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Review

Hypoglycemia unawareness prevention: Targeting glucagon production

Willis K. Samson [a,*], Lauren M. Stein [a], Mollisa Elrick [a], Alison Salvatori [a], Grant Kolar [b], John A. Corbett [c], Gina L.C. Yosten [a]

[a] Department of Pharmacology and Physiology, Saint Louis University School of Medicine, St. Louis, MO 63104, United States
[b] Department of Pathology, Saint Louis University School of Medicine, St. Louis, MO 63104, United States
[c] Department of Biochemistry, Medical College of Wisconsin, Milwaukee, WI, United States

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.

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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.

Contents

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 additional hypoglycemic events during which those compensatory mechanisms fail to be activated [3,5,12]. This apparent

* Corresponding author at: Department of Pharmacology and Physiology, Saint Louis University, 1402 South Grand Boulevard, St. Louis, MO 63104, United States.
E-mail address: samsonwk@slu.edu (W.K. Samson).

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unawareness of the 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. 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].

3. 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.

4. 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 leads 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