Drugs of the Future 2004, 29(8): 835-841

CCC: 0377-8282/2004

Review Article

Role of the glucagon receptor in glucose homeostasis: a therapeutic target to improve glycemic control in type 2 diabetes

Kyle W. Sloop* and M. Dodson Michael

Endocrine Discovery, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285, USA.
**Correspondence: e-mail: sloop_kyle_w@lilly.com*

CONTENTS

Abstract

Glucagon and insulin are the primary counterregulatory hormones that control glucose homeostasis. Disruption of the ratio of these hormones has been implicated in the etiology of type 2 diabetes mellitus, especially as pancreatic β-cell function deteriorates and prandial insulin secretion is progressively delayed. As a result, hepatic glucose production is no longer suppressed, contributing significantly to hyperglycemia. Thus, strategies to modulate glucagon activity have been proposed as new therapeutic approaches to improve glucose control in diabetic patients. Peptide glucagon antagonists, antiglucagon antibodies and low-molecular-weight glucagon receptor (GCGR) antagonists have shown glucose-lowering efficacy in acute studies, providing proof-of-concept validation that inhibiting this pathway impacts glycemia in diabetes. Recently, the use of gene knockout and antisense oligonucleotide (ASO) technology has demonstrated the potential long-term effects of modulating glucagon action. Importantly, administration of GCGR ASOs in rodent models of diabetes has provided key information about the effects of inhibiting the GCGR in this disease state. Although strategic drug discovery questions still remain that will impact the pursuit of antiglucagon compounds, the successful development of such molecules may offer diabetic patients better options to improve glucose control.

Introduction

Pioneering work throughout the 20th century led to the identification and understanding of the physiology of the polypeptide hormone glucagon. The first clue to glucagon's existence appeared when pancreatic extracts were shown to cause hyperglycemia and mobilize liver glycogen stores during periods of fasting (1, 2). Elegant cross-circulation experiments using dogs suggested that hypoglycemia triggered the release of glucagon from the pancreas (3-5). The purification and crystallization of glucagon (6) allowed the determination of its amino acid composition and primary structure (7) and enabled the production of highly purified glucagon, which was used to generate antiglucagon antibodies. Importantly, this allowed the development of a radioimmunoassay (RIA) to measure circulating glucagon (8-10). The glucagon RIA and a similar assay to measure insulin (11) were utilized to elucidate much of our fundamental understanding of glucose homeostasis (reviewed in 12). The identification of genomic and cDNA sequences coding for the glucagon receptor (GCGR), a type 2 seven-transmembrane G-protein-coupled receptor belonging to the secretin family (13-16), ultimately led to the generation of *Gcgr* knockout mice (17, 18) and enabled the generation of screening assays to facilitate the search for inhibitory compounds to block glucagon action for the treatment of diabetes.

Role of glucagon in glucose homeostasis

Coordinating plasma levels of glucagon and insulin is critical to maintaining euglycemia. Glucagon is the major counterregulatory hormone to insulin in glucose homeostasis (reviewed in 19, 20). In response to hypoglycemia, this 29-amino-acid polypeptide is released from pancreatic α-cells into the portal vein to stimulate hepatic glucose production. Glucagon binds to its receptor on the plasma membrane of hepatocytes to activate adenylate cyclase, which generates intracellular cAMP (21). Protein kinase A then activates signal transduction

pathways to stimulate glycogenolysis and gluconeogenesis, while inhibiting glycogen synthesis (reviewed in 22).

Insulin counteracts the glucagon regulation of hepatic glucose metabolism by activating phosphodiesterases, thereby decreasing cAMP levels, stimulating phosphorylation of glycogen synthase kinase-3, which prevents inhibition of glycogen synthase, and phosphorylating the Forkhead transcription factor FOXO1, leading to suppression of phosphoenolpyruvate carboxykinase and glucose-6-phosphatase. The net result of increasing the insulin:glucagon ratio is an increase in glycogen synthesis and an inhibition of glycogenolysis and gluconeogenesis. Under normal conditions, glucagon secretion is regulated by gradual changes in systemic glucose and insulin concentrations.

