Autoregulation of Glucose Production

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Glucose itself regulates endogenous glucose production independently of changes in glucoregulatory hormones. In addition, acute stimulation of gluconeogenesis does not increase net glucose production. This indicates autoregulation of glucose production. Glucokinase plays a role in this process by allowing hepatic glucose sensing.

The liver plays a major role in glucose homeostasis by releasing into the systemic circulation the exact amount of glucose required to match extrahepatic glucose utilization and maintain plasma glucose concentrations within tight normal limits. It performs this task by mobilizing glucose stored within hepatocytes as glycogen and/or by converting lactate, glycerol, and amino acids into glucose (gluconeogenesis). The net glucose release is the result of these two simultaneously ongoing processes and has to be very accurately regulated. If glucose production by the liver exceeds glucose requirements, plasma glucose inevitably rises. Alternatively, if glucose production does not meet glucose requirements, plasma glucose concentrations falls, and this eventually leads to brain energy shortage due to hypoglycemia.

After an overnight fast, the liver is the sole organ actually releasing glucose into the systemic circulation. The kidney also produces small amounts of glucose. Only during prolonged fasting (several days) does the kidney contribute a net amount of glucose into the systemic circulation. Glycogenolysis and gluconeogenesis each contribute ~50% of hepatic glucose output. However, the exact contribution of each of these two processes in glucose production remains controversial (3).

After ingestion of a mixed meal, the systemic appearance of exogenous glucose issued from the digestion and absorption of dietary carbohydrate exceeds the glucose requirements of the whole organism, and a transient rise in glycemia ensues. During this period, hepatic glucose production shuts down and part of the exogenous glucose reaching the portal vein is actually taken up by liver cells to replenish hepatic glycogen stores. Postprandial stimulation of insulin secretion and inhibition of glucagon secretion are involved in this process. Hyperinsulinemia inhibits both glycogenolysis and gluconeogenesis and stimulates glycogen synthesis. At the same time, glucagon secretion decreases. Since glucagon stimulates glycogenolysis and gluconeogenesis in postabsorptive conditions, this suppression of plasma glucagon also results in an
inhibition of glucose production. In addition, the rise in portal glucose concentration stimulates hepatic glucose uptake and inhibits glycogenolysis. The decrease in plasma free fatty acid concentration secondary to the inhibition by insulin of adipose tissue lipolysis also contributes to the inhibition of endogenous glucose output (1). This postprandial switch of the liver from a net glucose producer to a glucose-utilizing organ contributes to limiting hyperglycemia. Alternatively, when glucose requirements are increased as a result of stimulated extrahepatic glucose utilization, such as during exercise that requires enhanced uptake of glucose by skeletal muscle, the liver acutely increases its glucose production to prevent the occurrence of hypoglycemia (14). This is attained by simultaneous inhibition of insulin secretion, stimulation of glucagon and adrenal catecholamine hormones, and activation of sympathetic hepatic nerves.

As stated above, insulin, glucagon, and catecholamines play major roles in glucose homeostasis. Other hormones, in particular adrenal steroids and human growth hormone, are also involved. During insulin-induced hypoglycemia, the liver acutely increases its glucose production rate to match insulin-stimulated glucose utilization and raises plasma glucose to normal levels. This hepatic response to hypoglycemia is secondary mainly to concomitant increases in the plasma concentrations of glucagon, adrenaline, glucocorticoid, and growth hormone. The signal to increase the rate of secretion of these hormones is cerebral glucopenia, through both activation of efferent nerves to the endocrine pancreas and adrenal medulla and release of corticotropin-releasing hormone and growth hormone-releasing hormone secondary to cerebral glucopenia. Only in the case of glucagon is there a direct effect of hypoglycemia on pancreatic α-cells. However, this sole peripheral effect appears responsible for only a minor portion of hypoglycemia-induced hyperglucagonemia. This clearly illustrates that the autonomic nervous system actively participates in the control of glucose homeostasis as well.

Nonendocrine control of glucose production

There is also evidence that the liver itself actively participates in the control of its own glucose metabolism independently of endocrine and neural factors. This concept of hepatic autoregulation of glucose production is supported by several sets of observations, which will be briefly reviewed here.

Several experiments indicate that liver cells respond to acute changes in plasma glucose concentration by altering their rate of glucose production. An increase in plasma glucose concentration elicited by infusion of exogenous glucose decreased endogenous glucose production in dogs. This was observed even when hyperinsulinemia and hypoglucagonemia were prevented by infusion of somatostatin to inhibit endogenous secretion of these hormones, together with exogenous glucagon and insulin infusions to maintain their basal concentrations. Alternatively, hypoglycemia stimulated glucose production in humans even when hyperglucagonemia was prevented by somatostatin infusion, and the effects of adrenal catecholamines were inhibited by β-adrenergic antagonists. This effect was observed only during severe hypoglycemia (less than 2.0 mmol/l) but was absent at higher glucose concentrations (reviewed in Ref. 2).

