Glucagon and regulation of glucose metabolism

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Jiang, Guoqiang, and Bei B. Zhang. Glucagon and regulation of glucose metabolism. Am J Physiol Endocrinol Metab 284: E671–E678, 2003; 10.1152/ajpendo.00492.2002.—As a counterregulatory hormone for insulin, glucagon plays a critical role in maintaining glucose homeostasis in vivo in both animals and humans. To increase blood glucose, glucagon promotes hepatic glucose output by increasing glycogenolysis and gluconeogenesis and by decreasing glycogenesis and glycolysis in a concerted fashion via multiple mechanisms. Compared with healthy subjects, diabetic patients and animals have abnormal secretion of not only insulin but also glucagon. Hyperglucagonemia and altered insulin-to-glucagon ratios play important roles in initiating and maintaining pathological hyperglycemic states. Not surprisingly, glucagon and glucagon receptor have been pursued extensively in recent years as potential targets for the therapeutic treatment of diabetes.

glucagon; diabetes; metabolism; glucose homeostasis

GLUCAGON IS A 29-AMINO ACID peptide hormone processed from proglucagon. Proglucagon is expressed in various tissues (e.g., brain, pancreas, and intestine) and is proteolytically processed into multiple peptide hormones in a tissue-specific fashion. For example, proglucagon is processed into functional glucagon-like peptides-1 and -2 by subtilisin-like proprotein convertases PC1–3 in intestinal L cells (75), and it is processed into functional glucagon by PC2 in the pancreatic α-cells (32, 74, 76). Glucagon acts via a seven-transmembrane G protein-coupled receptor consisting of 485 amino acids (45). To date, glucagon-binding sites have been identified in multiple tissues, including liver, brain, pancreas, kidney, intestine, and adipose tissues (12, 20). A whole body of literature exists on the structure and the expression of glucagon and glucagon receptor genes, but this topic will not be covered in the present review.

Glucagon is released into the bloodstream when circulating glucose is low. The main physiological role of glucagon is to stimulate hepatic glucose output, thereby leading to increases in glycemia. This provides the major counterregulatory mechanism for insulin in maintaining glucose homeostasis in vivo. In the present review, we will discuss evidence supporting the critical role of glucagon in glycemic control, the molecular mechanisms by which glucagon regulates glucose metabolism, the abnormality of glucagon signaling in diabetic states, and the potential of antagonizing glucagon receptor for the treatment of type 2 diabetes.

GLUCAGON IS A KEY REGULATOR OF GLUCOSE HOMEOSTASIS IN VIVO

Glucagon plays a key role in glucose metabolism in vivo. Administration of exogenous glucagon increases glucose levels in fasted or fed animals (63, 96), and similar observations were made in humans (29, 42, 57). Consistent with its role as a counterregulatory hormone of insulin, glucagon raises plasma glucose levels in response to insulin-induced hypoglycemia (29). In fact, glucagon administration is used clinically to treat hypoglycemia in humans (14, 29, 35). Numerous ex vivo or in vitro studies have directly demonstrated that glucagon stimulates glucose output from intact perfused rat livers (7, 28, 43) resulting from increases in both glycogenolysis and gluconeogenesis. Similarly, glucagon also stimulates glucose output from primary hepatocytes in culture (60, 92, 93).

Several lines of evidence indicate that glucagon is a sensitive and timely regulator of glucose homeostasis in vivo. Small doses of glucagon are sufficient to induce significant glucose elevations (35, 57, 63). The effect of glucagon can occur within minutes and dissipate rapidly (27). Glucagon is secreted from islets in a pulsatile fashion (65), and such pulsatile deliveries of glucagon are more effective in inducing hepatic glucose output in vitro, ex vivo, and in vivo (49, 66, 92). Conversely, there is ample evidence demonstrating that inhibition of glucagon signaling in vivo leads to a reduction in plasma glucose, or hypoglycemia. It was shown that administration of polyclonal glucagon-neutralizing antibodies abolished the hyperglycemic response to exogenous glucagon in animals (83). A similar observation was made using a high-affinity monoclonal anti-glucagon antibody (11). Additionally, the monoclonal antibody reduced ambient blood glu-
cose by neutralizing endogenous glucagon in normal or diabetic animals (9–11). In these experiments, the glucagon antibodies reduced free glucagon in circulation to undetectable levels (9–11).