Dysregulation of glucagon in type 2 diabetes

In the 1970s, Unger hypothesized that disruption of the glucagon-insulin bihormonal relationship contributed to the manifestation of diabetes mellitus (23). Several studies have since tested this hypothesis, and accumulating evidence supports a pathophysiological role for glucagon in diabetes. For example, type 2 diabetics have higher levels of basal glucagon compared to normal subjects (24), which contribute to increased hepatic glucose output (25). Data from a 3-year study following postmenopausal women suggested that increased glucagon secretion is the strongest predictor for developing glucose intolerance (26). Other studies indicate that type 2 diabetics fail to appropriately decrease glucagon levels following food consumption (27). Dysregulation of glucagon secretion postprandially likely contributes to hyperglycemia in these individuals, especially impacting patients with increasing degrees of insulin resistance. Additionally, it appears that the lack of glucagon suppression worsens glucose tolerance in type 2 diabetics (27).

Overall, it appears that an elevation of circulating glucagon is a pathophysiological factor in the etiology of diabetes. Thus, intervention to suppress glucagon activity may improve insulin action in the liver to restore normal hepatic glucose metabolism, thereby decreasing hyperglycemia in diabetic patients.

Gcgr knockout mice

Recently, the generation of *Gcgr* knockout mice has provided important clues to the potential physiological effects of antagonizing the GCGR. Although plasma glucose and insulin levels are reduced in *Gcgr* null mice, glucose homeostasis appears to be relatively normal (17, 18). *Gcgr* knockout mice have elevated plasma levels of glucagon and glucagon-like peptide (GLP-1), develop enlarged pancreata with increased glucagon and GLP-1 content, and display pancreatic α-cell hyperplasia (17, 18). Interestingly, α-cell hyperplasia is also present in *prohormone convertase 2* knockout mice. This phenotype likely results from the inability to convert proglucagon to glucagon, thus leading to a loss of circulating glucagon (28, 29). Exogenous glucagon replacement therapy via micro-osmotic pump reverses and reduces α-cell hyperplasia in these animals (30).

Overall, these gene-deletion studies provide information on the physiological effects that may result from completely abolishing glucagon signaling in normal mice, indicating that both glucagon and its receptor must be functional in order to maintain a feedback loop that restrains α-cell growth and subsequent glucagon secretion. However, studies in diabetic animals lacking the GCGR will directly evaluate the effect of a loss of glucagon signaling on hyperglycemia. Furthermore, chronic *in vivo* studies with efficacious competitive and noncompetitive GCGR antagonists will be necessary to guide fundamental strategies to optimize compounds targeting the GCGR.

Inhibitors of glucagon action

Several groups have pursued the development of glucagon antagonists as a potential therapy for the treatment of diabetes. The majority of these efforts have focused on identifying low-molecular-weight GCGR antagonists, both competitive (31, 32) and noncompetitive (33, 34). Others have evaluated potent peptide glucagon antagonists and antiglucagon monoclonal antibodies. Unfortunately, cross-species differences in the amino acid sequence of the GCGR have hampered efforts to discover molecules possessing both potent binding affinity and favorable bioavailability. The generation of transgenic mice expressing the human GCGR (35) may aid future discovery efforts.

Peptide GCGR antagonists and antiglucagon monoclonal antibodies

Initial proof-of-concept studies in models of type 1 diabetes tested peptide glucagon antagonists and high-affinity antiglucagon monoclonal antibodies. These efforts provided the first data supporting the idea that inhibiting glucagon action might be a viable strategy for decreasing blood glucose levels. The glucagon analogue [1-*N* α-trinitrophenylhistidine, 12-homoarginine]glucagon lowered blood glucose in rats made diabetic with streptozotocin (STZ) (36). Recently, optimization efforts with the peptide [des-His^{1}, des-Phe^{6},Glu^{9}]glucagon-NH_2 (37, 38) have been pursued. Similarly, glucagon immunoneutralization lowered blood glucose levels in STZ-treated rats (39) and alloxan-induced diabetic rabbits (40). Furthermore, *ob*/*ob* mice administered antiglucagon antibodies had significantly lower glucose and triglyceride levels (41). These data support the pursuit of glucagon antagonists as antihyperglycemic agents. The natural product skyrin, a fungal bisanthroquinone that inhibits

