Blood Glucose Regulates the Effects of Insulin and Counterregulatory Hormones on Glucose Production In Vivo

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SUMMARY

Continuous, low dose, insulin infusion in conscious dogs produced moderate hypoglycemia but only a transient fall in glucose production that rose towards preinfusion levels 20 to 30 min before any detectable increase in plasma counterregulatory hormones. Addition of epinephrine or glucagon to the insulin infusion prevented the fall in glucose production throughout the experiment but only partially diminished the hypoglycemic response. When hypoglycemia was prevented by a variable glucose infusion, neither epinephrine nor glucagon was able to counteract the suppressive effect of insulin on glucose output. These findings suggest that a fall in blood glucose per se may reverse insulin-induced inhibition of glucose production independent of a rise in counterregulatory hormones and that the insulin antagonist effect of counterregulatory hormones is modulated, at least in part, by blood glucose concentration. DIABETES *28*:533–536, June 1979.

The counterregulatory response of the liver to insulin-induced hypoglycemia is a homeostatic process of importance, aimed at maintaining adequate amounts of glucose to ensure brain function. While hormonal control of this process was investigated extensively, the role of blood glucose concentration, as suggested by Soskin and co-workers[1] over 40 yr ago, remains unestablished. Studies of the perfused liver in vitro suggest the existence of a glucostatic system by which the liver may adapt the rate of glucose output to changes in glucose concentration independent of hormonal modulation.[2–4] Recent studies employing tracer[5] and splanchnic balance[6] techniques indicate that hyperglycemia per se inhibits hepatic glucose output in vivo in the presence of basal insulin levels. These observations raise the question of whether the blood glucose concentration regulates hepatic glucose output in vivo in circumstances of hypoglycemia. The present study was consequently undertaken to answer the following questions: (1) Is the counterregulation of insulin-induced hypoglycemia an entirely hormone-dependent process or does the liver possess an autoregulatory mechanism that responds to hypoglycemia per se? (2) Does blood glucose concentration exert any influence on the anti-insulin effect of counterregulatory hormones (e.g. epinephrine and glucagon) on hepatic glucose output in vivo?

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METHODS

28 experiments were performed on normal conscious dogs (23–31 kg) after a 15- to 18-h overnight fast. A polyethylene catheter was inserted percutaneously into an iliac vein or into a femoral artery (glucose clamp studies) for blood sampling. A cephalic vein was cannulated similarly for infusion of (3-^{3}H)-glucose and glucoregulatory hormones. A priming dose of tracer (30 μCi) was injected intravenously at t = −2 h, followed by a constant infusion (0.25 μCi/min), which was continued throughout the experimental period. An equilibration period of two hours was allowed before perturbing the system with glucoregulatory hormones to ensure that glucose's specific activity had reached a stable plateau.

Two series of experiments were performed. In the first, a constant infusion of crystalline porcine insulin was administered for two hours at a rate of 0.7 $mU \cdot kg^{-1} \cdot min^{-1}$. In the second group of experiments, the same dose of insulin was infused in combination with epinephrine (0.1 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or beef and pork glucagon (3 $ng \cdot kg^{-1} \cdot min^{-1}$). The latter studies were then repeated in dogs receiving an infusion of glucose at a variable rate to maintain plasma glucose at preinfusion levels throughout the infusion of glucoregulatory hormones. This was achieved by monitoring the plasma glucose concentration at 5-min intervals and adjusting the rate of glucose

TABLE 1
Plasma insulin concentration (μU/ml) during intravenous infusion of insulin in normal dogs

0 min*	20 min	40 min	60 min	80 min	100 min	120 min
16 ± 2	47 ± 4	50 ± 3	53 ± 5	55 ± 5	53 ± 5	52 ± 4

* Control values represent the mean of three observations on each subject before insulin administration.

infusion according to the servo-control negative feedback principle (glucose clamp technique).[7] The infusates were freshly prepared in sterile saline. Human serum albumin (250 mg/dl) was added to the insulin and glucagon solutions to prevent adherence to glassware and tubing, and ascorbic acid (30 mg/dl) was added to the epinephrine solution to protect against oxidation.

