Renal Glucose Production During Insulin-Induced Hypoglycemia

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Recent in vivo studies have rekindled interest in the role of the kidney in glucose metabolism. We therefore undertook the present study to evaluate the contribution of the kidney to systemic glucose production and utilization rates during insulin-induced hypoglycemia using arteriovenous balance combined with a tracer technique. Ten days after the surgical placement of sampling catheters in the right and left renal veins and femoral artery and of an infusion catheter in the left renal artery of dogs, systemic and renal glucose kinetics were measured with the peripheral infusion of [6-^{3}H]glucose. Renal blood flow was determined with a flowprobe. After baseline, six dogs received 2-h simultaneous infusions of peripheral insulin (4 mU · kg^{-1} · min^{-1}) and left intrarenal [6,6-^{2}H]dextrose (14 μmol · kg^{-1} · min^{-1}) to achieve and maintain left renal normoglycemia during systemic hypoglycemia. Arterial glucose decreased from 5.3 ± 0.1 to 2.2 ± 0.1 mmol/l; insulin increased from 46 ± 5 to 1,050 ± 50 pmol/l; epinephrine increased from 130 ± 8 to 1,825 ± 50 pg/ml; norepinephrine increased from 129 ± 6 to 387 ± 15 pg/ml; and glucagon increased from 52 ± 2 to 156 ± 12 pg/ml (all $P < 0.01$). Systemic glucose appearance increased from 16.6 ± 0.4 μmol · kg^{-1} · min^{-1} in the baseline to 24.2 ± 0.6 μmol · kg^{-1} · min^{-1} during hypoglycemia when endogenous glucose production was 10.2 ± 1.0 μmol · kg^{-1} · min^{-1} ($P < 0.01$). In the baseline, the liver accounted for 80% (13.3 ± 0.8 μmol · kg^{-1} · min^{-1}) and each kidney contributed 10% (1.6 ± 0.2 μmol · kg^{-1} · min^{-1}) to endogenous glucose production. During hypoglycemia, however, hepatic glucose production decreased to 4.0 ± 0.4 μmol · kg^{-1} · min^{-1}, whereas right renal glucose production doubled to 3.2 ± 0.2 μmol · kg^{-1} · min^{-1} ($P < 0.01$). Left renal glucose production was 17 ± 2 μmol · kg^{-1} · min^{-1}, 14 of which were derived from the exogenous infusion. These results indicate that glucose production by the kidney is stimulated by counterregulatory hormones and represents an important component of the body's defense against insulin-induced hypoglycemia.
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The role of the kidney in glucose metabolism is not well understood. Substantial in vitro evidence indicates that the kidney is capable of both glucose production and utilization (1–5). Krebs and colleagues (1,2) have demonstrated that cells of the proximal convoluted tubule are able to efficiently convert 3-carbon precursors to glucose. At the same time, cells of the distal nephron and those in the interstitial medulla are very active in glucose uptake, glycogen storage, and glucose oxidation (3–5). The in vivo data, however, are less conclusive. Most review articles and textbooks emphasize the dominant role of the liver in regulating glucose appearance (6–8) and indicate that the kidney does not contribute significantly to glucose production, except in prolonged fasting (9) and perhaps uncontrolled diabetes (10). We have recently demonstrated that postabsorptive renal glucose production accounts for ~30% of systemic glucose appearance in dogs (11). These findings have been subsequently confirmed in postabsorptive humans (12). Of additional interest, glucose production by the kidney, analogous to the liver, appears to be suppressed by insulin (11) and stimulated by catecholamines (12). The observations that hypoglycemic episodes in hospitalized patients occur in nondiabetic individuals with renal insufficiency (13) and that hypoglycemia is relatively common among uremic patients subject to protein-calorie deprivation (14) underscore the potential importance of the kidney in glucose homeostasis. These findings have raised the possibility that renal glucose production and utilization may play an important role in glucose counterregulation. The present studies were therefore undertaken to evaluate the contribution of the kidney to systemic glucose production and utilization rates during insulin-induced hypoglycemia using arteriovenous balance combined with a tracer technique.