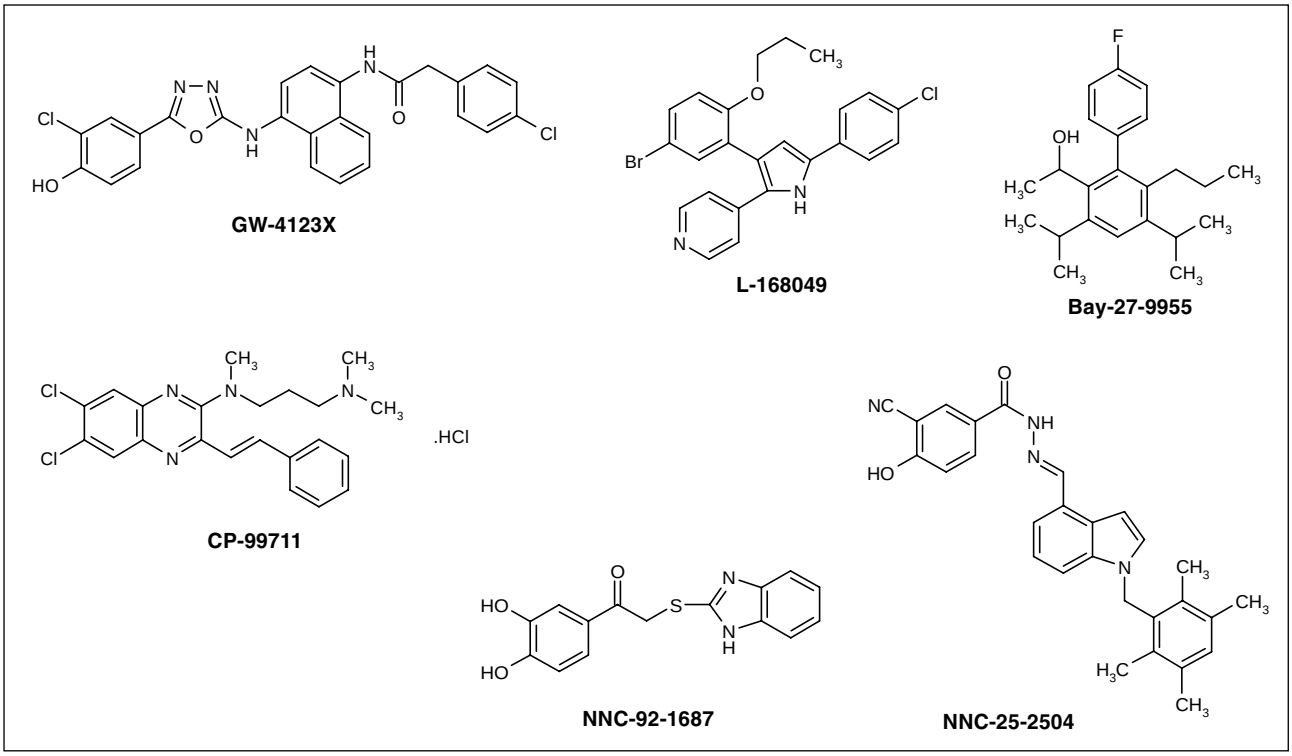

Fig. 1. Chemical structures of low-molecular-weight glucagon receptor inhibitors.

glucagon-stimulated cAMP production by uncoupling GCGR from adenylate cyclase, has also been evaluated (42).

Low-molecular-weight GCGR antagonists

High-throughput screening and/or targeted structure-activity relationship (SAR) medicinal chemistry strategies have been pursued vigorously with the hope of identifying orally active, nonpeptide, low-molecular-weight GCGR inhibitors (reviewed in 43). One of the first compounds to be identified, NNC-92-1687 (Fig. 1) (44), was generated via SAR efforts exploring derivatives of the quinoxaline CP-99711 (Fig. 1) (45). Compounds in a series of triarylpyrrole GCGR antagonists, typified by L-168049 (Fig. 1), act as noncompetitive antagonists of glucagon action by increasing the rate of dissociation of glucagon from its receptor (34).

Other discovery efforts have resulted in the identification of molecules of diverse chemical structures, including series of triarylimidazole molecules (46), 2-pyridyl-3,5-diarylpyrroles (33), 5-hydroxyalkyl-4-phenylpyridines (47) and alkylidene hydrazide derivatives (31). Although several of these molecules possess attractive *in vitro* characteristics highlighted by high receptor binding affinities, data have not been reported indicating whether any of these or related compounds possess the *in vivo* pharmacokinetic and pharmacodynamic properties necessary to lower glucose in diabetic rodents or human patients.

The small-molecule GCGR inhibitor to advance the farthest is Bay-27-9955 (Fig. 1), a competitive GCGR antagonist that is effective in blocking the glucose response to a glucagon challenge in healthy humans (48). Unfortunately, *in vivo* pharmacokinetic and chronic efficacy data in diabetic models or human patients treated with this compound have not been disclosed.

