Maintenance of the postabsorptive plasma glucose concentration: insulin or insulin plus glucagon?

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Raju, Bharathi, and Philip E. Cryer. Maintenance of the postabsorptive plasma glucose concentration: insulin or insulin plus glucagon? Am J Physiol Endocrinol Metab 289: E181–E186, 2005; doi:10.1152/ajpendo.00460.2004.—The prevalent view is that the postabsorptive plasma glucose concentration is maintained within the physiological range by the interplay of the glucose-lowering action of insulin and the glucose-raising action of glucagon. It is supported by a body of evidence derived from studies of suppression of glucagon (and insulin, among other effects) with somatostatin in animals and humans, immunoneutralization of glucagon, defective glucagon synthesis, diverse mutations, and absent or reduced glucagon receptors in animals and glucagon antagonists in cells, animals, and humans. Many of these studies are open to alternative interpretations, and some lead to seemingly contradictory conclusions. For example, immunoneutralization of glucagon lowered plasma glucose concentrations in rabbits, but administration of a glucagon antagonist did not lower plasma glucose concentrations in healthy humans. Evidence that the glycemic threshold for glucagon secretion, unlike that for insulin secretion, lies below the physiological range, and the finding that selective suppression of insulin secretion without stimulation of glucagon secretion raises fasting plasma glucose concentrations in humans underscore the princiacy of insulin in the regulation of the postabsorptive plasma glucose concentration and challenge the prevalent view. The alternative view is that the postabsorptive plasma glucose concentration is maintained within the physiological range by insulin alone, specifically regulated increments and decrements in insulin, and the resulting decrements and increments in endogenous glucose production, respectively, and glucagon becomes relevant only when glucose levels drift below the physiological range. Although the balance of evidence suggests that glucagon is involved in the maintenance of euglycemia, more definitive evidence is needed, particularly in humans.

glucose metabolism; hypoglycemia; diabetes

Given evidence of absolute or relative hyperglucagonemia in patients with type 1 and type 2 diabetes (27, 29, 37, 38), Unger and Orci (48) first proposed that glucagon, in the setting of absolute or relative insulin deficiency, plays a role in the pathogenesis of diabetes. Their bihormonal hypothesis was based on three lines of evidence: 1) endogenous hyperglycemia had never been observed in the absence of glucagon; 2) when both insulin and glucagon were suppressed with somatostatin, hyperglycemia did not occur, and 3) somatostatin-induced suppression of glucagon reduced glycemia in humans with diabetes. With respect to the last, somatostatin has been shown to reduce, but not normalize, fasting and postprandial hyperglycemia (15) and to delay increments in plasma glucose (as well as in plasma nonesterified fatty acids, glycerol, and β-hydroxybutyrate) concentrations following withdrawal of insulin in patients with type 1 diabetes (14). Although these findings are entirely consistent with a role of glucagon in the pathogenesis of hyperglycemia (and ketosis) in type 1 diabetes, control studies with somatostatin administration and replacement of glucagon documenting the reversal of these differences would have strengthened the interpretation that the observed effects of somatostatin were the result of lower glucagon levels. Furthermore, there is evidence that the glycemic response to somatostatin is biphasic in nondiabetic individuals, with an initial decrease in glucose production (22, 41, 44) and plasma glucose (23, 41, 44), followed by an increase in glucose production (22, 41, 44) and plasma glucose (23, 41, 44); i.e., hyperglycemia does occur in the absence of glucagon and when both insulin and glucagon are suppressed. Nonetheless, the bihormonal hypothesis has been supported by evidence that reduced glucagon action lowers plasma glucose concentrations in animal models of diabetes, as mentioned later. To our knowledge, comparable evidence has not been forthcoming in human diabetes. Diabetes aside, what is the physiological role of glucagon, vis-a-vis insulin, in maintenance of the postabsorptive plasma glucose concentration in nondiabetic individuals?