RESEARCH DESIGN AND METHODS

Animals and surgery. After approval by the Institutional Animal Care and Use Committee at State University of New York, Stony Brook, studies were performed in six 20- to 25-kg male mongrel dogs. Ten days before the experiment, a laparotomy was performed under halothane anesthesia. Silastic sampling catheters (0.040 in [internal diameter] × 0.085 in [external diameter]) were inserted into the left and right renal veins, and a doppler flowprobe (2R, Transonic Systems, Ithaca, NY) was placed around the right renal artery. In addition, a small (0.027 × 0.047 in) non-obstructive infusion catheter was inserted in the midportion of the left renal artery. An arterial sampling catheter was inserted in the aorta via left femoral artery. All catheters were filled with heparinized saline, ligated, and tunneled to a subcutaneous pocket, as previously described (11).

Experimental protocol. After an overnight fast, catheters were taken out, and an infusion catheter was inserted into the precava via a lateral saphenous

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SA, specific activity; RPF, renal plasma flow.

TABLE 1
Plasma glucose concentrations in postabsorptive (baseline) and hypoglycemic (study) dogs

	Artery		Right renal		Left renal	
Dog	Baseline	Study	Baseline	Study	Baseline	Study
1	5.2 ± 0.1	2.2 ± 0.1	5.4 ± 0.1	2.5 ± 0.1	5.3 ± 0.1	5.4 ± 0.2
2	5.3 ± 0.1	2.2 ± 0.1	5.4 ± 0.2	2.4 ± 0.2	5.3 ± 0.2	5.5 ± 0.2
3	5.2 ± 0.1	2.2 ± 0.1	5.3 ± 0.1	2.5 ± 0.1	5.3 ± 0.1	5.5 ± 0.1
4	5.2 ± 0.1	2.2 ± 0.1	5.2 ± 0.1	2.5 ± 0.2	5.3 ± 0.1	5.4 ± 0.2
5	5.3 ± 0.1	2.1 ± 0.1	5.3 ± 0.1	2.4 ± 0.2	5.4 ± 0.1	5.4 ± 0.2
6	5.3 ± 0.1	2.2 ± 0.1	5.3 ± 0.2	2.5 ± 0.1	5.3 ± 0.2	5.4 ± 0.1
Mean	5.3 ± 0.02	2.2 ± 0.02*	5.3 ± 0.03	2.5 ± 0.02†	5.3 ± 0.01	5.4 ± 0.02

Note that left renal-vein glucose concentration reflects left renal plasma normoglycemia attained by direct intrarenal dextrose infusion. *$P < 0.01$ vs. baseline; †$P < 0.01$ vs. baseline and arterial concentration.

vein. At 0800 ($t = -120$ min), a primed constant systemic infusion of [6-^{3}H]glucose (10 μCi, 0.20 μCi/min) was started and continued to the end of the study. The flowprobe was connected to a transducer, and renal blood flow was recorded continuously throughout the study. Baseline femoral artery, right and left renal-vein blood samples were obtained every 10 min from –30 to 0 min for measurements of plasma glucose concentration and specific activity (SA), insulin, glucagon, catecholamines, and microhematocrit. After completion of baseline collections, a 2-h constant peripheral insulin infusion (4 mU · kg^{-1} · min^{-1}) simultaneous with constant left intrarenal [6,6-^{2}H]dextrose infusion (14 μmol · kg^{-1} · min^{-1}) were started. These rates were selected to produce concomitant systemic hypoglycemia of ~40 mg/dl (2.2 mmol/l) and left renal plasma normoglycemia of ~90 mg/dl (5.0 mmol/l), assuming a unilateral renal plasma flow (RPF) of 5 ml · kg^{-1} · min^{-1} (11). Blood samples were obtained again every 10 min between 90 and 120 min for the same measurements as in the baseline period. Additionally, femoral artery and left renal-vein blood samples were obtained for the determination of the plasma enrichment of [6,6-^{2}H]glucose. At the end, the dog was killed and the positions of the catheters were verified.