As discussed previously, glucagon is processed from proglucagon in pancreatic α-cells by PC2 (32, 74, 76). In PC2-null (PC2−/−) mice, circulating glucagon was undetectable due to a severe defect in the processing of proglucagon (30). Interestingly, PC2−/− mice had reduced fasting blood glucose as well as improved glucose tolerance. Moreover, PC2−/− mice had significant α-cell hypertrophy, which was consistent with the compensatory response for the lack of functional glucagon. Whereas the correlation between the hypoglycemia phenotype and the lack of circulating glucagon in the PC2−/− mice is consistent with a major role of glucagon in glycemic control, the proposal is complicated by the finding that the mice were also defective in processing proinsulin to insulin (30, 32). It was recently shown, however, that glucagon replacement via micro-osmotic pump corrected hypoglycemia and α-cell hypertrophy in the PC2−/− mice, proving an unequivocal role of glucagon in glucose homeostasis in vivo (91).

A small acidic protein, 7B2, is exclusively localized to neuroendocrine tissues, and it binds to and activates PC2 (62). It was shown that 7B2-null mice displayed hypoglycagonegic as well as hypoglycemia (94). Finally, mice lacking the glucagon receptor gene (GCGR−/−) exhibited a phenotype of decreased glycemia under both fed and fasting states compared with control mice. No overt hypoglycemia was observed in GCGR−/− mice under ambient conditions, and these mice also had improved glucose tolerance (67). Together, these results support an important role of glucagon in glycemic control in vivo.

**MOLECULAR MECHANISM FOR GLUCAGON-MEDIATED GLUCOSE REGULATION**

Glucagon signals through its receptor on the cell surface (Fig. 1). The binding of glucagon to the extracellular loops of the glucagon receptor results in conformational changes of the latter, leading to subsequent activation of the coupled G proteins. At least two classes of G proteins are known to be associated with and involved in the signal transduction of the glucagon receptor, namely Gsα and Gq. The activation of Gsα leads to activation of adenylate cyclase, increase in intracellular cAMP levels, and subsequent activation of protein kinase A (PKA). The activation of Gq leads to the activation of phospholipase C, production of inositol 1,4,5-triphosphate, and subsequent release of intracellular calcium (12, 21). We will focus the discussion on how glucagon regulates hepatic glucose output by activating PKA, leading to changes in glycogenolysis, glycogenesis, gluconeogenesis, and glycolysis.

Potentiation of glycogenolysis. Overall, glucagon signaling promotes glycogenolysis and, at the same time, inhibits glycogen synthesis in the liver (Fig. 2). Upon glucagon stimulation, activated PKA phosphorylates glycogen phosphorylase kinase. The activated glycogen phosphorylase kinase subsequently phosphorylates serine-14 residue on glycogen phosphorylase, leading to its activation. Finally, the activated glycogen phosphorylase phosphorylates glycogen, resulting in increased glycogen breakdown.

![Glucagon-signaling pathway](image-url)
Glycogenolysis and the production of glucose 6-phosphate (G-6-P). G-6-P is then converted into glucose by glucose-6-phosphatase (G-6-Pase), increasing the glucose pool for hepatic output (47, 50). In addition to activating the PKA-glycogen phosphorylase kinase-glycogen phosphorylase cascade, glucagon has been shown to increase G-6-Pase activity (3, 4, 82). Recent studies suggest that the upregulation of G-6-Pase by glucagon is at least partially due to increased transcription of the G-6-Pase gene in a PKA-dependent fashion involving peroxisome proliferator-activated receptor-γ coactivator-1 (PGC-1), a nuclear transcriptional factor coactivator (95).

