; 310:R143–155.
5. Gerich JE, Mookan M, Veneman T, Korytkowski M, Mitrakou A. Hypoglycemia unawareness. *Endocrine reviews*. 1991; 12:356–371. [PubMed: 1760993]
6. Hua Y, Ma H, Samson WK, Ren J. Neuronostatin inhibits cardiac contractile function via a protein kinase A- and JNK-dependent mechanism in murine hearts. *American journal of physiology Regulatory, integrative and comparative physiology*. 2009; 297:R682–689.
7. Itoh M, Mandarino L, Gerich JE. Antisomatostatin gamma globulin augments secretion of both insulin and glucagon in vitro: evidence for a physiologic role for endogenous somatostatin in the regulation of pancreatic A- and B-cell function. *Diabetes*. 1980; 29:693–696. [PubMed: 6160072]
8. Jacobson L, Ansari T, McGuinness OP. Counterregulatory deficits occur within 24 h of a single hypoglycemic episode in conscious, unrestrained, chronically cannulated mice. *American journal of physiology Endocrinology and metabolism*. 2006; 290:E678–684. [PubMed: 16533951]

9. Klaff LJ, Taborsky GJ Jr. Pancreatic somatostatin is a mediator of glucagon inhibition by hyperglycemia. *Diabetes*. 1987; 36:592–596. [PubMed: 2883057]
10. Klaff LJ, Taborsky GJ Jr. Role of pancreatic somatostatin in determining glucagon response to arginine and morphine. *The American journal of physiology*. 1987; 252:E751–755. [PubMed: 2884887]
11. Lipska KJ, Warton EM, Huang ES, Moffet HH, Inzucchi SE, Krumholz HM, Karter AJ. HbA1c and risk of severe hypoglycemia in type 2 diabetes: the Diabetes and Aging Study. *Diabetes care*. 2013; 36:3535–3542. [PubMed: 23900589]
12. McCoy RG, Van Houten HK, Ziegenfuss JY, Shah ND, Wermers RA, Smith SA. Increased mortality of patients with diabetes reporting severe hypoglycemia. *Diabetes care*. 2012; 35:1897–1901. [PubMed: 22699297]
13. Miller ME, Bonds DE, Gerstein HC, Seaquist ER, Bergenstal RM, Calles-Escandon J, Childress RD, Craven TE, Cuddihy RM, Dailey G, Feinglos MN, Ismail-Beigi F, Largay JF, O'Connor PJ, Paul T, Savage PJ, Schubart UK, Sood A, Genuth S. The effects of baseline characteristics, glycaemia treatment approach, and glycated haemoglobin concentration on the risk of severe hypoglycaemia: post hoc epidemiological analysis of the ACCORD study. *BMJ (Clinical research ed)*. 2010; 340:b5444.
14. Otlivanchik O, Sanders NM, Dunn-Meynell A, Levin BE. Orexin signaling is necessary for hypoglycemia-induced prevention of conditioned place preference. *American journal of physiology Regulatory, integrative and comparative physiology*. 2016; 310:R66–73.
15. Pawson AJ, Sharman JL, Benson HE, Faccenda E, Alexander SP, Buneman OP, Davenport AP, McGrath JC, Peters JA, Southan C, Spedding M, Yu W, Harmar AJ, Ne I. The IUPHAR/BPS Guide to PHARMACOLOGY: an expert-driven knowledgebase of drug targets and their ligands. *Nucleic acids research*. 2014; 42:D1098–1106. [PubMed: 24234439]
16. Powell AM, Sherwin RS, Shulman GI. Impaired hormonal responses to hypoglycemia in spontaneously diabetic and recurrently hypoglycemic rats. Reversibility and stimulus specificity of the deficits. *The Journal of clinical investigation*. 1993; 92:2667–2674. [PubMed: 8254023]
17. Raju B, Cryer PE. Loss of the decrement in intraislet insulin plausibly explains loss of the glucagon response to hypoglycemia in insulin-deficient diabetes: documentation of the intraislet insulin hypothesis in humans. *Diabetes*. 2005; 54:757–764. [PubMed: 15734853]
18. Reiband HK, Schmidt S, Ranjan A, Holst JJ, Madsbad S, Norgaard K. Dual-hormone treatment with insulin and glucagon in patients with type 1 diabetes. *Diabetes/metabolism research and reviews*. 2015; 31:672–679. [PubMed: 25533565]
19. Salvatori AS, Elrick MM, Samson WK, Corbett JA, Yosten GL. Neuronostatin inhibits glucose-stimulated insulin secretion via direct action on the pancreatic alpha-cell. *American journal of physiology Endocrinology and metabolism*. 2014; 306:E1257–1263. [PubMed: 24735892]
20. Samson WK, Zhang JV, Avsian-Kretchmer O, Cui K, Yosten GL, Klein C, Lyu RM, Wang YX, Chen XQ, Yang J, Price CJ, Hoyda TD, Ferguson AV, Yuan XB, Chang JK, Hsueh AJ. Neuronostatin encoded by the somatostatin gene regulates neuronal, cardiovascular, and metabolic functions. *The Journal of biological chemistry*. 2008; 283:31949–31959. [PubMed: 18753129]
21. Taniguchi H, Utsumi M, Hasegawa M, Kobayashi T, Watanabe Y, Murakami K, Seki M, Tsutou A, Makimura H, Sakoda M, Baba S. Physiologic role of somatostatin. Insulin release from rat islets treated by somatostatin antiserum. *Diabetes*. 1977; 26:700–702. [PubMed: 326606]
22. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *The Journal of clinical investigation*. 2012; 122:4–12. [PubMed: 22214853]
23. van Bon AC, Luijck YM, Koebrugge R, Koops R, Hoekstra JB, DeVries JH. Feasibility of a portable bihormonal closed-loop system to control glucose excursions at home under free-living conditions for 48 hours. *Diabetes technology & therapeutics*. 2014; 16:131–136. [PubMed: 24224750]
24. Yosten GL, Pate AT, Samson WK. Neuronostatin acts in brain to biphasically increase mean arterial pressure through sympatho-activation followed by vasopressin secretion: the role of melanocortin receptors. *American journal of physiology Regulatory, integrative and comparative physiology*. 2011; 300:R1194–1199.

25. Yosten GL, Redlinger LJ, Samson WK. Evidence for an interaction of neuronostatin with the orphan G protein-coupled receptor, GPR107. *American journal of physiology Regulatory, integrative and comparative physiology*. 2012; 303:R941–949.
26. Zhu X, Hu N, Chen X, Zhu MZ, Dong H, Xu X, Luo F, Hua Y, Nair S, Samson WK, Xiong L. Neuronostatin attenuates myocardial contractile function through inhibition of sarcoplasmic reticulum Ca^{2+} -ATPase in murine heart. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology*. 2014; 33:1921–1932.
27. Zoungas S, Patel A, Chalmers J, de Galan BE, Li Q, Billot L, Woodward M, Ninomiya T, Neal B, MacMahon S, Grobbee DE, Kengne AP, Marre M, Heller S. Severe hypoglycemia and risks of vascular events and death. *The New England journal of medicine*. 2010; 363:1410–1418. [PubMed: 20925543]

Highlights

- Hypoglycemia unawareness following a severe bout of hypoglycemia can be fatal.
- Unawareness results in a failure of counter regulatory mechanisms (glucagon and epinephrine).
- Neuronostatin, produced in pancreatic delta cells, stimulates glucagon production and release.
- Neuronostatin may provide protection against hypoglycemia during episodes of unawareness.