Hepatic glucose output is the sum of two metabolic processes that can release glucose into the systemic circulation: glycolysis and gluconeogenesis. However, experiments performed in both animals and humans indicate that a rise in one of these processes will not lead to an equivalent rise in net glucose output. It was observed nearly 30 years ago that hepatic gluconeogenesis can be stimulated acutely by infusion of exogenous glycerol but that such stimulation of gluconeogenesis did not lead to an increase in glucose production or in plasma glucose concentration (15). More recently, it was similarly observed that gluconeogenesis could be stimulated in healthy human volunteers by infusion of lactate or fructose. In these experiments, stimulation of gluconeogenesis failed to increase glucose production or glyceremia even when changes in plasma insulin and glucagon concentrations were prevented by infusion of somatostatin, together with replacement of basal insulin and glucagon levels (7, 11) (Fig. 1). Absence of increase in glucose production during lactate infusion was also observed during β-adrenergic blockades, indicating that this process was not due to changes in β-adrenergic sympathetic tone (5). Conversely, acute inhibition of gluconeogenesis by ethanol in diabetic patients did not lower glucose production or glyceremia (8). Together, these experiments indicate that net glucose output has its own regulation and is not merely the result of single changes in glycolysis and gluconeogenesis. The experimental evidence points to an autoregulatory process that adapts net glucose output to the prevailing glucose needs of acute experimental changes in gluconeogenesis. The mechanisms by which this autoregulation is attained during acute stimulation of gluconeogenesis by gluconeogenic precursors remain speculative (Fig. 2). An
inhibition of net glycogenolysis is likely. Since there is evidence that hepatic glycogen is simultaneously synthesized and broken down, such an inhibition of net glycogenolysis may be explained by either inhibition of glycogenolysis, stimulation of glycolysis, or both. Alternatively, inhibition of net gluconeogenesis from endogenous substrates could be involved. Here, again, there is simultaneous gluconeogenesis and glycolysis (cycling between phosphoenol pyruvate and pyruvate and between fructose-6-phosphate and fructose-1,6-biphosphate), and either inhibition of regulatory gluconeogenic steps or stimulation of glycolysis may actually be involved.

Autoregulation of hepatic glucose production requires an accurate and sensitive hepatic glucose sensing sensitive to extracellular glucose concentration to adapt liver glucose metabolism to prevailing conditions. The presence in the liver cells of the glucose transporter isoform GLUT2 and glucose phosphorylating enzyme hexokinase IV, or glucokinase, is likely to serve this purpose. GLUT2 (10) is a glucose transporter protein constitutively present at the cell surface and characterized by a high Michaelis-Menten constant ($K_m$) for glucose and high transport capacity. This property makes it a possible glucose-sensing element in liver cells. It is, however, also the main glucose transporter protein in hepatocytes and hence is directly involved in hepatic glucose release. The putative role of GLUT2 remains sparsely documented. It was recently reported that mice lacking hepatic GLUT2 protein had modest insignificant elevations of fasting plasma glucose. Surprisingly, basal glucose production and its stimulation by glucagon were normal, indicating the existence of alternative glucose release (4).

Glucokinase is also characterized by a high $K_m$ for glucose and by the absence of feedback inhibition by its product glucose-6-phosphate (6). The presence of these two proteins ensures that glucose transport and phosphorylation to glucose-6-phosphate is proportional to hepatic blood glucose concentrations over the full physiological range. Glucokinase gene is expressed in pancreatic $\beta$-cells, in which it plays a major role in glucose-insulin secretion coupling. In pancreatic $\beta$-cells, glucokinase gene is regulated by insulin itself. In contrast, expression of the glucokinase gene in liver cells is mainly controlled by insulin, and hence liver glucokinase activity is likely to be decreased in insulin-deficient individuals. Due to these unique properties of GLUT2 and glucokinase, it can be expected that these proteins are involved in signaling the amount of glucose to be released by the liver to meet the organism’s requirements.

MODY2: a rare form of inherited diabetes characterized by an elevated glucose production with normal autoregulation

Maturity onset diabetes of the young (MODY) is a rare form of diabetes mellitus characterized by onset at or before 25 years of age and an autosomal dominant mode of transmission, indicating a monogenic defect. Several subgroups of MODY secondary to mutations of distinct genes have been identified (12). MODY2 corresponds to mutations of the glucokinase gene located on chromosome 7q. Several mutations have been identified that lead to a decrease in the maximal velocity of the enzyme or an increase in its $K_m$ for glucose. Homozygous mutations are lethal, and heterozygous mutations lead to a mild form of diabetes or impaired glucose tolerance. At the level of pancreatic $\beta$-cells, the mutations result in an impaired glucose-induced insulin secretion. Due to decreased intracellular glucose phosphorylation, a higher-than-normal glycemia is required to activate intracellular glucose metabolism and produce a given insulin secretion. However, over 24-h periods, MODY2 patients have normal insulin secretion rates, secondary to moderate increases in glycemia.