Inhibition of glycogenesis. In addition to promoting glycogenolysis, glucagon inhibits glycogen synthesis by regulating glycogen synthase in the liver (Fig. 2). Glycogen synthase plays a key role in glycogen synthesis by catalyzing the transfer of glucosyl residue from UDP-glucose to a nonreducing end of the branched glycogen molecule. Like glycogen phosphorylase kinase and phosphorylase, glycogen synthase is regulated by phosphorylation but in an opposite fashion. Glucagon induces glycogen synthase phosphorylation and inhibits glycogen synthase activity in the liver (2, 22, 72). Glycogen synthase is phosphorylated at multiple sites by several serine/threonine kinases, including PKA. It has been suggested that coordinated phosphorylation of glycogen synthase by multiple kinases could lead to graded inactivation of glycogen synthase. Inactivation of glycogen synthase reduces glycogen synthesis and, accordingly, increases the pool of glucose for hepatic output into blood (73).

Potentiation of gluconeogenesis. In addition to affecting glycogen metabolism, glucagon regulates blood glucose by affecting glucose metabolism, specifically by increasing gluconeogenesis and decreasing glycolysis (Fig. 3). Phosphoenolpyruvate carboxykinase (PEPCK) catalyzes the conversion of oxaloacetate into phosphoenolpyruvate, an early and rate-limiting step in the pathway of hepatic gluconeogenesis (Fig. 3). Glucagon treatment has been shown to increase the PEPCK mRNA level in the liver or hepatocytes (6, 19, 44). Recent studies suggest that PKA activation by cAMP promotes the transcription of the PEPCK gene (95).

Fig. 2. Regulation of glycogen metabolism by glucagon in the liver. Diagram outlines the effects of glucagon on glycogenolysis and glycogenesis in the liver. Overall directions of glycogenolysis and glycogenesis pathways are indicated with arrows located at the top and bottom portions of the diagram. GCG, glucagon; G-1-P, glucose 1-phosphate; G-6-P, glucose 6-phosphate; GP, glycogen phosphorylase; GPK, glycogen phosphorylase kinase; GS, glycogen synthase; GK, glucose kinase; HK, hexose kinase; +, promoted by glucagon; −, inhibited by glucagon.

Fig. 3. Regulation of glucose metabolism by glucagon in the liver. Diagram outlines the mechanisms by which glucagon regulates glycolysis and gluconeogenesis in the liver. Overall directions of the glycolysis and gluconeogenesis pathways are indicated with arrows located at the left and right of the diagram. F-6-P, fructose 6-phosphate; F(1,6)P2, fructose-1,6-bisphosphate; F(2,6)P2, fructose-2,6-bisphosphate; PEP, phosphoenolpyruvate; PFK1, phosphofructokinase-1; FBPase-1, fructose-1,6-bisphosphatase; FBPase-2, fructose-2,6-bisphosphatase; PK, pyruvate kinase. Reactions occurring inside or outside of mitochondria are indicated. Details on how glucagon affects the processes are described in the text. Arrow with dotted line indicates that there are intermediate reactions omitted in the figure for the sake of simplicity.
leads to phosphorylation of cAMP response element-binding (CREB) protein. The phosphorylated CREB protein in turn binds to a CAMP-responsive element in the promoter region of the transcriptional coactivator PGC-1 gene and upregulates PGC-1 transcription. PGC-1 and the nuclear transcription factor hepatocyte nuclear factor-4 (HNF-4) act together to increase the transcription of the PEPCK gene (34, 36, 90, 95). Given that glucagon activates PKA, such a pathway is likely to be responsible for glucagon-mediated upregulation of PEPCK transcription and activity, leading to increased gluconeogenesis in the liver.