The prevalent view is that the postabsorptive arterial plasma glucose concentration is maintained within the physiological range [72–108 mg/dl (4.0–6.0 mmol/l) with a mean of 90 mg/dl (5.0 mmol/l) in humans] by the interplay of the glucose-lowering action of insulin (specifically, suppression of endog-
enous glucose production) and the glucose-raising action of glucagon (specifically, stimulation of endogenous glucose production) (4, 18). However, although insulin secretion responds to fluctuations in the plasma glucose concentration within the physiological range [e.g., the arterialized venous glycemic threshold for a decrease in insulin secretion as glucose levels decline is 80–85 mg/dl (4.4–4.7 mmol/l) (7, 10, 43)], the glycemic threshold for an increase in glucagon secretion of 65–70 mg/dl (3.6–3.9 mmol/l) (7, 10, 16, 28, 43) lies below the physiological range in healthy humans. Although the latter threshold was defined initially under hyperinsulinemic conditions (7, 10, 28, 43), it has been supported under relative or absolute hypoinsulinemic conditions in humans (2, 16) and in dogs (11). For example, during recovery from hypoglycemia, plasma glucagon concentrations (as well as plasma epinephrine, cortisol, and growth hormone concentrations) fell to baseline levels when plasma glucose concentrations rose to a controlled plateau of 77 ± 2 mg/dl (4.3 ± 0.1 mmol/l), whereas insulin secretion rates remained suppressed and calculated hepatic portal insulin concentrations were at or below baseline levels in healthy humans (16). Similarly, after a 3-day fast, plasma glucagon concentrations appeared to be elevated when the plasma glucose concentration was 64 ± 2 mg/dl [3.6 ± 0.1 mmol/l, and plasma insulin and C-peptide levels were suppressed by 66 and 70%, respectively (2)]. Despite a higher postabsorptive physiological range, a similar pattern occurs in dogs (11). A 10 mg/dl (0.6 mmol/l) decrease in the mean arterial plasma glucose concentration, produced by phosphorylase inhibition, suppressed arterial and hepatic portal venous plasma insulin concentrations maximally, but arterial and hepatic portal venous plasma glucagon concentrations were no different from those during an otherwise identical euglycemic control study (11). [Those authors interpreted a statistically significant increase from baseline in mean arterial plasma glucagon of 8 pg/ml and in mean portal plasma glucagon of 13 pg/ml at 30 min as a response to developing hypoglycemia (glucose decrement 10 mg/dl), but the arterial and portal plasma glucagon concentrations at that time point were superimposed on those during a euglycemic control study.] A further decrease in the mean arterial glucagon concentration to >20 mg/dl (1.1 mmol/l) below baseline raised arterial and hepatic portal venous glucagon concentrations (11). Although hormones exert “tonic” influences on metabolic processes, they cause changes in their metabolic target activities through changes in their concentrations at target tissue cellular receptors. Thus taken together, these data (2, 7, 10, 11, 16, 28, 43) are seemingly inconsistent with the putative role of glucagon in maintenance of the postabsorptive plasma glucose concentration (4, 18).

The alternative view is that the postabsorptive arterial plasma glucose concentration is maintained within the physiological range by insulin alone, specifically regulated increments and decrements in insulin secretion, and the resulting decrements and increments in endogenous glucose production, respectively, and that glucagon becomes relevant only when glucose levels drift below the physiological range. Insulin both suppresses glucose production and stimulates glucose utilization, but glucagon only stimulates glucose production, largely by stimulating glycogenolysis (4, 8). There are several caveats that need to be considered in the interpretation of studies of the physiological role of glucagon. First, it has long been recognized that antibodies used to measure plasma glucagon concentrations recognize species in addition to biologically active 3,483-Da glucagon (46). Therefore, an unknown fraction of measured plasma glucagon concentrations may not be biologically active glucagon. Second, glucagon, like insulin, is a potent hormone (24, 30). Therefore, small changes in glucagon concentrations might have biological effects. Third, glucagon is secreted into the hepatic portal circulation, and exerts its glucose-raising actions on hepatic glucose production, and is partially cleared from the circulation by the liver (4). Therefore, it is the hepatic portal venous glucagon concentration that is most relevant biologically. That cannot be readily measured in humans, making studies in experimental animals particularly important. Fourth, the stimulatory effect of glucagon on glucose production is transient (4, 8); therefore, changes over time need to be considered. Fifth, it is conceivable that basal glucagon levels are necessary to tonically counteract the effects of basal insulin (5). Nonetheless, if glucagon secretion was less sensitive than that of insulin to physiological changes in the plasma glucose concentration, the dynamics of glucoregulation could be affected primarily, or even exclusively, by changes in insulin secretion.

In the paragraphs that follow, we first review the evidence for the prevalent view that the postabsorptive plasma glucose concentration is maintained within the physiological range by the interplay of the glucose-lowering action of insulin and the glucose-raising action of glucagon (4, 18). We then summarize the effects of changes in insulin without changes in glucagon. Glucoregulation involves a complex array of hormonal, neural, and substrate effects. Detailed discussion of these mechanisms is beyond the scope of this review; our focus is on the relative roles of insulin and glucagon in maintenance of the postabsorptive plasma glucose concentration.