Most recently, new work within an alkylidene hydrazide series has led to the identification of a molecule possessing attractive metabolic and physicochemical properties (32). NNC-25-2504 (Fig. 1) is able to block glucagon-stimulated glucose production from isolated primary hepatocytes and is also active in blocking an acute glucagon challenge in Sprague-Dawley rats (32). Also, GW-4123X (Fig. 1) has been reported to decrease fasting plasma glucose and inhibit the rise in plasma glucose in response to a glucagon bolus when administered intravenously to rats (49). Chronic studies in diabetic rodents or humans are needed to further evaluate these molecules.

GCGR antisense oligonucleotides

Due to the lack of potent GCGR inhibitors needed for chronic studies, 2*'-O*-(2-methoxy)ethyl-modified phosphorothioate GCGR antisense oligonucleotides (ASOs) were recently used to determine if inhibiting glucagon action would decrease hepatic glucose output and

hyperglycemia in rodent models of type 2 diabetes (50, 51). Exploiting modified ASOs of this class to inhibit the GCGR is advantageous because these compounds effectively decrease expression of targeted genes in tissues such as the liver (52, 53). In addition, these molecules possess extended half-lives that minimize dosing regimens (53), and the inherent specificity and *in vivo* stability of antisense inhibitors enable characterization of the biological effects of inhibiting the GCGR in models of type 2 diabetes.

In diabetic rodents, whereas hyperglycemia continued to worsen over time in untreated controls, animals treated with GCGR ASOs showed a marked reduction in plasma glucose, an 85-95% reduction in liver GCGR mRNA, and a concomitant decrease in [^{125}I]-glucagon binding to liver membranes. Consistent with blocking glucagon action, GCGR ASO therapy decreased glycogenolytic and gluconeogenic enzyme gene expression and prevented glucagon-mediated hepatic glucose production (51).

Since glucagon is believed to be essential for maintaining fasting and postprandial glucose homeostasis, GCGR ASOs have the potential to induce hypoglycemia, yet periods of fasting of up to 24 h resulted in a moderate 10-30% reduction in plasma glucose, without manifesting adverse levels of hypoglycemia. It is likely that GCGR ASO-treated animals avoid hypoglycemia during fasting by maintaining normal catecholamine- and glucocorticoid-stimulated hepatic glucose production, in addition to possessing low numbers of residual GCGRs.

In addition to dramatic effects on glucose, GCGR ASO treatment resulted in marked hyperglucagonemia in both normal and diabetic rodents (50, 51). This level of hyperglucagonemia is similar to that observed in *Gcgr* knockout mice (17, 18). Elevated concentrations of plasma glucagon in GCGR ASO-treated rodents were accompanied by pancreatic α-cell hypertrophy and α-cell hyperplasia in some models (50, 51). It appears that both glucagon and its receptor must be functional in order to maintain a feedback loop that restrains α-cell growth and subsequent glucagon secretion. The exact nature of this feedback loop is unclear, but it is not believed to be driven by hypoglycemia (54). Because very high levels of serum glucagon are present in animals receiving GCGR ASOs, it is possible that these concentrations might induce hyperglycemia. It is therefore significant that at no time during active treatment or washout did animals with hyperglucagonemia exhibit hyperglycemia (50, 51).

Similar to *Gcgr* null mice, rodents treated with GCGR ASOs had 10-20-fold elevated plasma concentrations of active GLP-1 (GLP-1[7-36 amide] and GLP-1[7-37]) derived from islets as opposed to the intestine. The striking increase in active GLP-1 induced by GCGR ASO therapy would be expected to enhance glucose-stimulated insulin secretion *in vivo*. Indeed, intraperitoneal glucose challenge experiments with normoglycemic and diabetic rats undergoing treatment with saline or GCGR ASOs demonstrated reduced glucose excursions in normal and diabetic GCGR ASO-treated rats, along with a robust insulin response. In diabetic rats, GCGR ASO treatment normalized fasting glucose and the glucose excursion curve; furthermore, basal insulin levels were elevated by GCGR ASO treatment, and there was a more pronounced insulin response to a glucose challenge (51). Because of the central roles that the liver and pancreas play in the control of glucose homeostasis, directly targeting these tissues with therapeutic agents has the potential to modify or delay the progression of type 2 diabetes. Molecules such as the GCGR ASOs that induce antidiabetic effects at both of these metabolic sites may offer patients more effective treatment options.