Plasma glucose was determined on a Beckman glucose analyzer. Methods used for the determination of plasma immunoreactive insulin and glucagon (using Unger's antibody 30K) and glucose's specific activity have been described previously.[8] Plasma epinephrine and norepinephrine were measured by an isotope derivative method[9] and plasma cortisol by a fluorometric method.[10] Glucose output was calculated in the steady state by the isotope dilution principle, while the changes during hormonal perturbation were monitored as a continuous function of time by use of the derivative form of Steele's equation.[11] This method was validated recently for both steady and nonsteady states.[12] In the glucose clamp experiments, the rate of glucose output was calculated by subtracting the rate of exogenous glucose infusion from the rate of total glucose inflow determined by (3-^{3}H)glucose (vide supra). The glucose output data generated in this study are presented in a separate communication.[13]

Statistical analyses were performed with the Student's *t*-test (the paired *t*-test was used when applicable).[14] Data in the text are presented as the means ± SE.

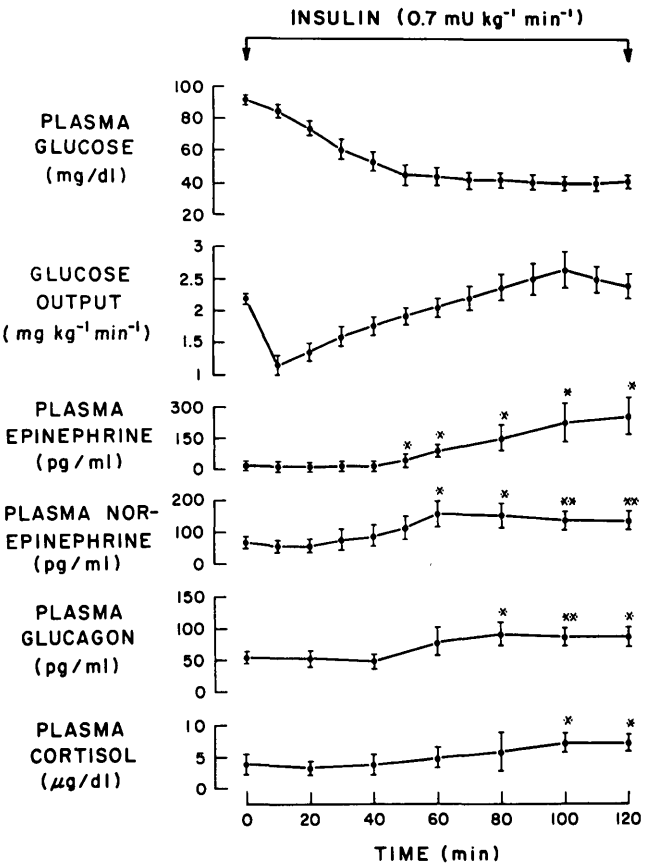
RESULTS

Changes in glucose output and counterregulatory hormones during constant insulin infusion. During the insulin infusion, plasma insulin levels increased rapidly and then stabilized at levels of 50–55 μU/ml throughout the remainder of the study (Table 1). As shown in Figure 1, the insulin infusion produced a progressive fall in plasma glucose, which stabilized after 50 to 60 min at levels 55% below base line. Glucose output fell by 50% at 10 min but then rose progressively, reaching preinfusion levels by 50 min. Glucose production at 30 min and thereafter was significantly greater than that observed at 10 min ($P < 0.01$). Plasma epinephrine and norepinephrine increased significantly at 50 and 60 min, respectively, while a significant rise in glucagon and cortisol levels was observed only after 80 and 100 min, respectively. Thus, the reversal of insulin-induced inhibition of glucose output began 20 to 30 min before an increase in plasma counterregulatory hormones was detectable.