EVIDENCE FOR THE PREVALENT VIEW

As noted earlier, infusion of somatostatin, which suppresses the secretion of insulin and glucagon, among other effects, causes a transient reduction in endogenous glucose production (22, 41, 44) and plasma glucose concentrations (23, 41, 44) in overnight-fasted humans. But these are followed by an increase in glucose production (22, 41, 44) and an increase in plasma glucagon concentrations above baseline levels (23, 41, 44), despite continued suppression of plasma insulin and glucagon concentrations. Although these findings suggest an initial tonic effect of basal glucagon to support the postabsorptive plasma glucose concentration, it appears that suppression of insulin is the dominant glycemic effect of somatostatin, and therefore insulin is the primary determinant of the postabsorptive plasma glucose concentration.

In the context of the physiology of glucose counterregulation (8), the mechanisms that prevent or rapidly correct hypoglycemia when glucose levels drift below the physiological range, data from several studies are germane to the biological role of glucagon in maintenance of the plasma glucose concentration within the physiological range. These include studies of the impacts of suppression of endogenous glucagon secretion in experimental animals (5) and humans (40), immunoneutralization of glucagon (3), defective synthesis of biologically active glucagon (12, 49), diverse mutations (1, 17, 25, 26), and absent (13, 32) or reduced (21, 45) glucagon receptors in experimental
animals and of glucagon antagonists in experimental animals (19), cells (33), and humans (35).

**Suppression of glucagon with insulin replacement.** Infusion of somatostatin, which suppresses the secretion of both insulin and glucagon among other effects, with basal insulin replacement by infusion reduced mean postabsorptive plasma glucagon concentrations by ~37% in dogs (5) and by ~30% in humans (40) as a result of reduced rates of endogenous glucose production. Notably, mean plasma glucose concentrations plateaued at ~65 mg/dl (3.6 mmol/l) in the human study (40); they did not fall to frankly hypoglycemic levels because of the glucose-raising actions of epinephrine (8, 40). Although these data suggest that basal glucagon levels support endogenous glucose production and thus plasma glucose concentrations in the postabsorptive state, that interpretation is predicated on the biological precision of the basal insulin replacement. Given the potency of the hormone, even slight insulin overreplacement could have caused the observed decrements in glucose production and plasma glucose. Furthermore, glucagon replacement, during somatostatin infusion with insulin replacement, was not studied. Finally, these experimental conditions eliminated glucose-regulated changes in insulin secretion and, therefore, could have overestimated the impact of suppression of glucagon secretion.

**Immunoneutralization of glucagon.** Administration of a monoclonal antibody to glucagon lowered plasma glucose concentrations in nondiabetic and alloxan-diabetic rabbits (3). In nondiabetic animals, mean plasma glucose concentrations decreased by ~30%. Again, as in the somatostatin studies just summarized, plasma glucose levels then plateaued. In moderately severe diabetic animals, those that survived without insulin therapy, mean plasma glucose concentrations decreased by ~50%. In both groups, plasma insulin concentrations decreased as plasma glucose levels declined. In severely diabetic, insulin-treated animals, mean plasma glucose concentrations decreased by ~40%, with no change in plasma insulin levels. These data seemingly provide strong support for a role of glucagon in maintenance of the postabsorptive glucose concentration. Nonetheless, since the neuroendocrine status of the rabbits under the study conditions was not reported, it is conceivable that the observed impact of glucagon antibody administration was on stimulated, rather than basal, glucagon secretion.

**Defective glucagon synthesis.** Prohormone convertase 2-null (PC2−/−) mice do not process proglucagon and therefore have little or no biologically active glucagon (12, 49). Their mean blood glucose concentrations have been found to be ~100 mg/dl (5.6 mmol/l), lower than those [~150 mg/dl (8.3 mmol/l)] in wild-type mice (12). However, PC2−/− mice also do not process other prohormones, including proinsulin, and have elevated proinsulin levels. Notably, glucagon replacement studies did not support the possibility that the lower blood glucose concentrations were simply the result of glucagon deficiency. Glucagon infusion in a dose of 0.5 μg/h, which raised plasma glucagon concentrations 20-fold compared with those in wild-type mice, was required to raise blood glucose concentrations in PC2−/− mice to levels comparable to those in wild-type mice; a glucagon infusion dose of 0.25 μg/h did not raise blood glucose concentrations in PC2−/− mice (49). Clearly, physiological glucagon concentrations did not correct the abnormality of glucose metabolism. Pharmacological glucagon levels were required.