Target-related development hurdles

Because glucagon's primary physiological role is to help maintain fasting and postprandial glucose homeostasis, target-related effects may impact compound advancement. For example, similar to other antihyperglycemic molecules, inhibiting glucagon action has the inherent potential to induce hypoglycemia. Similarly, in some instances, the impact of losing glucagon signaling on hepatic glycogenolysis may result in the occurrence of hepatomegaly due to excessive liver glycogen stores. However, excessive hepatic glycogen accumulation has not been observed in either *Gcgr* knockout mice or animals treated with GCGR ASOs. Furthermore, adverse hypoglycemia has not been observed in these animals even when challenged with periods of prolonged fasting. However, a potentially unacceptable finding observed in both *Gcgr* knockout mice and animals undergoing GCGR ASO therapy is the possible disruption of a feedback loop between the liver and pancreas that appears to result in a compensatory expansion of pancreatic α-cells and hyperglucagonemia (17, 18, 50, 51).

The likelihood of these effects occurring may be a function of the types of molecules that ultimately advance. For example, competitive and noncompetitive GCGR inhibitors may display different degrees of efficacy and will likely vary in their specific target-related antihyperglycemic effects. A possible advantage of developing competitive GCGR antagonists is that patients would likely maintain responsiveness to normal rises in plasma glucagon, thus decreasing the risk of hypoglycemia. These molecules may blunt the hyperglycemic effect of chronically elevated glucagon that has been shown to occur in diabetics (24), and may be especially effective in patients that fail to suppress glucagon following meals (27).

Conversely, if a compensatory increase in glucagon secretion occurs immediately upon any degree of GCGR antagonism, a competitive compound may not possess effective glucose-lowering efficacy, and thus, a noncompetitive inhibitor would be desirable. *Gcgr* null mice and animals undergoing GCGR ASO therapy provide potential insight into the likely physiological effects of noncompetitive GCGR compounds. It is encouraging that during a 24-h fast, wild-type animals undergoing GCGR ASO

treatment only experienced ~15% decrease in blood glucose levels. In addition, levels of liver glycogen in GCGR ASO-treated mice were not higher than those in untreated animals in the fed state (51). These data indicate that, as opposed to animals or humans with glycogen storage disease due to a deficiency of glucose-6-phosphatase (55, 56), counterregulatory signals such as glucocorticoids and catecholamines likely compensate for decreased glucagon signaling to maintain circulating glucose levels and hepatic glycogen stores within normal ranges.

Summary and future directions

The signal transduction pathways involved in the actions of glucagon and insulin have been under investigation for much of the past century. Significant advances in our fundamental understanding of the roles that these hormones play in glucose homeostasis have been facilitated by the development of a radioimmunoassay, molecules possessing inhibitory characteristics and technologies to decrease the expression of targeted genes. Because of its role in regulating glucose metabolism, the glucagon pathway is being targeted to develop novel antidiabetic molecules. Although proof-of-concept studies provide important target validation evidence that inhibiting glucagon action will improve glycemia, key questions remain that will likely impact the successful clinical development of such molecules. Issues such as strategic decisions to pursue the identification of competitive or non-competitive GCGR inhibitors are critical at early stages. In addition, because of cross-species amino acid differences in the GCGR, it is essential to implement creative screening designs and SAR schemes that enable preclinical advancement of molecules possessing potent activity at the human receptor. If successful, an effective anti-glucagon molecule will likely improve glucose control in diabetic patients, while avoiding the hypoglycemia, weight gain and fluid retention often observed with other therapies.

Acknowledgements

We wish to thank José Caro, Steve Jacobs and members of our laboratories for their advice, guidance and continuous support.

References

1. Murlin, J.R., Clough, H.D., Gibbs, C.B.F., Stokes, A.M. *Aqueous extractions of pancreas. I. Influence on the carbohydrate metabolism of depancreatized animals*. J Biol Chem 1923, 56: 253-96.

2. Kimball, C.P., Murlin, J.R. *Aqueous extractions of pancreas. III. Some precipitation reaction of insulin*. J Biol Chem 1924, 58: 337-46.

3. Foa, P.P., Weinstein, H.R., Smith, J.A. *Secretion of insulin and of a hyperglycemic substance studied by means of pancreatic-femoral cross-circulation expirements*. Am J Physiol 1949, 157: 197-204.