Fructose-1,6-bisphosphatase (FBPase-1) catalyzes the hydrolysis of the C-1 phosphate in fructose-1,6-bisphosphate [F(1,6)P2], converting F(1,6)P2 into fructose 6-phosphate (F-6-P), an important step in gluconeogenesis (Fig. 3). FBPase-1 is allosterically inhibited by fructose-2,6-bisphosphate [F(2,6)P2]. The level of F(2,6)P2 is regulated by the bifunctional enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase (PFK-2/FBPase-2) encoded in a single polypeptide. Although F(2,6)P2 is produced by phosphorylation of F-6-P by PFK-2, it is converted back to F-6-P by FBPase-2. Upon glucagon stimulation, activated PKA phosphorylates serine-36 in the FPK2/FBPase-2 polypeptide. This phosphorylation leads to simultaneous inhibition of the PFK-2 and activation of FBPase-2. This in turn reduces intracellular levels of F(2,6)P2, thereby relieving the inhibition of FBPase-1 and promoting gluconeogenesis (51, 64, 71). G-6-Pase promotes gluconeogenesis by converting G-6-P to glucose, the last step of the pathway (Fig. 3). As discussed previously on the role of G-6-Pase on glycogenolysis (Fig. 2), glucagon has been shown to increase G-6-Pase expression and activity (3, 4, 82, 95). Such upregulation of G-6-Pase should promote gluconeogenesis as well as glycogenolysis.

**Inhibition of glycolysis.** In addition to increasing gluconeogenesis, glucagon inhibits glycolysis. Phosphofructokinase-1 (PFK-1) phosphorylates the C-1 position of F-6-P, converting F-6-P into F(1,6)P2, an early and rate-limiting step in glycolysis. Similar to FBPase-1, PFK-1 is also allosterically regulated by F(2,6)P2 but in a reciprocal fashion. Although FBPase-2 is allosterically inhibited by F(2,6)P2, PFK-1 is allosterically activated by F(2,6)P2 (64, 70). By reducing F(2,6)P2 levels as described above in Inhibition of glycolysis, glucagon inhibits FPK1 activity and therefore inhibits glycolysis (16, 89).

Pyruvate kinase catalyzes the transfer of the phosphate group from phosphoenolpyruvate to ADP, producing pyruvate and ATP, the last step in the glycolysis pathway. Glucagon inhibits pyruvate kinase by several mechanisms. Glucagon activates PKA, which in turn phosphorylates pyruvate kinase. Phosphorylation inhibits pyruvate kinase, since the phosphorylated kinase is more readily inhibited allosterically by alanine and ATP and is, at the same time, less readily activated allosterically by F(1,6)P2 (70). Glucagon also inhibits transcription of the pyruvate kinase gene and increases the degradation of pyruvate kinase mRNA (70). The inhibition of pyruvate kinase by glucagon results in decreased glycolysis and increased gluconeogenesis.

**GLUCAGON AND GLUCAGON RECEPTOR IN DIABETES**

There is ample evidence suggesting that glucagon plays an important role in initiating and maintaining hyperglycemic conditions in diabetic animals and humans. Insulin and glucagon are the key regulatory hormones for glucose homeostasis. The absolute levels and, even more so, the ratios of the two hormones are tightly regulated in vivo, depending on nutritional status. It has been reported that the absolute levels of glucagon or the ratios of glucagon to insulin are often elevated in various forms of diabetes in both animal and human subjects (12, 85, 86). Diabetes is also one of the diseases associated with glucagonoma, a glucagon-secreting tumor derived from pancreatic islet α-cells (18). Chronic hyperglucagonemia is correlated with and is at least partially responsible for increased hepatic glucose output and hyperglycemia in type 2 diabetes (23).

It is controversial whether the number of glucagon receptors is altered in diabetic states. Most studies, however, appear to suggest that the number of glucagon receptors is reduced in diabetic subjects. Interestingly, even in the presence of fewer glucagon receptors, the ability of glucagon to stimulate cAMP production may remain unchanged or even be elevated (12). This may be at least partially explained by the observation that the activation of adenylate cyclase by glucagon involves only 20% of glucagon receptors (8).