Analytical techniques. Plasma glucose concentration was measured with a Glucose Analyzer (Beckman, Fullerton, CA). Plasma [^{3}H]glucose SA was determined in deproteinized plasma (15) after deionization with ion exchange resins. Plasma insulin (16) and glucagon (17) were determined by radioimmunoassays, and catecholamines were determined by a radioenzymatic method (18). [^{2}H]glucose atoms percent excess (APE) was determined by gas chromatography/mass spectometry (GC/MS) (19) after derivatization with butane boronic acid in pyridine and acetic anhydride (20).

Calculations. Unilateral RPF was calculated by the following equation:

$$RPF = RBF \cdot (1 - Hct) \quad (1)$$

where RBF is the renal blood flow readings from the flowprobe measured in milliliters per minute and Hct is the microhematocrit value. Left and right RPF were assumed to be identical based on published data using either doppler or indocyanine green infusion (11). Systemic and renal glucose kinetics were calculated as previously described (11), except that in the experimental period, left renal fractional extraction of glucose was calculated according to the formula:

$$FE_g = \frac{\{([Glu]_a \cdot PE_a \cdot RPF) + (IR\ INF)\} - \{([Glu]_{rv} \cdot PE_{rv} \cdot RPF)\}}{\{([Glu]_a \cdot PE_a \cdot RPF) + (IR\ INF)\}} \quad (2)$$

where [Glu] is the plasma glucose concentration, PE is the plasma [^{2}H]glucose enrichment, RPF is the renal plasma flow, and IR INF equals intrarenal [^{2,2}H]glucose infusion rate measured in micromoles per kilogram per minute. The use of monocompartmental equations can be associated with as much as 20% underestimation of the rate of the appearance of glucose under conditions in which there are large and rapid changes in plasma SA, such as during hyperinsulinemia (21,22). However, since isotopic steady state was approximated during the last 30 min of the hypoglycemic period when statistical comparisons were made, underestimation of overall glucose appearance was minimized.

Statistics. Data are expressed as means ± SE. Paired two-tailed Student's t tests were used to compare data obtained at baseline with those from the study period. All P values below 0.05 were considered statistically significant.

RESULTS

Unilateral RPF was 4.4 ± 0.4 ml · kg^{-1} · min^{-1} in the baseline and did not change (4.8 ± 0.2 ml · kg^{-1} · min^{-1}, NS) during hypoglycemia. Arterial plasma glucose decreased from a mean baseline value of 5.3 ± 0.1 to 2.2 ± 0.1 mmol/l ($P < 0.01$) during the last 30 min of the experimental period. Plasma insulin increased from 46 ± 5 to 1,050 ± 50 pmol/l; epinephrine increased 15-fold from 130 ± 8 to 1,825 ± 50 pg/ml; norepinephrine increased threefold from 129 ± 6 to 387 ± 15 pg/ml; and glucagon increased threefold from 52 ± 2 to 156 ± 12 pg/ml during hypoglycemia (all $P < 0.01$). Systemic glucose appearance increased from 16.1 ± 0.4 μmol · kg^{-1} · min^{-1} in the baseline period to 24.2 ± 0.6 μmol · kg^{-1} · min^{-1} ($P < 0.01$) during hypoglycemia, when endogenous glucose appearance was 10.2 ± 1.0 μmol · kg^{-1} · min^{-1}.