4. Foa, P.P., Santamaria, L., Weinstein, H.R., Berger, S., Smith, J.A. *Secretion of the hyperglycemic-glycogenolytic factor in normal dog*. Am J Physiol 1952, 171: 32-6.

5. Foa, P.P., Galansino, G., Pozza, G. *Glucagon, a second pancreatic hormone.* Rec Prog Horm Res 1957, 13: 473-510.

6. Staub, A., Sinn, L., Behrens, O.K. *Purification and crystallization of hyperglycemic-glycogenolytic factor.* Science 1953, 117: 628-9.

7. Bromer, W.W., Sinn, L.G., Staub, A., Bherens, O.K. *The amino acid sequence of glucagon.* J Am Chem Soc 1956, 78: 3858-9.

8. Unger, R.H., Eisentraut, M., Weinstein, H.R., McCall, M.S., Keller, S., Lanz, H.C., Madison, L. *Glucagon antibodies and their role use for immunoassay for glucagon*. Proc Soc Exp Biol Med 1959, 102: 621-3.

9. Unger, R.H., Eisentraut, M., McCall, M.S., Madison, L., Sims, K.R., Timm, L., Patman, L. *Glucagon antibodies and an immunoassay for glucagon*. J Clin Invest 1961, 40: 1280-9.

10. Unger, R.H., Eisentraut, M., McCall, M.S., Madison, L., Sims, K.R., Patman, L. *Measurements of endogenous glucagon in plasma and the influence of blood glucose concentration upon its secretion.* J Clin Invest 1962, 41: 682-9.

11. Yalow, R.S., Berson, S.A. *Assay of plasma inuslin in human subjects by immunological methods.* Nature 1959, 184: 1648-9.

12. Jiang, G., Zhang, B.B. *Glucagon and regulation of glucose metabolism*. Am J Physiol Endocrinol Metab 2003, 284: E671-8.

13. Jelinek, L.J., Lok, S., Rosenberg, G.B. et al. *Expression cloning and signaling properties of the rat glucagon receptor*. Science 1993, 259: 1614-6.

14. MacNeil, D.J., Occi, J.L., Hey, P.J., Strader, C.D., Graziano, M.P. *Cloning and expression of a human glucagon receptor.* Biochem Biophys Res Commun 1994, 198: 328-34.

15. Lok, S., Kuijper, J.L., Jelinek, L.J. et al. *The human glucagon receptor encoding gene: Structure, cDNA sequence and chromosomal localization*. Gene 1994, 140: 203-9.

16. Burcelin, R., Katz, E.B., Charron, M.J. *Molecular and cellular aspects of the glucagon receptor: Role in diabetes and metabolism*. Diabetes Metab 1996, 22: 373-96.

17. Parker, J.C., Andrews, K.M., Allen, M.R., Stock, J.L., McNeish, J.D. *Glycemic control in mice with targeted disruption of the glucagon receptor gene*. Biochem Biophys Res Commun 2002, 290: 839-43.

18. Gelling, R.W., Du, X.Q., Dichmann, D.S. et al. *Lower blood glucose, hyperglucagonemia, and pancreatic alpha cell hyperplasia in glucagon receptor knockout mice*. Proc Natl Acad Sci USA 2003, 100: 1438-43.

19. Unger, R.H., Orci, L. *Glucagon and the A cell: Physiology and pathophysiology (first two parts).* New Engl J Med 1981, 304: 1518-24.

20. Unger, R.H., Orci, L. *Glucagon and the A cell: Physiology and pathophysiology (second of two parts).* New Engl J Med 1981, 304: 1575-80.

21. Rodbell, M., Krans, H.M., Pohl, S.L., Birnbaumer, L. *The glucagon-sensitive adenyl cyclase system in plasma membranes*

of rat liver. IV. Effects of guanylnucleotides on binding of 125I-glucagon. J Biol Chem 1971, 246: 1872-6.

22. Stevenson, R.W., Steiner, K.E., Davis, M.A., Hendrick, G.K., Williams, P.E., Lacy, W.W., Brown, L., Donahue, P., Lacy, D.B., Cherrington, A.D. *Similar dose responsiveness of hepatic glycogenolysis and gluconeogenesis to glucagon in vivo*. Diabetes 1987, 36: 382-9.