**Diverse mutations.** Mice lacking the Kir6.2 pore-forming subunit of ATP-sensitive K+ (K_{ATP}) channels (Kir6.2−/−) were found to have reduced glucagon responses to (and delayed glucose recovery from) insulin-induced hypoglycemia (25). However, their fasting blood glucose and plasma insulin (26) and plasma glucagon (25) concentrations were not different from those of Kir6.2+/+ controls. Muscarinic acetylcholine receptor M3 subtype-null mice (M3−/−) were found to have reduced plasma glucagon (and insulin) concentrations (9). Their fed plasma glucose concentrations were reduced, but the animals were hypophagic and had lower body weights than wild-type controls. Fasting plasma glucagon concentrations were similar in M3−/− and M3+/+ mice. Gastrin-deficient (gastrin−/−) mice were found to have lower fasting blood glucose (and plasma insulin) concentrations than gastrin+/+ mice (1). Fasting plasma glucagon concentrations were similar in both groups, but the glucagon response to (and glucose recovery from) insulin-induced hypoglycemia was reduced in the gastrin−/− animals. Mice homozygous for a null mutation in the transcription factor hepatic nuclear factor-3α (HNF3α) (HNF3α−/−) died with growth retardation and hypoglycemia at 10–14 days of postnatal life (17). They were found to have low plasma glucose, insulin, and glucagon concentrations on postnatal day 8. The findings of delayed glucose recovery from and reduced glucagon responses to hypoglycemia in the Kir6.2−/− and gastrin−/− animals are consistent with the role of glucagon in the correction of hypoglycemia (8). However, fasting plasma glucagon concentrations were normal in the Kir6.2−/− and gastrin−/− mice. Lower fasting glucagon levels were not associated with lower glucose levels. Although the lower fasting glucose levels could have been the result of reduced glucagon secretion in the HNF3α−/− mice, or perhaps even in the gastrin−/− mice, glucagon replacement was not shown to raise glucose concentrations in either model. Thus none of these models, Kir6.2−/−, M3−/−, gastrin−/−, or HNF3α−/−, documents a causal relationship between deficient glucagon secretion and reduced postabsorptive glucose concentrations definitively.

**Absent or reduced glucagon receptors.** Glucagon receptor-null (GR−/−) mice have been reported to have lower postabsorptive plasma glucagon concentrations than GR+/+ mice, although that was not apparent in the baseline glucose tolerance test plasma glucagon concentrations which were identical (32). Furthermore, plasma insulin concentrations were not different, a finding seemingly inconsistent with lower plasma glucose concentrations. Notably, those authors concluded that “Despite a total absence of glucagon receptors, these animals maintained near-normal glycemia.” In another report of GR−/− mice, blood glucose concentrations were found to be lower throughout the day (except at 2400) at 10–12 wk of age and in the fasting state at 22–24 wk of age (13).

In lean nondiabetic mice, reduced hepatic glucagon receptor expression, produced by administration of a glucagon receptor antisense oligonucleotide, reduced fed blood glucose concentrations by 8% but had no effect on fasting blood glucose concentrations (21). [The increase in blood glucose from its nadir after insulin injection was reduced as expected, given the role of glucagon in the correction of hypoglycemia (8).] In obese diabetic (db/db) mice, reduced hepatic glucagon receptor
expression reduced fed blood glucose concentrations by 24% but raised fed plasma insulin concentrations 2.3-fold. It reduced fasting blood glucose concentrations by 28% without raising plasma insulin concentrations (21). These data implicate glucagon in the pathogenesis of fasting hyperglycemia in diabetic mice but do not support a role for glucagon in maintenance of the postabsorptive blood glucose concentration in nondiabetic mice. In another study, again in contrast to its effects in diabetic animals, antisense oligonucleotide-induced suppression of glucagon receptor expression, although it lowered insulin and raised glucagon levels, did not lower plasma glucose concentrations in nondiabetic rats (45).

Glucagon antagonists. A peptide glucagon antagonist has been reported to lower blood glucose concentrations in anesthetized streptozotocin-diabetic rats (19), a nonpeptide antagonist to reduce glucagon actions on cells in vitro (33), and another nonpeptide antagonist to reduce glycemic responses to infused glucagon in healthy humans (35). Notably, however, the last glucagon antagonist had no effect on postabsorptive rates of endogenous glucose production or plasma glucose concentrations over the 120-min interval between drug administration and initiation of glucagon infusion, the time frame that included maximum plasma concentrations of the antagonist (35). Taken at face value, the latter data do not support the view that glucagon plays an important role in maintenance of the postabsorptive plasma glucose concentration in humans. Perhaps, however, the antagonist was not sufficiently potent or was not administered in a sufficiently potent dose.