Individual animal data for plasma glucose concentrations in the arterial and left and right renal-vein plasma are summarized in Table 1. Plasma glucose was consistently higher in both right and left renal veins than in the artery during hypoglycemia. Plasma [^{3}H]glucose SA was constant in the baseline and during the last 30 min of the experimental period indicating that steady-state had been reached. Plasma [^{3}H]glucose SA decreased from 1,031 ± 97 to 683 ± 67 dpm/μmol in the artery, from 1,007 ± 86 to 559 ± 44 dpm/μmol in the right renal vein, and from 967 ± 82 to 134 ± 25 dpm/μmol in the left renal vein in the baseline and experimental periods, respectively (all $P < 0.01$). In the experimental period, plasma enrichment of [^{2,2}H]glucose was 0.3 ± 0.03% in the artery and 0.7 ± 0.07% in the left renal vein. In the baseline period, left renal glucose production was equal to glucose utilization (1.7 ± 0.4 vs. 1.7 ± 0.2 μmol · kg^{-1} · min^{-1}), and net renal glucose balance was neutral –0.2 ± 0.2 μmol · kg^{-1} · min^{-1}. During systemic hypoglycemia, glucose balance across the left kidney switched to a net output of 15.2 ± 0.5 μmol · kg^{-1} · min^{-1}. Hepatic and right and left renal glucose production rates are depicted in Fig. 1. The rate of glucose production by the left kidney increased to 17.5 ± 1.0 μmol · kg^{-1} · min^{-1}, of which 14.0 μmol · kg^{-1} · min^{-1} represented exogenous dextrose infusion and 3.5 ± 0.5 μmol · kg^{-1} · min^{-1} ($P < 0.05$) were actually produced by the left kidney; left renal glucose utilization increased to 2.3 ± 0.5 μmol · kg^{-1} · min^{-1} ($P < 0.05$). Right renal glucose production was equal to glucose utilization (1.6 ± 0.2 vs. 1.4 ± 0.2 μmol · kg^{-1} · min^{-1}) in the baseline, and net renal glucose balance was neutral –0.3 ± 0.2 μmol · kg^{-1} · min^{-1}. Systemic hypoglycemia was associated with an increase in the rate of glucose production across the right kidney to 3.2 ± 0.2 μmol · kg^{-1} · min^{-1} ($P < 0.05$); the rate of glucose utilization (1.9 ± 0.2 μmol · kg^{-1} · min^{-1}, NS) did not change. As a result, glucose balance across the right kidney switched to a net output

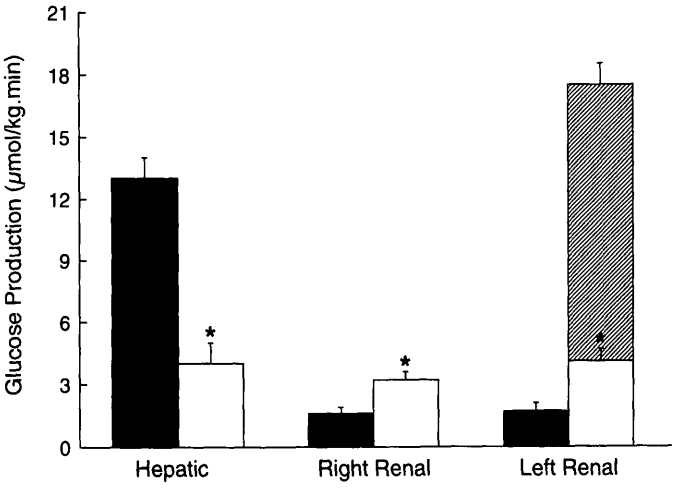

FIG. 1. Hepatic and right and left renal glucose production during baseline (■) and during the last 30 min of systemic hypoglycemia (□). Exogenous dextrose infusion (14 μmol · kg^{-1} · min^{-1}) is depicted above the horizontal line. In the baseline, the liver accounts for ~80% (13 μmol · kg^{-1} · min^{-1}) and each kidney ~10% (1.6 μmol · kg^{-1} · min^{-1}) of systemic glucose production. During hypoglycemia, however, endogenous glucose production accounts for ~10.2 μmol · kg^{-1} · min^{-1} of the total systemic glucose appearance (~24.2 μmol · kg^{-1} · min^{-1}). Hepatic glucose production decreases to ~4.0 μmol · kg^{-1} · min^{-1}, and the liver is responsible for 40% of glucose production. On the other hand, both kidneys contribute 60% of endogenous glucose production (~3.2 μmol · kg^{-1} · min^{-1} for each kidney). *P < 0.01 vs. baseline.

of 1.1 ± 0.1 μmol · kg^{-1} · min^{-1} (P < 0.05); the contribution of the kidney to systemic glucose appearance was 6.2 ± 1.1 μmol · kg^{-1} · min^{-1}.