Effect of blood glucose concentration on the anti-insulin action of epinephrine and glucagon.
As shown in Figure 2A, during the combined infusion of epinephrine and insulin, plasma glucose concentration fell after 50 min, reaching values 35% below base line by the end of the infusion period. The fall in glucose output, observed with infusion of insulin alone (Figure 1), was blocked by the addition of epinephrine. In contrast, when euglycemia was maintained by exogenous glucose, endogenous glucose output fell after 40 min and was suppressed by 85% ($P < 0.001$) by the end of the experimental period. Plasma insulin levels (15 to 20 μU/ml in the basal state) rose to 52 ± 8 μU/ml during epinephrine plus insulin and to 58 ± 6 μU/ml when glucose was added. Plasma glucagon concentration was not altered markedly from basal values (48 ± 8 pg/ml) either during the infusion of insulin and epinephrine or when euglycemia was maintained by addition of exogenous glucose (P = NS).

Similar results were obtained with the combined infusion of glucagon and insulin (Figure 2B). When these hormones were infused without glucose, moderate hypoglycemia developed gradually (45 mg/dl at 120 min). The addition of glucagon completely prevented the suppressive effect of insulin on glucose output (Figure 1). Plasma epinephrine (36 ± 9 pg/ml) increased slightly (35 to 40%), but not significantly ($P < 0.1$), only after 100 min, and plasma norepinephrine (104 ± 16 pg/ml) was unchanged. In contrast, when hypoglycemia was prevented by a variable glucose infusion, glucagon produced only a short-lived (for 20 to 30 min) increase in glucose output, which then fell

FIGURE 1. Effects of intravenous insulin infusion on plasma levels of glucose, epinephrine, norepinephrine, glucagon and cortisol, and hepatic glucose output in seven normal dogs (mean ± SE). The control values (t = 0) represent the mean of three base-line determinations. One or two asterisks indicate a P-value <0.05 or <0.01, respectively, when compared with control values (paired *t*-test).



to levels 75% below base-line ($P < 0.001$). In these experiments, plasma insulin and glucagon levels (54 ± 5 μU/ml and 154 ± 16 pg/ml) were comparable to those observed when insulin and glucagon were infused without glucose (50 ± 5 μU/ml and 160 ± 18 pg/ml).

DISCUSSION

The present data demonstrate that the reversal of insulin-induced inhibition of hepatic glucose output is initiated before circulating counterregulatory hormones rise, thus suggesting that the decline in blood glucose concentration has a stimulatory effect on hepatic glucose output through a mechanism that does not require elevated levels of circulating counterregulatory hormones. Previous studies in man showed an earlier increase in plasma catecholamines (25 to 30 min) and a delayed reversal of glucose output that was temporally related to the rise in catecholamines. However, in those experiments, pharmacologic doses of insulin (0.15 U/kg as a bolus) were employed, which produced a much greater rate of fall in glucose concentration.[15] Our data also reveal that epinephrine or glucagon (in the doses employed) counteract only transiently the inhibitory action of insulin on glucose output when the blood glucose concentration is maintained constant. On the other hand, if blood glucose is allowed to fall, the anti-insulin effect of these hormones becomes sustained. Thus, the blood glucose concentration determines if such doses of epinephrine or glucagon have a transient or a sustained effect in reversing insulin-induced inhibition of hepatic glucose production. These findings are in accord with previous studies demonstrating that the inhibitory effect of insulin on hepatic glucose output is sustained only when hypoglycemia is prevented by exogenous glucose[16,17] and suggests that glucose administration sustains the action of insulin primarily by preventing hypoglycemia rather than by preventing a rise in glucagon or epinephrine, a phenomenon that normally accompanies a decline in blood glucose concentration.