In normal animal and human subjects, the levels of insulin increase immediately after a meal, whereas the levels of glucagon decrease. In type 2 diabetic subjects, however, the postprandial secretion of insulin is delayed and depressed, whereas that of glucagon is not suppressed or is even elevated (5, 13, 54, 61). Such abnormality in insulin and glucagon secretion is associated with and predictive of glucose intolerance in type 2 diabetic human subjects (1, 53). The cause-and-effect relationship between hyperglucagonemia and hyperglycemia is strongly implied in studies showing that suppression of postprandial hyperglucagonemia corrects postprandial hyperglycemia in type 2 diabetic subjects (78). A lack of suppression of hyperglucagonemia has also been shown to contribute to postprandial glucose intolerance in type 1 diabetes (26). Although hyperglucagonemia results in glucose intolerance in diabetic subjects with impaired insulin secretion or in normal subjects whose insulin secretion is experimentally blocked, it does not produce the same effects when insulin secretion is intact (i.e., in normal healthy subjects) (77, 79, 84). Taken as a whole, the discussion above indicates that hyperglucagonemia plays an important role in initiating and maintaining hyperglycemia when combined with delayed or deficient insulin secretion, as in the cases of type 1 and type 2 diabetes.

In addition to the epigenetic effects of hyperglucagonemia on hyperglycemia, genetic polymorphism of the glucagon receptor has been reported to be associated with type 2 diabetes. A single heterozygous mis-
sense mutation in exon 2 of the glucagon receptor gene that changes a glycine to a serine (Gly408Ser) has been found to be associated with type 2 diabetes in some French populations. The mutant receptor was shown to have a reduced affinity to bind to glucagon and to produce cAMP in response to glucagon stimulation (33). The significance of such a mutation in diabetes is likely to be limited, since it is not associated with diabetes in most other studies in various populations (41, 81).

GLUCAGON AND GLUCAGON RECEPTOR AS THERAPEUTIC TARGETS FOR TYPE 2 DIABETES

Glucagon and glucagon receptor represent potential targets for the treatment of diabetes (97). Over the last two decades, encouraging progress has been made in attempting to normalize hyperglycemia by antagonizing glucagon signaling through use of glucagon-neutralizing antibodies, peptide glucagon analogs, and nonpeptide, small-molecule glucagon receptor antagonists.

Glucagon-neutralizing antibodies. As discussed previously, high-affinity glucagon-neutralizing antibodies can effectively reduce free glucagon and, at the same time, glycemia in animal models (9–11). It is therefore possible that high-affinity and high-titer humanized glucagon-neutralizing antibodies may prove useful as therapy for diabetes.

Antagonistic glucagon peptide analogs. Extensive efforts have been made to generate linear and cyclic peptide glucagon analogs. Compared with the wild-type glucagon, some of these peptide analogs have been shown to have distinct properties in terms of their ability to bind to the glucagon receptor and affect glucagon-stimulated cAMP production. They act as pure agonists, partial agonists/antagonists, or pure antagonists of the glucagon receptor (25, 39, 40). It was first reported that [1-natrinitrophenylhistidine,12-homoarginine]-glucagon, a potent antagonistic glucagon analog, significantly decreased hyperglycemia in streptozotocin-induced diabetic rats in vivo (46). [des-His1,Glu9]-glucagon amide, another potent antagonistic glucagon analog, was also found to completely block exogenous glucagon-induced hyperglycemia in normal rabbits and to block hyperglycemia due to endogenous glucagon in streptozotocin-induced diabetic rats (87). Finally, similar glucose-lowering effects have been reported for another antagonistic glucagon analog, [des-His1,des-Phe6,Glu9]-glucagon-NH2 (88). It is therefore possible that such antagonistic peptide glucagon analogs may also have therapeutic potentials.