EFFECTS OF CHANGES IN INSULIN WITHOUT CHANGES IN GLUCAGON

Given this background, we used the $K_{ATP}$ channel agonist diazoxide to test the hypothesis that selective suppression of insulin secretion, without stimulation of glucagon secretion, raises the postabsorptive plasma glucose concentration in healthy humans (36). Both intravenous and oral diazoxide caused dose-related increments in plasma glucose and serum nonesterified fatty acid concentrations. Plasma insulin and C-peptide concentrations decreased after intravenous diazoxide and appeared to decrease, despite increasing glucose levels, after oral diazoxide. Plasma glucagon concentrations were unaltered, and the glucagon response to intravenous arginine was not reduced, following diazoxide. Plasma epinephrine concentrations increased slightly, but not to biologically effective concentrations (6), after intravenous but not after oral diazoxide. There were diazoxide dose-related increments in plasma norepinephrine concentrations, undoubtedly a reflection of a compensatory sympathetic neural response to the vasodilatory effect of the drug, but such hemodynamic sympathetic neural activation does not raise postabsorptive plasma glucose or nonesterified fatty acid concentrations (20, 31). Plasma cortisol and growth hormone concentrations were also unaltered. Finally, nonesterified fatty acid elevations did not raise plasma glucose concentrations over the time frame studied (39, 47). Thus selective suppression of insulin secretion, without stimulation of glucagon secretion, raises postabsorptive plasma glucose concentrations. Conversely, it is clear from the clinical treatment of diabetes in pancreatectomized patients, who have no biologically active glucagon, that selective increments in insulin decrease plasma glucose concentrations without suppression of glucagon secretion. Furthermore, in patients with type 1 diabetes, transplanted pancreatic islets provide glucose-regulated decrements in insulin, but no increments in glucagon, as glucose levels decline (34), yet prevent hypoglycemia (42). These data underscore the primacy of regulated insulin secretion in the regulation of the postabsorptive plasma glucose concentration.

COMMENT

Given the primacy of regulated insulin secretion in the regulation of postabsorptive carbohydrate (and lipid) metabolism, what is the role of glucagon? Specifically, is maintenance of the postabsorptive plasma glucose concentration the result of the interplay of the glucose-lowering action of insulin (specifically, suppression of endogenous glucose production) and the glucose-raising action of glucagon (specifically, stimulation of endogenous glucose production) (4, 18)? Alternatively, is maintenance of the postabsorptive plasma glucose concentration the result of regulated increments and decrements in insulin secretion and the resulting decrements and increments in endogenous glucose production, respectively? The latter, but not the former, construct is consistent with the evidence that the glycemic threshold for alterations in insulin secretion lies within the physiological postabsorptive plasma glucose concentration range, whereas that for increased glucagon secretion lies below the physiological range (2, 7, 10, 11, 16, 28, 43), as discussed earlier. If glucagon secretion does not change with fluctuations in plasma glucose concentrations within the physiological range, how can increments in glucagon defend against declining glucose levels within that range?

As discussed earlier, there is a body of evidence, from studies of the impacts of suppression of endogenous glucagon secretion in experimental animals and humans, of immunoneutralization of glucagon, of defective synthesis of biologically active glucagon, of diverse mutations and absent or reduced glucagon receptors in experimental animals, and of glucagon antagonists in experimental animals, cells, and humans, interpreted to support a role of glucagon in maintenance of the postabsorptive plasma glucose concentration. However, as also discussed earlier, many of these studies are open to alternative interpretations. The notion that the postabsorptive plasma glucose concentration is normally maintained within the physiological range primarily by regulated variations in insulin secretion alone is plausible and consistent with most available data. If that were exclusively the case, the role of glucagon would be relegated to defense against plasma glucose concentrations that drift below the physiological postabsorptive range, i.e., the prevention or correction of hypoglycemia (8). The balance of evidence suggests that glucagon is involved in the maintenance of euglycemia, perhaps by tonically counteracting the effects of basal insulin. Nonetheless, more definitive evidence is needed, particularly in humans. Clearly, the physiology need not be dichotomous; both insulin and glucagon could be involved. If so, it would appear that insulin stands higher in the hierarchy of redundant mechanisms that maintain the postabsorptive plasma glucose concentration, as it does in those that prevent or rapidly correct hypoglycemia when plasma glucose concentrations drift below the physiological postabsorptive range (8).
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