The mechanism whereby hypoglycemia contributes to reversal of insulin inhibition of glucose production remains to be established. However, two possibilities are the most likely. First, hypoglycemia may trigger a sympathetic signal that leads to intrahepatic release of norepinephrine. This possibility is not excluded by our findings of unchanged levels of norepinephrine during the first 60 min of the insulin infusion, since the rapid processes of local reuptake and inactivation could have easily prevented the catecholamine from being released in measurable amounts into the bloodstream.[18] Furthermore, this interpretation is consistent with recent studies demonstrating an important role for the sympathetic nervous system in counteracting the hypoglycemic effect of insulin.[19,20] A second possibility is that hypoglycemia acts directly on the liver, inducing rapid changes in the activity of the enzymes regulating glucose output.[21] Alternatively, a delayed fall in portal insulin concentration as a consequence of a fall in endogenous insulin secretion (a result of hypoglycemia) could conceivably contribute to the rebound increase in glucose production. However, it is unlikely that portal insulin levels decreased markedly or returned to basal values, since peripheral insulin concentrations during the insulin infusion exceeded those observed normally in the portal circulation,[22] remained constant throughout the entire study (Table 1), and were not noticeably different whether or not euglycemia was maintained by infusion of exogenous glucose. Nevertheless, a small decline in portal insulin may have resulted, but it was not reflected in peripheral insulin levels in view of the normal portal-peripheral gradient of 2.5 to 3.1.[22] Whatever the explanation, our data indicate that the rebound in glucose output may occur independent of a rise in counterregulatory hormones.

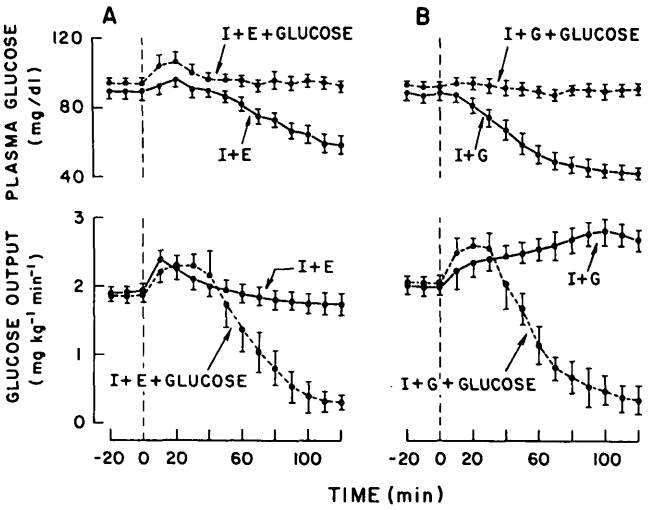


FIGURE 2. A. Changes in plasma glucose and hepatic glucose output (mean ± SE) during infusion of insulin (I) and epinephrine (E) with (four dogs) and without (six dogs) the addition of exogenous glucose to maintain euglycemia. B. Changes in plasma glucose and hepatic glucose output during infusion of insulin (I) and glucagon (G) with (five dogs) and without (six dogs) the addition of exogenous glucose to maintain euglycemia.

A role for blood glucose concentration in the regulation of hepatic glucose output was suggested by previous studies of the perfused liver.[2-4] However, such a role has not been assessed adequately in the intact animal, mainly because of the difficulty in distinguishing the effects of glucose per se from those of the hormonal alterations that inevitably follow deviations from normoglycemia. In the present study we circumvented this difficulty by analyzing the temporal relationships between the changes in hepatic glucose production and plasma counterregulatory hormones during the gradual decline in blood glucose caused by the low dose insulin infusion. In addition, using the glucose clamp technique,[7] we examined the interaction between insulin and epinephrine or glucagon on hepatic glucose output at different plasma glucose levels. The data generated by this approach provide evidence that glucoregulation in vivo is not purely hormonal in nature and that the hormonal influences on the glucoregulatory system are modulated in part by the product of the regulatory process, namely, the changes in blood glucose concentration.

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