DISCUSSION

The present studies confirm previous findings in dogs (11) and in humans (12), indicating that postabsorptive renal glucose production is responsible for ~20% of systemic glucose production. In addition, our studies demonstrate that glucose production by the kidney increases significantly during insulin-induced hypoglycemia. Normalization of renal plasma glucose concentration during hyperinsulinemia does not restore renal glucose production to postabsorptive rates. Even though these results contrast with the prevailing notion that endogenous glucose production is equal to hepatic glucose production under conditions of hypoglycemia (23–25), the limitations of previously used techniques to address these questions have not been sufficiently emphasized. Using a combination of arteriovenous difference and tracer technique, our data provide strong evidence that the kidney becomes an important source of glucose in conditions of hypoglycemia, thus complementing previous findings in hypoglycemic dogs (23,24) and in humans (25). A discrepancy of ~1 mg · kg^{-1} · min^{-1} between systemic glucose appearance measured by tracer dilution technique (~3.0 mg/kg^{-1} · min^{-1}) and net hepatic glucose balance measured by arteriovenous difference (~2.0 mg · kg^{-1} · min^{-1}) in the postabsorptive period was readily apparent in data published by Frizzell et al. (23). This difference was further enhanced to ~1.5 mg · kg^{-1} · min^{-1} (~6.0 vs. ~4.5 mg · kg^{-1} · min^{-1}) during hypoglycemia, which suggested the possibility of an extra-hepatic source of glucose during hypoglycemia (24). Similar analyses in human data are lacking because the independent contribution of hepatic and renal gluconeogenesis and glycogenolysis to systemic glucose appearance in conditions of hypoglycemia have not been examined (25).

The biochemical capacity of the kidney to produce glucose is not in question (1–3). Several recent in vivo publications have expanded on the original work published by Cahill (9), emphasizing the role of the kidney in glucose metabolism during hyperinsulinemia (11) and in conditions of high circulating catecholamines (12). Moreover, in view of the fact that renal insufficiency is associated with frequent hypoglycemic episodes (13,14), it is not surprising that the kidney is responsible for a considerable fraction of endogenously produced glucose during hypoglycemia. Nonetheless, the large contribution of the kidney to glucose production during hypoglycemia (~6.2 μmol · kg^{-1} · min^{-1}) should be viewed in the context of its net contribution to plasma glucose, which is significantly attenuated (~2.4 μmol · kg^{-1} · min^{-1}) by simultaneous renal glucose utilization (~3.8 μmol · kg^{-1} · min^{-1}). Our observation that normalization of renal plasma glucose is unable to restore glucose production by the left kidney to postabsorptive rates strongly suggests that counterregulatory hormones such as catecholamines and glucagon exert a stimulating effect on renal glucose production that overrides insulin's inhibitory effect. These findings are in agreement with recently published data in humans, demonstrating that epinephrine infusion enhances renal glucose production (12), consistent with the idea that adrenergic stimulation of lipolysis may contribute to increased glucose production during insulin-induced hypoglycemia (26). However, because plasma glucose concentration in the renal vein exceeds arterial glucose instantly (i.e., within 10 min of the decline in arterial glucose concentration [data not shown]), the possibility that glucose itself exerts an independent acute regulatory effect on renal glucose production should not be ruled out. Whether glucose released by the kidney during hypoglycemia derives predominantly from conversion of glycerol, lactate, or amino acids to glucose in the proximal tubules (1–3) cannot be determined from our studies.

In summary, we have demonstrated that the kidney is responsible for ~60% of systemic glucose production during insulin-induced hypoglycemia in dogs. Normalization of renal plasma glucose in the presence of hyperinsulinemia, hyperglucagonemia, and elevated plasma catecholamines does not restore renal glucose production to postabsorptive rates. We conclude that glucose production by the kidney is stimulated by counterregulatory hormones and represents an important component of the body's defense against insulin-induced hypoglycemia.

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