Nonpeptide, small-molecule glucagon receptor antagonists. Discovery and development of nonpeptidyl glucagon receptor antagonists of diverse structures have been reported over recent years (15, 17, 24, 48, 52, 55, 56, 58, 59, 68, 69). Although some of the earlier antagonists are less potent with high-micromolar IC50 in inhibiting either the binding of glucagon to the glucagon receptor or the potential of glucagon to stimulate cAMP production (59, 68), many of the recent antagonists are much more potent with nanomolar IC50. Some of these potent antagonists have also been shown to effectively lower fasting blood glucose (56) as well as to block exogenous glucagon-stimulated elevation of blood glucose in animal models in vivo (55). Most recently, Bay 27–9955, an orally absorbed and potent glucagon receptor antagonist, has been shown to block glucagon-induced elevation of blood glucose in humans (69).

The published antagonists appear to act via distinct mechanisms. Skyrin, one of the earlier antagonists, appeared to functionally inhibit glucagon-stimulated cAMP production and glycogenolysis without affecting glucagon binding (68). Other antagonists inhibit both the binding and the function of glucagon. Some of the antagonists may act in a noncompetitive fashion (15), whereas others have been shown to be competitive inhibitors (55, 56). Glucagon receptor antagonists have been shown to exhibit species specificity. For example, some antagonists were more potent toward human than murine glucagon receptor (15) and vice versa (55).

In this regard, mice expressing human glucagon receptors generated with a direct replacement vector should prove highly valuable in evaluating the in vivo efficacy of glucagon receptor antagonists for potential uses as therapy in humans (80).

Targeting glucagon and/or glucagon receptor for the treatment of diabetes is appealing for several reasons. As discussed previously, it is well established that glucagon is one of the key hormones regulating glucose homeostasis, and its deregulation contributes to hyperglycemia in various types of diabetes. Additionally, the published literature has provided strong pharmacological validation that suppression of glucagon signaling alleviates hyperglycemia in both animals and humans.

There are several potential concerns regarding such an approach. Given that glucagon plays a key role in inducing blood glucose elevation, it is possible that its inhibition may result in hypoglycemia. In this respect, it is encouraging that GCGR−/− mice have somewhat lower glycemia but are not hypoglycemic (67). GCGR−/− mice have pancreatic α-cell hypertrophy and are extremely hyperglucagonemic (67). PC2−/− mice also have pancreatic α-cell hypertrophy (30, 32). These observations clearly indicate a compensatory mechanism. It therefore remains to be seen whether antagonists will trigger similar compensation, leading to hyperglucagonemia and eventually the loss of efficacy of glucagon receptor antagonists in long-term treatment. Finally, it also remains to be seen whether glucagon receptor antagonists will result in unfavorable accumulation of lipids in the liver. It is known that glucagon reduces lipogenesis by multiple mechanisms. For instance, by increasing gluconeogenesis and decreasing glycolysis, glucagon inhibits lipogenesis by decreasing 3-carbon substrates available for fatty acid synthesis (85). In fact, glucagon has been proposed as a therapy for fatty livers (37, 38). Once again, however, mice deficient in glucagon receptor have normal lipids (67).
SUMMARY AND PERSPECTIVE

During the last two decades, significant progress has been made in understanding the biological function of glucagon, the regulation of expression and secretion of glucagon, the interaction of glucagon with its G protein-coupled receptor, and the role of glucagon in modulating key transcription factors and metabolic enzymes. Studies using transgenic and knockout mouse models and human subjects further revealed the pivotal role of glucagon in the control of glucose homeostasis in health and under disease states. Antagonizing glucagon action by neutralizing the hormone or blocking the action of the glucagon receptor may represent a new avenue for intervention of diabetes and related metabolic disorders.

We apologize to the many investigators whose work was not cited owing to space limitation. We thank colleagues at Merck Research Laboratories for insightful discussions.

REFERENCES