23. Unger, R.H. *Letter: Glucagon in pathogenesis of diabetes*. Lancet 1975, 1: 1036-42.

24. Reaven, G.M., Chen, Y.D., Golay, A., Swislocki, A.L., Jaspan, J.B. *Documentation of hyperglucagonemia throughout the day in nonobese and obese patients with noninsulin-dependent diabetes mellitus.* J Clin Endocrinol Metab 1987, 64: 106-10.

25. Baron, A.D., Schaeffer, L., Shragg, P., Kolterman, O.G. *Role of hyperglucagonemia in maintenance of increased rates of hepatic glucose output in type II diabetics*. Diabetes 1987, 36: 274-83.

26. Larsson, H., Ahren, B. *Glucose intolerance is predicted by low insulin secretion and high glucagon secretion: Outcome of a prospective study in postmenopausal Caucasian women.* Diabetologia 2000, 43: 194-202.

27. Shah, P., Vella, A., Basu, A., Basu, R., Schwenk, W.F., Rizza, R.A. *Lack of suppression of glucagon contributes to postprandial hyperglycemia in subjects with type 2 diabetes mellitus.* J Clin Endocrinol Metab 2000, 85: 4053-9.

28. Furuta, M., Yano, H., Zhou, A., Rouille, Y., Holst, J.J., Carroll, R., Ravazzola, M., Orci, L., Furuta, H., Steiner, D.F. *Defective prohormone processing and altered pancreatic islet morphology in mice lacking active SPC2*. Proc Natl Acad Sci USA 1997, 94: 6646-51.

29. Furuta, M., Zhou, A., Webb, G., Carroll, R., Ravazzola, M., Orci, L., Steiner, D.F. *Severe defect in proglucagon processing in islet A-cells of prohormone convertase 2 null mice*. J Biol Chem 2001, 276: 27197-202.

30. Webb, G.C., Akbar, M.S., Zhao, C., Swift, H.H., Steiner, D.F. *Glucagon replacement via micro-osmotic pump corrects hypoglycemia and alpha-cell hyperplasia in prohormone convertase 2 knockout mice*. Diabetes 2002, 51: 398-405.

31. Ling, A., Plewe, M., Gonzalez, J. et al. *Human glucagon receptor antagonists based on alkylidene hydrazides.* Bioorg Med Chem Lett 2002, 12: 663-6.

32. Madsen, P., Ling, A., Plewe, M. et al. *Optimization of alkylidene hydrazide based human glucagon receptor antagonists. Discovery of the highly potent and orally available 3-cyano-4-hydroxybenzoic acid [1-(2,3,5,6-tetramethylbenzyl)-1H-indol-4-ylmethylene]hydrazide*. J Med Chem 2002, 45: 5755-75.

33. de Laszlo, S.E., Hacker, C., Li, B. et al. *Potent, orally absorbed glucagon receptor antagonists.* Bioorg Med Chem Lett 1999, 9: 641-6.

34. Cascieri, M.A., Koch, G.E., Ber, E. et al. *Characterization of a novel, non-peptidyl antagonist of the human glucagon receptor*. J Biol Chem 1999, 274: 8694-7.

35. Shiao, L.L., Cascieri, M.A., Trumbauer, M., Chen, H., Sullivan, K.A. *Generation of mice expressing the human glucagon receptor with a direct replacement vector.* Transgenic Res 1999, 8: 295-302.

36. Johnson, D.G., Goebel, C.U., Hruby, V.J., Bregman, M.D., Trivedi, D. *Hyperglycemia of diabetic rats decreased by a glucagon receptor antagonist*. Science 1982, 215: 1115-6.

37. Ahn, J.M., Medeiros, M., Trivedi, D., Hruby, V.J. *Development of potent truncated glucagon antagonists*. J Med Chem 2001, 44: 1372-9.

38. Ahn, J.M., Medeiros, M., Trivedi, D., Hruby, V.J. *Development of potent glucagon antagonists: Structure-activity relationship study of glycine at position 4.* J Pept Res 2001, 58: 151-8.

39. Brand, C.L., Rolin, B., Jorgensen, P.N., Svendsen, I., Kristensen, J.S., Holst, J.J. *Immunoneutralization of endogenous glucagon with monoclonal glucagon antibody normalizes hyperglycaemia in moderately streptozotocin-diabetic rats.* Diabetologia 1994, 37: 985-93.