The glucokinase mutations at the origin of MODY2 are also expressed in liver cells. Decreased hepatic glucokinase activity can be documented by a decreased rate of cycling between glucose and glucose-6-phosphate, a futile cycle dependent on the activity of both glucokinase and glucose-6-phosphatase (9). As a probable consequence of decreased hepatic glucokinase activity, postprandial glucose uptake and glycogen synthesis are reduced in MODY2 patients compared with healthy subjects (13). Patients with MODY2 have normal absolute rates of fasting glucose production compared with healthy subjects. They also have normal insulin concentrations. These apparently normal values of glucose production and insulin concentrations are observed, however, in the presence of (moderate) hyperglycemia, which by itself should lead to suppression of endogenous glucose production and hyperinsulinemia. It was indeed observed that
healthy subjects had a 75% lower glucose production when studied at similar glycemia and portal insulin concentrations than MODY2 patients, indicating a defective inhibition of glucose production by glycemia in MODY2 (Fig. 3). To further assess the regulation of glucose production in MODY2, we recently monitored glucose production and counterregulatory hormone secretion during insulin-induced hypoglycemia (Guenat et al., unpublished observations). It was observed that hyperinsulinemia inhibited glucose production to the same extent in MODY2 patients and healthy subjects when glucose concentrations were maintained at their basal fasting values. When glycemia was allowed to drop progressively by reducing glucose infusion, endogenous glucose production rose at a glucose concentration that was definitely higher (~90 mg/dl) in MODY2 patients than in healthy controls (~60 mg/dl). There was also an increase of the glucose concentration threshold at which glucagon secretion was triggered (75 mg/dl vs. 60 mg/dl). This indicates that regulation of glucose production was essentially similar in MODY2 patients and healthy subjects, except that the curve linking glucose production to glucose concentrations was shifted to the right in MODY2. This is likely to indicate that decreased glucokinase activity, by impairing hepatic liver sensing, increased the set point at which glycemia is regulated. It is possible that increased sensitivity of pancreatic α-cells or of glucosensitive cells within the central nervous system to hypoglycemia contributed to this counterregulatory response to hypoglycemia in MODY2.

Conclusions

On the basis of the experiments depicted above, several conclusions can be made. First, glucose production is not only controlled by endocrine and neural factors but also by regulatory processes that take place within the liver. This allows the liver to match hepatic glucose production with extrahepatic glucose utilization with minimal changes in glycemia under most physiological conditions. Glucose sensing by liver cells is required for this autoregulatory process. Hepatic glucokinase is involved in hepatic glucose sensing, and the glycemic set point at which glucose production is (auto) regulated is increased when its activity is decreased secondary to heterozygous mutations, as in MODY2. Second, glucose production does not change when gluconeogenesis is acutely stimulated or inhibited. This indicates that net glycogenolysis changes in the opposite direction. It also indicates that the release of systemic glucose is regulated per se, i.e., independently of the individual regulations of gluconeogenesis and glycogenolysis, in the short term at least. The observations made in patients with glucokinase gene mutations and in healthy subjects receiving exogenous gluconeogenic substrate suggest that one or several intrahepatic metabolites of glucose are instrumental in the autoregulation of glucose production. Glucose-6-phosphate is a good candidate for this role. Decreased glucokinase activity is expected to lower intracellular glucose-6-phosphate at any given glucose concentrations, and this may secondarily relieve inhibition of glycogenolysis and/or gluconeogenesis. Administration of gluconeogenic substrate will stimulate glucose-6-phosphate synthesis. The resulting increase in glucose-6-phosphate may in turn inhibit glycogenolysis. This role of glucose-6-phosphate as an intrahepatic regulator of glucose production remains hypothetical, however, and other intrahepatic metabolites may be involved.

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This article will focus mainly on the O2-sensing process that leads to the activation of the O2-sensor and the regulatory transcription factor(s), several factors modulating gene activity in response to the ambient PO2. Changes in ambient PO2 need to be sensed to allow long-term adaptation of cellular functions via the O2-sensing system. The O2 sensor and the regulatory transcription factor(s) activate gene activity. Along the signaling cascade between the O2-sensor and the transcription factor, several reactions, within seconds or minutes, by modification of enzyme activities and the long-term adaptation of cellular functions via regulation of gene expression. This O2-sensing system should consist of the sensor proper, from ROS are produced in mitochondria during respiration when O2 is not completely reduced to water. The resulting oxygen intermediates are the superoxide anion radical (O2−), hydrogen peroxide (H2O2), and hydroxyl radical (OH•). Peroxynitrite (ONOO−) is considered to be a member of the ROS family as well. ROS are highly reactive intermediates that can damage membranes, oxidize proteins, and mutate DNA. These hazardous reactions are often referred to as oxidative stress, resulting in cell damage. However, in a limited fashion ROS might have regulatory functions other than damage. Since the production of ROS increases proportionally with the O2 tension, they are ideal candidates to act as messengers in oxygen-dependent signal transduction.

O2 sensing is a biological principle that developed during evolution; O2 radicals and iron appear to be involved in the O2-sensing process. The Fenton reaction for triggering gene expression could be the key event in the O2 signaling pathway. The generation of OH• from H2O2 in an iron-dependent perinuclear Fenton reaction for triggering gene expression could be the key event in the O2 signaling pathway. Oxygen radicals as messengers in oxygen-dependent gene expression.

References