40. Brand, C.L., Jorgensen, P.N., Svendsen, I., Holst, J.J. *Evidence for a major role for glucagon in regulation of plasma glucose in conscious, nondiabetic, and alloxan-induced diabetic rabbits.* Diabetes 1996, 45: 1076-83.

41. Brand, C.L., Hansen, B., Groneman, S., Boysen, M., Holst, J.J. *Sub-chronic glucagon neutralisation improves diabetes in ob/ob mice.* Diabetes 2000, 49: A81.

42. Parker, J.C., McPherson, R.K., Andrews, K.M. et al. *Effects of skyrin, a receptor-selective glucagon antagonist, in rat and human hepatocytes*. Diabetes 2000, 49: 2079-86.

43. McCormack, J.G., Westergaard, N., Kristiansen, M., Brand, C.L., Lau, J. *Pharmacological approaches to inhibit endogenous glucose production as a means of anti-diabetic therapy*. Curr Pharm Des 2001, 7: 1451-74.

44. Madsen, P., Knudsen, L.B., Wiberg, F.C., Carr, R.D. *Discovery and structure-activity relationship of the first non-peptide competitive human glucagon receptor antagonists*. J Med Chem 1998, 41: 5150-7.

45. Collins, J.L., Dambek, P.J., Goldstein, S.W., Faraci, W.S. *CP-99,711: A non-peptide glucagon receptor antagonist*. Bioorg Med Chem Lett 1992, 2: 915-8.

46. Chang, L.L., Sidler, K.L., Cascieri, M.A. et al. *Substituted imidazoles as glucagon receptor antagonists.* Bioorg Med Chem Lett 2001, 11: 2549-53.

47. Ladouceur, G.H., Cook, J.H., Doherty, E.M., Schoen, W.R., MacDougall, M.L., Livingston, J.N. *Discovery of 5-hydroxyalkyl-4-phenylpyridines as a new class of glucagon receptor antagonists*. Bioorg Med Chem Lett 2002, 12: 461-4.

48. Petersen, K.F., Sullivan, J.T. *Effects of a novel glucagon receptor antagonist (Bay 27-9955) on glucagon-stimulated glucose production in humans*. Diabetologia 2001, 44: 2018-24.

49. Handlon, A.L., Akwabi-Ameyaw, A., Brown, K. et al. *Glucagon receptor antagonists for the treatment of type 2 diabetes*. 226th ACS Natl Meet (Sept 7-11, New York City) 2003, Abst MEDI-164.

50. Liang, Y., Osborne, M.C., Monia, B.P., Bhanot, S., Gaarde, W.A., Reed, C., She, P., Jetton, T.L., Demarest, K.T. *Reduction in glucagon receptor expression by an antisense oligonucleotide ameliorates diabetic syndrome in db/db mice.* Diabetes 2004, 53: 410-7.

51. Sloop, K.W., Cao, J.X., Siesky, A.M. et al. *Hepatic and glucagon-like peptide-1-mediated reversal of diabetes by glucagon receptor antisense oligonucleotide inhibitors*. J Clin Invest 2004, 113: 1571-81.

52. Zhang, H., Cook, J., Nickel, J., Yu, R., Stecker, K., Myers, K., Dean, N.M. *Reduction of liver Fas expression by an antisense oligonucleotide protects mice from fulminant hepatitis*. Nat Biotechnol 2000, 18: 862-7.

53. Yu, R.Z., Zhang, H., Geary, R.S., Graham, M., Masarjian, L., Lemonidis, K., Crooke, R., Dean, N.M., Levin, A.A. *Pharmacokinetics and pharmacodynamics of an antisense phosphorothioate oligonucleotide targeting Fas mRNA in mice.* J Pharmacol Exp Ther 2001, 296: 388-95.

54. Vincent, M., Guz, Y., Rozenberg, M., Webb, G., Furuta, M., Steiner, D., Teitelman, G. *Abrogation of protein convertase 2 activity results in delayed islet cell differentiation and maturation, increased alpha-cell proliferation, and islet neogenesis*. Endocrinology 2003, 144: 4061-9.

55. Lei, K.J., Shelly, L.L., Pan, C.J., Sidbury, J.B., Chou, J.Y. *Mutations in the glucose-6-phosphatase gene that cause glycogen storage disease type 1a*. Science 1993, 262: 580-3.

56. Yang Chou, J., Mansfield, B.C. *Molecular genetics of type 1 glycogen storage diseases.* Trends Endocrinol Metab 1999, 10: 104-13.